IONTOPHORETIC TRANSPORT MECHANISMS ACROSS SKIN

by

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This dissertation has been read by each member of the following supervisory committee and by majority vote has been found to be satisfactory.

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To the Graduate Council of the University of Utah:

I have read the dissertation of Sandra Marie Sims in its final form and have found that (1) its format, citations and bibliographic style are consistent and acceptable; (2) its illustrative materials including figures, tables, and charts are in place; and (3) the final manuscript is satisfactory to the supervisory committee and is ready for submission to The Graduate School.

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ABSTRACT

Iontophoresis is the process of increasing the penetration rate of ions into or through a membrane by the application of an external electric field across the membrane. The Nernst-Planck theory has been used to describe the flux enhancement of ions across membranes during iontophoresis. This theory neglects secondary effects such as convective solvent flow and permeability increases due to membrane alterations which result from the applied field. Therefore, the mechanisms whereby charged and uncharged solutes transport across skin under the influence of an applied electric field were investigated.

The modified Nernst-Planck theory, which includes convective solvent flow contributions to the total flux of ions, has been used to describe the flux enhancement of neutral (glucose) and charged (tetraethylammonium ion and salicylate ion) solutes during iontophoresis. Using glucose and a model, net negatively charged membrane (Nuclepore®), solvent flow was shown to be in the direction of counterion (cation) movement. An estimate of the solvent flow velocity was obtained from the glucose flux using the modified Nernst-Planck theory. This velocity value was then used to predict the flux enhancements of a monovalent cation and anion. Comparisons between the theoretical prediction and experimental data using tetraethylammonium ion (cation) and salicylate ion (anion) showed good agreement.

A physical model was developed to gain a more mechanistic understanding of the solvent flow process. The Poisson-Boltzmann equation, which relates the surface potential to the surface charge density, was solved numerically for the radial electric potential profile within a cylindrical pore. The potential profile was then used with the equations of fluid motion to predict the electro-osmotic velocity. The membrane pore partitioning behavior
for the tetraethylammonium and salicylate ions was then predicted at selected ionic strengths using the best estimates of the electrical potential profile. The agreement between experimental and theoretical partition coefficient values was very good.

Iontophoretic studies employing human skin demonstrated that there were both electro-osmosis effects and field induced membrane alterations in addition to direct electric field effects on ion transport. Membrane alterations occurred at 1000 mV but not at lower voltages. These alterations reversed after removal of the applied voltage. Mannitol flux enhancement in human skin showed only a 20-25% solvent flow contribution to the modified Nernst-Planck flux for monovalent cations and anions. Experiments with tetraethylammonium and salicylate ions were in good agreement with the predictions of the modified Nernst-Planck equation when membrane alteration effects were taken into consideration.
To my mother,
Eleanor Snider Sims,
and
my father,
Edward Bruce Sims
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LIST OF SYMBOLS

\( a \)  pore radius, cm
\( a' \)  effective pore radius, cm
\( A \)  surface area, cm\(^2\)
\( c \)  concentration, gm. moles/cm\(^3\)
\( \Delta C \)  concentration difference between donor and receiver chambers
\( d \)  hard sphere diameter, cm
\( D \)  diffusion coefficient, cm\(^2\)/s
\( e \)  electronic charge
\( E \)  enhancement factor due to applied electrical field
\( E \)  applied field per unit length, V/cm
\( f \)  force, dyne
\( F \)  Faraday's constant, coulombs/equivalent
\( F \)  electric field vector
\( I \)  ionic strength, M
\( J \)  flux, gm. moles/cm\(^2\) \cdot s
\( K \)  dimensionless constant
\( K(r) \)  partition coefficient at point \( r \)
\( i \)  length of cylindrical pore, cm
\( n \)  concentration, molecules/m\(^3\)
\( n \)  number of experiments
\( N \)  Avagadro's number
\( p \)  total pressure, dynes/cm\(^2\)
\( P \)  permeability coefficient, cm/s
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<tbody>
<tr>
<td>Q</td>
<td>receiver side radioactivity, dpm</td>
</tr>
<tr>
<td>r</td>
<td>radius, cm</td>
</tr>
<tr>
<td>R</td>
<td>gas constant, joule/g · mole °K</td>
</tr>
<tr>
<td>Rc</td>
<td>radius of curvature</td>
</tr>
<tr>
<td>t</td>
<td>time, s</td>
</tr>
<tr>
<td>T</td>
<td>temperature, °K</td>
</tr>
<tr>
<td>u</td>
<td>mobility, cm/s · dyne</td>
</tr>
<tr>
<td>v</td>
<td>velocity, cm/s</td>
</tr>
<tr>
<td>x</td>
<td>direction of flux</td>
</tr>
<tr>
<td>Δx</td>
<td>thickness of the membrane, cm</td>
</tr>
<tr>
<td>Xs</td>
<td>fraction of undissociated species</td>
</tr>
<tr>
<td>z</td>
<td>valency</td>
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**Greek letters**

<table>
<thead>
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<tr>
<td>β</td>
<td>partition coefficient</td>
</tr>
<tr>
<td>ε</td>
<td>bulk dielectric constant</td>
</tr>
<tr>
<td>γ</td>
<td>activity coefficient</td>
</tr>
<tr>
<td>ΔΨ</td>
<td>potential difference, millivolts</td>
</tr>
<tr>
<td>η</td>
<td>solvent viscosity, gm/cm · s</td>
</tr>
<tr>
<td>κ</td>
<td>Debye-Huckel reciprocal length, cm⁻¹</td>
</tr>
<tr>
<td>μ</td>
<td>chemical potential, joule/gm · mole</td>
</tr>
<tr>
<td>μ</td>
<td>electrochemical potential, joule/gm · mole</td>
</tr>
<tr>
<td>ν</td>
<td>partial molal volume, cm³</td>
</tr>
<tr>
<td>ρ</td>
<td>excess charge density</td>
</tr>
<tr>
<td>σ</td>
<td>surface charge density, C/m²</td>
</tr>
<tr>
<td>Ψ</td>
<td>electric potential, volts</td>
</tr>
<tr>
<td>Ψ₀</td>
<td>surface potential</td>
</tr>
<tr>
<td>Ω</td>
<td>electrical resistance, ohms</td>
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xvii
\( \zeta \)  
zeta potential, volts

\( \nabla \)  
Laplacian operator

**Subscripts**

1  
referenced to an initial passive permeability coefficient

2  
referenced to a second passive permeability coefficient

0  
initial passive value

0'  
second passive value

BL  
boundary layer

d  
donor side

d'  
donor side electrolyte gap

DE  
dermis/epidermis

G  
Glucose

i,j  
species

L  
lipoidal pathway

M  
membrane

P  
aqueous pore pathway

r  
receiver side

r'  
receiver side electrolyte gap

SC  
stratum corneum

T  
total

+  
cation

-  
anion

\( \Delta \Psi \)  
during application of voltage

**Superscripts**

0  
standard state

-  
property in the membrane phase
## LIST OF ABBREVIATIONS

<table>
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<tr>
<td>PBS</td>
<td>phosphate buffered saline</td>
</tr>
<tr>
<td>mCi</td>
<td>milliCuries</td>
</tr>
<tr>
<td>MW</td>
<td>molecular weight</td>
</tr>
<tr>
<td>μl</td>
<td>microliter</td>
</tr>
<tr>
<td>ml</td>
<td>milliliter</td>
</tr>
<tr>
<td>mV</td>
<td>millivolt</td>
</tr>
<tr>
<td>dpm</td>
<td>disintegrations per minute</td>
</tr>
<tr>
<td>P-value</td>
<td>permeability coefficient</td>
</tr>
<tr>
<td>E-value</td>
<td>enhancement factor</td>
</tr>
<tr>
<td>Pe-value</td>
<td>Peclet number</td>
</tr>
<tr>
<td>σ-value</td>
<td>surface charge density</td>
</tr>
<tr>
<td>PB</td>
<td>Poisson-Boltzmann</td>
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CHAPTER 1

INTRODUCTION

Iontophoresis is the process of increasing the rate of penetration of ions into or through a tissue by the application of an external electric field across the tissue. Such a definition identifies iontophoresis as a particular class of the more general problem of transport of ions across membranes in the presence of an electric field. This enables the fundamental thermodynamic treatments of this problem developed in the fields of electrochemistry (Bockris and Reddy, 1970), membrane biophysics (Schultz, 1980), and physiology (Curran and Schultz, 1968) to be applied to iontophoresis. These fields have traditionally been concerned with the problem of ion diffusion under the influence of an electric field.

The transdermal delivery of many ionized drugs at therapeutic levels is not possible because their rate of diffusion under a concentration gradient through the "barrier layer," the stratum corneum, is too slow. By superposition of an electric potential gradient on the existing chemical potential gradient for diffusion, iontophoresis can enhance the flux of ionized drugs into the skin. The magnitude of flux enhancement is dependent upon the magnitude of the applied potential gradient. Thus, it may be possible to elevate the flux of ionized drugs to therapeutic levels by iontophoresis. Furthermore, iontophoresis is a noninvasive technique that could greatly expand the classes of compounds currently considered to be candidates for administration via the transdermal route. In the development of transdermal drug delivery systems, iontophoresis could serve as a substitute for chemical penetration enhancers eliminating those problems associated with the presence of penetration enhancers. Alternatively, iontophoresis could synergize with a chemical penetration enhancer to potentially permit transdermal delivery of high molecular
weight polypeptides. While much of the early work on iontophoretic drug delivery was geared towards achieving localized therapeutic effect (Comeau et al., 1973; Gangarosa et al., 1974), this technique has been increasingly investigated as a tool for systemic therapy (Okabe et al., 1986; Siddiqui et al., 1987).

While the utility and potential of iontophoretic transdermal drug delivery are beyond dispute, little information of a fundamental nature is available on the physical and chemical properties controlling the iontophoretic mechanisms. This is admittedly a very complex problem involving interactions between the drug and its vehicle electrolyte or buffer, partitioning of the drug between its vehicle and skin, and finally diffusion through a highly heterogeneous membrane under the combined influence of chemical and electrical potential gradients. Once the factors controlling iontophoretic mechanisms are better understood and quantified, iontophoresis could become a basis for the design of future controlled release drug delivery systems.

1.1 Overview of Clinical Iontophoretic Studies

Iontophoresis has been used in the treatment of a variety of disease states. Applications of iontophoresis in the treatment of skin diseases (e.g., hyperhidrosis, plantar warts, herpes simplex) have been reviewed by Sloan and Soltani (1986). Duke-Elder (1962) has discussed iontophoretic treatments in ophthalmology. Other areas of use include topical application of steroids to areas of inflammation (Glass et al., 1980), cancer chemotherapy (Beving et al., 1990), diagnosis of cystic fibrosis (Glikfeld et al., 1989), treatment of periodontal disease (Ciancio, 1985), angina/hypertension (Okabe et al., 1985), burns (Glass et al., 1980; Rapperport, 1965), and various types of anesthesia (Russo et al., 1980; Karlin, 1989; Bezzant et al., 1988; Comeau et al., 1973). Recent reviews of other biomedical applications of iontophoresis are available (Tyle, 1986; Banga and Chien, 1988). In addition, Tyle (1986) has given an overview of commercially available iontophoretic devices.
Iontophoresis currently is being investigated as a means of delivering peptides (Burnette and Marrero, 1986; Bodde et al., 1980; Srinivasan et al., 1989; De Koninck and Henry, 1989; Pettit and Mueller, 1989) and small proteins (Siddiqui et al., 1987; Srinivasan et al., 1989). Additionally, transdermal iontophoretic treatment of various systemic disease states in animal models continue, for example, the very recent work of Wearley and Chien (1990) in which the anti-AIDS (Auto-Immune Difficiency Syndrome) drug azidothymidine was iontophoresed across hairless mouse skin.

The transdermal route of iontophoresis is of special interest due to its non-invasive nature and the possibility of treating systemic as well as topical diseases. In the present work, the primary focus was on transdermal iontophoretic drug delivery. Specifically of interest were the mechanisms by which an applied electric field caused an increase in the fluxes of charged and uncharged solutes across skin. To this end, it was necessary, first, to understand the structure of skin.

1.2 Anatomy of the Skin

The skin is composed of three distinct layers: the epidermis, dermis, and panniculus adiposus (Montagna and Parakkal, 1974; Junqueira et al., 1983). The epidermis is a multilayered tissue consisting of two main cell types: keratinocytes and melanocytes. During maturation, keratinocytes lose their nuclei and become highly ordered, keratin filled cells. The anucleated outermost layer (10-50 mm thick) of the epidermis is called the stratum corneum. It is the primary barrier to the penetration of drugs and toxins and also acts to prevent loss of fluids from the body (Scheuplein and Blank, 1971; Brown et al., 1988). The stratum corneum is a heterogeneous structure containing about 40% protein, 40% water, and 15 to 20% lipids. Structurally, it is comprised of a layered, close packed array of dead, partially desiccated, keratinized cells cemented together by intercellular lipids; the intercellular phase is clearly distinguishable from the intracellular phase. Protein is present in both phases, but the lipids are largely concentrated in the
extracellular phase. The stratum corneum is pierced by appendages, such as sweat glands and hair follicles. The appendages account for relatively small total fractional area (\( @ 10^{-3} \)). This does not represent an open-pore area, but an area characterized by the diffusivity of hair and the cells comprising the sweat ducts.

Below the stratum corneum lies the living epidermis, a layer of rapidly proliferating, nucleated cells (Scheuplein and Blank, 1971). The dermis is located below the living epidermis. It is composed of connective tissue, nerves, blood and lymph vessels, glands, appendages and a few cells such as mast cells, fibroblasts and histiocytes. The dermis functions to support and bind the epidermis to the underlying subcutaneous tissue.

Underlying the dermis is the panniculus adiposus or subcutaneous tissue. It functions to bind the skin (dermis/epidermis) to subjacent organs, making it possible for the skin to slide over them (Junqueira et al., 1983; Montagna and Parakkal, 1974).

1.3 Theory of Ion Transport

The physical-chemical description of ion transport under the influence of an applied electric field was established by Nernst and Planck (Aveyard and Hayden, 1970; Bockris and Reddy, 1970). This problem has also been approached from the viewpoint of irreversible thermodynamics (Burnette and Marrero, 1986; Banga and Chien, 1988; Pikal, 1990). However, this approach requires a knowledge of cross-coefficients both in the presence and absence of an electric field. These cross-coefficients are difficult to determine experimentally, especially if the membrane is altered during iontophoresis. Therefore, in order to gain insight into the mechanism of ion transport under the influence of an electric field in a well defined system, the Nernst-Planck theory has been employed in the studies presented herein. Since iontophoresis is the process whereby an external electric field is used to increase the flux of ions across a tissue, the equations of ion transport should be
applicable. A discussion on the derivation of the Nernst-Planck theory of ion motion, similar to that offered by Schultz (1980), follows.

1.3.1 Electrochemical potential gradient

The electrochemical potential of a species is defined as (Castellan, 1983):

\[ \mu_i = \mu_i^0 + z_i F \Psi \]  
(1.1)

where \( \mu_i \) is the electrochemical potential of species \( i \), \( \mu_i^0 \) the chemical potential, \( z_i \) the charge on \( i \), \( F \) the Faraday’s constant and \( \Psi \) the electric potential. The electrochemical potential is the partial molal free energy of species \( i \) and combines the partial molal free energy change resulting from a change in the chemical composition of the system (the chemical potential \( \mu_i^0 \)) as well as from the addition of charge to a system whose electrical potential is \( \Psi \) when \( z_i \neq 0 \).

In this treatment, it is implicitly assumed that solutions are ideal, i.e., for a system at constant temperature \( T \),

\[ \mu_i = (\mu_i^0)_T + RT \ln c_i + z_i F \Psi + v_i p, \]  
(1.2)

where \( \mu_i^0 \) is the standard state chemical potential, \( v_i \) the partial molal volume, \( p \) the total pressure, \( c_i \) the concentration of species \( i \), and \( R \) the universal gas constant. Furthermore, the term \( v_i p \) for most solutes is about three orders of magnitude smaller than the other terms and its contribution to \( \mu_i \) can be neglected. Therefore,

\[ \mu_i = (\mu_i^0)_T + RT \ln c_i + z_i F \Psi \]  
(1.3)

1.3.2 Nernst-Planck model

Consider a system where 1 cm\(^3\) of solution containing \( n \) solute particles lies adjacent to 1 cm\(^2\) of membrane surface, as illustrated in Fig. 1.1. It is assumed that the solution is perfectly stirred and that the diffusion process is controlled by the membrane resistance. The number of particles that will cross the membrane in the \( x \) direction per unit time is simply:

\[ J_i = c_i v_i \]  
(1.4)
Figure 1.1: A 1 cm$^3$ volume of solution adjacent to a membrane with a surface area of 1 cm$^2$. (Adapted from Schultz, 1980)
where $J_i$ is the steady state flux of solute and $v_i$ the velocity of each solute particle. At steady state, the velocities are constant. Under these conditions, the total force acting on a particle is zero, so that the driving force $f_i$ is balanced by the retarding frictional forces. For example, for diffusion of a spherical particle with radius $r_i$ in a fluid whose viscosity is $\eta$, the velocity is found to be:

$$v_i = \frac{1}{6\pi \eta r_i} f_i = u_i f_i$$  \hspace{1cm} (1.5)$$

where the proportionality constant $u_i$ is the velocity per unit force and is defined as the mobility. Thus, $u_i$ is inversely proportional to the viscosity of the surrounding fluid and the radius of the particle. Equation 1.5 follows from the Stokes-Einstein relationship (see for example Bockris and Reddy, 1970).

In general, force may be defined as the rate of change of energy with distance so that the force acting on a mole of matter is simply the gradient of the free energy per mole or the gradient of the electrochemical potential. This gradient is the driving force for diffusion. Thus,

$$f_i = \frac{d\mu_i}{dx}$$  \hspace{1cm} (1.6)$$

Combining Eqns. 1.4, 1.5, and 1.6, results in:

$$J_i = -c_i u_i \frac{d\mu_i}{dx}$$  \hspace{1cm} (1.7)$$

Equation 1.7 is referred to as the Nernst-Planck equation and is the most common starting point for the formal description of diffusion. The negative sign is included to make $J_i$ positive when $(d\mu/dx)$ is negative, i.e., the flow is defined as positive when it takes place from a region of higher electrochemical potential to one of lower electrochemical potential.

The Nernst-Planck equation assumes that $J_i$ is driven entirely by its conjugate driving force (the electrochemical potential gradient) and is not affected by other flows or forces. In particular, Eqn. 1.7 assumes that there is no convective flow of species $i$ resulting from interaction with the flow of solvent.
Combining Eqns. 1.3 and 1.7 results in an expression for diffusion across a membrane at constant temperature:

\[ J_i = -\bar{c}_i u_i \left[ \frac{RT}{dx} \ln \bar{c}_i + \frac{z_i F d\bar{\Psi}}{dx} \right] \]  

(1.8)

where a bar over a symbol represents the quantity in the membrane phase, as opposed to the quantity in the bulk fluid on either side of the membrane, which is represented without bars over the symbol. This has been illustrated in Fig. 1.2. At steady state, \( J_i \) is the same at all points within the membrane (the unsteady state case has been treated by Keister and Kasting, 1986). Using the Einstein relationship \( D_i = RTu_i \), where \( D_i \) is the diffusion coefficient, Eqn. 1.8 can be rearranged to:

\[ J_i = -\bar{D}_i \left[ \frac{d\bar{c}_i}{dx} + \frac{\bar{c}_i z_i F d\bar{\Psi}}{RT dx} \right] \]  

(1.9)

Assuming that \( \bar{D}_i \) is constant throughout the membrane, Eqn. 1.9 can be integrated across the thickness of the membrane for specific cases.

1.3.3 The constant-field equation

When there is an electrical potential difference across the membrane \( \Delta \Psi \neq 0 \), integration of Eqn. 1.8 is quite complex. At steady state \( J_i \) is constant. However, to integrate Eqn. 1.9, the dependence of \( \bar{\Psi} \) on \( x \) within the membrane (i.e., the electrical potential profile) must be known. The simplest and most frequently used assumption is the "constant field" assumption suggested by Goldman (1943), that is, \( \bar{\Psi} \) is a linear function of \( x \) or that \( (d\bar{\Psi}/dx) = (\Delta \bar{\Psi}/\Delta x) \) so that \( \bar{\Psi}_x = (\Delta \bar{\Psi}/\Delta x)_x \), where \( x \) is some point in the membrane between 0 and \( \Delta x \). With this assumption, Eqn. 1.9 becomes a linear, inhomogeneous, constant coefficient, first order differential equation and can be readily solved. The complete solution, for the boundary conditions indicated in Fig. 1.2, is (Bockris and Reddy, 1970b):

\[ J_i = -\bar{D}_i z_i F \Delta \Psi \left[ \bar{c}_i \exp \left( \frac{z_i F \Delta \Psi}{RT} \right) - \bar{c}_i, d \right] \]  

\[ \frac{RT \Delta x}{\exp \left( \frac{z_i F \Delta \Psi}{RT} \right) - 1} \]  

(1.10)
Figure 1.2: Steady state diffusion across a membrane. (Adapted from Schultz, 1980)
Equation 1.10 is frequently referred to as the Goldman (1943) equation or the constant-field flux equation and is in terms of intramembrane properties. In an effort to relate $J_i$ to the measurable properties of the external solution, Hodgkin and Katz (1949) assumed that the partition coefficient $\beta$ across the two membrane-solution interfaces is concentration independent so that

$$\bar{c}_{i,d} = \beta c_{i,d} \quad \text{and} \quad \bar{c}_{i,r} = \beta c_{i,r}$$  \hspace{1cm} (1.11)

If the translocation of the ion through the membrane is slow compared to the rate at which the ion can partition across the interface, so that the interfacial distribution can be considered to be an equilibrium distribution, then it can be shown that (Schultz, 1980):

$$(\bar{\Psi}_r - \bar{\Psi}_d) = (\Psi_r - \Psi_d) = \Delta \Psi$$  \hspace{1cm} (1.12)

Under these conditions:

$$J_i = -P_i K \left( \frac{c_{i,d} - c_{i,r} \exp (K)}{1 - \exp (K)} \right)$$  \hspace{1cm} (1.13)

where $P_i$ is the permeability coefficient defined as:

$$P_i = \frac{D_i \beta_i}{\Delta x}$$  \hspace{1cm} (1.14)

and $K$ is a dimensionless constant as follows:

$$K = \frac{z_i F \Delta \Psi}{RT}$$  \hspace{1cm} (1.15)

Equation 1.13 is often referred to as the Goldman-Hodgkin-Katz (GHK) equation and has been widely used for the description of ion transport across biological membranes. Implicit in the GHK equation is the so-called independence principle – the movement of a single ion at a point within the membrane is influenced only by the electrochemical potential gradient of the ion at that point, and is independent of the presence of other ions of the same or different species.

Several other approaches have been taken toward the integration of the Nernst-Planck equation. These solutions are more complex, do not offer any a priori advantages
over the constant field assumption and are infrequently encountered. These approaches are discussed by Lakshminarayanaiah (1969).

1.3.4 Particular cases of the GHK equation

The GHK equation (Eqn. 1.13) may be simplified under various conditions. The following approximations to the GHK equation are found to be useful for the description of experimental iontophoretic flux data.

1. If $\Delta \Psi = 0$ (no electric potential gradient), then Eqn. 1.13 should simplify to the corresponding solution for passive diffusion. Taking the limit of $J_i$ as $\Delta \Psi$ tends to 0 in Eqn. 1.13,

$$\lim_{\Delta \Psi \to 0} J_i = J_i \big|_{\Delta \Psi = 0} = P_i (c_{i,d} - c_{i,r})$$

which is the appropriate equation for passive, steady state diffusion. For the case where sink conditions ($c_{i,r} = 0$) prevail on the receiver side,

$$J_i \big|_{\Delta \Psi = 0} = P_i c_{i,d}$$

Equation 1.17 is valid for diffusion of an uncharged species (which is unaffected by the electric field) and can be readily derived from Eqn. 1.8 with $z_i = 0$.

2. In both in vivo and in vitro iontophoresis situations, sink conditions usually prevail ($c_{i,r} = 0$). For this case, Eqn. 1.13 reduces to

$$J_i = -P_i c_{i,d} \left( \frac{K}{1 - \exp(K)} \right)$$

Equation 1.18 is a very good approximation to Eqn. 1.13 in most cases if the applied voltage is sufficient. For example, even when $c_{i,d} = c_{i,r}$, if $\Delta \Psi = -0.1$ V, $z_i = +1$, and $T = 37^\circ$C ($310^\circ$K), then Eqn. 1.18 differs from Eqn. 1.13 by approximate 1%. In practical terms, this means that the electrical potential gradient dominates the chemical potential gradient for diffusion of ions.
3. An enhancement factor $E$ can be defined to quantify the influence of the applied potential gradient on the ion flux as compared to the ion flux in the absence of the applied voltage:

$$ E = \frac{\text{flux with applied voltage}}{\text{flux without applied voltage}} = \frac{J_i}{J_i |\Delta \psi = 0} $$

or

$$ E = \frac{-K}{1 - \exp(K)} $$

(1.19)

The exponential term in Eqn. 1.19 decays very rapidly to zero. For example, for a monovalent cation ($z_i = +1$) at $\Delta \psi = -0.1$ V and $T = 37^\circ$C ($310^\circ$K), the exponential term is only $\sim 0.01$. Hence for all practical purposes

$$ E = -K = -\frac{z_i F \Delta \psi}{RT} $$

(1.20)

Therefore, the enhancement due to the applied electric field is directly proportional to the applied voltage drop and the charge on the ion. Equation 1.20 allows the prediction of the flux enhancement due to an applied voltage drop across a membrane, relative to passive diffusion alone.

1.3.5 Assumptions of the GHK equation

At first glance, there are several assumptions in the derivation of Eqns. 1.13 - 1.20 which appear to limit its utility. It is instructive to examine the implications of these assumptions in greater detail, in order to better understand the applicability of the flux enhancement ratio, Eqn. 1.20, to iontophoresis situations.

1.3.5.1 Structure of the skin

The pathways of iontophoretic permeation through the skin are not well understood and need more detailed investigation. It has been reported that solutes diffuse through sweat ducts or hair follicles substantially faster than through the intact stratum corneum (Scheuplein, 1967). If this is the case, then the lag time, defined as the time required to
reach steady state, should be much smaller for diffusion through appendages than through
the intact stratum corneum. Therefore, appendageal diffusion must predominate during the
unsteady state period. However, the large diffusivity in the appendages is more than
compensated for by their small effective fractional area. At steady state, the difference in
surface area between the two routes can compensate for the difference in the diffusion
coefficient and support the concept of intrinsically slow diffusion occurring throughout the
bulk of the stratum corneum (Scheuplein, 1967). The often cited work of Abramson and
Gorin (1940) as "proof" that iontophoresis is through appendageal diffusion should be
viewed in light of this apparent conflict between steady state and nonsteady state diffusion.
Abramson and Gorin (1940) demonstrated that penetration through sweat ducts under a
potential gradient could occur within 1-5 minutes with no comparable transport through the
stratum corneum within this period. Clearly, all that their work demonstrates is that
appendageal diffusion dominates during the initial stages (the nonsteady state).

The body of knowledge on the effect of ion size, degree of hydration, charge and
potential difference is insufficient to draw specific conclusions regarding the pathways of
the diffusion process. In the light of this, it is best to interpret steady state experiments as
diffusion through the entire skin surface characterized by an average permeability
coefficient.

1.3.5.2 Effect of solution nonidealities

In the derivation of the GHK flux equation (Eqn. 1.13), it is assumed that the
solutions behave ideally, i.e., there are no solute-solute interactions. To account for
possible interactions among ions, Eqn. 1.3 for the electrochemical potential of species i
should be modified to (Bockris and Reddy, 1970):

\[ \mu_i = (\mu_i^0)_T + RT \ln \xi_i + z_iF\Psi + RT\ln \eta_i \]  (1.21)
where $\gamma_i$ is the activity coefficient of species $i$ and absorbs all the nonidealities. Combining Eqns. 1.7 and 1.21, the expression for the steady state flux across a membrane (corresponding to Eqn. 1.9) can be written as:

$$J_i = -D_i \left[ \frac{d\tilde{c}_i}{dx} + \frac{\tilde{c}_i z_i F d\tilde{V}}{RT \frac{d\tilde{V}}{dx}} + \frac{\tilde{c}_i}{\gamma_i} \frac{d\gamma_i}{dx} \right]$$

(1.22)

Since the activity coefficient is only a function of concentration, which varies with distance, Eqn. 1.22 may be rewritten using the chain rule of differentiation as:

$$J_i = -D_i \left[ \frac{\tilde{c}_i z_i F d\tilde{V}}{RT \frac{d\tilde{V}}{dx}} + \frac{d\tilde{c}_i}{dx} \left( 1 + \frac{\tilde{c}_i}{\gamma_i} \frac{d\gamma_i}{dx} \right) \right]$$

(1.23)

If the variation of the activity coefficient is not significant over the concentration difference which produces diffusion, then $(\tilde{c}_i / \gamma_i) \left( \delta \gamma_i / \delta \tilde{c}_i \right) \ll 1$, and for all practical purposes Eqn. 1.23 is identical to Eqn. 1.9.

It can be shown (Bockris and Reddy, 1970), in diffusion between solutions which have a large concentration difference, such as 0.1 M to 0.01 M, that the activity coefficient correction term in Eqn. 1.23 is on the order of a few percent. Furthermore, in most in vitro iontophoresis situations, the electrolyte solutions in the donor and receiver chambers are identical and the drug is present on the donor side in tracer levels. The nonidealities are primarily due to electrostatic or coulombic interactions between charged species. Therefore, the coulombic interactions of the drug ion are most likely with the foreign electrolyte ions, rather than with other drug ions (which are present only in tracer levels). Since there is no gradient of the ionic concentration across the membrane, the activity coefficient correction term may be insignificant and the use of ideal solution expression for $\mu_i$ is unlikely to result in any significant error. In any iontophoresis situation where the major current carrying species is an electrolyte such as sodium chloride, the drug ion is present in relatively small concentrations. Therefore, the activity coefficient correction term is likely to be small and may be neglected without sacrificing accuracy to a significant degree.
1.3.5.3 Solvent transport

In the absence of an external pressure gradient and/or osmotic pressure gradient across the membrane, there are no conjugate intensive property gradients to cause convective flow of solvent across the membrane. However, solvent transport may still result from nonconjugate driving forces, e.g., current induced net flow of water (Barry and Hope, 1969). Such instances have been reported in the literature (Gangarosa et al., 1980; Burnette and Marrero, 1986). There are no pressure (total or osmotic) gradients in the present experimental system, except perhaps current induced effects, because the two chambers of the diffusion cell (see Appendix A) contain the same buffer with tracer levels of permeant added to the donor side. Therefore, as a first approximation, solvent flow effects have been assumed to be negligible.

1.3.5.4 Constant field assumption

It should be stressed that Eqns. 1.10 - 1.14 apply only when the electrical potential profile across the membrane is linear (the Goldman assumption). If the membrane has a high density of fixed charges, then the net electric potential at any point in the membrane is determined by the applied electric field across the membrane and by a component arising from the net charge residing in the membrane. If, however, the net fixed charge density of the membrane is low and the electrical double layer is sufficiently thin, then the curvature of the electrical potential profile (d²Ψ/dx²) is likely to be small and it can be approximated by a linear profile without too much error. Conditions under which the constant field assumption may not hold are discussed by Schultz (1980).

1.4 Recent Studies on Iontophoretic Flux Enhancement

Only recently have efforts been made to understand the underlying mechanisms of iontophoretic flux enhancement. As yet, little information of a fundamental nature is available on the physical and chemical properties controlling iontophoretic drug delivery.
1.4.1 Constant current versus constant voltage iontophoresis

According to the Nernst-Planck theory (see Eqns. 1.7 and 1.8), the electrochemical potential drop is the driving force for ion transport under an applied electric field. However, most of the experimental work in iontophoresis has been done using two-electrode, constant current devices. In the two electrode system, the current between the electrodes (and consequently, the current through the membrane, which is in series) is maintained at a constant value. This is done by appropriately adjusting the voltage drop across the electrodes. In these systems, the actual voltage drop across the membrane is unknown (see Appendix A). The voltage drop is applied across all the resistances in series, thus, it is incorrect to assume the voltage drop across the electrodes is the same as the voltage drop across the membrane. Therefore, in a two electrode, constant current system, the driving force is unknown and the Nernst-Planck equations may not be accurately used.

Keister and coworkers (1987) devised a constant current four point probe system with voltage probes placed close to the opposite sides of the membrane to measure the voltage drop across the membranes. In this case, the voltage probes must be designed to eliminate the contributions from the two electrode-electrolyte interfaces. This is schematically illustrated in Fig. 1.3, which shows how the measured potential difference is likely to be distributed in the region between the two electrodes. The two electrode-electrolyte interfaces can contribute significantly to the measured potential drop and the magnitude of their contribution is a function of the magnitude of the current flowing in the circuit. The contributions from these two interfaces must be eliminated to precisely measure the voltage drop across the membrane (or alternately to maintain a set voltage drop across the membrane).

Constant voltage experiments have been achieved in which the potential drop due to the reference electrodes is eliminated by using Luggin capillaries (Masada et al., 1986, 1989; Srinivasan et al., 1990). This system involves a four electrode potentiostat system
Figure 1.3: Diagram to illustrate how the total potential difference is distributed in the region between the two electrodes.
which maintains a fixed, known, voltage drop across the membrane and is described in
detail in Appendix A.

1.4.2 Iontophoretic flux enhancement studies

Masada et al. (1986; 1989) recognized that the driving force for ion flux was the
voltage drop across the membrane. These authors examined the predictions of the Nernst-
Planck theory using a four electrode constant voltage potentiostat system (see Appendix A).
Results of studies utilizing tetraethylammonium and citrate ions as model solutes
demonstrated the usefulness of the theory, especially at low voltages, for both
hairless mouse skin and cellophane membranes. These and other studies (Mathot et al.,
1989; Srinivasan et al., 1989b) showed that the flux of the cationic solute was greater than
the Nernst-Planck prediction, while the flux of model anions was less than prediction.
Keister and Kasting (1986) also proposed using the Nernst-Planck theory to describe
iontophoretic enhancement of ions across skin. In addition, these authors examined the
effects of the electric field on the lagtime, defined as the time to reach steady state. Lagtime
was predicted to be reduced during iontophoresis. Experiments with ethanehydroxy-
diphosphonate (EHDP) and hairless mouse skin (Kasting et al., 1988) showed acceptable
agreement with the Nernst-Planck theory at low voltages (≤250 mV), however, similar to
the report of Masada et al. (1989), significant deviations occurred above 500 mV. This
was attributed to membrane damage caused by the higher applied voltages. Membrane
damage effects on hairless mouse skin were further examined by Srinivasan et al. (1989).
In these studies, in which glucose was the solute, each skin sample was used as its own
control and a passive permeability coefficient was determined before and after application
of voltage. Results indicated that irreversible membrane damage occurred at all applied
voltages to hairless mouse skin during iontophoresis.

In addition to flux contributions arising from membrane alterations, solvent flow
was found to occur during iontophoresis. Studies by Pikal and Shah (1986) in which the
volume flow was measured across hairless mouse skin, showed that solvent flow was in the same direction as the current (anode to cathode for a negatively charged membrane). Srinivasan et al. (1989) further confirmed the directional nature of solvent flow by measuring the flux enhancement of a neutral solute, glucose, across hairless mouse skin during iontophoresis. Glucose flux was enhanced in the anode to cathode polarity but was retarded in the cathode to anode polarity. Several other reports have shown increased flux of various uncharged solutes during iontophoresis. For example, Burnette and Marrero (1986) found that the flux of thyrotropin releasing hormone (TRH) was greater in the unprotonated state than the protonated state and suggested this was due to convection. Gangarosa et al. (1980) noted that the fluxes of 9-β-D-arabinofuranosyladenine, water and thymidine were increased in both the anode to cathode polarity (150-500%) and the cathode to anode polarity (78-300%). Flux enhancement was also demonstrated for glycine, glucose, and tyrosine (Pikal and Shah, 1990), small chain n-alkanols (Del Terzo et al., 1989), and mannitol (Burnette and Ongpipattanakul, 1987; Sims et al., 1990b).

These studies have given valuable insight into some of the phenomena occurring during iontophoresis. In addition to the direct electric field effects, membrane alterations and convective solvent flow have been found to occur during iontophoresis. However, a mechanistic interpretation for these events has not been advanced. Electro-osmosis has been suggested (Pikal and Shah, 1986; Burnette and Marrero, 1986) as the mechanism for convective solvent flow, but has not been quantitatively described from thermodynamic principles. Also, there are virtually no reports on the physical-chemical properties controlling iontophoresis in human skin.

1.5 Conclusions

Recognition of the applied voltage drop across a membrane as the driving force for the transport of charged species through it opens up new approaches to model iontophoretic transport of ionic drugs across membranes. The Nernst-Planck model predicts that the flux
enhancement is directly proportional to the voltage drop across the membrane and the charge on the ion (see Eqn. 1.24). However, the Nernst-Planck theory does not take into account secondary effects such as membrane alterations and convective solvent flow. Thus, to determine if the Nernst-Planck theory accurately describes ion transport during iontophoresis, each contribution to the total flux enhancement, i.e., current/voltage induced membrane alterations, convective solvent flow, and direct electric field effects, must be determined separately.

The four electrode potentiostat system developed by Masada and coworkers (1986) allows precise control of the voltage drop across a membrane and enables identification of experimental conditions under which the membrane is not damaged by the applied field. Model membranes, which would not be susceptible to alteration by an applied field, along with neutral solutes, may also be used to examine the solvent flow effects. Electro-osmotic theory may be employed in an effort to quantify the solvent flow effects and to identify the mechanism of the flow. Lastly, the application of the physical-chemical models must be extended to human skin and the separate contributions to the overall flux enhancement determined.
CHAPTER 2

STATEMENT OF THE PROBLEM

Efforts to understand the fundamental processes that govern the transport of ions across skin under the influence of an applied electric field have shown that the basic Nernst-Planck theory, which describes the direct field effect upon the ions, may be useful in describing the flux enhancement of ions during iontophoresis. However, the likely importance of secondary contributions to the total flux enhancement, such as convective solvent flow and, in biological membranes, permeability increases due to membrane alterations in the presence of the applied electric field, has been suggested. These may account for the observed discrepancies between the Nernst-Planck predictions and experimental observations. These discrepancies include the flux enhancement of uncharged solutes (relative to passive diffusion) and the greater enhancement seen with cationic solutes over that observed for anionic solutes in iontophoresis experiments.

It is the goal of this project to define and to investigate the mechanisms by which charged and uncharged solutes are transported across skin under the influence of an externally applied electric field. Preliminary experiments, employing the butyrate ion as the solute and hairless mouse skin as the model membrane, support the existence of convective solvent flow and membrane alterations during iontophoresis. However, the large variabilities among the skin samples and the irreversible nature of the membrane alterations have precluded using hairless mouse skin for quantitative studies. Accordingly, the Nuclepore® membrane (a well defined model system which, as will be seen, mimics human skin in many ways) was chosen for studies to be carried out along with the human skin investigations. Iontophoresis studies with the Nuclepore® membrane were expected to be especially valuable in developing an understanding (at the quantitative level) of the
issues related to electro-osmotic (solvent flow) effects upon the transport of neutral and charged solutes during iontophoresis. Thus, these studies were to provide the solid reference base for the interpretation of the data on neutral and charged solute transport across human skin during iontophoresis.

The following were the specific objectives of this research:

1. To carry out iontophoresis flux experiments using the Nuclepore® membrane with a model neutral permeant (glucose), a model cationic permeant (tetraethylammonium ion) and a model anionic permeant (salicylate ion);

2. To investigate the theory for iontophoresis (the modified Nernst-Planck theory) which takes into account solvent flow and to examine whether solvent flow may explain all of the experimental Nuclepore® membrane transport data;

3. To investigate the theory for electro-osmosis which would involve calculations using the Poisson-Boltzmann equations and the equations for fluid motion in cylindrical pores and to test the hypothesis that solvent flow during iontophoresis is consistent with the classical theory of electro-osmosis;

4. To apply the above findings in interpreting data obtained from iontophoresis experiments using the human epidermal membrane and employing a neutral permeant (mannitol), a cationic permeant (tetraethylammonium ion), and an anionic permeant (salicylate ion) as probes.

The knowledge gained from these studies will be invaluable in understanding the mechanisms of drug transport across skin under the influence of an applied electric field.
CHAPTER 3

PRELIMINARY STUDIES USING HAIRLESS MOUSE SKIN

3.1 Introduction

While there have been many clinically oriented iontophoresis studies reported in the literature, efforts needed to understand the fundamental physical chemistry involved in iontophoresis are just beginning (Srinivasan et al., 1990; Burnette and Bagniefski, 1988; Pikal and Shah, 1986; Schultz, 1980; Keister and Kasting, 1986; Goldman, 1943). An understanding of the mechanism of charged and uncharged solute transport across skin, both with and without an applied electric field, is of fundamental importance. A knowledge of the type of diffusion pathway, its limitations with regard to the size of the permeant molecule or ion, its charge status, and how each pathway can be manipulated by an external electric field, would aid both the understanding of iontophoresis, and the development of iontophoresis for drug delivery. By relating the iontophoretic fluxes to the properties of both membrane and drug, it may be possible to extrapolate these results beyond experimental systems to practical iontophoretic drug delivery devices.

Several studies have provided a foundation for the understanding of the movement of molecules and ions in the stratum corneum, the barrier layer of skin. Scheuplein (1967) addressed the issue that shunts such as hair follicles and sweat glands may be a route of solute diffusion. He suggested that shunts may be important for solute transport, especially during the transient period prior to steady state. However, transport through the intact stratum corneum (i.e., the transepidermal route) generally appears to be the dominant pathway of solute diffusion in stratum corneum. Various mechanisms and models have been proposed for the transport of solutes across the bulk stratum corneum. Michaels et al. (1975) proposed a "brick and mortar" type model of the skin in which a solute could
penetrate by only two routes: one required alternate passage through protein (corneocyte regions) and lipid phases (intercellular lipid regions) and the other transit only through a continuous lipid phase. This model cannot accommodate ionic solutes which presumably diffuse only through an aqueous path. Recently, Ghanem et al. (1987) have discussed a model in which the stratum corneum is treated as the diffusional barrier with parallel lipid and aqueous pore pathways for diffusion. This model provides a continuous aqueous route and could, therefore, permit ionic solutes to penetrate.

In this chapter, the parallel pathway model is examined as a means of quantifying the iontophoretic transport of a weak electrolyte. In this model, it is assumed that the electric field is able to enhance the movement of ions along the pore pathway and experimental data obtained with a weak electrolyte (butyric acid) are employed to test this aspect of the model.

3.2 Theory

3.2.1 Physical model of the skin

In this section a physical model is presented for the transport of a weak electrolyte across skin both with and without the influence of an applied electric field. The model is shown in Fig. 3.1. It consists of the stratum corneum in series with a porous matrix, the dermis-epidermis. The stratum corneum is comprised of parallel lipoidal and aqueous pore pathways for diffusion. It is assumed (a) only nonionized species are able to partition into and be transported through the lipoidal phase, and (b) both ionized and nonionized species penetrate the aqueous route with essentially equal facility (i.e., with essentially the same permeability coefficient).

For the steady state situation we may write for the total flux \( J_T \) of a weak electrolyte:

\[
J_T = A P_T \Delta C
\]

(3.1)

where \( P_T \) is the total (or effective) permeability coefficient of a weak electrolyte; \( A \) is the
Figure 3.1: Schematic diagram of the physical model for the diffusion of charged and uncharged species across skin. The stratum corneum consists of parallel lipoidal and aqueous pore pathways that are in series with the porous dermis-epidermis layer.
area for diffusion and \( \Delta C \) is the difference between the donor and receiver chamber permeant concentration. Also:

\[
J_T = \frac{dQ}{dt}
\]  (3.2)

where \( dQ/dt \) is the steady-state slope of the amount of species transported from donor to receiver chamber as a function of time. Therefore:

\[
P_T = \frac{1}{A \Delta C} \frac{dQ}{dt}
\]  (3.3)

For the model presented in Fig. 3.1, \( P_T \) may be separated into contributions from the stratum corneum and the dermis-epidermis (resistance in a series):

\[
\frac{1}{P_T} = \frac{1}{P_{SC}} + \frac{1}{P_{DE}}
\]  (3.4)

where \( P_{SC} \) is the permeability coefficient of stratum corneum and \( P_{DE} \) is the permeability coefficient of the dermis-epidermis combination. Furthermore, for the situation of parallel lipoidal and aqueous pore pathways in the stratum corneum,

\[
P_{SC} = X_S P_L + P_p
\]  (3.5)

where \( P_L \) is the permeability coefficient for the lipoidal pathway, \( P_p \) is the permeability coefficient for the aqueous pore pathway, and \( X_S \) the fraction of undissociated species.

Combining Eqn. 3.4 and 3.5:

\[
\frac{1}{P_T} = \frac{1}{P_p + X_S P_L} + \frac{1}{P_{DE}}
\]  (3.6)

Except for extremely lipophilic permeants, \( P_{DE} \) is generally very large compared to \( P_{SC} \), i.e., the stratum corneum is usually the primary diffusional barrier. In this case, \( 1/P_{DE} \) can be neglected and Eqn. 3.6 becomes:

\[
P_T = P_p + X_S P_L
\]  (3.7)

As will be seen in the present studies using butyric acid, \( P_{DE} \) is indeed much greater than \( P_{SC} \) for hairless mouse skin, and therefore Eqns. 3.7 is a good approximation.
3.2.2 Enhancement of ion transport via an applied electric potential

While the total passive permeability coefficient may be described by Eqn. 3.7, the contribution to transport from an applied voltage drop across the skin requires further elaboration. It is assumed that only the undissociated species enters the lipid domain whereas both ionized and nonionized species may permeate the pore pathway. Further, the electric field is assumed to influence only the charged species in the pore pathway. For this situation, the following equation for the permeability coefficient for a weak electrolyte solute in the presence of an electric field may be written:

\[ P_T = XSP_L + XSP_P + E(1 - XS)P_P \]  

(3.8)

where \( E \) is the enhancement factor for the ionized species due to the applied electric potential drop across the skin. The enhancement factor is defined as (Srinivasan et al., 1990):

\[ E = J_{\Delta \Psi}/J_0 \]  

(3.9)

where \( J_{\Delta \Psi} \) and \( J_0 \) are the fluxes of the ionized species with and without the applied voltage, respectively. Assuming that solvent flow effects are negligible, it can be shown (Srinivasan et al., 1990; Masada et al., 1989) from the Nernst-Planck equation with the constant field assumption that (see Section 1.3.4):

\[ E = \frac{-K}{1 - \exp(K)} \]  

(1.19)

where \( K \) is a dimensionless constant previously defined as:

\[ K = \frac{-z_iF\Delta \Psi}{RT} \]  

(1.15)

Here, \( z_i \) is the charge on species \( i \), \( F \) the Faraday constant, \( \Delta \Psi \) the electric potential drop, \( R \) the gas constant and \( T \) the absolute temperature. Equations 3.8, 3.9, 1.19, and 1.15 may be used to predict \( P_T \) when \( P_L, P_P \) and \( X_S \) are known. Such calculations are shown for butyric acid in Fig. 3.2, where \( P_T \) values are presented as a function of pH for passive diffusion and diffusion under applied voltages of 250 and 500 millivolts (mV).
Figure 3.2: Theoretical butyric acid permeability coefficient-pH profiles for passive diffusion (using Eqn. 3.7) and for applied voltage drops of 250 and 500 mV (using Eqn. 3.8). $P_p = 1.8 \times 10^{-8}$ cm/sec; $P_L = 1.3 \times 10^{-6}$ cm/sec; $E_{250} = 9.4$; $E_{500} = 18.7$; $pK_a \approx 4.85$ at 37°C.
3.3 Experimental

3.3.1 Materials

Iontophoresis studies were conducted with radiolabeled \([1-^{14}C]\) butyric acid (specific activity 15 mCi/mmol) and \([3-^{3}H]\) glucose (specific activity 13.5 Ci/mmol) obtained from New England Nuclear Corporation (Boston, MA). Butyrate (pKa = 4.85) was chosen as a model anion because the fraction dissociated is pH dependent, thus allowing assessment of the electric field effects on both the lipid and pore pathway permeabilities. Glucose was used as a model nonelectrolyte to independently assess "membrane damage" and to obtain insight into solvent flow effects.

Buffers ranged in pH from 2.4 to 9.5. Buffer composition (McKenzie, 1969) varied with pH and in all cases NaCl was the dominant salt species. Chemicals were reagent grade and used as received. All buffers had an ionic strength of 0.1 and were prepared in distilled, deionized water.

3.3.2 Iontophoresis apparatus

Skin permeabilities were measured using a four-electrode potentiostat system which maintains a constant voltage drop across a membrane mounted in a two-chamber diffusion cell. Details of this system are provided in Appendix A where it is compared to the conventional two electrode, constant current device.

3.3.3 Iontophoresis experiments with hairless mouse skin

Following cervical dislocation (Durrheim et al., 1980) of the hairless mouse (8-12 week old hairless mouse skin; SKH-HR-1), abdominal skin was excised and mounted between the two half cells with the stratum corneum facing the donor side. The two Luggin capillaries were positioned on each side with their tips very close to the membrane on either side (see Appendix A). The receiver chamber was filled with buffer of the pH to be tested. The donor solution contained the same buffer premixed with tracer levels of radiolabeled solute. The electrolyte solutions in the Luggin capillaries were the same as that
in the receiver chamber. This was done to ensure that there was no interfacial potential drop between capillary solution and the cell solution, thus the applied voltage drop is the voltage drop across the membrane (see Appendix A). Samples (100μl) were withdrawn from the donor side at the beginning and end of the experiment for analysis. The difference in the counts (dpm) between these two samples were always negligible.

Preliminary experiments indicated that the electric field may cause alterations in the membrane. To monitor and better understand the changes in the intrinsic transport properties caused by the electric field, each experiment involved three stages (Fig. 3.3). Briefly, a passive permeability coefficient was first determined in which $P_T = P_{T,0}$. In the second stage an electric field was applied and the total permeability coefficient due to this potential drop was determined, i.e., $P_T = P_{T,ΔΨ}$. Finally, a second passive permeability coefficient was determined, i.e., $P_T = P_{T,0}$. A more precise description of each stage follows.

Stage I involved the measurement of the initial passive permeability of total butyrate and/or glucose through hairless mouse skin. After assembly, the experimental system was allowed to equilibrate for 6 to 7 hours and reach steady state. Five 1 ml samples were withdrawn at 30 minute intervals from the receiver chamber. Each sample was mixed with 10 ml Opti-Fluor (Packard Instrument Co.) scintillation fluid and counted on a Beckman Liquid Scintillation Counter (Model LS-7500). The samples were replaced with fresh buffer. The initial passive permeability coefficient, $P_{T,0}$, was calculated from Eqn. 3.3.

During Stage II, which lasted one hour, the iontophoretic permeability coefficient, $P_{T,ΔΨ}$, was determined for the permeant at an applied voltage drop, $ΔΨ$, across the skin. After the final sampling in Stage I, the cells were connected to the potentiostat (JAS Instrumental Systems, Inc., Salt Lake City, UT) and a fixed voltage drop was applied across the skin (cathode on donor side, anode on receiver side). Four 1 ml samples were taken at 15 minute intervals from the receiver side and analyzed as in Stage I. Samples
Figure 3.3: Typical permeation profile for a three-stage experiment across hairless mouse skin: (■) butyric acid; (□) glucose.
were replaced with fresh buffer. $P_{T,\Delta \Psi}$ was calculated using the last three points of Stage II (Fig. 3.3) from Eqn. 3.3 as before. The cell current was monitored continuously and the applied voltage was confirmed via a voltmeter (Beckman Industrial Model 310).

At the end of Stage II, the receiver chamber was flushed three times, then refilled with fresh buffer. After 1 hour, the Stage III passive permeability coefficient, $P_{T,0'}$, was determined as before.

The total time for each experiment was, at most, 11-12 hours. Passive experiments at each pH indicated that the barrier properties of the skin remained intact during the entire experiment. Other authors (Bond and Barry, 1988; Gordon, 1980) have also shown that hairless mouse skin retains its barrier function in aqueous buffer over this time range. At the end of each experiment the pH in both chambers was checked. The pH varied only 0.2-0.3 pH units in extreme cases.

3.4 Results

3.4.1 Total butyrate transport

Figure 3.3 presents the result of a typical three stage, dual label, permeation experiment. The best fit straight line (neglecting the first point in each stage as being part of lagtime) for each stage was used to calculate the total permeability coefficient, $P_T$. Table 3.1 lists the experimentally determined permeability coefficients for total butyrate for each stage of the experiment as a function of pH and applied voltage drop. In Table 3.2 these data are converted to the total butyrate effective enhancement factors, $E_1$ and $E_2$, as defined by the following equations:

$$E_1 = \frac{P_{T,\Delta \Psi}}{P_{T,0}} \quad (3.10)$$

$$E_2 = \frac{P_{T,\Delta \Psi}}{P_{T,0'}} \quad (3.11)$$

$E_1$ represents the enhancement of butyric acid under the influence of the voltage, $\Delta \Psi$, over the initial passive value ($P_{T,0}$), while $E_2$ is the enhancement based on $P_{T,0'}$ as the "control." Also shown in Table 3.2 is the ratio $E_1/E_2$ which is a measure of the membrane
Table 3.1
Experimental permeability coefficients for butyric acid with hairless mouse skin

<table>
<thead>
<tr>
<th>pH</th>
<th>No. of Expts., n</th>
<th>PT,0</th>
<th>PT,ΔΨ</th>
<th>PT,0'</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.</td>
<td>ΔΨ = 250 mV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td>3</td>
<td>13 ± 4.5</td>
<td>12 ± 1.0</td>
<td>12 ± 2.0</td>
</tr>
<tr>
<td>3.6</td>
<td>4</td>
<td>7.6 ± 4.5</td>
<td>14 ± 8.0</td>
<td>14 ± 3.0</td>
</tr>
<tr>
<td>4.8</td>
<td>5</td>
<td>5.9 ± 4.0</td>
<td>8.2 ± 2.7</td>
<td>8.2 ± 2.2</td>
</tr>
<tr>
<td>6.0</td>
<td>3</td>
<td>0.2 ± 0.8</td>
<td>1.2 ± 1.2</td>
<td>0.7 ± 0.3</td>
</tr>
<tr>
<td>7.5</td>
<td>3</td>
<td>0.2 ± 0.0</td>
<td>1.6 ± 0.7</td>
<td>0.5 ± 0.2</td>
</tr>
<tr>
<td>9.0</td>
<td>3</td>
<td>0.4 ± 0.3</td>
<td>2.7 ± 1.8</td>
<td>0.7 ± 0.6</td>
</tr>
<tr>
<td>B.</td>
<td>ΔΨ = 500 mV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.4</td>
<td>4</td>
<td>14 ± 3.0</td>
<td>32 ± 13</td>
<td>21 ± 3.0</td>
</tr>
<tr>
<td>3.6</td>
<td>4</td>
<td>5.7 ± 4.1</td>
<td>14 ± 5.0</td>
<td>12 ± 5.0</td>
</tr>
<tr>
<td>4.8</td>
<td>3</td>
<td>6.6 ± 3.5</td>
<td>17 ± 6.0</td>
<td>12 ± 7.0</td>
</tr>
<tr>
<td>5.5</td>
<td>3</td>
<td>2.8 ± 0.4</td>
<td>11 ± 4.0</td>
<td>3.9 ± 0.7</td>
</tr>
<tr>
<td>6.5</td>
<td>4</td>
<td>0.3 ± 0.1</td>
<td>8.4 ± 4.8</td>
<td>1.9 ± 1.0</td>
</tr>
<tr>
<td>7.5</td>
<td>5</td>
<td>0.2 ± 0.1</td>
<td>2.5 ± 0.6</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>9.5</td>
<td>6</td>
<td>0.5 ± 0.3</td>
<td>3.0 ± 10</td>
<td>2.6 ± 1.0</td>
</tr>
</tbody>
</table>

\(a\) Mean ± S.D.
Table 3.2
Enhancement factors for butyric acid with hairless mouse skin

<table>
<thead>
<tr>
<th>pH</th>
<th>E₁</th>
<th>E₂</th>
<th>E₁/E₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.4</td>
<td>1.1 ± 0.5</td>
<td>1.0 ± 0.1</td>
<td>1.0 ± 0.6</td>
</tr>
<tr>
<td>3.6</td>
<td>2.0 ± 0.5</td>
<td>1.0 ± 0.3</td>
<td>2.3 ± 1.0</td>
</tr>
<tr>
<td>4.8</td>
<td>1.8 ± 1.0</td>
<td>1.1 ± 0.4</td>
<td>1.8 ± 1.0</td>
</tr>
<tr>
<td>6.0</td>
<td>6.0 ± 5.2</td>
<td>1.6 ± 1.0</td>
<td>3.9 ± 1.9</td>
</tr>
<tr>
<td>7.5</td>
<td>7.0 ± 2.6</td>
<td>3.6 ± 0.9</td>
<td>2.0 ± 0.5</td>
</tr>
<tr>
<td>9.0</td>
<td>9.0 ± 5.2</td>
<td>7.4 ± 7.5</td>
<td>1.6 ± 0.6</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.4</td>
<td>2.4 ± 0.7</td>
<td>1.5 ± 0.5</td>
<td>1.6 ± 0.3</td>
</tr>
<tr>
<td>3.6</td>
<td>3.2 ± 1.4</td>
<td>1.4 ± 0.8</td>
<td>2.4 ± 0.8</td>
</tr>
<tr>
<td>4.8</td>
<td>2.8 ± 0.7</td>
<td>1.8 ± 0.8</td>
<td>1.7 ± 0.5</td>
</tr>
<tr>
<td>5.5</td>
<td>4.0 ± 1.3</td>
<td>2.8 ± 0.9</td>
<td>1.4 ± 0.1</td>
</tr>
<tr>
<td>6.5</td>
<td>22 ± 7.1</td>
<td>4.4 ± 1.7</td>
<td>6.2 ± 0.9</td>
</tr>
<tr>
<td>7.5</td>
<td>14 ± 6.9</td>
<td>6.4 ± 1.7</td>
<td>2.2 ± 0.8</td>
</tr>
<tr>
<td>9.5</td>
<td>54 ± 19</td>
<td>12 ± 3.7</td>
<td>4.5 ± 2.6</td>
</tr>
</tbody>
</table>

a Mean ± S.D.
alteration (probed by using butyric acid as the permeant) caused by the applied electric field.

3.4.2 Glucose transport

Table 3.3 lists the experimentally determined total permeability coefficients \( P_{0,G}, P_{A\psi,G}, P_{0,G'} \) for glucose across hairless mouse skin at various pH values. Table 3.4 shows the enhancement values for glucose, \( E_G1 \) and \( E_G2 \), which are defined as follows:

\[
E_G1 = \frac{P_{A\psi,G}}{P_{0,G}}
\]
\[
E_G2 = \frac{P_{A\psi,G}}{P_{0,G'}}
\]

The ratio \( E_G1/E_G2 \) (Table 3.4) is a measure of the membrane alteration caused by the electric field. Since it was assumed that glucose is transported only along the pore pathways (for all pH conditions), this ratio was expected to be a general measure of the irreversible alteration in the pore pathway caused by the applied voltage. The ratio \( E_G1/E_G2 \) was, therefore, expected to be different from the ratio \( E_1/E_2 \), except at high pH conditions where the butyrate ion transport was expected to be much more important than the non-ionized butyric acid transport across skin.

3.5 Discussion

3.5.1 Comparison of results with theoretical prediction

Considering a meaningful approach to analyze the experimental results with the model (i.e., Eqns. 3.8, 3.9, 1.19, and 1.15), one is faced with the problem of the significant membrane alterations caused by the applied voltage in most instances (Tables 3.2 and 3.4). An important question was whether \( P_{T,0}, P_{T,0'} \), or some average should be used as the reference for calculating the enhancement (e.g., \( E_1 \) versus \( E_2 \)). In an attempt to gain some insight on the matter, the passive glucose permeability coefficients were determined as a function of the time of the application of voltage. Figure 3.4 shows how "membrane damage" may vary with the time of the application of the voltage over a 60 minute time period.
Table 3.3
Experimental permeability coefficients for glucose with hairless mouse skin

<table>
<thead>
<tr>
<th>pH</th>
<th>No. of Expts., n</th>
<th>Permeability Coefficient&lt;sup&gt;a&lt;/sup&gt; (x10&lt;sup&gt;8&lt;/sup&gt; cm/sec)</th>
<th>P&lt;sub&gt;0,G&lt;/sub&gt;</th>
<th>P&lt;sub&gt;ΔΨ&lt;/sub&gt;</th>
<th>P&lt;sub&gt;0,G'&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.6</td>
<td>6</td>
<td>4.5 ± 2.2</td>
<td>30 ± 18</td>
<td>24 ± 25</td>
<td></td>
</tr>
<tr>
<td>4.8</td>
<td>3</td>
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<td>4.0 ± 3.0</td>
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<tr>
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<td>0.9 ± 0.5</td>
<td>0.6 ± 0.3</td>
<td>1.5 ± 1.1</td>
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<tr>
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<td>2.2 ± 0.4</td>
<td>1.5 ± 0.6</td>
<td>3.7 ± 1.7</td>
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<tr>
<td>9.0</td>
<td>6</td>
<td>2.5 ± 2.1</td>
<td>1.8 ± 1.8</td>
<td>5.2 ± 3.1</td>
<td></td>
</tr>
<tr>
<td>B.</td>
<td></td>
<td></td>
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<tr>
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<td>10 ± 9.0</td>
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<td>1.2 ± 0.3</td>
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<td>6.2 ± 1.4</td>
<td>3.1 ± 1.3</td>
<td></td>
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</tbody>
</table>

<sup>a</sup> Mean ± S.D.
Table 3.4
Enhancement factors for glucose with hairless mouse skin

<table>
<thead>
<tr>
<th>pH</th>
<th>E&lt;sub&gt;G1&lt;/sub&gt;</th>
<th>E&lt;sub&gt;G2&lt;/sub&gt;</th>
<th>E&lt;sub&gt;G1&lt;/sub&gt;/E&lt;sub&gt;G2&lt;/sub&gt;</th>
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</thead>
<tbody>
<tr>
<td>A.</td>
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<tr>
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<td>2.0 ± 1.0</td>
<td>4.9 ± 4.1</td>
</tr>
<tr>
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<td>1.6 ± 0.3</td>
<td>1.5 ± 0.7</td>
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<td>0.8 ± 0.6</td>
<td>0.6 ± 0.3</td>
<td>1.6 ± 0.8</td>
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<tr>
<td>7.5</td>
<td>0.7 ± 0.2</td>
<td>1.1 ± 0.9</td>
<td>1.1 ± 0.7</td>
</tr>
<tr>
<td>9.0</td>
<td>0.7 ± 0.4</td>
<td>1.4 ± 1.7</td>
<td>1.4 ± 1.2</td>
</tr>
<tr>
<td>B.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.6</td>
<td>5.6 ± 1.2</td>
<td>3.0 ± 0.9</td>
<td>1.9 ± 0.1</td>
</tr>
<tr>
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<td>1.6 ± 0.5</td>
<td>2.9 ± 1.3</td>
</tr>
<tr>
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<td>1.4 ± 0.5</td>
<td>2.8 ± 1.0</td>
</tr>
<tr>
<td>6.5</td>
<td>2.6 ± 1.6</td>
<td>1.5 ± 0.4</td>
<td>1.9 ± 1.3</td>
</tr>
<tr>
<td>7.5</td>
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<td>0.6 ± 0.2</td>
<td>4.3 ± 3.1</td>
</tr>
<tr>
<td>9.5</td>
<td>1.3 ± 0.7</td>
<td>0.2 ± 0.1</td>
<td>5.6 ± 2.0</td>
</tr>
</tbody>
</table>

a Mean ± S.D.
Figure 3.4: Glucose $E_{G1}/E_{G2}$ ratio as a function of the time voltage was applied across hairless mouse skin. (□) pH 7.5, 250 mV; (■) pH 7.5, 500 mV; (△) pH 9.5, 500 mV.
Although the variabilities are large, these results suggest that membrane damage in hairless mouse skin occurs early and the effect tends to plateau between 30 and 60 minutes. Because of these data, it was judged that $P_{T,0'}$ would be better than $P_{T,0}$ (or the average of the two) in comparing the experimental results with theory. Accordingly, the $P_{T,0'}$ values were used to deduce the $P_L$ and the $P_P$ values for use in Eqns. 3.7 and 3.8.

As can be seen from Figs. 3.5 and 3.6, there is semiquantitative agreement between experiment and theory. At low pH, the theory predicts that transport would involve predominantly the undissociated species diffusing along the lipid pathway, and therefore, there should be little or no iontophoretic enhancement. Here, there is good agreement between experiment and theory, and the experimental permeability coefficients approach the same asymptote with or without the applied voltage. At high pH (>7.4), the pore pathway should dominate and the differences between $P_{T,\Delta\Psi}$ and $P_{T,0'}$ become large. Under high pH conditions, the experimental and the theoretical enhancement values are seen to agree within about a factor of two or better.

3.5.2 Membrane alteration due to the applied voltage

Membrane damage during iontophoresis has been defined as an increase in the passive permeability of a membrane (Srinivasan et al., 1990). Alteration in the membrane barrier properties has been suggested by many authors. Bellantone et al. (1986) saw a varying increase in benzoic acid flux through hairless mouse skin after termination of an applied current. Burnette and Bagniefski (1988) demonstrated that the impedance of hairless mouse skin was generally lower and $\text{Na}^+$ flux was higher after iontophoresis. Similarly, human skin was shown to have a lower impedance and higher $^{3}\text{H}_2\text{O}$ flux after iontophoresis (Burnette and Ongpipattanakul, 1988). These studies indicate that skin undergoes some change due to iontophoresis such that the passive permeability of the skin is increased.
Figure 3.5: Experimental butyric acid $P_P$ (■) and $P_{\Delta \psi}$ (○) as a function of pH with hairless mouse skin. Solid lines represent the theoretical prediction.

$P_P = 4.5 \times 10^{-8}$ cm/sec; $P_L = 1.2 \times 10^{-6}$ cm/sec; $E_{250} = 9.4$; $\Delta \Psi = 250$ mV; $pK_a = 4.85$. 
Figure 3.6: Experimental butyric acid $P_\gamma$ (■) and $P_{A\Psi}$ (□) values as a function of pH with hairless mouse skin. Solid lines represent the theoretical prediction. $P_p = 4.0 \times 10^{-8}$ cm/sec; $P_{L} = 1.2 \times 10^{-6}$ cm/sec; $E_{500} = 18.7$; $A\Psi = 500$ mV.
Tables 3.2 and 3.4 present the \( \frac{E_1}{E_2} \) and the \( \frac{E_{G1}}{E_{G2}} \) ratios which represent membrane damage when butyric acid and glucose, respectively, are used as the probes. The glucose probes the aqueous pore pathway over the entire pH range, while butyric acid probes both the lipid and pore pathways, depending upon the pH (Eqns. 3.7 and 3.8). Tables 3.2 and 3.4 show that there is significant membrane alteration at both 250 and 500 mV. Because of large variability in the data, it is difficult to judge (a) whether there is less damage in the lipid pathway over the pore pathway, and (b) whether the damage in the pore pathway is dependent on pH. The data do indicate, however, that at high pH (especially at 500 mV) the \( \frac{E_{G1}}{E_{G2}} \) and \( \frac{E_1}{E_2} \) results correlate well; this is consistent with the expectation (Eqn. 3.8) that, at high pH, the pore pathway will dominate in butyric acid permeation. The data also indicate that at high pH and 500 mV, membrane damage is greater than at low pH and lower voltages. This latter thought is consistent with the high baseline passive value seen in Fig. 3.8.

### 3.5.3 Solvent flow effects due to the applied potential drop

Also of importance in these experiments is the possible existence of solvent flow. Solvent flow during iontophoresis has been suggested in several recent studies. Gangarosa et al. (1980) demonstrated that the permeation of nonelectrolytes through hairless mouse skin can be enhanced by iontophoresis due to convective flow. Pikal and Shah (1986) suggested bulk fluid flow by electro-osmosis as the mechanism by which iontophoretic enhancement of the permeability of uncharged species occurs. Glikfeld et al. (1988) reported measurable volume changes in both diffusion cell chambers when a current of 0.5 mA was applied to the system. Charged species may also be affected by solvent flow. Solvent flow is generally believed to occur in the direction of counter ion flow (Pikal and Shah, 1986; Gangarosa, 1980).

In the present study, where the cathode is placed in the donor chamber and the anode in the receiver chamber, it was thought that glucose transport under the influence of
the electric field would be retarded relative to passive glucose transport and that such information could be used to assess solvent flow during iontophoresis. Unfortunately, the data in Table 3.4 show considerable variability, such that a rigorous interpretation of the data is not possible. It would be instructive, however, to attempt an upper limit estimation of the solvent flow effects upon the butyrate ion transport, employing the data in Table 3.4. Incorporation of solvent flow effects in the Nernst-Planck equation (Lakshminarayanan, 1969) results in an equation similar to Eqn. 1.15 but with a correction term for solvent flow (Srinivasan and Higuchi, 1990). If an $E_G2$ value of 0.2 (see Table 3.4) is used to estimate solvent flow, one finds that the correction for the butyrate ion enhancement is 10-20%. This estimate suggests that solvent flow effects are relatively small and consistent with the views of other investigators (Pikal and Shah, 1986; Srinivasan et al., 1989; Burnette and Ongpipattanakul, 1987).

3.6 Conclusions

The experimentally determined pH profiles for the iontophoretic transport through hairless mouse skin of butyric acid agree semiquantitatively with the proposed parallel lipid-aqueous pore pathway model for the transport of ions across skin. Glucose flux enhancements, used to assess solvent flow effects in the cathode to anode polarity during iontophoresis, were less than one. This indicated that solvent flow opposed the flux in this polarity. An estimate of the solvent flow contribution showed it to be a secondary effect as compared to the direct electric field effects.

Irreversible membrane damage occurred at all pH's and at both 250mV and 500mV. Significant variability among hairless mouse skin samples, as well as the membrane damage due to the field, precluded a quantitative assessment of the solvent flow and direct electric field effects. Therefore, to establish a more quantitative understanding of the direct field effects and the secondary effects, studies using a model membrane, which would not be altered by the electric field, would be useful.
CHAPTER 4

MODEL MEMBRANE STUDIES

4.1 Introduction

The Nernst-Planck equation (Section 1.3), which is the starting point for the formal description of ion transport under the influence of an electrochemical potential gradient (Schultz, 1980; Masada et al., 1985; Keister and Kasting, 1986; Srinivasan et al., 1990), has been used by many investigators to model iontophoretic transport. This model assumes that the iontophoretic flux is driven entirely by the primary or conjugate driving force (the electrochemical potential gradient) and ignores the contributions of secondary effects such as current induced convective solvent flow (Burnette and Marrero, 1986; Srinivasan et al., 1989a; Mathot et al., 1989) and, in biological membranes, permeability increase due to the applied electrochemical potential gradient (Srinivasan et al., 1989a, 1989b; Sims and Higuchi, 1990). As a consequence of these assumptions, this model predicts that the iontophoretic flux enhancement (relative to passive diffusion alone) is independent of the sign of the permeant's charge. This model also predicts that the flux of uncharged solutes should not be affected by iontophoresis.

Studies with model cations and anions have shown, however, that the enhancement of cations is much greater than that of anions (Mathot et al., 1989; Sims and Higuchi, 1990; Srinivasan et al., 1989b). Also, the transport of uncharged solutes in skin was observed to be enhanced during iontophoresis (Gangarosa et al., 1980; Burnette and Marrero, 1986; Burnette and Ongpipattanakul, 1987; Srinivasan et al., 1989a). It has been suggested that the increase in flux, relative to passive flux, of the neutral solutes is due to convective solvent flow. The directionality of flow in skin was demonstrated from volume flow studies (Pikal and Shah, 1986) and from iontophoretic transport studies using glucose.
(Srinivasan et al., 1989a), where the glucose flux was shown to be enhanced when the polarity was anode to cathode but inhibited when the polarity was reversed.

To account for convective solvent flow and the asymmetry between cation and anion enhancement, the Nernst-Planck equation was modified by the addition of a linear flow velocity term (Srinivasan and Higuchi, 1989; 1990). The new model predicts 1) an asymmetry in the enhancement of cations and anions and 2) uncharged molecules are enhanced or retarded depending on the polarity of the applied electric field.

While the modified Nernst-Planck theory predicts the observed trends, it does not address the mechanism(s) governing the flow. An electric field applied parallel to a charged surface (such as a pore wall) can induce movement of liquid adjacent to the surface, a process known as electro-osmosis (Hunter, 1981; Aveyard and Haydon, 1973). Skin has been shown to be a net negatively charged membrane (Burnette and Ongpipattandul, 1987). Thus, it is reasonable to expect electro-osmosis to affect solute transport across skin under an applied electric field. As solvent flow has been shown to be directional, the question of cation/anion enhancement asymmetry may be expected to be due, at least in part, to electro-osmotic flow.

Since uncharged solutes are not directly affected by the electric field, the solvent flow velocity may be determined from the iontophoretic enhancement of a neutral molecule. Thus, from neutral solute transport enhancement experiments, the direct field effects on ion transport enhancement may be separated from the convective flow contribution. The asymmetry in cation and anion iontophoretic enhancement may then be assessed in light of the directionality of solvent flow. The mechanism of flow may also be investigated using the Helmholtz-Smoluchowski theory for electro-osmosis.

As shown in Chapter 3, permeability measurements through hairless mouse skin have been shown to be highly variable (Sims and Higuchi, 1990; Srinivasan et al., 1989a). Additionally, the application of an electric field may alter the stratum corneum permeability (Sims and Higuchi, 1990). In order to demonstrate the underlying fundamentals of the
model, a model membrane system, the Nuclepore® membrane, has been employed. Nuclepore® is a coated polycarbonate membrane with essentially straight, aqueous filled pores of known dimensions (Appendix B). It has been shown to possess a small, net negative, surface charge (Meares and Page, 1972; Ibanez and Tejerina, 1982; Keesom et al., 1988).

In this chapter glucose will be used to examine the convective flow contribution to iontophoretic flux enhancement. A method for separating solvent flow from the direct electric field effects on ion transport will be demonstrated. Cation and anion enhancement asymmetry is then addressed once the solvent flow velocity is determined from the flux enhancement of glucose. The mechanism of convective flow is also examined via the Helmholtz-Smoluchowski theory of electro-osmosis.

4.2 Theory

4.2.1 Modified Nernst-Planck equations

Eqn. 1.9 has been modified to include a solvent flow velocity term. For this case, the steady-state flux, \( J \), of a permeant, \( i \), having charge \( z_i \) and effective diffusion coefficient \( D \), through a porous membrane of thickness \( \Delta x \), is given by (Srinivasan and Higuchi, 1990):

\[
J = -D[(dC/dx) + (z_iF/C/RT)(d\Psi/dx)] \pm vC \quad (4.1)
\]

where \( C \) is the permeant concentration, \( \Psi \) the electric potential at any point \( x \) in the membrane, \( F \) the Faraday constant, \( R \) the gas constant, \( T \) the absolute temperature and \( v \) the average solvent velocity. The term \( vC \) is a measure of the transport of permeant resulting from convective solvent flow. For a net negatively charged membrane, the convective flow would be expected to assist the transport of a positively charged solute (+vC) and impede that of a negatively charged solute (-vC) (Srinivasan and Higuchi,
1990). This equation has been solved using the Goldman (1943) assumption of constant electric field within the membrane (Schultz, 1980; Srinivasan and Higuchi, 1990).

An enhancement factor, $E$, defined as the ratio of iontophoretic flux ($I_{\Delta \psi}$) at an applied voltage $\Delta \psi$ across the membrane to the passive flux ($I_{o}$) can be obtained from Eqn. 4.1 for cations ($E_+$) and anions ($E_-$):

$$E_+ = -K \left[ 1 - \frac{Pe}{K} \right] / \left[ 1 - \exp \left( K \left( 1 - \frac{Pe}{K} \right) \right) \right]$$ (4.2)

$$E_- = -K \left[ 1 - \frac{Pe}{K} \right] / \left[ 1 - \exp \left( K \left( 1 + \frac{Pe}{K} \right) \right) \right]$$ (4.3)

where

$$K = \frac{z_i F \Delta \psi / RT}{\Delta x / D}$$ (1.20)

and

$$Pe = (\nu \Delta x / D)$$ (4.4)

Equations 4.2 and 4.3 give iontophoretic flux enhancements due to both a direct field (Nernst-Planck) effect and a solvent flow effect. The Peclet number ($Pe$) characterizes the effect of convective solvent flow on the flux of the permeant, while $K$ involves the direct field effects. Equation 4.2 for cation enhancement reflects the additive effects of convective flow and direct field effects, while Eqn. 4.3 for anion enhancement reflects the opposing solvent flow effects. Qualitatively, predictions from Eqns. 4.2 and 4.3 are consistent with published results for cations and anions (Mathot et al., 1989; Srinivasan et al., 1989b).

Assuming the direct electric field effect applies only for charged permeants, any enhancement in the flow of uncharged solutes can be assumed to be due to only convective flow. The enhancement factor (due only to the solvent flow) can be obtained by letting $K = 0$ (i.e., $z = 0$) in Eqns. 4.2 and 4.3 (Srinivasan and Higuchi, 1990):

anode to cathode: \hspace{2cm} E = \frac{Pe}{[1 - \exp(-Pe)]} \hspace{2cm} (4.5)

cathode to anode: \hspace{2cm} E = -\frac{Pe}{[1 - \exp(Pe)]} \hspace{2cm} (4.6)

Equation 4.5 predicts enhancement factors greater than 1 for the anode to cathode polarity, while Eqn. 4.6 predicts $E < 1$ for the cathode to anode polarity. This is consistent with published experimental enhancement values (Sims et al., 1989; Mathot et al., 1989; Srinivasan et al., 1989a).
If a charged solute and a neutral probe solute have approximately the same diffusion coefficient and if the electrical double layer thickness (1/κ) is small compared to pore dimensions, then the Peclet number for the two solutes can be assumed to be equal under the same experimental conditions. From the experimentally determined enhancement factor for the charged solute and the Peclet number determined using the neutral solute, the asymmetry in cation and anion enhancement may be assessed.

4.3 Experimental

4.3.1 Materials

Iontophoresis studies were conducted with the following permeants: [3-3H]-glucose (specific activity 13.5 Ci/mmol), [1-14C]-mannitol (specific activity 55.0 mCi/mmol), [7-14C]-salicylic acid (specific activity 56.1 mCi/mmol), and [1-14C]-tetraethylammonium bromide (TEAB) having a specific activity of 3.0 mCi/mmol obtained from New England Nuclear Corporation, Boston, MA. Glucose (MW 180.2) was used as a model uncharged solute to assess the convective flow component of total flux across a membrane. Salicylate (MW 138.1; pKa 2.97) was used as a model anion and the tetraethylammonium ion (MW 130.3) as a model cation.

Standard phosphate buffered electrolyte solutions (PBS, pH 7.5) were prepared in distilled, deionized water. The ionic strengths used in the studies were 0.001, 0.01, and 0.10M. The 0.1M PBS solution was made with 4.68 gm NaCl, 1.63 gm Na2HPO4 · 7H2O and 0.14 gm NaH2PO4 · H2O per liter of buffer. The lower ionic strength solutions were obtained by 10 and 100 fold dilution of the 0.1M solution to give 0.01M and 0.001 M solutions, respectively. In all cases NaCl was the dominant salt species. Chemicals were reagent grade and used as received.

Nuclepore® membranes having pore radii of about 75Å and a porosity of 0.001 were obtained from Nuclepore Corporation, Pleasanton, CA. These membranes have a polyvinylpyrrolidone coated, polycarbonate backbone and have been shown to have a
small, net negative, surface charge density (Meares and Page, 1972; Ibanez and Tejerina, 1982; Keesom et al., 1988). Details of the model membrane are provided in Appendix B.

4.3.2 HPLC Analysis

HPLC analysis of glucose was carried out to monitor possible decomposition of glucose during iontophoresis. An Aminex HPX-87H column (BioRad, Richmond, CA) was used and the mobile phase was 0.01 N H$_2$SO$_4$ at a flow rate of 0.4 ml/min. at 25°C. Detection was by ultraviolet light at a wavelength of 193nm.

4.3.3 Iontophoresis Apparatus

Membrane transport rates were measured using a four electrode potentiostat system (JAS Instrumental Systems, Inc., Salt Lake City, UT) which has been described in Appendix A. Either Pt or Ag-AgCl counter electrodes were used. Ag-AgCl electrodes, prepared by the method of Uzgiris (1980), were used to minimize pH variations. Temperature was maintained at 37°C by circulating water (Brinkmann RM-6 model circulating bath, American Scientific).

4.3.4 Iontophoresis experiments with Nuclepore® membranes

To attain an adequate membrane controlled situation, it was necessary to use 50 Nuclepore® membranes stacked together. The resistance of this "plug" was about 1.5 kilo-ohms (Appendix B) which is the same order of magnitude seen with hairless mouse skin (Chapter 3). In each experiment, Nuclepore® membranes presoaked in PBS were assembled such that there were no air bubbles between individual membranes. The assembled membrane was mounted between the half-cells and the Luggin capillaries were inserted. The Luggin capillaries were filled with the same buffer as used in the donor and receiver chambers. Five milliliter (ml) of phosphate buffered saline, pH 7.5, was pipetted into the receiver chamber. The radiolabelled permeant (tracer level) was premixed in the same PBS solution before pipetting 5 ml of solution into the donor chamber.
A typical experiment involved three stages. Stage I involved the determination of the passive permeability coefficient ($P = P_o$). One ml samples were withdrawn from the receiver chamber at predetermined time intervals and replaced with fresh buffer each time. For Stage II, the four electrode potentiostat was connected to the diffusion cell system as described in Appendix B. Usually, permeation runs were carried out consecutively for four different voltages (125, 250, 500, and 1000 millivolts), each for a duration of up to 60 minutes. During each voltage run, one ml samples were taken from the receiver chamber at predetermined time intervals and replaced with fresh buffer each time. Also, the current was continually monitored during Stage II. The pH of the solution in each chamber was measured before and after each applied voltage period. Also, the receiver chamber was flushed and the donor concentration was checked between the successive increases in applied voltage. At the end of Stage II, both chambers were flushed and refilled. Finally, in Stage III, the system was again allowed to equilibrate and a second passive permeability coefficient was determined.

The samples were mixed with 10 ml of scintillation cocktail (Opti-Fluor, Packard Instrument Co.) and were assayed on a Beckman Liquid Scintillation Counter, Model LS-7500. The data were plotted as $Q$, the cumulative disintegrations per minute (dpm) transported into the receiver compartment, as a function of time, $t$. The permeability coefficient, $P$, was calculated for each of the voltage runs and for the passive permeation stages from:

$$P = \frac{1}{\Delta C \cdot A} \frac{dQ}{dt}$$

(3.3)

where $A (0.7 \text{ cm}^2)$ is the area for diffusion, $\Delta C$ is the concentration difference across the membrane, and $dQ/dt$ is the steady state slope.
4.4 Results and Discussion

4.4.1 Glucose experimental results and calculations

Results of a typical experiment with glucose are presented in Fig. 4.1. In this experiment, the polarity was anode to cathode (i.e., the anode was in the donor chamber) and the voltages applied were 125, 250, 500, and 1000 mV. The slopes (dQ/dt) were determined from such data for both polarities (anode-to-cathode and cathode-to-anode), for each of the voltages, and for the three ionic strength solutions.

The experimental P-values, the E-values, and the Peclet numbers (Pe) for all of the glucose experiments were obtained in the following manner. Equation 3.3 was used to calculate the P-values and these are tabulated in Table 4.1. The E-values were calculated for each experiment by dividing the P-values for each voltage by P₀ (the passive P-value), and the averages of these E-values are presented in Table 4.2. Finally the Peclet numbers were calculated from the E-values in Table 4.2 using Eqns. 4.5 and 4.6 and assuming that D was constant throughout the experiment. The Peclet numbers are given in Table 4.3.

The experimental variabilities were generally rather large, and this is reflected in the substantial standard deviations of the P-values in Table 4.1. In most cases, the values for the Stage I and the Stage III passive permeability coefficients in a given experiment were close. When, however, these two values in an experiment differed by a factor of two or more, the entire experiment was discarded and not included in calculating the results presented in Table 4.1.

Tables 4.1 and 4.2 clearly show that the two polarities give opposite effects with regard to the influence of the applied voltage. For the anode to cathode polarity, the E-values were always greater than unity and the P-values and the E-values increased essentially linearly with increasing applied voltage. The opposite was true in the case of the cathode to anode (i.e., the cathode was in the donor chamber) experiments: the P-values and the E-values decreased with increasing voltage and, at the highest voltages (500mV and 1000mV), the P-values were indistinguishable from zero.
Figure 4.1: Results from a typical three stage permeation experiment using Nuclepore® membranes and 0.1M phosphate buffered saline. (○) Stage I passive; Stage II: (●) 125 mV; (△) 250 mV; (▲) 500 mV; (□) 1000 mV; (■) Stage III passive. The polarity in this experiment was anode to cathode.
Table 4.1
Glucose permeability coefficients from Nucleapore® experiments as a function of polarity, ionic strength, and applied voltage drop

<table>
<thead>
<tr>
<th>Ionic Strength (M)</th>
<th>Passive ( (P_0) )</th>
<th>125 mV</th>
<th>250 mV</th>
<th>500 mV</th>
<th>1000 mV</th>
</tr>
</thead>
<tbody>
<tr>
<td>( 0.001 )</td>
<td>3.1 ± 1.0</td>
<td>7.7 ± 1.5</td>
<td>14 ± 4.1</td>
<td>30 ± 8.6</td>
<td>34 ± 17</td>
</tr>
<tr>
<td>( 0.01 )</td>
<td>5.1 ± 1.6</td>
<td>14 ± 2.1</td>
<td>23 ± 7.8</td>
<td>54 ± 17</td>
<td>110 ± 16</td>
</tr>
<tr>
<td>( 0.10 )</td>
<td>2.8 ± 1.7</td>
<td>5.8 ± 4.0</td>
<td>10 ± 9.2</td>
<td>31 ± 21</td>
<td>63 ± 33</td>
</tr>
<tr>
<td>( 0.001 )</td>
<td>1.8 ± 0.24</td>
<td>0.49 ± 0.23</td>
<td>0.22 ± 0.07</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>( 0.01 )</td>
<td>3.2 ± 0.62</td>
<td>1.7 ± 0.79</td>
<td>0.31 ± 0.14</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>( 0.10 )</td>
<td>4.7 ± 0.43</td>
<td>2.2 ± 0.14</td>
<td>0.88 ± 0.32</td>
<td>**</td>
<td>1.5 ± 0.26</td>
</tr>
</tbody>
</table>

\( a \) Mean ± S.D.; \( n \geq 3 \)

** Permeability coefficients were indistinguishable from zero.
Table 4.2
Enhancement factor (E) for transport of glucose across Nuclepore® membranes during iontophoresis

<table>
<thead>
<tr>
<th>Ionic strength (M)</th>
<th>125 mV</th>
<th>250 mV</th>
<th>500 mV</th>
<th>1000 mV</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.</td>
<td>Anode to cathode</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.001</td>
<td>2.7 ± 0.8</td>
<td>4.9 ± 1.6</td>
<td>11 ± 4.4</td>
<td>22 ± 16</td>
</tr>
<tr>
<td>0.01</td>
<td>3.2 ± 1.3</td>
<td>5.1 ± 2.5</td>
<td>9.9 ± 4.1</td>
<td>17 ± 10</td>
</tr>
<tr>
<td>0.10</td>
<td>2.1 ± 0.5</td>
<td>3.4 ± 1.1</td>
<td>11 ± 2.4</td>
<td>24 ± 10</td>
</tr>
<tr>
<td>B.</td>
<td>Cathode to anode</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.001</td>
<td>0.29 ± 0.15</td>
<td>0.12 ± 0.023</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>0.01</td>
<td>0.58 ± 0.36</td>
<td>0.10 ± 0.04</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>0.10</td>
<td>0.47 ± 0.04</td>
<td>0.19 ± 0.07</td>
<td>**</td>
<td>**</td>
</tr>
</tbody>
</table>

*a Mean ± S.D.; n ≥ 3
** Permeability coefficients were indistinguishable from zero.
Table 4.3

Peclet numbers (Pe) determined from glucose enhancement factors as a function of ionic strength and applied voltage

<table>
<thead>
<tr>
<th>Applied Voltage (mV)</th>
<th>Peclet Number (Pe)$^{a}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.001 M</td>
</tr>
<tr>
<td>A. Anode to cathode</td>
<td></td>
</tr>
<tr>
<td>125</td>
<td>2.5 ± 0.8</td>
</tr>
<tr>
<td>250</td>
<td>4.9 ± 1.6</td>
</tr>
<tr>
<td>500</td>
<td>11 ± 4.4</td>
</tr>
<tr>
<td>1000</td>
<td>22 ± 16</td>
</tr>
<tr>
<td>B. Cathode to anode</td>
<td></td>
</tr>
<tr>
<td>125</td>
<td>2.1 ± 0.70</td>
</tr>
<tr>
<td>250</td>
<td>3.3 ± 0.10</td>
</tr>
<tr>
<td>500</td>
<td>**</td>
</tr>
<tr>
<td>1000</td>
<td>**</td>
</tr>
</tbody>
</table>

$^{a}$Mean ± S.D.; $n \geq 3$

** Permeability coefficients were indistinguishable from zero.
In one case, 0.1M PBS and 1000mV, the average P-value (n=4) appeared to be greater than zero (see Table 4.1). However, an HPLC check indicated that the maximum amount of any radiochemical impurity in the receiver chamber at the end of the run would be the order of 10% which would be too small to account for the greater than zero P-value. Subsequently, experiments under the same conditions were carried out with $^{14}$C-mannitol that showed zero flux.

Typical current measurements taken during the application of voltage are shown in Figure 4.2 for each ionic strength. The current increased linearly with increasing applied voltage and was very constant during the length of each voltage run.

4.4.2 Consistency of the glucose results with the Helmholtz-Smoluchowski theory

Electro-osmosis has been suggested as a possible mechanism for the convective flow in skin (Pikal and Shah, 1986, 1990; Burnette and Marrero, 1986; Srinivasan and Higuchi, 1990). Electro-osmosis occurs when the solid (pore wall) remains stationary and the ions, moving in response to an applied electric field, drag the solvent along with them (Hunter, 1981). The classical, quantitative theory for electro-osmosis was established by Smoluchowski (1921) for the case when the pore radius, a, is much larger than the thickness of the electrical double layer (see Appendix C). Using a Boltzmann distribution to describe the charge distribution within the pore and Poisson's equation to relate potential to charge density in the electrical double layer (see, for example, Bockris and Reddy, 1970; Hunter, 1981; Hiemenz, 1986), one may obtain an equation for the flow velocity, $v$:

$$v = (\varepsilon F \zeta) / 4\pi \eta$$

(4.7)

where $\varepsilon$ is the bulk dielectric constant, $F$ is the electric field vector, $\eta$ the viscosity of the bulk phase, and $\zeta$ the zeta potential.

The zeta potential is defined as the potential at the plane of shear of the liquid. Assuming a low surface charge density and neglecting ion-binding reactions, $\zeta$ may be
Figure 4.2: Current profiles for typical Nuclepore® experiments at each ionic strength and applied voltage. (●) 0.10M PBS; (○) 0.01M PBS; (▲) 0.001M PBS.
approximated by the surface potential, $\Psi_0$, (Keesom et al., 1988; Hunter, 1980; Ottewill et al., 1960). The surface charge density, $\sigma$, can then be related to the surface potential via:

$$\zeta = \Psi_0 = \frac{4\pi \sigma}{\varepsilon \kappa}$$  \hspace{1cm} (4.8)

$\kappa$ is the Debye-Huckel reciprocal length and $1/\kappa$ is a measure of the electrical double layer thickness. It is defined as:

$$\kappa = \left(\frac{8\pi e^2 I N}{1000 \varepsilon k T}\right)^{1/2}$$  \hspace{1cm} (4.9)

where $e$ is the electronic charge, $I$ is the ionic strength of the buffer (dominated by a symmetrical electrolyte, e.g., NaCl), $N$ is Avogadro's number, $k$ is the Boltzmann constant and $T$ is the absolute temperature. Substituting Eqn. 4.8 into Eqn. 4.7 and using the constant field assumption of Goldman (1943) results in an electro-osmotic velocity expression:

$$v = \frac{\sigma}{\kappa \eta} (\Delta \Psi / \Delta x)$$  \hspace{1cm} (4.10)

Equation 4.10 is expected to be strictly valid when $1/\kappa << a$.

In Fig. 4.3, the $Pe$ values for 0.10M ionic strength taken from Table 4.3 are plotted against the applied voltage. Here $1/\kappa = 10\AA$ and, therefore, $1/\kappa << a$. As can be seen, $Pe$ increases approximately linearly with increasing applied voltage. It is also seen that the effect is independent of polarity. Thus, it appears that the solvent flow velocities determined from glucose transport experiments are in agreement with the predictions of the Helmholtz-Smoluchowski theory (Eqn. 4.10).

For ionic strengths of 0.01M and 0.001M, $1/\kappa$ values are about 30\AA{} and 100\AA{}, respectively. For these cases (see Table 4.3) in which $1/\kappa = a/3$ and $1/\kappa = a$, the solvent flow velocities are essentially indistinguishable from those for $1/\kappa << a$.

In Table 4.4, the average $\sigma$ values calculated using Eqn. 4.10 and the velocity values from Table 4.3 (see Eqn. 4.4) are presented and compared to $\sigma$ values reported by Meares and Page (1972). In the Meares and Page report $\sigma$ was determined by electro-
Figure 4.3  Peclet number ($Pe$) determined for glucose in 0.1M PBS versus the applied voltage drop across the membrane. (○) anode to cathode; (●) cathode to anode.
Table 4.4

Estimates of the surface charge density and surface potential of Nuclepore® membranes as a function of ionic strength

<table>
<thead>
<tr>
<th>Ionic Strength (M)</th>
<th>$\kappa a$</th>
<th>Surface Charge Density (x10$^3$ C/m$^2$)</th>
<th>Surface Potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Present</td>
<td>Meares &amp; Page$^b$</td>
<td>Present$^a$</td>
</tr>
<tr>
<td>0.001</td>
<td>0.8</td>
<td>39$^c$</td>
<td>0.9 ± 0.5</td>
</tr>
<tr>
<td>0.01</td>
<td>2.4</td>
<td>123$^c$</td>
<td>2.7 ± 0.6</td>
</tr>
<tr>
<td>0.10</td>
<td>7.7</td>
<td>265$^d$</td>
<td>7.9 ± 2.3</td>
</tr>
</tbody>
</table>

a; Mean ± S.D.; $n \geq 3$; $\varepsilon = 78$; $D = 6 \times 10^{-10}$ m$^2$/s; $\Delta x = 3 \times 10^{-4}$ m$^2$.
c; $a = 0.38$ microns

d; $a = 0.26$ microns
membranes at various ionic strength NaCl solutions. Nuclepore® membranes having different pore sizes are manufactured by the same process, therefore, it is not unreasonable to compare the surface characteristics of the small and large pore membranes. In all of the Meares and Page work, $\kappa a \gg 1$ (see Table 4.4), thus electro-osmotic theory should be applicable at all ionic strengths. Despite the differences in experimental methods, at the 0.10M ionic strength where $\kappa a > 1$ for both methods, the $\sigma$ values calculated from glucose transport are comparable to the analogous $\sigma$ values obtained from electro-osmotic flow measurements. At lower ionic strengths, the Meares and Page's values are expected to be good estimates since $\kappa a \gg 1$. The $\sigma$ values calculated from Eqn. 4.10 are within a factor of two of those obtained from flow studies at both 0.01M and 0.001M ionic strengths.

The $\sigma$ value may be used to estimate the surface potential from Eqn. 4.8. These potential values are shown in Columns 6 and 7 of Table 4.4 for both the present study and for the Meares and Page (1972) work, respectively. Equation 4.8 is valid only when $\kappa a \gg 1$ and the surface potential is small; thus, the theory should not be used for the case of 0.001M ionic strength in small pores ($\kappa a = 0.8$). At higher ionic strength, the surface potential is found to be small and justifies the use of the low potential approximation.

Rice and Whitehead (1965) have reported a correction factor, $F(r)$, for the electro-osmotic volume flow for the case when the double layer thickness and the pore radius, $a$, are of similar size:

$$F(r) = 1 - \frac{2I_1(\kappa a)}{\kappa a I_0(\kappa a)} \quad (4.11)$$

where $I_j$ are the zero-order ($j=0$) and first-order ($j=1$) modified Bessel functions of the first kind. Table 4.5 shows the corrected surface potential for both the present work and that of Meares and Page (1972). When $\kappa a \gg 1$, $F(r)$ tends to 1 and the result is Eqn. 4.8. Thus the correction factor makes very little difference in the Meares and Page work and for the 0.10M ionic strength calculation from glucose transport (Table 4.4). At 0.01M ionic strength, the estimated surface potential increases by a factor of three and may violate the
Table 4.5

Estimates of the surface potential of Nuclepore® membranes correcting for \( \kappa a = 1 \)

<table>
<thead>
<tr>
<th>Ionic Strength (M)</th>
<th>Corrected Surface Potential using Eqn. 4.11 (mV)\textsuperscript{a}</th>
<th>Corrected Surface Charge Density using Eqn. 4.8 (x 10(^3) C/m(^2))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Present</td>
<td>Meares &amp; Page\textsuperscript{b}</td>
</tr>
<tr>
<td>0.001</td>
<td>(-178 \pm 83)</td>
<td>(-19.1)</td>
</tr>
<tr>
<td>0.01</td>
<td>(-34 \pm 17)</td>
<td>(-14.5)</td>
</tr>
<tr>
<td>0.10</td>
<td>(-15 \pm 4.5)</td>
<td>(-11.5)</td>
</tr>
</tbody>
</table>

\(*; \text{ see text}\)

\(\text{a; Rice, C.L. and Whitehead, R.,} \textit{J. Phys. Chem.}, 69 (1965) 4017-4024.}\)

\(\text{b; Meares, P. and Page, K.R.,} \textit{Phil. Trans. Royal Soc.}, 272 (1972) 1-46.\)
low potential approximation and the validity of Eqn. 4.8 which, in general, is true for $\Psi_0 \leq 25\text{mV}$. When the correction factor (Eqn. 4.11) is applied for the 0.001M case, the resulting surface potential is about -180mV. Since Eqn. 4.11 is valid only in the low potential approximation, it is clear that the above calculations are not correct for the 0.001M ionic strength. To avoid this problem, the Poisson-Boltzmann equation must be numerically solved to find the potential, $\Psi$, as a function of distance, $r$, from the wall of a cylindrical pore.

The "corrected" surface charge density may be calculated from the surface potentials in Table 4.5 and Eqn. 4.8. These $\sigma$-values are shown in Columns 4 and 5 of Table 4.5 for the present work and that of Meares and Page, respectively. The changes in the surface charge density reflect the changes in the surface potential. For large $\kappa a$, the $\sigma$-values remain unchanged. The $\sigma$-values at 0.01M ionic strength in small pores has increased by more than twofold and, for the 0.1M case, there has been only a slight increase in the surface charge density. As the surface potential is very large at 0.001M, Eqn. 4.8 has not been used to estimate the surface charge density.

4.4.3 Evaluation of the convective flow contribution to cation and anion transport

Glucose was used to estimate the solvent flow contribution to the transport of ions during iontophoresis. By assuming the diffusion coefficients and molecular weights of model charged solutes are similar to that of glucose, the solvent flow velocity determined from glucose may be used as an estimate of the flow for the charged species. From Eqn. 1.20, $K$ for monovalent anions and cations may be calculated for each voltage drop: 125mV, $K = -4.7'$; 250mV, $K = -9.4'$; 500mV, $K = -18.7'$; 1000mV, $K = -37.4'$. E-values for cations and anions may now be predicted from Eqns. 4.2 and 4.3, respectively, using the experimentally determined Pe values for glucose (see Table 4.3). The experimentally determined P-values for TEA$^+$ ion (a monovalent cation) and salicylate ion (a monovalent
Table 4.6

Tetraethylammonium ion and salicylate ion permeability coefficients from Nuclepore® experiments as a function of ionic strength and applied voltage drop

<table>
<thead>
<tr>
<th>Ionic Strength (M)</th>
<th>Passive (P₀)</th>
<th>125mV</th>
<th>250mV</th>
<th>500mV</th>
<th>1000mV</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>Passive (P₀)</td>
<td>125mV</td>
<td>250mV</td>
<td>500mV</td>
<td>1000mV</td>
</tr>
<tr>
<td>A. Tetraethylammonium ion (TEA⁺)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.001 5</td>
<td>7.4 ± 2.4</td>
<td>35 ± 18</td>
<td>63 ± 42</td>
<td>190 ± 78</td>
<td>*</td>
</tr>
<tr>
<td>0.01 5</td>
<td>1.2 ± 0.2</td>
<td>8.4 ± 1.3</td>
<td>18 ± 2.0</td>
<td>37 ± 5.0</td>
<td>61 ± 17</td>
</tr>
<tr>
<td>0.10 5</td>
<td>0.5 ± 0.1</td>
<td>3.0 ± 1.1</td>
<td>7.7 ± 1.9</td>
<td>14 ± 5.0</td>
<td>31 ± 11</td>
</tr>
<tr>
<td>B. Salicylate ion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.001 2</td>
<td>0.01 ± 0.01</td>
<td>0.03 ± 0.03</td>
<td>0.02 ± 0.01</td>
<td>0.35 ± 0.43</td>
<td>*</td>
</tr>
<tr>
<td>0.01 3</td>
<td>0.2 ± 0.1</td>
<td>0.5 ± 0.3</td>
<td>0.6 ± 0.3</td>
<td>1.8 ± 0.6</td>
<td>4.3 ± 0.7</td>
</tr>
<tr>
<td>0.10 2</td>
<td>0.4 ± 0.1</td>
<td>1.2 ± 0.2</td>
<td>2.2 ± 0.3</td>
<td>5.2 ± 0.8</td>
<td>13 ± 1.0</td>
</tr>
</tbody>
</table>

a; Mean ± S.D.
b; n = 2
*; Unable to measure the permeability coefficient.
anion) are shown in Table 4.6 for each of the three ionic strengths. The experimental value in Stage II to the P-value in Stage I. These E-values have been compared to the predictions of Eqns. 4.2 and 4.3 in each of the three ionic strength buffers in Figs. 4.4, 4.5, and 4.6. The Nernst-Planck prediction (without solvent flow) is also shown in each figure for comparison.

It is clearly shown in these figures that the cation and anion behave asymmetrically, i.e. the cation enhancement is above the prediction of the Nernst-Planck theory while the anion enhancement is below the prediction. At the high ionic strengths, both the salicylate and TEA+ enhancement factors are much better described by the curves which include the solvent flow velocity effects (Figs. 4.4 and 4.5), especially at the lower applied voltages. The solvent flow at 0.01 and 0.1M ionic strengths may increase or decrease the flux of the charged solute by as much as 50%. At 0.001M PBS, the salicylate experiments were difficult to carry out due to very long times to reach steady state (> 24 hours). The enhancement factors for salicylate seem to follow the prediction of the modified Nernst-Planck theory at 0.001M ionic strength. The model anion is not favored to enter the negatively charged pores and the additional inhibition due to solvent flow further decreases the salicylate flux. However, the TEAB enhancement may be described by the Nernst-Planck equation without solvent flow. This may indicate solvent flow is a small, secondary effect relative to the applied field at this ionic strength for cations which would be favored to enter the negatively charged pores.

4.5 Conclusion

The enhancement of monovalent anions and cations is best described by accounting for a convective flow component to total flux enhancement. The equations for electro-osmotic velocity describe the convective flow term in high ionic strength buffer which implies electro-osmosis is one mechanism by which convective flow occurs.
Figure 4.4: TEAB (○) and salicylate (●) enhancement factors in 0.10M PBS as a function of applied potential. The solid line is the Nernst-Planck prediction without accounting for convective flow. (---) represents the prediction of Eqn. 4.2 for a monovalent cation and (.....) represents the prediction of Eqn. 4.3 for a monovalent anion.
Figure 4.5: TEAB (○) and salicylate (●) enhancement factors in 0.01M PBS as a function of applied potential. The solid line is the Nernst-Planck prediction without accounting for convective flow. (- - -) represents the prediction of Eqn. 4.2 for a monovalent cation and (······) represents the prediction of Eqn. 4.3 for a monovalent anion.
Figure 4.6: TEAB (○) and salicylate (●) enhancement factors in 0.001M PBS as a function of applied potential. The solid line is the Nernst-Planck prediction without accounting for convective flow. (- - -) represents the prediction of Eqn. 4.2 for a monovalent cation and (⋯⋯) represents the predictions of Eqn. 4.3 for a monovalent anion.
The proposed theory is able to predict trends in the permeability of uncharged molecules during iontophoresis in both the anode to cathode and the cathode to anode polarities. However, to further understand the electro-osmotic flow, the Poisson-Boltzmann equation, in cylindrical coordinates, must be solved numerically.
CHAPTER 5

IONIC PARTITION COEFFICIENTS AND ELECTRO-OSMOTIC FLOW IN CYLINDRICAL PORES: COMPARISON OF THE PREDICTIONS OF THE POISSON-BOLTZMANN EQUATION WITH EXPERIMENT

5.1 Introduction

Recent investigations of the physical-chemical parameters related to the transport of ions across porous, charged membranes, both with and without the influence of an external electric field, have demonstrated that the net charge of the membrane may play a very important role in governing the transport of charged and uncharged molecules (Burnette and Marrero, 1986; Kasting and Keister, 1989; Srinivasan and Higuchi, 1990; Sims et al., 1990a,b; Keister and Kasting, 1986). Specifically, the passive flux of a monovalent cation, at tracer levels, across a negatively charged membrane (Nuclepore® membrane) was found to increase as the ionic strength of the medium was lowered, while the passive flux of a monovalent anion was found to decrease with decreasing ionic strength (see Table 4.6). Neutral solutes, such as glucose, were found to be unaffected by the ionic strength (see Table 4.1). This behavior shows that more of the counterion (positively charged cation), in this case, is partitioning into the pore than the co-ion (anion) as the ionic strength is lowered. Selective partitioning of one type of permeant over another into a membrane (permselectivity of a membrane) depends on the electrical potential profile in the pore, which depends on the sign of the charge and charge density of the pore "wall" and the ionic strength of the electrolyte in the pore. The surface charge density may be related to the electric potential in the pore by means of the Poisson-Boltzmann equation (Bockris and Reddy, 1970; Hunter, 1981; Adamson, 1990). The Poisson-Boltzmann equation has been solved analytically for the case of low surface potential (< 25mV) and the pore radius, a,
much larger than the electrical double layer thickness, $1/\kappa$ (Rice and Whitehead, 1965; Kobatake and Fujita, 1964; Hunter, 1970). However, these approximations limit the use of the results to conditions of high ionic strength and, thus, the evaluation of the difference in the transport of cations and anion at low ionic strength is compromised (Sims et al., 1990a).

In previous work with model Nuclepore® membranes (Sims et al., 1990a), human skin (Sims et al., 1990b), and hairless mouse skin (Srinivasan et al., 1989; Burnette and Ongpipattanakul, 1987; Pikal and Shah, 1986, 1990), convective solvent flow was found to be directional (in the direction of the counter-ion flow). In these studies, it was assumed that uncharged permeants would not be directly affected by the applied electric potential, but would be transported by the field induced convective solvent flow. Electro-osmosis is thought to occur in the electrical double layer where hydrated counterions move in response to the applied electric field, causing a net transport of solvent due to the excess of one ion over the other within the double layer. Thus, the direction of electro-osmotic flow depends on the sign of the net charged surface and on the ionic strength of the electrolyte (Hunter, 1970). In Chapter 4, the linearized Poisson-Boltzmann equation was employed to estimate the surface charge density from the measured flux of an uncharged solute. This analysis assumed a low surface potential and $\kappa a > 1$; therefore, the calculations for the surface charge density at 0.001M ($\kappa a < 1$) and, perhaps, 0.01M ionic strength where $\kappa a = 2.4$, may have incurred a significant error.

An accurate analysis of transport data at the low ionic strengths should involve the complete Poisson-Boltzmann equation without limitations on the magnitude of the surface potential and $\kappa a$. Once the potential as a function of radial distance in the pore is known, a more realistic prediction of the permselectivity (or partitioning) and the electro-osmotic velocity behavior may be possible under all experimental conditions.

In the present work, the transport pathway for charged and uncharged solutes is modeled as a right cylindrical pore and, assuming a value for the surface charge density, a
numerical solution to the Poisson-Boltzmann equation (in cylindrical coordinates) is used to calculate the radial potential profile. This radial potential profile is then used to estimate the electro-osmotic velocity through the membrane. Employing glucose flux as a measure of the solvent flow, experimental velocity data was obtained at various ionic strengths using a membrane with well characterized cylindrical pores (Nuclepore® membrane). This data provides electro-osmotic velocities to which the theoretical predictions may be compared. The surface charge density giving best agreement between theory and experiment is taken to be the correct charge density. This prediction may then be used to estimate the partition coefficient of monovalent cations and anions as a function of ionic strength. The surface charge density is a property of the membrane and, thus, should be independent of the ionic strength and a single value should provide good agreement between the theoretically calculated and the experimentally estimated partition coefficients and electro-osmotic velocities.

5.2 Theory

5.2.1 Pore geometry

Following the arguments of Weaver (1989), the transport of charged solutes across a membrane is assumed to occur via an aqueous pore pathway in which the pores are idealized as right cylinders of length, \( l \), and radius, \( a \). The assumptions of the model are: a) the length of the pore is large compared to the radius, thus end effects may be neglected, b) the surface of the pore possesses a net negative charge which is assumed to be homogeneously distributed, and c) the distance between pores is assumed to be large, thus, the transport across a single pore may be used to represent transport across the membrane.

A schematic diagram of the cylindrical pore is shown in Fig. 5.1. In this representation, an idealized electrolyte solution composed of hard spheres having a diameter, \( d \), and valence, \( z \), are dispersed in a medium of uniform dielectric constant, \( \varepsilon \), within the pore. Due to their finite size, the ions in solution have a distance of closest
Figure 5.1: Schematic diagram of a cylindrical pore of radius, $a$, and length, $l$. The effective radius is $a'$ and $d$ is the diameter of ions in the electrolyte solution. A hypothetical potential profile is also shown. (Adapted from Weaver, 1989)
approach of d/2 to the pore surface. Thus, the electrolyte solution is contained in an
effective volume of $2\pi a'^3$ where $a' = a - d/2$.

5.2.2 Poisson-Boltzmann Equation

Most theoretical treatments of surface electrical phenomena utilize Poisson's
equation which relates the average electrostatic surface potential, $\Psi_0$, to the excess charge
density, $\rho(r)$, in the electrolyte solution near the surface (Bockris and Reddy, 1970; 
Weaver, 1989; Adamson, 1990):

$$\nabla^2 \Psi = \frac{-4\pi \rho(r)}{\varepsilon} \quad (5.1)$$

$\nabla^2$ is the Laplacian operator whose form depends on the geometry of the surface and $\varepsilon$ is
assumed to be the dielectric constant of bulk water.

The excess charge density at a distance $r$ from a charged surface is related to the
thermodynamic properties of the electrolyte through a Boltzmann distribution (Bockris and
Reddy, 1970; Adamson, 1990):

$$\rho(r) = \sum n_i^0 e z_i \exp(-z_i e \Psi(r)/kT) \quad (5.2)$$

where $n_i^0$ is the bulk concentration of the $i$-th species (molecules/m$^3$), $e$ is the electronic
charge, $z_i$ is the valence of species $i$, $\Psi(r)$ is the electrical potential at $r$, $k$ is the Boltzmann
constant, and $T$ is the absolute temperature. For a 1:1 electrolyte where $n = n_+ + n_-$ and
$z_+ = z_- = z$, Eqn. 5.2 takes the form:

$$\rho(r) = -2ne \sinh(-e \Psi(r)/kT) \quad (5.3)$$

Substituting Eqn. 5.3 into Eqn. 5.1 results in the Poisson-Boltzmann (PB) equation which
is the starting point for most theoretical descriptions of electrical phenomena at interfaces
(Weaver, 1989; Adamson, 1990):

$$\nabla^2 \Psi = \frac{8\pi ne \sinh(-e \Psi(r)/kT)}{\varepsilon} \quad (5.4)$$
The PB equation can be solved analytically only for the case of a planar surface (Bockris and Reddy, 1970; Adamson, 1990). Other analytic solutions have been obtained for both the planar and cylindrical geometries once an approximation to the potential has been made (Weaver, 1989a). These solutions are limited by the assumptions, in many cases, of low surface potential and thin electrical double layer thickness, 1/K, compared to the pore radius (Rice and Whitehead, 1965; Kobatake and Fujita, 1964; Smith and Deen, 1980, 1983; Koh and Anderson, 1975). Weaver (1989) has reported a numerical solution to Eqn. 5.4 which does not involve these limiting assumptions. The following discussion highlights the equations and boundary conditions used to numerically solve the rigorous Poisson-Boltzmann equation.

In the present application in which the potential profile in the pore is assumed to have cylindrical symmetry, the appropriate form of the Laplacian is:

$$\nabla^2 \Psi = \frac{1}{r} \frac{d}{dr} \left( r \frac{d\Psi}{dr} \right)$$  \hspace{1cm} (5.5)

In the systems under investigation, the electrical potential is produced by the surface charge density, \(\sigma\), which may result from ionization of surface sites, adsorption of ions and/or image forces (Weaver, 1989). Since it is the form of the potential extending into the diffuse layer of charge that is of interest, \(\sigma\) is assumed to be the net charge resulting from the surface forces and the adjacent Stern layer. The surface charge density is assumed to be smeared out uniformly over the wall of the cylinder. The electroneutrality condition requires that the sum of the charges in the electrolyte and on the cylinder wall be zero:

$$\int_0^{a'} \rho(r) 1 \ 2\pi r \ dr \ + \ \sigma 2\pi a = 0$$  \hspace{1cm} (5.6)

Substituting Eqn. 5.1 into Eqn. 5.6 and integrating over the radial area results in the boundary condition:

$$\frac{d\Psi}{dr} \bigg|_{r=a'} = -\frac{4\pi a}{ea'}$$ \hspace{1cm} (5.7)
The second boundary condition arises from the assumption of cylindrical symmetry which requires the potential to be a minimum at the axis of the cylinder \((r = 0)\):

\[
\left. \frac{d\Psi}{dr} \right|_{r=0} = 0
\]  

Eqns. 5.7 and 5.8 are valid boundary conditions for any form of the potential chosen. In these studies, the full Poisson-Boltzmann equation for cylindrical conditions (Eqn.5.4) was solved numerically (Weaver, 1989) for various values of \(\sigma\) and ionic strength, \(I\), keeping a constant. The radial potential profile, \(\Psi(r)\), obtained from Eqn. 5.4 was then used to estimate the electro-osmotic velocity for each experimental condition.

### 5.2.3 Electro-osmotic velocity

The electro-osmotic velocity may be predicted from the equation of fluid motion in the presence of an applied potential gradient, \(E\) (Rice and Whitehead, 1965):

\[
\frac{d^2v}{dr^2} + \frac{1}{r} \frac{dv}{dr} = -\frac{E\rho(r)}{\eta}
\]  

where \(v\) is the electro-osmotic velocity and \(\eta\) is the coefficient of viscosity. In this expression the velocity was assumed to be only in the axial direction and pressure gradients are negligible. The boundary conditions necessary to solve Eqn. 5.9 are:

\[
v(a) = 0
\]  

and

\[
\left. \frac{dv}{dr} \right|_{r=0} = 0
\]  

Equation 5.10 expresses the velocity at the pore surface while Eqn. 5.11 requires the parabolic velocity profile to have a maximum at the center of the pore. Substituting Eqn.5.3 into Eqn. 5.9 results in:

\[
\frac{dv^2}{dr^2} + \frac{1}{r} \frac{dv}{dr} = -\frac{E^2n e}{\eta} \sinh[-ze\Psi(r)/kT]
\]  

Thus, the electro-osmotic velocity is related to the radial potential profile determined from the PB equation. Once the velocity profile in the pore is known, the average velocity can be readily calculated.
The electro-osmotic velocity may be estimated experimentally from the flux of an uncharged solute, such as glucose, through a pore under the influence of an externally applied electric field (Srinivasan et al., 1989; Sims et al., 1990a,b). The flow velocity calculated using Eqn. 5.12 and the PB equation for \( \Psi(r) \) may then be compared to the experimental velocity values. If the experimentally and theoretically determined electro-osmotic velocities are similar, then the chosen \( \sigma \)-value is assumed to be the best estimate of the surface charge density under the experimental conditions. To further substantiate the calculated \( \sigma \)-value, the partition coefficient may be determined and compared to experimental measurements.

5.2.4 Partition coefficient

The equilibrium distribution of a solute between the bulk solution and any point \( r \) is usually expressed in terms of the partition coefficient, \( K(r) \), or equilibrium pore-to-bulk concentration ratio (Davidson et al., 1987; Smith and Deen, 1980; Malone and Anderson, 1978):

\[
K(r) = \frac{n_i(r)}{n_i^0}
\]  

(5.13)

where \( n_i(r) \) is the average concentration of the \( i \)-th species at point \( r \) in the pore. The pore concentration may be expressed as a Boltzmann distribution (Adamson, 1990) assuming radial diffusion is negligible compared to axial diffusion (Smith and Deen, 1980; Koh and Anderson, 1975; Anderson and Quinn, 1974 Deen, 1987; Brenner and Gaydos, 1977):

\[
n_i(r) = n_i^0 \exp[-z_i e \Psi(r)/kT]
\]  

(5.14)

Substituting Eqn. 5.14 into Eqn. 5.13 and integrating the resulting expression for \( K(r) \) over the radial area of the pore (normalized by the total area) gives the average dimensionless partition coefficient, \( \beta_i \):

\[
\beta_i = \frac{2}{(a')^2} \int_0^{a'} r \exp[-z_i e \Psi(r)/kT] \, dr
\]  

(5.15)
Equation 5.15 may be solved numerically once the potential, \( \Psi(r) \), is known from the Poisson-Boltzmann equation (Eqn. 5.4). The experimentally determined ratio of the passive permeability coefficient of a monovalent cation to that of a monovalent anion (i.e., \( \beta = \beta_c/\beta_a \)) gives an estimate of the effective partition coefficient for Nuclepore membranes (\( a = 75\text{Å} \)) over the range of ionic strengths of interest. The ratios of partition coefficients calculated using Eqn. 5.15 and the potential profile from the PB equation may then be compared to those estimated from experimental data. As with the electro-osmotic velocity calculations, if the experimentally and theoretically determined partition coefficients are similar, then the chosen \( \sigma \)-value is assumed to be an acceptable approximation for the experimental conditions.

The differences observed between the transport of cations and anions during iontophoresis (Mathot et al., 1989; Sims et al., 1990a,b) reflect the surface charge density on the surface of the pore. The surface charge density should be a property of the membrane and should be independent of ionic strength assuming there is negligible ion adsorption from solution (Westermann-Clark and Anderson, 1983). The theoretical and experimentally predicted electro-osmotic velocities and partition coefficients are used to determine a representative \( \sigma \)-value for the Nuclepore® membranes. These \( \sigma \)-values are determined for those experimental conditions in which differences in the passive transport between monovalent cations and anions were observed to be a function of ionic strength. This process enables the independence of \( \sigma \) on ionic strength to be assessed also.

5.3 Experimental

5.3.1 Materials

Radiolabeled solutes used in the transport experiments were \([1-^{14}\text{C}]\) tetraethylammonium bromide (specific activity 3.0 mCi/mmol), \([7-^{14}\text{C}]\) salicylic acid (specific activity 56.1 mCi/mmol), and \([3-^{3}\text{H}]\) glucose (specific activity 13.5 Ci/mmol) obtained from New England Nuclear Co. (Boston, MA). Phosphate buffered saline
(PBS), pH 7.5, was used in all experiments (Sims et al., 1990a). The ionic strengths examined in this study were 0.001, 0.01, and 0.10 M. Polycarbonate membranes, which are described in Appendix B, were obtained from Nuclepore Corp. (Pleasanton, CA). These membranes have been shown to possess a small, net negative, surface charge (Meares and Page, 1972; Bisio et al, 1980; Ibanez and Tejerina, 1982; Keesom et al, 1988; Sims et al., 1990a) with a reported pKa of about 4 (Bisio et al, 1988).

5.3.2 Apparatus

Side-by-side, two chamber diffusion cells were used in the passive transport studies. Each half-cell was stirred at a fixed rate (150 rpm) by a motor driven propeller. The volume of each chamber was 2 ml.

The electro-osmotic velocity studies were conducted with the four electrode potentiostat system (JAS Instrumental Systems, Inc., Salt Lake City, UT) which has been described in detail in Appendix A.

5.3.3 Methods

5.3.3.1 Electro-osmotic velocity determination

The flux of an uncharged molecule is not expected to be directly affected by an applied electric field. The neutral solute transport may, however, be influenced by the field induced solvent flow or electro-osmosis. In Chapter 4 of this thesis, glucose flux was used as a measure of the convective solvent flow velocity through 50 Nuclepore® membranes. Glucose permeability coefficients were determined under passive conditions and under the influence of an applied electric field (in both the anode to cathode and the cathode to anode polarity). From the experimentally determined flux enhancement, defined as the ratio of the flux with applied field to the flux without the field, an estimate of the solvent flow velocity was obtained from the relationships (Srinivasan and Higuchi, 1990):

\[
\text{anode to cathode: } \frac{E}{E_0} = \frac{P_e}{1 - \exp(-Pe)}
\] (4.6)
cathode to anode: \[ E = -Pe / [1 - \exp(Pe)] \] (4.7)
where \[ Pe = \frac{v \Delta x}{D} \] (4.5)

and \( \Delta x \) is the membrane thickness and \( D \) is the diffusion coefficient of the permeant.

Details of the experimental methods and results are found in Chapter 4.

5.3.3.2 Passive transport experiments

In the passive transport experiments, either 1, 10, or 50 Nuclepore® membranes, presoaked in the appropriate buffer, were mounted between the half-cells (Sims et al., 1990a) such that no air bubbles were present between membranes. The receiver chamber was filled with 2 ml of PBS (of specified ionic strength). Tracer levels of the selected permeant was premixed in the same ionic strength PBS prior to pipetting 2 ml of the solution into the donor chamber. The assembled cell was placed in a water bath maintained at 37°C by a Haake circulating water bath (Model D1, American Scientific). At timed intervals 0.25 ml samples (during the 1 and 10 membrane studies) or 0.5 ml samples (during the 50 membrane studies) were withdrawn from the receiver chamber and replaced with equal volume of fresh buffer. The samples were mixed with 10 ml of scintillation cocktail (Opti-Fluor, Packard Instrument Co.) and were assayed on a Beckman Liquid Scintillation Counter, Model L.S-7500. The data were plotted as \( Q \), the cumulative (corrected for sampling effects) disintegrations per minute (dpm) transported into the receiver chamber, as a function of time, \( t \). Permeability coefficients, \( P \), were calculated for each experiment from:

\[ P = \frac{1}{A \Delta C} \frac{dQ}{dt} \] (3.3)

where \( dQ/dt \) is the steady-state slope, \( A \) is the area for diffusion (0.7 cm²), and \( \Delta C \) is the concentration difference across the membrane. The experimental partition coefficient was calculated by taking the ratio of the passive permeability coefficient of TEAB to the passive permeability coefficient of salicylate at each ionic strength and for each set of membranes:
5.3.3.3 Theoretical Simulations

Equation 5.4 was solved using a FORTRAN program, developed by Weaver (1989), which was run on a COMPAQ 386 personal computer equipped with an Intel 80386 main processor and a 80387 co-processor. Details of the program are found elsewhere (Weaver, 1989). Parameter values were as follows: ionic strengths of 0.001, 0.01, and 0.10M, pore radius of 75Å, dielectric constant of 78, temperature 37°C, and the hard sphere diameter, d, of the ions was assumed to be 4Å, although its exact value is not critical for the parameter considered here (Weaver, 1989). The numerical solution was tested to meet the boundary conditions specified by Eqns. 5.7 and 5.8 to within 10⁻⁴%.

5.3.3.4 Partition coefficient and velocity profile calculations

Equations 5.12 and 5.15 were solved for the partition coefficients and electro-osmotic velocity profile, respectively, using Microsoft Excel, version 2.2A. These programs were run on a Macintosh SE personal computer. Details of these program algorithms may be found in Appendix D.

5.4 Results and Discussion

5.4.1 Electro-osmotic velocity

Electro-osmosis occurs when the solid (pore wall) remains stationary and the ions, moving in response to an applied electric field, drag the solvent along with them (Hunter, 1970). Glucose flux was used in these studies to experimentally probe the solvent flow velocity. It was assumed that glucose would move at the same rate as the solvent when the electric field was applied to the system. The results of the glucose transport studies have been reported previously (Chapter 4). The velocity calculated from the glucose data using Eqns.4.5 and 4.6 are shown in Table 5.1 for the anode to cathode polarity, as a function of
Table 5.1
Electro-osmotic velocities calculated from glucose flux enhancement

<table>
<thead>
<tr>
<th>Applied Voltage (mV)</th>
<th>Electro-osmotic Velocity (x 10^6 m/s)^a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.001M</td>
</tr>
<tr>
<td>125</td>
<td>5.2 ± 2.0</td>
</tr>
<tr>
<td>250</td>
<td>9.8 ± 3.2</td>
</tr>
<tr>
<td>500</td>
<td>22 ± 8.8</td>
</tr>
<tr>
<td>1000</td>
<td>44 ± 32</td>
</tr>
</tbody>
</table>

^aMean ± S.D.; Data from Sims et al., 1990a.
ionic strength and the applied voltage drop. It is to these velocity values that the theoretically calculated average velocities from Eqn. 5.12 were compared.

The velocity calculation involves choosing a $\sigma$-value for which the potential profile is generated from the Poisson-Boltzmann equation (Eqn. 5.4). The potential profile is then used to determine the velocity from Eqn. 5.12. Since there are variations in the experimental velocities (Table 5.1), $\sigma$-values were chosen such that the low, mid, and high experimental velocity values (mean ± one standard deviation) were matched for each ionic strength and applied voltage drop. The resultant $\sigma$-values estimated from these calculations are reported in Table 5.2 along with the average $\sigma$-values for each ionic strength. The surface charge density is constant at 0.001M and 0.01M ionic strengths. A slightly higher $\sigma$-value was obtained at 0.1M; due to the variation in the data, it is not possible to say that this value is different from the $\sigma$-values at the lower ionic strengths. The average $\sigma$-values at each ionic strength (Table 5.2) have been used to estimate the average partition coefficient from Eqn. 5.15 and compared to experimentally determined partition coefficients.

5.4.2 Passive transport studies

Results of typical tetraethylammonium bromide (TEAB) and salicylate transport experiments at 0.10M ionic strengths are shown in Fig. 5.2 for the 50 membrane case. As can be seen, the amount in the receiver chamber increases linearly with time after an initial lagtime has elapsed. In previous work using 50 Nuclepore® membranes (Sims et al., 1990a) it was difficult to obtain accurate passive permeation data at the lowest ionic strength of 0.001M. Therefore, the number of membranes was varied in the present study to gain better precision in the passive measurements. Similar linear results were also obtained in experiments at the lower ionic strengths using 50 membranes and at each ionic strength for the experiments employing 1 and 10 membranes. From such plots, the
Table 5.2

Estimated surface charge density based on electro-osmotic velocity data
as a function of ionic strength and applied voltage drop

<table>
<thead>
<tr>
<th>Applied Voltage (mV)</th>
<th>Surface Charge Density&lt;sup&gt;a&lt;/sup&gt; &lt;br&gt;σ, (x 10&lt;sup&gt;3&lt;/sup&gt; C/m&lt;sup&gt;2&lt;/sup&gt;)</th>
<th>0.001M</th>
<th>0.01M</th>
<th>0.10M</th>
</tr>
</thead>
<tbody>
<tr>
<td>125</td>
<td>5.3 ± 2.1</td>
<td>7.1 ± 3.0</td>
<td>7.0 ± 2.0</td>
<td></td>
</tr>
<tr>
<td>250</td>
<td>4.7 ± 1.4</td>
<td>5.7 ± 2.8</td>
<td>9.0 ± 4.0</td>
<td></td>
</tr>
<tr>
<td>500</td>
<td>5.5 ± 2.3</td>
<td>5.5 ± 2.5</td>
<td>11 ± 3.5</td>
<td></td>
</tr>
<tr>
<td>1000</td>
<td>5.5 ± 4.0</td>
<td>4.9 ± 2.8</td>
<td>10 ± 5.5</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>5.3 ± 2.5</td>
<td>5.8 ± 2.8</td>
<td>9.4 ± 3.8</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Mean ± S.D.
Figure 5.2: Results of a typical passive permeation experiment using 50 Nuclepore® membranes in 0.1M ionic strength buffer. (●) tetraethylammonium ion; (○) salicylate ion.
permeability coefficient was calculated using Eqn. 3.3 and the best fit slope from each experiment, allowing 30 minutes for lag time.

Boundary layer effects on the calculated permeability coefficient, \( P \), were assessed by treating the membrane and boundary layer as resistance in series and solving for the permeability coefficient of the membrane, \( P_M \):

\[
\frac{1}{P} = \frac{1}{P_M} + \frac{1}{P_{BL}} \quad (5.21)
\]

It was assumed that there should be a linear relationship between the \( P \)-values determined with 1 membrane and that determined with 10 and 50 membranes (see Appendix B) and that \( P_{BL} \) is the same for all experiments. Therefore, \( P_M \) of 1 membrane should be 10 fold higher than that of 10 membranes (and 50-fold higher than that of 50 membranes). Using this assumption, the boundary layer permeability coefficient, \( P_{BL} \), was determined as that value which gave a linear relationship between \( P_M \) for 1, 10, and 50 membranes. A value of \( 2.2 \times 10^{-4} \text{ cm/sec} \) was estimated from this procedure. As expected, the correction mainly affected the \( P \)-values obtained with TEAB through one membrane at the lower ionic strengths: over 200\% increase in the \( P \)-values at 0.001M and about 60\% increase at 0.01M. Under all other conditions, there was less than 20\% change in the experimentally calculated \( P \)-values and when 50 membranes were used, the correction was less than 4\%.

Hence, the permeability coefficients determined from the one membrane studies, while being presented, have not been used further in the estimation of the average partition coefficient.

The average passive \( P \)-values of TEAB and salicylate through 1, 10, and 50 Nuclepore\textsuperscript{®} membranes (corrected for the boundary layer) are shown as a function of ionic strength in Figs. 5.3, 5.4, and 5.5, respectively. As can be seen from Figs. 5.3-5, the shape of the curves in all three cases are the same, i.e., the passive \( P \)-values of TEAB increase as the ionic strength is decreased, while salicylate \( P \)-values decrease as the ionic strength is decreased.
Figure 5.3: Passive permeability coefficients determined using one Nuclepore® membrane versus ionic strength. Boundary layer correction was $2.2 \times 10^{-4}$ cm/sec. (●) Tetraethylammonium ion; (○) salicylate ion.
Figure 5.4: Passive permeability coefficients determined using ten Nuclepore® membrane versus ionic strength. Boundary layer correction was $2.2 \times 10^{-4}$ cm/sec. (●) Tetraethylammonium ion; (○) salicylate ion.
Figure 5.5: Passive permeability coefficients determined using fifty Nuclepore® membrane versus ionic strength. Boundary layer correction was $2.2 \times 10^{-4}$ cm/sec. (●) Tetraethylammonium ion; (○) salicylate ion.
The transport data may be interpreted by examining the nature of the pore surface-electrolyte interface. A net negatively charged membrane, such as Nuclepore® (Bisio, et al., 1980; Ibanez and Tejerina, 1980), should preferentially attract cations into the area adjacent to the charged pore wall with the result that an electrical double layer is formed (Bockris and Reddy, 1970). The thickness of this double layer is described by the Debye-Huckel reciprocal length, $1/\kappa$, and is dependent on the ionic strength, $I$, of the electrolyte solution:

$$\kappa = \left[ \frac{8\pi e^2 N}{1000 e k T} \right]^{1/2} \quad (4.9)$$

where $N$ is Avogadro's number and all other terms have been previously defined. As the ionic strength decreases, the electrical double layer thickness increases: at 0.1M, $1/\kappa = 10\AA$, at 0.01M, $1/\kappa = 30\AA$, and at 0.001M, $1/\kappa = 100\AA$. Thus, the layer in which cations would accumulate, and from which most anions would be excluded, increases as the ionic strength is lowered. The effective partition coefficient, defined as the ratio of the cation passive $P$-value to the anion passive $P$-value, is expected to be a measure of the selectivity of the membrane. Thus, in the situation where the double layer is thin, both cation and anion may be expected to enter the pore equally and the partition coefficient would be about 1.0. The ratio calculated from the transport experiments are presented in Table 5.3. As can be seen, $\beta = 1$ at 0.1M ionic strength under all experimental conditions. As the ionic strength is decreased, the double layer becomes thicker and the pore becomes increasingly permselective to the cations. This is reflected in the increasing difference between the passive $P$-values of TEAB and salicylate at lower ionic strengths in Figs. 5.3, 5.4, and 5.5, and in the increasing effective partition coefficients in Table 5.3.

In Table 5.3, the number of membranes does not appear to affect the effective partition coefficient at 0.01 or 0.10M ionic strength; at 0.001M there appears to be a trend toward increasing $\beta$ with increasing number of membranes, however, this was believed to be artificial and the error in the data suggest there is little difference due to the number of
Table 5.3
Effective partition coefficients as a function of ionic strength

<table>
<thead>
<tr>
<th>Ionic Strength (M)</th>
<th>Effective Partition Coefficient, $\beta^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 Membrane $^b$</td>
</tr>
<tr>
<td>0.001</td>
<td>33 ± 19</td>
</tr>
<tr>
<td>0.01</td>
<td>3.3 ± 0.9</td>
</tr>
<tr>
<td>0.10</td>
<td>1.0 ± 0.3</td>
</tr>
</tbody>
</table>

$^a$Mean ± S.D.; $n \geq 3$
$^b$Partition coefficient values were not used in further calculations.
membranes. Due to the large boundary layer effect on the one membrane permeability coefficient determination, the average value of $\beta$ (at each ionic strength) from the experiments in which 10 and 50 membranes were used was assumed to be the best estimate of the partition coefficient. Theoretical calculations using Eqn. 5.15 and the average $\sigma$-value (Table 5.2) for each ionic strength were compared to the experimentally determined partition coefficients. The results of these calculations are shown in Table 5.4.

As can be seen in Table 5.4, the average theoretically determined $\beta$ are in good agreement with the experimental partition coefficients, however, the variability in the theoretical calculation increases as the ionic strength is lowered. This is caused by the sensitivity of the partition coefficient calculation to changes in the $\sigma$-value. Selective partitioning occurs into the double layer. At 0.10M ionic strength, where $1/\kappa = 10\,\text{Å}$, the partitioning occurs close to the pore wall and the effects of $\sigma$ fall off relatively rapidly leaving the bulk of the pore equally available to both cations and anions. Therefore, at 0.1M ionic strength, large variations in the $\sigma$-value cause only small changes in $\beta$. As the ionic strength is lowered, the electrical double layer encloses more of the pore volume and partitioning occurs over more of the pore region. Under these conditions, small changes in the $\sigma$-value at 0.01M and 0.001M ionic strength cause large changes in $\beta$ (Table 5.4).

5.4.3 Potential profiles determined from the Poisson-Boltzmann equation

Once the best estimate of the surface charge density has been obtained, the surface potential may be calculated from Eqn. 5.4, along with the radial potential profile within the cylindrical pore. The surface potential, $\Psi_0$, was determined using the average $\sigma$-values from Table 5.2 and are presented in Column 5 of Table 5.4.

Figures 5.6, 5.7, and 5.8 show the radial potential profiles determined using the overall average $\sigma$-value (0.0068 C/m$^2$) from Table 5.2 for 0.001, 0.01, and 0.10M ionic strength, respectively. In these figures, the numerical solution to the Poisson-Boltzmann
Table 5.4
Partition coefficient predicted from the Poisson-Boltzmann equation

<table>
<thead>
<tr>
<th>Ionic Strength (M)</th>
<th>Partition Coefficient</th>
<th>Average Surface Charge Density, σ (x 10^3 C/m^2)</th>
<th>Average Surface Potential, Ψ_0 (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.001</td>
<td>50 ± 11</td>
<td>230 ± 230</td>
<td>5.3 ± 2.5</td>
</tr>
<tr>
<td>0.01</td>
<td>3.5 ± 0.8</td>
<td>4.7 ± 2.3</td>
<td>5.8 ± 2.8</td>
</tr>
<tr>
<td>0.10</td>
<td>1.1 ± 0.2</td>
<td>1.3 ± 0.2</td>
<td>9.4 ± 3.8</td>
</tr>
</tbody>
</table>

^a Mean ± S.D.
^b Average experimentally determined partition coefficients from 10 and 50 membrane data.
^c Predicted partition coefficient from Eqn. 5.15.
^d Estimate of the surface charge density used in Eqn. 5.4 (from Table 5.2).
^e Determined from the Poisson-Boltzmann equation (Eqn. 5.4).
Figure 5.6: Potential profile in a cylindrical pore of radius 75 Å at 0.10 M ionic strength. The numerical solution and the linearized solution of the Poisson-Boltzmann equation are indistinguishable from each other. ($\sigma = 0.0068 \text{ C/m}^2$)
Figure 5.7: Potential profile in a cylindrical pore of radius 75Å at 0.01M ionic strength. (---) Numerical solution of the Poisson-Boltzmann equation. (- - -) Low potential solution $(\sigma = 0.0068 \text{ C/m}^2)$
Figure 5.8: Potential profile in a cylindrical pore of radius 75Å at 0.001M ionic strength. (---) Numerical solution of the Poisson-Boltzmann equation. (- - -) Low potential solution. ($\sigma = 0.0068 \text{ C/m}^2$)
equation is compared to that of the linearized Poisson-Boltzmann equation (Rice and Whitehead, 1965; Weaver, 1989). The potential profiles are all qualitatively similar; the profiles are parabolic with a maximum at the pore axis (a=0). The linearized form of the PB equation assumes a low surface potential and that $\kappa a \gg 1$, thus it is expected to be valid only in the case of 0.1M ionic strength. The linearized solution and numerical solution are nearly indistinguishable at 0.1M ionic strength where $\kappa a = 7.7$ as seen in Fig. 5.6. Thus, the linearized PB equation appears to be a good approximation under conditions where $\kappa a \gg 1$. As the ionic strength is lowered to 0.01M ($\kappa a = 2.4$), there is less agreement between the numerical and linearized solutions (Fig. 5.7); the linearized PB equation overestimates the potential by about 5% under these conditions. At 0.001M ionic strength ($\kappa a = 0.8$), the linearized form of the PB equation is not expected to be valid. Figure 5.8 clearly shows large overestimation of the potential profile by the approximate form as compared to the numerical solution.

In Chapter 4, the surface charge density was estimated from glucose transport data and the Helmholtz-Smoluchowski theory of electro-osmosis. The surface potential was then calculated from Eqn. 4.8. This process is strictly valid only when $\kappa a \gg 1$. Thus, even at 0.1M ionic strength ($\kappa a = 7.7$) there may be considerable error in the calculation of $\sigma$ (and, hence, $\Psi_0$). Rice and Whitehead (1965) derived a correction factor for the surface potential for the case when $\kappa a = 1$ (see Eqn. 4.11). Using the velocity data in Table 5.1 and the correction factor, the average surface potential was estimated to be $-15 \pm 4.5$ mV at 0.1M, $-34 \pm 17$ mV at 0.01M, and $-178 \pm 83$ mV at 0.001M (see Table 4.5). The surface potential values at 0.1M and 0.01M ionic strength are very close to the Poisson-Boltzmann estimate while the value at 0.001M was greatly overestimated, as shown in Table 5.4. It appears, therefore, that the Helmholtz-Smoluchowski approximation gives adequate agreement at 0.1M and 0.01M ionic strength when the surface potential is low.
5.4.4 Ionic strength dependence of electro-osmotic velocity

The experimental velocities determined from glucose flux did not indicate an ionic strength dependence (Table 5.1). However, the equation of fluid motion (Eqn. 5.13) includes two terms, the bulk ion concentration and the potential profile, which are functions of ionic strength (see Eqn. 5.4). To further investigate the ionic strength dependence, the electro-osmotic velocity was predicted for each ionic strength and applied voltage drop using the overall average $\sigma$-value of $0.0068 \text{ C/m}^2$. The results of this calculation, shown in Table 5.5, predict a very small ionic strength effect between 0.001M and 0.01M and less than a factor of two difference between 0.001M and 0.1M ionic strengths. Therefore, it seems probable that the experimental glucose studies were not of sufficient accuracy to demonstrate the small differences in velocity between the tested ionic strengths.

5.4.5 Assumptions of the electro-osmotic velocity measurements

Glucose flux enhancement has been used as an experimental measure of the convective solvent flow velocity. It has been assumed that glucose dissolves into the solvent and moves at the same rate as the solvent under passive and iontophoretic (applied electric field) conditions. As the solubility of glucose is very high (82 grams in 100 grams of water), this assumption seems to be reasonable (Dean, 1973).

Using the flux of an uncharged solute to estimate the solvent flow velocity also involves the assumption of a negligible radial diffusion gradient within the pores. Since glucose is uncharged, the pore surface charge should not affect its distribution within the pore. Therefore, under passive conditions, glucose does not have a radial concentration gradient and will exhibit "plug" flow. When an electric field is applied and the solvent begins to move, the situation changes depending on the ionic strength and surface charge density. Figure 5.9 illustrates the electro-osmotic velocity profiles at 0.1, 0.01, and 0.001M ionic strengths for $\sigma = 0.0068 \text{ C/m}^2$. At 0.10M the velocity profile is essentially
Table 5.5
Electro-osmotic velocity predicted from the equation of fluid motion as a function of ionic strength and applied voltage

<table>
<thead>
<tr>
<th>Applied Voltage (mV)</th>
<th>Electro-osmotic Velocity Calculated from Eqn. 5.13 (x 10^6 m/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.001M</td>
</tr>
<tr>
<td>125</td>
<td>7</td>
</tr>
<tr>
<td>250</td>
<td>14</td>
</tr>
<tr>
<td>500</td>
<td>25</td>
</tr>
<tr>
<td>1000</td>
<td>49</td>
</tr>
</tbody>
</table>

\(\sigma = 0.0068 \text{ C/m}^2\)
Figure 5.9: Electro-osmotic velocity profile in a cylindrical pore (determined from the equation of fluid motion) as a function of ionic strength. The surface charge density was kept constant at 0.0068 C/m². (---) 0.1M; (...) 0.01M; (- - -) 0.001M.
"plug" flow, i.e., most of the fluid moves at the same rate. As the ionic strength is lowered, the profiles become more parabolic and the fluid at the center moves faster than the fluid near the wall. This velocity gradient causes an increase in the solute concentration at the pore center, thus establishing radial diffusion from the pore center towards the pore wall. The effect of radial diffusion is to decrease the parabolic nature of the flow and acts to return the system to "plug" flow. Therefore, the use of glucose flux as a measure of solvent flow should give a good estimate of the solvent flow velocity.

In the integration of Eqn. 5.9, the viscosity coefficient was assumed to be constant and equal to its bulk value. It has been shown that when an electric field is applied to a porous system, the apparent viscosity of the pore fluid is greater than the bulk viscosity value (Koh and Anderson, 1975; Hunter, 1981). This "electro-viscous" effect is greatest for very small $\kappa a (<1)$. For aqueous potassium chloride systems, a maximum of 28% increase in the bulk solution viscosity has been found (Koh and Anderson, 1975). This possible increase in pore viscosity is within the experimental variations obtained from the glucose experiments at 0.001M (where $\kappa a = 0.8$). Therefore, the assumption of constant viscosity has been utilized.

5.4.6 Assumptions of the Poisson-Boltzmann equation

The Poisson-Boltzmann equation (Eqn. 5.4) has been solved numerically assuming the value of the dielectric constant, $\varepsilon$, is constant and identical with that of the bulk electrolyte solution. Solvent molecules are subject to polarization (Davies and Rideal, 1961) from large electric fields ($5 \times 10^6$ V/cm). The solvent molecules may align themselves near the electrified interface and this increased molecular ordering may cause a decrease in the dielectric constant (Koh and Anderson, 1975). The surface potential of Nuclepore® is very small (Table 5.4), therefore; it has been assumed that the value of the dielectric constant remains constant and equal to the bulk value.
The surface potential in a cylindrical pore may be determined from the Poisson-Boltzmann equation (Eqn. 5.4). As noted by Koh and Anderson (1975), it is the zeta potential, defined as the electrostatic potential at the shear plane in the liquid adjacent to the pore wall, that is related to the electro-osmotic velocity (Hunter, 1981). In the electro-osmotic velocity calculation, the zeta potential has been identified with the surface potential, i.e., the plane of shear has been assumed to be identical with the solid/liquid interface. This assumption, in general, is believed to be valid for systems in which the surface potential is small and the ionic strength is less than 0.1M (Davies and Rideal, 1961). Since these conditions have been essentially realized in the Nuclepore® system, this assumption is believed to be reasonable.

5.5 Conclusions

The numerical solution to the full Poisson-Boltzmann equation may be used, in conjunction with experimental electro-osmotic velocity data and the equations of fluid motion, to estimate the surface charge density of Nuclepore® membranes. The surface charge density was found to be essentially independent of ionic strength. Once the surface charge density was established, the partition coefficient was predicted using the radial potential profile determined from the Poisson-Boltzmann equation. The agreement between experimentally determined partition coefficients at 0.1, 0.01, and 0.001M ionic strengths and the prediction based on the Poisson-Boltzmann equation was very good.

A comparison of the potential profiles determined from the low potential approximation of the Poisson-Boltzmann equation (linearized PB equation) to those determined numerically shows that the linearized form gives excellent agreement at 0.1M ionic strength, but there is less agreement as the ionic strength is lowered and $1/\kappa$ becomes comparable to the pore radius.
CHAPTER 6

HUMAN SKIN STUDIES

6.1 Introduction

The Nernst-Planck model assumes the iontophoretic flux is controlled entirely by the primary driving force (the electrochemical potential gradient) and neglects flux contributions from secondary effects. Secondary effects include current induced convective solvent flow (Burnette and Marrero, 1986; Srinivasan et al., 1989a; Mathot et al., 1989; Sims and Higuchi, 1990) and, in biological membranes, permeability increases due to the applied electric field (Srinivasan et al., 1989a; Sims and Higuchi, 1990).

Modification of the Nernst-Planck equation to include a linear convective flow term (Srinivasan and Higuchi, 1990; Sims et al., 1990a) has enabled an evaluation of the current induced solvent flow contribution. This new model predicts 1) an asymmetry in the enhancements of cations and anions and 2) uncharged molecules are enhanced or retarded depending on the polarity of the applied electric field.

Nuclepore® membrane, having net negatively charged pores, was used previously (Chapter 4) to examine the modified Nernst-Planck model (Sims et al., 1990a). Glucose, a neutral, polar solute, was employed as a probe permeant and its flux was used as a measure of the solvent flow velocity through the membrane under an applied field. Results of these studies showed that glucose flux was enhanced when the system polarity was anode to cathode. When the polarity was reversed (cathode to anode), glucose flux was opposed by the solvent flow and resulted in flux inhibition. The solvent flow velocity was found proportional to the magnitude of the applied voltage at high ionic strength which indicated electro-osmosis as one mechanism by which solvent flow occurs. Also, by including the
solvent flow contribution, the experimental enhancement factors for monovalent cations and anions were found to be in good agreement with the Nernst-Planck prediction.

In this chapter, the convective solvent flow contribution to total flux enhancement during iontophoresis across human epidermal membrane is examined. Due to its high aqueous solubility, mannitol (a neutral, polar solute) has been used as a probe permeant and its flux provides a measure of the solvent flow component. In skin there is the possibility for voltage induced membrane alterations. Therefore, experiments were designed to determine if alterations occurred and the extent to which membrane changes were reversible.

6.2 Theory

As in the Nuclepore studies using glucose (Chapter 4), the flux of uncharged permeants is assumed to be affected indirectly by the applied electric field via convective solvent flow (Srinivasan and Higuchi, 1990; Sims et al., 1990a). For a porous, net negatively charged membrane, the solvent flow will be in the anode to cathode direction (Srinivasan et al., 1989a). Thus, depending on the polarity of the applied electric field, the solvent flow will either enhance or impede the flux of an uncharged molecule across the membrane. For neutral solutes the enhancement factors were defined in Chapter 4 as (Srinivasan and Higuchi, 1990; Sims et al., 1990a):

\[ E = \frac{Pe}{1 - \exp(-Pe)} \] (4.5)  

where \( Pe = \frac{v \Delta x}{D} \) (4.4)

anode to cathode: \[ E = Pe / [1 - \exp(-Pe)] \] (4.5)

and cathode to anode: \[ E = -Pe / [1 - \exp(Pe)] \] (4.6)

where \( Pe = (v \Delta x/D) \) (4.4)

and \( v \) is the solvent flow velocity, \( \Delta x \) is the membrane thickness and \( D \) is the solute diffusion coefficient. The Peclet number (Pe) characterizes the effect of convective solvent flow on the flux of the permeant. Equation 4.5 predicts enhancement factors greater than 1 for the anode to cathode polarity while Eqn. 4.6 predicts \( E < 1 \) for the cathode to anode polarity.
Some additional comment on the significance of Eqn. 4.4 will be briefly discussed. For charged membranes (which is the case of interest), Eqns. 4.4-4.6 are most easily understood when pore sizes are large compared both to permeant molecule size and to the electrical double layer thickness. In this limiting case, solvent flow due to electro-osmosis may be estimated by determining the transport enhancement of a neutral molecule such as mannitol. For this situation, solvent flow should be essentially "plug" flow and mannitol should be effective as a "marker" for the solvent, i.e., as a result of mannitol's high aqueous solubility, its flux should be representative of the solvent flow. These ideal conditions were believed met in the studies (Sims et al., 1990a) with the Nuclepore® membrane (Chapter 4): in 0.10M ionic strength (electric double layer thickness around 10Å) and membrane pore size of 150Å diameter. When electrical double layer thickness is not small compared to pore size, plug flow no longer holds, radial diffusion may become important (Deen, 1987; Anderson and Quinn, 1974), and the meaning of v in relation to the probe permeant as a marker for solvent flow becomes less clear. Finally, when pore sizes are comparable to molecular dimensions, the use of a permeant such as mannitol as a marker for solvent flow should be highly questionable.

6.3 Experiment

6.3.1 Materials

All permeants were obtained from New England Nuclear (Boston, MA): [1-14C]-mannitol (specific activity 55.0 mCi/mmol), [7-14C]-salicylic acid (specific activity 56.1 mCi/mmol), and [1-14C]-tetraethylammonium bromide (specific activity 3.0 mCi/mmol). Phosphate buffered saline (PBS), ionic strength 0.1M, pH 7.5, was used in all experiments (Sims et al, 1990a). Sodium azide (0.1%, Baker) was added to the buffer as a bacteriostatic agent.

Human skin was obtained from Ohio Valley Tissue and Skin Bank (Cincinnati, OH). The epidermal membrane was heat separated from the dermis by placing the full
thickness skin in a 60°C waterbath for 1 minute. The epidermal membrane, which includes the stratum corneum and part of the epidermis, was then peeled away from the underlying dermis and stored at -20°C until used. The pretreatment of the human skin has been detailed in Appendix E.

6.3.2 Methods

All experiments were conducted with the four electrode potentiostat system (JAS Instrumental Systems, Inc., Salt Lake City, UT) as discussed in Appendix A. After visual inspection for the absence of holes, the epidermal membrane was mounted between the half-cells with the stratum corneum facing the donor chamber. An additional check for small holes in the membrane was made by tilting the cell toward the donor side and adding 2 ml of PBS to the receiver chamber. The cell remained in this position for 15-20 minutes. If buffer was then found in the donor chamber, the skin was discarded and the procedure repeated with a new piece of skin. Once an intact skin sample was selected, the Luggin capillaries were inserted into the half-cells. The Luggin capillaries were filled with the same buffer as used in the donor and receiver chambers. The receiver chamber was filled with 5 ml of PBS. Tracer level of the selected permeant was pre-mixed in PBS prior to pipetting 5 ml of the solution into the donor chamber.

A typical experiment involved four experimental stages. In Stage I, a passive transport run was carried out by taking 1 ml samples from the receiver chamber at predetermined time intervals and replacing with 1 ml of fresh PBS. At the end of Stage I, the entire contents of the receiver chamber were removed and replaced with fresh PBS. A fixed voltage drop was applied across the membrane during Stage II. The current was continuously monitored during this period and one ml samples were taken from the receiver chamber and replaced with fresh PBS. At the end of Stage II, the voltage was turned off and both donor and receiver chambers were flushed and refilled with their respective solutions. In Stage III (during the 8 hours directly after turning off the voltage drop), a
second passive transport run was carried out. Finally, in Stage IV (during the 16 to 22 hour period after termination of the voltage), a third passive run was conducted.

The samples from each of the runs were mixed with 10 ml of scintillation cocktail (Opti-Fluor, Packard Instrument Co.) and were assayed on a Beckman Liquid Scintillation Counter, Model LS-7500. The data were plotted as $Q$, the cumulative disintegrations per minute (dpm) in the receiver chamber, as a function of time, $t$. Permeability coefficients, $P$, were calculated for each stage from:

$$P = \frac{1}{A \Delta C} \frac{dQ}{dt}$$  \hspace{1cm} (3.3)

where $dQ/dt$ is the steady state slope, $A$ is the area for diffusion, and $\Delta C$ is the concentration difference across the membrane. Receiver solution samples were also checked by HPLC to assure no significant radiochemical impurities were present.

In some experiments the current at an applied voltage drop of 250mV was periodically measured during Stages I, III, and IV. This was done as an independent measure of membrane alteration (by comparing this current prior to and directly after Stage II) and of reversibility over time (by showing this current returning to its initial value determined in Stage I). The voltage was applied across the membrane for a very brief time.

6.4 Results and Discussion

6.4.1 Skin sample selection criteria

Skin samples were selected on the basis of visual examination and through the tilting process described in Methods (Section 6.3.2). Membranes which showed no gross holes by either of these methods were used in this study. Subsequent mannitol transport studies showed wide variability in the passive permeability coefficient (95% of the skin samples had passive $P$-values in the range $3 \times 10^{-7}$ cm/s to $3 \times 10^{-9}$ cm/s). A passive permeability coefficient of the order of $10^{-8}$ cm/sec was considered to be representative of undamaged human epidermal membrane (Ghanem et al., 1989). This criteria seems to be
consistent with the recent report of Kasting and Bowman (1990). In some cases, however, skin samples having ten fold higher permeability coefficients were used. Since these membranes did not have visible holes, the results have, with some reservations, been included herein.

6.4.2 125mV and 250mV experimental results

Membrane alteration effects were found to occur at 1000mV, but not at the lower voltages (125mV and 250mV). Therefore, the low voltage data will be discussed separately from the 1000mV data.

6.4.2.1 Mannitol results

Typical results of mannitol transport experiments are shown in Fig. 6.1 for an anode to cathode (i.e., the anode in the donor chamber) polarity (at 125mV) run and in Fig. 6.2 for a cathode to anode (i.e., the cathode in the donor chamber) polarity (at 250mV) run. As can be seen, the data plots are essentially linear in all cases and, permeability coefficients were calculated for all of the experiments from the best-fit slope (dQ/dt) for each stage using Eqn. 3.3. These P-values are presented in Table 6.1.

The electrode polarity effects are seen by comparing the Stage II P-values with the passive P-values in Table 6.1. For the anode to cathode situation, the Stage II P-values are always larger than the passive P-values and this effect is greater at 250mV than at 125mV. On the other hand, for the cathode to anode situation, the Stage II P-values are smaller than the passive P-values, and at 250mV the Stage II slope of the data plots were all indistinguishable from zero. This is clearly demonstrated in Fig. 6.2. For a net negatively charged membrane, the solvent flow opposes the concentration gradient in the cathode to anode polarity (Pikal and Shah, 1986; Sririvasan et al., 1989a; Sims et al, 1990a). Since mannitol is uncharged, this results in an inhibition of the flux compared to the passive mannitol flux (Stages I, III, or IV). In the opposite polarity, i.e. anode to cathode, the solvent flow is in the same direction as the concentration gradient and the result is
Figure 6.1: Typical results of a mannitol transport experiment during which 125mV was applied across the human skin membrane in the anode to cathode polarity (run 4-1AC). (o) Stage I; () Stage II; () Stage III; (■) Stage IV.
Figure 6.2: Typical results of a mannitol transport experiment during which 250mV was applied across the human skin membrane in the cathode to anode polarity (run 3-2CA). (○) Stage I; (●) Stage II; (□) Stage III; (■) Stage IV.
Table 6.1

Mannitol permeability coefficients from individual experiments at 125mV and 250mV across human epidermal membrane

<table>
<thead>
<tr>
<th>Applied Potential (mV)</th>
<th>Run #</th>
<th>Stage I</th>
<th>Stage II</th>
<th>Stage III</th>
<th>Stage IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>125</td>
<td>1-1AC</td>
<td>1.3</td>
<td>1.6</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>125</td>
<td>2-1AC</td>
<td>0.9</td>
<td>1.4</td>
<td>1.9</td>
<td>1.3</td>
</tr>
<tr>
<td>125</td>
<td>3-1AC</td>
<td>64</td>
<td>90</td>
<td>58</td>
<td>33</td>
</tr>
<tr>
<td>125</td>
<td>4-1AC</td>
<td>5.5</td>
<td>8.8</td>
<td>5.0</td>
<td>4.8</td>
</tr>
<tr>
<td>250</td>
<td>1-2AC</td>
<td>7.9</td>
<td>42</td>
<td>11</td>
<td>18</td>
</tr>
<tr>
<td>250</td>
<td>2-2AC</td>
<td>1.6</td>
<td>2.9</td>
<td>2.0</td>
<td>2.3</td>
</tr>
<tr>
<td>250</td>
<td>3-2AC</td>
<td>0.7</td>
<td>5.8</td>
<td>2.2</td>
<td>2.1</td>
</tr>
<tr>
<td>125</td>
<td>1-1CA</td>
<td>1.3</td>
<td>1.2</td>
<td>1.4</td>
<td>1.5</td>
</tr>
<tr>
<td>125</td>
<td>2-1CA</td>
<td>4.5</td>
<td>2.3</td>
<td>5.4</td>
<td>5.9</td>
</tr>
<tr>
<td>125</td>
<td>3-1CA</td>
<td>2.8</td>
<td>0.9</td>
<td>3.8</td>
<td>2.1</td>
</tr>
<tr>
<td>250</td>
<td>1-2CA</td>
<td>1.4</td>
<td>*</td>
<td>2.0</td>
<td>1.9</td>
</tr>
<tr>
<td>250</td>
<td>2-2CA</td>
<td>5.9</td>
<td>*</td>
<td>6.3</td>
<td>4.9</td>
</tr>
<tr>
<td>250</td>
<td>3-2CA</td>
<td>1.7</td>
<td>*</td>
<td>2.0</td>
<td>1.6</td>
</tr>
</tbody>
</table>

*Permeability coefficient during Stage II in the cathode to anode polarity at 250mV was indistinguishable from zero.
enhancement of the flux relative to the passive flux. The results of these studies support the idea of human skin having a net negative charge (Burnette and Marrero, 1986).

Membrane alteration was assessed during the low voltage runs by determining the passive permeability coefficients before (Stage I) and after (Stages III and IV) application of a voltage drop in Stage II. These passive P-values, in general, indicated that there were little or no irreversible changes taking place in these experiments. Additionally, the currents monitored during Stage II remained essentially constant at both 125 and 250mV as shown in Fig. 6.3. This constant current behavior has also been observed with Nuclepore® membranes in similar experiments (see Fig. 4.2). Nuclepore® membranes remain unaltered by an applied electric field under the conditions employed herein (Section 4.4.2). The constant current therefore implies that the epidermal membrane did not change during the application of low voltages in Stage II. Thus both the transport data and the current data suggest that the epidermal membrane is not significantly altered by the applied field at either 125 or 250mV. Results reported (Kasting and Bowman, 1990) using a low constant current (corresponding to low voltages) seem to support the findings of this study. Observations of the current-voltage relationship for various skin samples by Kasting and Bowman (1990) showed a "stable voltage" for current values between 0 and ±10µA with "little evidence for the production of irreversible or slowly reversible changes in the skin."

6.4.2.2 TEAB and salicylate

Typical results of transport experiments in which 250mV were applied during Stage II are shown in Fig. 6.4 for TEAB and in Fig. 6.5 for salicylate. As can be seen, the data plots in each case are essentially linear and the permeability coefficients were calculated for all experiments from the best-fit slope (dQ/dt) for each stage using Eqn. 3.3. These P-values are presented in Table 6.2.

Comparison of the TEAB Stage II P-values with the passive P-values (Stage I, III, or IV) shows about a ten-fold enhancement in the Stage II value, except in run 2-T. The
Figure 6.3: Current density-time profiles during Stage II for representative experiments at 125 and 250mV. (●) 125mV, anode to cathode (run 4-1AC); (■) 125mV, cathode to anode (run 2-1CA); (□) 250mV, anode to cathode (run 2-2AC); (○) 250mV, cathode to anode (run 3-2CA).
Figure 6.4: Typical results of a TEAB experiment in which 250mV was applied across the human skin membrane during Stage II (run 4-T). Top plot shows (○) Stage I, (●) Stage II, (□) Stage III, and (■) Stage IV. Bottom plot is an enlargement of Stage II.
Figure 6.5: Typical results of a salicylate experiment in which 250mV was applied across the human skin membrane during Stage II (run 3-S). Top plot shows (○) Stage I, (●) Stage II, (▲) Stage III, and (■) Stage IV. Bottom plot is an enlargement of Stage II.
Table 6.2

Individual TEAB and salicylate experimental permeability coefficients determined for runs in which 250mV was applied across human epidermal membranes in Stage II

<table>
<thead>
<tr>
<th>Run #</th>
<th>Permeability coefficient, $P$, ($\times 10^7$ cm/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stage I</td>
</tr>
<tr>
<td>A. Tetraethylammonium bromide (TEAB)</td>
<td></td>
</tr>
<tr>
<td>1-T</td>
<td>0.38</td>
</tr>
<tr>
<td>2-T</td>
<td>0.19</td>
</tr>
<tr>
<td>3-T</td>
<td>10</td>
</tr>
<tr>
<td>4-T</td>
<td>1.5</td>
</tr>
<tr>
<td>5-T</td>
<td>0.38</td>
</tr>
<tr>
<td>6-T</td>
<td>2.3</td>
</tr>
<tr>
<td>B. Salicylic Acid</td>
<td></td>
</tr>
<tr>
<td>1-S</td>
<td>3.0</td>
</tr>
<tr>
<td>2-S</td>
<td>0.93</td>
</tr>
<tr>
<td>3-S</td>
<td>1.9</td>
</tr>
</tbody>
</table>
passive P-value in Stage I of run 2-T was considered acceptable, as was the current profile obtained in Stage II (see below); therefore, for no apparent reason, this run appears to be an outlier from the remaining experiments (Table 6.2). The Nernst-Planck equation (without solvent flow effects) predicts an enhancement factor of 9.4 at 250mV for a monovalent ion (Srinivasan et al., 1990a; Sims and Higuchi, 1990). If solvent flow effects were important at 250mV, then the TEAB flux would be increased over and above the increase caused by the applied electric field since the solvent flow would be in the same direction as the field (anode to cathode in a net negatively charged, porous, membrane). The TEAB results indicate solvent flow is a secondary effect at the low applied voltage.

Similar comparison of the Stage II P-values of salicylate to the passive P-values for each run indicates an average enhancement of the salicylate flux of about 6 (± 3) due to the applied field (versus 9.4 predicted by the Nernst-Planck equation). Solvent flow would oppose the electric field (cathode to anode polarity) in the salicylate runs. Thus, the small negative deviation from the Nernst-Planck prediction for the salicylate runs again suggests solvent flow effects are secondary to the direct electric field effects.

Membrane alteration was assessed during the low voltage runs by determining the passive permeability coefficient before (Stage I) and after (Stages III and IV) application of a voltage drop in Stage II. These passive P-values for both TEAB and for salicylate were, in general, found to be comparable for a given experiment, indicating that there were little of no irreversible changes taking place during these experiments. Additionally, the currents monitored during Stage II remained essentially constant, as shown in Fig. 6.6. This constant current behavior is similar to that reported with Nuclepore® membrane (Fig. 4.2) and in the human epidermal membrane experiments using mannitol at low voltages (Fig. 6.3) and, again, supports the idea of an unchanging membrane. As with the mannitol experiments, both the transport data and current data suggests that the epidermal membrane is not significantly altered by an applied field of 250mV.
Figure 6.6: Current density-time profiles for individual experiments at 250mV. Salicylate runs: (x) 1-S; (+) 2-S; (•) 3-S. TEAB runs: (○) 1-T; (●) 2-T; (□) 3-T; (■) 4-T; (△) 5-T; (▲) 6-T.
6.4.3 1000mV Experimental results

6.4.3.1 Mannitol results

The results of the mannitol runs at 1000mV were significantly different from those at the two lower voltages. There was no "typical" experiment. However, there appeared to be two extreme cases: in the anode to cathode polarity the amounts of mannitol transported across the membrane into the receiver chamber during Stage II either increased linearly or non-linearly, the latter suggesting membrane alterations taking place during the run. Representative plots of each of these are shown in Figs. 6.7 (linear) and 6.8 (non-linear). The Stage II data plots have been enlarged to show that the slope changes with time in the case of 2-3AC, but not for 4-3AC.

As in the case of the low voltages, the passive permeability coefficients were calculated from the best-fit slopes for Stages I, III, and IV. Experimental protocol precluded the taking of early time points; thus, in some cases, the errors were rather large in the determination of the Stage I passive permeability coefficients. The passive P-values are shown in Columns 2, 5, and 6 of Table 6.3 for each experiment.

The evaluation of the Stage II P-values was made difficult by the nonlinear behavior (flux increasing with time) in two out of the five mannitol runs. In these nonlinear cases, two P-values were calculated from the Stage II data. An initial P-value, $P_{\Delta \psi, i}$, was determined from the initial slope of the amount in the receiver chamber over time and a final P-value, $P_{\Delta \psi, f}$, from the limiting slope at the end of Stage II. These two P-values were the same in runs where the increase in the amount in the receiver over time was linear (Fig. 6.7). The Stage II P-values are presented in Columns 3 and 4 of Table 6.3. Runs 1- and 2-3AC show $P_{\Delta \psi, f}$ increasing three to four times over that of the respective $P_{\Delta \psi, i}$. The increasing $P_{\Delta \psi}$-value is indicative of membrane alteration having taken place during application of the field. Figure 6.9 shows the current profiles during Stage II for the runs at 1000mV. As can be seen, for the two experiments (1- and 2-3AC) which showed significant increases in $P_{\Delta \psi}$ during Stage II, there were also significant increases
Figure 6.7: Representative results of a mannitol transport experiment in which the skin was not altered during the application of 1000mV in the anode to cathode polarity (run 4-3A). Inset is an enlargement of Stage II. (○) Stage I; (●) Stage II; (□) Stage III; (■) Stage IV.
Figure 6.8: Representative results of a mannitol transport experiment in which the skin was altered during the application of 1000 mV in the anode to cathode polarity (run 1-3AC). Inset is an enlargement of Stage II. (○) Stage I; (●) Stage II; (□) Stage III; (■) Stage IV.
Table 6.3
Mannitol permeability coefficients determined from individual experiments at 1000mV across human epidermal membrane.

<table>
<thead>
<tr>
<th>Expt</th>
<th>Stage I</th>
<th>Permeability coefficients, P, (x10^7); cm/sec</th>
<th>Stage II</th>
<th>Stage III</th>
<th>Stage IV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Stage I</td>
<td>PΔψ, i</td>
<td>PΔψ, f</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. Anode to cathode</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-3AC</td>
<td>0.21</td>
<td>1.9</td>
<td>7.5</td>
<td>0.44</td>
<td>0.24</td>
</tr>
<tr>
<td>2-3AC</td>
<td>0.44</td>
<td>1.9</td>
<td>6.3</td>
<td>0.42</td>
<td>0.32</td>
</tr>
<tr>
<td>3-3AC</td>
<td>2.1</td>
<td>19</td>
<td>18</td>
<td>1.2</td>
<td>2.0</td>
</tr>
<tr>
<td>4-3AC</td>
<td>0.43</td>
<td>3.5</td>
<td>3.1</td>
<td>0.58</td>
<td>0.47</td>
</tr>
<tr>
<td>5-3AC</td>
<td>0.71</td>
<td>4.0</td>
<td>2.8</td>
<td>0.55</td>
<td>0.77</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. Cathode to anode</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-3CA</td>
<td>3.0</td>
<td>*</td>
<td>*</td>
<td>20</td>
<td>5.3</td>
</tr>
<tr>
<td>2-3CA</td>
<td>3.2</td>
<td>*</td>
<td>*</td>
<td>4.0</td>
<td>3.6</td>
</tr>
<tr>
<td>3-3CA</td>
<td>0.2</td>
<td>*</td>
<td>*</td>
<td>0.2</td>
<td>0.3</td>
</tr>
</tbody>
</table>

* Permeability coefficient during Stage II in the cathode to anode polarity at 1000mV was indistinguishable from zero.
Figure 6.9: Current density-time profiles for each of the mannitol runs in which 1000mV, anode to cathode, was applied during Stage II. ( ) run 1-3AC; ( ) run 2-3AC; ( ) run 3-3AC; ( ) run 4-3AC; ( ) run 5-3AC.
(factor of 2 to 4) in the current during Stage II. The remaining experiments (4-, 5-, and 6-3AC) showed relatively constant $\Delta \Psi$ values as well as current profiles which indicated only small increases (15-20%) in current over time during Stage II.

In the cathode to anode polarity, the Stage II $P$-values were all found to be indistinguishable from zero. Solvent flow in the direction opposite to that of the electrochemical potential gradient appeared to completely inhibit the transport of mannitol across the membrane. Passive permeability coefficients in Stages I, III, and IV were calculated in the same manner as above and are shown in Table 6.3.

Evidence for membrane alterations at high voltages ($\geq 1.0V$) may be found in the work of Kasting and Bowman (1990). These authors carried out constant current studies and measured the voltage as a function of current. At low current/voltage, the current versus voltage profiles were linear; at high voltages ($\geq 1.0V$) these profiles became distinctly nonlinear, in very good agreement with the present findings.

6.4.3.2 TEAB and salicylate results

Results of representative TEAB and salicylate transport experiments in which 1000mV was applied in Stage II are shown in Figs. 6.10 and 6.11, respectively. As can be seen, the slopes in Stage I, III, and IV are linear, and thus, the $P$-value was easily calculated from the best-fit slope in each period using Eqn. 3.3. Experimental protocol precluded obtaining data points during early times, and thus there were larger uncertainties in the Stage I passive $P$-values. These $P$-values are shown in Columns 2, 5, and 6 of Table 6.4 for each experiment.

In most of the 1000mV runs, the amount of permeant (TEAB and salicylate) transported across the membrane into the receiver chamber during Stage II increased non-linearly (Figs. 6.10 and 6.11). As in the experiments using mannitol, this non-linearity suggested membrane alterations were taking place during the run. For this situation, two $P$-values were calculated from the Stage II data as described above. The Stage II $P$-values
Figure 6.10: Results of a representative TEAB-human skin experiment in which 1000mV was applied during Stage II (run 3-T). Top plot shows (○) Stage I, (●) Stage II, (□) Stage III, and (■) Stage IV. Bottom plot is an enlargement of Stage II.
Figure 6.11: Results of a representative salicylate-human skin experiment in which 1000mV was applied during Stage II (run 8-S). Top plot shows (o) Stage I, (●) Stage II, (○) Stage III, and (■) Stage IV. Bottom plot is an enlargement of Stage II.
Table 6.4

individual TEAB and salicylate experimental permeability coefficients
determined for runs in which 1000mV was applied in Stage II
across human epidermal membrane

<table>
<thead>
<tr>
<th>Run #</th>
<th>Stage I</th>
<th>$P_{\Delta \psi, i}$</th>
<th>$P_{\Delta \psi, f}$</th>
<th>Stage III</th>
<th>Stage IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Tetraethylammonium bromide (TEAB)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7-T</td>
<td>4.6</td>
<td>150</td>
<td>190</td>
<td>4.3</td>
<td>4.6</td>
</tr>
<tr>
<td>8-T</td>
<td>4.8</td>
<td>230</td>
<td>320</td>
<td>4.2</td>
<td>4.8</td>
</tr>
<tr>
<td>9-T</td>
<td>1.4</td>
<td>290</td>
<td>440</td>
<td>12</td>
<td>1.8</td>
</tr>
<tr>
<td>10-T</td>
<td>2.2</td>
<td>290</td>
<td>620</td>
<td>6.5</td>
<td>2.9</td>
</tr>
<tr>
<td>11-T</td>
<td>9.8</td>
<td>610</td>
<td>1200</td>
<td>19</td>
<td>6.3</td>
</tr>
<tr>
<td>B. Salicylic Acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-S</td>
<td>0.70</td>
<td>8.4</td>
<td>26</td>
<td>0.91</td>
<td>1.0</td>
</tr>
<tr>
<td>5-S</td>
<td>2.0</td>
<td>27</td>
<td>65</td>
<td>1.6</td>
<td>0.82</td>
</tr>
<tr>
<td>6-S</td>
<td>2.0</td>
<td>120</td>
<td>290</td>
<td>4.3</td>
<td>3.4</td>
</tr>
<tr>
<td>7-S</td>
<td>3.3</td>
<td>170</td>
<td>270</td>
<td>2.1</td>
<td>5.1</td>
</tr>
<tr>
<td>8-S</td>
<td>0.95</td>
<td>12</td>
<td>160</td>
<td>1.5</td>
<td>3.0</td>
</tr>
</tbody>
</table>
are presented in Columns 3 and 4 of Table 6.4 for both TEAB and salicylate. It is clearly shown that the $P_{\Delta \Psi, f}$-value increased over that of the respective $P_{\Delta \Psi, i}$-value in each run. The increasing $P_{\Delta \Psi}$-value is indicative of membrane changes having occurred during the application of 1000 mV.

Figure 6.12 shows the current profiles during Stage II for the runs at 1000 mV. These data may be considered in two groups: runs in which the current was relatively stable, i.e., less than twofold change (runs 7-T, 8-T, 4-S, and 5-S) and runs in which there were more than twofold change in the current (runs 6-, 7-, 8-S and 9-, 10-, 11-T). The small changes in the current during Stage II of the first group are reflected by the Stage II $P$-values in Table 6.4; TEAB runs (7- and 8-T) show a 70-80% increase in the current and about a 40% increase in the $P_{\Delta \Psi, f}$-value over the respective $P_{\Delta \Psi, i}$-value, while the salicylate runs (4- and 5-S), which have current changes of about twofold, show a similar increase in $P_{\Delta \Psi, f}$ over $P_{\Delta \Psi, i}$. The fact that these skin samples have relatively stable current profiles may indicate either a slowly changing membrane, a membrane which was not changed, or a membrane which was rapidly altered at the outset of Stage II (prior to the first sample) by the applied field; once in the altered state, the membrane may then continue to change slowly over the remaining time course of Stage II.

The second group of experiments, in which there was greater than two-fold difference in the current, showed much larger increases (than in the first group) in $P_{\Delta \Psi, f}$ over $P_{\Delta \Psi, i}$ (Table 6.4) with the greatest difference occurring in run 8-S: a 13-fold difference in the Stage II $P$-values and about a 5-to-10 fold difference in the current. From the current and transport data, it appears that there were more extensive alterations during Stage II in these experiments.

6.4.4 Reversibility of membrane alteration

The large changes seen during Stage II (see Tables 6.3 and 6.4) were not reflected in passive permeability coefficients determined at some long time after Stage II. It is seen
Figure 6.12: Current density-time profiles for individual experiments at 1000mV. Salicylate runs: (△) 4-S; (●) 5-S; (●) 6-S; (○) 7-S; (+) 8-S. TEAB runs: (○) 7-T; (●) 8-T; (□) 9-T; (■) 10-T; (△) 11-T.
in Tables 6.3 and 6.4 that the Stage I and Stage IV passive P-values are essentially the same in nearly all experiments. Reversibility of electric field effects on human skin have been reported (DeNuzzio and Berner, 1990), and the present results support the view that membrane alterations which take place during Stage II are reversible in 16 to 22 hours.

Current measurements (at 250mV) during Stages III and IV provide additional insights into the recovery kinetics after significant membrane alteration during Stage II. The current values taken at different times during Stages III and IV are shown in Table 6.5 for several experiments. These data may be interpreted as follows. The current values at the beginning of Stage III are large compared to those for Stage I in all of the cases except 5-3AC. It is believed (based on current measurements during Stage II) that, in each of these cases, there were significant membrane changes during Stage II, and, at the beginning of Stage III, the membranes are still in the perturbed state. In experiments 2-3CA and 5-S the recovery back to the Stage I state is faster than for the other experiments. Experiments 2-3CA and 5-S are nearly fully recovered by the middle of the Stage III., however, the recovery for runs 1-3CA and 6-, 7-, and 8-S is slower. For all experiments, however, the current values during Stage IV indicate essentially full recovery.

In the case of experiment 5-3AC, the current value at the beginning of Stage III indicates that little or no membrane alteration had taken place during Stage II, and this is in agreement with the 5-3AC current pattern for Stage II (see Fig.6.9, which shows constancy of the current during Stage II).

6.4.5 Possible mechanism(s) of membrane alteration

Reversible pore formation has been suggested as a mechanism by which an electric field increases the permeability of some lipid bilayers (depending on their chemical composition) (Kinosita and Tsong, 1977; Benz and Zimmermann, 1981; Chernomordik et al., 1983; Glaser et al., 1988), erythrocyte cell membranes (Kinosita and Tsong, 1977; Serpersu et al, 1985), and mesophyll protoplast cell membranes (Zimmermann and
Table 6.5

Current measured at 250mV during Stages I, III, and IV for four different salicylate and three different mannitol experiments in which 1000mV was applied during Stage II

<table>
<thead>
<tr>
<th>Stage</th>
<th>5-S</th>
<th>6-S</th>
<th>7-S</th>
<th>8-S</th>
<th>5-3AC</th>
<th>1-3CA</th>
<th>2-3CA</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0.0023</td>
<td>0.0078</td>
<td>0.0083</td>
<td>0.0023</td>
<td>0.019</td>
<td>0.14</td>
<td>0.13</td>
</tr>
<tr>
<td>III, initial</td>
<td>0.0083</td>
<td>0.041</td>
<td>0.039</td>
<td>0.022</td>
<td>0.023</td>
<td>0.64</td>
<td>0.82</td>
</tr>
<tr>
<td>III, middle</td>
<td>0.0026</td>
<td>0.017</td>
<td>0.014</td>
<td>0.0077</td>
<td>0.020</td>
<td>0.35</td>
<td>0.18</td>
</tr>
<tr>
<td>IV</td>
<td>0.0027</td>
<td>0.013</td>
<td>0.012</td>
<td>0.0056</td>
<td>0.019</td>
<td>0.16</td>
<td>0.13</td>
</tr>
</tbody>
</table>
Vienken, 1982). The mechanism of pore formation is still unclear but several theories have been offered. It has been suggested that a high electric field intensity causes local compression of the membrane (electromechanical stress) which leads to thinning of the membrane (Zimmermann and Vienken, 1982; Benz and Zimmermann, 1980). Reorganization of the membrane structure may then occur to form the transient pores, e.g., by inducing a looser packing of the lipids (Sugar, 1979). Teissie and Tsong (1981) have suggested a "gating" mechanism in which the polar head groups of the phospholipids act as electrical dipoles. These dipoles are thought to be reoriented by an externally applied electric field. The existence of spontaneous pores within the membrane due to structural defects has also been advocated (Teissie and Tsong, 1981). These pores are thought to be stabilized due to lowered activation energy for pore formation by an electric field. More recently, Glaser et al. (1988) have used planar lipid bilayers to show pore formation may involve at least two steps. First, the formation of hydrophobic pores and secondly, inversion of the pore edge to form a hydrophilic pore. It is interesting to note that all of these mechanisms are voltage dependent, i.e. both the magnitude and duration of the applied voltage determines the size, quantity, and reversibility of the pore formation.

While the present studies with human skin do not allow for the determination of the mechanism of membrane alteration, there is some basis to believe reversible pore formation may be occurring when an electric field is applied across the epidermal membrane.

6.4.6 Current as a measure of the passive permeability coefficient

In some of the experiments the current was periodically measured at an applied voltage of 250mV during Stages I, III, and IV. Figure 6.13 shows these current values plotted against their respective passive P-values. Data presented here involved only those experiments where there was little or no membrane alteration during Stage II; therefore, it is believed that current values are paired with the appropriate passive P-values.
Figure 6.13: Passive permeability coefficients versus the current measured at 250mV in each stage. Open symbols: data taken before (Stage I) and after (Stages III and IV) several 1000mV (Stage II) experiments. Closed symbols: data taken before (Stage I) and after (Stages III and IV) several 125mV (Stage II) experiments. (○, ●) Stage I P-value; (□, ■) Stage III P-value; (△, ▲) Stage IV P-value. Data taken from experiments where there were little or no membrane alterations during Stage II. Area of membrane is 0.7 cm².
Figure 6.13 shows that there is a clear linear relationship between the current and the passive permeability coefficient. The data scatter is believed to be primarily due to the error in the P-value determinations. This linear relationship demonstrates that the transport pathway for a highly polar molecule such as mannitol is the same as the pathway for electrical conduction, i.e., a pore pathway is the pathway for both processes. It is interesting to note that Kasting and Bowman (1990) have shown a similar type of plot; these authors plotted the passive P-values of Na⁺ versus membrane resistance and obtained an essentially linear relationship.

In Figure 6.14, the results of additional experiments are presented to show that the linear relationship seen in Fig. 6.13 is rather general for the present apparatus. As expected from Ohm’s law, it is seen that the initial currents determined at 1000mV (measured 10-15 minutes after the start of Stage II) divided by four and currents measured at 125mV multiplied by two also fall on or near the best-fit line when plotted against their respective passive P-values (Stage I). This is an indication that the membrane is not rapidly altered at the beginning of Stage II (at 1000mV), but continues to change throughout the period. Also, the data point taken from a study with the synthetic Nuclepore® membrane falls on the line (see Fig. 4.2). Finally, one example (taken from experiment 2-3CA, Table 6.5) is presented showing that, when there is significant membrane alteration (during Stage II) the pairing of the passive P-value with the current measurement can be a problem: it is seen here that the current measured at the beginning of Stage III gives a data point (i.e., 0.82mA) that falls far away from the best fit line; however, if the current value taken from the midpoint of Stage III is used, this data point (0.18mA) falls much closer to the best-fit line.

The preceding discussion points out that the electrical current may be a reliable measure of the passive permeability coefficient associated with the pore pathway. It may
Figure 6.14: The Stage I passive permeability coefficients are plotted against the initial current measured during (●, ○) 1000mV runs (divided by 4) and (□) 125mV runs (multiplied by 2). The solid line is the best-fit line from Figure 6.13. (▲, △) experiment 2-3CA (see Table 6.5). (x) Passive P-value from a Nuclepore experiment versus the current at 250mV (Chapter 4). Open symbols are for mannitol runs and closed symbols are for TEAB runs. Area of membrane is 0.7 cm².
be used to monitor membrane changes during iontophoresis. Also, the current may be used to help preselect skin samples for dermal and transdermal transport research purposes.

Not shown in Figs. 6.9 and 6.12 are the current values at time zero, i.e., when the voltage was first applied. It is evident, however, that a rapid increase in the current occurred within the first 30 minutes of Stage II in many of the runs, suggesting that significant membranes changes occurred during this time period, in these instances. It is from the data within this time frame that the initial Stage II P-value was calculated; thus, while it was thought that the $P\Delta \psi_{i}$-value might reflect a relatively unaltered membrane, it is clear that some membrane alteration effects were included in the $P\Delta \psi_{i}$-values.

6.4.7 Convective solvent flow through human skin

An enhancement factor, $E$, has been defined (Srinivasan et al., 1990) as the ratio of the flux with an electric field to the flux without the field (passive flux) and is given as:

$$E = \frac{P\Delta \psi}{P} \quad (6.1)$$

$P\Delta \psi$ is the Stage II permeability coefficient and $P$ is the appropriate passive P-value from Stage I, III, or IV. The average mannitol enhancement factors for the 125 and 250mV cases were calculated using Eqn. 6.1 and the Stage II and IV P-values (see Table 6.1). In the 1000mV cases, the initial Stage II P-value, $P\Delta \psi_{i}$, was used in Eqn. 6.1 to calculate the E-value (see Table 6.3). Thus, the E-values, presented in Table 6.6, are not expected to include contributions from membrane alteration effects or, at least, the effects of membrane alterations were expected to be minimal for these E-values. The Stage IV passive P-value was chosen as the reference P-value (denominator in Eqn. 6.1) because it was felt that the uncertainties in the slope determination for Stage I P-values were generally greater than those for Stage IV. As can be seen, qualitatively, the results follow the predictions of Eqns. 4.5 and 4.6, i.e., $E>1$ in the anode to cathode polarity and $E<1$ in the opposite polarity.
Table 6.6
Mannitol enhancement factors determined from
human epidermal membrane experiments

<table>
<thead>
<tr>
<th>Applied Voltage (mV)</th>
<th>n</th>
<th>Enhancement Factor, E&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. Anode to cathode</td>
<td></td>
<td></td>
</tr>
<tr>
<td>125</td>
<td>4</td>
<td>1.3 ± 0.4</td>
</tr>
<tr>
<td>250</td>
<td>3</td>
<td>2.1 ± 0.7</td>
</tr>
<tr>
<td>1000</td>
<td>5</td>
<td>7.0 ± 2.9</td>
</tr>
<tr>
<td>B. Cathode to anode</td>
<td></td>
<td></td>
</tr>
<tr>
<td>125</td>
<td>3</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td>250</td>
<td>3</td>
<td>*</td>
</tr>
<tr>
<td>1000</td>
<td>3</td>
<td>*</td>
</tr>
</tbody>
</table>

<sup>a</sup>: Mean ± S.D.

* Permeability coefficient during iontophoresis (Stage II) was indistinguishable from zero.
The Peclet number, $Pe$, which includes the solvent flow velocity term (see Eqn. 4.4), may be calculated from the mannitol $E$-values in Table 6.6 using Eqns. 4.5 and 4.6. As can be seen in Fig. 6.15, the $Pe$-values calculated using the results in the anode to cathode polarity are proportional to the applied voltage. In the cathode to anode experiments, the Stage II $P$-values for the 250 and 1000mV runs were very low and could not be distinguished from zero; however, the $Pe$-value for the 125mV case was found to fall within experimental error on the best-fit line for the anode to cathode results, this in accordance with theoretical expectation, i.e., the magnitude of the electro-osmotic velocity should be independent of the system polarity. It should be mentioned that the very low flux results for the 250mV and the 1000mV experiments in the cathode to anode case are consistent with predictions based on the anode to cathode data: very low $P$-values of the order of $10^{-9}$ and $10^{-10}$ cm/sec for the 250 and 1000mV cases, respectively, were expected.

Assuming the $Pe$ value calculated from the mannitol transport enhancement is essentially the same as that for $\text{TEA}^+$ and the salicylate ion (this is believed to be a reasonably good assumption: at high ionic strengths, $\text{TEA}^+$, salicylate and mannitol—all have comparable passive permeability coefficients in porous membrane transport experiments (Sims et al., 1990a)), enhancement factors may be predicted using Eqns. 4.2 and 4.3 for $\text{TEA}^+$ and salicylate ion, respectively.

As with the mannitol $E$-values, enhancement factors for $\text{TEAB}$ and salicylate were determined by dividing the Stage II $P$-value (at 250mV) or the initial Stage II $P_{\Delta \psi}$-value (at 1000mV) by the passive Stage IV $P$-value and these are presented in Table 6.7. Initially, it was thought that by using the early time data from Stage II that the $E$-values would not include the effects of membrane alteration. However, during that time, as noted above, the membrane appeared to have been changing somewhat.

In Fig. 6.16, the $\text{TEAB}$ and salicylate $E$-values from Table 6.7 are plotted against the predicted curves from Eqns. 4.2 and 4.3. As can be seen, the $E$-values at 250mV are close to the predicted values; however, the 1000mV data show significant deviations.
Figure 6.15: Peclet number calculated from mannitol enhancement factors using Eqn. 4.5 in the anode to cathode polarity (●) and Eqn. 4.6 in the cathode to anode polarity (○).
Table 6.7
TEAB and salicylate enhancement factors determined from human epidermal membrane experiments

<table>
<thead>
<tr>
<th>Applied Potential (mV)</th>
<th>No. of Expts, n</th>
<th>Enhancement factor, $E^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. Tetraethylammonium bromide (TEAB)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>250</td>
<td>6</td>
<td>8.1 ± 6.3</td>
</tr>
<tr>
<td>1000</td>
<td>5</td>
<td>88 ± 50</td>
</tr>
<tr>
<td>B. Salicylate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>250</td>
<td>3</td>
<td>5.7 ± 1.7</td>
</tr>
<tr>
<td>1000</td>
<td>5</td>
<td>23 ± 15</td>
</tr>
</tbody>
</table>

$^a$ Mean ± S.D.
Figure 6.16: TEAB (●) and salicylate (○) enhancement factors compared to the predicted curves from Eqn. 4.2 for cations (...) and Eqn. 4.3 for anions (- - -). The solid curve is the Nernst-Planck prediction without solvent flow. Mannitol flux was used as a measure of solvent flow (Pe) across human epidermal membrane in Eqns. 4.2 and 4.3.
These deviations are believed to be, at least in part, due to the use of $P_{\Delta \Psi_1}$ values which insufficiently minimized the effects of membrane alterations in the calculation of $E$. In order to further explore this question, average $E$-values were calculated from experiments in which the Stages I and IV $P$-values were very close and in which the current remained relatively low and stable during Stage II (see Figs. 6.6 and 6.12). These $E$-values are plotted against the predictions of Eqns. 4.2 and 4.3 in Fig. 6.17. Although the variability is high, it appears that if membrane alteration effects could be eliminated as a factor, the agreement between experiment and theory would be quite good.

Electro-osmosis has been suggested as the mechanism for convective solvent flow in skin (Pikal and Shah, 1986; Burnette and Marrero, 1986; Srinivasan and Higuchi, 1990). The classical theory of electro-osmosis in porous membranes was developed by Smoluchowski (1921) (see Appendix C) for the case when the pore radius, $a$, is much larger than the thickness of the electrical double layer, $1/\kappa$ (Bockris and Reddy, 1970; Hunter, 1981; Hiemenz, 1986). Combining the Smoluchowski equation with the constant field assumption of Goldman (1943) results in an expression for the electro-osmotic velocity (Sims et al., 1990a):

$$v = \left(\frac{\sigma}{\kappa \eta}\right) \frac{\Delta \Psi}{\Delta x} \quad (4.10)$$

where $\sigma$ is the surface charge density of the "pore wall," $\kappa$ is the Debye-Huckel reciprocal length, $\eta$ is the bulk viscosity, and $\Delta \Psi/\Delta x$ is the electric field. Equation 4.10 has been derived assuming a low surface potential and is expected to be strictly valid when $1/\kappa\ll\sigma$ (in the present studies, $1/\kappa$ is around 10Å).

In human stratum corneum the pore size is unknown and it is likely that a range of pore sizes exists. Thus, in considering electro-osmosis in skin, it is necessary to keep in mind that there may be pores smaller than or comparable in size to the electric double layer thickness. Therefore, in order to develop the more general theory applicable to stratum corneum, the situation where $1/\kappa$ is of comparable magnitude to the pore radius must be
Figure 6.17: TEAB (●) and salicylate (○) enhancement factors calculated from the experiments exhibiting the least amount of membrane alteration in the initial phase of Stage II. (....) predicted enhancement from Eqn. 4.2 for monovalent cations; (- - -) predicted enhancement from Eqn. 4.3 for monovalent anions. The solid line is the Nernst-Planck prediction without solvent flow.
considered and the Poisson-Boltzmann equation must be solved numerically. It is believed, nevertheless, that the direct proportionality of \( v \) with respect to \( \Delta \Psi / \Delta x \) is general and this relationship is seen with the present data (Fig. 6.15). The theoretical considerations have been found useful in qualitatively describing the solvent flow velocity through skin during iontophoresis and implicate electro-osmosis as one mechanism by which solvent flow occurs.

The volume flow across the epidermal membrane is of practical importance. In these studies, using tracer levels of mannitol, the volume flow when 1000mV was applied, anode to cathode, in Stage II was found to be the order of 1\( \mu \)l/hr/cm\(^2\) for tissue samples having passive P-values of the order of \( 10^{-8} \) cm/s. These studies indicate that solvent flow effects, alone, probably impact in only a secondary manner in the iontophoretic transport of ionic species.

6.5 Conclusions

Human epidermal membrane undergoes continuous membrane alteration at high applied voltages (1000mV) but not at 250mV. These changes appear to reverse over time once the voltage drop is removed. The membrane alterations caused an additional increase in the enhancement factors for the transport of ionic species over that expected from the combined applied field and solvent flow contributions. This phenomena may be important for the delivery, by iontophoresis, of both charged and uncharged drug molecules.

Solvent flow, as measured by an increase in mannitol flux, occurs in human skin during the application of an electric field. The effect on ion transport is relatively small compared to the direct electric field effects. However, coupled with membrane alteration, the solvent flow effects may become significant.
CHAPTER 7

SUMMARY

The goal of this project was to investigate the mechanisms by which charged and uncharged solutes are transported across skin under the influence of an externally applied electric field. Preliminary studies with hairless mouse skin using a neutral permeant (glucose) and an anionic permeant (butyrate ion), demonstrated that solvent flow occurred during iontophoresis. Irreversible membrane alterations due to the applied field were also observed. These alterations and the significant membrane-to-membrane variabilities seen with hairless mouse skin precluded a quantitative analysis of the solvent flow effects.

Subsequently, the modified Nernst-Planck theory was critically examined using the model Nuclepore® membrane. Iontophoresis experiments at 125, 250, 500, and 1000mV for both the anode to cathode and the cathode to anode polarities were carried out at three ionic strengths (0.1, 0.01, and 0.001M). Studies with the neutral solute, glucose, showed little influence of the ionic strength upon glucose flux enhancement (relative to passive diffusion) at all applied voltages for both polarities. In the anode to cathode polarity, the glucose enhancement factor increased linearly with the applied voltage, attaining a maximum value of about 20 at 1000mV. In the cathode to anode polarity, glucose E-values decreased with increasing applied voltage; at 500 and 1000mV, the glucose fluxes were indistinguishable from zero. These results demonstrated the directional nature of the solvent flow (in the direction of the counterion flow) and showed that the flux of a solute may be impeded or enhanced depending on the polarity of the system. An estimate of the solvent flow velocity was made from the glucose E-values using the modified Nernst-Planck theory. The velocity thus determined was then used for predicting the E-values for
TEAB and for the salicylate ion. Comparisons between the experimental and predicted E-values for TEAB and salicylate showed that the agreement was quite good.

A physical model approach was developed in order to gain a more detailed mechanistic understanding of the solvent flow process during iontophoresis. A numerical method was employed to solve the Poisson-Boltzmann equation for the potential, $\Psi$, in a cylindrical pore as a function of the pore radial distance, $r$. The potential profile was then incorporated into the equation of motion for fluid flow in a cylindrical pore. These equations were solved numerically for the linear solvent flow velocities for various conditions of surface charge density, ionic strength, and applied voltage drop. An estimate of the surface charge density, $\sigma$, of the Nuclepore® membrane was obtained via a matching process whereby the $\sigma$-value was varied until the predicted velocity was the same as the experimentally determined velocity. The $\sigma$-values were found to be essentially independent of ionic strength. The partition coefficients at 0.1, 0.01, and 0.001M ionic strengths was then predicted using the $\sigma$-values determined from the velocity calculation. The agreement between the predicted and the experimentally determined partition coefficients was very good.

Iontophoresis studies with human epidermal membrane were conducted with mannitol (neutral solute), TEAB (cation), and salicylate ion (anion). These experiments involved four stages: Stage I was a passive period, Stage II involved application of a voltage drop, and Stages III and IV were passive periods directly after (Stage III) and 16-24 hours after (Stage IV) the end of Stage II. In many of the experiments at 1000mV there were significant, nonlinear, increases (2 to 10 fold) in the flux during Stage II. At lower voltages (125 and 250mV), the flux increased linearly in all experiments. The current was found to mimic the permeation data, i.e., in experiments where the flux increased nonlinearly, the current also increased nonlinearly and by about the same factor. The nonlinear behavior was interpreted to mean membrane alterations were occurring to the skin during Stage II. In nearly all cases, the passive fluxes determined in Stage IV were
essentially the same as that determined during Stage I. These results imply that membrane alterations were reversible.

Mannitol flux enhancement was used in the human skin experiments as a measure of the solvent flow contribution to the flux enhancement. Mannitol E-values were about one-half of that observed with the Nuclepore® system. This corresponds to a 20-25% solvent flow contribution to the fluxes of TEAB and salicylate ions. Comparison of the experimental E-values for TEAB and salicylate ion with those predicted by the modified Nernst-Planck equations show that there is generally good agreement between experiment and theory.

The results of this project provide a basic understanding of the mechanisms by which charged and uncharged solutes transport across skin under an applied electric field. This information may be used to further investigate aspects of iontophoretic drug delivery across human skin such as the coupling of iontophoresis with chemical penetration enhancers and the possibility of using the reversible membrane alteration effects at high voltages to increase the flux enhancement of solutes.
APPENDIX A

THE FOUR ELECTRODE POTENTIOSTAT SYSTEM
The four electrode potentiostat system for a two chamber diffusion cell was developed to allow the potential drop across the membrane to be precisely controlled. It is a modification of a four electrode potentiostat widely used in electrochemistry (Masada et al., 1989). Figure A.1 is an illustration of the four electrode potentiostat system. The heart of the system is the potentiostat, which maintains the potential drop across two Luggin capillary probes (which are placed very close to the membrane on either side) at the desired value. This value may be static or programmed. Reactions which may occur at the driving electrodes, and subsequent potential losses are thus eliminated in this system.

A.1 Diffusion Cells and Luggin Capillaries

The diffusion cell and Luggin capillary were made of glass. Figure A.2 is a sketch of one half-cell. The cells were assembled with the membrane sandwiched between the two half-cells. The area for mass transfer is 0.7 cm². Each half-cell had a volume of ~5 ml. Each half-cell had a water jacket and the cells were maintained at 37°C by circulating water from a constant temperature bath. The cell contents were stirred by propellers in each half-cell rotating at 150 rpm.

In the four electrode potentiostat system, each half cell has two electrodes – a counter and a reference electrode. Either a platinum or silver-silver chloride wire serves as the counter electrode and a Luggin capillary serves to probe and define the controlled region and contains the reference electrode. The Luggin capillary consists of a long thin glass capillary placed very close to the membrane (see Fig. A.2). It is filled with the same electrolyte solution as in the two half cells. A calomel electrode is used as reference for the potentiostat. The voltage drop between the Luggin capillary tips is controlled at the set value by driving the required current through the counter electrodes. The value of the voltage drop selected across the capillary tips is displayed on a digital voltmeter (DVM). The voltage drop across the counter and reference electrodes can also be independently measured by connecting an external DVM to the appropriate terminals in Fig. A.1a. Only
Figure A.1: Four electrode potentiostat system. (a) Schematic illustration; (b) Equivalent circuit diagram.
Figure A.2: Sketch of a half-cell with its associated Luggin capillary.
negligible current can flow through the reference electrodes because they represent very high impedance inputs. The maximum output of the potentiostat is one ampere.

The use of the Luggin capillary, filled with the same electrolyte as in the half cell, eliminates any interfacial potential drop close to the membrane. So the potential drop across the Luggin capillary tips is the potential drop that actually exists in the solution across the tips, which are placed very close to the membrane. An equivalent circuit diagram for the four electrode iontophoresis system is shown in Fig. A.1b. Since the Luggin capillary tips are not exactly touching the membrane surface, there is an electrolyte filled gap between the capillary tip and the membrane surface. The resistance of this electrolyte gap on either side of the membrane is labeled $\Omega_d'$ and $\Omega_r'$. The resistance of this electrolyte gap ($\Omega_d' + \Omega_r'$ in Fig. A.1b) can be readily estimated by clamping the two together without a membrane in between. The electrolyte filled capillaries are placed in each half-cell and the two chambers filled with electrolyte. The current in the diffusion cell is monitored as a function of the voltage drop applied across the Luggin capillary tips. Figure A.3 shows a typical current-voltage profile obtained when the electrolyte is phosphate buffered saline, isotonic, pH 7.4. The current-voltage profile is linear and the resistance of the electrolyte gap is about 6.5 ohms.

Membrane resistances are typically in the kilo-ohm range, so that the resistance of the electrolyte gap is negligible compared to the resistance of the membrane. Consequently, the voltage drop across this electrolyte gap is negligibly small and the voltage drop applied across the capillary tips is, for all practical purposes, the voltage drop across the membrane. Since the resistance of the electrolyte gap is negligible compared to that of the membrane, slight variations in the placement of the capillary tips from run to run will not affect the results significantly. Membrane damage is understood to mean significant change in the resistance (and consequently the permeability coefficient) of the membrane during the course of an experiment (Srinivasan et al., 1990a; Srinivasan et al., 1989). The flux enhancement equation (Eqn. 1.20) clearly assumes that there is
Figure A.3: Typical current-voltage profile obtained for a phosphate buffered saline solution, isotonic, pH 7.4.
no change in the membrane due to the applied electric field. It is particularly important to ensure this in experiments with skin. Artificial membranes are less likely to be affected by the electric field.

With the four electrode iontophoresis system, it is possible to monitor the integrity of the membrane during the course of an experiment. The applied voltage drop across the membrane remains constant throughout. Therefore if the membrane breaks down, the current across the membrane will change markedly. In a two electrode system, the current across the membrane is held constant even if the membrane breaks down and there is no built in device for monitoring the condition of the membrane.

A.2 The Four Electrode Potentiostat

A simplified block diagram of the electronic circuit of the four electrode potentiostat is shown in Fig. A.4. It illustrates the working principles of the instrument. The basic unit is manufactured by JAS Instruments, Salt Lake City, UT, with some modifications carried out at the Department of Chemistry, University of Utah.

The potentiostat is represented by the operational amplifiers OA 1-3. It can internally generate any voltage in the range ± 4.000 V. The value selected is displayed on a DVM display. When this voltage versus ground is applied to the input of OA 1, the same voltage of the opposite sign appears at the contact to the reference electrode RE 1, while the contact to the reference electrode RE 2 is always held at virtual ground. Because each reference electrode is connected to the high impedance input of an operational amplifier (OA 2 or OA 3), only a negligible current can flow through each of them. Thus, the potential difference between the reference electrodes (which are connected to the Luggin capillaries) is then controlled in a well defined manner.

The current flowing through the cell and consequently the membrane is supplied by the outputs of the operational amplifiers OA 1 and OA 3. Operational amplifiers typically output currents in the milliampere range, while cell resistances are typically in the 10 ohms
Figure A.4: Block diagram of the electronic circuit of the four electrode potentiostat.
to 10 kilo-ohm range. To increase the available current (and thus to make the instrument more generally useful), a current booster (a push-pull amplifier) has been added at the output of OA 1 to enable the potentiostat to deliver a maximum current of 1 ampere. The value of the current flowing through the membrane is measured as the floating voltage drop across the resistor $\Omega_2$ by a current follower and displayed digitally. An analog voltage signal is also provided at a socket for hookup to a recorder. This gives out $\pm 10$ V corresponding to the full scale value of the current range.

Thus, the four electrode potentiostat system is ideally suited to maintain a constant voltage drop across a membrane in a two chamber diffusion cell and thus test the flux enhancement equations developed from the Nernst-Planck theory.

A.3 Two Electrode Constant Current Potentiostat

Figure A.5a is a schematic representation of a two electrode, constant current system (Srinivasan et al., 1990a). The membrane is clamped in between two half-cells, one of which is the donor and the other the receiver compartment. Each half cell is filled with electrolyte, well mixed by a stirrer, and has a sampling port. The ionic drug is present in the donor chamber. An electrode (usually a platinum wire) is placed in each half cell and the polarity adjusted according as the drug ion in the donor chamber is positively charged or negatively charged. If, for example, the drug ion is positively charged, then the electrode in the donor chamber is kept at a positive potential with respect to the electrode in the receiver chamber, as shown in Fig. A.5a. An electrolytic conductor obeys Ohms law for small electric fields, and under steady state conditions, it can be represented in an electrical circuit (in which there is only a dc source) by a resistor (Bockris and Reddy, 1970). Thus, an equivalent circuit for this system is shown in Fig. A.5b, where $\Omega_d$, $\Omega_r$, and $\Omega_M$ are the resistances of the donor electrolyte, receiver electrolyte and membrane, respectively. In the two electrode system, the current between the electrodes (and consequently the current through the membrane, which is in series) is maintained at a
Figure A.5: Two electrode, constant current system. (a) Schematic diagram; (b) Equivalent circuit diagram.
constant value. This is done by appropriately adjusting the voltage drop across the electrodes. The voltage drop that exists across the membrane is not known.

The voltage drop applied across the two electrodes is not the voltage drop that exists across the membrane. It is the voltage drop across all the resistances in series (the two electrode-electrolyte interfaces, the electrolyte in the two compartments plus the membrane). Assuming that the voltage drop across the electrodes is the voltage drop that appears across the membrane is incorrect.
APPENDIX B

NUCLEPORE® MEMBRANE
The Nernst-Planck theory assumes that the membrane of interest is homogeneous with well defined pathways for diffusion. Therefore, in order to examine the predictions of the Nernst-Planck equations, a model membrane was employed which embodied the essential characteristics of skin (the actual membrane of interest) but was also homogeneous and had well defined pore geometry. Skin is known to be a net negatively charged membrane with a pKa of about 4. Additionally, ions are assumed to transport across skin along an aqueous pathway (as opposed to a hydrophobic pathway). Thus, the model membrane chosen was the Nuclepore® membrane made by Nuclepore Corporation (Pleasanton, CA). The physical characteristics were sufficiently close to those of skin while the track-etching procedure used by the manufacturer assured a known pore shape, size and porosity.

B.1 Manufacturing Process

The Nuclepore® membranes used in these studies were prepared from solution cast Bisphenol A polycarbonate films. The polycarbonate films undergo a track-etching procedure involving nuclear fission with a U235 laser which weakens some of the polycarbonate bonds in its pathway. The film is then etched with a very strong caustic which attacks the weakened bonds; the time and temperature of caustic exposure determines the pore size. Since polycarbonate is very hydrophobic, the membranes are coated with polyvinylpyrrolidone (PVP) to render them hydrophilic. PVP does not covalently bond to the polycarbonate and is assumed to enter the pores in a more or less molecular monolayer. Air flow studies are used to verify the pore size (within -20% to +0% of specification) after PVP coating. Thus, the track-etching method provides membranes with straight, cylindrical pores of known dimensions.

B.2 Physical Characteristics

The smallest pore size membrane available was chosen for the present studies. The nominal physical characteristics provided by Nuclepore Corp. for these membranes were:
Scanning electron microscopy (SEM) of the membrane surface was inconclusive as to the exact shape of the pores due to the very small pore dimensions and porosity. SEM micrographs of larger pore membranes demonstrate, in general, the circular geometry of the pore openings (Nuclepore Corp.). Some authors have reported small deviations from the nominal pore size and porosity values based on SEM (Cheng et al., 1989; Bisio et al., 1980). In addition, the manufacturer allows a tolerance of up to 13° from the normal to the membrane face. Transmission electron microscopy (TEM) was used to examine vertical sections of these membranes. Straight cylindrical pores traversing the width of the membrane were observed (Fig. B.1). The pore channels appeared to have essentially equal radii along their length, however, this was difficult to ascertain with confidence as only a few of the channels were scanned.

The resistance of individual Nuclepore® membranes was found to be about 30 ohms (Sims et al., 1990a) while the surrounding electrolyte had a specific conductivity of about 0.01 ohm\(^{-1}\) cm\(^{-1}\) (assuming a cylindrical geometry). In order to have a membrane controlled situation (see Appendix A), it was necessary to stack 50 Nuclepore® membranes together to form a “plug.” The current-voltage profile of the 50 membrane plug at 0.001, 0.01, and 0.1 M ionic strengths was compared to the profiles determined for 1 membrane and a 10 membrane plug at 0.1 M ionic strength in Fig. B.2. As can be seen, the current in the 1 and 10 membrane examples rises quickly with small increases in voltage and, at relatively low applied voltages, approaches a maximum value. The current in the 50 membrane case increases linearly with increasing voltage over the entire voltage range of interest. This linearity implies Ohm’s law is obeyed and the resistance may be calculated directly as the slope of the current-voltage plot; the resistance of the 50 membrane plug was found to be about 1.5 kilo-ohms in 0.1 M ionic strength, as expected from
Figure B.1: Transmission electron microscopy (TEM) picture of the pore channel in a Nuclepore® membrane. Radius of the pore is 75Å. Magnification X 38,456.
Figure B.2: Current-voltage profiles for (o) 1, (+) 10, and (●) 50 Nuclepore® membranes in 0.10M ionic strength buffer. The current-voltage profiles of 50 membranes in (■) 0.01M and in (□) 0.001M ionic strength buffers have been enlarged to show their linear slopes.
The permeability coefficient of glucose was determined using 1, 10 and 50 membranes during iontophoresis at several applied voltages. These P-values are shown in Fig. B.3 as a function of the applied voltage drop. Again, the 1 and 10 membrane cases rise quickly and approach a maximum value at relatively low applied voltages. The 50 membrane examples increases linearly, as with the current, over the entire voltage range of interest. Thus, the model membrane studies described herein were conducted with 50 Nuclepore® membranes stacked together such that no air bubbles were trapped between membranes (Sims et al., 1990a).

Nuclepore® membranes have been shown to have a net negatively charged pore surface (Meares and Page, 1972; Ibanez and Tejerina, 1982; Keesom et al., 1988; Bisio et al., 1980; Sims et al., 1990a). Since the surface charge density cannot be measured directly, it has been inferred from electro-osmotic flow studies (Meares and Page, 1972), streaming potential measurements (Keesom et al., 1988), membrane and diffusion potential studies (Ibanez and Tejerina, 1982), and neutral solute flux (Sims et al., 1990a). All of these methods invoked the Gouy-Chapman double layer theory and relate the surface charge density to a measurable quantity, such as volume flow or streaming potential via the Helmholtz-Smoluchowski theory, as discussed in Appendix C. While all of the various methods of estimating the surface charge density give very similar results (about -0.003 C/m²), an ionic strength dependence was also noted. Keesom et al. (1988) postulated that this may be due to the ionic strength dependent charge densities of the diffuse double layer.

The surface characteristics of Nuclepore® membranes were further probed by Bisio et al. (1980) who reported a pKa of about 4. This pKa-value is similar to that reported for human skin (Burnette and Marrero, 1986). Additionally, the zeta potential was measured as a function of pH by Keesom et al. (1988). These authors found a sigmoidal shaped curve below pH 6 and a constant zeta potential above pH 6. This suggested the presence of at least one acidic surface group which dissociated completely above pH 6. In these studies, the maximum zeta potential, -25mV, indicated a very sparsely charged surface.
Figure B.3: Glucose permeability coefficients determined using (○) 1, (■) 10, and (△) 50 Nuclepore® membranes.
B.3 Source of the Membrane Charge

The pore surface charge on Nuclepore® membranes may originate from ion adsorption from the surrounding electrolyte, surface ionizable groups, and/or image charging. Meares and Page (1972) suggested Cl- adsorption to the polycarbonate backbone as the source of the pore wall charge. However, this seems unlikely as Cl- is not expected to actively adsorb to this polymer. Keesom et al. (1988) and Bisio et al. (1980) observed a pKa of 3.9 for Nuclepore® membranes which is in the range found for bulk carboxylic acids, thus suggesting the formation of carboxyl groups during the track-etching process. Further substantiation of surface carboxyl groups was provided using ESCA which indicated about 2.5% of the detectable surface carbon was in the potentially hydrolyzable form of carboxyl groups (Keesom et al., 1988). This corresponds to the low charge densities estimated by many authors (Meares and Page, 1972; Ibanez and Tejerina, 1982; Sims et al., 1990).

Another possible source of the pore surface charge may be image charges which arise at an interface between an insulator, such as a polymer, that has a dielectric constant, \( e_1 \), smaller than that of the electrolyte solution, \( e_2 \) (Williams, 1975; De Levie, 1981). A schematic diagram that illustrates image charging at an electrolyte-insulator (also called a dielectric) interface is shown in Fig. B.4. In this figure, an ion of radius \( r/2 \) is located at point \( q \), a distance \( d \) from the boundary. The fictitious image charge resides at point \( q' \). The result of image charging in this situation is to repel ions from the interface and to cause a decrease in the dielectric constant close to the interface (De Levie, 1981; Williams, 1975).

Image forces have been found to occur when a charged body nears a lipid bilayer (Honig, 1986; Edmonds, 1988). Image energy calculations applicable to lipid bilayers have been developed by many authors, however, the agreement between them is not perfect (Honig, 1986). Other areas in which image charges have been studied include semiconductor films (Aharoniun et al., 1988), surface phenomena (Outhwaite, 1970; Vorotyntsev and Ivanov, 1988), and the transport of electrons and protons across
Figure B.4: Schematic diagram of an image charge at an electrolyte-insulator (or dielectric) interface. (Adapted from Williams, 1975)
biological membranes (Zimangi and Garab, 1989). In the Nuclepore® membrane, the pore wall contains a fixed negative charge. Thus, image charges could arise from the ions in solution and from the fixed charges on the pore wall. The interaction of these image charges with the electrostatic attraction of the fixed charges with counterions in solution becomes very complex. Qualitatively, there will be increased ordering of the electrolyte around each fixed ion, with the result that counterions will predominate near the wall. Far away from the fixed ion, the image charge from solution may polarize the membrane surface, however, since both positive and negative ions may cause polarization, the net effect may be small.

In the present study it is the form of the surface potential extending into the double layer, which results from the charged surface, that is of interest. Therefore, the surface charge density is assumed to be due to the combination of electrostatic and image forces. It is also assumed that the surface charge density is smeared out uniformly over the wall of the pore.
APPENDIX C

HELMHOLTZ-SMOLUCHOWSKI THEORY OF ELECTRO-OSMOSIS
Electro-osmosis occurs when a solid (pore wall) remains stationary and the ions, moving in response to an applied electric field, drag the solvent along with them (Hunter, 1981). This electrokinetic phenomena relies on the existence of an electrical double layer at the solid-electrolyte interface in which there is a predominance of one type of ion (counterion) over another (co-ion). Smoluchowski (1921) developed the theory of electro-osmosis for the case where the thickness of the double layer (1/k) is negligible compared to the radius of curvature, $R_c$, of the surface. This theory was based on the Gouy-Chapman diffuse double layer model of ions at an electrified interface. Therefore, in the following, a brief description of the Gouy-Chapman model and an outline of the derivation of the Helmholtz-Smoluchowski equation for electro-osmotic velocity has been provided.

### C.1 Gouy-Chapman Diffuse Double Layer Model

The Gouy-Chapman model is shown in Fig. C.1. The model consists of an infinite planar surface carrying a smeared surface charge in contact with an electrolyte solution containing ions which are considered as point charges (Aveyard and Haydon, 1973). The distribution of ions normal to the surface is described by a Boltzmann distribution:

$$n_i(x) = n_i^0 \exp[-z_i e/kT (\Psi(x) - \Psi(\infty))]$$ (C.1)

where $n_i(x)$ is the number of ions per unit volume of species $i$ at a distance $x$ and potential, $\Psi(x)$, from the surface and $n_i^0$ is the number of ions per unit volume at $x = \infty$ and potential, $\Psi(\infty)$. Poisson's equation is used to relate the electrical potential to the space charge density, $\rho(x)$. For a 1:1 electrolyte in one dimension (Adamson, 1990):

$$\frac{d^2 \Psi(x)}{dx^2} = -\frac{4\pi \rho(x)}{\epsilon}$$ (C.2)

and

$$\rho(x) = -2n_i^0 e \sinh[z_i e \Psi(x)/kT]$$ (C.3)

Combining Eqns. C.1-C.3 and using the boundary conditions:

$$\Psi(x) \to 0 \text{ as } x \to \infty$$ (C.4)

and

$$\left. \frac{d\Psi(x)}{dx} \right|_{x=\infty} = 0$$ (C.5)
Figure C.1: Schematic diagram of ion distribution near a negatively charged surface according to the Gouy-Chapman model.
and the condition that the charge on the surface, \( \sigma \), must be balanced by the charge in the electrolyte solution, i.e.,

\[
\sigma = -\int_0^\infty \rho(x) \, dx
\]  
(C.6)

allows for the integration of Eqn. C.2 (Aveyard and Haydon, 1973; Hiemenz, 1986; Adamson, 1990). The final form of the Gouy-Chapman equation (in one dimension) is then:

\[
\sigma = \left[ \frac{2n^0e^2kT}{\pi} \right]^{1/2} \sinh[ze\Psi_0/2kT]
\]  
(C.7)

where \( \Psi_0 \) is the surface potential.

C.2 Helmholtz-Smoluchowski Theory

When an electric field, \( F \), is applied, the ions in the diffuse layer will experience a force parallel to the surface and will transmit this force through friction to the surrounding solvent (Aveyard and Haydon, 1973; Bockris and Reddy, 1970; Hiemenz, 1986; Hunter, 1981). Figure C.2 is a schematic diagram of the forces acting on the interfacial region. At steady state, the frictional force is balanced by the electrical force, i.e.:

\[
F \rho(x) \, dx = \eta \frac{d^2v(x)}{dx^2} \, dx
\]  
(C.8)

where \( \eta \) is the bulk viscosity and \( v(x) \) is the velocity of the solution parallel to the surface. On substituting for \( \rho(x) \) from Eqn. C.2 and assuming \( \varepsilon \) and \( \eta \) are constants, Eqn. C.8 may be integrated to give (Hiemenz, 1986):

\[
-eF \frac{d\Psi}{dx} = \eta \frac{dv}{dx}
\]  
(C.9)

Assuming that at the surface of shear, \( \Psi = \zeta \) and \( v = 0 \) and that at \( x = \infty \), \( \Psi = 0 \) and \( v = v \), Eqn. C.9 may be integrated to give:

\[
v = \frac{\varepsilon \zeta F}{4\pi\eta}
\]  
(C.10)
Figure C.2: Schematic diagram of the forces acting on the charged interface between a solid surface and an electrolyte solution under the influence of an applied electric field.
Eqn. C.10 is known as the Helmholtz-Smoluchowski equation; $\zeta$ is the zeta potential and $v$ is the electrokinetic or electro-osmotic velocity.
The average partition coefficient and average electro-osmotic velocity were calculated using Microsoft Excel, version 2.2A software and run on a Macintosh SE personal computer. The calculation procedures are illustrated below.

D.1 Partition Coefficient

The expression for the average partition coefficient, $\beta_i$, in a cylindrical pore is:

$$\beta_i = \frac{2}{(a')^2} \int_0^{a'} r \exp(-BP(r)) dr$$  \hspace{1cm} (D.1)

where $a'$ is the effective pore radius, $P(r)$ is the radial potential profile defined in Chapter 5, and $B$ is a constant defined as:

$$B = \frac{zF}{kT}$$  \hspace{1cm} (D.2)

The integral in Eqn. D.1 may be solved numerically once the potential, $P(r)$, is known for each $r$. Given this function (Weaver, 1989), the integration may be approximated as a summation over the pore. In the case of cylindrical pores, cylindrical shells of thickness, $\Delta r$, are summed:

$$\beta_i = \frac{2}{(a')^2} \sum_r r \exp(-BP(r)) \Delta r$$  \hspace{1cm} (D.3)

In the present case, $r$ and $P(r)$ were obtained by numerically solving the Poisson-Boltzmann equation in cylindrical coordinates (Weaver, 1989). The parameters used to solve Eqn. D.3 were:

- $a' = 7.3 \times 10^{-9} \text{ m}$
- $T = 37^\circ \text{C} = 310 \text{ K}$
- $z = \pm 1$
- $B = \pm 0.03743 \text{ mV}^{-1}$
D.2 Electro-osmotic Velocity Profile

The average solvent flow velocity or electro-osmotic velocity, \( v \), was determined by solving the equation of fluid motion in cylindrical coordinates (Rice and Whitehead, 1965):

\[
\frac{d^2v}{dr^2} + \frac{1}{r} \frac{dv}{dr} = -\frac{2neE}{\eta} \sinh[ze\Psi(r)/kT]
\]  
(D.4)

where \( n \) is the bulk concentration of ions, \( e \) is the electronic charge, \( E \) is the applied electric field divided by the membrane thickness, \( \Delta x \), and \( \eta \) is the bulk viscosity. To numerically solve this equation, the radial potential profile must be known from the Poisson-Boltzmann equation. Once this is obtained, Eqn. D.4 may be solved by making the following substitutions:

\[
H = r \frac{dv}{dr}
\]  
(D.5)

and

\[
\rho(r) = \frac{-2neE}{\eta} \sinh[ze\Psi(r)/kT]
\]  
(D.6)

Substituting Eqns. D.5 and D.6 into Eqn. D.4:

\[
dH = r \rho'(r) \, dr
\]  
(D.7)

Integration of Eqn. D.7 from the centerline of the pore \(( r=0 )\) to any point \( r \) yields:

\[
H(r) - H(0) = \int_0^r r \rho'(r) \, dr
\]  
(D.8)

The boundary conditions for equation D.1 require that the \( v = 0 \) at \( r = a' \) and that \( dv/dr = 0 \) when \( r = 0 \). Thus, from the definition of \( H \) (Eqn. D.5), \( H(0) \) must also be zero, and Eqn. D.8 reduces to:

\[
H(r) = \int_0^r r \rho'(r) \, dr
\]  
(D.9)

An expression for the velocity may be obtained by rearranging equation D.5:

\[
dv = \frac{H}{r} \, dr
\]  
(D.10)

Integration of equation D.10 from \( r = 0 \) to \( r = r \) results in:
\[ v(r) - v(0) = \int_{0}^{r} \frac{H}{r} \, dr \quad (D.11) \]

Using L'Hopital's Rule, in the limit at \( r \to 0 \), \( H/r \) goes to zero, thus the integrand in Eqn. D.11 does not go to infinity. Equations D.9 and D.11 may then be solved numerically by summing the contributions of cylindrical shells of width, \( \Delta r \), from \( r = 0 \) to \( r = a' \), as with the partition coefficient. Parameter values used in these calculations were:

\[ \eta = 7 \times 10^{-4} \text{ kg/m s} \]
\[ T = 37°C = 310 \text{ K} \]
\[ z = \pm 1 \]
\[ \Delta x = 3 \times 10^{-4} \text{ m} \]

The concentration of ions, in units of \( \# \) ions per \( m^3 \), depended on the ionic strength of interest and the magnitude of the applied electric field was varied from 125mV up to 1000mV.
APPENDIX E

PRETREATMENT AND STORAGE OF HUMAN SKIN
Human skin obtained from Ohio Valley Tissue and Skin Bank (Cincinnati, OH) undergoes the following sequence of steps from surgical excision (cadaver) to storage.

1. The skin area is shaved and scrubbed with betadine solution (an iodine-polyvinylpyrrolidone complex) to disinfect the skin prior to excision and dermatomining. A mineral oil scrub applied prior to dermatomining lubricates the skin.

2. After dermatomining, the skin is placed in phosphate buffered saline, pH 7.4, containing some antibiotics such as penicillin and streptomycin to prevent degradation.

3. The skin is then prepared for freezing by washing first with sterile saline and then with the preservative solution (10% glycerine in saline). The skin is frozen in stages to the final temperature of -120°C.

The epidermal membrane, which consists of the stratum corneum and part of the epidermis, was heat separated from the underlying dermis at TheraTech, Inc. (Salt Lake City, UT), using the following procedure.

1. The full thickness skin was immersed for 1 minutes in a 60°C waterbath after which the epidermal tissue was separated from the dermis.

2. The tissue was then stored at -20°C until used.
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