International Journal of Pharmaceutics, 49 (1989) 57–62
Elsevier

IJP 01643

Examination of iontophoretic transport of ionic drugs across skin: baseline studies with the four-electrode system

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(Received 24 February 1987)
(Revised version received 30 April 1988)
(Accepted 21 June 1988)

Key words: Iontophoresis; Four-electrode system; Enhancement factor

Summary

Recognition of the voltage drop across a membrane as the driving force for the flux of ions through it opens up new approaches to model iontophoretic transport of ionic drugs across skin. A theoretical equation relating the flux enhancement (relative to the passive diffusion flux) to the applied voltage drop across a membrane is given. A 4-electrode potentiostat system, ideally suited for maintaining a constant voltage drop across a membrane in a two-chamber diffusion cell is described. It is shown that the predictions of the flux enhancement equation are in reasonable agreement with the experimental results obtained with the four electrode potentiostat system for an artificial membrane and for hairless mouse skin.

Introduction

Iontophoresis can be defined as 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. Iontophoresis is being increasingly investigated as a technique for enhancing the penetration of ionic drugs across skin (Tyle, 1986). However, these studies have not provided data relating the actual potential drop across the membrane to the iontophoretic flux enhancement achieved.

In this communication, a theoretical equation for the prediction of ionic flux enhancement due to an applied voltage drop across a membrane is given, based on the fundamental thermodynamic properties of the system. A novel, 4-electrode potentiostat system for precisely maintaining a constant voltage drop across a membrane in a two-chamber diffusion cell is outlined. The theoretically predicted flux enhancements are compared with the experimentally measured values for various applied voltage drops across a cellophane membrane and excised hairless mouse skin for model ionic solutes.

Theory

The most common starting point for the formal description of diffusion is the Nernst–Planck

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equation, which, for ideal solutions, is given as (Schultz, 1980):

$$J_i = -D_i\left[\frac{dc_i}{dx} + \frac{c_i z_i F}{RT}\cdot\frac{d\Psi}{dx}\right] \quad (1)$$

where J_i is the steady-state flux of species i, D_i the diffusivity of i, c_i the concentration of i, z_i the charge on i, Ψ the electric potential, F the Faraday's constant, R the gas constant and T the absolute temperature.

For the case of a linear electric field across a membrane, it can be shown that (Schultz, 1980; Srinivasan et al., 1988) the enhancement factor E (relative to passive diffusion) is given by:

$$E = \frac{-K}{1-\exp(K)} \quad (2)$$

where

$$K = \frac{z_i F \Delta\Psi}{RT} \quad (3)$$

Eqn. 2 states that the enhancement due to the applied electric field is directly proportional to the voltage drop and the charge on the ion and predicts the flux enhancement for a given species for various applied voltage drops across the membrane.

The assumptions required to derive Eqn. 2 from Eqn. 1 are described in detail elsewhere (Schultz, 1980; Srinivasan et al., 1988). The implications of the assumptions are discussed in detail in Srinivasan et al. (1988).

Experimental section

Materials

Tetraethylammonium bromide (TEAB) and citric acid were selected as model ionic solutes for this study. [1-^{14}C]TEAB (4.7 mCi/mmol) and [1,5-^{14}C]citric acid (54.5 mCi/mmol) were obtained from New England Nuclear Co., Boston, MA, with stated radiochemical purity of > 98%. Ethyl alcohol (200 proof, dehydrated, U.S.P.) was obtained from U.S. Industrial Chemicals Co., Tuscola, IL. All other reagent grade chemicals were obtained from American Scientific Products, McGraw Park, IL, and were used as received.

Membranes

Full thickness skin was freshly excised from the abdomen of 2–4 month-old male hairless mouse (SKH/HR1) according to the procedure of Durrheim et al. (1980). Cellophane membrane was obtained from Transylwrap, Denver, CO. These membranes are 25 μm thick and are coated with a thin film of polyvinyl dichloride or saran to render the surface hydrophobic. Consequently, the membrane does not swell appreciably.

The 4-electrode potentiostat

The 4-electrode potentiostat for a two-chamber diffusion cell is a modification of a system widely used in electrochemistry (Samec et al., 1977). Fig. 1 is a schematic diagram of the 4-electrode potentiostat and the two-chamber diffusion cell. The potentiostat maintains the potential drop across the two Luggin capillary probes (which are placed very close to the membrane on either side) at the desired value by driving the required current through the cell with the help of the counter electrodes. Fig. 2 shows one half-cell of the two-chamber diffusion cell with its associated Luggin capillary and counter electrode.

The 4-electrode potentiostat system is described in detail in Srinivasan et al. (1988), who also compare it to the conventional two-electrode, con-

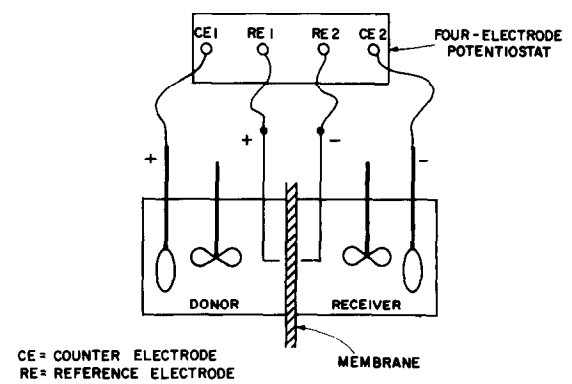

Fig. 1. Schematic of diffusion cell and the 4-electrode system for iontophoretic transport studies.

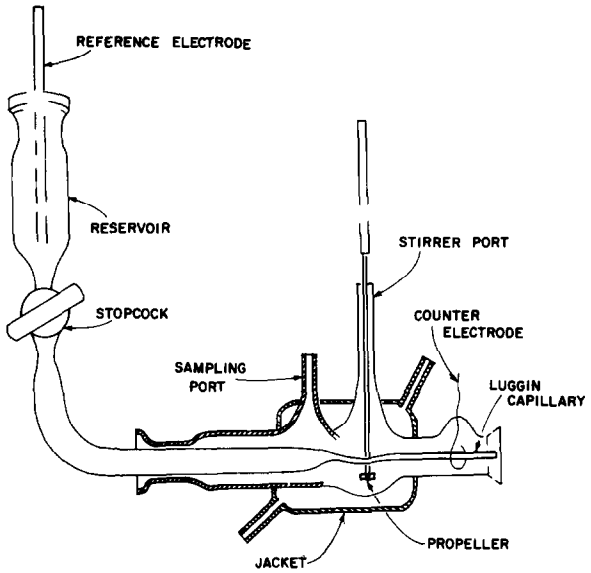

Fig. 2. Schematic of the diffusion half-cell with its associated Luggin capillary and counter electrode.

stant current system and discuss its advantages. The 4-electrode system was developed by Masada et al. (1985).

In a two-electrode system the current is maintained constant by the driving system, while the membrane resistance determines the voltage drop across the membrane. This voltage drop is unknown. In a 4-electrode potentiostat system, the voltage drop *across* the membrane is maintained constant and the current through the membrane is also simultaneously monitored. Thus the 4-electrode potentiostat system provides additional information that permits a more rigorous test of the predictions of the Nernst–Planck equation. This is not possible with a conventional two-electrode system.

Experimental procedure

The experimental protocol, sampling and assay technique and data analysis procedure are described elsewhere (Srinivasan et al., 1988). Briefly, the diffusion cell was assembled with the membrane and Luggin capillaries in place. The donor and receiver compartment were filled with the appropriate solution. The depletion of permeant in the donor side was determined to always be insignificant so that sink conditions could be assumed to prevail during the experiment. At predetermined times, 1 ml samples were withdrawn from the receiver side for assay and replaced with 1 ml buffer. The passive permeability coefficient P_0 (without iontophoresis) was first determined for a membrane. Then different voltage drops were applied across the same membrane (0.125 V, 0.25 V, 0.5 V, etc.) one after the other and the permeability coefficient determined at each applied voltage drop. The experimental enhancement factors were calculated as follows:

$$E = P_{\Delta\Psi}/P_0 \tag{4}$$

where $P_{\Delta\Psi}$ is the permeability coefficient at an applied voltage drop $\Delta\Psi$ across the membrane.

Results and Discussion

Iontophoresis of TEAB

Table 1 summarizes the permeability coefficients and enhancement factors of TEA ion through cellophane at different applied voltage drops across the membrane. The corresponding results for TEA ion through hairless mouse skin are summarized in Table 2. The experimentally observed enhancement factors are plotted as a function of the applied voltage drop in Fig. 3 for cellophane and hairless mouse skin. The dashed line is the theoretical prediction of Eqn. 2.

The enhancement factor for hairless mouse skin shows good agreement with the theoretical prediction of Eqn. 2 up to 0.5 V. The observed enhancement factor at 1.0 V is significantly higher than the theoretical prediction, possibly due to skin

TABLE 1

Iontophoresis of TEAB through cellophane

Medium: PBS isotonic, pH 6.0; Temperature: 37 °C.

Voltage (V)	P (cm/s)	E (exp.)	E (theory)
0	1.24×10^{-7}	–	–
0.125	7.24×10^{-7}	5.8	4.71
0.250	1.54×10^{-6}	12.4	9.35
0.500	3.97×10^{-6}	32.0	18.69
1.000	9.18×10^{-6}	74.0	37.38

TABLE 2

Iontophoresis of TEAB through hairless mouse skin

Medium: PBS isotonic, pH 6.0; Temperature: 37 °C.

Voltage (V)	P (cm/s)	*E* (exp.)	*E* (theory)
0	1.43×10^{-7}	–	–
0.125	5.88×10^{-7}	4.1	4.71
0.250	1.43×10^{-6}	10.0	9.35
0.500	3.13×10^{-6}	21.9	18.69
1.000	8.92×10^{-6}	61.0	37.38

damage. Solvent transport effects may also be a contributing factor to this effect, as suggested by Gangarosa et al. (1980) and Burnette and Marrero (1986). The enhancement factor for cellophane also shows good agreement with the theory up to 0.25 V, and then begins to deviate.

Ionic strength of the medium

The effect of ionic strength on iontophoresis was studied by varying the sodium chloride concentration of the medium. Fig. 4 shows the enhancement factor for TEA ion through hairless mouse skin at 3 different NaCl concentrations. It appears that the ionic strength has no significant effect on iontophoretic transport up to about 0.5 V. At higher voltage drops, increasing the ionic strength seems to cause a greater positive deviation from the theoretical predictions.

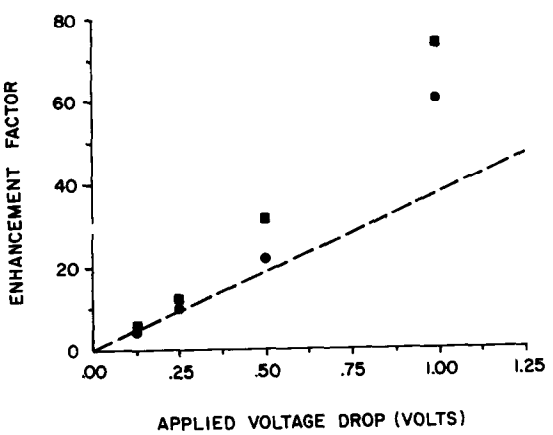

Fig. 3. The relationship between the applied voltage drop and the enhancement factor for iontophoretic transport of TEA ion at 37 °C. The dashed line is the theoretical prediction of Eqn. 2. Key: ●, hairless mouse skin; ■, cellophane membrane.

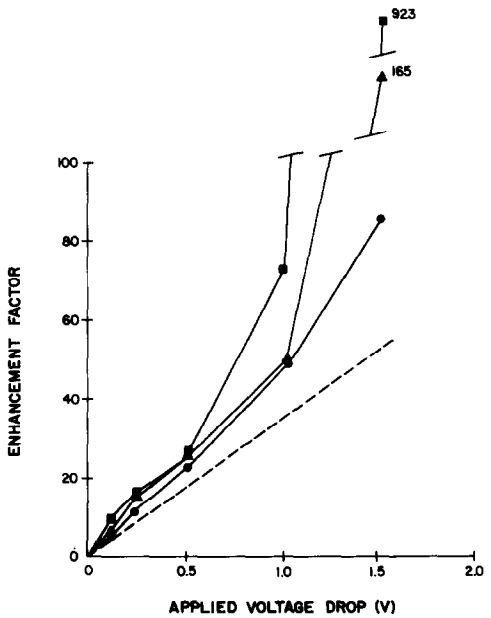

Fig. 4. The effect of ionic strength of the medium on the enhancement factor for iontophoretic transport of TEA ion through hairless mouse skin at 37 °C. The dashed line is the theoretical prediction of Eqn. 2. Key: ●, NaCl 0.45%; ▲, NaCl 0.9%; ■, NaCl 1.8%.

Skin damage

In order to test if the hairless mouse skin is damaged at higher voltage drops and hence the observed large positive deviations, voltage drop was applied in cycles (0–0.25 V and 0–1.5 V). A cycle consisted of 4.0 h of total duration with 3.0 h without voltage and 1.0 h with voltage. Results shown in Fig. 5A indicate that a similar flux pattern was observed in each 0–0.25 V cycle. In the case of 0–1.5 V cycles, however, as seen in Fig. 5B, the flux at 1.5 V in the second cycle increased remarkably, and P_0, the permeability coefficient at 0.0 V, in the third cycle was about 185 times larger than P_0 in the first cycle indicating an irreversible damage to the skin at this voltage. These data also suggest that the observed skin damage is a result of the magnitude of the applied voltage drop and its duration. The ionic strength of the medium seems to enhance skin damaging effect with increasing ionic strength, as seen from larger than expected deviations at higher voltage drops from theoretical predictions (Fig. 4).

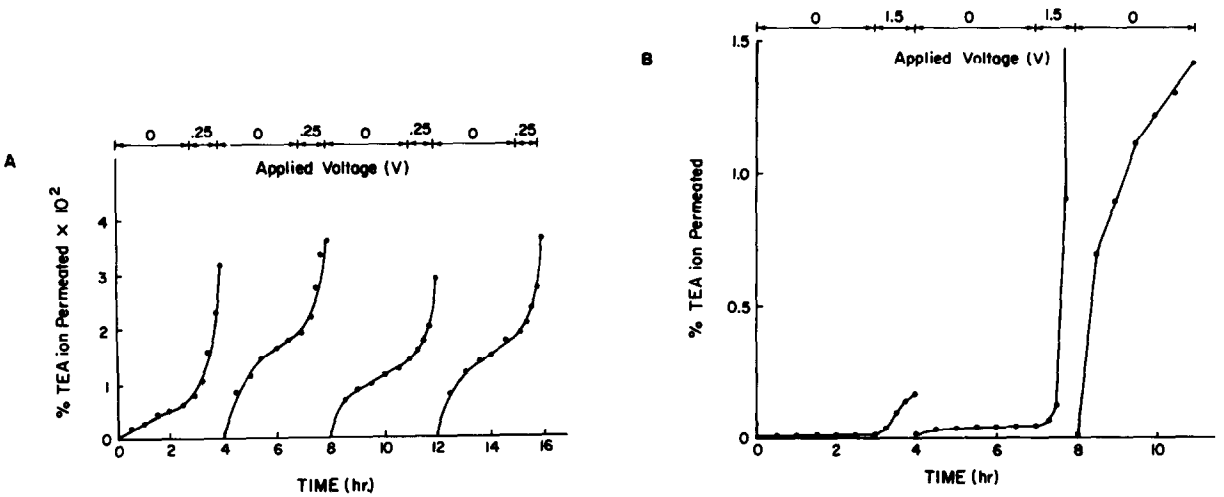

Fig. 5. Cyclic iontophoretic permeation of TEA ion in saline through the hairless mouse skin at 37 °C. Key: (A) 0–0.25 V cycle test; (B) 0–1.5 V cycle test.

Non-aqueous medium

Results of iontophoretic transport through hairless mouse skin in a non-aqueous medium (ethanol) are shown in Fig. 6. P_0 of TEAB at 37 °C in ethanol is 5.21×10^{-6} cm/s, 177 times larger than that observed in saline. The enhancement factor followed the predicted values reasonably well up to 1.5 V.

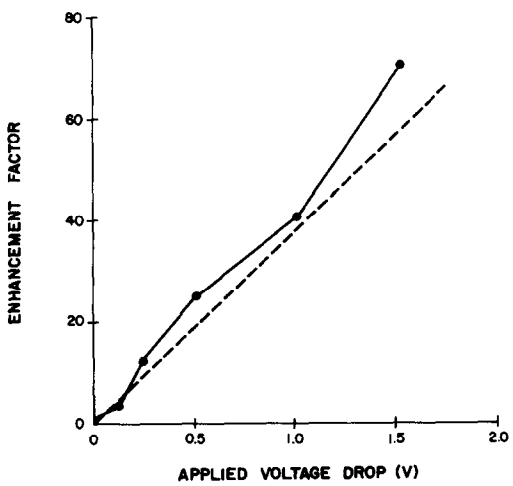

Fig. 6. The relationship between the applied voltage drop and the enhancement factor (E) for the iontophoretic transport of TEA through the hairless mouse skin at 37 °C, in ethylalcohol. The dashed line is the theoretical prediction of Eqn. 2.

Iontophoretic transport of a trivalent negative ion

As a model negatively charged ion, citric acid ionizes in PBS, pH 8.0 isotonic buffer used as medium into a trivalent ion. The citric acid flux enhancement results with hairless mouse skin as a function of the applied voltage drop are plotted in Fig. 7. The enhancement factor shows an abrupt departure from the theoretical predictions beginning at applied voltage drops > 0.125 V and shows significant positive deviations, probably due to skin damage and solvent transport effects.

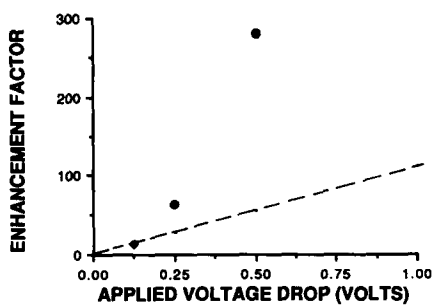

Fig. 7. The relationship between the applied voltage drop and the enhancement factor (E) for iontophoretic transport of citric acid in pH 8.0 isotonic buffer solution at 37 °C. The dashed line is the theoretical prediction of Eqn. 2.

Conclusion

These studies illustrate the utility of an equation for predicting the flux enhancement of ions across membranes (relative to passive diffusion), due to an applied voltage drop across the membrane. This work also shows the usefulness of a newly developed 4-electrode system for carrying out iontophoretic studies under conditions where the voltage drop across the membrane is well-defined to elicit fundamental information regarding factors controlling iontophoresis.

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