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Iontophoretic transport of weak electrolytes through the excised human stratum corneum

OVAIS SIDDIQUI*, MICHAEL S. ROBERTS†, ALAN E. POLACK, *School of Pharmacy, University of Tasmania, Hobart, Tasmania, Australia 7001*

Abstract—The effect of iontophoresis on the rate of permeation of a number of therapeutically active weak acids and bases through excised human stratum corneum has been examined, over a range of pH values. It has been shown that the amount of ionized drug species present in the drug solution is an important factor in the delivery of acids and bases by this route and that the molecular weight of the drug does not influence the rate of delivery by iontophoresis.

Iontophoresis (ITP), ion-transfer under the influence of electrical current, has been demonstrated to be a useful technique to enhance the permeation rate of a variety of drugs through the skin (Siddiqui et al 1985a, 1987; Burnette & Marrero 1986; Bellanto et al 1986; Chien et al 1987; Sanderson et al 1987).

An in-vitro technique can be used to determine the effect of iontophoresis on the rate of permeation of drug(s) through the skin and to optimize the iontophoretic parameters promoting the absorption of a drug(s) through the skin. In-vitro studies may be able to predict the possible degree of enhancement which is likely to be achieved when a drug is administered in-situ, by a transdermal mechanism for local or systemic effect (Siddiqui et al 1985a, 1987; Keister & Kasting 1986; Chien et al 1987).

We have reported previously that the increased local anaesthetic effect found following iontophoretic delivery of lignocaine in-situ, was pH dependent (Siddiqui et al 1985a). The fact that lignocaine, at pH 5 to 6, was effective clinically may have been due to the presence of a high proportion of the ionized species of lignocaine in the delivery solution. In the present work, the same in-vitro technique has been used to study the effect of pH on the rate and extent of permeation of a variety of other weak electrolytes over a range of molecular weight (MW) during ITP.

Materials and methods

Materials. Salicylic acid BP (MW 138, acid, pK_a 3·0, 13·4), Aspirin BP (MW 180, acid, pK_a 3·5) and Ephedrine hydrochloride BP (MW 202, base, pK_a 9·6) were obtained from Evans Medical Ltd, UK. Pilocarpine hydrochloride (MW 245, base, pK_a 1·6, 6·9) was obtained from Macfarlane Smith Ltd, UK. Chlorpromazine hydrochloride (MW 355, base pK_a 9·3) and chlorpheniramine maleate (MW 391, base, pK_a 9·2) were gifts from May and Baker Australia Pty Ltd, and Glaxo Australia Pty Ltd., respectively. Methotrexate (MW 454, acid, pK_a 4·3, 5·5) was obtained from Sigma Chemical Company, USA. The type of buffers and the radioactive materials used were the same as reported previously (Siddiqui et al 1985a, b).

Permeation Studies. Details of the permeation studies, with and without ITP have been described previously (Siddiqui et al 1985a). The stratum corneum was prepared as follows: Samples of human skin, including the subcutaneous fat approximately 25 cm by 6 cm, were removed from the mid-abdominal region of a male Caucasian cadaver aged 60 years within 48 h of death and stored at −20°C. The subcutaneous fat was trimmed and the method of Kligman & Christophers (1963) was used to remove epidermis and stratum corneum. The transparent sheet of stratum corneum obtained was washed three times with water, dried overnight at room temperature (20°C) and stored at −20°C. The stratum corneum was allowed to thaw overnight at room temperature and was re-hydrated by immersion in water for 1 h (Swarbrick et al 1982) before being placed in the permeation cell with the dermal side towards the receptor compartment.

For each experiment, the donor compartment contained a solution of the drug in a buffer at one of several pH values. A variety of pH values was used in order to obtain different degrees of ionization for each substance. The buffer systems used were as previously described (Siddiqui et al 1985a, b).

The current density of the conventional DC power source used was 0·23 (±0·02) mA cm^{-2}; that is application of 1 mA of DC current to the skin with the surface area of 4·5 cm^2. The ITP was applied for an arbitrarily selected time of 3 min every h for 4–7 h, depending on the time required to attain a steady state permeation rate. The importance of ITP variables in skin transport are discussed elsewhere (Siddiqui 1988; Siddiqui et al 1985a, 1987).

Preliminary work using stratum corneum prepared as described previously (Siddiqui et al 1985a) and the same cells has shown an inter-specimen variation in permeation rate of 15% (n = 10) and an intra-specimen variation of 5% (n = 6) (Siddiqui 1985). Individual data points reported here are the mean of the results for two separate runs.

The integrity of the stratum corneum was examined using the method of Siddiqui et al (1985a).

Methods of analysis. The concentrations of methotrexate in aqueous samples were measured by radioactive counting and/or HPLC, as described previously (Siddiqui et al 1985b). The concentrations of salicylic acid (λ = 237 nm), aspirin (λ = 237 nm), ephedrine hydrochloride (λ = 251 nm), pilocarpine hydrochloride (λ = 215 nm), chlorpromazine hydrochloride (λ = 254 nm) and chlorpheniramine maleate (λ = 262 nm) in aqueous solution were determined spectrophotometrically (Beckman DB-G) by reference to a calibration plot which was linear in each case. The method of preparation of the stratum corneum decreased the likelihood of skin components leaching into the receptor compartment where they could have interfered with the analytical procedures.

Data analysis. The steady state flux (J_{ss}) was determined according to Siddiqui et al (1985a). The fraction change in (steady state) flux is defined as the ratio (J_{ss} with ITP-J_{ss} without ITP)/J_{ss} with ITP. To determine the importance of the extent of ionization of a drug solution in transdermal delivery, with and without ITP application, a standard one way analysis of variance was performed with a factorial arrangement of treatments, where the factors are the pH and the steady state flux (with and without ITP). The log transformation of these fluxes was required to equalize variances between the treatments. A test of least significant difference (l.s.d.) between the two values

* Current address: Rutgers University, College of Pharmacy, New Jersey, USA.
† Current address: Department of Pharmacy, University of Otago Medical School, Dunedin, New Zealand.
Correspondence to: A. E. Polack, School of Pharmacy, University of Tasmania, Hobart, Tasmania, Australia 7001.

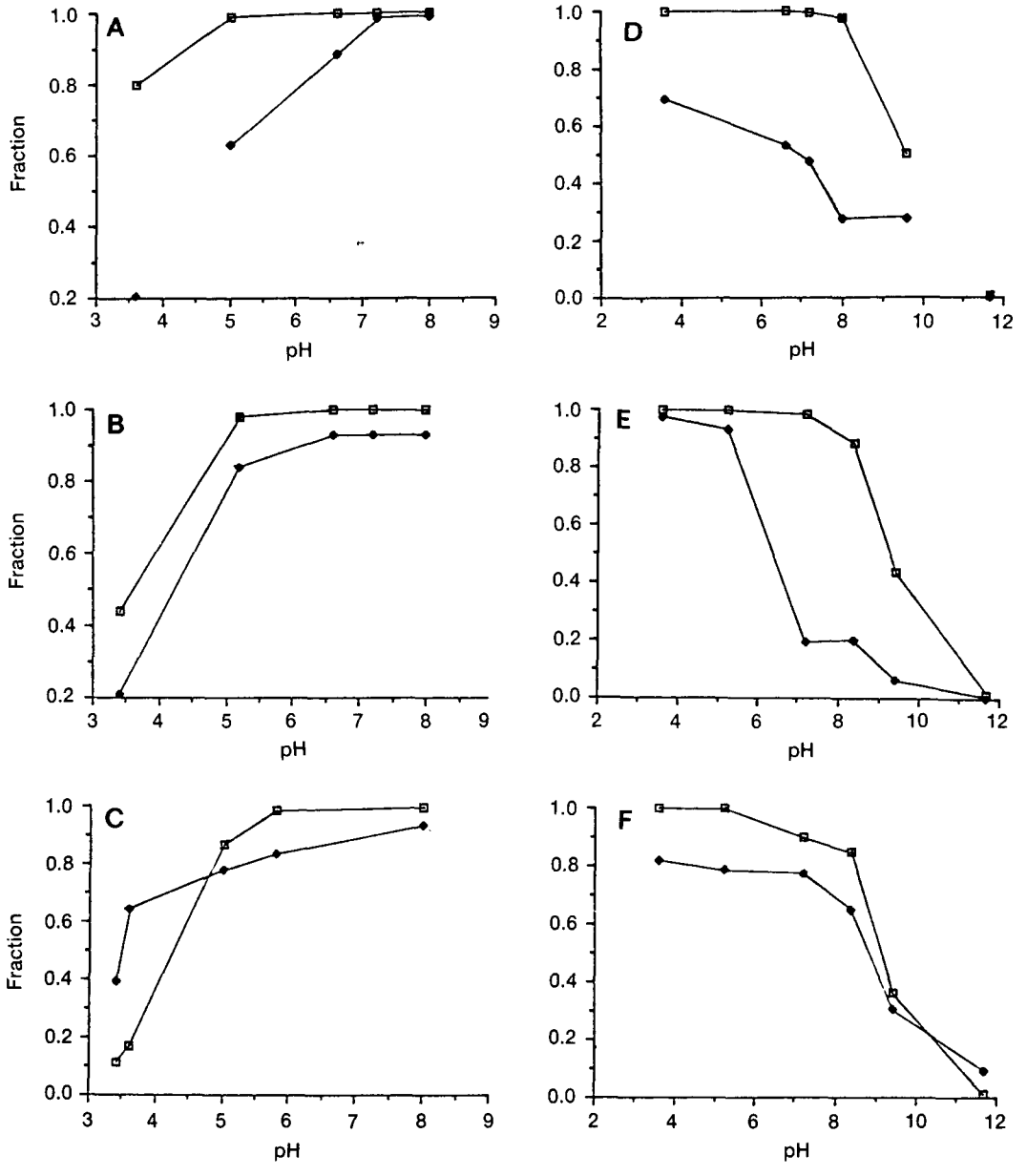

FIG. 1. Effect of ionization on steady state flux at various pH values (A) salicylic acid; (B) acetylsalicylic acid; (C) methotrexate; (D) ephedrine hydrochloride; (E) chlorpromazine hydrochloride; (F) chlorpheniramine maleate. □ Fraction ionized; ◆ Fraction change in steady state flux.

of fluxes at a particular pH value, with and without ITP, was also carried out (Siddiqui et al 1985a).

Results and discussion

Fig. 1. shows the effect of ionization on the degree of enhancement of J_{ss} due to iontophoresis under various pH conditions for each of the weak electrolytes used.

The rate of in-vitro permeation of pilocarpine hydrochloride, with and without ITP, was carried out at pH values of 3·6 and 8·0 only. As the pH is increased from 3·6 to 8·0 there is a 99% reduction in the fraction of the ionized species of the pilocarpine hydrochloride present in solution. Consistent with the other electrolytes, the fraction change in steady state flux is reduced from 0·812 to 0·308, respectively.

The results of statistical analysis for all the substances used are summarized in Table 1. There is a significant difference in the

Table 1. Statistical analysis of the data to show the effect of ITP and the importance of ionization on skin permeation rates.

Drug	pH range	F	df	*P*	S/NS[1]
Salicylic acid	3·6–8·0	1494	4/10	0·001	S
Aspirin	3·4–8·0	581	4/10	0·001	S
Ephedrine hydrochloride	3·6–11·7	6999	5/12	0·001	S/NS[2]
Chlorpromazine hydrochloride	3·6–11·7	11483	5/12	0·001	S/NS[3]
Chlorpheniramine hydrochloride	3·4–11·7	349	5/12	0·001	S/NS[4]
Methotrexate	3·4–8·0	1034	4/10	0·05	S
Pilocarpine	3·4–8·0	870	1/4	0·001	S

[1] S/NS = Significant or not significant at the value of *P* shown.
[2] S: pH 3·6–8·0; NS: pH 9·6–11·7.
[3] S: pH 3·6–7·2; NS: pH 8·4–11·7.
[4] S: pH 3·4–9·4; NS: pH 11·7.

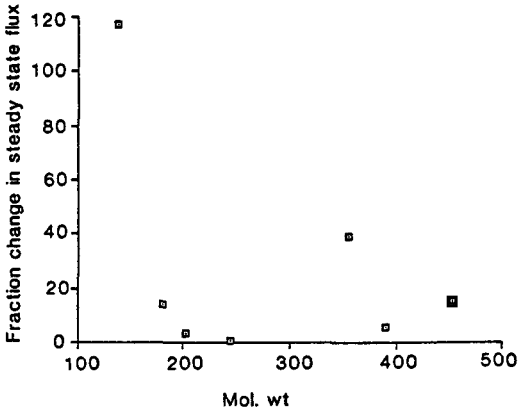

FIG. 2. Relationship between molecular weight and fraction change in steady state flux at pH of maximum ionization for seven substances.

steady state fluxes with and without ITP for each of the substances used at the *P* level shown in the Table.

The exposure of the stratum corneum to aqueous solutions of different pH values, with and without ITP, did not have any effect on the permeability of tritiated water over the pH range 3·4–11·0 suggesting that the integrity of the stratum corneum did not change when ITP was applied with a current density of 0·23 mA cm^{-2}.

Previous work has demonstrated that iontophoretic delivery of lignocaine through the skin is pH dependent and that the unionized species can also permeate during ITP (Siddiqui et al 1985a). The present results confirm that the extent of the ionization of weak electrolytes is also important in obtaining an increase in the fraction change in flux during ITP. Other parameters which control the conventional direct current iontophoretic delivery of the drugs through the skin are the ionic strength and conductivity of the solution, the charge density of the substance at the pH used, the duration of ITP application and the magnitude of current or current density (Chien et al 1987; Siddiqui et al 1987; Liu et al 1988).

Gangarosa et al (1978) reported that at higher pH values the hydrochloride salts of local anaesthetics conducted best when the positive charge on the molecules was dominant. Similarly, methotrexate was reported to have high conductivity because of the presence of a glutamate residue with two negative centres. This might be one of the reasons why lignocaine and methotrexate show higher permeation under ITP than other weak electrolytes (Table 1, Fig. 1).

The magnitude of the fraction change in flux will depend on the physicochemical properties of the particular weak electrolyte. ITP is found to be more effective over a wider pH range for the acidic weak electrolytes than for the basic weak electrolytes used during this work. However, each of the drug used showed a substantial difference in the rate of permeation with and without ITP, at pH values that ensured more than 25% ionization of the substance in aqueous solutions.

The substances used showed a reduction in lag times when ITP was used. For methotrexate the lag time at pH 5·0 and 8·0 without ITP was 4 and 11·3 h, respectively; this was reduced to approximately 1 and 5 h, respectively, with ITP. Similarly, for pilocarpine hydrochloride the lag times were reduced on ITP application from approximately 4 h and 1 h to 1 h and less then 10 min at pH 3·6 and 8·0, respectively. This general reduction in lag times during ITP suggests that the transport of the ionic species of weak electrolytes through the excised human stratum corneum may occur via the hair follicles, sweat ducts, apocrine and/or exocrine glands, all of which breach the stratum corneum and are referred to as "shunt" pathways. It is also possible that the "intercellular" route may be an additional pathway for the penetration of these ionic species (Siddiqui et al 1985a, 1987)

The actual sizes of the substances used here as represented by their molecular weight does not appear to be a determinant for the iontophoretic delivery of the weak electrolytes used in this work. The relationship between molecular weight and the fraction change in flux, at the pH at which the substance is at its maximum level of ionization in the present work, is shown in Fig. 2. Previous work has demonstrated that macromolecules with molecular weights up to 6000 can be delivered through the skin by iontophoresis (Burnette & Marrero 1986; Siddiqui et al 1987; Chien et al 1987; Liu et al 1988).

The present work on model weak electrolytes suggests that the amount of ionized species in the drug solution is one of the important considerations in the transdermal delivery of weak electrolytes for local or systemic effect. This information could be useful in the possible transdermal iontophoretic delivery of weak electrolytes and peptide/protein drugs.

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