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Effect of current density on the iontophoretic permeability of benzyl alcohol and surface characteristics of human epidermis

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Abstract

Benzyl alcohol (BA), a non-electrolyte, was selected as a probe permeant to investigate its iontophoretic transport through human epidermis. The flux of BA and [^{3}H]water were greater during anodal than cathodal iontophoresis. Increases in current density enhanced the permeability coefficient of BA during iontophoresis. The greater permeability of BA during anodal iontophoresis than cathodal may be due to permselective nature of human epidermis at physiological pH 7.4 for positively charged buffer ions and hence, associated [^{3}H]water and BA. Thus, it is possible to enhance and control the transdermal transport of non-electrolyte by iontophoresis. Scanning electron microscopy of the human epidermis treated with iontophoresis showed greater loosening of epidermal cells and distention in intercellular space with increasing current densities.

Keywords: Transdermal; Iontophoresis; Benzyl alcohol; Current density; Human epidermis; Scanning electron microscopy

1. Introduction

Iontophoresis uses a potential difference between two electrodes to transport solutes across epithelia. The process causes an increased penetration of ionized substances into tissues with the assistance of electric current (Keister and Kasting, 1986; Masada et al., 1989; Srinivasan et al., 1989a; Sims et al., 1991; Singh and Roberts, 1995; Singh and Bhatia, 1996; Bhatia et al., 1997). Iontophoresis appears to be a safe technique for transdermal delivery of solutes (Singh and Maibach, 1993; Singh et al., 1994a,b). It has been

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reported that the penetration of non-electrolytes can be increased by iontophoresis (Gangarosa et al., 1980; Burnette and Ongpipattanakul, 1987; Srinivasan et al., 1989b; Pikal and Shah, 1990; Roberts et al., 1990; Sims et al., 1991; Delgado-Charo and Guy, 1994; Singh et al., 1994c). The above investigators postulated that buffer ions were transported and co-transported water and other non-electrolytes during iontophoresis.

Inada et al. (1994) found that decreases in human epidermal membrane electrical resistance induced by the applied voltage were accompanied by proportional increases in membrane permeability. However, the effect of iontophoresis on the surface characteristics of human skin has not been adequately focused. The visualization of the changes in the surface characteristics of skin helps in predicting the possible mechanism of transport (Singh and Singh, 1995; Ganga et al., 1996). In this study, current density and the type of iontophoresis were varied in an evaluation of the transport of BA across human epidermis. Scanning electron microscopy (SEM) was used to visualize changes in the surface characteristics of the epidermis due to iontophoresis.

2. Materials and methods

2.1. Materials

Benzyl alcohol and sodium chloride were supplied by Ranbaxy Laboratories Ltd. (S.A.S. Nagar, Punjab) and Glaxo Laboratories Ltd. (Bombay), respectively. [^{3}H]water (25 mCi$\cdot$g^{-1}) was obtained from NEN Research Products (Wilmington). The chemicals used for tissue fixation were sodium cacodylate (Romali, Bombay), gluteraldehyde solution 25% (S.D. Fine Chem Pvt. Ltd., Boisar) and osmium tetroxide (Loba Cheme Industrial Co., Bombay). Other chemicals used were trisodium citrate (Glaxo Laboratories Ltd., Bombay), glycine (Glaxo Laboratories Ltd., Bombay), disodium hydrogen phosphate (E. Merck Pvt. Ltd., Bombay) and potassium dihydrogen orthophosphate (Central Drug House Pvt. Ltd., New Delhi).

2.2. Methods

2.2.1. Preparation of buffers

The donor solution buffer was prepared by mixing equal proportion of 30 mM each of trisodium citrate, disodium hydrogen phosphate and glycine. The pH of buffer was adjusted to 7.4 by adding 1 M sodium hydroxide or 1 M hydrochloric acid. The receiver fluid 0.2 M phosphate buffer (pH 7.4) was prepared by mixing 50 ml of 0.2 M potassium dihydrogen orthophosphate and 39.1 ml of 0.2 M sodium hydroxide to produce 200 ml with deionized water. Osmolar concentration of donor and receiver buffer was 810 mOsM and 800 mOsM, respectively. The capacity of donor buffer was 0.064 at pH 7.4. The buffer capacity (0.064) of the donor buffer was sufficient to resist changes in pH during iontophoresis.

2.2.2. Preparation of human epidermis

Skin samples from 21–50 years old cadavers of either sex, including subcutaneous fat, approximately 25 × 6 cm, were removed from the mid-abdominal region within 24 h of death. The subcutaneous fat was trimmed and the method of Kligman and Christophers (1963) was adopted to remove the epidermis. The epidermis was washed with water, dried overnight at room temperature and stored at −20°C (Siddiqui et al., 1985). The epidermis was used within two weeks. This is satisfactory method of storage for human skin (Harrison et al., 1984). The epidermis was rehydrated by immersing in water for 1 h (Swarbrick et al., 1982) before placing into diffusion cells for in vitro transport studies.

2.2.3. In vitro transport studies

The method for in vitro transport studies was similar to the one described in our earlier work (Singh et al., 1992). The donor and receiver compartments contained 3 ml of 0.5% w/v benzyl alcohol solution in donor buffer (pH 7.4) and receiver buffer (pH 7.4), respectively. In water transport experiments, 0.1 mCi$\cdot$ml^{-1} of [^{3}H]water in donor buffer was used as donor solution. The surface area of the epidermis exposed to the solution was 2.83 cm^2. Platinum

electrodes and d.c. constant current source were used for iontophoresis. Anodal iontophoresis was performed by inserting anode in the donor compartment and cathode in the receiver compartment. Cathodal iontophoresis was carried out by reversing the electrodes polarity.

Samples were withdrawn at regular intervals from the receiver compartment (0.5 ml) for analyses of the BA concentration and the same volume was replaced by the receiver fluid. Samples (0.1 ml) were taken from the donor compartment before and after the experiment to make sure that the decrease in donor concentration did not exceed 10% in order to maintain receiver drug concentration at a level less than 10% of donor concentration (Roberts et al., 1990). The samples were diluted appropriately and the absorbance was measured at 215 nm spectrophotometrically (Beckman Model-24 spectrophotometer, USA).

Samples containing [^{3}H]water were assayed by liquid scintillation counting. Each sample was mixed with 10 ml scintillation fluid (Econosafe®, Research Products International Corp., USA) and counted in a liquid scintillation counter (Packard, Tri Carb 2100 TR, USA). In vitro transport experiments were carried out in triplicate for each condition studied. After the experiment, skin samples were used for scanning electron microscopy.

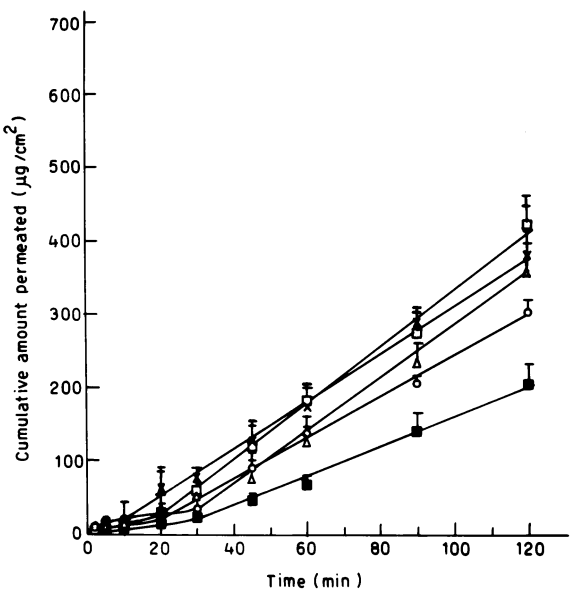


Fig. 1. Transport of benzyl alcohol through human epidermis during anodal iontophoresis at different current densities (mA/cm^2). Key: (■) passive, 0.0 mA/cm^2; (○) iontophoresis, 0.28 mA/cm^2; (△) iontophoresis, 0.35 mA/cm^2; (×) iontophoresis, 0.53 mA/cm^2; (□) iontophoresis, 1.05 mA/cm^2.

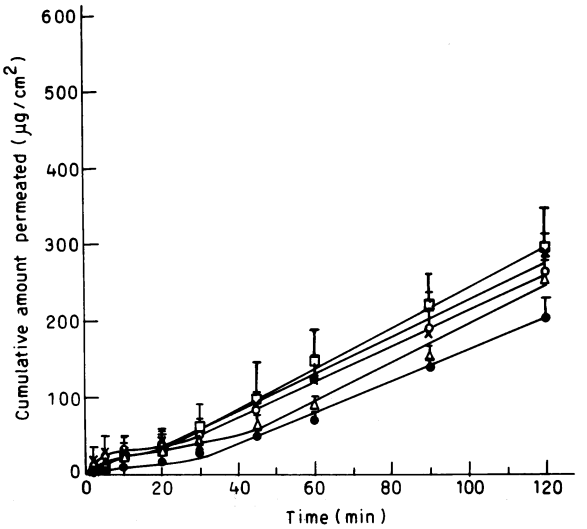


Fig. 2. Transport of benzyl alcohol through human epidermis during cathodal iontophoresis. Key: (●) passive, 0.0 mA/cm^2; (○) iontophoresis, 0.28 mA/cm^2; (△) iontophoresis, 0.35 mA/cm^2; (×) iontophoresis, 0.53 mA/cm^2; (□) iontophoresis, 1.05 mA/cm^2.

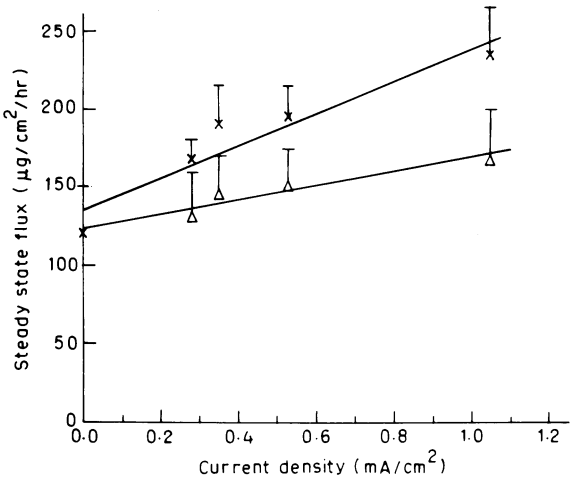


Fig. 3. Effect of current density on benzyl alcohol flux through human epidermis. Key: (×) anodal iontophoresis; (△) cathodal iontophoresis.

Table 1
Permeability coefficient (K_p) and enhancement factor (E_f) of benzyl alcohol at pH 7.4 during anodal and cathodal iontophoresis at different current densities

Current density (mA/cm^2)	Permeability coefficient [(cm/h) $\times 10^{-2}$]		E_f	
	Anodal	Cathodal	Anodal	Cathodal
0.00	2.41 ± 0.63	2.41 ± 0.63	—	—
0.28	3.35 ± 0.30	2.60 ± 0.24	1.39	1.08
0.35	3.83 ± 0.62	2.89 ± 0.59	1.80	1.20
0.53	3.88 ± 0.60	3.01 ± 0.56	1.85	1.25
1.05	4.70 ± 0.60	3.36 ± 0.55	1.98	1.39

E_f, iontophoretic permeability coefficient/passive permeability coefficient

2.2.4. Scanning electron microscopy of human epidermis

The epidermal samples were fixed for 4 h at 4°C in a 2.5% w/v gluteraldehyde in 0.1 M cacodylate buffer (pH 7.2). The samples were washed three times in 0.1 M cacodylate buffer (pH 7.2) for 10 min each to remove excess fixative. Post fixation was performed for 1 h at 4°C in 1% w/v osmium tetraoxide solution in 0.1 M cacodylate buffer (pH 7.2). After rinsing two times in buffer, these specimens were dehydrated by placing into graded ethanol solutions and critically dried in a critical point drier (Balzer's Union M 9202, Liechtenstein). The samples were then mounted on a clean aluminum stubs with silver PAG-915 and coated with gold palladium alloy (160 angstrom thickness) on a sputter coater. Specimens were then observed under scanning electron microscope (Phillips SEM 515, Holland) and photographed.

2.2.5. Data analysis

The BA or [^{3}H]water concentration was corrected for sampling effects according to the equation described by Hayton and Chen (1982):

$$C_n^1 = C_n(V_T/V_T - V_S)(C_{n-1}^1/C_{n-1}) \quad (1)$$

Where C_n^1 is the corrected concentration of the nth sample; C_n the measured concentration of BA or [^{3}H]water in the nth sample, C_{n-1} the measured concentration of the BA or [^{3}H]water in the $(n-1)$th sample; V_T the total volume of the receiver fluid, V_S the volume of the sample drawn. The cumulative amount of BA or [^{3}H]water transported per unit surface area is plotted against time and slope of the linear portion of the plot was determined to get the steady state flux (J_{SS}). The permeability coefficient (K_p) was calculated as:

$$K_p = J_{SS}/C_V \quad (2)$$

Where C_V is the donor concentration of BA.

2.2.6. Statistical analysis

The results are expressed as mean $\pm$ S.D. of three determinations. The Comparisons were made with Student's t-test (one-tail). A probability value of less than 0.05 was considered significant.

3. Results

The transport of BA increased with increasing current density during both types of iontophoresis (Figs. 1 and 2). Fig. 3 and Table 1 depict the effect of current density on the flux and permeability coefficient of BA, respectively. The flux of BA increased linearly with current density during both types of iontophoresis (anodal, $r = 0.95$; and cathodal, $r = 0.97$). However, cathodal iontophoretic fluxes were not significantly different (t-test, $p > 0.05$) than the passive flux.

Figs. 4 and 5 show the transport of [^{3}H]water during anodal and cathodal iontophoresis, respectively. Table 2 depicts values of the [^{3}H]water flux through human epidermis during anodal and cathodal iontophoresis. We observed a greater transport and flux of [^{3}H]water with increasing current density during both the anodal and catho-

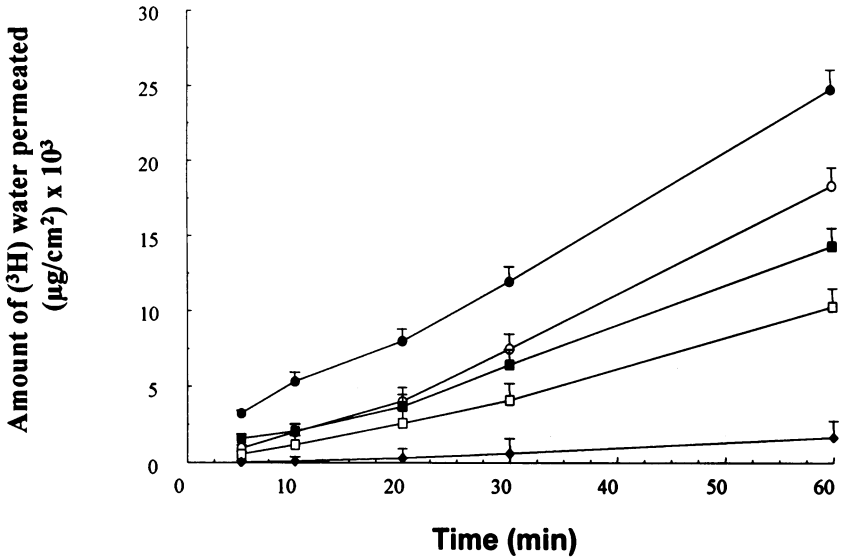


Fig. 4. Transport of [^{3}H]water through human epidermis during anodal iontophoresis at different current densities (mA/cm^2). Keys: (◆) passive, 0.0 mA/cm^2; (□) iontophoresis, 0.28 mA/cm^2; (■) iontophoresis, 0.35 mA/cm^2; (○) iontophoresis, 0.53 mA/cm^2; (●) iontophoresis, 1.05 mA/cm^2.

dal iontophoresis. Also, the flux of [^{3}H]water was greater during anodal than cathodal iontophoresis at each current density studied (Table 2).

SEM microphotographs are given in Fig. 6A–E. The normal untreated human epidermis consists of closely united assembly of squames (Fig. 6A). During passive diffusion, the epidermal cells are densely packed, no intercellular gap is visible and only few cells are desquamated (Fig. 6B). There is no any change in the epidermal surface at current density 0.28 mA/cm^2 (Fig. 6C). SEM of the sample treated at 0.53 mA/cm^2 shows loosened epidermal surface with notable intercellular gaps (Fig. 6D). Microphotograph at current density 1.05 mA/cm^2 (Fig. 6E) shows thick lines on the epidermal surface. This indicates that creasing of the surface has started and triangular areas are being formed at current density of 1.05 mA/cm^2.

4. Discussion:

The present results demonstrate that the penetration of non-electrolyte, BA, can be enhanced through human epidermis by iontophoresis. The linear relationship exists between current density and flux. The enhancement in the flux is more during anodal iontophoresis due to increased penetration of water and greater convective solvent flow in the direction of the flow of current. In this case, the iontophoretic movement of hydrated ions seems to be the suitable explanation for greater water movement and penetration of BA during iontophoresis. It has been shown that uncharged substances, such as urea and sugar, are transported in an electrical field in vitro (Moore, 1955). The above explanation supports our findings of transport enhancement of BA during iontophoresis than passive diffusion. It may be assumed that BA moves passively in association with water or with moving ions.

The hydrated ion hypothesis of water movement during iontophoresis predicts that both the positive and negative ions would carry water during iontophoresis. At physiological pH, the epidermis is negatively charged and is permselective for positively charged buffer ions. Thus, the greater movement of positively charged buffer ions explains the greater water and hence, the passively associated BA transport through human epidermis during anodal than cathodal iontophoresis. Several investigations suggest that ion-

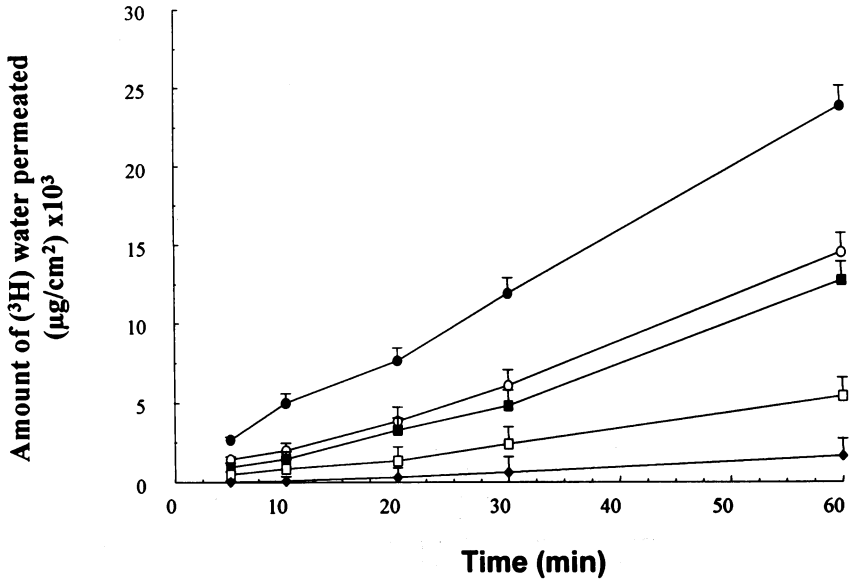


Fig. 5. Transport of [^{3}H]water through human epidermis during cathodal iontophoresis at different current densities (mA/cm^2). Keys: (◆) passive, 0.0 mA/cm^2; (□) iontophoresis, 0.28 mA/cm^2; (■) iontophoresis, 0.35 mA/cm^2; (○) iontophoresis, 0.53 mA/cm^2; (●) iontophoresis, 1.05 mA/cm^2.

tophoretic transport of solutes occurs through aqueous shunt routes such as skin appendages (Burnette and Ongpipattanakul, 1987; Cullander and Guy, 1992). However, paracellular route of iontophoretic transport has also been demonstrated (Monteiro-Riviere et al., 1994). Recently, physiological structures associated with iontophoretic paths in hairless mouse skin and two cultured skin models have been reported (Lee et al., 1996). Hair follicles are the predominant transport path in hairless mouse skin. For cultured skin models, which have no appendages, paracellular iontophoretic transport through lipid bilayer regions is the predominant transport path. The relevant literature strongly supports the importance of pores in the current assisted transdermal flux of solutes (Burnette and Bagniefski, 1988). However, the true identity of the pores has not been definitively elucidated through complementary morphological studies. One cannot ignore the possibility that molecular sized pores may be present or may be formed in the lipid matrix of the stratum corneum during iontophoresis treatment (Wearley et al., 1989). Electroporation—formation of transient pores—has been suggested as a phenomenon which occurs in lipid bilayers (Prausnitz et al., 1992).

Table 2
Flux of [^{3}H]water at pH 7.4 during anodal and cathodal iontophoresis

Current density [mA/cm^2]	Flux [(µg/cm^2/h) × 10^{-2}]	
	Anodal	Cathodal
0.00	0.14 ± 0.10	0.14 ± 0.21
0.28	0.86 ± 0.35	0.44 ± 0.25
0.35	1.17 ± 0.56	0.96 ± 0.59
0.53	1.54 ± 0.63	1.13 ± 0.55
1.05	2.03 ± 0.60	1.86 ± 0.62

It is well-known that polar, but neutral, compounds can be delivered at higher rates by iontophoresis. The net convective flow of volume (electroosmosis) from anode to cathode leads to enhanced transport of dissolved polar (but uncharged) solutes in the same direction (Kim et al., 1993). We also found greater [^{3}H]water and BA flux enhancement during anodal than cathodal iontophoresis. Cathodal BA flux should be retarded, relative to passive transport, by a net

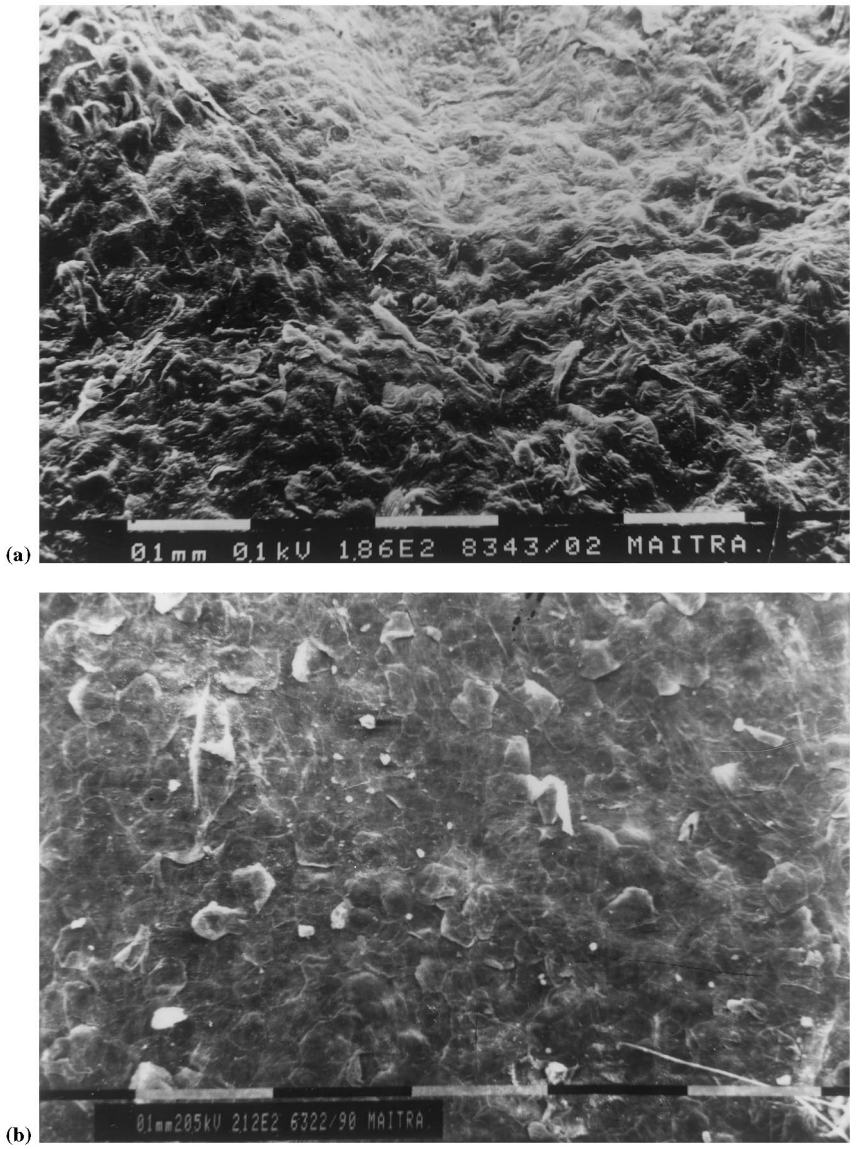


Fig. 6. Scanning electron microphotographs of epidermal surface of human skin: (A) untreated skin; (B) passive diffusion; (C) iontophoresis at 0.28 mA/cm^2 current density; (D) iontophoresis at 0.53 mA/cm^2 current density; and (E) iontophoresis at 1.05 mA/cm^2 current density. Magnification: $\times 200$.

volume flow in the opposite direction. However, our findings suggest an increase but not significantly different ($p > 0.05$) cathodal BA flux than the passive (no-current) flux. Slight increases in cathodal flux of polar but uncharged solutes such as mannitol (Kim et al., 1993) and glucose (Srinivasan et al., 1989b) have been reported.

Iontophoresis perturbs the passive permeability barrier of the membrane (Delgado-Charo and Guy, 1994). The greater cathodal BA flux over passive flux may be due to epidermal barrier perturbation by iontophoretic current. Inada et al. (1994) found that the rate of decrease in resistance was strongly dependent upon the applied voltage.

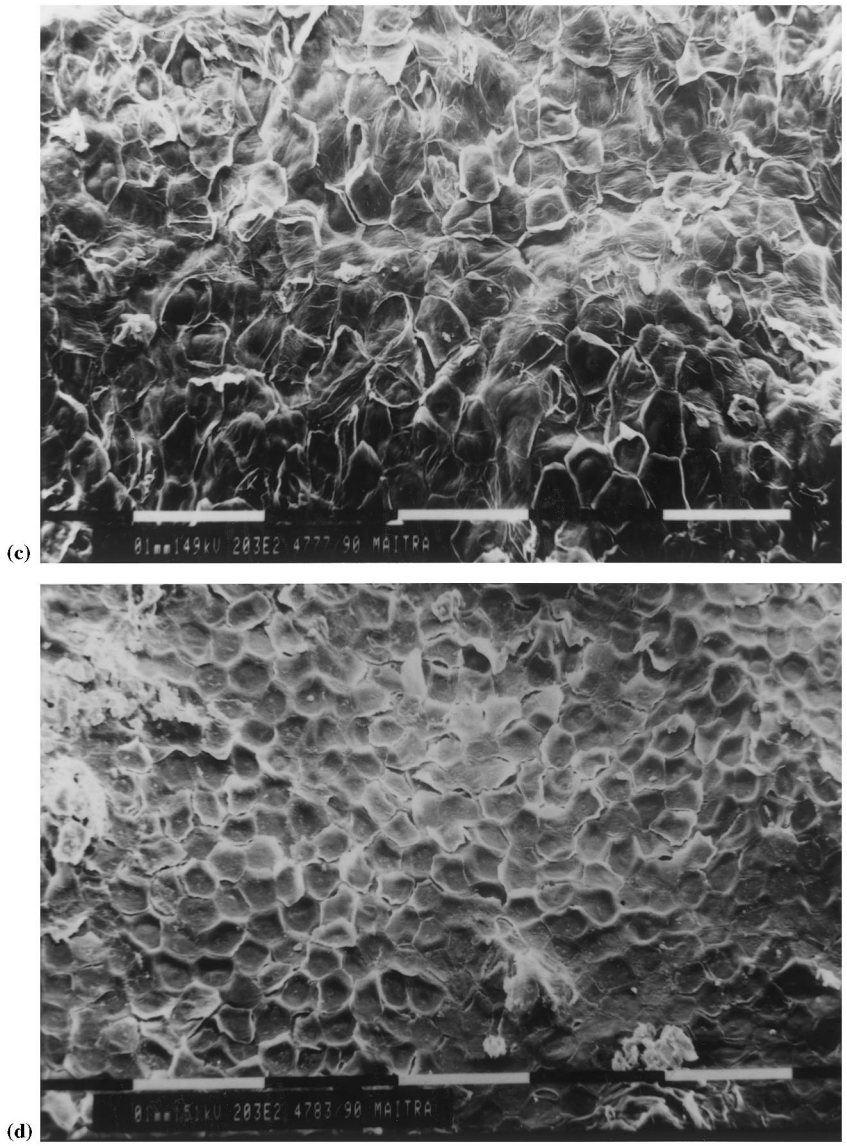


Fig. 6. (*Continued*)

The above also examined the hypothesis that decreases in human epidermal membrane electrical resistance induced by the applied voltage are accompanied by proportional increases in human epidermal membrane permeability. The findings strongly support the view that the human epidermal membrane alterations induced by electric field result in pore formation and expected changes in membrane permeability. Thus, greater cathodal flux of BA with increasing current density may be due to greater reduction in epidermal membrane resistance possibly due to additional pores formation or enlargement of pores.

The thick lines in Fig. 6E may be the point of flexion of the epidermal surface. Appearance of these lines suggests that the stress due to current has increased beyond structural limit of the epidermal surface and the creasing of the surface

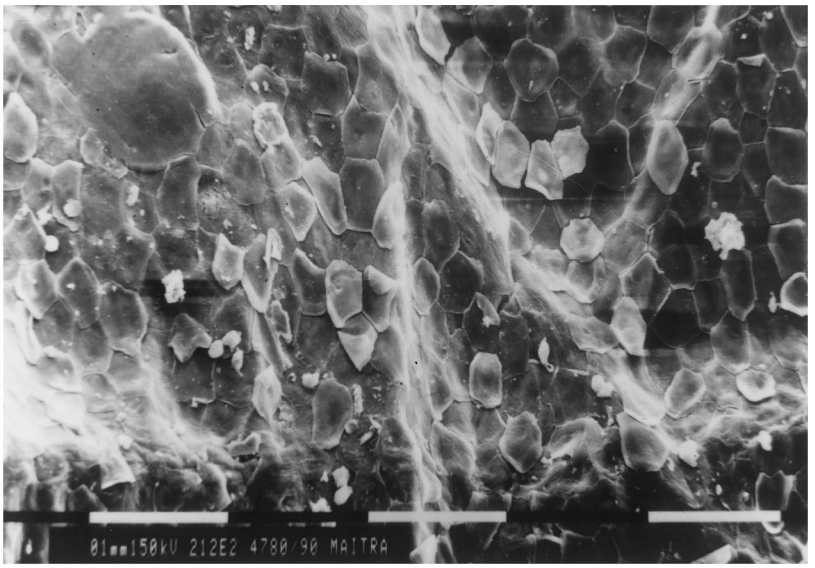
(e) 

Fig. 6. (*Continued*)

may begin at these lines to distribute the stress more evenly by increasing the surface area of the epidermal surface (Hashimoto, 1974; Millington and Wilkinson, 1983).

In summary, the permeability coefficient of BA through human epidermis increased with increasing current density. Iontophoretic movement of hydrated buffer ions is suitable explanation for greater permeability of BA. We assume that BA moves in association with water of hydration of moving ions. The greater permeability of BA during anodal iontophoresis than cathodal may be attributable to the permselective nature of human epidermis at pH 7.4 for positively charged ions and passively associated BA. Thus, it is possible to enhance and control the iontophoretic transdermal transport of non-electrolytes. SEM of human epidermis treated with iontophoresis shows loosening of epidermal cells and increase in the intercellular gap with increasing current densities.

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