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Short-term iontophoretic and post-iontophoretic transport of model penetrants across excised human epidermis

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ABSTRACT

The effect of short-term current application (0.4 mA for 10 min) on the epidermal transport of two model penetrants (butyl paraben, BP; caffeine, CF) of differing lipohilicity was investigated and compared to that produced by employing an established method of skin penetration enhancement (delipidisation). The aim was to investigate the mechanism of enhancement and route of skin permeation associated with each penetrant and mode of treatment. Franz cell diffusion experiments were conducted using human epidermal sheets and a saturated buffer solution (pH 7.4) of the respective penetrant, at a pseudo-finite dose. The effects of electrode type (anodal or cathodal) and current treatment protocol (iontophoresis or post-iontophoresis) on solute permeation was found not to be significantly different ($p > 0.05$). However, in contrast to BP, a significant increase in CF transport (3–5-fold) relative to untreated skin was observed when iontophoretic/post-iontophoretic treatment protocols were employed. The use of delipidised skin was found to enhance the permeation of both model penetrants to an extent greater than iontophoresis (BP: 3-fold; CF: 24-fold). Results from this study suggest that the permeation of the more hydrophilic CF across the skin, unlike BP, may involve multiple pathways. Electroperturbation of the epidermis was confirmed as the mechanism responsible for enhancing CF transport when electrical current was applied. Iontophoretic and post-iontophoretic enhancement may serve as a potential approach to enhance the topical delivery of CF in cosmetic or dermatological treatments (anti-cellulite, viral infections and psoriasis).

1. Introduction

Attempts to reduce temporarily the barrier nature of the skin in order to deliver therapeutic amounts of medicaments by (trans)dermal therapy has resulted in the emergence of two broad strategies, involving either the use of either passive (e.g. chemical enhancers, supersaturated systems, liposomes) or physical (electrical and mechanical) methods (Barry, 2001; Brown et al., 2006). One such physical method that has been become increasingly popular over the years is iontophoresis (Kalia et al., 2004). Iontophoresis is defined as the use of electric current to drive ionic and possibly non-ionic molecules across cell membranes (Singh et al., 1998; Guy et al., 2000, 2001). The potential use of iontophoresis as a skin permeation enhancement strategy has been studied extensively by previous workers (Banga, 1998; Guy et al., 2000; Kalia et al., 2004). For example, the delivery of opioids (Gupta et al., 1998; Subramony et al., 2006), local anaesthetics (Riviere et al., 1992; Subramony et al., 2006) and antivirals (Volpato et al., 1995; Stagni et al., 2004) from such iontophoretic systems/devices has been reported previously. The iontophoretic devices currently on the market and those in development have been reviewed elsewhere, alongside the benefits and limitations associated with this mode of delivery (Kalia et al., 2004; Brown et al., 2006). The mechanisms which account for this mode of delivery are electromigration (electrorepulsion and electroosmosis) (Delgado-Charro and Guy, 2001) and electroperturbation (Craane-van Hinsberg et al., 1997a). Electrorepulsion involves the repulsion of charged molecules across membranes under an electromotive force when in contact with an electrode of similar (current) charge. Electroosmosis may be defined as a current-induced volume flow. Such fluid volume flow or water movement is sometimes referred to as "iontohydrokinesis" and is responsible for dragging ionic or neutral molecules across the membrane in the direction of the current (Gangarosa et al., 1980; Pikal, 1992; Banga, 1998). Electroperturbation refers

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to the induced skin permeabilization as a result of its exposure to current. Perturbation of the skin by such a mechanism has the potential to enhance the passive percutaneous absorption of charged and uncharged molecules (Inada et al., 1994; Prausnitz, 1996). The passive skin permeability of electrolytes (Santi et al., 2003) and non-electrolytes (Wang et al., 1993; Singh et al., 1998) has been shown to be enhanced by skin perturbation induced by applying electric current. However, whilst such a mechanism might be beneficial in enhancing delivery across skin, it is also imperative that the barrier reverts to its original status on termination of the applied current (Turner et al., 1997).

In order to optimise iontophoresis as a mode of enhancing the skin absorption of compounds, ideally the effects of electromigration should be separated from those of electroperturbation. Previous diffusion studies (Akomeah et al., 2004; Nanayakkara et al., 2005; Akomeah et al., 2006) have hypothesised that the use of model penetrants (butyl paraben and caffeine) which traverse the SC barrier via one or a combination of the different skin permeation routes (transcellular, intercellular and transappendageal pathways) (Roberts et al., 2002) may enable an estimate to be made of the relative importance of each route to the overall transport process of each compound. The use of such model penetrants will therefore offer an insight into how electroperturbation and/or electromigration affect the potential skin permeation pathways.

The limitations of most iontophoretic experiments include the use of long current treatment times (often over 2 h) and an infinite dose method to assess solute permeability across skin. An infinite dose application may not mimic in-use conditions, where a thin film (finite dose) of the drug product is usually applied to the skin. It was therefore planned to employ a finite dose methodology and a shorter iontophoretic treatment in this study. Such conditions would also be expected to reduce the likelihood of potential damage to the skin and or irritation induced by long-term iontophoresis (Cullander, 1992). Short-term iontophoresis may also be advantageous when a localised effect is desirable due to increased drug penetration, large skin drug depot, short lag times and ease of compliance (Sintov and Brandys-Sitton, 2006).

The aim of this study was therefore to investigate the effect of short-term iontophoresis on the skin permeation of two model penetrants applied in finite doses, namely caffeine (charged) and butyl paraben (uncharged) using iontophoretic and post-iontophoretic protocols as well as different electrodes (cathodal and anodal). It was planned to compare any enhancement effect associated with the iontophoretic and post-iontophoretic transport of the model penetrants to a part-characterised method of skin penetration enhancement involving skin delipidisation (removal of SC lipid) (Rastogi and Singh, 2001a, b), since this would serve as a positive control.

2. Materials and methods

2.1. Materials

Butyl paraben, BP (MW 194.23; pK_a 8.47; log P (octanol/water partition coefficient) 3.57), Caffeine, CF (MW 194.19; pK_a 10.4; log P −0.07) and triethylamine were purchased from Sigma Chemical Co. (Dorset, UK). Orthophosphoric acid and potassium dihydrogen orthophosphate (KH_2PO_4) were obtained from BDH laboratories supplies (Loughborough, UK). Phosphate buffered saline (PBS, 0.01 M, pH 7.4) was prepared using Dulbecco 'A' tablets supplied by Oxoid Ltd. (Basingstoke, UK). Deionised water was prepared using an Elgstat water purifier, Option 3A, Elga Ltd. (Elga, Buckinghamshire, UK). All solvents involved in this study were of HPLC grade and purchased from Rathburn Chemicals Ltd. (Walkerburn, Scotland).

2.2. Preparation of saturated solutions

An excess amount of each penetrant was introduced into a glass vial containing approximately 10 ml of PBS at 32 °C (a temperature which corresponds to that at the skin surface in the Franz cell, when receptor chamber is maintained at 37 °C). The suspension was stirred overnight, after which samples were removed and filtered using a Whatman® 0.2 μm, 13 mm disposable syringe filter (Whatman International Ltd., Maidstone, UK). The filtered solution was then used for the in vitro skin diffusion experiments.

2.3. Preparation of epidermal sheets

Fresh, surgically excised samples of human skin were obtained directly after abdominoplastic surgery with informed consent and approval from the Research Ethics Committee of King's College London. Subcutaneous fat was carefully removed from the skin sample using forceps and a scalpel. Following removal of the fat, individual portions of skin were immersed in water at 60 °C for 45 s (Kligman and Christophers, 1963). The skin was then pinned, dermis side down, on a corkboard and the epidermis (comprising stratum corneum and epidermal layer) gently removed from the underlying dermis by rolling the membrane using the thumb or middle finger. The dermis was then discarded and the epidermal membrane floated onto the surface of water and taken up onto a Whatman no.1 filter paper (Whatman international, Maidstone, UK). The resultant epidermal sheets were blotted dry with tissue paper and stored flat, wrapped in aluminium foil at 4–8 °C until use.

2.4. Electrochemical stability studies

Effect of current (0.4 mA) on the stability of the permeant was examined over a 10 min period (corresponding to the maximum duration of current exposure to skin) using the method described by Nakakura et al. (1996). The experiment was conducted using 500 μl of the standard concentrations of the permeant in PBS (1–50 μg ml^{-1}). Electrochemical stability of three replicates of each concentration was examined at 32 °C. Control experiments involved the use of a standard solution of the penetrant and no applied current.

2.5. Skin treatment and in vitro permeation studies

2.5.1. Iontophoretic and post-iontophoretic procedures

A schematic representation of the iontophoretic set up used in this study is shown in Fig. 1. A constant direct current (DC) was supplied using an automatic crossover power supply (Model APH 1000M, Kepco Inc., Flushing, USA) which provides a flexible programmable voltage/current source. Constant current iontophoresis using stainless steel electrodes and a 0.4 mA current limit was maintained throughout the study (usually 10 min). The iontophoretic set-up employed in this study was designed to mimic that reported previously by Morrel et al. (2006). The electrode in contact with skin, comprised a disc or plate (surface area of ca. 0.65 cm^2) in order to cover the total effective diffusional area of skin attached to the Franz cell. Anodal treatment was conducted with the anode electrode plate in contact with the skin, whilst the opposite electrode (cathode) in the form a wire was inserted into the receptor chamber to complete the circuit (Fig. 1). Cathodal treatment was conducted by reversing the electrode polarity. The current treatment protocols employed were as follows (a) iontophoresis; where a saturated solution of the penetrant was administered simultaneously with application of the current or (b) post-iontophoresis; where a saturated solution of

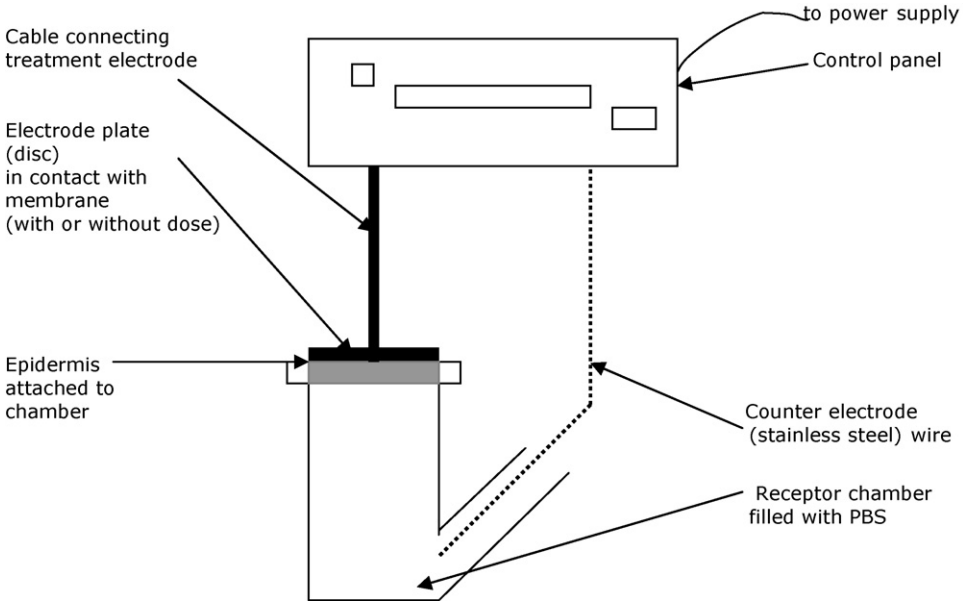

Fig. 1. Schematic illustration of iontophoresis setup.

the penetrant was applied immediately after skin exposure to current.

2.5.2. Skin delipidisation procedure

Delipidised skin was prepared by immersing discs of epidermal sheets (prepared as in Section 2.3 and cut by means of a cork borer) of surface area ~1 cm^2 in chloroform and methanol (2:1, v/v) for 40 min (Rastogi and Singh, 2001a, b). The skin was then removed from the solvent, blotted dry with tissue and then vacuum-dried (650 mmHg) at 25 °C for 1 h to remove any remaining organic solvent. The delipidised skin was then used for the in vitro skin diffusion studies.

2.5.3. Franz cell experiments (pseudo-finite dose)

Diffusion experiments were conducted using calibrated receptor chambers of the Franz diffusion cells of surface area (0.65 cm^2) and volume (1.8 ml). Treated and untreated (intact) epidermal sheets (skin–filter paper composite) were cut into circular discs (1 cm^2) by means of a cork borer. The filter paper used as support for the epidermal disc was then wetted using PBS and gently peeled away. The epidermis was then attached to the receptor chamber (Fig. 1) using cyanoacrylate adhesive (ensuring that there was no contact between the adhesive and effective surface area for diffusion). This was to guarantee that the membrane was firmly attached to the receptor chamber since the donor chamber and clamp could not be employed for this experimental procedure. After allowing the adhesive to dry for 15 min, the receptor was then filled with PBS. The effectiveness of the seal was confirmed by inverting the cell to ensure that there was no leakage of receptor fluid from the region of contact between skin and adhesive. The cells containing magnetic stirrers were then immersed into a water bath at 37 °C. After equilibration for 30 min, a target dose of 20 ± 2 mg cm^{-2} contained within a saturated system of the model penetrant (with suspended solute particles) in PBS was then introduced to the effective skin surface area using a previously calibrated positive displacement pipette (Gilson Pipetman® 20 μl, Anachem UK Ltd.). Iontophoretic/post-iontophoretic procedures and control experiments (involving intact, untreated skin or delipidised skin, where appropriate) were conducted as described. The experiments lasted for a maximum period of 2 h, with samples being removed after 30 and 120 min from the sampling arm of the receptor chamber. Epidermal sheet corresponding to the same donor was used throughout the study except where otherwise stated.

2.6. HPLC analysis of model permeants

Chromatographic analysis of BP and CF was conducted as already described (Akomeah et al., 2004). CF analytical detection was enhanced where applicable by increasing the injection volume to 50 μl and using a wavelength of 215 nm.

2.7. Microscopy studies

The effect of electric current on the epidermal membrane was assessed by scanning electron microscopy, where the integrity of iontophoretic treated and untreated (control) samples were compared. Samples were fixed by immersion in 2% formaldehyde/2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) overnight. A 5 mm × 5 mm square of skin was pinned to a thin piece of cork in order to keep it flat during processing. Samples were immersed for 20 min in each of 30, 50, 70, 95, 100, 100 and 100% (v/v) acetone in water, then dried using liquid carbon dioxide in a Polaron E3000 critical point drier. The samples were then removed from the cork and mounted on 12.5 mm aluminium pin stubs using double sided adhesive carbon pads. Samples were sputter-coated with approximately 20 nm of gold in a Polaron E5100 sputter-coater and examined and photographed using a Philips SEM501B scanning electron microscope.

2.8. Data treatment and statistical analysis

Data were expressed as amount of permeant present in receptor fluid after a known time interval. Enhancement factors (EF) after specified time intervals were calculated as follows:

$$\mathrm{EF} = \frac{\text{receptor amount}_{(\text{treated skin})}}{\text{receptor amount}_{(\text{untreated skin})}}$$

Experimental data reported represent the mean values (n = 4–5) ± standard deviation (S.D.). Statistical analysis was

Table 1
Effect of short-term iontophoretic and post-iontophoretic protocols on skin permeation of BP.

	Amount of BP in receptor with time ($\mu g\,cm^{-2}$)	
	30 min	120 min
Untreated	$2.03 \pm 1.42^{(n=4)}$	$3.16 \pm 1.2^{(n=4)}$
Iontophoresis (anodal)	$2.12 \pm 1.71^{(n=5)}$	$3.48 \pm 1.77^{(n=4)}$
Post-iontophoresis (anodal)	$2.69 \pm 1.06^{(n=4)}$	$4.46 \pm 1.51^{(n=4)}$
Iontophoresis (cathodal)	$1.48 \pm 0.60^{(n=4)}$	$2.75 \pm 0.82^{(n=4)}$
Post-iontophoresis (cathodal)	$1.89 \pm 0.78^{(n=4)}$	$2.75 \pm 0.99^{(n=4)}$

Data represent mean ± S.D. n: number of skin sections used per experiment. Current treatment (0.4 mA) was applied to the skin for 10 min during the iontophoretic or post-iontophoretic protocols.

conducted using Student's t-test, significant differences were accepted when $p \leq 0.05$.

3. Results

3.1. Penetrant solubility and electrochemical stability

The saturated solubility ($mg\,ml^{-1}$) of CF and BP in PBS at 32 °C was 25.64 ± 0.21 and 0.26 ± 0.01, respectively. The passage of electric current through standard solutions of the model penetrant in PBS was found not to affect the chemical stability of the penetrant significantly ($p \leq 0.05$). HPLC chromatograms (data not shown), did not display any unknown or unidentified peaks. The average recovery values (%) were 100.97 ± 1.57 and 98.42 ± 2.86 for BP and CF, respectively for the penetrant concentrations employed in the stability study after the exposure to electric current. Degradation of any of the penetrants when 0.4 mA was applied to 500 μl of penetrant solution for 10 min was therefore considered negligible.

3.2. In vitro skin permeation studies

3.2.1. Effect of electrode charge and treatment protocol on skin permeation

The effect of anodal and cathodal electrodes using the different treatment protocols on the amount of penetrant delivered to the receptor at 30 min and 120 min is shown for all the model penetrants (Tables 1 and 2). There were no differences ($p > 0.05$) in the amounts of BP which had permeated across untreated skin and after iontophoresis (whether anodal or cathodal) at either of the time points (Table 1).

Unlike the paraben, CF could not be detected in the receptor (CF limit of detection, 0.09 $\mu g\,ml^{-1}$) when no iontophoresis was employed until 120 min after application of the penetrant

Table 2
Effect of short-term iontophoretic and post-iontophoretic protocols on skin permeation of CF.

	Amount of CF in receptor with time ($\mu g\,cm^{-2}$)	
	30 min	120 min
Untreated	–	$0.64 \pm 0.17^{a(n=4)}$
Iontophoresis (anodal)	$1.54 \pm 0.84^{+(n=4)}$	$2.33 \pm 1.08^{+(n=4)}$
Post-iontophoresis (anodal)	$1.52 \pm 0.90^{+(n=4)}$	$3.16 \pm 2.37^{(n=4)}$
Iontophoresis (cathodal)	$0.71 \pm 0.16^{+(n=5)}$	$1.98 \pm 1.43^{(n=4)}$
Post-iontophoresis (cathodal)	$0.96 \pm 0.45^{+(n=5)}$	$2.25 \pm 0.77^{+,\times(n=5)}$

Data represent mean ± S.D. n: number of skin sections used per experiment.

[a] CF detection possible only after 120 min.

[+] Significantly different ($p < 0.05$) from the amount of penetrant in receptor when untreated skin is used.

[×] Significantly different from the amount in receptor at 30 min using similar treatment protocol. Current treatment (0.4 mA) was applied to the skin for 10 min during the iontophoretic or post-iontophoretic protocols.

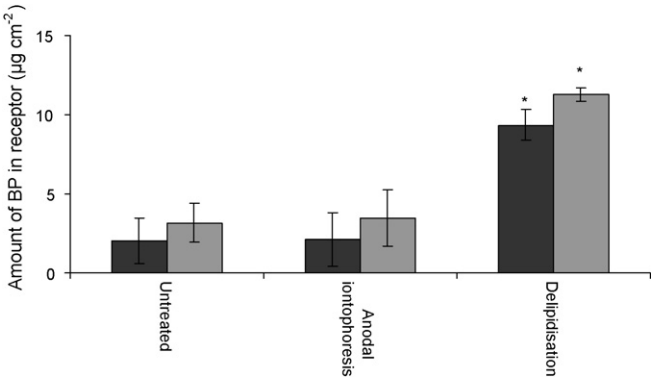

Fig. 2. The effect of different modes of enhancement on the amount of BP in receptor after a diffusional period of (■) 30 min and (▒) 120 min across human epidermis. *Significantly different from untreated control at equivalent diffusional period ($p \leq 0.05$). Anodal iontophoretic treatment (0.4 mA) was performed for a duration of 10 min. Data represent mean ± S.D. ($n \geq 4$).

(Table 2). A significant enhancement in permeation parameters (in terms of both a reduced lag time and increased amount in the receptor) was observed for CF when iontophoresis was utilised, demonstrated by the ability to detect and accurately quantify the amount of CF in the receptor after 30 min. Significant differences were however not observed in CF permeation between anodal and cathodal iontophoresis ($p > 0.05$) although the trend was for a greater amount to penetrate faster after anodal iontophoresis (Table 2).

Upon comparing the effects of treatment, i.e. iontophoresis with post-iontophoresis (Tables 1 and 2), it is evident that the different current treatment protocols had a similar effect on the in vitro absorption of the model penetrants. In the iontophoretic and post-iontophoretic studies, the amount of penetrant in the receptor chamber after 120 min was found to be generally higher than that recorded at 30 min. However, significant differences in skin permeation, as determined by the relative amounts in the receptor compartment after these two time points, were not observed, except when post-iontophoresis (cathodal) was employed for CF ($p \leq 0.05$) (Table 2).

3.2.2. Comparison of the effects of short-term iontophoresis and skin delipidisation on epidermal transport

Since the charge on the electrode had no significant effect on permeation (Tables 1 and 2), the application of anodal iontophoresis alone was selected for use in this part of the study. There were no significant differences in amounts of BP in the receptor compartment when the skin was either untreated or subjected to anodal iontophoresis within the experimental period of 120 min (Fig. 2), as was observed previously (Table 1). However, delipidisation significantly enhanced the absorption of BP compared to anodal iontophoretic treatment ($p \leq 0.05$). Significant differences in the amount of BP in the receptor after 30 min and 120 min compared to the control skin samples were observed. An EF of approximately 3.5 was recorded for delipidisation after 120 min.

As previously observed, analytical quantification of CF in the receptor chamber was not possible at 30 min, when untreated skin was employed (Fig. 3). Delipidisation and anodal iontophoresis were found to significantly enhance CF permeation ($p \leq 0.05$) at both sampling time points. Significant differences in these two enhancement methods on CF penetration were only observed after an experimental duration of 120 min, when an EF of approximately 24 and 5 was recorded for delipidisation and anodal iontophoresis respectively.

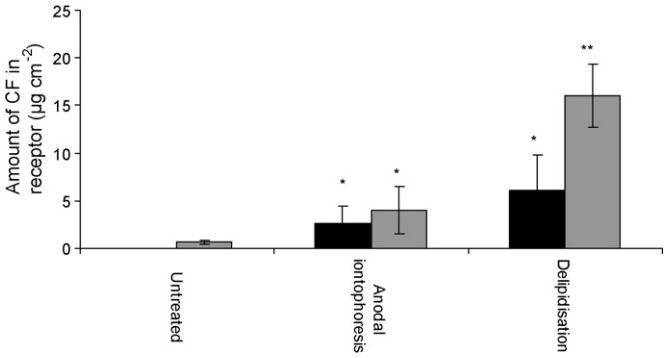

Fig. 3. The effect of different modes of enhancement on the amount of CF in receptor after a diffusional period of (■) 30 min and (▒) 120 min across human epidermis. *Significantly different from untreated skin. **Significantly different from anodal iontophoresis at equivalent diffusional period ($p \leq 0.05$). Anodal iontophoretic treatment (0.4 mA) was performed for a duration of 5 min. Data represent mean ± S.D. ($n \geq 4$). Skin used in this experiment was different from that employed in experiments represented by the data in Tables 1 and 2.

3.2.3. *Effect of electric current on epidermal membrane*

The scanning electron micrographs (Fig. 4a–d) depict the effect of current application on the surface characteristics of human epidermis. The degree of perturbation was found to be greater for iontophoretic treated skin than untreated skin. With iontophoretic treated skin, lines of disruption or weakness were apparent and a higher extent or degree of scaling of the skin was observed (Fig. 4c and d). The latter was associated with the loosening of epidermal cells providing a generally rougher surface. The untreated surface appeared to be completely intact, with tightly packed cells (honeycomb structure) and minimal or no lines of weakness observed (Fig. 4a and b).

4. Discussion

In this current study, the effect of electrical parameters (electrode charge and treatment protocol) on skin penetration using a short-term current treatment (0.4 mA, 10 min) was investigated and compared to other established modes of skin penetration enhancement such as delipidisation (Rastogi and Singh, 2001a, b). The contribution from the different iontophoretic mechanisms (electrotransport and electroperturbation) underlying transport of the model penetrants was also considered. The similarity in penetrant molecular weight allowed the effect of other solute properties (charge and/or lipophilicity) on iontophoretic and post-iontophoretic transport and skin delipidisation to be investigated via the 'pseudo-finite' dose method, which was employed to simulate "in use" conditions (Akomeah et al., 2008).

Penetrants involved in the study were found to be chemically stable (recovery ≥ 98%) during co-administration of dose with an applied current. At the pH (7.4) employed in this study, 99% of CF in solution was positively charged (cationic) whilst butyl paraben was predominantly unionised (≤9% of the parabens existed in the anionic form). As a result of the neutrality (unionised state) of the paraben any enhancement in permeation will be due to one or a combination of the following mechanisms: (i) cathodal iontophoresis, which would enhance the permeation of the small fraction of anionic forms of the parabens via electrorepulsion, (ii) electroosmosis during anodal iontophoresis, which would enhance the skin absorption of non-ionized paraben and (iii) electroperturbation during anodal or cathodal iontophoresis, which might increase

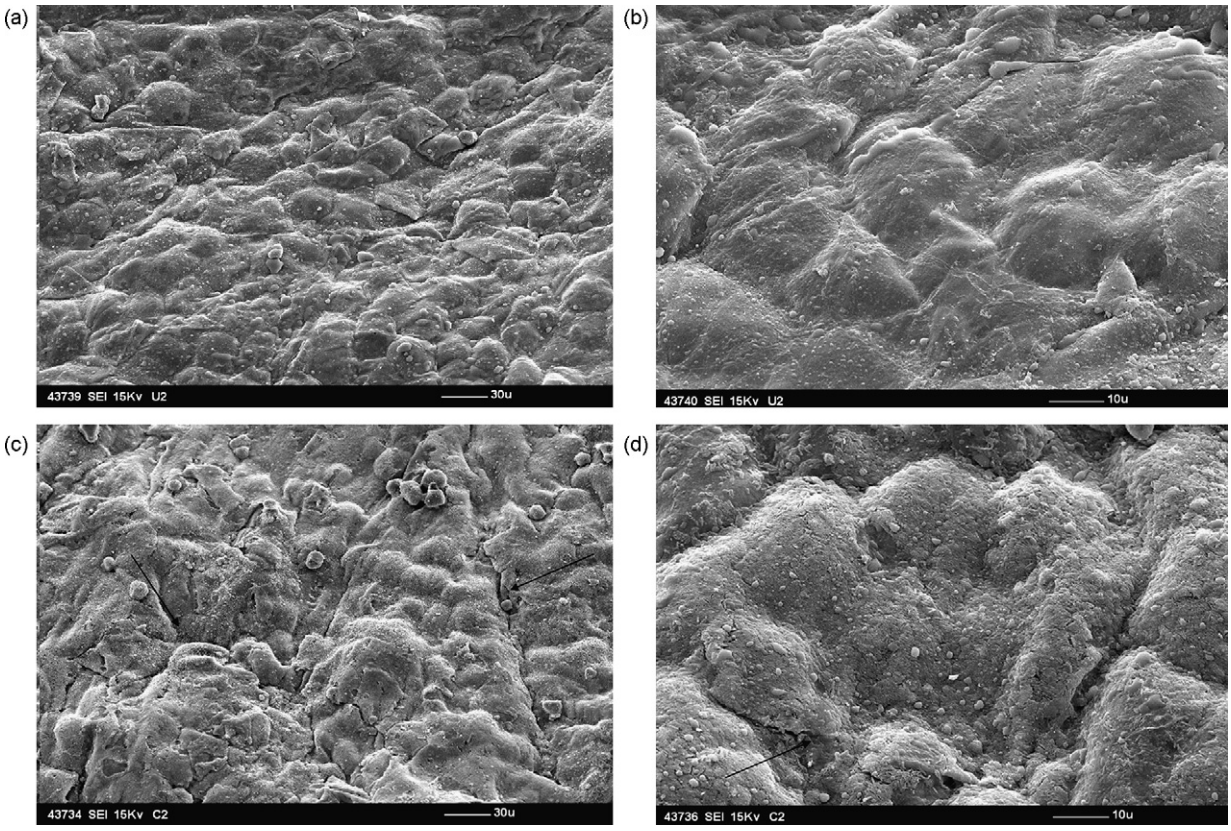

Fig. 4. Scanning electron micrographs of the surface of human epidermis: (a) untreated (bar = 30 μm); (b) untreated (bar = 10 μm); (c) iontophoretic treated (bar = 30 μm), 0.4 mA for 10 min; and (d) iontophoretic treated (bar = 10 μm), 0.4 mA for 10 min. Arrows represent lines/regions where disruption has occurred.

passive transport. Due to the positively charged nature of CF, electrorepulsion via anodal iontophoresis or electroperturbation has the potential to increase its epidermal transport.

Generally, the amount of each model penetrant transported across the epidermis after short-term current exposure was not significantly affected by the electrode charge (anodal or cathodal) and treatment protocols (iontophoretic or post-iontophoretic) (Tables 1 and 2) employed in this study. However, the exposure of electrical current to the skin (irrespective of electrode charge and treatment protocol) allowed detectable concentrations of CF to be monitored after 30 min of the application of a dose comprising a saturated solution of the penetrant, whereas no CF was detectable in the receptor after the same time interval when untreated skin was employed. A significant increase in the in vitro permeation of CF in response to the use of post- and iontophoresis was thus observed. The increase in iontophoretic and post-iontophoretic transport of CF coupled with the insignificant differences in amount of penetrant in receptor observed for both treatment protocols in (Tables 1 and 2) suggest electroperturbation to be responsible for a majority of the enhanced permeation observed for CF. Any contribution from electrorepulsion or electroosmosis is small in comparison to this epidermal disruptive mechanism. The effect of electroperturbation appears to be confirmed by the SEMs of the epidermal surface (Fig. 4a–d). This may be as a consequence of the direct contact between the treatment electrode and membrane in the Franz diffusion cell, resulting in perturbation of the epidermis as observed by loosening of the cells, lines/regions of disruption and a much rougher skin surface. Previous experiments (Wang et al., 1993; Singh et al., 1998) reported similar effects of electric current on the surface characteristics of epidermal membrane. Results from this study suggest that electroperturbation can contribute significantly to the iontophoretic enhancement of charged species across the skin.

The anticipated increase in butyl paraben skin penetration upon using iontophoresis, which might be attributable to the mechanisms of electrotransport and electroperturbation were however not observed. Previous studies by Craane-van Hinsberg et al. (1997a, b) using calorimetry and X-ray diffraction, showed that electroperturbation of the skin can cause a decrease in skin lipid transition temperature. Since the resistance and the capacitance properties of human skin lie in the intercellular lipids, perturbation of this region would be expected to enhance skin permeation of topically applied charged and uncharged species. Paraben (butyl and methyl) permeation across skin has been proposed to occur via mainly the intercellular lipid pathway (Kitagawa et al., 1997), however as indicated electroperturbation did not result in an enhanced permeation for BP, as found when delipidised skin was employed. Iontophoresis and post-iontophoresis studies in our laboratories involving methyl paraben ($\log P$ 1.96) yielded results (data not shown) similar to those reported for BP here, under the same experimental conditions. Therefore, the results obtained in the present study suggest that for uncharged molecules with good passive skin permeability the use of iontophoretic treatment may not necessarily offer any advantage due to the higher lipoidal affinity of such compounds relative to charged species (Del Terzo et al., 1989).

The observed iontophoretic and post-iontophoretic enhancement of CF permeation compared to butyl paraben further implies differences in the permeation pathway or kinetics of the two classes of compounds during iontophoresis. It is possible that iontophoretic skin treatment renders any aqueous pathways or so-called pores (Cullander, 1992), which are claimed to serve as the route for ion permeation across SC, more accessible to the transport of more water soluble permeants. CF permeation might therefore be expected to be absorbed predominantly via such routes in contrast to BP. Whilst the intercellular lipid provides the major pathway for all permeants, ionic permeants will revert to other routes, providing least resistance in the SC barrier. Such pathways are very important for permeation of polar, hydrophilic compounds and may include defects or pores in the SC (Mitragotri, 2003), as well as diffusion via the skin appendages (hair follicles) (Illell et al., 1991; Meidan et al., 2005). As a result of the hydrophilic nature of CF, there is the potential for multiple pathways being involved in its transfer across the SC (Akomeah et al., 2006).

The effect of delipidisation on the skin permeation of butyl paraben was found to be significantly higher ($p \leq 0.05$) than that which resulted after iontophoretic treatment, although for CF significant differences were not observed between these two techniques after 30 min Delipidisation can remove approximately 80–90% of SC lipids and also result in the creation of cavities within the intercellular pathway (Rastogi and Singh, 2001a, b) and protein components of the SC (Williams and Barry, 2004). Such artificially created "polar" pathways which are not present in normal SC may decrease the resistance associated with the skin transport of topically applied solutes (once the SC barrier is impaired). The degree of enhancement observed as a consequence of skin delipidisation was found to be greater for CF than BP (Figs. 2 and 3). This reflects the ease in which CF traverses the epidermis, as a result of the elimination of the continuous and tortuous pathway associated with the SC lipid barrier and CF's exploitation of newly available transport routes. Delipidised skin is non-rate limiting and can be likened to a cellulose (porous) membrane, this implies that the relatively higher solubility of CF (25.64 mg ml^{-1}) in the topical dose introduced on the epidermal surface compared to BP (0.26 mg ml^{-1}) will result in a higher concentration of the former in the Franz cell receptor chamber when the SC barrier is removed.

This present study has provided evidence that the electrotreatment protocols (iontophoresis or post-iontophoresis) employed may not necessarily enhance the skin permeation of all topically applied solutes, possibly as a consequence of the short duration of the applied current (0.4 mA, 10 min) and/or the unfavourable physicochemical properties of the applied compounds (as appears to be the case for butyl paraben). The results also suggest that alternative route(s) other than the intercellular lipid pathway may play an important role in the skin transport of CF during iontophoresis. Electroperturbation was found to be the main mechanism responsible for the enhancement of CF across the epidermis in this study. Such enhancement using short-term iontophoresis may be critical in the use of CF for cosmetic and dermatological purposes such as cellulite treatment (Rawlings, 2006; Velasco et al., 2008), viral infections (Yoshida et al., 1996; Yamamura et al., 1996; Hamuy and Berman, 1998) and psoriasis (Vali et al., 2005).

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