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Combined effects of iontophoretic and chemical enhancement on drug delivery
II. Transport across human and murine skin

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Abstract

This paper reports measurements of the release characteristics of the model drug salbutamol from a liquid crystalline vehicle across both human and hairless murine skin in vitro. The use of oleic acid and iontophoresis as penetration enhancement techniques, used separately and simultaneously, was also investigated.

Over a period of 12 h, salbutamol base did not diffuse from the vehicle across excised human skin while, in contrast, over a period of 2 h, the drug passively transported across hairless murine skin. The diffusion co-efficient for the drug in this tissue was estimated to be $4.54 \pm 0.60 \times 10^{-9}$ cm^2 s^{-1} with a permeability co-efficient of $7.03 \pm 0.83 \times 10^{-7}$ cm s^{-1}. A current of density of 0.39 mA cm^{-2} facilitated a significant transport of salbutamol from the liquid crystalline vehicle across excised human skin but with a small (<0.1) transport number. The quantity of salbutamol transported across excised hairless murine skin under the same conditions was significantly greater with a transport number of 0.68. The alteration of the permeability of the tissue was less than that of the human skin and a full recovery of the pre-iontophoretic permeability of murine skin was consistently observed. The incorporation of either oleic or lauric acid into the monoglyceride component of the vehicle at a concentration of 0.1 M had a marked effect on the transport of salbutamol across both human and murine skin. The initial passive permeation of the drug across the skin was not affected but the rate of drug delivery during iontophoresis was typically observed to increase by a factor greater than two. The post-iontophoretic transport of salbutamol across either tissue was also substantially enhanced in the presence of the fatty acid. The analogous use of stearic acid did not significantly influence the iontophoretic or the post-iontophoretic transport of salbutamol across excised human skin. The investigation also revealed a synergistic combination of the fatty acid and anodal iontophoresis to enhance the in vitro transport of other drug substances, including nicotine and diltiazem hydrochloride across murine skin. Oleic acid increased both the iontophoretic and post-iontophoretic transport of nicotine, so that the enhancement of drug delivery was greater than that caused by the current alone. The investigation also indicated that the barrier properties of the skin recover following the constant current iontophoresis in the presence of oleic or lauric acids.

Keywords: Iontophoresis; Transdermal; Enhancer; Oleic acid; Salbutamol

1. Introduction

This paper follows a recent study (Nolan et al., 2003), that described the effects of the simultaneous chemical and electrical enhancement of the delivery of salbutamol from a liquid crystalline vehicle across an artificial non-rate-limiting membrane. The methodology used involved initial measurements of the passive rates of transport of the drug from the cubic liquid crystalline phase of an amphiphilic monoolein vehicle. The results of control experiments were then compared with the analogous rates measured when the vehicles contained added concentrations of chemical penetration enhancers such as oleic or lauric fatty acids. The passive release of the salbutamol was found to adhere to matrix diffusion control and the addition of the oleic acid was shown to increase the lipophilicity of the cationic drug due to ion-pairing with the anionic enhancer so that its partitioning into the aqueous part of the vehicle and subsequent

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release across the membrane was significantly reduced. The delivery of the salbutamol from the simple gel was significantly enhanced by iontophoresis with the rates being approximately proportional to the currents achieved. The data also clearly indicated the iontophoretic process to be considerably less efficient in the presence of buffer ions due to reductions in the transport numbers of the drug ions. Addition of either fatty acid to the electrically assisted device resulted in a substantial enhancement of the delivery rates. The principal factors governing the efficiency of the iontophoretic delivery were identified as being the concentrations of extraneous ions in the system and the mobility of the drug ion itself through the vehicle and across the membrane.

Here we report on an analogous investigation in which drug transport from the same liquid crystalline gels is studied in vitro across human and nude murine skins. The passive and iontophoretic skin permeation of salbutamol base, nicotine, diltiazem hydrochloride and sodium diclofenac are all measured and the study includes a comparison between drug transport across excised human and nude murine skins. The presence of oleic acid in the *stratum corneum* has been shown to facilitate an increased in vitro permeation for many drugs including salicyclic acid, estradiol, 5-fluorouracil and piroxicam (Cooper, 1984; Goodman and Barry, 1989; Francoeur et al., 1990). The technique described in Nolan et al. (2003) by which both the drug and the fatty acid enhancer are incorporated into the liquid crystalline vehicle could provide a better method of enhancing drug delivery across the skin. The main objective of this current work is, therefore, to assess the possibility of utilising such complex vehicles to carry out transdermal delivery and to develop a system in which the vehicle itself enhances the iontophoretic transport. To this end the effects of separately incorporating oleic, lauric or stearic acids into the liquid crystalline vehicle on drug transport through the skin are quantified as are the synergistic effects when this drug delivery is simultaneously electrically assisted.

2. Experimental

Details of the diffusion cells used to study the in vitro transport and of the preparation of the liquid crystalline vehicles were given in Nolan et al. (2003). Sodium diclofenac, which is a potent synthetic non-steroidal anti-inflammatory agent that also has analgesic properties, and stearic acid (99%) were obtained from Sigma Chemicals. The nature and sources of all the other chemicals used were also listed in Nolan et al. (2003).

The preparation of the human cadaver skin (HS) was based in part on the procedure described by Kligman and Christophers (1963). It was used as full thickness skin because the presence of both the epidermis and dermis makes for a more realistic model for in vivo drug transport. The electrical resistance of full thickness skin has been observed to be comparable with that of excised epidermal tissue (Nolan et al., 1993) and it is easier to manipulate. Human skin samples were prepared for in vitro use by removing the subcutaneous fat. The majority of the experiments involved the use of the skin as it was received. However due to the irregular availability of donors, some samples were carefully dried, stored at 271 K and then reconstituted prior to use. Kasting and Bowman (1990) have verified that human skin stored in this manner is entirely suitable for iontophoretic studies.

Shah–Shah hairless mice (HMS) were supplied by the Bioresources Unit, Trinity College, Dublin. After sacrifice, the skin was carefully removed and the loosely attached brown fat was removed from the underside. The skin was used immediately upon removal from the animal.

The basic experimental protocols used in both the passive and iontophoretic drug release studies have been described in Nolan et al. (2003). However the nature of the membranes being used here necessitated the following changes to these procedures. The monoglyceride liquid crystalline vehicles were prepared using isotonic phosphate buffer (IPBS) as the solvent: the same solution was employed as the receptor medium. Again, each experiment consisted of three distinct stages. Stage I entailed the passive steady state permeation of the selected drug through a skin sample. For human skin the duration of this stage was 12 h with samples taken at regular intervals during the initial and final 2 h periods. Because murine skin is reported to retain its barrier properties only for up to 20 h (Srinivasan et al., 1989a) the total duration of each transport study on this tissue was limited to 18 h. A considerably shorter time of 2 h was therefore assigned to stage I. Stage II, for both types of skin, consisted of a 6 h period at a constant iontophoretic current of 1.0 mA through the drug delivery system. These studies predominantly involved the passage of an anodal current but when anionic drugs were used the current was switched to be cathodal. These protocols were adopted following evidence from preliminary experiments that showed that exposure to 0.39 mA cm^{-2} for 6 h was required to establish steady-state drug transport across human skin. Gay et al. (1992) used similar conditions. Platinum was used as the sole electrode material because it had been shown in Nolan et al. (2003) to produce a more efficient iontophoretic delivery from the liquid crystalline vehicle being used here. It was also shown that the pH changes expected in the vehicle would be too small to cause damage to skin. During stage III the rate of passive drug delivery after cessation of iontophoresis was assessed. The quantity of drug transported into the receptor in the 4 h immediately after iontophoresis was used to estimate a rate of drug transport. As in stage I, the duration of this passive period was varied with the skin types: for human skin stage III lasted 20 h, for nude murine skin it was limited to 10 h.

With the exception of sodium diclofenac the analytical procedures for the HPLC determinations of each drug have been given in Nolan et al. (2003). Sodium diclofenac was also analysed using the Waters HPLC system. The mobile phase comprised 400 cm^3 of 50 mM orthophosphoric acid and 600 cm^3 acetonitrile. The flow rate was 1.0 cm^3 min^{-1}, the wavelength was 276 nm and the observed retention time was 5.2 min.

3. Results

3.1. Passive transport of salbutamol base across excised skin

The quantities of salbutamol base (Q' in mg) crossing the excised human skin during each stage of the delivery protocol are

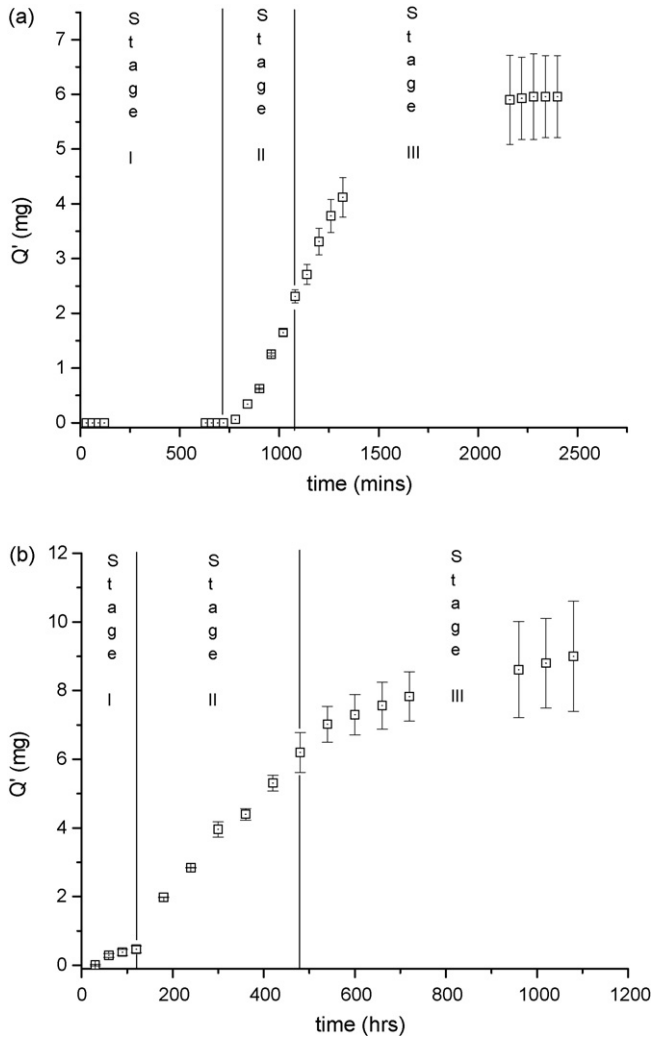

Fig. 1. (a) The quantities of salbutamol (Q') transported across excised full thickness human skin, from the monoglyceride liquid crystalline vehicle, before (I), during (II) and after iontophoresis (III). The initial drug loading of the vehicle was 28 mg cm^{-3} and a constant current of 0.39 mA cm^{-2} was used in stage II. (b) Analogous data for transport across excised nude murine skin. The data are, in each case, the means of six separate determinations using different skin samples: the errors are the standard errors of the means.

shown in Fig. 1a. During the 12 h of stage I no passive transport was detected in any of the skin samples studied. Investigations by Bannon (1989) and Carr (1992) also failed to detect any passive transport of salbutamol sulphate, from either a hydrophilic gel or a monoglyceride formulation, across excised human epidermal tissue. In contrast, as shown in Fig. 1b, the salbutamol base was found to passively cross the hairless murine skin during the initial 2 h period. A lag time, t_L, of 30 min was estimated from these data by extrapolation of the steady state profile to the time axis. Assuming a thickness, h, of the murine skin of 70 μm (Bronaugh et al., 1982) the diffusion coefficient of the drug, D_M, through that membrane was estimated as $4.54 \pm 0.60 \times 10^{-9}$ cm^2 s^{-1} using the equation Flynn et al. (1974):

$$t_L = \frac{h^2}{6D_M} \quad (1)$$

There is considerable evidence that the diffusional resistance of murine skin is located within the *stratum corneum* (Burnette and Bagniefski, 1988; Francoeur et al., 1990; Knutson et al., 1990). The value of the permeability coefficient, K_P, for salbutamol in hairless murine skin estimated using Fick's first law of diffusion and the data measured in STAGE I, is $7.03 \pm 0.83 \times 10^{-7}$ cm s^{-1}. This corresponds to an average flux of 1.12×10^{-3} mg cm^{-2} min^{-1} over a period of 24 h. This is larger than the value of 3.5×10^{-4} mg cm^{-2} min^{-1} reported by Gokhale et al. (1992) for similar transport from a delivery device described simply as a polymeric film. It is possible that the higher rate of transport of the salbutamol observed here is the result of increased partitioning of the drug from a predominantly lipophilic vehicle into the murine *stratum corneum*. Aungst et al. (1990) reported increased partitioning of both acidic and basic drugs into excised human *stratum corneum* as the fatty acid content of the vehicle was increased. Differences in drug transport similar to those reported here have previously been observed between the behaviours of human and nude murine skins. The latter has sometimes been used as a model for human skin (Stoughton, 1975; Durrheim et al., 1980) but its permeability to a number of substances, including benzoic acid, acetylsalicyclic acid and nitroglycerine, has been observed to be substantially greater than that of human skin (Bronaugh et al., 1982; Roberts and Mueller, 1990). Barry (1986) has suggested that the use of murine skin may overestimate the permeation of human skin by as much as 7-fold.

3.2. Iontophoretic transport of salbutamol base across excised skin

Preliminary iontophoretic experiments revealed that at a current density of 0.20 mA cm^{-2}, the flux of the salbutamol from the vehicle across human skin was minimal and for some samples none was detected. The application of constant current iontophoresis at 0.39 mA cm^{-2} substantially increased the transport of the drug through both human and murine skins relative to that which had occurred passively. As illustrated in Fig. 1b the quantity of salbutamol delivered across murine skin during iontophoresis (stage II) was reasonably linear with time. Analysis of the data revealed an iontophoretic rate of drug delivery of $5.4 \pm 0.021 \times 10^{-3}$ mg cm^{-2} min^{-1} thus indicating that the rate of transport across the skin had been increased by a factor of four over its passive rate. In contrast, the corresponding section of the profile relevant to the iontophoretic transport of salbutamol across human skin in Fig. 1a curves upwards. However, during the latter 5 h of stage II the quantity of salbutamol transported across the tissues becomes approximately linear with time; the rate of this delivery is $3.2 \pm 0.030 \times 10^{-3}$ mg cm^{-2} min^{-1}. This value is a considerable enhancement over a rate of drug delivery of 4.8×10^{-4} mg cm^{-2} min^{-1} evident during the first hour of stage II, and is a more realistic representation of the rate of transport of this drug across human skin during iontophoresis. The rates of salbutamol transport relevant to each stage of the investigation across the two membranes and under all the conditions studied are given in Table 1.

Table 1
The rate at which salbutamol is delivered across the skin in vitro, before (R_P stage I), during (R_I stage II) and after iontophoresis (R_P' stage III): n indicates the number of experimental determinations and the surface are of the gel is 2.54 cm^2

Vehicle type	Gel solvent	Skin type	n	Current density ($mA\,cm^{-2}$)	Stage I 10^2 $R_P \pm$ S.E. ($mg\,min^{-1}$)	Stage II 10^2 $R_I \pm$ S.E. ($mg\,min^{-1}$)	Stage III 10^2 $R_P' \pm$ S.E. ($mg\,min^{-1}$)
Liquid crystalline gel	IPBS	HS	6	0.39	Not detected	0.81 ± 0.078	0.76 ± 0.086
Liquid crystalline gel with 0.1 M oleic acid	IPBS	HS	6	0.39	Not detected	1.94 ± 0.20	1.66 ± 0.195
Liquid crystalline gel with 0.1 M lauric acid	IPBS	HS	6	0.39	Not detected	1.90 ± 0.19	1.50 ± 0.171
Liquid crystalline	IPBS	HS	3	0.39	Not detected	1.26 ± 0.16	0.44 ± 0.028
Liquid crystalline gel with 0.1 M stearic acid	IPBS	HS	3	0.39	Not detected	1.65 ± 0.12	0.43 ± 0.02
Liquid crystalline gel	IPBS	HMS	6	0.39	0.30 ± 0.03	1.38 ± 0.085	0.45 ± 0.04
Liquid crystalline gel with 0.1 M oleic acid	IPBS	HMS	6	0.39	0.33 ± 0.04	2.2 ± 0.155	1.98 ± 0.20
Liquid crystalline gel	H_2O	HS	–	0.09	Not detected	0.50[a]	0.057[a]

Data relevant to the transport of the salbutamol sulphate from other investigations is also included.
[a] Refers to a study by Carr (1992).

The transport numbers for the delivery of salbutamol across both skin types were calculated from the iontophoretic rate of drug delivery on the basis of the method used by Burnette and Ongpipattanakul (1987). During iontophoresis it is likely that there are contributions to the observed flux of salbutamol from the electromigration of the ionised drug, from electroosmosis and from enhanced diffusion. The iontophoretic process is further complicated by the presence of extraneous buffer ions in the solvent domain of the liquid crystalline vehicle. As a consequence, it is likely that the transport number may not be an accurate reflection of the fraction of the current carried by the drug ion during iontophoresis. However, the values of the transport number calculated here are less by a factor of five than those obtained for the iontophoretic release of the drug from the same delivery vehicle across a non-rate limiting membrane (Nolan et al., 2003). As anticipated, the skin severely restricts the delivery of the drug into the receptor solution. The data were also examined in terms of the drug fluxes at hourly intervals. Fluxes were calculated by determining the rates of iontophoretic drug transport on a point by point basis during stage II. The results are illustrated in Fig. 2. The flux of salbutamol across the murine tissue is observed to show an initial peak within the first hour of iontophoresis and continues to fluctuate about a value of $\sim 5.8 \times 10^{-2}\,mg\,cm^{-2}\,min^{-1}$: the significance of this will be discussed later. For human skin, a steady-state drug transport process sets in three and a half hours after iontophoresis has commenced at a lower rate of $\sim 2.5 \times 10^{-2}\,mg\,cm^{-2}\,min^{-1}$.

The voltage required to maintain the current at a density across human skin of $0.39\,mA\,cm^{-2}$ was found to increase by ~30% during the first 60 min of stage II from the initial ~6.0 V. It then declined slightly and remained constant. Alteration of the skin's permeability during iontophoresis may significantly contribute to the flux of both charged and uncharged species (Srinivasan et al., 1989b; Sims et al., 1992; Pikal and Shah, 1990).

The quantities of drug transported passively into the receptor solution during stage III, allows estimation of the increase in the skin's permeability as a consequence of iontophoresis having taken place, although there may also be some initial contributions from an increased reservoir effect. This analysis revealed that the drug is delivered across human skin at a rate of $3.0 \pm 0.34 \times 10^{-3}\,mg\,cm^{-2}\,min^{-1}$ during an initial post-iontophoretic period of 4 h, indicating that upon cessation of the current the rate of drug delivery is only minimally decreased. It would therefore seem that the flux of the drug across human skin during iontophoresis, is primarily facilitated by enhanced diffusion. In contrast, the delivery of the drug across murine skin is observed to decrease by $68 \pm 6.8\%$, during the same stage III time interval, to a rate of $1.8 \pm 0.16 \times 10^{-3}\,mg\,cm^{-2}\,min^{-1}$, a value representing approximately 50% increase over the initial rate evident during stage I. While these data indicate that the permeability of murine skin is increased during iontophoresis, the flux of the drug is predominantly electrically driven. This transport process is likely to involve the direct effect

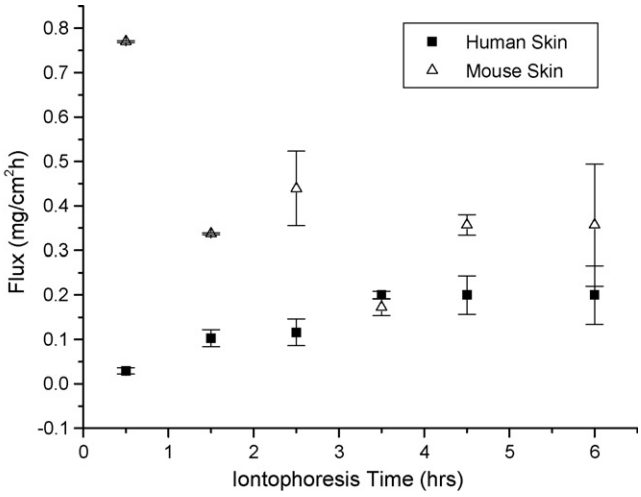

Fig. 2. The fluxes of salbutamol across both excised human and murine skin determined at hourly intervals during stage II iontophoresis at a current density of $0.39\,mA\,cm^{-2}$.

of the electrical field on the drug ion coupled with the indirect effect of electroosmosis. It has been observed that, for the iontophoretic transdermal transport of low and intermediate molecular weight cations, the electroosmotic contribution is less significant than the effects of direct electrostatic repulsion and the increase in the permeability of the skin (Srinivasan et al., 1989b; Sims et al., 1992; Banga et al., 1999). The findings of Burnette and Ongpipattanakul (1987) also support the hypothesis that this flux is proportional to the applied current density.

Human skin is known to display considerable inter-sample variation in both its physiochemical and transport properties (Behl et al., 1989; De Nuzzio and Berner, 1990; Sims et al., 1992). This phenomenon was observed in the present investigation during stages II and III of salbutamol transport across the tissue. The standard error on the quantity of drug transported into the receptor solution is indicated on each data point in Fig. 1. In terms of the drug permeability, the coefficients of variation indicate the inter-sample variation at ∼35%. However the intra-sample variation observed in the transport of salbutamol across different skin samples from the same individual is less (∼20%).

Examination of the data relevant to the iontophoretic transport of salbutamol across human skin may provide an indication of the time course during which the barrier function of the tissue changes. Analysis of data in Fig. 2 reveals that the flux of salbutamol across the tissue increases throughout the first half of stage II before a steady rate of drug transport is achieved. A comparison with the profile for the same process in Fig. 1a suggests that the changes in the barrier function during iontophoresis (Sims et al., 1991, 1992) may, because of the absence of any passive transport, be coupled here with other effects due to the absorption and partitioning of the salbutamol into the human skin. In contrast, the transport of salbutamol across murine skin is established during stage I so that the movement of the drug into the receptor medium can take place immediately when the iontophoretic potential is applied. Thus a linear increase in the quantity of the drug delivered is observed throughout stage II (Fig. 1b). This is also clearly illustrated in the first data point relevant to the iontophoretic flux of the drug across murine skin in Fig. 2 where the profile shows an initial peak which is likely due to the rapid depletion at the onset of iontophoresis of the drug reservoir established within the skin.

3.3. The passive and iontophoretic transport of salbutamol from liquid crystalline vehicles containing fatty acids

3.3.1. Human skin

Oleic acid, lauric acid and stearic acid were each incorporated into the oil component of the liquid crystalline vehicle at a concentration of 0.1 M. The mean ($n = 6$) transport of salbutamol across excised human skin from the oleic and lauric acid containing vehicles is illustrated in Fig. 3a and b. For comparison the drug transport across the same tissue from a conventional vehicle, which contained no additional fatty acid, is also shown. The presence of either fatty acid in the vehicle appears not to affect the passive permeation of the drug across the skin during stage I, no passive flux of the drug was detected. In contrast, the fatty acid has a considerable effect on the iontophoretic transport of the drug across the skin. The inclusion of oleic acid in the vehicle results in an enhanced iontophoretic transport of salbutamol throughout the 6 h period of stage II. The presence of lauric acid in the gel substantially enhances the delivery of the drug during the last 2 h of iontophoresis.

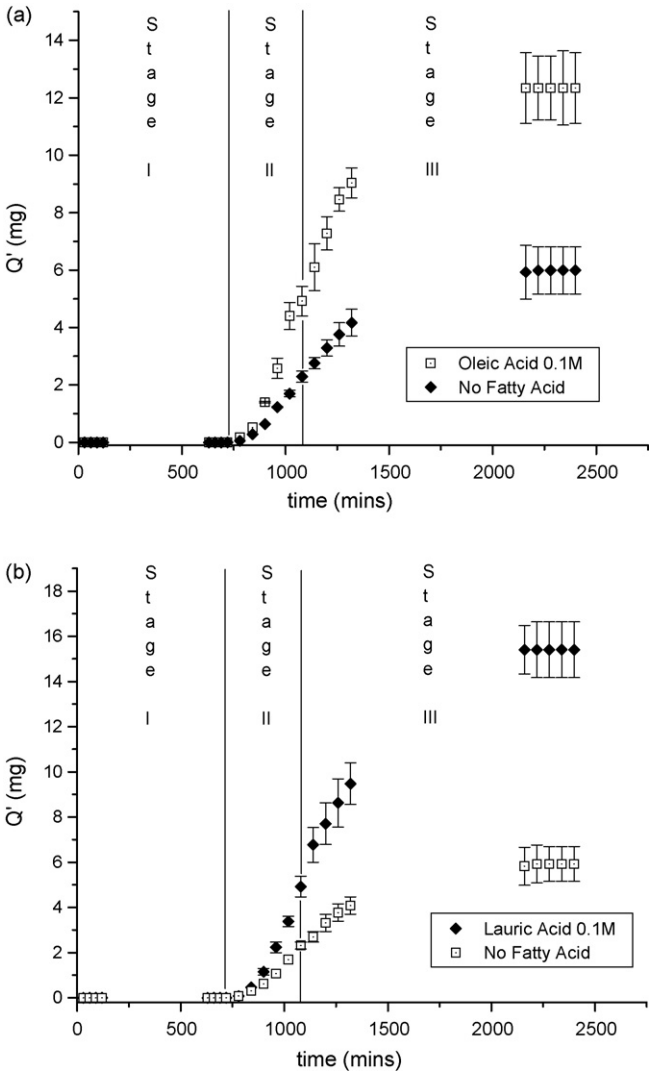

Fig. 3. The effects of incorporating (a) 0.1 M oleic acid and (b) 0.1 M lauric acid into the liquid crystalline vehicle, on the transport of salbutamol across excised human skin, before (I), during (II) and after (III) iontophoresis. The initial drug loading of the vehicle was 28 mg cm^{-3} and a constant current of 0.39 mA cm^{-2} was used in stage II. The data are the means of six separate determinations using different skin samples: the errors are the standard errors of the means.

For the transport of salbutamol from the conventional vehicle, the quantity of the drug delivered across human skin increases linearly with time during the later 5 h period of iontophoresis. The rate of drug transport during iontophoresis was estimated from the linear section of the profile. The values given in Table 1 indicate that, as a result of incorporation of the fatty acid into the vehicle, the iontophoretic delivery of the drug is enhanced by a factor of approximately 2.4. In the presence of either fatty acid the post-iontophoretic rate of drug delivery (stage III) is

more than twice that observed from the standard liquid crystalline vehicle ($P \leq 0.005$ for oleic acid; $P \leq 0.05$ for lauric acid). Relative to the delivery of the drug during stage II, the rate at which salbutamol is transported from the vehicle containing oleic acid across the tissue, is decreased by ∼15%. Similarly the rate of drug transport from the vehicle containing lauric acid is decreased by ∼10 to 20%. As noted previously, the rate at which salbutamol is transported from a conventional vehicle across excised human skin after iontophoresis also shows a small decrease. Human skin was observed to recover its pre-iontophoretic barrier properties during the extended period of stage III.

Experiments were also carried out in which stearic acid, a fully saturated C_{18} straight chain fatty acid, was incorporated into the liquid crystalline vehicle at a concentration of 0.1 M. The rates of drug transport during iontophoresis and after cessation of the current are shown in Table 1. The incorporation of stearic acid did not significantly enhance the transport of the drug across the skin during iontophoresis ($P \geq 0.10$). After cessation of the current this fatty acid did not seem to have any influence on the rate of drug transport across the tissue. The results indicate that stearic acid does not influence the permeability of the skin tissue. Aungst (1989) has suggested that stearic acid is too similar to the lipids present in normal *stratum corneum* to influence their packed structure and because of this it may not facilitate increased skin permeability.

3.3.2. Murine skin

The transport data for salbutamol across hairless murine skin from a liquid crystalline vehicle containing 0.1 M oleic acid during each stage of the experiment are included in Table 1. The fatty acid influences the transport of the drug across the murine skin in a manner similar to that which was observed for the human tissue. Oleic acid does not significantly influence the passive permeation of the drug during stage I: the rate at which the drug was transported over the 2 h period was observed to increase by ∼10%. However, the data presented in Table 1 indicate that the delivery of salbutamol to the receptor during iontophoresis was increased by some 60% ($P \leq 0.02$). The rate of transport decreased after cessation of the current but was greater by a factor of 4.4 ($P \leq 0.05$) over the corresponding rate in the absence of the acid. Murine skin was observed to regain its pre-iontophoretic permeability within a 10 h period after cessation of the current with complete recovery of the skin evident in every tissue sample investigated. The present investigation indicates that the incorporation of either oleic or lauric acid into the liquid crystalline vehicle most effectively enhances the iontophoretic and post-iontophoretic transport of salbutamol across the skin.

3.3.3. Polarity of transport cell

In an effort to further elucidate this process the transport of salbutamol across hairless murine skin was examined under cathodal iontophoretic conditions using a liquid crystalline vehicle containing 0.1 M oleic acid. A cathodic current density of 0.39 mA cm^{-2} was initially passed through the skin and the gel for a period of 3 h: the transport of the drug was then conducted passively for 2 h. Then the polarity of the electrodes was reversed and the drug transport effected by anodal iontophoresis at 0.39 mA cm^{-2} for 3 h. The control experiment involved the use of a standard liquid crystalline vehicle that did not contain additional fatty acid; otherwise the protocols were identical. The data relevant to the transport of salbutamol during each stage of delivery are given in Table 2. The presence of oleic acid in the gel does not influence the cathodal iontophoretic transport of the drug or the rate at which the drug passively permeates through the tissue. During cathodal iontophoresis the migration of the salbutamol cations present in the aqueous domain of the vehicle should be orientated away from the epidermis towards the negative electrode. The rate of drug transport during this period is reduced relative to the rate at which the drug is transported across this tissue passively. These results indicate that the permeability of the tissue is not altered to any significant extent.

3.4. The passive and iontophoretic transport of nicotine, diltiazem and diclofenac across hairless murine skin in the presence of oleic acid

The purpose of these measurements is to assess any synergy arising when the use of oleic acid and iontophoresis are combined to enhance the in vitro transdermal transport of other drug substances. Nicotine and diltiazem were chosen as model cationic drugs, as their passive release had also been examined previously (Nolan et al., 2003). Sodium diclofenac was selected as a model anionic drug on the basis that it is extensively ionised at physiological pH. The experimental protocol involved the incorporation of both the drug and fatty acid into the monoglyceride component of the liquid crystalline vehicle. The results obtained are summarized in Table 3. When the rates at which nicotine is passively transported during stage I are compared with data, taken from transport studies by Bannon (1989) and Carr (1992), in Table 4 the murine skin is seen to be up to eight times more permeable. The presence of oleic acid in the vehicle appears to slightly enhance the initial passive rate of nicotine delivery (not statistically significant $P \geq 0.50$). Application of a current density of 0.39 mA cm^{-2} enhances the rate of drug transport in the control experiment by $49 \pm 15\%$. An effective transport number was estimated for this process which indicated that the participation of the nicotine cation in the conduction process during iontophoresis is greater than that of the salbutamol ion. The rate of nicotine transport after cessation of the current was observed to decrease by $26 \pm 10\%$ as opposed to a decrease of 68% previously observed for salbutamol. Thus during iontophoresis the extent to which nicotine is electrically driven across the tissue is a secondary factor and the drug is predominantly transported by diffusion. Relative to the control experiment the presence of oleic acid in the vehicle increased the rate of iontophoretic drug delivery by a factor of 1.35 ($P \leq 0.05$; Table 3). The effective transport numbers for nicotine in Table 4 suggest that the fraction of the current carried by the drug is increased from ∼30 to ∼40% on incorporating the fatty acid into the vehicle. At physiological pH, nicotine is present as a free base and also as a mono-cation (Oakley and Swarbrick,

Table 2
The rates at which salbutamol is transported across murine skin during 3 h cathodal iontophoresis at a current density of 0.39 mA cm^{-2} (R_{Ic}): followed by 2 h passive diffusion (R_p), and finally during 3 h anodal iontophoresis at the same current density (R_{Ia})

Vehicle	Gel solvent	10^2 $R_{Ic} \pm$ S.E. (mg min^{-1})	10^2 $R_p \pm$ S.E. (mg min^{-1})	10^2 $R_{Ia} \pm$ S.E. (mg min^{-1})
Liquid crystalline gel	IPBS	0.170 ± 0.036	0.39 ± 0.052	0.855 ± 0.032
Liquid crystalline gel with 0.1 M oleic acid	IPBS	0.166 ± 0.040	0.41 ± 0.026	0.995 ± 0.022

The results are the mean of 6 experiments and the surface area of the gel is 2.54 cm^2.

Table 3
The rates at which nicotine, diltiazem and diclofenac are transported across murine skin before (R_P stage I), during (R_I stage II) and after iontophoresis (R_P' stage III)

Drug	Vehicle type	Gel solvent	Stage I 10^2 $R_P \pm$ S.E. (mg min^{-1})	Stage II 10^2 $R_I \pm$ S.E. (mg min^{-1})	Stage III 10^2 $R_P' \pm$ S.E. (mg min^{-1})
Nicotine	Liquid crystalline gel	IPBS	2.0 ± 0.15	2.97 ± 0.25	2.2 ± 0.14
Nicotine	Liquid crystalline gel with 0.1 M oleic acid;	IPBS	2.5 ± 0.14	4.00 ± 0.15	4.30 ± 0.20
Diltiazem HCl	Liquid crystalline gel	IPBS	Not detected	0.40 ± 0.028	0.55 ± 0.05
Diltiazem HCl	Liquid crystalline gel with 0.1 M oleic acid;	IPBS	Not detected	1.04 ± 0.05	0.52 ± 0.02
Diclofenac Na	Liquid crystalline gel	IPBS	Not detected	0.18 ± 0.007	0.17 ± 0.019
Diclofenac Na	Liquid crystalline gel with 0.1 M oleic acid;	IPBS	Not detected	0.19 ± 0.02	0.18 ± 0.03

The current density used in Stage II was 0.39 mA cm^{-2}.

1987). The increased iontophoretic transport of the drug may be a reflection of the interaction between the ionised oleic acid and buffer cations within the vehicle. An interaction of this nature would reduce the extent to which the current is carried across the vehicle and the skin by the extraneous ions. Consequently the participation of the drug ion in the conduction process should increase. This process has previously been examined in detail in relation to the iontophoretic release of salbutamol from the liquid crystalline delivery vehicle (Nolan et al., 2003). After cessation of the current the rate of nicotine transport was unaffected. The rate at which nicotine is transported across the tissue during the initial period of stage III exceeds that of the control experiment by a factor of 2 ($P \leq 0.05$). As previously discussed for salbutamol the post-iontophoretic transport of nicotine may involve the partitioning from a reservoir of drug, built up during stage II, out of the skin. The delivery of nicotine across murine skin during stages II and III could also be increased as a result of the fatty acid directly altering the permeability of the tissue. As nicotine can readily diffuse across the tissue the post-iontophoretic drug transport is likely to be significantly enhanced by the presence of the fatty acid in the skin. Throughout the duration of stage III the rate of nicotine transport continuously declined in the experiment involving oleic acid. Although the permeability of murine skin to nicotine approached that which was evident prior

Table 4
Effective transport numbers for nicotine, diltiazem and diclofenac during iontophoresis

Drug	Vehicle type	Gel solvent	Skin type	Current density (mA cm^2)	Stage II (transport number ± S.E.)
Nicotine	Liquid crystalline gel	IPBS	HMS	0.39	0.29 ± 0.01
Nicotine	Liquid crystalline gel with 0.1 M oleic acid;	IPBS	HMS	0.39	0.41 ± 0.02
Nicotine	Liquid crystalline gel	H_2O	HS	–	0.074[a]
Nicotine	4% Agar	H_2O	HS	–	0.41[b]
Diltiazem HCl	Liquid crystalline gel	IPBS	HMS	0.39	0.015 ± 0.001
Diltiazem HCl	Liquid crystalline gel with 0.1 M oleic acid	IPBS	HMS	0.39	0.046 ± 0.002
Diclofenac Na	Liquid crystalline gel	IPBS	HMS	0.39	0.01 ± 0.001
Diclofenac Na	Liquid crystalline gel with 0.1 M oleic acid	IPBS	HMS	0.39	0.01 ± 0.002

Data relevant to the transport of nicotine from other investigations are also included.
[a] Refers to a study by Carr (1992).
[b] Refers to a study by Bannon (1989).

to iontophoresis a full recovery of the tissue was not observed. A good recovery of the initial permeability of the tissue to nicotine was, however, observed in the control experiment.

The results obtained using diltiazem are also included in Table 3 and compared with analogous data for other penetrants in Table 4. In contrast to nicotine and salbutamol, this drug does not passively diffuse through the murine tissue. In the control experiment iontophoresis facilitated a minimal level of diltiazem transport across the skin. Nolan et al. (2003) observed that diltiazem was not readily released from the hydrophobic component of the liquid crystalline vehicle and argued that the drug does not actively partition from the hydrophobic regions of the gel into the aqueous region. The limited iontophoretic transport of diltiazem is most likely a reflection of the poor partitioning, electromigration and skin permeability of this bulkier drug species. In the 3 h period after cessation of the current the rate of drug transport is observed not to decrease relative to that evident during stage II. This suggests that diltiazem was not primarily electrically driven across the skin during stage II but rather that the drug transport was the result of diffusion where the iontophoretic process had increased the skin permeability. In contrast, when oleic acid was incorporated into the delivery vehicle, the iontophoretic transport of diltiazem was increased by a factor of approximately 2.6 ($P \leq 0.02$). After cessation of the current, the rate of drug transport was identical to that of the control experiment. Thus the fatty acid facilitated the increased participation of the drug in the conduction process during stage II. The data relevant to the iontophoretic transport of salbutamol and nicotine indicate that the incorporation of oleic acid into the vehicle can affect drug transport during stage III. However this was not the case for diltiazem. Six hours after cessation of the current no further transport of the drug across the tissue was evident in either the control experiment or that involving the fatty acid. This recovery of the skin may again be considered an indication of the basic non-permeability of the skin to the drug.

The results obtained with diclofenac sodium are summarized in Table 3. Diclofenac was not passively transported across the skin during stage I but cathodal iontophoresis for the 6 h period of stage II facilitated drug delivery. The literature relevant to cathodic iontophoretic drug transport across the skin is somewhat conflicting. For example, Bannon (1989) reported that benzoic acid was not transported across excised human epidermis by means of an electrophoretic current whereas Gay et al. (1992) observed the delivery of piroxicam across hairless murine skin in vitro, to be significantly enhanced by cathodal iontophoresis. In this study the rate of diclofenac transport after cessation of the current indicates that during stage II approximately 5% of the drug was electrically driven across the tissue. Thus the transport of diclofenac during iontophoresis is predominantly due to diffusion. It is likely that the transfer of the anionic drug across the tissue during stage II is limited due to the presence of smaller extraneous buffer cations in the delivery vehicle. The process is also likely to be severely restricted by the permselectivity of the tissue towards cations at physiological pH. The presence of oleic acid in the vehicle does not influence the passive or the iontophoretic transport of diclofenac across the skin.

These data indicate that a synergistic effect between the current and the fatty acid was evident only during anodal iontophoresis. With regard to the transdermal transport of nicotine and diltiazem no additional analysis of this process was undertaken. It would be expected, however, that in the presence of the fatty acid the enhanced drug transport during stage II and III is achieved in a manner similar to that previously discussed for salbutamol.

The incorporation of oleic acid into the liquid crystalline vehicle does not influence the transport of sodium diclofenac across murine skin during or after iontophoresis. However in these experiments the passage of the current across the assembly during stage II was maintained by the migration of anions away from the anode surface through the vehicle and the skin. Consequently an association between oleic acid and the buffer cation will not enhance the fraction of the current carried by the diclofenac anion across the skin. The data relevant to the cathodal iontophoresis of salbutamol suggest that the fatty acid anion is not directly transported into the skin. Similarly, it is likely that this is the case during the cathodal iontophoresis of sodium diclofenac. This is verified by the fact that the rate of drug transport during iontophoresis in the presence of the fatty acid and in the control experiment are very similar (Table 3) and thus the ionised lipophilic acid does not compete with the drug to carry the current. Furthermore, even as the iontophoretic process increases the permeability of the skin the diffusion of the associated fatty acid into the tissue is likely to be severely restricted. The application of a cathodic current across the vehicle and the skin will result in the migration of the buffer cations, present in the gel, away from the epidermal surface. Thus it is likely that any fatty acid which associates with these cations is transported away from the skin towards the negative electrode at the surface of the gel. At physiological pH the drug should be present in the aqueous domain of the vehicle as an anion which cannot associate with the ionised lipophilic acid.

4. Discussion

4.1. Factors affecting the iontophoretic transport of salbutamol base

As little or no passive transport of drug is reported here, with the exception of that across murine skin, the greater part of this discussion will focus on the factors which influence or control the enhanced delivery of the drug across human *stratum corneum*. Of significance are the ion selectivity of the skin and the facilitation of charge transport across the skin. The isotonic phosphate buffer solution used as the gel solvent provides a sodium ion concentration of 0.24 M. The drug was initially loaded into the monoglyceride component of each vehicle at a concentration of approximately 0.1 M. A consideration of these concentrations and of the ionic mobilities of the drug and buffer ions would suggest that the electromigration of the salbutamol cation through the gel will be restricted. De Nuzzio and Berner (1990) observed that, at physiological pH, human epidermal tissue displays selectivity for a range of simple monovalent cations, including Na^+. These authors also conclude that the skin is sig-

nificantly selective as to the size of the cation. In view of these findings, the transport of the current across the skin in the present work should be predominantly facilitated by the Na^+ ion. As the concentration of Na^+ ions present is so much in excess the process is likely to be sustained in this fashion throughout the 6 h period of stage II. Burnette and Marrero (1986) similarly report that the iontophoretic transport of thyrotropin releasing hormone across murine skin was severely impaired by the presence of buffer cations in the delivery vehicle.

The iontophoretic transport of salbutamol sulphate across human *stratum corneum* membranes has previously been investigated in this laboratory. These studies involved the use of hydrophilic 4% agar vehicles (Bannon, 1989) and monoglyceride liquid crystalline vehicles (Carr et al., 1997) to contain the drug. Thus, the presence of extraneous ions was not required to enhance the conductivity of the vehicle. Consequently these authors report a more efficient iontophoretic delivery of the sulphate salt of the drug. Furthermore, both Bannon and Carr observed that the iontophoretic flux of the drug was predominantly governed by the magnitude of the applied current. However, consistent with the results reported here, it was observed that the permeability of the *stratum corneum* was altered during iontophoresis and that this effect was reversible after a sufficient recovery time had elapsed.

4.2. The combined physical and chemical enhancement of the transdermal transport of salbutamol base

Of most significance here is the greater than additive effect of combining physical and chemical methods to enhance the transdermal delivery of salbutamol base. Among the factors to be considered, the effect of the chemical enhancer on the permeability of the skin is of primary importance. Thus it could be argued that the enhanced transport of salbutamol across the skin both during and after iontophoresis may partly involve the fatty acid increasing the permeability of the skin. The data presented by Nolan et al. (2003) suggest that the fatty acid incorporated into the delivery vehicle is unable to escape from the gel. Free or complexed fatty acid is not passively or actively transported from the hydrophobic regions of the liquid crystalline gel. It should however be emphasised that in the presence of a lipophilic membrane, such as the skin, the release of the fatty acid from the vehicle may be facilitated.

Oleic acid and lauric acid have previously been observed to enhance the in vitro skin transport of a range of drug substances including caffeine, naphazoline, indomethacin, 6-mercaptopurine (Green et al., 1989; Aungst et al., 1990; Goa and Singh, 1998). These enhancing effects are observed using a wide variety of vehicles. Most recently oleic acid has been shown to have enhancing activity on transdermal flux of sumatriptan (Femenía-Font et al., 2005). An investigation by Oh et al. (2001) demonstrated that permeation-enhancing effects of saturated fatty acids increased in the following order: C10\C12\C14\C16\C18. The saturated fatty acid, however, did not significantly shorten the lag time regardless of the carbon chain length. Meanwhile, similar to saturated lauric acid (C12), unsaturated oleic acid (C18) dramatically enhanced the skin permeability coefficient of Melatonin more than 950-fold from a propylene glycol vehicle. Wang et al. (2005) found that oleic acid in mineral oil yielded fast permeation of Physotigmine with a short lag time and also from propylene glycol vehicles (Wang et al., 2004). Similarly, oleic acid has also been shown to produce the largest skin reservoir of the drug flurbiprofen when incorporated into hydrogels (Fang et al., 2003). It has been proposed that both lauric and oleic acid may undergo an ion-pairing process with cationic drug species which may result in increased partitioning of the drug into the skin (Green and Hadgraft, 1987; Green et al., 1988; Fini et al., 1999). Enhanced skin transport facilitated by the fatty acid is frequently rationalised on the basis of the lipophilic enhancer increasing the diffusivity of the drug within the *stratum corneum* (Golden et al., 1987; Goodman and Barry, 1989; Higo et al., 1993). This may be achieved through the fatty acid disrupting the packing integrity of the intercellular lipids (Barry, 1987; Mak et al., 1990; Potts et al., 1991). Touitou et al. (2001) have shown that a 10% ethanolic solution of oleic acid has a major effect on the morphology of mouse skin, most notably a dramatic decrease in the density of Langerhans cells and dendrecites.

There is an increasing number of investigations, e.g., Kalia and Guy (1997) and Choi et al. (1999), in which the combined use of transdermal penetration enhancers and electrical assistance to increase drug transport across the skin have been reported. An approach which was initially used by Srinivasan et al. (1989a) involved pre-treatment of the skin with absolute ethanol followed by iontophoresis. It was observed that the permeability of insulin was dramatically increased. It has been suggested that ethanol can remove the *stratum corneum* lipids (Bommannan et al., 1991; Goa and Singh, 1998). Thus during iontophoresis the transport of insulin will be enhanced as the barrier function of the outer layer of the epidermis has been decreased. Other investigations have involved the application of the drug and enhancer to the skin together from a single solvent vehicle. The results have been positive and suggest that in vivo it may be possible to further enhance drug transport over that which is achieved by iontophoresis alone (Su et al., 1994). Data presented by Padmanabhan and Surnam (1991) indicate that pre-treatment of excised pig skin with 1% oleic acid in an ethanol–water solution facilitates an increased steady-state rate of drug transport during iontophoresis. However, when the iontophoretic transport of the drug takes place with the fatty acid present in the donor solution the rate of drug delivery is increased over and above this by a factor of 2.4. In agreement with the results of the present study, this would appear to indicate that the effect of the fatty acid is predominantly manifested during iontophoresis.

Finally, the present investigation indicates, through the assessments made of the continued transport of the drug during stage III, that the combined effects of the fatty acid and iontophoresis on both human and murine skin are reversible. Furthermore, this recovery was found to require a time period similar to that which was needed for the tissue to recover from the effects of iontophoresis alone. This indicates that the synergism of iontophoresis and penetration enhancement does not have any long term effect on the skin. Mak et al. (1990) observed

that human skin in vivo recovers from the effects of oleic acid treatment within a 24 h period.

5. Conclusions

The experiments reported here show that over a period of 12 h, salbutamol base does not diffuse form the hydrophobic region of monoglyceride liquid crystalline vehicle through excised human skin. In contrast, the drug was passively transported, over a period of 2 h, from a similar vehicle across hairless murine skin. Thus, excised murine skin is more permeable than is the human tissue.

The application of a current density of 0.39 mA cm^{-2} facilitated an appreciable level of salbutamol transport from the liquid crystalline vehicle across excised human skin. Analysis of the transport of salbutamol after cessation of the current revealed that, during iontophoresis drug delivery was primarily achieved by enhanced diffusion due to alteration of the permeability of the tissue. The initial barrier properties of human skin to the drug were observed to slowly recover in the 16 h period after iontophoresis and the relevant data indicate an almost complete recovery of the tissue. At a similar current density the quantity of salbutamol transported across excised murine skin was significantly greater. In this case the direct effect of the electrical field was a primary factor governing the transport of salbutamol across the rodent tissue with 68% of salbutamol delivery being electrically driven making the enhanced diffusion of the drug a secondary factor. The incorporation of either oleic or lauric acid into the monoglyceride component of the vehicle at a concentration of 0.1 M, had a profound effect on the transport of salbutamol across both human and murine skin. The initial passive permeation of the drug across the skin was not affected. The rate of drug delivery during iontophoresis was typically observed to increase by a factor of greater than two. This is possibly a reflection of the influence of the fatty acid on the conduction process within the vehicle. It has been suggested that the fatty acid, through an association with the extraneous cations present in the vehicle, reduces the portion of the current carried by these ions. The post-iontophoretic transport of salbutamol across either tissue was also substantially enhanced in the presence of the fatty acid. The incorporation of stearic acid into the liquid crystalline vehicle did not significantly influence the iontophoretic or the post-iontophoretic transport of salbutamol across excised human skin.

A combination of fatty acid and anodal iontophoresis also synergistically enhance the in vitro transport of nicotine and diltiazem hydrochloride. across murine skin. With regard to nicotine the effect of oleic acid was to increase both the iontophoretic and post-iontophoretic transport, so that the enhancement of drug delivery was greater than that caused by the current alone. In the case of diltiazem oleic acid enhanced the extent to which the drug is electrically driven across the tissue. Oleic acid did not influence the post-iontophoretic transport of the drug across the tissue. In contrast, the incorporation of oleic acid into the liquid crystalline vehicle did not affect the transport of sodium dicolfenac across hairless murine skin.

Finally, the investigation indicates that the barrier properties of the skin essentially recover to their initial values following the application of constant current iontophoresis in the presence of oleic or lauric acid.

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