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Iontophoretic and chemical enhancement of drug delivery Part I: Across artificial membranes

L.M.A. Nolan [a,1], J. Corish [a,*], O.I. Corrigan [b], D. Fitzpatrick [a,2]

[a] *Department of Chemistry, Trinity College, Dublin 2, Ireland*
[b] *Department of Pharmaceutics and Pharmaceutical Technology, Trinity College, Dublin 2, Ireland*

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Abstract

This paper reports on measurements of the release characteristics of the model drug salbutamol base from a liquid crystalline vehicle across a non-rate limiting synthetic membrane. The measured passive release rates were compared with analogous behaviour: (i) when a penetration enhancer such as oleic acid was incorporated into the vehicle; (ii) when the release was iontophoretically assisted; and (iii) when the penetration enhancer and iontophoretic assistance were used simultaneously. The effects of using isotonic phosphate buffer solution as the aqueous domain of the vehicle and in the receptor were also separately assessed. The passive release from the standard system was consistent with matrix diffusion control. The addition of oleic acid indicated association of the drug with the fatty acid so that its release into an aqueous medium was significantly retarded. With buffer ions present in the vehicle the release rate increased consistent with reduced association, and when phosphate buffer was used as a receptor medium the release rate exceeded that of the standard vehicle due to an ion exchange process. The delivery of salbutamol from the fatty acid containing systems was substantially enhanced by iontophoresis and the rates were shown to be approximately proportional to the assisting currents. The data clearly indicate the iontophoretic process to be significantly less efficient in the presence of buffer ions but with the iontophoretic delivery rates being enhanced by the presence of a fatty acid.

Keywords: Buffer; Enhancers; Iontophoresis; Liquid crystals; Oleic acid; Salbutamol; Transdermal

1. Introduction

The success of transdermal drug delivery systems depends on the ability of the drug molecules to penetrate the skin and reach the circulatory system in sufficient quantities for them to be therapeutic. For most drug substances the outermost layer of the skin, the *stratum corneum*, presents a barrier to drug transport that prevents the attainment of a therapeutic systemic level. As a consequence there has been intensive research into the development of strategies to increase, in a controlled and reversible fashion, the permeability of the barrier (Hadgraft, 1999). Among these strategies, iontophoresis, a technique involving the use of an electric current to drive ionised drug molecules across the skin, and chemical penetration enhancement, whereby chemical agents are used to modify the barrier properties of the tissue, have been most extensively investigated.

* Corresponding author. Tel.: +353-1-6081776; fax: +353-1-6712826.
E-mail address: jcorish@tcd.ie (J. Corish).
[1] Present address: Department of Health, Hawkins House, Dublin 2, Ireland.
[2] Present address: Department of Chemistry, University College Cork, Cork, Ireland.

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During iontophoresis an electrical potential gradient is used to effect drug transport. A particular advantage of the technique is that the rate of drug delivery may potentially be regulated by controlling the current through the device (Burnette and Marrero, 1986). Furthermore, iontophoresis may serve to reduce the intra and inter subject variability in the rates of drug delivery through the skin (Tyle, 1986). It is generally agreed that during iontophoresis ion transport takes place across the skin through the paths of least resistance. These pathways include hair follicles, sweat ducts and imperfections in the tissue structure (Abramson and Gorin, 1940; Burnette and Marrero, 1986). The enhanced transport is due to electrorepulsion and electroosmosis (Guy et al., 2000). The other contributing factors, which affect the flux of both charged and non-charged species, are alteration of the skin's intrinsic permeability (Burnette, 1989). Increases in skin permeability that occur during iontophoresis have also been interpreted as an alteration in the porosity of the *stratum corneum* induced by the applied field, due to electrostatic repulsion between neighbouring dipoles in the protein structure (Chien et al., 1989).

Chemical penetration enhancement refers to the process whereby chemical agents are used to modify the barrier properties of the *stratum corneum* and ultimately enhance the transdermal delivery of drug substances. Penetration enhancers may be applied to the skin prior to application of the drug, co-applied with the drug or used in the vehicle matrix (Goodman and Barry, 1989; Aungst et al., 1990). Ideally, the effects of the penetration enhancer on the skin should be reversible: its effect should also be non-toxic, non-allergenic and non-irritant (Turunen and Urtti, 1992).

For many years, investigators have examined not only the effectiveness of a wide variety of chemical species in increasing the absorption of drugs through the *stratum corneum*, but also their possible invasive potential: this field has recently been reviewed by Moser et al. (2001). Initial research focused upon the use of polar solvents such as DMSO, DMF and short chain alcohols (Allenby et al., 1969). More recently naturally occurring compounds, including terpenes from the chemical classes of hydrocarbons, alcohols, ketones and oxides (Barry and Williams, 1989; Morimoto et al., 1993) and long-chain fatty acids when applied to the skin in a suitable co-solvent such as propylene glycol have been used. The extent of the enhancement is dependent upon drug solubility, the length of the alkyl chain and its degree of unsaturation and the position and orientation of the double bond (Goodman and Barry, 1989; Aungst et al., 1990; Francoeur et al., 1990; Cooper, 1984; Aungst, 1989).

In recent years investigators have found that in vitro iontophoresis in the presence of a variety of chemical enhancers results in a synergistic effect that dramatically increases drug flux across the skin (Francoeur and Potts, 1990; Padmanabhan and Surnham, 1991; Gay et al., 1992). Similar results have also been observed in vivo using mice (Su et al., 1994). Combined physical and chemical penetration enhancement could have the distinct advantage that it could permit iontophoretic drug delivery under milder conditions of electrical potential and current density, thereby minimising side effects such as skin damage. The initial aim of this work is to explore the combined effect of electrical current and enhancing fatty acids on drug release from a liquid crystal-based medium across an artificial membrane, thus avoiding the complications of enhancement due to altered bio-membrane permeability. As iontophoresis was to be employed, a porous membrane is necessary. Visking, a non-rate limiting synthetic membrane that would allow the release characteristics of the vehicles to be determined while, at the same time, supporting them above the liquid receptor, was chosen for this purpose.

Oleic acid, a fatty acid extensively ionised at physiological pH, was selected as the principal penetration enhancer to investigate the effect of simultaneous iontophoretic assistance and chemical enhancement of drug delivery. Salbutamol base was used as a model drug. Its co-administration with theophylline in transdermal patches has recently been reported (Murthy et al., 2001).

The vehicle chosen was the cubic liquid crystalline phase of the amphiphilic monoolein, which at 313 K will incorporate a water content of 35% and also fatty acids. Furthermore, the cubic phase of monoolein has been observed to incorporate, at levels up to 20%, a large range of drugs of very different sizes and polarities, including proteins and oligopeptides, without experiencing a phase change (Ericsson et al., 1991; Burrows et al., 1994). The release kinetics of a range of substances from this matrix have been observed to conform to standard diffusion models (Engström,

1990; Carr et al., 1997). The latter study also showed that this monoglyceride delivery vehicle is suitable for use during iontophoretic transdermal drug transport.

2. Materials and methods

2.1. Vehicles

The monoglyceride vehicles were made in accordance with the method detailed by Carr et al. (1997) from Myverol^{TM} supplied by Eastman Kodak (Ireland). This system has a monoglyceride/water ratio of 2.5:1. Carr et al. (1997) showed that this composition maximised the release of model drug compounds. Myverol powder (4.7 g) was melted at 323 K, the accurately weighed requisite quantity of drug was then placed in the oil, the melt stirred to ensure uniform distribution and 1.88 cm^3 of water or phosphate buffer added. The liquid crystalline system that immediately formed was again stirred. For vehicles containing the fatty acid enhancers the required weight of the acid was added to the Myverol melt and stirred until dissolved before the addition of the salbutamol. The liquid crystalline vehicle was semi-solid in consistency and was syringed in 2 cm^3 quantities onto the Visking membrane. The thickness of the resulting disks, which were retained in place by a teflon collar on the top of the diffusion cell, was approximately 8 mm: their cross-sectional area was 2.54 cm^2.

2.2. Chemicals and membranes

Salbutamol base BP (molecular weight 239.3 amu) was supplied by K. and K. Greff, England. All the salts ($Na_2HPO_4 \cdot 12H_2O$; $NaH_2PO_4 \cdot 2H_2O$ and NaCl) used to prepare the physiologically adjusted isotonic phosphate buffer solutions (IPBS) were Analar Grade and were supplied by Reidal de Haen. The oleic acid and lauric acids were supplied by Sigma–Aldrich and were >99% pure. The water used was distilled and then purified in a Milli-Q Water purification system to a final resistivity of 18 $M\Omega\,cm^{-1}$.

The Visking membranes were 18/32 cellulose dialysis tubing supplied by the Visking Co., Chicago, IL. This membrane has been reported to have an average pore size of 2.4 nm (Corrigan et al., 1980) and an average thickness of 20 μm (Bannon, 1989). It was pre-treated by repeated immersions in frequently changed boiling water (Molyneux and Frank, 1961) to remove any soluble material such as glycerol or sulphur compounds.

2.3. Drug solubility studies

The solubility studies were conducted using a sealed vial method. An excess of salbutamol was weighed (approximately three times the expected solubility) and placed in a 10 cm^3 ampoule. The ampoule was covered to prevent exposure to light and placed in a Heto shaker water bath at 120 cycles/min and at 310 K. Solubility studies were carried out at least in triplicate and the dilution medium was either water, physiologically adjusted phosphate buffer (pH 7.4) or isotonic saline. The drug was allowed to equilibrate with the medium for periods of 24 and 28 hours. Following this the ampoule was broken and the contents filtered using 2 μm membrane filters. Several 1 cm^3 aliquots were taken and analysed by HPLC.

The solubility of the drug in the Myverol material was assessed in the following manner. Approximately 6 g of Myverol was weighed and melted using a water bath at 323 K. The Myverol was maintained at this temperature and a small quantity of drug added. This mixture was stirred continuously and the temperature of the bath raised at intervals of 3 °C until the cloudiness disappeared as the drug dissolved. The temperatures and quantities of the drug dissolved were recorded. Following this a further quantity of drug was added. The procedure was repeated continuously until the temperature of the monoglyceride reached approximately 358 K. These solubility determinations were carried out in triplicate.

2.4. Release studies

The diffusion cells were custom made modified Franz cells and have been previously described in detail (Bannon et al., 1987). They were of single compartment design with a volume of approximately 60 cm^3. The Visking membrane was secured across the top by means of a teflon collar and a star-headed magnetic follower, which was carefully controlled to avoid vortex formation, was used to stir the receptor fluid. The cells were immersed in a thermostatted water bath maintained at 310 K. Samples of volume 1.0 cm^3

were withdrawn by pipette through a port for analysis and were replaced by drug free receptor solution.

When iontophoretic assistance was provided the anode was placed on top of the drug loaded gel and the cathode in the receptor fluid just below the Visking membrane. Most of these experiments were conducted using pure (99%) Pt electrodes but Ag anodes and Ag/AgCl cathodes were used in a limited number of cases. The Ag/AgCl electrodes were prepared by the method of Burnette and Bagniefski (1988). Once time had been allowed for equilibrium, the typical protocol for the iontophoretic experiments involved passive drug delivery for a period of two hours followed by six hours electrically assisted delivery. The appropriate dc potential was applied and the cell was run galvanostatically, using a custom built controller, at currents in the range 0.25–1.0 mA. Both the applied voltage and the currents were monitored continuously during this delivery period. When the applied voltage was switched off the cell again operated passively.

2.5. Analysis

All analyses were made with HPLC using reverse phase chromatography with a Bondpak C_{18} Radialpak 8 mm × 10 mm column. Detection was by a Waters model 486 tunable UV absorbance detector at a wavelength of 276 nm. The Waters 746 data module integrator used an external standard method to calculate the drug content of each sample. The reproducibility of this method was 0.1% relative standard deviation.

3. Results and discussion

3.1. Passive release of salbutamol base

The cumulative quantities of salbutamol base released passively from the monoglyceride liquid crystalline vehicles using water as a solvent, across the membrane are plotted versus time in Fig. 1. The data are consistent with the Higuchi equation, Eq. (1) below, (Higuchi, 1960, 1961, 1963) when fitted using non-linear regression. In the Higuchi equation

$$q = Kt^{1/2} \quad (1)$$

t is the time, q is the quantity of drug released per unit area of the system (mg cm^{-2}), K is the release rate constant and is equal to $2C(D/\pi)^{1/2}$ where C is the initial drug concentration (mg cm^{-3}) and D the diffusion coefficient of the drug in the vehicle (cm^2 s^{-1}). Values of K for the Higuchi rate constants for the drug in the liquid crystalline vehicles are shown in Table 1. These are up to seven-fold lower than the values previously reported for salbutamol sulphate dissolved

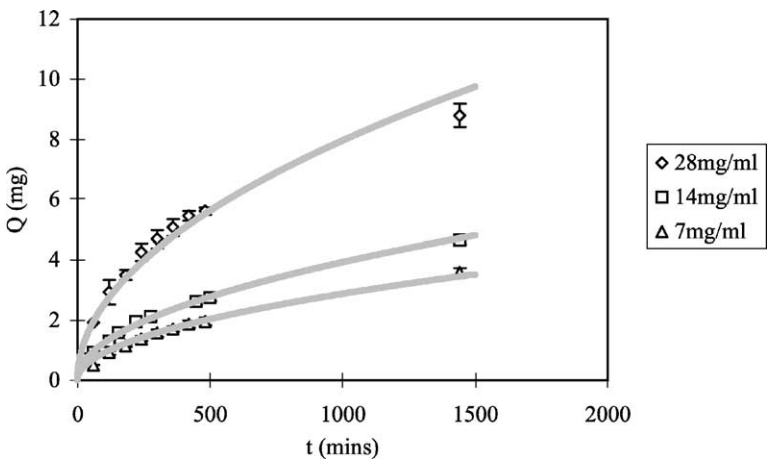

Fig. 1. The quantities of salbutamol base released from the liquid crystalline vehicle with a surface area of 2.54 cm^2, Q (mg), across Visking into an aqueous receptor plotted vs. elapsed time and fitted to the Higuchi equation. The data are the mean of six determinations, the error shown is the standard deviation of the mean.

Table 1
Experimentally measured values of K (the Higuchi rate constant) for salbutamol base from various gel formulations

Drug	Vehicle type	Vehicle solvent	Receptor medium	K ($\times 10^3$, mg cm^{-2} s$^{-1/2}$)
Salbutamol base (determined at gel loadings over range 7–28 mg ml^{-1})	Liquid crystalline 30% solvent	H_2O	H_2O	13.4 ± 0.9
Salbutamol base (determined at gel loading of 28 mg ml^{-1} only)	Liquid crystalline 30% solvent	IPBS	H_2O	13.6 ± 0.6
Salbutamol base (determined at gel loading of 28 mg ml^{-1} only)	Liquid crystalline 30% solvent	H_2O	IPBS	14.06 ± 0.95
Salbutamol base (determined at gel loading of 28 mg ml^{-1} only)	Liquid crystalline 30% solvent	IPBS	IPBS	16.31 ± 1.35
Salbutamol base (determined at gel loadings over range 14–28 mg ml^{-1})	4% agar	H_2O	H_2O	25.9 ± 2.07

The data relevant to this investigation represent the mean of at least four separate determinations.

either in the highly aqueous 4% agar gel (Bannon, 1989) or in the aqueous domain of the liquid crystalline phase (Carr et al., 1997). Thus, it appears that salbutamol base, in contrast to salbutamol sulphate, partitions significantly into the hydrophobic domain of the vehicle and this retards its release. The solubility of salbutamol base in Myverol was determined using the van't Hoff isochore method at 6.58 mg cm^{-3} at 310 K. The aqueous solubility is 20.72 mg cm^{-3} and these values, taken with the measured partition coefficient, suggest that at the 28 mg cm^{-3} loading the vehicles were saturated with the drug while those loaded at 14 mg cm^{-3} were close to saturation.

This could account for the rapid delivery of salbutamol at the two highest loadings studied, which may be due to the release of drug present on the surface of the vehicle.

3.2. Effect of buffering the vehicle and receptor medium

Studies were undertaken in which either the liquid crystalline vehicle and/or the receptor medium contained isotonic phosphate buffer (pH 7.4) instead of triply distilled water. The presence of the buffer aided the release of the drug and drug transport was found to be most enhanced when buffer was used as both the receptor medium and as the aqueous phase of the gel (Fig. 2). The generally more rapid release of drug on inclusion of electrolytes is likely due to facilitation of transfer of unionized drug from the hydrophobic domain to the aqueous phase resulting in more rapid diffusion in the base.

3.3. Effect of addition of oleic acid to the oil phase of the vehicles

Oleic acid was incorporated at a concentration of 0.1 M into the hydrophobic region of the liquid crystalline vehicle. Initial measurements involved the use of water as both the gel solvent and receptor medium. The study was then extended to assess the effect of also including buffer ions in one or both of these parts of the system. The results for the simple aqueous system are shown in Fig. 3(a) where they are compared with the analogous system without the fatty acid. Release profiles for the systems containing the phosphate buffer in the vehicle and/or the receptor medium are shown in Fig. 3(b). The stoichiometric ratio of drug to fatty acid in all of these systems was 1:1. It is clear that in the presence of the oleic acid the release of the drug is reduced. For the unbuffered system this effect is most pronounced: After 24 hours only 4.7 mg of salbutamol base was released compared with 8.8 mg of salbutamol base when the oleic acid was not present. The pK_a of salbutamol base is 9.5 and that of oleic acid at 5.4. The pH of the Myverol-based gel was measured at 7.4 so that both the drug and the acid would be highly ionised in this medium (Padmanabhan and Surnham, 1991). Ion association between these species would be expected to occur and concentration of the drug available to diffuse across the Visking membrane and into the receptor medium is effectively reduced. The incorporation of the oleic acid results in a decrease in the rate of release by a factor of three after eight hours (Fig. 3(a)). This suggests that the driving force for drug release, the concentration of free salbutamol

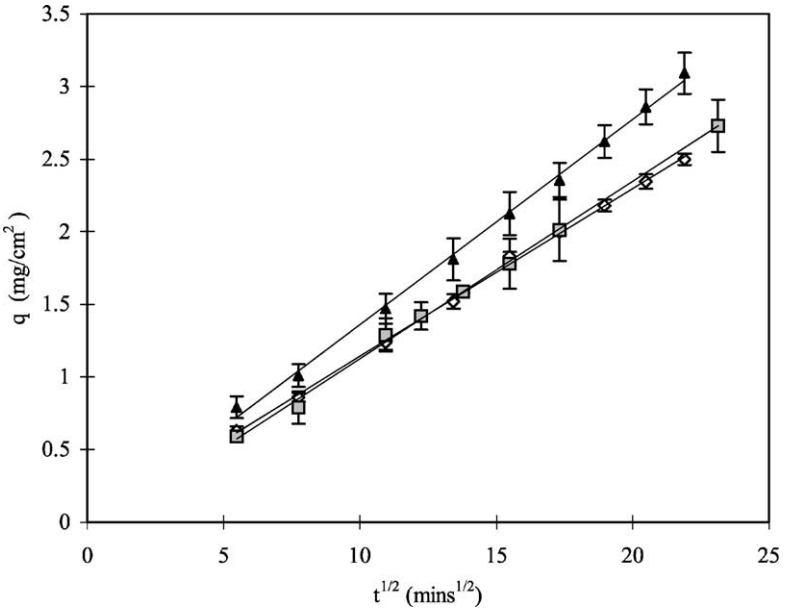

Fig. 2. The effect on the release rates of salbutamol base from the liquid crystalline vehicle of the addition of isotonic phosphate buffer (pH 7.4) to both/either the aqueous phase of the gel and the receptor medium (gel/receptor medium, ◇ = buffer/water; □ = water/buffer; ▲ = buffer/buffer). The data presented represent an average of five separate determinations: the error is the standard deviation of the mean. The drug loading in each case was 28 mg cm^{-3}.

base, is approximately one-third of that available in the absence of the fatty acid.

When the oleic acid was added to a buffered gel the rate of release of the drug into water was also less than that from the analogous completely water-based system but enhanced relative to that from the water-based system containing no fatty acid (Fig. 3(a) and (b)). The addition of the buffer ions to the vehicle could give rise to a number of interactions. There are ions present that can associate with the polar head groups of the hydrophobic acid and drug ions. The interaction of sodium ions with the ionised acid could result in the formation of sodium oleate. Conversely, anionic buffer species may associate with the ionised drug either in the interfacial regions of the bilayer structure or simply within the aqueous domain. The overall effect is likely to be competition and so a reduction in the association between the drug and fatty acid.

After eight hours, the extent of salbutamol release from both buffered and non-buffered vehicles containing oleic acid more than doubles when the phosphate buffer is used as the receptor medium (Fig. 3(b)). In fact, the drug release in the experiments in which a buffer receptor is used is greater with the oleic acid than without. In the case where there are no buffer ions initially in the vehicle it may be envisaged that as buffer ions diffuse from the receptor into the vehicle they initiate an 'exchange' type process with the fatty acid associated drug species present. With buffer in the vehicle at least some of the Na^+ ions are likely to be associated with the oleic acid so that a gradient in Na^+ concentration relative to that in the receptor will exist and diffusion from the receptor will again take place though to a lesser extent. The presence of the fatty acid in the liquid crystalline vehicle may result in an increased partitioning of the drug from the gel. As a result of buffer ions diffusing from the receptor medium it is likely that the ratio between the concentration of free and oleic acid associated drug species in the vehicle is greatly increased. The presence of oleic acid at the relatively high concentration of 0.1 M will further increase the oil/water ratio of the liquid crystalline structure. The non-complexed drug may preferably partition from the more hydrophobic vehicle into the membrane and ultimately into the receptor medium.

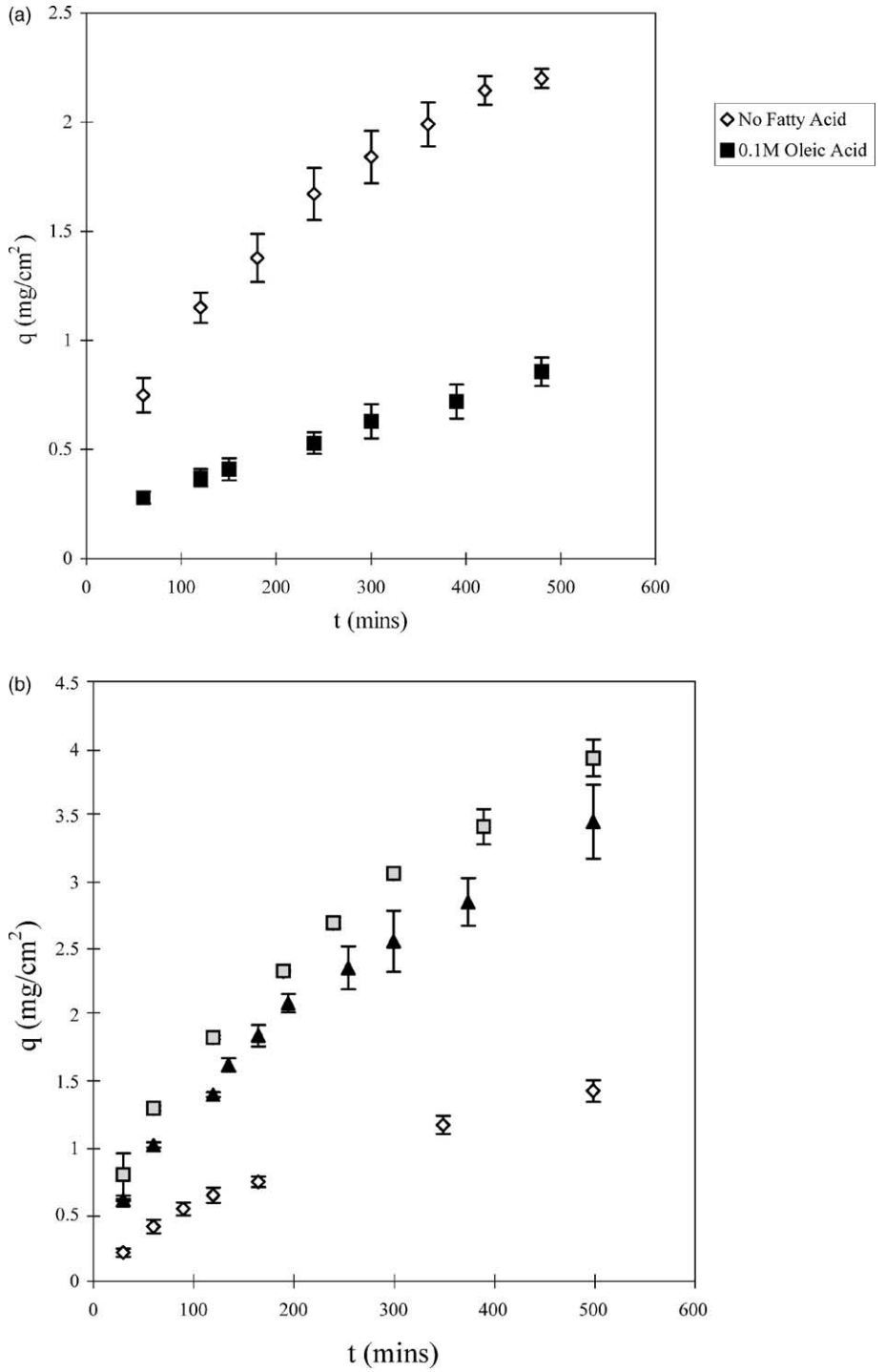

Fig. 3. (a) The effect on the passive transport of salbutamol from liquid crystalline vehicles containing 28 mg cm^{-3} of the drug of the incorporation of 0.1 M oleic acid into the hydrophobic domain of the gel. (b) Release rates from vehicles similar to (a) but into which phosphate buffer (pH 7.4) has been added to both/either the aqueous phase of the gel and the receptor medium (gel/receptor medium, ◇ = buffer/water; □ = water/buffer; ▲ = buffer/buffer). All the data shown represent the mean of five separate determinations: the error on the quantity of drug released is the standard deviation of the mean.

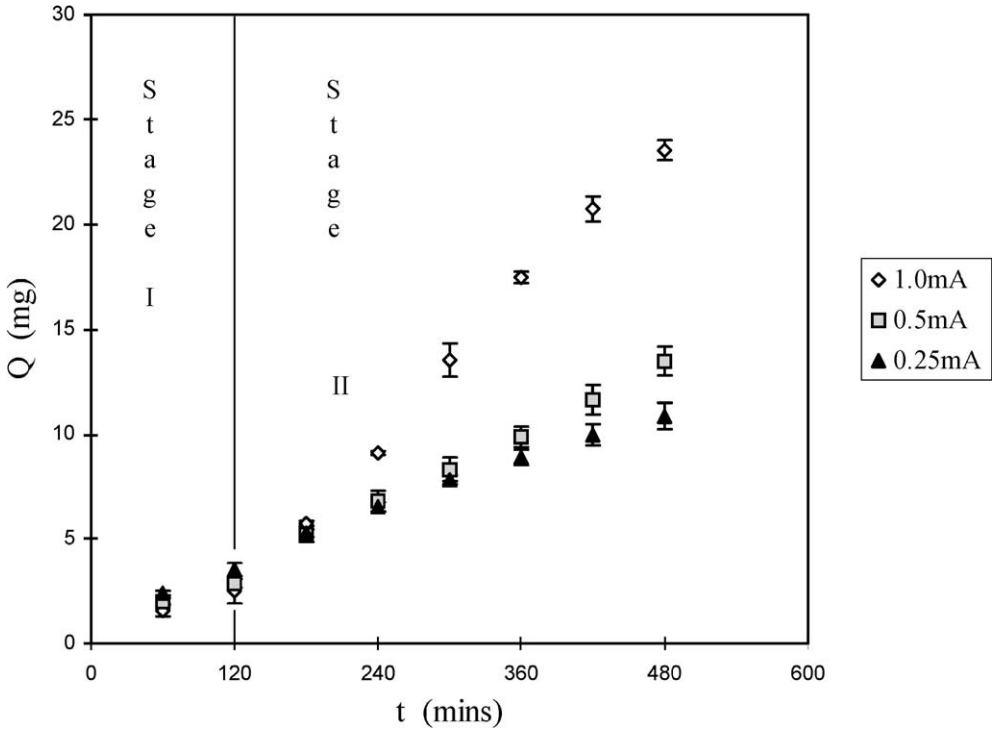

Fig. 4. The quantities of salbutamol released from the liquid crystalline vehicle during a two-hour passive period, (I), followed by six-hour iontophoresis (II) at the currents shown. The release was across a Visking membrane, the initial loading of the vehicle was 28 mg cm^{-3} and the data represent the mean of six separate determinations with the errors on *Q* being the standard deviations of the means.

The release of the drug was then studied as the stoichiometric ratio of drug to fatty acid was varied in the sequence 2:1, 1:1 and 1:2. The decrease in drug transfer that was observed is consistent with an increase in association between the two species. An analogous effect, due to an additive, was observed by Swarbrick and Siverly (1992). They reported that the diffusion of promicromil from an oil-based liquid crystalline vehicle was reduced when the concentration of lipophilic surfactant incorporated into the structure was increased.

3.4. Iontophoretically assisted release of salbutamol base

The effects on the delivery of salbutamol base from the liquid crystalline gel across Visking of iontophoretic assistance at 0.25, 0.5 and 1.0 mA using platinum electrodes are shown in Fig. 4. These currents correspond to current densities of 0.10, 0.20 and 0.40 mA cm^{-2}, respectively. The protocol used was two hours passive release (Stage I) followed by six hours galvanostatic iontophoretic assistance (Stage II). The vehicle solvent used in most of these iontophoretic experiments was phosphate buffer, as water-based vehicles were found to require significantly higher potentials to maintain the required currents. Water was used consistently as the receptor throughout the iontophoretic study to remove the possibility of participation by extraneous ions from the receptor solution in the conduction process. The passage of a constant current through similar gels loaded with different drug concentrations was found to substantially enhance the release of the drug over the corresponding passive release. The drug release profiles became approximately linear with time and the resulting release rates and transport numbers are listed in Table 2. The transport number is defined as the fraction of the total charge carried by that species: the quantity of salbutamol base transported

passively was subtracted in making this calculation.

As is implied before, the electrical resistance of the completely water-based systems was considerably larger than that of the systems that contained buffer solutions. However, despite the fact that the potentials required to maintain the current through vehicles in which water is used as the gel solvent were higher than can be expected to be tolerated therapeutically, iontophoretically assisted delivery was measured at current levels of 0.25, 0.4 and 0.5 mA with an initial drug loading of 28 mg cm^{-3}. The profiles were linear and, as is evident from Table 2, the process was more efficient that the corresponding transport from the gel using IPBS as solvent. This is in agreement with the suggestion by Degim et al. (1998) that the presence of small inorganic ions in the transdermal system can reduce the iontophoretic delivery of the target drug.

3.5. Effect of electrode type

A second iontophoretic system employing silver and Ag/AgCl electrodes, as had previously been shown to maximise delivery from a number of devices (Phipps et al., 1989), was also studied. The anode material in contact with the vehicle was silver foil and with the Ag/AgCl cathode in the receptor solution. Because silver is electrolysed at a lower potential than water this system, in principle, eliminates the iontophoretically generated 'anode ion'. The currents used and the protocols followed were the same as those for the first system studied (cf. Table 2) and the profiles showed that the delivery of salbutamol was reduced throughout Stage II at each current level. This effect was most significant at the highest 1.0 mA current where the iontophoretic rate of delivery is seen to decrease by a factor of almost two (cf. Table 2). Although this may appear surprising in the context of the previously quoted literature reports, it can be rationalised on the basis that the partitioning of the salbutamol base from the Myverol component into an aqueous solution should increase as the pH of the aqueous solution is decreased. Iontophoresis in the presence of platinum electrodes may synergistically involve two effects: enhanced delivery of salbutamol from the aqueous domain owing to electromigration of the drug and, as the pH of the region is reduced, increased partitioning of the drug from the oil regions. The pH of the aqueous domain at any instant during Stage II is at least two units below the pK_a of the drug. As a result the drug partitioning from the oil component would tend to ionise and participate in the conduction process. In contrast, iontophoresis involving the silver anode is effectively limited to the concentration of the salbutamol that partitions into the aqueous domain at pH 7.4.

The effectiveness of iontophoresis in transporting the drug molecule can be expressed in terms of the transport number of the drug species. For a system such as is being used here, where the vehicle contains buffer ions or the anode generated ion can enter the system during iontophoresis, the transport number must be determined from the measured rates of electrically assisted drug delivery at each known current. The data in Table 2 show that the most efficient transport from the systems containing both IPBS and water-based gels occurs at a current of 0.25 mA. This may be due to the minimal liberation of H^+ ions from the anode at the lower potentials required to maintain the smaller 0.25 mA current. Detailed analyses also showed a lesser participation by Na^+ ions at this current (Degim et al., 1998). Also the transport numbers measured from the water-based gels are all higher, as would be expected, than the corresponding values from gels containing buffer ions. The transport numbers for the 14 mg cm^{-3} drug loading indicate that the efficiency of the iontophoretic process is impaired by the reduced quantity of drug that is present. The transport numbers in Table 2 are considerably higher than those previously reported for the transport of salbutamol sulphate from 4% agar (0.4) and from Myverol/water formulations (0.47) (Bannon, 1989; Carr et al., 1997). This may reflect the fact that iontophoresis with platinum electrodes ultimately enhances the partitioning of the drug from the monoglyceride component of the vehicle into the aqueous domain.

3.6. Effect on iontophoretic delivery of the addition of fatty acids to vehicles

Vehicles containing 0.1 M fatty acid were prepared as previously described. Drug was loaded at both 14 and 28 mg cm^{-3} to give stoichiometric ratios of fatty acid to drug of 2:1 and 1:1, respectively. The gel solvent was isotonic buffer and to ascertain if the effects

Table 2
The rates of iontophoretic delivery and the transport numbers for the delivery of salbutamol base from the liquid crystalline vehicles at each of the currents used

Drug loading ($mg\,cm^{-3}$)	Current (mA)	Electrode material	Gel solvent	Standard vehicle 10^2 release rate at Stage II ($mg\,min^{-1}$)	Vehicle plus 0.1 M oleic acid 10^2 release rate at Stage II ($mg\,min^{-1}$)	Vehicle plus 0.1 M lauric acid	Standard vehicle transport number	Vehicle plus 0.1 M oleic acid transport number	Vehicle plus 0.1 M lauric acid transport number
28	1.0	Pt	IPBS	6.09	8.69	10.45	0.44	0.63 ($E_R = 1.40$)	0.76 ($E_R = 1.70$)
28	0.50	Pt	IPBS	2.73	6.14	5.85	0.44	0.92 ($E_R = 2.20$)	0.88 ($E_R = 2.20$)
28	0.25	Pt	IPBS	1.87	2.48	3.01	0.56	0.74 ($E_R = 1.30$)	0.90 ($E_R = 1.60$)
14	1.0	Pt	IPBS	5.22	7.91	8.10	0.38	0.57 ($E_R = 1.50$)	0.59 ($E_R = 1.60$)
14	0.5	Pt	IPBS	2.05	3.92	4.07	0.31	0.59 ($E_R = 1.90$)	0.61 ($E_R = 2.00$)
14	0.25	Pt	IPBS	1.12	–	–	0.34	–	–
28	0.5	Pt	H_2O	4.97	7.38	–	0.75	1.11 ($E_R = 1.50$)	–
28	0.4	Pt	H_2O	3.45	6.00	–	0.62	1.10 ($E_R = 1.80$)	–
28	0.25	Pt	H_2O	3.00	4.07	–	0.92	1.20 ($E_R = 1.30$)	–
28	1.0	Ag, Ag/AgCl	IPBS	2.93	7.81	–	0.20	0.57 ($E_R = 2.90$)	–
28	0.5	Ag, Ag/AgCl	IPBS	1.96	4.28	–	0.29	0.64 ($E_R = 2.20$)	–
28	0.25	Ag, Ag/AgCl	IPBS	1.41	1.75	–	0.42	0.53 ($E_R = 1.30$)	–

The enhancement ratio (E_R shown in brackets) refers to the ratio between the transport number obtained for the transport of the drug from the standard vehicle which contains no fatty acid and that obtained from the vehicle containing the fatty acid. The values shown are the means of at least five separate determinations: the standard deviation of the mean in each case was less than 5%.

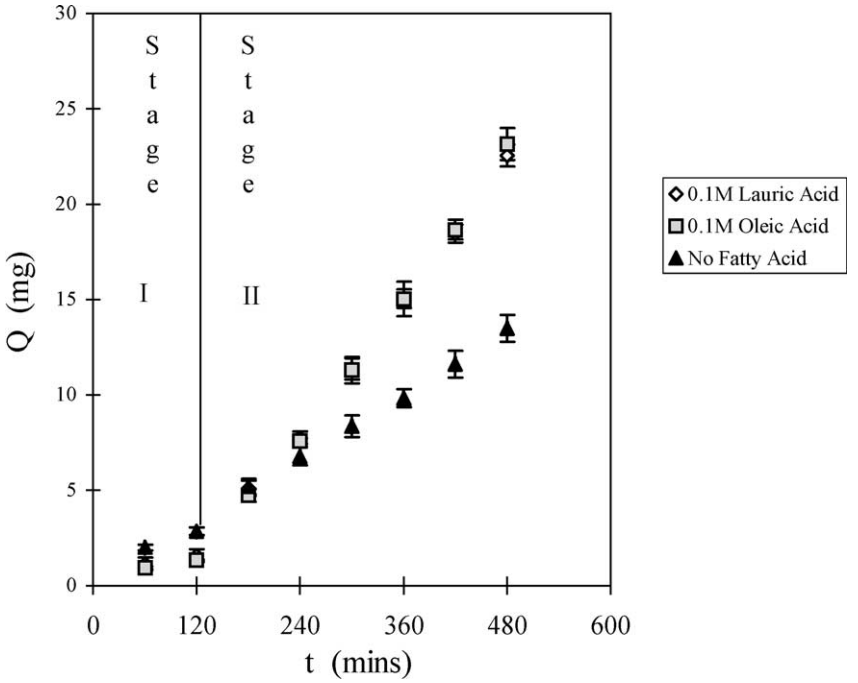

Fig. 5. The quantities of salbutamol base released prior to and during iontophoresis at 0.5 mA from liquid crystalline vehicles containing 0.1 M fatty acids (as indicated). The initial drug loading was 28 mg cm^{-3}. The data presented represent the mean of six separate determinations: the errors on the quantities released are the standard deviations.

observed were unique to oleic acid, the study was extended to assess the effects of the addition of lauric acid. The protocol was unchanged from that employed for the previously described iontophoretic experiments.

Release profiles at 0.5 mA from vehicles containing 28 mg cm^{-3} of the drug and 0.1 M fatty acids as indicated are shown in Fig. 5 and are typical of all the data measured. The presence of either fatty acid in the vehicle had been shown to result in a depressed passive rate of release of the drug. In contrast, the iontophoretic release is considerably augmented at the higher current levels in Stage II. The profiles for this electrically assisted release then become approximately linear with time.

The rates of drug delivery from the liquid crystalline vehicles containing oleic and lauric acids are given in Table 2. The optimum enhancement is achieved from the vehicles containing 0.1 M fatty acid and with a current of 0.5 mA. Here the delivery of the drug is approximately doubled. The data suggest that the enhancing effects of lauric acid may exceed those of oleic acid. The relative enhancements in the rates of release during the iontophoretic periods are seen to be comparable for the two drug loadings investigated. It is also evident from Table 2 that the iontophoretic delivery of salbutamol from the vehicle incorporating oleic acid is enhanced to an even greater extent when the solvent domain of the gel does not contain buffer ions (Degim et al., 1998). The transport numbers in Table 2 reveal that the efficiency of iontophoretic drug release from the Myverol/water liquid crystalline vehicle was increased at each current level in the presence of a fatty acid. For example, the transport numbers at 0.50 and 0.25 mA are increased by approximately 80% when oleic acid is incorporated into this vehicle. The fact that the transport numbers are greater than unity is probably because each figure is augmented by increased electroosmotic transport in the presence of the fatty acids. These complex fatty acid-containing liquid crystalline systems, with hydrophobic and hydrophilic domains, behave in a permselective manner. The immobilised fatty acid groups are likely to contribute a negative charge within

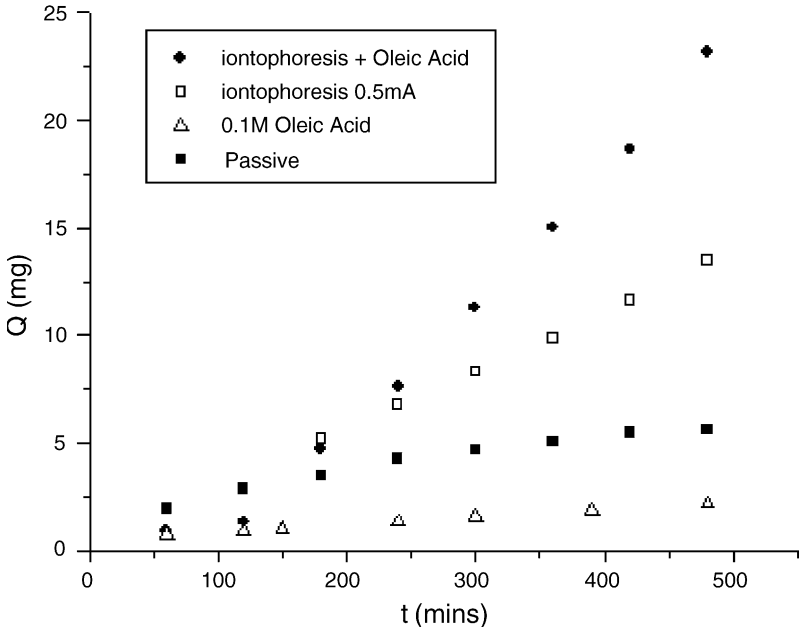

Fig. 6. Comparison of the rate of passive diffusion of salbutamol base with those observed using iontophoresis, the addition of oleic acid and a combination of these two enhancements. All experiments were conducted using a drug loading of 28 mg ml^{-1}, (0.1 M), concentration.

the matrix so that the effect is similar to that occurring in skin.

Measurements in these systems were also made using silver (anode) and Ag/AgCl (cathode) electrodes, with isotonic phosphate buffer as the gel solvent. The data in Table 2 show that the rates of iontophoretic delivery were dramatically increased at both 1.00 and 0.50 mA currents when (0.1 M) oleic acid was incorporated into the vehicle. When either oleic or lauric acids were added to the device the iontophoretic delivery of salbutamol was substantially enhanced. The effect was found in all the systems investigated. A transport number of 0.88 was measured for the release of salbutamol at 0.5 mA from a device containing 0.1 M lauric acid using platinum electrodes. This is a two-fold increase in iontophoretic efficiency as a result of the addition of the fatty acid. Furthermore, the association between the fatty acid and cationic species in the gel is not maintained when the electric field is applied to drive the iontophoresis. This explanation is supported by the fact that the efficiency of iontophoretic transport is maximised for a delivery device in which the drug is predominantly present as an associated species. After six hours of iontophoresis at 1.0 mA the data show that some 50% of the salbutamol present in the vehicle had been released. For a vehicle in which the initial drug loading was 14 mg cm^{-3}, almost all of the salbutamol was released and the corresponding curve was seen to level off: the gel contained 0.1 M fatty acid and had isotonic buffer solution as solvent.

Fig. 6 summarises the interplay of the effects of iontophoretic assistance and the addition of fatty acid to the release of salbutamol base across the synthetic membrane from the liquid crystalline gel. The fatty acid alone decreases transport whereas the process is enhanced under iontophoretic conditions. However, it is clear that when both the chemical and physical treatments are combined a greater than expected enhancement is observed. The slightly greater than linear relationship of the iontophoretic drug delivery with time, evident in Figs. 5 and 6, may be due to an increased mobility of the drug owing to the increased fluidity of the entire vehicle in the presence of oleic acid. Even greater enhancement ratios might be expected from the simultaneous use of the electrical current and fatty acids across a skin barrier since the latter are known to reduce the barrier function of the skin.

The work reported here shows that the efficiency of iontophoretic drug delivery is governed primarily by the concentration of extraneous ions in the system and

the mobility of the drug ion itself through the vehicle and across the membrane. The extent to which such drug delivery can be enhanced is likely to depend on the interplay of these effects.

The buffer and, for systems utilising platinum electrodes, the H^+ ions generated during iontophoresis are the two sources of extraneous ions. The inclusion of the fatty acid will have a number of implications for the concentrations of extraneous ions present in the vehicle. The data pertaining to the passive release from the analogous systems indicate that a substantial fraction of the oleic acid may be associated with Na^+ buffer ions. This interaction may prevent the participation of the ion in the conduction process. Consequently, the portion of the current carried by the drug would increase to compensate for the effect. The results also clearly indicate increased iontophoretic efficiency when water was used as the gel solvent and the aqueous domain did not contain buffer ions.

Increased iontophoretic drug delivery on the basis of trapping the ion generated at the anode by negative drug counter ions in the device has previously been rationalised (Phipps and Untereker, 1988; Phipps et al., 1989). The transport numbers obtained for the iontophoretic release of the drug, from a delivery device containing 30% water using platinum electrodes are consistently greater than unity. Although these values may be augmented by electroosmotic contributions to the drug flux during iontophoresis, they may also reflect the affinity of the fatty acid for the proton generated at the anode. However, as the iontophoretic delivery of the drug was enhanced when silver and Ag/AgCl electrodes were used, trapping of the H^+ ion is not likely to be the only effect involved.

Thus, the enhanced iontophoretic drug delivery observed cannot be simply accounted for by removal of the extraneous ions from the conduction process by the fatty acid, and the subsequent increased mobility of the drug ion. It would appear that the mobility of the drug is affected in a more complex manner. The acid may be considered to be immobile and to be confined to the lipophilic domain of the vehicle. If during iontophoresis the affinity of the fatty acid for the Na^+ and H^+ exceeds that of the drug, the iontophoretic mobility of salbutamol should increase to compensate for the effect. A similar phenomenon has been observed during the electro-migration of Na^+ and H^+ in a cation exchange column using Pt electrodes (Spiegler and Croyell, 1952). The resin is considered to remain stationary during the process and the conduction to be maintained entirely by the cations. The H^+ ions introduced at the anode efficiently displace the Na^+ ions initially present on the beads. The resin, owing to its affinity for the proton, was thus considered to increase the mobility of the Na^+ ions. In the present system during iontophoresis, all the cations in the liquid crystalline delivery vehicle will migrate through the aqueous region away from the anode. It may be envisaged that the smaller ion species (Na^+ and H^+) quickly displace the drug cation from the fatty acid. This process may not deplete these ions from the system but rather delay their participation in the conduction process as they in turn are displaced from the acid by the continued migration of positive ions from the anode. Subsequently, the electro-migration of the displaced drug should proportionally increase to compensate for the slower electro-migration of the competitive extraneous ions. Alternately, the fatty acid may expedite the increased mobility of the drug through structural alteration of the delivery vehicle. Fatty acids such as oleic, stearic and arachidonic acids have been shown to enhance the conductivity of Na^+ and K^+ channels present in skeletal muscle cells (Wallert et al., 1991; Wieland et al., 1992). Furthermore, it has been proposed that this takes place by means of a physiological mechanism whereby the fatty acid alters the lipid environment of the membrane surrounding the channel (Ordway et al., 1989). Oleic and lauric acids may also disrupt the packing and as a consequence increase the fluidity of the lipid bilayers in human *stratum corneum* thus acting as penetration enhancers for this membrane (Barry, 1991). By analogy with these mechanisms, it is possible that the fatty acid could disorder the hydrophobic region of the liquid crystalline vehicle. During iontophoresis the mobility of the drug may be enhanced if the process takes place through a more expansive hydrophobic region.

4. Conclusions

The passive release of salbutamol base from the liquid crystalline vehicle was found to adhere to matrix diffusion control. The lower transport observed compared to the analogous transport from a 4% agar gel, may be attributed to the partitioning and solubility

characteristics of the drug. The effect of the addition of oleic acid to the device indicates association with the fatty acid so that its release into an aqueous medium is significantly reduced. When buffer ions are present in the system this association is reduced and when the phosphate buffer was used as a receptor medium the release rate exceeded that of the standard vehicle containing no fatty acid and approached that for the release of salbutamol from 4% agar.

The delivery of salbutamol from these systems was substantially enhanced by iontophoresis. Because of the participation of extraneous ions in the conduction process, the transport numbers for salbutamol base were generally observed to decrease as the current was increased. The rates of iontophoretic delivery were approximately proportional to the currents achieved. The data clearly indicate the iontophoretic process to be significantly less efficient in the presence of buffer ions: at the 0.5 mA level the transport number is reduced to 0.41 compared to 0.75 for the device containing water as the solvent in the gel. The corresponding transport number when Ag/AgCl electrodes were used fell to 0.29 so that for this particular system the platinum electrode was found to be the most suitable. The pH changes that occur in the device when the platinum electrode is used may alter the partitioning of the basic drug in the aqueous regions of the vehicle. Iontophoretic delivery rates were enhanced by the presence of a fatty acid.

The results reported here characterise the passive and iontophoretic delivery of salbutamol across a non rate-limiting artificial membrane from a monoglyceride liquid crystalline vehicle. The study has also determined separately the effects on these release and transport processes of the inclusion of fatty acids capable of penetration enhancements into the systems and of the application of iontophoretic assistance. Finally, it has investigated the synergistic effects of simultaneous chemical and electrical enhancement. A future paper will report on the use of these systems to deliver salbutamol and other drug molecules across human and murine skin.

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