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International Journal of Pharmaceutics 325 (2006) 90–98
INTERNATIONAL JOURNAL OF PHARMACEUTICS
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Release characteristics of anionic drug compounds from liquid crystalline gels III. Chemical and iontophoretic enhancement of delivery across non-rate-limiting membranes

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Received 28 February 2006; received in revised form 9 June 2006; accepted 17 June 2006
Available online 8 July 2006

Abstract

This paper investigates the release and transport of a range of anionic drugs from liquid crystalline gels using chemical and physical enhancement techniques. Previous papers [Fitzpatrick, D., Corish, J., 2005. Release characteristics of anionic drug compounds from liquid crystalline gels. I. Passive release across non-rate limiting membranes. Int. J. Pharm. 301, 226–236; Fitzpatrick, D., Corish, J., 2006. Release characteristics of anionic drug compounds from liquid crystalline gels. II. The effects of ion pairing and buffering on the passive delivery of anionic drugs across non rate-limiting membranes. Int. J. Pharm.] have reported on the passive release profiles and those resulting from the incorporation of a chemical enhancer in the vehicle. This paper investigates the behaviour of the system under iontophoretic conditions and also under those of combined physical and chemical enhancement. The data presented here are directly comparable to previous work by Nolan et al. [Nolan, L.M.A., Corish, J., Corrigan, O.I., Fitzpatrick, D., 2003. Iontophoretic and chemical enhancement of drug delivery. Part I. Across artificial membranes. Int. J. Pharm. 12, 41–55; Nolan, L.M.A., Corish, J., Corrigan, O.I., Fitzpatrick, D., 2006. Combined effects of iontophoretic and chemical enhancement on drug delivery. II. Transport across human and hairless murine skin. Int. J. Pharm., submitted for publication] which investigated the behaviour of cationic compounds under analogous conditions.

The iontophoretic release of diclofenac in the presence of model enhancers is thoroughly investigated. It is also shown that a range of anionic drug molecules undergo an electrochemical change during the course of the experiments which leads to their poor detection. This may be a factor in the under reporting of iontophoretic delivery of anionic drugs in the literature. However, it has been shown that the transport of the drugs is greatly enhanced by the application of an iontophoretic current. Results of combined enhancement studies provide a positive basis on which to proceed with *in vitro* studies of the system across human skin.

Keywords: Diclofenac; Liquid crystalline gel; Transdermal; Buffering; Iontophoresis; Combined enhancement methods

1. Introduction

The search to understand and elucidate the interactions of ionic molecules with bio-membranes continues to be an important research objective across many fields from the delivery of anionic non-steroidal anti-inflammatory drugs to cationic antimicrobial peptides (Powers et al., 2005).

In order to fully understand and characterise the release of a compound from a vehicle, it is necessary to first determine the transport of the drug from the vehicle under non-rate limiting conditions. This is especially the case when the optimisation of drug transport is sought under potentially enhancing iontophoretic conditions. The data presented here present a clear picture of the behaviour of the liquid crystalline vehicle under iontophoretic conditions and also determine the optimum system which is required to successfully investigate the transport of anionic drugs across human skin.

The use of artificial membranes in characterising vehicles for transdermal delivery is common whether to achieve non-rate

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doi:10.1016/j.ijpharm.2006.06.048

limiting conditions or to investigate enhancer effects. Maitani et al. (1996) used ethanol treated and untreated silicone membranes to investigate the transport of the acid and sodium salt of diclofenac to mimic lipid and pore pathways in the skin. Iordanskii et al. (2000), have used skin imitating hydrophobic carbosil membranes for modelling the transport of propranolol.

In the last decade it has been more clearly shown that the process often referred to as iontophoresis is more realistically the sum of several mechanistically different components, namely, electrorepulsion, electroosmosis and electroporation. It has also become evident that these components can work with and against each other depending on the nature of the drug to be transported. For example, electrorepulsion works to assist the transport of ionised drugs whereas electroosmosis favours large uncharged molecules (Pikal, 1992; Guy et al., 2000). Electroosmosis can be defined as bulk fluid flow which occurs when a voltage difference is imposed across a charged membrane. Electroosmosis always occurs in the same direction as the flow of counter ions. This term is also referred to as convective solvent flow.

Many models have been adapted to try and predict the rate of drug delivery using the applied potential or the delivery current as independent variables. Unfortunately, like many other predictive models, these models do not have closed form equations unless gross simplifications are made. Probably the most accurate model to describe iontophoresis is the Nernst–Planck flux equation (Pikal, 1992). However, most treatments of the problem of drug flux across skin using the Nernst–Planck approach ignore the electroosmotic contribution.

Data presented here also illustrate the complexity of the behaviour of the vehicle under combined physical and chemical enhancements. The ultimate aim of this and similar investigations is to make iontophoretic devices more accessible and acceptable to the marketplace. A combination of chemical and physical enhancements may lead to 'milder' and more patient friendly administration of drug compounds in a tailored manner.

2. Experimental

For the most part the experimental procedure is similar to that outlined in previous papers (Fitzpatrick and Corish, 2005, 2006) and only a short summary and a description of the iontophoretic experiments are presented here. Experiments consisted of studies in which the drug compound of interest was transported from a solid gel vehicle through a synthetic membrane into a Franz like cell (Franz, 1978). The synthetic membrane used was Visking™ which acted as a non-rate limiting barrier and so was utilized to ascertain the release characteristics of various drugs from the gel. The liquid crystalline gel used was formed using Myverol®, a commercially available emulsifier to form an oil/water mixture. The cell is suspended at neck height in a thermostatic water bath at 310 K. Samples are taken at regular intervals, through the stoppered port, and analysed using HPLC to determine the concentration of drug. For chemical enhancement trials the enhancer is also incorporated into the gel, along with the drug.

In the iontophoretic experiments an electrode was placed in the receptor solution directly below the membrane and another was placed on the upper surface of the gel. The constant direct current was supplied by battery or mains operated adjustable power supplies that were custom built for the work. For investigations of synergistic methods of enhancement, a combination of the above procedures was adopted.

2.1. Materials

The following analar grade chemicals were supplied by Sigma–Aldrich. Sodium diclofenac (2-(2,6-dichloroanilino)-phenyl acetate sodium) has a molecular weight of 318.1 and a melting point of 559 K. (Note the melting point of the acid-form is approximately 429–431 K and has a molecular weight of 296.2.) Indomethacin, [1-(4-chlorobenzoyl)-5-methoxy-2-methylindol-3-y] sodium is a pale yellow odourless solid. The compound is decomposed by light. Indomethacin exhibits polymorphism; one form melts at about 428 K and the other at about 435 K. It may exist as a mixture of both forms, which melts between these two temperatures. Salicylic acid (2-hydroxybenzoic acid) and its derivatives is one of the earliest known and most widely used NSAID. The sodium salt is a white odourless compound. Finally, sodium benzoate was also used as an anionic model compound. Although sodium benzoate is not a therapeutic compound, it was used because of its similarities to the other model compounds and its ready availability. It is commonly used as a food preservative.

Analar grade chemical enhancers and candidate chemical enhancers were supplied by Sigma–Aldrich Chemicals. These were benzyldimethyldodecyl ammonium bromide (BDDAB) and oleic acid. Potassium bromide and potassium chloride (both 99% purity) were also supplied by Sigma–Aldrich Chemicals. The following chemicals were supplied by Riedal-de-Haan: sodium dihydrogenphosphate and di-sodium hydrogenphosphate (both 98% purity), as well as the following analytical grade solvents and acids: acetone, acetonitrile, methanol, sulphuric acid, nitric acid and phosphoric acid.

Myverol 18–92 was donated by Eastman Chemicals (UK) Limited. Myverol is derived from rapeseed oil and consists mainly of monoglycerides. The material contained less than 5% diglycerides. Myverol was used as the main constituent in the preparation of the liquid crystalline delivery vehicles. Myverol is an off-white, odourless semi-solid and is a dispersing or foaming agent. Its melting point is 313 K and forms a liquid crystalline gel above 303 K on addition of water.

A non-crystalline hydrogel was used for the purpose of comparison. This was purified agar which was supplied by Oxoid (code L28) as yellowish granules. It is produced by using hot water extraction of selected seaweed to yield a polysaccharide mixture of agarose and agaropectin.

2.2. Preparatory methods

A complete description of the preparation of the liquid crystalline gels, buffer solutions, mobile phase and Visking membranes has been detailed in Fitzpatrick and Corish (2005).

2.2.1. Preparation of diffusion cells

Single compartment Franz-like diffusion cells were the standard apparatus for all experiments. The Visking membrane was securely held on the top of the Franz diffusion cell using Parafilm™ wrapped around the neck of the cell. IPBS or triply distilled water was placed in the receptor port of the cell. The 1.8 ml of the viscous gel-containing drug was syringed onto the membrane and covered with Parafilm™ to prevent water loss. The receptor port of the cell was then topped up to exclude air bubbles. The cell was placed in a thermostated water bath at 310 K with which it quickly equilibrated. The first sample was taken after half an hour and subsequently on the hour up to 8 h and also after 23 and 24 h had elapsed.

The procedure for the iontophoretically-assisted experiments was the same except that an electrode (cathode) was placed on top of the gel before the application of Parafilm™. The anode was permanently fixed in the receptor compartment of the cell just below the membrane. Platinum electrodes (1 cm^2, 99% purity) were used in all experiments.

3. Results and discussion

3.1. Iontophoretically assisted transport of anionic drug molecules across Visking membrane from a LCG

The use of iontophoresis as a physical enhancement technique is well described in the literature (Smith and Maibach, 1995). The suitability of sodium diclofenac for enhanced drug delivery by this method is due to the negative charge of the carboxylate group of the molecule in the ionised state. Such charged moieties will be expected to carry a proportion of the charge when a current is established and to move towards the electrode of opposite charge, in this case the anode. The ionised drug, having a negative charge, will be repelled away from the negatively charged electrode by electrorepulsion, which is the principal driving force of electrically assisted transport. Previous work in this laboratory (Carr et al., 1997) has shown that Visking membrane does not significantly affect the diffusion of a range of drugs from the vehicle. Consequently, it was assumed that neither would the membrane influence the iontophoretically-assisted release of the drug from the delivery device. The parameters to be investigated include: the effect of buffering; the current density employed and the nature of the drug molecule with specific emphasis on sodium diclofenac.

The initial investigations of the release of sodium diclofenac used water as the gel solvent and also in the receptor medium. Triply distilled water was used to avoid the possibility that extraneous ions could participate in the conduction process across the system. This water/water protocol has also been found to give the most significant release in passive studies with sodium diclofenac (Fitzpatrick and Corish, 2005). Nevertheless, the four possible permutations, using water and buffer alternately in the gels and in the receptor, were investigated to obtain a full picture of the behaviour of the system.

The systems were allowed to operate passively in stage I for 2 h before current was switched on between the electrodes (stage II). Potential and current were both monitored during the course of experiments using a digital multimeter and all experiments were carried out in triplicate. The standard current used throughout the investigation was 0.5 mA (0.2 mA/cm^2 for the membrane), which is within generally accepted levels of tolerance for iontophoretically assisted transdermal transport (Smith and Maibach, 1995).

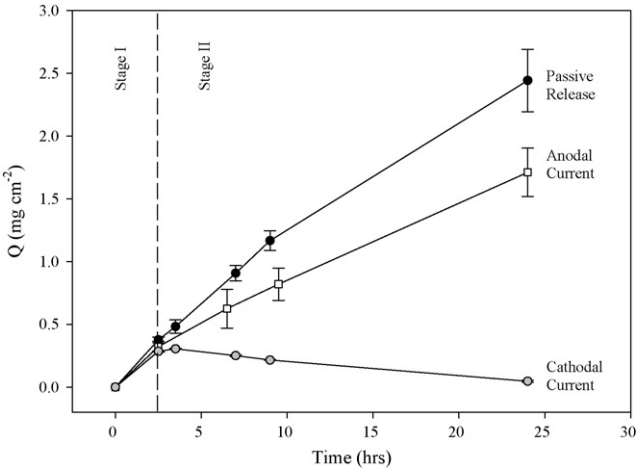

Fig. 1. Comparison of the rates of the release of sodium diclofenac from the LCG across Visking membrane passively and with cathodal and anodal iontophoretic assistance. All concentrations of the drug in the gel were 0.1 M.

The iontophoretic release of sodium diclofenac from liquid crystalline vehicles in comparison to its analogous passive release is illustrated in Fig. 1.

Surprisingly, the release of sodium diclofenac was found not to be enhanced by the current. In fact, the complete opposite effect to that expected was observed. The initial release of the drug, during the passive stage I, was as expected and corresponded to the release rates measured in earlier experiments (Fitzpatrick and Corish, 2005). When the potential was applied in stage II, further release of the drug was inhibited and, even more surprisingly, the concentration of the drug which has diffused into the receptor cell gradually became lower so that it was, apparently, being removed from the receptor medium. Due to this unexpected release profile observed using cathodal iontophoresis the polarity of the electrodes was reversed and the profile for anodal iontophoresis measured. These experiments were carried out with all other conditions the same as those used for cathodal iontophoresis and the results are also shown in Fig. 1.

From the profile of the anodic iontophoretic data in Fig. 1, there is evidence that the application of a constant current noticeably decreases the release of drug compared to its passive release, although this decrease is not as substantial as that for cathodal iontophoresis. A decrease in release was anticipated because the drug would be expected to be attracted towards the anode, which in the experimental configuration used was on top of the vehicle.

The total drug delivery rate from an iontophoretic system, R, can be divided into two components: R_p due to the chemical potential gradient and R_i due to the electric potential gradient. The driving forces resulting from the chemical and electrical potentials act simultaneously. Consequently, the following

expression can be written for the total delivery rate, R:

$$R = R_p + R_i \quad (1)$$

The iontophoretic transport rate, R_i, may be simply expressed as the product of the current, i, and the iontophoretic flux, F_i, defined as the amount of drug (on a weight basis) delivered per unit time, per unit current:

$$R_i = iF_i \quad (2)$$

Consequently, if R_p is constant, R may be expected to vary linearly with respect to the current, as illustrated by the combination of Eqs. (1) and (2):

$$R = R_p + iF_i \quad (3)$$

Linear relationships between the release rate of the drug and the iontophoretic current flowing have been observed and reported for iontophoretic drug delivery (Bannon et al., 1988). The suppressed release of sodium diclofenac under anodal iontophoresis is ~30% less than that of passive release after 24 h. It may be expected that under cathodal iontophoretic assistance that the assisted release of the drug would also be of the order of 30% or greater than that of its passive release. This increase would be due to molecules, under passive and iontophoretic delivery, moving in the same direction rather than opposing directions to each other, as is the case under anodal conditions.

Having confirmed that the intended polarity had been used in all of the experiments, the experimental iontophoretic conditions that gave rise to the very reduced release profiles of sodium diclofenac after 24 h of cathodal current were closely examined. It is known that the electrolysis of water occurs at an electrical potential of ≥2 V at a platinum electrode. With cell potentials significantly in excess of this value (up to 24 V), the production of hydronium and hydroxide ions in this system is almost certain. Although the pH of the system will not change due to the formation of equal amounts of positive and negative ions. It is possible that in the presence of a large concentration of hydronium ions that the acid-form of the drug may form. Another possibility under these iontophoretic conditions is the oxidation of the diclofenac anion.

To investigate these possibilities a voltammogram was taken using the drug itself as the supporting electrolyte and with platinum electrodes (99.99% purity). The voltammogram indicated that the oxidation of sodium diclofenac occurred at a potential of 0.7 V, which is in agreement with the recorded value (Merck index). The reduction of the drug takes place at a potential of −0.4 V. The over-potential is ~1.0 V indicating that the reaction is non-reversible under cathodal iontophoretic conditions.

The unexpected release profile of sodium diclofenac under iontophoretic conditions was then further investigated. First the iontophoretic process was simplified to its most basic components by placing both electrodes in a specially prepared 0.1 M solution of sodium diclofenac in triply distilled water. This solution was not stirred after the dissolution of the drug. As soon as the current was established it was noticed that a precipitate formed on the anode. The precipitate continued to form over a period and eventually caked over the whole electrode. Under the cathodal iontophoretic protocol used here, the anode was placed in the receptor medium. Because the product was insoluble it was assumed that the acid-form of diclofenac was being produced at the anode or perhaps a complex of diclofenac due to oxidation.

The product formed in the simple experiment was collected, dried and prepared for NMR analysis by dissolving it in deuterated DMSO. Mass spectrometry, IR and melting point determination were also used in its identification. A comparison of ^{1}H NMR (400 MHz, DMSO) spectra of sodium diclofenac and the collected product indicated that the product which was collected was diclofenac acid. Melting point tests provided confirmatory evidence that the product was the acid-form of the drug. The electrolysis product melted at a temperature of 431 K, which is the literature value of the melting point of the acid (Merck index). By comparison the melting point of the sodium salt is 559 K. Other confirmatory tests such as IR and mass spectrometry also indicated the presence of the acid-form of the drug.

This result raised the question as to what had been taking place during the iontophoretic experiments. In the simple electrolysis experiment the acid collected on the anode whereas in standard iontophoretic experiments there was no apparent formation of this product. Even though the quantity expected would be far below that produced from a 0.1 M solution the product might still be expected to have been visible. The simple experiment was carried out again but on this occasion the solution was continuously stirred. This stirring of the solution had a significant effect. Instead of the acid forming at the anode, the anode remained clear but the solution was slightly cloudy and developed a very fine particulate suspension. At the end of the experiment the solution was allowed to stand to allow the particulate matter to collect at the bottom of the beaker. This precipitate was then collected; analysed using the techniques previously described and was also identified as diclofenac acid.

In light of these results, the full-scale iontophoretic experiments were repeated and when they had been completed the receptor cells were inspected for precipitate. After standing overnight a small quantity of fine precipitate was indeed evident at the bottom of all three cells.

Reversing the polarity in the cell turned out to be a useful indicator that the intended polarity had certainly been used in the initial experiments. The simple electrolysis experiments described above prove beyond doubt that diclofenac diffuses from vehicles in substantial quantities under iontophoretic conditions but undergoes an electrochemical change to diclofenac acid during the experiment. As diclofenac acid is practically insoluble in aqueous media it forms very fine particulates, which eventually precipitate. This fact explains why the drug is not detected using standard sampling techniques and HPLC.

An interesting example of an unrecognised electrochemical iontophoretic change in diclofenac has been identified in the literature (Fang et al., 1999). In this paper, diclofenac is transported iontophoretically across rat skin *in vivo*. A microdialysis tube was placed under the skin with circulating receptor fluid. Platinum electrodes were used during the study. Fang et al. graphically illustrated how the transport of diclofenac peaks after the first hour of iontophoresis and decreases rapidly

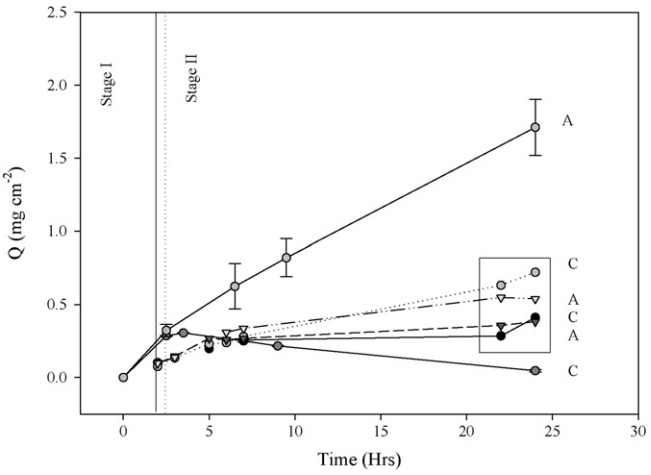

Fig. 2. The effect of buffering the receptor medium on both anodal (A) and cathodal (C) iontophoretic release at a current of 0.5 mA of sodium diclofenac from the LCG across Visking. The box in the graph encloses diffusion profiles where buffer is used as the receptor medium.

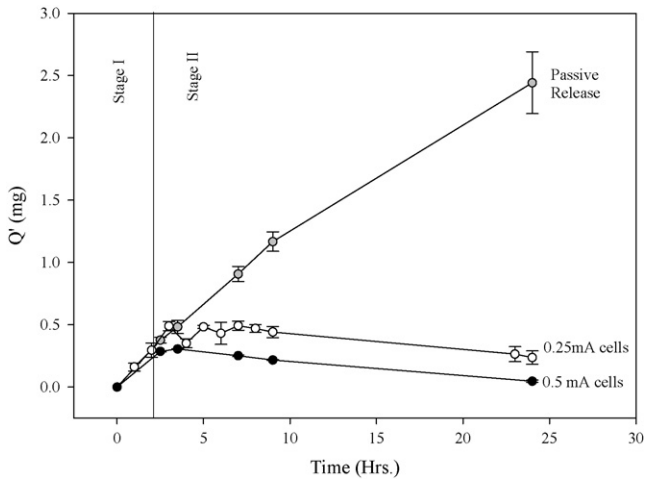

Fig. 3. The iontophoretic release of sodium diclofenac from LCG at current levels of 0.25 and 0.5 mA across Visking compared to its passive release. All concentrations of the drug in the gel were 0.1 M.

thereafter 5 h before the application of current was terminated. The authors attributed the early peak in iontophoretic transport of diclofenac to delivery through shunt routes which is amplified due to the higher number of hair follicles in furry rat skin than those present in human skin. However, the decrease in detection may also be attributed to detection problems as the sodium salt of the drug is converted to the acid-form.

3.2. The effect of buffering on the iontophoretic delivery of sodium diclofenac

Buffering the receptor medium was shown to have significantly reduced the passive release of sodium diclofenac from the LCG (Fitzpatrick and Corish, 2005). The effect of buffering under iontophoretic conditions is shown in Fig. 2.

Experiments were carried out using both cathodal and anodal currents. It is evident that the detection of the diclofenac anion delivered under cathodal conditions is better due to the presence of the buffer ions in the receptor solution. The dashed box in the graph encloses diffusion profiles where buffer is the receptor medium. All the data points in the box are seen to lie between those for iontophoretic delivery with both polarities but without any buffer in the receptor medium.

The buffer has a dual effect in these experiments. Firstly, the figure shows that for buffered cells with cathodal assistance the detection of diclofenac is significantly increased, it had been found to be negligible after 24 h in the absence of buffer. This increase is due to the buffer action, which reduces the amount of hydronium ions in solution so that less of the drug anions are removed as precipitate in the acid-form. Secondly, the graph shows that for buffered cells with anodal assistance the detection of diclofenac is significantly reduced from that in the analogous case without the buffer present. This effect had been noted already (Fitzpatrick and Corish, 2005) where the passive release of sodium diclofenac was seen to be smaller due to the reduced ion gradient within the vehicle between the two domains of the gel.

The fact that the data for cathodal and anodal assisted delivery in which the receptor solution is buffered, fall and overlap in the same portion of the graph is due to this complex interplay of effects.

3.3. The effect of current, applied potential and vehicle type on the iontophoretic release of sodium diclofenac

Another variable which is expected to have an effect on the iontophoretic release of sodium diclofenac is the magnitude of the current flowing. The iontophoretic release profiles of diclofenac from liquid crystalline gels with current of 0.25 and 0.5 mA are shown in Fig. 3 where they are compared to the analogous passive release.

The graph indicates a five-fold increase in release when the current is halved from 0.5 to 0.25 mA. Although the current was decreased by half, the applied potential required to maintain a constant current was still significantly above the potential at which electrolysis of water occurs, i.e., 2 V. A reduction of the formation of diclofenac acid would not be expected to occur until the electrical potential used to give iontophoretic assistance was reduced to less than 2.0 V. This could not be tested in practice because at such low potentials it was not possible to establish a current through the gel. This resistance of the LCG gel has been previously reported (Nolan et al., 2006).

Iontophoretic studies were also carried out using a 4% agar hydrogel as the delivery vehicle. It was expected because of the significant passive release of diclofenac from agar (>70%, Fitzpatrick and Corish, 2005) that the iontophoretic delivery of the drug might proceed more quickly than its conversion to the acid-form therefore improving detection levels. The iontophoretic (0.5 mA) release profile was found to be identical in character to the corresponding data shown in Fig. 3 for the release of sodium diclofenac from liquid crystalline gels. The only difference was that the scale of release from the agar was very significantly greater. In these experiments, a substantial amount of precipitate was recovered from the bottom of

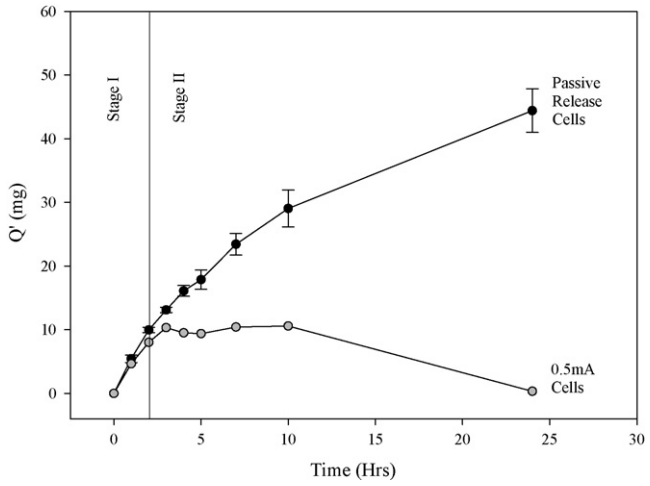

Fig. 4. The iontophoretic release of sodium diclofenac from 4% agar at a current intensity of 0.5 mA across Visking in comparison to passive release from the same vehicle. The initial concentration of the drug in the gel was 0.1 M.

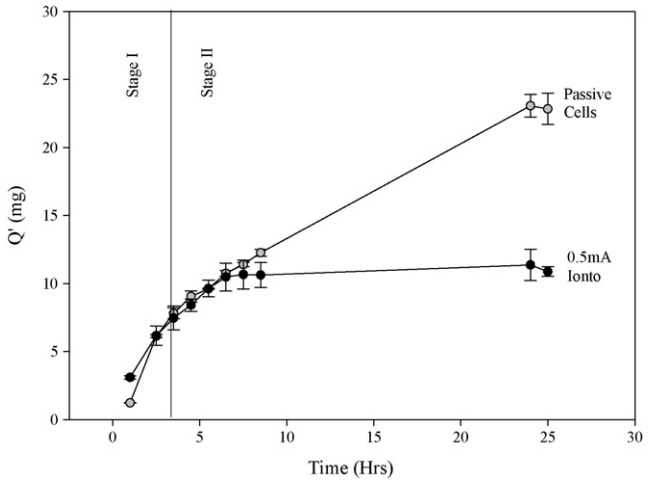

Fig. 5. The iontophoretic (0.5 mA) release of sodium indomethacin (0.1 M) from LCG across Visking in comparison to that of passive release. The initial concentration was 0.1 M.

the cells and again identified through analysis as diclofenac acid.

The precipitate was collected from iontophoretic cells by filtration 11.7 mg were collected from one cell, which represents to 32% of initial drug loading of 50.4 mg of sodium diclofenac. Although this figure is half of that reported for passive release (Fitzpatrick and Corish, 2005) it must be taken into account that the vehicle dehydrates rapidly under iontophoretic conditions which may cause the release of the drug to become inhibited. Although this retrieval of product was not quantitative it demonstrated a 25% increase in the amount of drug detected compared to standard sampling techniques involving HPLC detection. This result may indicate that the iontophoretic transport of anionic drugs from LCG will equally have a release of at least 32% after 24 h compared to the negligible level detected thus far. In the absence of total recovery radiolabelled studies it is difficult to directly quantify release from the LCG. As is shown in Fig. 4, these results also suggest that the rate of conversion of the drug to the acid-form will occur as rapidly as, or more rapidly than its diffusion from the vehicle.

During the period between 4 and 10 h after the experiment is started the level of diclofenac measured in the receptor compartment was observed to remain more or less constant. This would suggest that the rate of diffusion from the gel is equal to the rate of conversion of the drug during this time period. The total conversion to the acid-form occurs at some point between 10 and 24 h—overnight in the case of these experiments.

3.4. Comparison of iontophoretic release of similar anionic drug molecules

The results reported so far have been confined to sodium diclofenac. Experiments reported in this section were broadened to investigate the effect of iontophoresis on a range of anionic drug molecules. The molecules under investigation were those whose passive release rates from liquid crystalline gels, have been already measured (Fitzpatrick and Corish, 2005), these were sodium indomethacin, sodium salicylate and sodium benzoate. As all these model drugs are salts of carboxylic acids, it is anticipated that they will behave similarly to sodium diclofenac under analogous iontophoretic conditions.

The iontophoretic release of sodium indomethacin from the liquid crystalline gel is shown in Fig. 5.

The profiles during the initial 2-h passive periods (stage I) are seen to be similar for all the cells. The profiles run concurrently for a further 2-h period during stage II. After this point the iontophoretically assisted cells show a levelling off in the detection of the drug. This deviation from the expected enhanced profile was also attributed to an electrochemical change of the drug to its acid-form. Similar profiles were obtained for sodium salicylate and sodium benzoate. The assisted transport of sodium salicylate over the first six time points in stage II of Fig. 6 best demonstrates an equilibrium between the transport of the drug into the receptor medium and its conversion to the acid-form.

The rate of transport in seen to increase rapidly during the 2–4 h period in which the current is applied. The quantity of

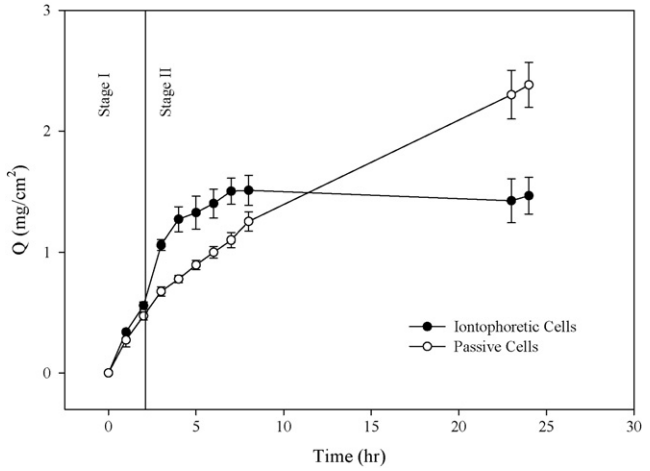

Fig. 6. The iontophoretic (0.5 mA) release profiles of sodium salicylate (0.1 M) from LCG across Visking in comparison to passive release. The surface area of the membrane = 2.54 cm^{-2}.

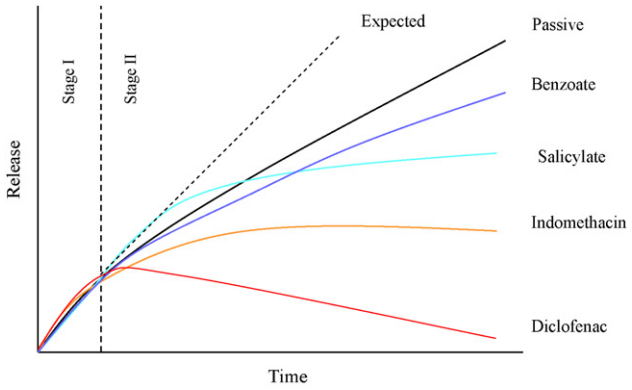

Fig. 7. An illustrated diagram of the comparative iontophoretic release of anionic drugs and sodium benzoate from LCG across Visking during a 24 h time period. Each iontophoretic profile is shown relative to a common generic passive curve. All vehicle concentrations were 0.1 M and current assistance were 0.1 M and 0.5 mA, respectively.

drug transported is over and above that which is transported passively in the same time period. After this time the rate slows down and eventually reaches a steady state over the course of the experiment.

Fig. 7 illustrates the relative iontophoretic release rates of the drugs measured in relation to their passive profiles. It shows that none of the iontophoretic release profiles of the sodium salts of the drugs are as large as their passive release profiles, i.e., there is no enhancement of release apparent. A decrease occurs shortly after the current is established in all cases with the exception of sodium salicylate which shows enhancement over a short period of time. The percentage reductions in the iontophoretic release for each drug compared to its passive release are benzoate (15%), salicylate (30%), indomethacin (65%) and diclofenac (99%). These data reveal an emerging trend for the drugs under investigation. It should be noted that this trend is inversely related to the solubility of the acid-form of each drug in aqueous solution, i.e., the greater the solubility of the acid the less the percentage reduction observed.

Further evidence towards understanding the electrochemical change of the drugs was provided by monitoring the pH and current profiles during the experiments. Over the course of the experiments the pH of the receptor medium changed from an initial value of 6.8 during stage I to a final pH of ~9.0 at the end of stage II. This indicated that hydronium ions produced by electrolysis were far fewer in solution at the end of the experiment. The formation of the acid-form of the drug at the anode would remove hydronium ions leaving an excess of hydroxide ions in solution from the electrolysis of water.

Secondly, it was noted that as the experiments progressed the current gradually decreased. An increasingly greater potential was required to maintain the current at the stated values, from 7.0 V initially to >20.0 V by the end of the experiment. This is again consistent with a reduction in the numbers of drug and hydronium ions available to carry the current as the experiments proceed.

The effects of buffering in experiments reported to date are worth noting. Buffering the system is now known to have three effects depending on the type of experiment. Previous work (Fitzpatrick and Corish, 2005) recognised that buffering reduced the ion gradient between the oil and aqueous domains within the vehicle thus reducing the release of sodium diclofenac. During chemical enhancer studies (Fitzpatrick and Corish, 2006) the buffer was found to reduce the degree of ion-paring between the drug and model enhancer molecules in the vehicle, thus increasing the amount of drug released and finally in iontophoretic studies buffer has been shown to reduce the amount of acid formation in the receptor therefore increasing the amount of drug detected by the standard methods of analysis.

4. The simultaneous physical and chemical enhancement of the transport of sodium diclofenac from a LCG across Visking membranes

Nolan et al. (2006) found that the combined use of chemical (oleic acid) and iontophoretic enhancements significantly increased the delivery of salbutamol base across the skin. Because it has now been established by the work reported here that anionic drugs are released at an enhanced rate from the vehicle by iontophoresis (albeit subsequently electrochemically modified in the receptor), enhanced transport across full thickness skin should also be observed for anionic drugs.

As determined by Nolan et al. (2006), some synergy should also be expected so that the enhancement when the chemical and physical techniques are applied simultaneously may be even greater than the sum of those observed when each method is used on its own. Oleic acid has been shown not to ion-pair with anionic moieties (Fitzpatrick and Corish, 2006). Because of the absence of ion pairing, there may be a greater enhancement than was observed by Nolan et al. (2006) in the case of oleic acid and salbutamol base.

Examples of simultaneous physical and chemical enhancement are now numerous in the transdermal literature. Among those more relevant to this study are the investigations by Oh et al. (1998), which showed a synergistic enhancement of AZT in the presence of oleic acid and iontophoretic assistance. Also relevant are investigations by Fang et al. (1998), which provided evidence for the enhanced transdermal delivery of enoxacin (an anionic drug) by the combined use of benzalkonium chloride and iontophoresis. These experiments were carried out from a vehicle at pH 10. The mechanistic interactions of many enhancers with bio-membranes are poorly understood. Thennarasua et al. (2005), for example, have investigated in detail the affects of lipopeptides on membranes. They have found mechanisms such as induced positive curvature strain on membranes and also the formation of lipid peptide complexes on the surface of the membranes which result in transient pores. More detailed studies such as these are required to elucidate the interaction of enhancers under iontophoretic conditions.

It was anticipated that ion pairing of BDDAB and sodium diclofenac within the vehicle leading to non-release of the drug would produce the same difficulty under iontophoretic assistance as has been encountered in the passive experiments

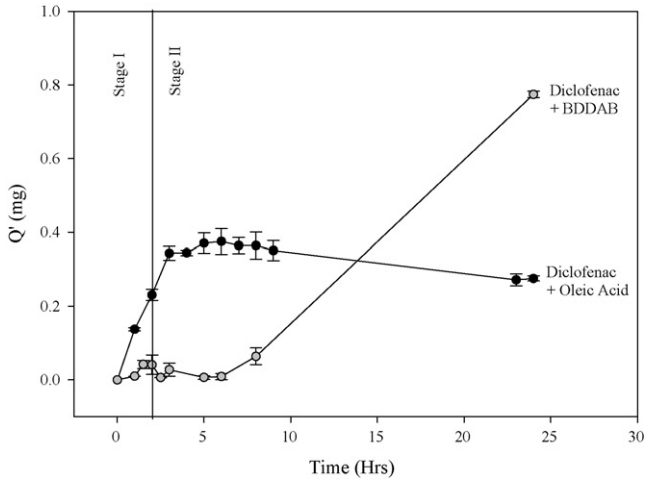

Fig. 8. A comparison of the iontophoretic release of sodium diclofenac across Visking from the LCG incorporating oleic acid or BDDAB. All concentrations are 0.1 M with 0.5 mA current.

(Fitzpatrick and Corish, 2006). The iontophoretic release profiles of sodium diclofenac from a liquid crystalline gel containing BDDAB are shown in Fig. 8.

The profile shows a negligible release in stage I and during the following 6 h of stage II of the experiment. The increase evident in the release rate after an eight our period of the experiment was not expected. There are several possible reasons for this delayed increase in the amount of drug detected in the receptor medium. The most plausible explanation is that the production of ions from the electrolysis of water reduces the ion pairing between the drug and enhancer within the vehicle. This in turn increases the release of drug from the vehicle in the same manner in which buffer ions have been shown to act. After the hydronium and hydroxide ions are produced at the anode they must diffuse upwards into the vehicle to have an effect, hence, the delayed increase. It is possible that the presence of BDDAB reduces the amount of diclofenac being converted to the acid-form in the receptor by acting as a base within the vehicle. In earlier experiments there was no diclofenac evident in the receptor after 24 h under iontophoretic conditions alone, cf. Fig. 1.

Although the release after 24 h is only 27% of that released under passive conditions, the results are encouraging. It can be assumed that additional diclofenac in acid-form is also present in solution but not in sufficient quantities to form a quantifiable precipitate.

Experiments using combined physical and chemical enhancement were also carried out using oleic acid as the model enhancer. As oleic acid has been ruled out as the cause of the inhibited release of sodium diclofenac in previous experiments, it was deemed suitable for use in these investigations. Also oleic acid has been proven to be an effective enhancer by many researchers (Nolan et al., 2006; Oh et al., 1998). The iontophoretic release profile of sodium diclofenac in the presence of oleic acid in comparison to that when BDDAB was used as an enhancer is also shown in Fig. 8. The concentration of both drug and enhancer in each case was 0.1 M and the current flow was 0.5 mA.

The graph shows the model enhancers to have significantly different effects on the release of the drug under iontophoretic conditions. The profile of sodium diclofenac and oleic acid is similar to that observed in the absence of oleic acid except that the level of detection of diclofenac remains the same after the first hour of stage II and with only a slight decline by the end of the experiment. The levelling off of the quantity detected compared to that observed in the absence of the acid is probably due to the buffer action of oleic acid within the system. Oleic acetate will thus reduce the number of hydronium ions which may otherwise associate with sodium diclofenac resulting in a greater quantitative detection of the drug. The total quantity of sodium diclofenac detected after 24 h is 13.6% of that detected after the same period for passive release but also represents a 13-fold increase in detection compared to the same experiment in the absence of oleic acid. The effect of iontophoresis can be said to be significant for both enhancer systems. Although the true cumulative amount of drug delivered across the membrane was not accurately quantified, the significant increase observed in the course of these studies justify further studies investigating the transport of diclofenac across human skin.

5. Conclusions

Initial iontophoretic experiments resulted in an apparent decrease in the transport of the drug from the LCG across a non-rate limiting membrane compared to passive release. However, it was then shown analytically using NMR, and other analytical techniques, that sodium diclofenac converts to the acid form in the receptor medium and leads to precipitation. This results in a failure to detect its presence. Further investigations have shown that the drug actually undergoes significant transport. Accurate quantification of the amount transported proved difficult within the remit of the experimental protocol. However, sufficient transport was proven to suggest that *in vitro* investigations using human skin would be worthwhile.

The inclusion of buffer in the system resulted in a slight decrease in the formation of the acid-form of the drug due to the presence of additional ions. It must be noted that the acid-form of these drugs are equally therapeutically active as is the salt form.

Reducing the iontophoretic current by 50% was found to increase the quantity of drug available for detection five-fold although this still represents poor detection in comparison to that in passive release studies due to the electrochemically altered state of the drug. The iontophoretic release profile from aqueous agar gels was found to mimic those from the LCGs with similar problems in detection. A range of anionic drugs demonstrated similar behaviour under the same conditions and the amount of drug detectable was shown to be related to the solubility of the acid-form of the drug in the receptor medium.

The inclusion of BDDAB in the vehicle leads to an unexpected significant delivery of drug from the vehicle. Under passive conditions the inclusion of the potential enhancer results in no release (Fitzpatrick and Corish, 2006). This also indicates a decrease in the electrochemical change of diclofenac in the presence of BDDAB.

The inclusion of oleic acid in the vehicle stabilised the rate of formation of the acid-form of the drug but not to the same extent as BDDAB. Oleic acid is not capable of forming ion-pairs with anionic compounds.

Acknowledgement

We are grateful for the kind donation of Myverol® by the Eastman Chemicals Company.

References

Bannon, Y.B., Corish, J., Corrigan, O.I., Masterson, J.G., 1988. Iontophoretically-induced transdermal delivery of salbutamol. Drug Develop. Ind. Pharm. 14, 2151–2166.

Carr, M.G., Corish, J., Corrigan, O.I., 1997. Drug delivery from a liquid crystalline base across visking and human stratum corneum. Int. J. Pharm. 157, 35–42.

Fang, J.-Y., Lin, H.-H., Chen, H.-I., Tsai, Y.-H., 1998. Development and evaluation of transdermal delivery of enoxacin via chemical enhancers and physical iontophoresis. J. Control. Release 54, 293–304.

Fang, J.-Y., Sung, K.C., Lin, H.-H., Fang, C.-L., 1999. Transdermal iontophoretic delivery of diclofenac sodium from various polymer formulations *in vitro* and *in vivo* studies. Int. J. Pharm. 178, 83–92.

Fitzpatrick, D., Corish, J., 2005. Release characteristics of anionic drug compounds from liquid crystalline gels. I. Passive release across non-rate limiting membranes. Int. J. Pharm. 301, 226–236.

Fitzpatrick, D., Corish, J., 2006. Release characteristics of anionic drug compounds from liquid crystalline gels. II. The effects of ion pairing and buffering on the passive delivery of anionic drugs across non-rate-limiting membranes. Int. J. Pharm. 311, 139–146.

Franz, T.J., 1978. The finite dose technique as a valid *in vitro* model for the study of percutaneous absorption in man. Curr. Probl. Dermatol. 7, 58–69.

Guy, R., Kalia, Y.N., Delgado-Charro, M.B., Merino, V., Lopez, A., Marro, D., 2000. Iontophoresis: electrorepulsion and electroosmosis. J. Control. Release 64, 129–132.

Iordanskii, A.L., Feldstein, M.M., Markin, V.S., Hadgraft, J., Plate, N.A., 2000. Modeling of the drug delivery from a hydrophilic transdermal therapeutic system across polymer membrane. Eur. J. Pharm. Biopharm. 49, 287–293.

Maitani, Y., Shimada, K., Nagai, T., 1996. L-Menthol, oleic acid and lauricidin in absorption enhancement of free and sodium salt of diclofenac using ethanol treated silicone membrane as model for skin. Chem. Pharm. Bull. 44, 403–408.

Nolan, L.M.A., Corish, J., Corrigan, O.I., Fitzpatrick, D., 2003. Iontophoretic and chemical enhancement of drug delivery. Part I. Across artificial membranes. Int. J. Pharm. 12, 41–55.

Nolan, L.M.A., Corish, J., Corrigan, O.I., Fitzpatrick, D., 2006. Combined effects of iontophoretic and chemical enhancement on drug delivery. II. Transport across human and hairless murine skin. Int. J. Pharm., submitted for publication.

Oh, S.Y., Jeong, S.Y., Park, T.G., Lee, J.H., 1998. Enhanced transdermal delivery of AZT (Zidovudine) using iontophoresis and penetration enhancer. J. Control. Release 51, 161–168.

Pikal, M.J., 1992. The role of electroosmotic flow in transdermal iontophoresis. Adv. Drug Deliv. Rev. 9, 201–237.

Powers, J.-P.S., Tan, A., Ramamoorthy, A., Hancock, R.E.W., 2005. Solution structure and interaction of the antimicrobial polyphemusins with lipid membranes. Biochemistry 44, 15504–15513.

Smith, E.W., Maibach, H.I., 1995. Percutaneous Penetration Enhancers. CRC Press.

Thennarasua, S., Leea, D.-K., Tana, A., Prasad Karib, U., Ramamoorthy, A., 1711. Antimicrobial activity and membrane selective interactions of a synthetic lipopeptide MSI-843. Biochim. Biophys. Acta, 49–58.