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The effects of esterified solvents on the diffusion of a model compound across human skin: An ATR-FTIR spectroscopic study

W.J. McAuley[a,*], S. Chavda-Sitaram[a], K.T. Mader[b], J. Tetteh[b], M.E. Lane[a], J. Hadgraft[a]

[a] School of Pharmacy, University College London, UK
[b] Medway Sciences, University of Greenwich, UK

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ABSTRACT

Attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy has been used to investigate the effects of three fatty acid esters on skin permeation. Propylene glycol diperlargonate (DPPG), isopropyl myristate (IPM) and isostearyl isostearate (ISIS) were selected as pharmaceutically relevant solvents with a range of lipophilicities and cyanophenol (CNP) was used as a model drug. The resultant data were compared with that obtained when water was used as the solvent. The diffusion of CNP, DPPG and IPM across epidermis was successfully described by a Fickian model. When ISIS was used as a solvent Fickian behaviour was only obtained across isolated stratum corneum suggesting that the hydrophilic layers of the epidermis interfere with the permeation of the hydrophobic ISIS. The diffusion coefficients of CNP across epidermis in the different solvents were not significantly different. Using chemometric data analysis diffusion profiles for the solvents were deconvoluted from that of the skin and modelled. Each of these solvents was found to diffuse at a faster rate across the skin than CNP. DPPG considerably increased the concentration of CNP in the stratum corneum in comparison with the other solvents indicating strong penetration enhancer potential. In contrast IPM produced a similar CNP concentration in the stratum corneum to water with ISIS resulting in a lower CNP concentration suggesting negligible enhancement and penetration retardation effects for these two solvents respectively.

1. Introduction

The stratum corneum, the outermost layer of skin is the main barrier to the absorption of drug molecules across and into human skin. This barrier has important implications for the topical treatment of skin conditions as it may render a particular treatment ineffective. Modification of the barrier properties of the stratum corneum is often sought primarily to increase drug penetration and the use of chemical penetration enhancers in a formulation is perhaps the most common strategy used to achieve this. A variety of different types of molecules have been shown to have the potential to affect drug permeation including alcohols, esters and fatty acids (Gorukanti et al., 1999; Liu et al., 2009; Ogiso et al., 1995). These molecules modify the properties of the stratum corneum altering drug flux across the skin. However it is difficult to elucidate the mechanisms through which they exert their actions. Improved knowledge of how individual enhancers modify skin penetration would facilitate formulation design and perhaps also give insight into how combinations of enhancers can have synergistic effects greatly increasing skin penetration (Goldbergcettina et al., 1995), allowing this effect to be utilised in formulations.

Fatty acid esters have been commonly used as penetration enhancers, with IPM being the most commonly used (Goldbergcettina et al., 1995; Gorukanti et al., 1999; Kikwai et al., 2002; Leichtnam et al., 2006; Liu et al., 2006; Yamato et al., 2009). Other examples of such molecules which have been investigated for this effect include ethyl oleate, decyl oleate, propylene glycol laurate, propylene glycol monolaurate, propylene glycol monocaprylate/caprate, glyceryl monocaprylate/caprate and ISIS (Cornwell et al., 1998; Gwak and Chun, 2002; Kikwai et al., 2002; Liu et al., 2006; Ozawa et al., 1988; Takahashi et al., 1996). These molecules exhibit considerable variation in their physiochemical properties, differing in hydrocarbon chain length, number of hydrocarbon chains and polarity. The effect of these molecules as chemical penetration modifiers of drug transport is however variable. For example IPM as a sole agent may increase drug permeation significantly (Gorukanti et al., 1999) but the effect may be modest in other cases and some of the other esters such as propylene glycol laurate and glyceryl monocaprylate/caprate have been demonstrated to be superior to IPM for particular drug molecules (Cornwell et al., 1998; Gwak and Chun, 2002). Also in combination with other enhancers these esterified solvents have shown a synergistic penetration enhancement effect, though this is not always

* Corresponding author. Current address: School of Life and Medical Sciences, University of Hertfordshire, Hatfield, HERTS, AL10 9AB, UK. Tel.: +44 0 1707 281052.
E-mail address: w.j.mcauley@herts.ac.uk (W.J. McAuley).

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the case (Alberti et al., 2001; Gorukanti et al., 1999; Gwak and Chun, 2002; Liu et al., 2009). Better insight into the mechanism of action of these types of enhancers would facilitate their selection and help rationalise the topical formulation development process.

Diffusion across a membrane may be followed using ATR-FTIR spectroscopy which potentially offers improved mechanistic insight into the role of penetration enhancers on drug diffusion than can be gained with conventional in vitro diffusion experiments such as those performed using Franz cells. The technique makes it easier to separate the effect of the enhancer on the concentration and diffusion coefficient of the drug in the stratum corneum and can also give molecular insight into the mechanism of action. For example Harrison et al. (1996) have correlated the increase in the diffusion coefficient of a model compound, cyanophenol across the stratum corneum with increased fluidity of the intercellular lipids. The technique also allows the diffusion profile of the enhancer to be monitored and can give insight into drug transport mechanisms. Tantishaiyakul et al. (2004) used ATR-FTIR spectroscopy to follow the diffusion of ion pairs and McAuley et al. (2009) have reported the diffusion of hydrogen bonded species across model membranes.

In this study the effect of three fatty acid ester solvents on the transport of a model drug CNP across human epidermis have been investigated using ATR-FTIR spectroscopy. The esters examined, IPM, ISIS and DPPG are all used in topical formulations and have a range of polarities and hence should provide insight into the effects of these types of enhancers on drug transport across human skin.

2. Materials

CNP was obtained from Fisher Scientific (Loughborough, UK). IPM was obtained from Sigma–Aldrich (Poole, UK). ISIS was received as a gift from Uniqema (Gouda, The Netherlands) and DPPG was received as a gift from Gattefosse (Saint-Priest, France).

3. Methods

3.1. Tissue preparation

Dermatomed human thigh skin from a single female patient was obtained from the International Institute for the Advancement of Medicine. Separated epidermis was prepared by blunt dissection following heat separation. The dermatomed skin was defrosted for 2 h at room temperature before being immersed in a water bath at 60 °C for 1 min. The tissue was then pinned to a cork board and the epidermis was peeled away from the dermis using tweezers. Isolated stratum corneum was prepared by soaking the epidermis in phosphate buffered saline containing 0.0001% trypsin for 24 h at 37 °C (Pellett et al., 1997b). The stratum corneum was then rinsed thoroughly with deionised water. All skin tissue was stored frozen at −20 °C and allowed to thaw at room temperature prior to use.

3.2. ATR-FTIR spectroscopy studies

Diffusion experiments were conducted at ambient temperature (21 ± 2 °C) using a Nicolet Avatar 360 FTIR spectrometer fitted with a multibounce ATR accessory with a Zinc Selenide (ZnSe) crystal. The incident angle of the IR radiation was 45°. Ten scans were taken every 60 s with resolution of 2 cm^{-1} and an average spectrum was produced at each time point. All experiments were repeated in triplicate. Spectral analysis was performed using Opus® 5.5 software.

Epidermis was placed on the ZnSe crystal so that the stratum corneum was in direct contact with the crystal. Intimate contact between the epidermis and crystal was assessed visually. This type of experimental set up using epidermis for ATR-FTIR spectroscopic studies of diffusion has been described previously (Tetteh et al., 2009). An aluminium trough constructed specifically for the diffusion experiments was placed on top of the epidermis and was sealed with silicone grease. A saturated CNP solution was applied to the membrane and an aluminium lid was placed on top, again sealed with silicone grease.

When isolated stratum corneum was used it was placed on the ZnSe crystal so that the outer surface was in direct contact with the crystal, thus the stratum corneum was placed on the ATR crystal in the same orientation as in the epidermis experiment.

Saturated solutions of CNP in the three esters were prepared by constant agitation of excess of CNP in the solvent in the presence of an immiscible water layer for 48 h. The aqueous layer was then removed prior to the solution being used. The CNP solutions were equilibrated with water to prevent them from dehydrating the skin tissue and causing it to curl off the ATR crystal. The saturated solution of CNP in water was prepared by constant agitation of an excess of CNP in water for 48 h.

3.3. Data modelling

Assuming that the Beer–Lambert law applies, the increase in IR absorbance associated with either the drug or solvent molecule with time is directly related to the concentration of the species in the membrane. The increase in absorbance can therefore be modelled by fitting appropriate boundary conditions to Fick's second law,

$$\frac{\partial C}{\partial t} = -D\frac{\partial^2 C}{\partial x^2} \quad (1)$$

where C is the concentration of the diffusing species in the membrane, D is the diffusion coefficient of the diffusing species, x is the diffusional pathlength, and t is time. In this study the method of Laplace transformation was used to solve Eq. (1) to obtain diffusion coefficients for the permeating molecules across skin. This approach has been reported previously (McAuley et al., 2010). The diffusion coefficients were obtained by fitting the normalised data with Micromath Scientist® 3.0 for Windows, using the following Laplace transformation;

$$\frac{A}{A_\infty} = \frac{\overline{C}}{\overline{C}_\infty} = \frac{\cosh(h \cdot \sqrt{s/D})}{s} \quad (2)$$

where A is the absorbance, A_∞ is the absorbance at infinite time, $\overline{C}$ is the concentration in the Laplace domain, $\overline{C}_\infty$ is the concentration in the Laplace domain at infinite time, s is the Laplace variable and h is the diffusional pathlength. Statistical analyses were made using Graphpad Prism 5 software. Comparisons of the calculated diffusion coefficients were made using the Kruskal–Wallis test with post hoc comparison made using the Mann–Whitney U test. Significance was accepted at the $p \leq 0.05$ level.

3.4. Solubility studies

The solubilities of CNP at 21 ± 2 °C in water, IPM, DPPG and ISIS were measured by UV absorbance at 249 nm. Saturated solutions of the model drugs were prepared using constant agitation for 48 h. An aliquot of this was then centrifuged to separate any solid material and the supernatant was sampled, diluted as necessary and analysed.

3.5. Chemometric data analysis

Chemometric data analysis was used to obtain the diffusion profiles of the solvents. The multivariate target factor analysis approach was used to deconvolute the solvent profile from that of the skin and CNP. The analysis was conducted using InSight (InSight