JPP Journal of Pharmacy and Pharmacology

JPP 2008, 60: 1139–1147

Received April 23, 2008
Accepted May 13, 2008
DOI 10.1211/jpp.60.9.0004
ISSN 0022-3573

An investigation into solvent–membrane interactions when assessing drug release from organic vehicles using regenerated cellulose membranes

Monica L. Reid, Marc B. Brown, Gary P. Moss and Stuart A. Jones

Abstract

The influence of organic solvents on artificial membranes when assessing drug release from topical formulations is, generally, poorly characterised yet current guidelines require no characterisation of the membrane before, during or after an experiment. Therefore, the aim of this study was to determine the effect of solvent–membrane interactions when using in-vitro Franz cell methods for the assessment of corticosteroid release and to assess compliance or otherwise with Higuchi's equation. The rate of beclometasone dipropionate monohydrate (BDP) and betamethasone 17-valerate (BMV) release across a regenerated cellulose membrane (RCM), from both saturated solutions and commercial formulations, was determined. Increasing the ratio of organic solvent, compared with aqueous phase, in the donor fluid (DF) resulted in up to a 416-fold increase in steady-state flux. Further, alterations in the receiver fluid (RF) composition caused, in some cases, 337-fold increases in flux. Analysis indicated that the RCM remained chemically unchanged, that its pore size remained constant and that no drug partitioned into the membrane, regardless of the DF or RF employed. However, it was observed that the organic solvents had a thinning effect on the RCM, resulting in enhanced flux, which was potentially due to the variation in the diffusional path length. Such findings raise issues of the veracity of data produced from any membrane release study involving a comparison of formulations with differing solvent content.

Introduction

The topical delivery of a therapeutic agent can offer numerous advantages over other routes of administration. For example, problems such as adverse side-effects and first-pass metabolism can be avoided when the drug is applied directly to the target site as it does not need to be absorbed systemically. However, whether the desired site of action is within the skin to treat topical afflictions or through the epidermis to the bloodstream for transdermal application, delivering an adequate concentration of the drug can be problematic due to the relative impermeability of the outermost layer, the stratum corneum (Suhonen et al 1999).

To overcome this barrier, novel formulations have been developed that utilise high levels of organic solvents to solubilise and enhance the permeation of active ingredients. These formulations rely on solvents such as ethanol to either evaporate, and potentially increase the thermodynamic activity of the drug in the formulation, or to act as a penetration enhancer by changing the properties of the stratum corneum. Examples of novel formulations containing high amounts of organic solvents include a spray developed by Acrux Ltd (up to 60% organic solvent (Reed et al 2004)), Versafoam-HF developed by Connetics (up to 50% organic solvent (Abram 2004)) and Androgel developed by Unimed/Solvay Pharmaceuticals (up to 75% organic solvent (Dudley et al 2003)). In each of these formulations the high amounts of organic solvents are reported to facilitate comparatively high drug permeation. However, the effects of such solvents on the in-vitro test systems used in their optimisation has not been disclosed.

A number of different synthetic membranes have previously been used to screen topical formulations in-vitro. These include cellulose, polysulfone, ethyl (vinyl acetate) and silicone (Haigh & Smith 1994). The choice of membrane is often dependent upon the physicochemical properties of the drug and the objectives of the study. For example, when using a

Pharmaceutical Science Research Division, King's College, London, 150 Stamford St, London SE1 9NH, UK

Monica L. Reid, Stuart A. Jones

School of Pharmacy, University of Hertfordshire, College Lane, Hatfield, Hertfordshire, AL10 9AB, UK

Marc B. Brown, Gary P. Moss

Correspondence: S. A. Jones, Pharmaceutical Science Research Division, King's College, London, 150 Stamford St, London SE1 9NH, UK. E-mail: stuart.jones@kcl.ac.uk

confluent barrier, mass transport of a compound from a region of high concentration to a low concentration occurs as a result of partitioning into the membrane from the formulation, diffusion through the membrane and partitioning from the membrane into the receiver fluid. However, mass transport across a porous membrane is often simply due to diffusion, as the drug never partitions into the highly permeable material. Silicone is a non-porous, inert, lipophilic membrane, which allows for the in-vitro assessment of the mass transport of lipophilic drugs (Moser et al 2001), whereas cellulose membranes are used as porous supports to assess drug release from a formulation.

Higuchi's equation of mass transport is widely accepted as an appropriate model for drug diffusion across membranes (Equation 1):

$$J = \alpha DA/\gamma L \quad (1)$$

where J is the flux of the molecule, α is the thermodynamic activity of the drug in the donor solution, D is the diffusion coefficient of the drug, A is the effective cross-section area of the membrane, γ is the effective activity coefficient of the drug in the membrane and L is the barrier thickness (Higuchi 1960). This equation states that all saturated solutions of the same drug should provide the same rate of transport, and thus should be independent of the properties of the vehicle. However, this transport model makes several assumptions, including that only one compound is transported across the membrane, the membrane is the rate controlling barrier, thermodynamic activity is homogenous throughout the formulation, mass transfer occurs under sink conditions and the application vehicle does not affect the barrier. Although it is commonly assumed that in the design of most in-vitro permeation studies the methodology controls each of these variables, this assumption can be incorrect. While the determination of drug solubility (to maintain sink conditions), compound stability, the control of thermodynamic activity via drug saturation and the use of a single species is commonplace, the assessment of the effects of the experimental methodology on the membrane is rarely reported. For example, the European Centre for Validation of Alternative Methods and the Scale Up and Post Approval Changes (SUPAC) guidelines both act to standardise in-vitro diffusions experiments, yet no characterisation of the barrier before, during or after drug application is recommended (OECD 2004; US Dept of Heath and Human Services et al 1997). This is of particular concern as these guidelines are often used to help demonstrate bioequivalence or essential similarity between different topical medicines. In addition, there is a wealth of published studies that do not consider the effects of application solvent on the membrane. For example, Megrab et al (1995) designed a study to investigate the effects of supersaturation from various organic solvent mixtures across silicone membranes, while also utilising these solvents as the receiver fluid (RF), which SUPAC guidelines mention specifically as acceptable media when using hydrophobic drugs (US Dept of Heath and Human Services et al 1997). The organic solvent content ranged from 12% to 60%, but the effect of the solvent on the barrier properties was never reported (Megrab et al 1995). Similar studies have been performed with propylene glycol (Pellett et al 1994, 1997a; Schwarb et al 1999) and ethanol (Twist & Zatz 1988; Watkinson et al 1995), where the authors postulated that organic solvent–membrane interactions were the reason for drug-release trends that did not obey Higuchi's equation; however, the actual effect of the solvent on the membrane was never examined. Therefore, the phenomenon of solvent–membrane interactions, possibly resulting in membrane damage, has been widely postulated but seldom, if ever, investigated.

The aim of this study was to investigate if the use of regenerated cellulose membrane (RCM) was suitable to test the release of lipophilic drugs from topical formulations. Although RCM has previously been employed in topical-release studies, a systematic investigation of their use with vehicles containing high levels of organic solvents has not been performed. Betamethasone 17-valerate (BMV) and beclometasone dipropionate monohydrate (BDP) were chosen as model drugs because both have limited aqueous solubility ($<0.01\ mg\,mL^{-1}$) (Bronaugh & Stewart 1984). RCM was chosen as the membrane because it has reportedly excellent chemical resistance to a wide range of solvents and its porous hydrophilic nature removes the potential of any mass transfer caused by partitioning (Houk & Guy 1988; Dias et al 1999). Solvents with differing solubility parameters were used as a means of assessing the influence of such solvents on drug flux through model membranes (Dias et al 2007). Two methods were used to investigate the effects of organic solvents upon the RCM during the release studies. Firstly, dextran permeability was used to assess whether the pore size of the membrane was changing and, secondly, thickness measurements were recorded to assess membrane swelling. It was anticipated that the results for these two experiments would assist in determining the mechanism of membrane–solvent interaction, and also to indicate whether a quality control method for testing such effects is required in future studies.

Materials and Methods

Materials

BDP was purchased from Airfilco (UK) and BMV was purchased from Symbiotec (India). The BDP cream (Propaderm, 0.025% w/w) and BMV cream (Betnovate, 0.1% w/w) were obtained from GlaxoSmithKline (UK). The BMV mousse (Bettamousse, 0.1% w/w) was supplied by Celltech (UK). Ethanol (EtOH, 99.7–100% v/v) and sodium trihydrate acetate were purchased from BDH (UK). Acetonitrile (ACN; HPLC grade) was from Fisher Scientific (UK) and Brij 98 from Sigma Aldrich (UK). Poly (vinyl pyrrolidone) (PVP K90) was purchased from Fluka (Switzerland) and the phosphate-buffered saline tablets from Oxoid (UK). Regenerated cellulose dialysis tubing (12–14 k molecular weight cut-off (MWCO)) was purchased from Medicell International (UK).

Saturated solubility determination

BMV or BDP was added in excess to solutions and stirred for 72 h at ambient temperature. The solution was then centrifuged at 14000 rotations for 30 min (Biofuge pico Heraeus Instruments,

Germany), the supernatant was diluted and assayed by HPLC. Drug solubility was tested in 100% EtOH and 70:30 ACN–de-ionised water (DiH_2O) for both corticosteroids. Additionally, 0.1 M acetate buffer–EtOH–Brij 98 (78:20:2), at pH 4.5, was assessed for BMV and 0.18 M phosphate buffer–EtOH–Brij 98 (78:20:2), at pH 7.2, was tested for BDP. The calculated solubility parameters of the solutions were obtained using the Fedors method (Fedors 1974; Sloan et al 1986), whereby all solvents were assumed to have ideal behaviour.

Membrane thickness determination

Approximately 1 cm^2 of RCM was soaked in 5 mL of the chosen solvent system for 4 h (the duration of the release experiment). The membrane was removed and gently patted dry and measured immediately with a Vernier micrometer (Starrett, UK). Five measurements were made in five different, randomly selected sections of the membrane. For the diffusion studies of topical formulations, after the Franz cell was disassembled at 4 h the membrane was removed and gently patted dry to remove the formulation, and the membrane was measured.

Drug-release studies

The release experiments were carried out in previously calibrated upright Franz cells (MedPharm Ltd, UK) with an average receiver volume of 10.8 cm^3 and an average surface area of 2.1 cm^2. RCM was soaked in DiH_2O (conductivity 0.5–1 μS) for 30 min at 70°C and then rinsed with DiH_2O to remove any impurities. The membrane was then cut to fit the Franz cell with scissors and mounted on the cell with a 13-mm magnetic flea in the receiver chamber. The top of the cell was positioned over the membrane and the cell was sealed by wrapping parafilm around the two sections. The cell was then inverted, filled immediately with previously sonicated RF, checked for leaks and placed on a submersible stirring plate in a pre-heated water bath set at 37°C, to obtain 32°C at the membrane surface (Maddock & Coller 1933). The cells were left to equilibrate for 1 h before application of the donor solution, which consisted of either saturated solutions or semi-solid formulations (1 mL). Gels consisting of 0.1% w/w either BMV or BDP, 19.7% w/w PVP K90 and 80.2% w/w EtOH were stirred overnight before application. Other semi-solid dosage forms were discharged from their canisters or tubes, drawn up in a 1-mL plastic syringe, and applied to the surface of the Franz cell. Diffusion was monitored by removal of 1-mL samples out of the sampling arm of the cell, which was placed into an HPLC vial without dilution. One millilitre of thermostatically regulated RF was then added to the diffusion cell to replace the withdrawn sample.

HPLC analysis

Quantitative determination of the active substances was performed using an HPLC system consisting of a Waters 600E pump, a Waters 996 PDA Detector, Waters 717 Plus Autosampler coupled with Millennium^{32} Software, version 4.0. The mobile phase was ACN–DiH_2O (70:30) set at a flow rate of 1.0 mL min^{-1}. BDP was separated using a Waters Nova-Pak C18 150 mm × 4 μm stationary phase at room temperature with a 100-μL injection volume and UV detection at 254 nm. BMV was separated using a Hichrom ACE C18 150 mm × 4 μm column at room temperature using a 10-μL injection and UV detection at 239 nm. Peak retention times were 3.0 min for BMV and 3.1 min for BDP. Calibration curves were constructed from integrated peak areas for BDP and peak height for BMV from known concentrations of standards, as the peak height measurement was shown to be more accurate and precise for BMV than the peak area. Both methods were shown to be acceptable with regard to their accuracy, precision, linearity, detection and quantitation limits, in accordance with the International Conference on Harmonisation guidelines (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use 1996).

Dextran permeation

Fluorescein-isothiocyanate-labelled dextran (FITC-dextran), with average molecular weight of 4, 20 or 40 kDa (Sigma, UK), was dispersed in either 100% Tris buffer (pH 9.5) or EtOH–Tris buffer (50:50) (pH 9.5) for a 2 mg mL^{-1} solution. A 1-mL sample of each of these solutions was used as a donor solution. The receiver fluid was composed of an equivalent solvent to that used to make up the FITC-dextran donor solution. The Franz cells were assembled and placed in a pre-heated water bath, set at 37°C, as in the drug release studies. Five samples (each of 100 μL) were taken at the appropriate time points and an equivalent volume of thermostatically regulated RF was used to replace the withdrawn sample. The samples were then transferred to a black 96-well plate and fluorescence was measured by using a fluorometer (Cytofluor, Series 4000, Foster City, USA) with an excitation and emission wavelength of 485 and 530 nm, respectively. The dextran was quantified by comparison with a calibration curve of equivalent grade material in identical solvents (a method developed and validated by Grainger et al (2006)).

Data analysis

Cumulative amounts of drug (μg) penetrating the unit surface area of the membrane (cm^2) were corrected for sample removal and plotted against time (h) in Microsoft Excel 2003. Flux was taken from the line of best fit over at least five time points with a linearity of $r^2 \geq 0.97$, which was deemed as the apparent steady state flux (J). All data are expressed as mean ± standard deviation (s.d.) Statistical analysis was performed in SPSS, version 14.0, using analysis of variance and Student's *t*-test with the chosen level of significance at $P \leq 0.05$.

Results

Effect of donor and receiver fluid composition on drug release across regenerated cellulose membranes

The rate of mass transfer (flux) for two hydrophobic drugs, BMV and BDP, was determined across an RCM using different donor fluid (DF) and RF compositions of various

solubility parameters (Table 1). Dose depletion was not observed, and sink condition were maintained in the RF for each experiment. Hence, the plots of cumulative drug release against time were found to be linear over the course of the 4-h experiments. Regardless of the DF or RF employed, BDP had a significantly slower flux ($P \leq 0.05$) than BMV (Figure 1). For example, when ACN–DiH_2O (70:30) was employed as a DF and RF, BMV flux was $1129.6 \pm 114.0\ \mu g\,cm^{-2}h^{-1}$ while BDP flux was only $161.6 \pm 27.5\ \mu g\,cm^{-2}h^{-1}$. This was the case for all systems except where EtOH was used as both DF and RF and in this instance fluxes of both drugs were not significantly different ($P > 0.05$).

Increasing the solubility parameter of the DF, while keeping the RF constant, led to a significant decrease in flux of both BMV and BDP (Figure 1A). While keeping the DF composition constant with a solution of 100% EtOH, increasing the solubility parameter of the RF resulted in a decrease in flux for both drugs (Figure 1B). Flux was lower when either the DF or RF contained a high proportion of water (buffer–EtOH–Brij 98, 78:20:2). As the percentage of organic solvent was increased in either DF or RF, the flux increased. For example, as the percentage of organic solvent in the DF increased from 20% to 100% the flux of BMV increased from 43.3 ± 3.3 to $1432.2 \pm 168.1\ \mu g\,cm^{-2}\ h^{-1}$, and the flux of BDP increased from 1.4 ± 0.2 to $563.4 \pm 65.4\ \mu g\,cm^{-2}h^{-1}$ when the RF was maintained constant (Figure 1A). Likewise, increasing the proportion of organic solvent in the RF from 20% to 100% increased the flux of BMV from 31.7 ± 5.9 to $1735.2 \pm 162.1\ \mu g\,cm^{-2}h^{-1}$ and BDP from 4.4 ± 0.5 to $1493.4 \pm 257.9\ \mu g\,cm^{-2}h^{-1}$ when a constant DF was employed. Transport of both compounds was highest when water was absent from both the DF and RF.

Table 1 Results of saturated solubility studies with betamethasone valerate (BMV) or beclometasone dipropionate (BDP) in various solutions of different solubility parameters

Solution	Solubility parameter $(cal\,cm^{-3})^{1/2}$	Saturated solubility $(mg\,mL^{-1})$	
		BMV	BDP
Buffer–EtOH–Brij 98 (78:20:2)	21.0	1.1	0.06
EtOH–buffer (50:50)	18.1	6.5	N/A
ACN–DiH_2O (70:30)	15.4	82.8	10.4
EtOH–buffer (80:20)	14.8	67.8	N/A
EtOH	12.7	55.2	31.6

The solutions were mixtures of ethanol (EtOH), acetonitrile (ACN), de-ionised water (DiH_2O) with Brij 98 as a surfactant. The buffer system used with BMV was 0.1 M acetate buffer adjusted to pH 4.5; the buffer system used with BDP was 0.18 M phosphate buffer. N/A denotes test not performed.

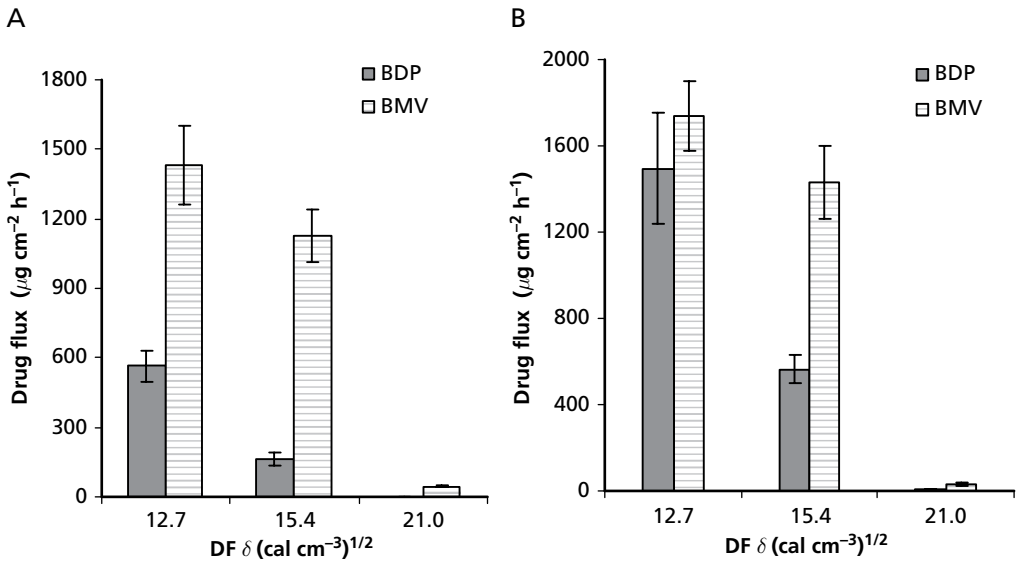

Figure 1 Effect of solubility parameter (δ) on steady-state flux of betamethasone valerate (BMV) and beclometasone dipropionate (BDP) through a regenerated cellulose membrane. Saturated donor fluids (DF) with different δ values were tested with an acetonitrile–water (70:30) receiver fluid (RF) (A). Saturated solution of ethanol was used as the DF with RFs of different δ values (B). Data are presented as mean ± s.d., n = 5.

Solvent effects on regenerated cellulose

The influence of solvent composition on pore size of the RCM when undertaking Franz cell permeation studies was examined using three different FITC-dextran molecular weight markers of 4, 20 and 40 kDa (Figure 2). This experiment was conducted with both the RF and DF of the Franz cells containing either 100% Tris buffer or Tris buffer–EtOH (50:50). A limitation of this test was that dextran was not soluble in 100% EtOH and as such was not tested, therefore the effect of a full solvent system on the membrane could not be examined. Altering the solvent system in which the mass transport experiment was performed caused no significant change ($P > 0.05$) in the amount detected in the RF of the three dextran markers after a period of 24 h. A significantly greater ($P \leq 0.05$) amount of the 4 kDa marker passed through the membrane, compared with either the 20 or 40 kDa marker. However, the amounts of both the 20 and 40 kDa markers that passed the barrier in 24 h were not significantly different from each other ($P > 0.05$).

Solvent effects on regenerated cellulose membrane thickness

The effect of the percentage of organic solvent in solution on the thickness of the RCM was examined (Figure 3). Increasing the amount of solvent (either EtOH or ACN) caused thinning of the RCM in a linear fashion ($r^2 = 0.94$), from $94.0 \pm 5.5\,\mu$m in 100% DiH_2O to $50.0 \pm 0.0\,\mu$m in 100% EtOH, with the effect becoming significant when the percentage of solvent was greater than 30% ($P \leq 0.05$). It was evident that this thinning of the membrane had a dramatic effect on the flux of BMV when these two parameters were compared for five different solvent systems: 100% EtOH, EtOH–acetate buffer (80:20), ACN–DiH_2O (70:30), EtOH–acetate buffer (50:50) and EtOH–acetate buffer–Brij 98 (78:20:2). It was seen that by increasing the proportion of organic solvent from 20% to 100%, a corresponding 40-μm decrease in the thickness of the RCM and a 542-fold increase in drug release was observed. As expected, drug release was shown to be inversely proportional to membrane thickness ($r^2 = 0.91$).

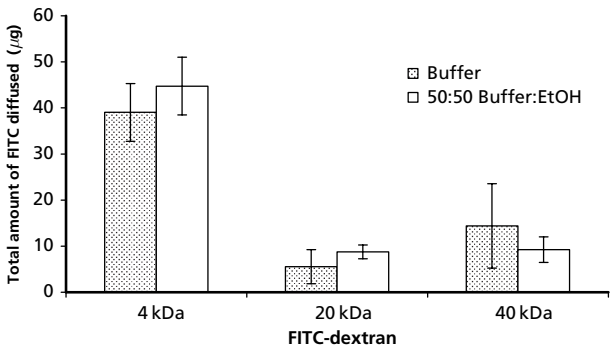

Figure 2 Comparison of the total amount of fluorescein-isothiocyanate-labelled dextran (FITC-dextran, 4 kDa, 20 kDa and 40 kDa) diffused at 24 h through a regenerated cellulose membrane when dissolved in Tris Buffer or Tris buffer–ethanol (EtOH) (50:50). Data are presented as mean ± s.d., n = 3–5.

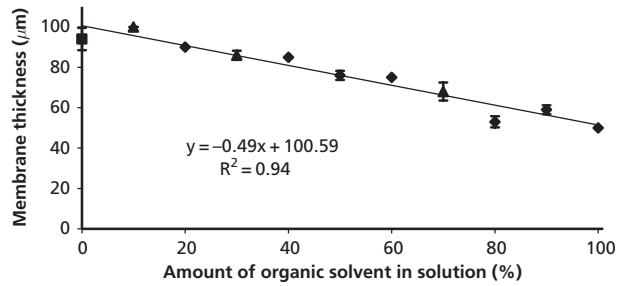

Figure 3 Effect of amount of organic solvents EtOH (diamonds), acetonitrile (triangles) and de-ionised water (squares) on the thickness of the regenerated cellulose membrane after 4 h. Organic solvent levels ranged from 0 to 100%. Data are presented as mean ± s.d., n = 5.

Membrane–solvent interactions effects on drug release from commercial topical formulations

Three topical formulations (gel, cream and mousse) containing BMV were tested in Franz cells using two different RFs (Figure 4). Membrane thickness was measured to determine the extent of the organic solvent effect. The membrane thickness varied from $88.0 \pm 4.5\,\mu$m for the ACN RF to $118.0 \pm 4.5\,\mu$m for the buffer RF. All formulations displayed a more rapid release when the ACN–DiH_2O (70:30) RF was employed, compared with the 0.1 M acetate buffer–EtOH–Brij 98 (78:20:2) RF. The total amount of drug released from the gel at 4 h was $757.7 \pm 42.4\,\mu g\,cm^{-2}$ using ACN–DiH_2O (70:30) RF, and this was significantly greater ($P \leq 0.05$) than the 0.1 M acetate buffer–EtOH–Brij 98 (78:20:2) RF ($149.3 \pm 24.6\,\mu g\,cm^{-2}$). The mousse released $84.3 \pm 8.6\,\mu g\,cm^{-2}$ compared with $96.3 \pm 29.3\,\mu g\,cm^{-2}$ from the two RFs. The mousse did not achieve an extended steady-state rate of release, which may be attributed to the dynamic nature of the foam after dose application. The cream released $33.3 \pm 5.3\,\mu g\,cm^{-2}$ in the ACN–DiH_2O (70:30) RF but no drug could be detected at 4 h in the buffer RF. Sink conditions were maintained in all RFs (maximum of 3% of saturated solubility). Only BMV mousse and cream in the ACN–DiH_2O (70:30) RF had statistically similar rates of release ($P > 0.05$). However, irrespective of the RF employed, the rank order of drug release rate from the formulations was gel > mousse > cream.

Two different topical formulations containing BDP were tested in Franz cells using two different RFs: ACN–DiH_2O (70:30) and 0.1 M acetate buffer–EtOH–Brij 98 (78:20:2) (Figure 5). The rate of drug release was faster from the gel than the cream in both RFs. Again, the cream showed no drug release at 4 h when the buffer RF was used. The total amount of drug released was significantly higher ($P \leq 0.05$) as the percentage of organic solvent increased. The gel released a total of $582.7 \pm 80.4\,\mu g\,cm^{-2}$ BDP using the ACN RF compared with $21.9 \pm 5.2\,\mu g\,cm^{-2}$ with the predominantly buffer RF. The BDP gel stopped releasing drug after 2 h. This was attributed to the drug in the receiver compartment exceeding a concentration that allows diffusion to occur under sink conditions.

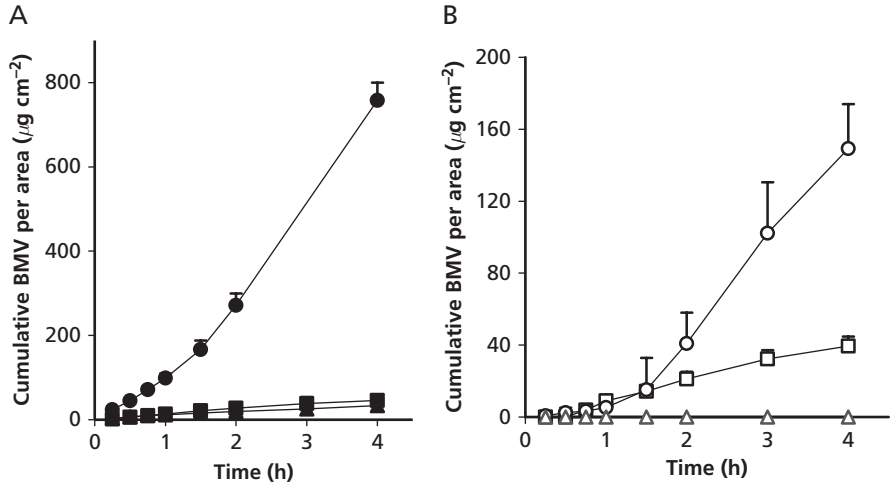

Figure 4 Comparison of betamethasone valerate (BMV) flux from gel (circles), mousse (squares) or cream (triangles), with two different receiver fluids: acetonitrile–water (70:30) (A) and 0.1 M acetate buffer–ethanol–Brij 98 (78:20:2) (B). Data are presented as mean ± s.d., n = 5.

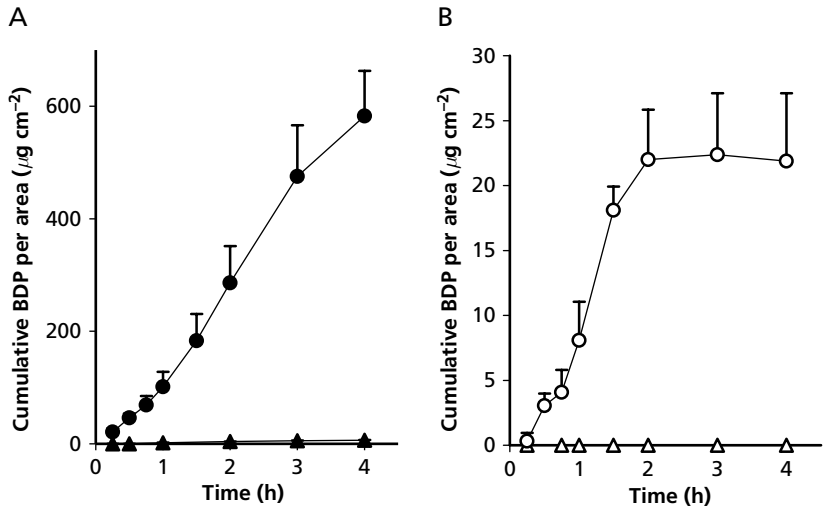

Figure 5 Comparison of beclometasone dipropionate (BDP) flux from gel (circles) or cream (triangles) into two different receiver fluids: acetonitrile–water (70:30) (A) and 0.18 M phosphate buffer–ethanol–Brij 98 (78:20:2) (B). Data are presented as mean ± s.d., n = 5.

Discussion

Corticosteroids typically display a low aqueous solubility. In this study the solubility of BDP was too low to be detected using the developed HPLC assay and BMV showed a maximum solubility of 1.45 $\mu g mL^{-1}$ in 0.18 M phosphate buffer (pH 7.2). The lack of aqueous solubility for these corticosteroid molecules meant that additional additives were required to solubilise BMV and BDP in the formulation vehicles to allow the in-vitro release experiments to be conducted under sink conditions. Additives typically used in Franz diffusion studies consist of organic solvents or solubilisers, such as non-ionic surfactants (Bronaugh & Stewart 1984). However, organic solvents and surfactants can interact with, and change the properties of, membranes (Coldman et al 1969). In addition, surfactants have the tendency to form micelles above a critical concentration, thereby effectively creating a two-phase system in which the drug must partition into for it to be solubilised in the solution (Shah et al 1989; Keyhanimorrison et al 1995; Crison et al 1996). Thus, selection of an appropriate solvent and membrane in a Franz cell system is essential to test the release of a corticosteroid from a topical formulation effectively.

Synthetic membranes are often used in topical drug release studies to study the release mechanisms of the formulation without the added complexity of a biological barrier, such as human or animal skin (Haigh & Smith 1994). RCM is a porous membrane and as such removes the potential of any influence of partitioning on flux seen in confluent membranes

such as silicone (Houk & Guy 1988; Dias et al 1999). By using a simpler membrane and potentially removing some of the variables related to mass transfer it was hoped that fewer complications would arise when examining the release of the drug from different solvent systems. In addition, RCM was employed as the barrier to overcome the issues of solvent selection because it has been reported to be resistant to the effects of a wide range of aqueous and organic solvents (Fink et al 2001; Medicell International Ltd 2007). It was anticipated that the excellent solvent resistance of this porous membrane would allow for successful use of solvents such as EtOH and ACN in the diffusion system. However, varying the solvent system in which the corticosteroid was presented to the RCM in the release studies significantly altered the flux of the compound. Since, according to Equation 1, all saturated solutions of a compound should give equivalent flux as they have the same thermodynamic activity (Higuchi 1960), it was proposed that altering the solvent system must be influencing the experimental set up such that all the assumptions associated with Higuchi's mathematical model were not being met. This was not thought to be due to violation of sink conditions, as they were maintained throughout the duration of the drug release studies.

Since volatile organic solvents were used in the study, one cause of the non-equivalent flux across various solvent systems may have been due to evaporation of the vehicle in which the drug is either dissolved or diffused into. Solvent evaporation from either the DF or RF can result in an alteration of the dynamics of the release experiment. For example, solvent evaporation in the DF can result in supersaturation, whilst solvent evaporation in the RF can lead to increased drug concentration (Coldman et al 1969). If the dynamics of the system were altered by the volatile solvents a non-linear, irreproducible rate of mass transfer should be observed in the release study. However, all the saturated solutions showed linear release over the time period studied. This indicates that the membrane is the rate limiting step of the drug flux (i.e. satisfying one of Higuchi's assumptions, that solvent evaporation was not leading to a supersaturated DF) (Davis & Hadgraft 1991). This is further substantiated by consideration of the release of the two corticosteroids examined using solvents, such as water, that are less likely to evaporate under the particular experimental conditions employed. In such cases, linear release profiles were observed but these systems did not result in equivalent fluxes being obtained.

There are numerous examples in the literature of organic solvents influencing drug permeation through both membranes and skin. For example, Babar et al (1999) reported that the addition of ethanol to a topical formulation resulted in a significant increase in drug flux. Similar results have also been reported when ethanol was used in the RF of Franz cell experiments (Bronaugh & Stewart 1984; Shah et al 1992; Keyhanimorrison et al 1995). Further, Watkinson et al (1995) found that drug saturated ethanol, PEG 400 and water solutions gave very different fluxes through a silastic membrane. Twist & Zatz (1988) also found that the higher the mole fraction of ethanol, the higher the percentage weight increase of the polydimethylsiloxane (PDMS) membranes and the faster the flux of parabens. Several studies that utilised ATR-FTIR of silicone membranes to study drug permeation from various solvents found that solvent–membrane interactions were predominant and heavily affected the flux of these compounds although no chemical changes in the membranes could be detected (Watkinson et al 1995; Pellett et al 1997b; Dias et al 2001). These authors speculated that the solvent was diffusing into the membrane and altering its properties, although the exact mechanism by which these solvents change the mass transport process through the membrane has not been explicitly identified.

One hypothesis that could explain the vehicle-dependent flux is 'solvent drag', where the solvent leaches into the membrane and increases the rate at which the drug partitions (Bendas et al 1995; Bowen & Heard 2006). It has been shown for PDMS membranes that flux of the solute is increased when greater amounts of solvent are absorbed by the membrane (Gelotte & Lostritto 1990; Twist & Zatz 1990). However, the lipophilic PDMS and the hydrophilic RCM have different structural and physiochemical characteristics. Cellulose membranes are porous scaffolds that are freely permeable to low-molecular-weight solutes, whereas PDMS membranes are confluent barriers. Hydrophilic and hydrophobic molecules can penetrate through cellulose because they are merely filtering through the pores. Therefore, the potential for solvent-induced partition effects with RCM is minimal, although hydrophilic molecules could interact with the membrane as it has a substantial number of OH binding sites (Murphy & de Pinho 1995; Dias et al 1998).

Another theory that could be used to explain the solvent-dependent flux observed in the RCM studies is that the solvents interact with the membrane, changing its physical characteristics and thus affecting the drug flux. Cellulose is composed of poly-β(1–4)-D-glucose chains and is naturally crystalline in nature. It is a straight-chain polymer and these chains adopt a rail-like conformation. Industrial regeneration from a viscous solution of cellulose creates the RCM (Tuwiner 1962). It has been seen previously that physical alteration of RCM can influence the flux of molecules (Craig & Konigsberg 1961). Obviously, the larger the pores become then the faster compounds could diffuse through the membrane (Lindau et al 1995). However, in this study, irrespective of the ratio of organic solvent, the amount of the FITC-dextran marker that passed through the membrane was identical. This was true for all three sizes employed (4, 20 and 40 kDa), which indicated that pore size remained unchanged and thus this was probably not the reason for the variations in flux. A sharp decrease in the amount of 20 and 40 kDa FITC-dextran released across the membrane compared with the 4 kDa FITC-dextran demonstrated that the RCM did retain its molecular weight selectivity properties, despite the presence of ethanol, and these results agree with the manufacturer's specifications (i.e. a 12 k to 14 k MWCO) (Medicell International Ltd 2007). However, as a small but significant amount (approximately 0.5% of the dose applied) of the 20 and 40 kDa markers did diffuse through the membrane, the ability of this method to detect small changes of pore size is questionable. It is accepted that the MWCO specification is not very precise and small defects in the membrane may have resulted in a small proportion of permeation, but as the amount of 20 and 40 kDa FITC-dextran that passed through the membrane was $1/10^{th}$ of that detected for the

4 kDa FITC-dextran this may suggest that the MWCO had been altered in the conditions that the membranes were used. This is not surprising as it is unlikely that the conditions employed in the release experiments are similar to the standard dialysis conditions employed by the manufacturer to test the membranes. Furthermore, it could be argued that the maintenance of the manufacturer's pore size specification in the Franz cell solvents is not essential. The relative pore size when switching between solvents in identical experimental conditions was shown to be similar in this work and this suggests no gross changes were occurring. While it would have been desirable to test the effect of 100% EtOH on pore size, this could not be done practically due to the inability of the alcohol to solubilise the dextran.

Despite these differences in drug flux from saturated solutions the RCM did show excellent chemical resistance. No chemical changes in the membrane could be detected after exposure to solvents. RCM was monitored by attenuated total reflectance Fourier transform infrared scanning and found to give identical scans despite being soaked for extended periods of time in different solvents (data not shown). No degradation of the RCM was observed as the release of the drug through the RCM was linear over the time period studied in all cases. If the membrane was slowly degrading or dissolving over time, there would be large increases of activity detected in the drug release profile and a plateau reached as an equilibrium between the DF and RF would occur rapidly if there were no membrane to separate them. This was not seen, lending evidence that the membrane was still intact and fully functional as a barrier.

In the absence of partitioning effects, the influence of volatile solvents, pore size alteration and chemical alteration it can be concluded that the variance of flux results mainly from changes in the thickness of the RCM. Water is a powerful swelling agent for cellulose, by means of breaking the H-bonds within the cellulose, expanding the amorphous regions and increasing the chain segmental motion by penetrating between the individual crystal units where cohesive forces are relatively weak (Tuwiner 1962). Water can even penetrate the crystalline sections, forming cellulose hydrate crystals. This creates a hydrophilic barrier through which the hydrophobic corticosteroids must pass and the membrane no longer represents a simple support scaffold. Conversely, when organic solvents were used as donor and receiver fluids and the solubility parameter decreased, the membrane became thinner in-situ. As the membrane becomes thinner (i.e. the diffusional path length shortens), it takes less time for the drug to traverse the membrane (Pellett et al 1997c; Iordanskii et al 2000; Shin & Lee 2002). The inverse, linear relationship between membrane thickness and BMV flux theoretically means the flux of the corticosteroid though the RCM can be normalised to eliminate the effects of path length variance. However, the model was not completely linear and this indicates that although membrane thickness is the predominant explanation other factors may also have an influence on this trend. As such, further work is required to apply the theories proposed here to a much wider set of compounds so that improvements in the accuracy of the linear model can be seen, which may allow any additional factors to be resolved. However, it was noted that there was a trend of decreasing solubility parameter value with membrane thinning, as less hydrophilic solutions caused the membrane pathway to shorten. This parameter could be used as a surrogate indicator to help predict membrane dehydration.

When comparing the commercial topical formulations, the rates of release were different and dependent on the RF composition. However, the order of release of the formulations was the same irrespective of RF constitution. The membrane thickness varied from $88.0 \pm 4.5\,\mu$m for the ACN RF to $118.0 \pm 4.5\,\mu$m for the buffer RF during the experiment. Hence, whilst the RF did have an effect on membrane thickness, using a consistent DF with different marketed products did allow a reliable comparison between the corticosteroid release from the different formulations. The more organic RF allowed better discrimination between formulations and maintained steady-state conditions for a longer period of time.

Conclusion

According to the manufacturer's specification, the RCM was expected to show 'good tolerance' with all the solvents employed in this study. Although, chemically, the membrane did appear to be resistant to the solvents, the extent of membrane swelling, and hence thickness, was solvent dependent. Increasing levels of organic solvent in the solutions used in the release studies decreased RCM thickness and as a result increased drug release. Measuring the membrane thickness is a simple procedure and can provide a great deal of information about the dynamics of the diffusion system, and there is no evidence in the literature of this routinely being performed. Thus, these results show that careful control of the membrane through the choice of solvent systems is critical to gain meaningful comparisons of drug release between formulations containing differing solvent content. It is the recommendation of the authors that the guidelines associated with the performance of drug-release testing (e.g. SUPAC or OECD, where no mention of ensuring membrane integrity throughout the experiment is mentioned) should be amended to address the issue of solvent–membrane compatibility.

References

Abram, A. Z. Mousse Composition. 09/719,662[6,730,288]. 2004. US. Ref Type: Patent

Babar, A., Ray, S. D., Patel, N. K., Plakogiannis, F. M., Gogineni, P. (1999) In vitro release and diffusion studies of promethazine hydrochloride from polymeric dermatological bases using cellulose membrane and hairless mouse skin. *Drug Dev. Ind. Pharm.* **25**: 235–240

Bendas, B., Schmalfuss, U., Neubert, R. (1995) Influence of propyleneglycol as cosolvent on mechanisms of drug transport from hydrogels. *Int. J. Pharm.* **116**: 19–30

Bowen, J. L., Heard, C. M. (2006) Film drying and complexation effects in the simultaneous skin permeation of ketoprofen and propylene glycol from simple gel formulations. *Int. J. Pharm.* **307**: 251–257

Bronaugh, R. L., Stewart, R. F. (1984) Methods for invitro percutaneous-absorption studies. 3. Hydrophobic compounds. *J. Pharm. Sci.* **73**: 1255–1258

Coldman, M. F., Poulsen, B. J., Higuchi, T. (1969) Enhancement of percutaneous absorption by use of volatile–nonvolatile systems as vehicles. *J. Pharm. Sci*. **58**: 1098–1102

Craig, L. C., Konigsberg, W. (1961) Dialysis studies. 3. Modification of pore size and shape in cellophane membranes. *J. Phys. Chem*. **65**: 166–172

Crison, J. R., Shah, V. P., Skelly, J. P., Amidon, G. L. (1996) Drug dissolution into micellar solutions: development of a convective diffusion model and comparison to the film equilibrium model with application to surfactant-facilitated dissolution of carbamazepine. *J. Pharm. Sci*. **85**: 1005–1011

Davis, A. F., Hadgraft, J. (1991) Effect of supersaturation on membrane transport: 1. Hydrocortisone acetate. *Int. J. Pharm*. **76**: 1–8

Dias, C. R., Rosa, M. J., de Pinho, M. N. (1998) Structure of water in asymmetric cellulose ester membranes: an ATR-FTIR study. *J. Membrane Sci*. **138**: 259–267

Dias, M., Farinha, A., Faustino, E., Hadgraft, J., Pais, J., Toscano, C. (1999) Topical delivery of caffeine from some commercial formulations. *Int. J. Pharm*. **182**: 41–47

Dias, M., Raghavan, S. L., Hadgraft, J. (2001) ATR-FTIR spectroscopic investigations on the effect of solvents on the permeation of benzoic acid and salicylic acid through silicone membranes. *Int. J. Pharm*. **216**: 51–59

Dias, M., Hadgraft, J., Lane, M. E. (2007) Influence of membrane-solvent-solute interactions on solute permeation in model membranes. *Int. J. Pharm*. **336**: 108–114

Dudley, R. E., Kottayil, S. G., Palatchi, O. (2003) Pharmaceutical composition and method for treating hypogonadism. [6,503,894]. 2003. Ref Type: Patent

Fedors, R. F. (1974) Method for estimating both solubility parameters and molar volumes of liquids. *Polymer Eng. Sci*. **14**: 147–154

Fink, H.-P., Weigel, P., Purz, H. J., Ganster, J. (2001) Structure formation of regenerated cellulose materials from NMMO-solutions. *Prog. Polymer Sci*. **26**: 1473–1524

Gelotte, K. M., Lostritto, R. T. (1990) Solvent interaction with polydimethylsiloxane membranes and its effects on benzocaine solubility and diffusion. *Pharm. Res*. **7**: 523–529

Grainger, C. I., Greenwell, L. L., Lockley, D. J., Martin, G. P., Forbes, B. (2006) Culture of Calu-3 cells at the air interface provides a representative model of the airway epithelial barrier. *Pharm. Res*. **23**: 1482–1490

Haigh, J. M., Smith, E. W. (1994) The selection and use of natural and synthetic membranes for in-vitro diffusion experiments. *Eur. J. Pharm. Sci*. **2**: 311–330

Higuchi, T. (1960) Physical chemical analysis of percutaneous absorption process from creams and ointments. *J. Soc. Cosmetic Chem*. **11**: 85–97

Houk, J., Guy, R. H. (1988) Membrane models for skin penetration studies. *Chem. Rev*. **88**: 455–471

International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (1996) Q2(R1): validation of analytical procedures: text and methodology

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

Keyhanimorrison, E., Nadkarni, S., Sakr, A. (1995) Optimization of an in-vitro method for evaluating the release of a novel retinoid from topical gels. *Pharm. Ind*. **57**: 414–419

Lindau, J., Jonsson, A. S., Wimmerstedt, R. (1995) The influence of a low-molecular hydrophobic solute on the flux of polysulphone ultrafiltration membranes with different cut-off. *J. Membrane Sci*. **106**: 9–16

Maddock, W. G., Coller, F. A. (1933) The role of the extremities in the dissipation of heat. *Am. J. Physiol*. **106**: 589–596

Medicell International Ltd (2007) Chemical tolerances of regenerated cellulose membrane http://www.visking.com/Visk_Tech_ChemTol.htm.

Megrab, N. A., Williams, A. C., Barry, B. W. (1995) Estradiol permeation through human skin and silastic membrane—effects of propylene-glycol and supersaturation. *J. Control. Release* **36**: 277–294

Moser, K., Kriwet, K., Froehlich, C., Naik, A., Kalia, Y. N., Guy, R. H. (2001) Permeation enhancement of a highly lipophilic drug using supersaturated systems. *J. Pharm. Sci*. **90**: 607–616

Murphy, D., de Pinho, M. N. (1995) An ATR-FTIR study of water in cellulose acetate membranes prepared by phase inversion. *J. Membrane Sci*. **106**: 245–257

OECD (2004) Skin absorption: in vivo method, No. 428. *OECD Guideline for the Testing of Chemicals*

Pellett, M. A., Davis, A. F., Hadgraft, J. (1994) Effect of supersaturation on membrane transport: 2. Piroxicam. *Int. J. Pharm*. **111**: 1–6

Pellett, M. A., Castellano, S., Hadgraft, J., Davis, A. F. (1997a) The penetration of supersaturated solutions of piroxicam across silicone membranes and human skin in vitro. *J. Control. Release* **46**: 205–214

Pellett, M. A., Watkinson, A. C., Hadgraft, J., Brain, K. R. (1997b) Comparison of permeability data from traditional diffusion cells and ATR-FTIR spectroscopy. 1. Synthetic membranes. *Int. J. Pharm*. **154**: 205–215

Pellett, M. A., Watkinson, A. C., Hadgraft, J., Brain, K. R. (1997c) Comparison of permeability data from traditional diffusion cells and ATR-FTIR spectroscopy. 2. Determination of diffusional pathlengths in synthetic membranes and human stratum corneum. *Int. J. Pharm*. **154**: 217–227

Reed, B. L., Morgan, T. M., Finnin, B. C. (2004) Dermal penetration enhancers and drug delivery systems involving the same. [6818226]. 2004. Ref Type: Patent

Schwarb, F. P., Imanidis, G., Smith, E. W., Haigh, J. M., Surber, C. (1999) Effect of concentration and degree of saturation of topical fluocinonide formulations on in vitro membrane transport and in vivo availability on human skin. *Pharm. Res*. **16**: 909–915

Shah, V. P., Konecny, J. J., Everett, R. L., Mccullough, B., Noorizadeh, A. C., Skelly, J. P. (1989) Invitro dissolution profile of water-insoluble drug-dosage forms in the presence of surfactants. *Pharm. Res*. **6**: 612–618

Shah, V. P., Elkins, J., Skelly, J. P. (1992) Relationship between in vivo skin blanching and in vitro release rate for betamethasone valerate creams. *J. Pharm. Sci*. **81**: 104–106

Shin, S. C., Lee, H. J. (2002) Controlled release of triprolidine using ethylene-vinyl acetate membrane and matrix systems. *Eur. J. Pharm. Biopharm*. **54**: 201–206

Sloan, K. B., Koch, S. A. M., Siver, K. G., Flowers, F. P. (1986) Use of solubility parameters of drug and vehicle to predict flux through skin. *J. Invest. Dermatol*. **87**: 244–252

Suhonen, T. M., Bouwstra, J. A., Urtti, A. (1999) Chemical enhancement of percutaneous absorption in relation to stratum corneum structural alterations. *J. Control. Release* **59**: 149–161

Tuwiner, S. (1962) Cellulose membranes. *Diffusion and membrane technology*. Reinhold Publishing Corporation, New York, pp 180–199

Twist, J. N., Zatz, J. L. (1988) Membrane solvent solute interaction in a model permeation system. *J. Pharm. Sci*. **77**: 536–540

Twist, J. N., Zatz, J. L. (1990) A model for alcohol-enhanced permeation through polydimethylsiloxane membranes. *J. Pharm. Sci*. **79**: 28–31

US Dept of Heath and Human Services, FDA, CDER (1997) Scale-up and postapproval changes: chemistry, manufacturing, and controls; in vitro release testing and in vivo bioequivalence documentation. *Guidance for Industry*

Watkinson, A. C., Joubin, H., Green, D. M., Brain, K. R., Hadgraft, J. (1995) The influence of vehicle on permeation from saturated solutions. *Int. J. Pharm*. **121**: 27–36