ELSEVIER

International Journal of Pharmaceutics 229 (2001) 87–94

international journal of pharmaceutics

www.elsevier.com/locate/ijpharm

UV-spectrophotometry study of membrane transport processes with a novel diffusion cell

K.T.E. Kierstan [a], A.E. Beezer [a,*], J.C. Mitchell [a], J. Hadgraft [a], S.L. Raghavan [a], A.F. Davis [b]

[a] *Medway Sciences, Natural Resources Institute (NRI), The University of Greenwich, Medway University Campus, Central Avenue, Chatham Maritime, Chatham, Kent ME4 4TB, UK*

[b] *SmithKline Beecham Consumer Healthcare, St. George's Avenue, Weybridge, Surrey KT13 ODE, UK*

Received 22 January 2001; accepted 13 July 2001

Abstract

A novel diffusion cell has been constructed which allows study of membrane diffusion processes without the need for sampling of the receiver compartment, that is highly sensitive and, being based around a diode array spectrophotometer also allows for continuous, real-time recording of multi-species concentration changes in the receiving compartment. The system is controlled to operate isothermally (via a Peltier control system) at temperatures between 15 and 85 °C. To examine the performance of this novel design, the transfer of tetracaine from a preparation in PEG 400 (20% tetracaine in PEG 400) has been studied. The results have been used to determine flux, lag time and related parameters. The performance of the novel cell is compared with results from traditional Franz cell diffusion studies.

Keywords: Novel diffusion cell; Membrane transport; Tetracaine

1. Introduction

Interest in topical delivery systems is considerable (Hadgraft, 1999) as is the interpretation and understanding of transport processes in skin. Recently this group has introduced (Wood et al., 1997) a new surface enhanced Raman spectroscopic method for following the initial stages of the transport process of compounds through model membranes. This technique does not, however, allow the accumulation of data that leads to interpretation of the developed steady-state processes. The novel diffusion cell reported here is an attempt to address this issue through design of a continuous recording, non-sampling, sensitive system.

The majority of published transport studies, particularly for skin permeation, involves the use of Franz-type diffusion cells (Franz, 1975). These consist of two compartments with a membrane clamped between the donor and receiver chambers. Such diffusion or permeation cells have a

* Corresponding author. Tel.: +44-1634-883-362; fax: +44-1634-883-044.

E-mail address: a.beezer@greenwich.ac.uk (A.E. Beezer).

0378-5173/01/$ - see front matter
PII: S0378-5173(01)00826-2

fixed volume of agitated donor and receptor solutions and can then be used to evaluate the time course for permeation of a compound through a membrane. Partition and diffusion coefficients can then be derived from the slope of the steady-state flux and the lag time, from appropriate solutions to Fick's law of diffusion (Williams and Barry, 1992).

Extrapolation of the steady-state portion of a graph showing the transport of a diffusant across a membrane against time, to the intercept on the abscissa gives the lag time, L. The lag time is related to the diffusion coefficient by:

$$L = \frac{h^2}{6D}, \quad (1)$$

where h is the membrane thickness and D is the diffusion coefficient.

The partition coefficient can then be calculated by rearranging Eq. (2).

$$J = \frac{DCK}{h}, \quad (2)$$

where J is the rate of transport (or flux) obtained from the linear part of the steady-state portion of a graph of the cumulative amount of drug crossing a unit area of membrane, against time. C is the concentration of the diffusant in the donor phase bathing the membrane and K is the membrane/donor partition coefficient. It is often difficult to get reproducible values of L because of the inherent variability in biological membranes. If there are enough data points in the non-steady-state region of the diffusion curve, it is possible to fit a full solution of Fick's second law (Eq. (2)) to give values of D and K, provided h is known (Diez-Sales et al., 1991). However, this may be problematic for membranes like skin where tortuous pathways exist. In this case, an accurate value of h is not available.

Franz cells attempting to imitate in vivo conditions may use a flow-through receptor fluid, imitating the blood supply, with an unstirred donor phase equivalent to a drug formulation (Flynn and Smith, 1971; Nguyen et al., 1993; Brown et al., 1987). Others require the removal of aliquots of the receiving solution, causing disruption to the system (Artursson, 1990; Flynn and Yalkowsky, 1972; Scheuplein, 1965; Mafune et al., 1998; Aguiar and Weiner, 1969; Guy and Fleming, 1979; Stehle and Higuchi, 1972; Sanghvi and Collins, 1993; Bosman et al., 1996). More sophisticated apparatus specifically attempts to minimise the variables and control the overall conditions resulting in reproducible results, representing in vivo conditions more closely.

Thus, an attempt has been made to construct a diffusion cell, which is simple to use, involves non-invasive sampling and provides reproducible results. This technique allows temperature, compartmental concentrations, stirring, dosage, donor or receptor pH and membrane integrity, area and thickness to be easily controlled. The analysis of permeant concentration as a function of time relies upon diode array UV-spectrophotometry.

A simple PTFE cap has been constructed (Fig. 1), which fits onto a UV-spectrophotometer quartz cuvette. The cuvette itself holds an appropriate receiver solution, which is constantly stirred by a magnetic flea. The PTFE cap holds the membrane securely, and possesses an injection port through which compounds or formulations can be applied to the upper membrane surface. Sampling is not required and aliquots do not have to be removed from the receiver solution; therefore, the system is not disrupted and sink conditions can be maintained throughout experimentation. As long as the compound under test gives an absorbance in the UV region of the

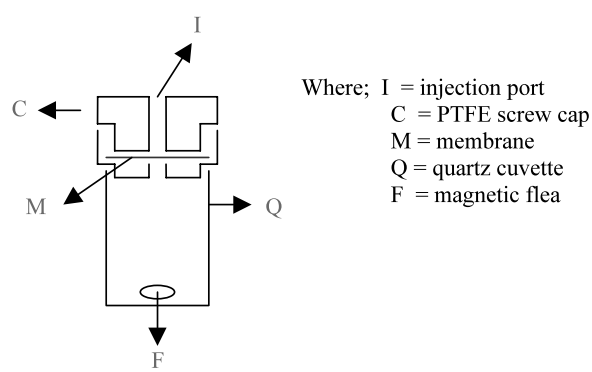

Fig. 1. A schematic diagram of the novel diffusion cell.

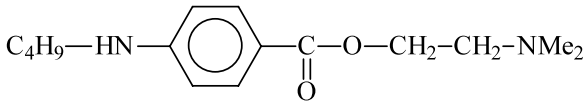

Fig. 2. Tetracaine.

spectrum, its lag time L, and rate of appearance can be determined. Also, by appropriate mathematical analysis, the diffusion and partition coefficients of the compound can be calculated by standard procedures using Eqs. (1) and (2) (Williams and Barry, 1992) or by a full solution to Fick's second law and curve fitting (Diez-Sales et al., 1991).

In order to assess the reproducibility of the novel diffusion cell, a model compound, tetracaine (Fig. 2), which has been well documented for its transport through membranes (Woolfson and McCafferty, 1993; McCafferty et al., 1988a,b; Miller et al., 1993a,b; Foldvari, 1994) was chosen (Fig. 2). Tetracaine is a topical anaesthetic, and has a calculated log P value of 3.75 (ACD software, Advanced Chemistry Development Inc., Toronto, Canada). It has a λ_{max} value of 310 nm in distilled water, though its absorbance spectrum, does have a significant pH dependence.

The spectrophotometer used in this study (Hewlett Packard Diode Array Spectrophotometer, HP 8453 fitted with Hewlett Packard Peltier temperature controller 89090A) has the ability to take readings from 190 to 1100 nm every 0.5 s, and therefore has the potential to monitor the absorbance at several wavelengths, at regular short intervals, over a set period of time. This allows mixtures to be monitored. For example, drug components, together with excipients or penetration enhancers can be monitored in this diffusion cell. This technique could also be beneficial for biological samples, where interfering species can be 'spectroscopically' subtracted using a second order derivative technique (Green and Hadgraft, 1988). Data analysis was conducted via the HP845x UV–Visible System Software.

2. Experimental section

2.1. Chemicals

Tetracaine, M_r, 264.4, ICN Biomedicals Inc.; polyethylene glycol (PEG), Av. M_r, 400.0, Sigma; distilled water, from a Merit water still W4000; high vacuum grease, Dow Corning; non-sterile, A class VI USP Medical Grade silicone rubber, 0.005 in. thickness, Technical Products, Inc., GA; isopropyl myristate (IPM), 98%, Aldrich.

2.2. Method

Twenty percent tetracaine in PEG 400 mixture was tested in the novel diffusion cell. Unbuffered sonicated distilled water was used as the receiver solution for simplicity. PEG was chosen as a carrier for tetracaine as it is spectrophotometrically silent and would not interfere with the signal from tetracaine. In addition, the use of PEG 400 allowed greater control over the concentration of tetracaine presented in the donor compartment. A 20% tetracaine formulation was a convenient concentration to use from which measurable data could be obtained. In order to make the 20% tetracaine in PEG mix, 200.0 mg of tetracaine was weighed on an analytical balance. One millilitre PEG 400 was added to the tetracaine using a Gilson pipette. The tetracaine was dissolved into the PEG with the aid of a Whirlimixer.

Sonicated distilled water was placed in a standard UV-quartz cuvette so that the meniscus came above the lip of the cuvette. A small magnetic flea was dropped into the cuvette, ensuring no air bubbles were introduced. A $\frac{1}{4}$ in. diameter disc of 0.005 in. silastic membrane was cut using a stainless steel cork borer. Stainless steel forceps were used to place the membrane, in the PTFE cap. The lid of the PTFE cap was screwed down onto the membrane gently so as to prevent puckering. The PTFE cap was then turned upside down. High vacuum grease was applied to the sealing edges of the cap to help prevent seepage of air into the system. A small length of silastic tubing was then attached to a sterile needle, which itself was connected to a 1.0-ml syringe. Degassed distilled water was applied to the underside of the

membrane ensuring no air bubbles were present. The PTFE cap was then placed on the quartz cuvette again ensuring no air bubbles were present in the cuvette or on the underside of the membrane. The diffusion cell was finally sealed with a thin strip of sealing film. The cell was placed in the UV-spectrophotometer and left to equilibrate at 25.0 °C and 200.0 rpm for 30 min.

A small length of silastic tubing was attached to another sterile needle attached to a 1.0-ml syringe. 0.03 ml of the 20% tetracaine in PEG 400 mixture was drawn up ensuring no air bubbles were present. The silastic tubing attached to the needle ensured that when the vehicle was applied, the membrane was not punctured in any way.

On the addition of the 20% tetracaine system, analysis of the receiver solution commenced. The absorbance of the receiver solution was monitored at 310 nm (tetracaine λ_{max} in distilled water) every 30.0 s over a 4-h period. The experiment was repeated ten times.

The value for the lag times L, was calculated by extrapolating the steady-state portion of the transport profile to an absorbance of 0. J was determined by calculating the slope of the steady-state region (Section 1). By carrying out a Beer-Lambert calibration experiment for tetracaine in distilled water, the J values were converted to μg cm^{-2} h^{-1}, enabling diffusion and partition coefficients to be calculated using Eqs. (1) and (2).

3. Results

3.1. Novel technique

Plots obtained from the UV-spectrophotometer (Fig. 3) for the transport of tetracaine across a silastic membrane from a PEG 400 formulation at 25.0 °C were, after an initial period, of zero order (Table 1).

L values varied quite significantly (1804 ± 670 s) and this maybe due to irregularities between membrane samples. However, J values are reproducible at 27.5 μg cm^{-2} h^{-1} (± 3.2).

The calculated diffusion coefficient D, from the average J value for 20% tetracaine in PEG 400 passing through a 0.005 in. dry silastic membrane

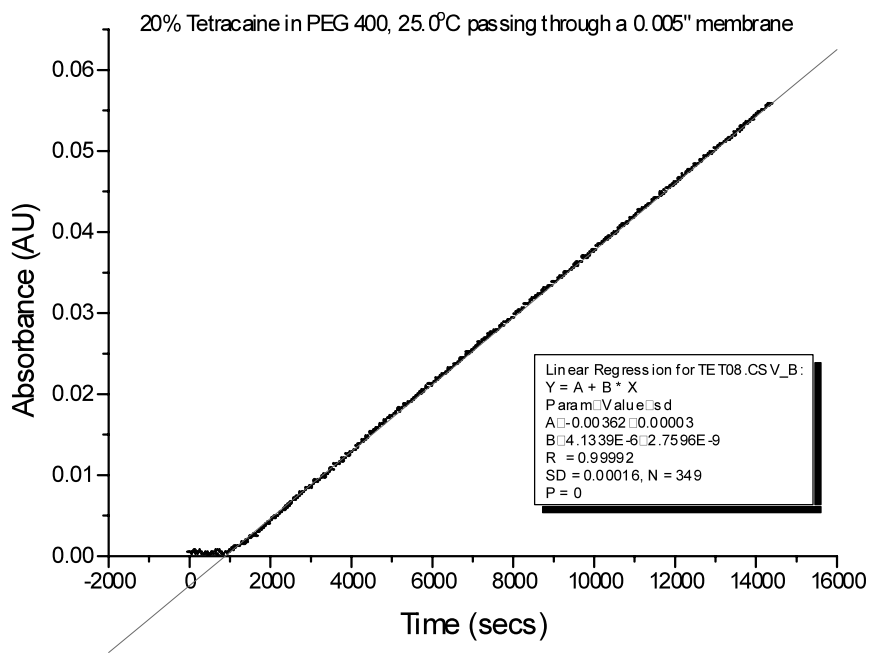

Fig. 3. Example transport profile.

Table 1
Results obtained from such plots as seen in Fig. 3

Experiment no.	Lag time, L (s)	Rate of appearance, J (AU s^{-1})	Rate of appearance, J (μg cm^{-2} h^{-1})
1	934.54	4.43×10^{-6}	24.80
2	1595.70	5.12×10^{-6}	28.66
3	1719.83	4.64×10^{-6}	25.98
4	2432.73	4.46×10^{-6}	24.97
5	901.20	4.15×10^{-6}	23.23
6	1925.86	5.53×10^{-6}	30.96
7	2130.77	5.20×10^{-6}	29.11
8	1902.58	5.03×10^{-6}	28.16
9	3113.98	4.65×10^{-6}	26.03
10	1386.67	6.00×10^{-6}	33.59
Average	1804	4.92×10^{-6}	27.5
S.D.	±670	$\pm 5.64 \times 10^{-7}$	±3.2

(i.e. non-conditioned), was 1.5×10^{-8} cm^2 s^{-1} ($\pm 4 \times 10^{-9}$ cm^2 s^{-1}).

The calculated partition coefficient K, from the average J value was 0.032 (± 0.018).

3.2. Traditional technique

These values were compared with those obtained using traditional Franz cell apparatus.

Conventional diffusion experiments were conducted using all glass Franz-type diffusion cells that have a receptor volume of ≈ 3.9 ml and a diffusional surface area of about 0.95 cm^2. The receptor chambers had side arms through which samples could be taken. To ensure sink conditions and to avoid bubble formation, sonicated deionised water was used as the receptor phase. The silastic membrane 0.005-in. thick, was cut to an appropriate size. Silicone grease was used to produce a leak-proof seal between the flanges of the two halves of the cell held together with a screw clamp. Twenty percent tetracaine in PEG 400 was introduced in the donor compartments and occluded using microscopic cover slips. The receptor compartment of the cells was maintained at 25.0 °C in a water bath. Magnetic followers were used to stir the receptor compartments. The arms were closed with caps to prevent evaporation. At 2-h intervals over 10 h, 0.4 ml of the receptor phase was removed and replaced with an equal volume of pre-thermostated receptor phase. The samples were assayed using UV-spectroscopy (Unicam UV–Visible spectrophotometer: samples were diluted appropriately prior to analysis following calibration using solutions of known concentration). The steady-state flux of tetracaine from the solution was determined by plotting the amount of tetracaine transported across the membrane against time and calculating the slopes for the steady-state region ($n = 4$).

With this traditional method measurements are taken every 2 h for 10 h. The initial processes of transport are therefore not monitored using this methodology and L values were not determined. This should be contrasted with the novel diffusion cell, where analysis starts from time zero and only lasts 4 h, with a reading taken every 2 min.

The traditional diffusion cell gives an average J value of 66 μg cm^{-2} h^{-1}, which is comparable with that obtained from the novel cell method. However, results are obtained much more quickly and in more detail without disruption to the system via the new technique reported here. The difference between this value and that obtained from the novel diffusion cell may be due to batch-to-batch variability in the silastic membrane samples used.

3.3. Hydrodynamic effects

To study the importance of the hydrodynamic effects of the novel diffusion cell, the system was tested further by varying the speed of the magnetic stirrer. The speed is controlled using the

UV-spectrophotometer software, and no attempt was made to verify the actual speed, only nominal speeds are reported here. The spectrophotometer has the potential to control the magnetic stirrer from 40 to 1000 rpm, with 200 rpm being the default speed.

Therefore, the transport of 20% tetracaine in PEG 400 at 25.0 °C was monitored at 0, 100, 300 and 400 rpm and compared with the initial 200 rpm results. Table 2 and Fig. 4 show the average values obtained from carrying out a diffusion experiment at each speed ($n = 4$).

It can be seen that between 100 and 400 rpm, there is no appreciable difference in the rate at which tetracaine passes through the membrane over the stirring range. As expected, for an unstirred receiver solution absorbance against time is varied and non-linear (data not presented nor displayed in the Figure). This is likely to be due to the non-homogeneity of the unstirred receiver solution. Plots from 100 to 400 rpm, however, are linear and reproducible, though the higher the stirring speed, the smaller the S.D. value for the rates of appearance. Similarly lag time values do not change over the stirring range but at 100 rpm they are varied and unreproducible as would be expected, but become more reproducible at more moderate stirring speeds.

3.4. Treated membranes

In many diffusion experiments the synthetic membranes used are not always dry but they are conditioned in the same way. Membranes are often pre-soaked in water or in IPM.

Such membranes were also tested in the novel diffusion cell. Membrane samples were soaked in water or IPM for 24 h before use. Zero order plots for the transport of 20% tetracaine in PEG 400 were obtained through treated membranes, J values being approximately twice that of those in which the formulation was applied to the dry membrane, shown in Fig. 4.

J values are reproducible at 43.5 μg cm^{-2} h^{-1} (± 5.0), through a membrane soaked in water, and 50.1 μg cm^{-2} h^{-1} (± 3.5), through a membrane soaked in IPM. Lag time values are 1525 s (± 505) and 5869 s (± 251), respectively.

4. Discussion and conclusion

In conclusion, the novel diffusion cell gives reproducible rate values for the transport of 20% tetracaine in PEG 400 across a 0.005 in. dry membrane at 25.0 °C and 200 rpm with small S.D. values. Varying the stirring speed of the magnetic flea from 100 to 400 rpm causes only a slight change in the rate of transport. However, if the receiver solution is not stirred, the non-homogeneity gives rise to irregular data, as expected. Soaking the silastic membrane in water or IPM shows an increase in transport.

Lag time values have large S.D. values associated with them. This in turn affects the S.D. associated with the derived values for the diffusion and partition coefficients.

The cell/spectrophotometer system has a high degree of sensitivity. On average, the percentage of tetracaine applied to the dry membrane that passes through during a 4-h period is 0.039%, in terms of concentration this is $\approx 2.23 \times 10^{-6}$ mol dm^{-3}. For a water or IPM soaked membrane, this percentage can increase to 0.082% (4.66×10^{-6} mol dm^{-3}).

Table 2
Rate of appearance and lag time values for 20% tetracaine passing through a 0.005-in. dry silastic membrane, at 25.0 °C, at varying stirring speeds

Stirrer speed (rpm)	Rate of appearance (AU s^{-1})	Rate of appearance (μg cm^{-2} s^{-1})	S.D. of rate of appearance (μg cm^{-2} s^{-1})	Lag time (s)	S.D. of lag time (s)
100	3.75×10^{-6}	21.0	±1.9	2081	±1721
300	3.96×10^{-6}	22.2	±1.2	1497	±194
400	3.38×10^{-6}	18.9	±0.5	2341	±210

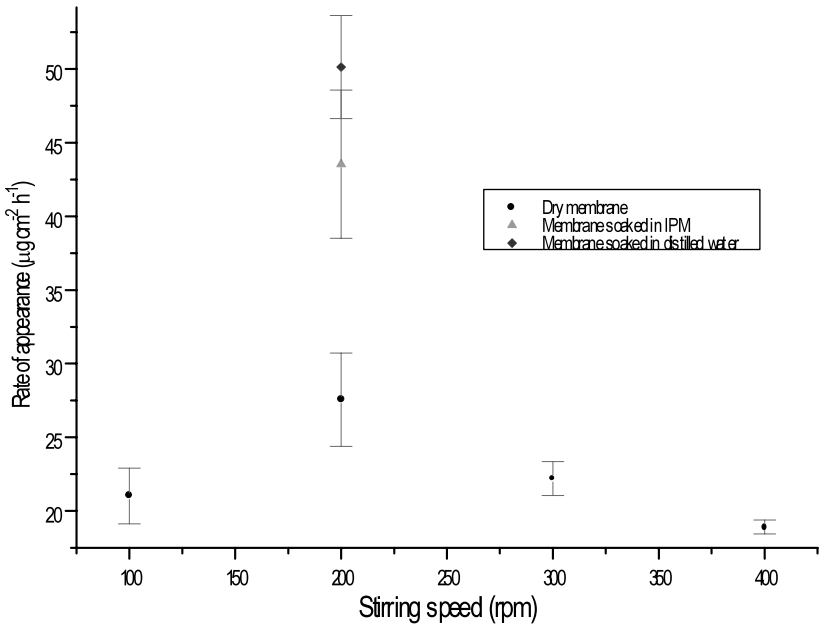

Fig. 4. The rate of appearance for 20% tetracaine in PEG 400 as a function of stirrer speed and through differently treated 0.005-in. silastic membranes at 25.0 °C.

The initial experiments carried out with the novel diffusion cell for use in a UV-spectrophotometer, have provided reproducible rates of appearance values for the transport of a 20% tetracaine in PEG 400 mixture through a silastic membrane. Lag time values are not so reproducible, but may be explained due to irregularities between membrane samples.

As the UV-spectrophotometer is connected to a Peltier heater, the temperature of the cell can easily be changed and maintained. Thus, temperature profiles can be constructed enabling activation energies to be calculated which in turn may provide information on the mechanism of transport, i.e. intracellular or intercellular diffusion in skin. Investigations on the effect of formulation, in terms of the presence of excipients and/or penetration enhancers as well as dosage volume are also a possibility.

This non-invasive method has great potential for easy and quick penetration analysis. The lag time and rate of appearance can easily be obtained from the raw data. The system does not require removal of samples for analysis as with other Franz cell type apparatus. It has the potential to be automated. The only constraint with this method, however, is the species under test must be detectable within the UV region of the spectrum.

This technique could be utilised to analyse a vast array of compounds, such as drugs, agrochemicals, different formulations of species, and the effects of penetration enhancers, through various membrane types, such as synthetic, animal, human skin, cultured cells, packaging materials or even leaf discs.

References

Aguiar, A.J., Weiner, M.A., 1969. Percutaneous absorption studies of chloramphenicol. J. Pharm. Sci. 58, 210–215.

Artursson, P., 1990. Epithelial transport of drugs in cell culture. I. A model for studying the passive diffusion of drugs over intestinal absorbtive (Caco-2) cells. J. Pharm. Sci. 79, 476–482.

Bosman, L.J., Lawant, A.L., Avegaart, S.R., Ensing, K., de Zeeuw, R.A., 1996. Novel diffusion cell for in vitro transdermal permeation, compatible with automated dynamic sampling. J. Pharm. Biomed. Anal. 14, 1015–1023.

Brown, L.R., Cline, L.F., Raleigh, C.L., Henry, M.B., 1987. Improved method for measuring in vitro diffusion of drugs through human skin. Controlled Release Technol. 113–119 (Chapter 8).

Diez-Sales, O., Copovi, A., Casabo, V.G., Herreaz, A., 1991. A modelistic approach showing the importance of the stagnant aqueous layers in in vitro diffusion studies, and in vitro–in vivo correlations. Int. J. Pharm. 77, 1–11.

Flynn, G.L., Smith, E.W., 1971. Membrane diffusion. I. Design and testing of a new multifeatured diffusion cell. J. Pharm. Sci. 60, 1713–1716.

Flynn, G.L., Yalkowsky, S.H., 1972. Correlation and prediction of mass transport across membranes. I. Influence of alkyl chain length on flux-determining properties of barrier and diffusant. J. Pharm. Sci. 61, 838–852.

Foldvari, M., 1994. In vitro cutaneous and percutaneous delivery and in vivo efficacy of tetracaine from liposomal and conventional vehicles. Pharm. Res. 11, 1593–1598.

Franz, T.J., 1975. Percutaneous absorption and the relevance of in vitro data. J. Invest. Derm. 64, 190–195.

Green, P.G., Hadgraft, J., 1988. The use of second-order derivative UV spectroscopy to monitor the percutaneous absorption of naphazoline and oxprenolol. Int. J. Pharm. 46, 193–198.

Guy, R.H., Fleming, R., 1979. The estimation of diffusion coefficients using the rotating diffusion cell. Int. J. Pharm. 3, 143–149.

Hadgraft, J., 1999. Passive enhancement strategies in topical and transdermal delivery. Int. J. Pharm. 184, 1–6.

Mafune, E., Takahashi, M., Taksugi, N., 1998. In vivo and in vitro evaluations of water-absorption properties of various ointments. Drug Dev. Ind. Pharm. 24, 51–56.

McCafferty, D.F., Woolfson, A.D., McClelland, K.H., Boston, V., 1988a. Comparative in vivo and in vitro assessment of the percutaneous absorption of local anaesthetics. Br. J. Anaesth. 60, 64–69.

McCafferty, D.M., Woolfson, A.D., McClelland, K.H., Boston, V., 1988b. Concentration–response analysis of percutaneous local anaesthetic formulations. Br. J. Anaesth. 61, 589–592.

Miller, K.J., Goodwin, S.R., Westermann-Clark, G.B., Shah, D.O., 1993a. Solubility and in vitro percutaneous absorption of Tetracaine from solvents of proylene glycol and saline. Int. J. Pharm. 98, 101–111.

Miller, K.J., Goodwin, S.R., Westermann-Clark, G.B., Shah, D.O., 1993b. Evaluation of local anaesthesia provided by transdermal patches containing different formulations of tetracaine. J. Pharm. Sci. 82, 1123–1125.

Nguyen, Q.T., Gref, R., Clement, R., Lenda, H., 1993. Differential permeation – Part I: A method for the study of solvent diffusion through membranes. Colloid Polymer Sci. 271, 1134–1142.

Sanghvi, P.P., Collins, C.C., 1993. Comparison of diffusion studies of hydrocortisone between the Franz cell and the enhancer cell. Drug Dev. Ind. Pharm. 19, 1573–1585.

Scheuplein, R.J., 1965. Mechanism of percutaneous adsorption. I. Routes of penetration and the influence of solubility. J. Investig. Dermatol. 45, 334–346.

Stehle, R.G., Higuchi, W.I., 1972. In vitro model for transport of solutes in three-phase system. II. Experimental considerations. J. Pharm. Sci. 61, 1931–1935.

Williams, A.C., Barry, B.W., 1992. Skin absorption enhancers. Critical Rev. Therap. Drug Carrier Syst. 9, 305–353.

Wood, E., Sutton, C., Beezer, A.E., Creighton, J.A., Davis, A.F., Mitchell, J.C., 1997. Surface enhanced Raman scattering (SERS) study of membrane transport processes. Int. J. Pharm. 154, 115–118.

Woolfson, A.D., McCafferty, D.F., 1993. Percutaneous local anaesthesia: drug release characteristics of the amethocaine phase-change system. Int. J. Pharm. 94, 75–80.