International Journal of Pharmaceutics, 3 (1979) 143–149

THE ESTIMATION OF DIFFUSION COEFFICIENTS USING THE ROTATING DIFFUSION CELL

RICHARD H. GUY and ROBERT FLEMING

Department of Pharmaceutical Chemistry, School of Pharmacy, University of London, 29/39 Brunswick Square, London WC1 (England)

(Received November 29th, 1978)
(Accepted January 8th, 1979)

SUMMARY

The rotating diffusion cell developed by Albery et al. (1976) has been used to estimate the diffusion coefficients of two esters of nicotinic acid in a selection of organic barriers. It is shown that the diffusion coefficients of these esters in some of the systems investigated are comparable to their values in the epidermis of human skin. The rotating diffusion cell is therefore proposed as a possible in vitro method for studying the percutaneous absorption of certain drugs.

INTRODUCTION

Having shown how a stable interface could be established between two liquids on the sinter of a Stokes cell (Stokes, 1950), Albery et al. (1974, 1976) developed the rotating diffusion cell to study further the kinetics of interfacial transfer. The cell uses the hydrodynamics of the rotating disc system (Riddiford, 1966) to impose a known pattern of convective diffusion on both sides of a Millipore filter; the latter being preferred to a sinter because its smaller thickness (0.15 mm compared with 3 mm) reduces the response time and enables faster interfacial transfer rates to be measured. The rotation of the filter establishes two stagnant diffusion layers of known thickness on either side of the filter. In all the systems described in this paper two interfaces are set up: the filter contains the organic phase with the aqueous phase above and below the filter.

The majority of systems studied previously (Albery et al., 1976; Albery and Hadgraft, 1979a) have employed isopropyl myristate (IPM) as the organic phase. The choice of IPM reflects a suggestion that it is a good model compound for skin lipids (Poulsen et al., 1968). Results are presented here which enable the diffusion coefficients of methyl and ethyl nicotinates in several different organic phases to be estimated; the values obtained are compared with available in vivo percutaneous absorption data.

MATERIALS AND METHODS

A cross-sectional diagram of the rotating diffusion cell is shown in Fig. 1; a detailed description has been given previously (Albery et al., 1976). The Millipore filter (F), which

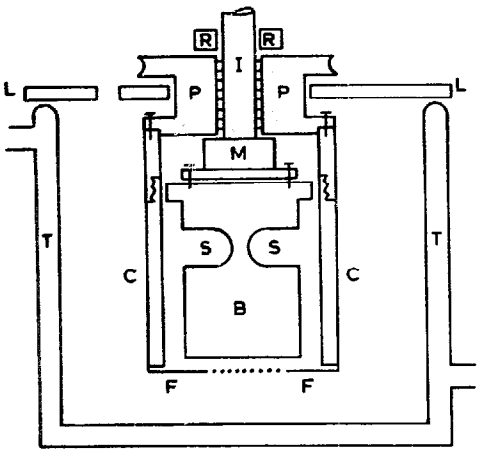

Fig. 1. Cross-sectional diagram of the rotating diffusion cell. The central cylinder (C) rotates within a thermostatted jacket (T). The treated Millipore filter (F) divides the cell into two compartments. Rotating disc hydrodynamics are established in the inner compartment by the incorporation of a PTFE baffle (B), which is positional rigidly by means of a hollow stainless steel shaft (I). The slots (S) in the baffle are necessary to achieve the correct hydrodynamic flow (see Albery and Hadgraft, 1979a). Rotation of the cell is achieved via the pulley (P) and vertical movement of the inner cylinder is prevented by the mantle (M) and a removable locking collar (R). The rotating assembly is attached to a perspex lid (L) wich prevents excessive evaporation. The lid is drilled to facilitate periodic sampling of the solution in the outer compartment.

s treated so that only the central dotted region remains permeable (see below), is mounted on the rotating perspex cylinder (C), thereby dividing the cell into two compartments. The stationary baffle (B) ensures that rotating disc hydrodynamics are established on the inside of the filter. The diffusion of the nicotinic acid esters from the inner to the outer compartment is followed at 37°C by removing samples periodically and measuring the concentration spectrophotometrically.

Partition coefficients for systems 1–3 and 6–8 are determined by shaking the system for about 24 h followed by spectrophotometric analysis of the solute. This procedure proved impossible for systems 4, 5, 9 and 10 for which the organic barrier consisted of dilute solutions of phospholipid in IPM. On vigorous shaking of these systems with aqueous solutions of nicotinate the lipid precipitated out in the aqueous phase. The partition coefficient values were therefore estimated from the relative solubilities of the nicotinates in IPM and in the lipid-containing IPM solutions.

All chemicals used are reagent grade and supplied commercially.

Filter preparation

A 47 mm diameter Millipore cellulose ester MF type filter (0.22 μm pore size) is first mounted on the detachable end of the perspex cylinder (C) using Millipore MF cement. The pores in the outer region of the filter are then collapsed (with a solution containing by volume 33% dioxan, 33% dichloroethane, 33% hexane and 1% water) to leave a white, opaque, permeable centre (approximately 1 cm in diameter) surrounded by the trans-

parent, impermeable, collapsed material. The filter is then treated with a few drops of 'Repelcote' [1] which renders it hydrophobic before it is impregnated with the organic phase; the latter is achieved by placing a few drops of the substance on the filter, waiting for a few minutes and then wiping any excess away with absorbent tissue.

THEORY

Detailed derivation of the equations used to analyze the results have already been given in the original paper on the rotating diffusion cell (Albery et al., 1976).

The rate of transfer (J mol^{-1}) of the diffusing species from the inner compartment to the outer can be expressed

$$J = kAc_I \tag{1}$$

where A is the area of the filter, c_I is the bulk concentration in the inner compartment and k for the systems described in this paper is given by:

$$\frac{1}{k} = \frac{2z_D}{D_{aq}} + \frac{2}{\alpha k_{-I}} + \frac{Kl}{\alpha D_{org}} \tag{2}$$

The three terms on the right-hand side of Eqn. 2 have the following significance:

(1) $2z_D/D_{aq}$ describes diffusion through the aqueous stagnant diffusion layers on either side of the filter, where z_D, the thickness of these layers, is given by the Levich equation (Levich, 1962)

$$z_D = 0.643\, w^{-1/2}\, \nu^{1/6}\, D_{aq}\, 1/3 \tag{3}$$

ν being the kinematic viscosity and w (revolutions per second) the rotation speed.

(2) $2/\alpha k_{-I}$ describes the interfacial transfer reactions (the factor of two appearing because there are two such reactions), where α (the value of which is 0.75 for a 0.22 μm filter) is the area of the pores of the filter divided by A. k_{-I} and K for a species M are described as follows

$$M(org) \underset{k_{-I}}{\overset{k_I}{\rightleftharpoons}} M(aq), \quad K = \frac{k_I}{k_{-I}}$$

(3) $Kl/\alpha D_{org}$ describes diffusion through the filter of thickness l where D_{org} is the diffusion coefficient of the solute in the organic phase. Because z_D is proportional to $w^{-1/2}$ as defined by the Levich equation, it follows from Eqn. 2 that a plot of k^{-1} against $w^{-1/2}$ should be a straight line having

$$\text{slope} = 1.286\, \nu^{1/6}\, D_{aq}^{-2/3} \tag{4}$$

[1] 2% solution of dimethyldichlorosilane in CCl_4 supplied by Hopkins and Williams Ltd., Chadwell Heath, Essex, England.

$$\text{intercept} = \frac{2}{\alpha k_{-1}} + \frac{Kl}{\alpha D_{org}} \quad (5)$$

On extrapolation to inifinite rotation speed ($z_D \rightarrow 0$), therefore, the term in Eqn. 2 corresponding to transport on either side of the filter disappears leaving the terms corresponding to diffusion through the filter and the interfacial transfer steps.

RESULTS AND DISCUSSION

The experimental results are summarized in Tables 1 and 2 and Figs. 2 and 3. For each system 1/k is plotted against $w^{-1/2}$ and values for the slope and intercept, which are described by Eqns. 4 and 5, respectively, are derived. It should first be observed that for all 10 systems the values of the measured slopes are in good agreement with those predicted theoretically. This shows that the correct hydrodynamic conditions for controlled transport have been achieved both above and below the rotating disc of filter paper.

Consideration of Eqn. 5 reveals that, having experimentally determined the numerical value of the intercept, there remain two unknowns on the right-hand side: namely k_{-I} and D_{org}. For system 1, D_{org} has been measured using the Stokes cell (Albery et al., 1976) and hence from the value of the intercept we find

$$k_{-I} = 85 \text{ mMs}^{-1}$$

This value is somewhat larger than that determined previously by Albery et al. (1976) of 46 mMs^{-1}. In order to estimate D_{org} for systems 2–5 we now assume that k_{-I} retains the

TABLE 1

RESULTS FOR METHYL NICOTINATE

System	Organic phase	K	[a] Slope/ $(\text{mMs}^{-1})^{-1}\text{s}^{-1/2}$	[b] Intercept/ $(\text{mMs}^{-1})^{-1}$	Estimated $D_{org}/10^{11}$ m^2s^{-1}
1	IPM	0.39	0.1008	0.1841	51.0 [c]
2	Tetradecane	1.60	0.0950	0.3219	110.2
3	Linoleic acid [d]	0.15	0.0980	0.2415	14.3
4	EL in IPM [e]	0.20	0.1031	0.2281	20.3
5	DPPC in IPM [f]	0.20	0.1117	0.2794	16.1

[a] The value predicted by the theory (Eqn. 4) is 0.1065 $(\text{mMs}^{-1})^{-1}\text{s}^{-1/2}$ (using $D_{aq} = 1.2 \times 10^{-9}$ m^2s^{-1} (Albery et al., 1976) and $\nu_{aq} = 0.67$ m^2Ms^{-1}).

[b] The value of the right-hand side of Eqn. 5.

[c] A measured value using the Stokes cell (Albery et al., 1976).

[d] 9,12-octadecadienoic acid ($C_{17}H_{31}CO_2H$) supplied by Koch-Light.

[e] An approximately 2% solution of egg lecithin (prepared by the method of Hanahan et al. (1952) and Singleton et al. (1962)).

[f] An approximately 1% solution of DL-$\beta\gamma$-dipalmitoyl-α-lecithin ($C_{40}H_{80}NO_8P$) supplied by Fluka A.G.

TABLE 2

RESULTS FOR ETHYL NICOTINATE

System	Organic phase	K	[a] Slope/ $(mMs^{-1})^{-1}s^{-1/2}$	[b] Intercept/ $(mMs^{-1})^{-1}$	Estimated $D_{org}/10^{11}$ m^2s^{-1}
6	IPM	0.11	0.1016	0.0735	46.0 [b]
7	Tetradecane	0.35	0.1094	0.1113	81.7
8	Linoleic acid	0.05	0.1112	0.1092	12.0
9	EL in IPM	0.08	0.1007	0.1265	15.9
10	DPPC in IPM	0.08	0.1001	0.1400	14.0

[a] The value predicted by Eqn. 4 is 0.1157 $(mMs^{-1})^{-1}s^{-1/2}$ (using $D_{aq} = 1.06 \times 10^{-9}$ m^2s^{-1}).
[b] Estimated from D_{aq} and the ratio of D_{aq}/D_{org} for methyl nicotinate in water and IPM.
[c] Note that for each organic phase the ratio $(D_{org})_{MeNic}/(D_{org})_{EtNic}$ has very nearly the same value (of about 1.1–1.3); this is in good agreement with Eqn. 6.

same value as determined in system 1. This assumption can be justified on the grounds that the organic phases chosen in this work have eseentially the same characteristics, and in particular they all contain a large saturated hydrocarbon component. Furthermore, it has been demonstrated (Albery et al., 1976) that the interfacial transfer term in Eqn. 5 invariably contributes less than 1/3 to the value of the measured intercept. For one

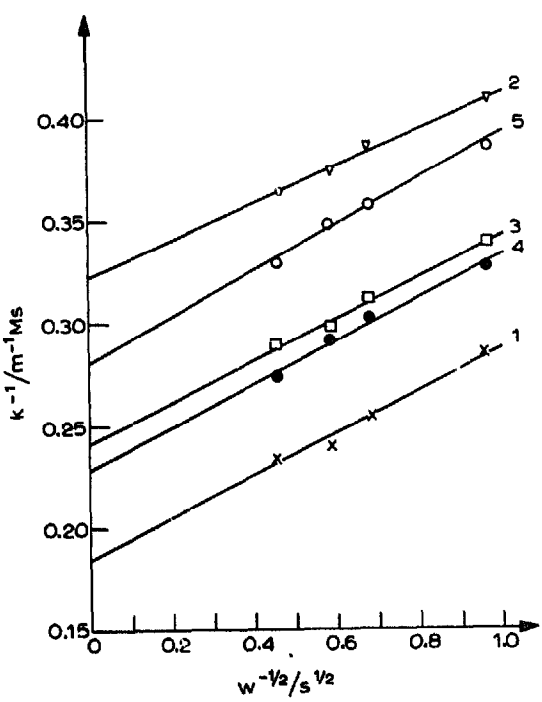

Fig. 2. Plots of k^{-1} against $w^{-1/2}$ for systems 1–5 (see Table 1, results for methyl nicotinate).

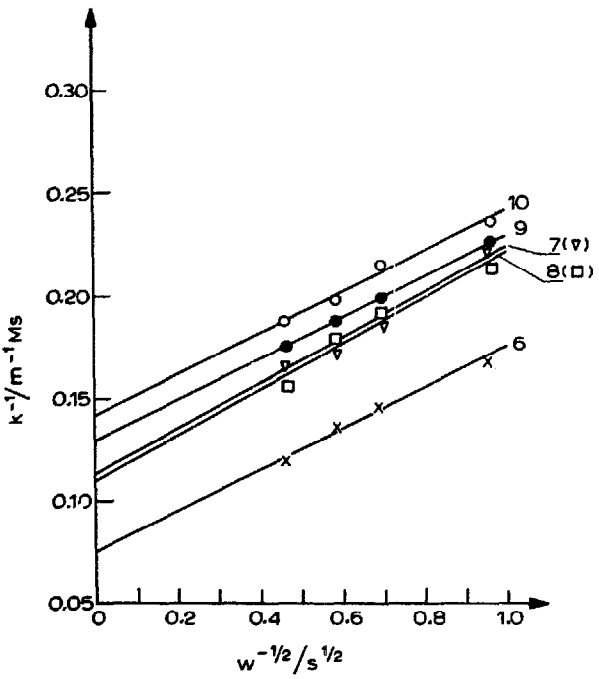

Fig. 3. Plots of k^{-1} against $w^{-1/2}$ for systems 6–10 (see Table 2, results for ethyl nicotinate).

particular solute, therefore, a change in the value of the intercept as the organic barrier is altered will essentially be due to a change in the term describing diffusion through the filter.

For ethyl nicotinate no independent measure of its aqueous diffusion coefficient has been made. However, a reliable estimate of this parameter can be found by extrapolation between experimentally determined values of D_{aq} for methyl, butyl and hexyl nicotinates (Albery et al., 1976). Using this D_{aq} (10.6×10^{-9} m^2s^{-1}) and the ratio D_{aq}/D_{IPM} for methyl nicotinate, we can find D_{IPM} for ethyl nicotinate from

$$\frac{(D_{aq})_{MeNic}}{(D_{aq})_{EtNic}} = \frac{(D_{IPM})_{MeNic}}{(D_{IPM})_{EtNic}} \qquad (6)$$

This method gives $(D_{IPM})_{EtNic} = 46 \times 10^{-11}$ m^2s^{-1} and hence, from the value of the intercept for system 6, we find that for ethyl nicotinate

$$k_{-I} = 104 \text{ mMs}^{-1}$$

As for methyl nicotinate, we assume that k_{-I} remains constant in the other systems (7–10) and thereby estimate the corresponding values for $(D_{org})_{EtNic}$.

The results in Tables 1 and 2 demonstrate that while tetradecane represents a weaker barrier than IPM, the two nitotinates diffuse much more slowly through the other organic phases. That tetradecane is the weakest barrier can be understood in terms of its saturated

hydrocarbon structure having little scope for interaction with the diffusing solutes. Similarly, it is hardly surprising that the dissolution of phospholipids in IPM improves its barrier capabilities. The reasons why linoleic acid should provide the greatest barrier to diffusion are less obvious, but chain length (C_{18} compared to C_{14} for IPM and tetradecane), the presence of two double bonds, and the change from an ester to an acid, must all be contributory factors.

The percutaneous absorption of methyl, butyl and hexyl nicotinates has been studied in vivo by observing the time taken for erythema to develop after their application (Albery and Hadgraft, 1979c; Albery et al. [2]). The mathematics describing the diffusion of these solutes through the skin into the blood stream have been solved (Albery and Hadgraft, 1979b) and their diffusion coefficients across the stratum corneum (which represents the major barrier to the diffusion of drugs through the skin) have been estimated to be of the order of 2×10^{-11} m^2 s^{-1}. The results obtained in this work suggest that linoleic acid or dilute solutions of phospholipid in IPM are better models of studying the diffusion of small solutes across the skin than IPM itself.

This information and use of the carefully controlled hydrodynamic conditions of the rotating diffusion cell, therefore, may well provide a useful technique for the in vitro study of the absorption of drugs through the skin.

ACKNOWLEDGEMENTS

The authors would like to thank Professor W.J. Albery and Dr. J. Hadgraft for their helpful criticisms and suggestions.

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