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Solute transport resistance at the octanol–water interface

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Summary

A rotating diffusion cell has been used to determine the kinetics and thermodynamics associated with the transport of methyl nicotinate (3-pyridine-carboxylic acid, methyl ester) across an aqueous phase/*n*-octanol liquid–liquid interface. The selection of the organic phase reflects the frequent successful use of octanol to simulate the properties of biomembranes and biophases in general. Hence the experiments are designed to probe, under carefully controlled conditions in the laboratory, a physicochemical event common to passive membrane transport and other pharmaceutically relevant partitioning processes. The results demonstrate that a significant free energy input is required for interfacial transfer and that the enthalpic and entropic contributions to the barrier depend upon the direction of partitioning. The kinetics of the liquid–liquid transfer process imply that interfacial transport has the potential to be rate-limiting in many biological and pharmaceutical systems.

Introduction

The relationship between lipophilic character and biological activity of drug molecules has been and remains a subject of considerable interest and importance (Hansch, 1969, 1979; Hansch and Clayton, 1973; Hansch and Dunn, 1972; Leo et al., 1971). Frequently addressed is the correlation of substrate bioactivity with an oil/water partition coefficient (e.g. Diamond and Wright, 1969; Wright and Di-

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amond, 1969a; 1969b). In the large majority of cases, the oil phase employed has been *n*-octanol and the distribution constants of numerous solutes between this organic liquid and water have been determined (Leo et al., 1971) and successfully related to biological or pharmacological observations. The reasons for the success of *n*-octanol as a model 'biophase' remain somewhat unclear; certainly the molecule has a substantial lipid portion and a hydrogen-bonding capability, characteristics common to both proteins and bilayer membranes. However, such a comparison, though true in a very simplistic sense, cannot provide more than a superficial explanation for the applicability of octanol in predicting in vivo behavior. Nevertheless, despite this lack of understanding, octanol continues to be used and remains a remarkably good simulation medium. Because of this fact, we have selected octanol as the organic liquid model to study in vitro a process of relevance to many biological and pharmaceutical systems, namely the transfer of substrate at an aqueous solution–lipid phase interface.

Solute interfacial transfer in two-phase liquid–liquid systems represents a physicochemical event that has proved difficult to quantify (Sears, 1973). This is the result of two stringent experimental criteria that must be satisfied for accurate determination of interfacial transfer rate constants: (i) the area of the interface must be accurately known; and (ii) so that solute interfacial concentrations may be related to their respective bulk phase values, the hydrodynamics of the transport process delivering solute to and removing solute from the interface must be understood and well-controlled. In the laboratory such conditions may be achieved and, most recently, a rotating diffusion cell has been successfully used to assess the kinetics and thermodynamics of phase transfer in a number of biologically relevant systems (Albery et al., 1976; Fleming et al., 1983; Guy and Hadgraft, 1981; Ahmed et al., 1982; 1983). The results have demonstrated that a substantial free energy barrier (~ 40 KJ $\cdot$ mol^{-1}) to interfacial transfer exists and this has led to a re-examination of the role of interfacial transport in determining, for example, the overall rate of membrane permeation and the kinetics of drug release from biphasic pharmaceutical delivery systems.

In this investigation, we consider the movement of a solute across an interface established between an aqueous phase and the ubiquitous biophase model, *n*-octanol. Rate constants at 5 temperatures and thermodynamic parameters are reported and the interpretation of the physicochemical information is discussed.

Materials and Methods

Interfacial transfer kinetics were measured with a rotating diffusion cell (RDC) (Albery et al., 1976). The technique has been described in detail elsewhere (Albery et al., 1976). In summary, an octanol-impregnated 0.22-μm pore size GS Millipore filter (treated so that only a small central region remained permeable) separated an aqueous inner compartment solution from an aqueous receptor phase, thereby establishing interfaces on both surfaces of the filter. The filter area and porosity defined the area of the interface and transport of solute towards and away from the

interfacial regions was described by rotating disc hydrodynamics.

It was found that the interfacial stability between an aqueous solution and *n*-octanol could be considerably enhanced by the presence of a high concentration of electrolyte in the former. Furthermore, improvement in this regard was also achieved by lowering the temperature of the system and, thus, we have performed kinetic measurements over the range 5–25°C.

The initial experimental configuration employed in this investigation was the following:

Inner compartment: 40 cm^3 of 0.1 M methyl nicotinate in 1 M aqueous potassium chloride (KCl);

Filter impregnated with *n*-octanol;

Outer compartment: 250 cm^3 of 1 M aqueous KCl solution.

The selection of methyl nicotinate (3-pyridine-carboxylic acid, methyl ester) as the transporting substrate was made because its frequent use in previous RDC work (Albery et al., 1976; Fleming et al., 1983; Guy et al., 1982a, b, c) provided certain already determined physicochemical characteristics and RDC information with which to compare the results from this system. To check that a high aqueous phase ionic strength did not alter interfacial transfer kinetics for this solute, experiments were performed in which the above design was altered such that the filter was impregnated with a different organic phase, isopropyl myristate (IPM). The results were then compared with published RDC data (Fleming et al., 1983; Guy et al., 1982c) for the transport of methyl nicotinate from water, through IPM, into water.

At each of the temperatures considered, solute flux from the inner compartment aqueous phase to the outer was monitored as a function of cell rotation speed by periodically removing 3 cm^3 samples from the outer solution and measuring the concentration by UV spectrophotometry. Interpretation of the data obtained followed published procedures (Albery et al., 1976).

Octanol/aqueous KCl partition coefficients (K) of methyl nicotinate at the appropriate temperatures, which were required for analysis of the results, were found classically by shaking continuously an aqueous solution of solute with an equal volume of octanol for approximately 48 h and then analyzing for the substrate spectrophotometrically.

To obtain interfacial transfer rate constants it was also necessary to know the diffusion coefficient of methyl nicotinate in both the aqueous and organic phases at each temperature. Aqueous diffusion coefficients (D_{aq}) have been reported down to 20°C (Albery et al., 1976; Fleming et al., 1983; Guy et al., 1982c) and were checked with a filter paper diaphragm diffusion cell (Cadman et al., 1981; Kreevoy and Wewerka, 1967). Below 20°C, D_{aq} was evaluated using the data at higher temperatures and the Stokes-Einstein relation in a standard procedure (Tanford, 1961). The diffusion coefficient of the substrate in octanol (D_o) at 25°C was also measured with the diaphragm diffusion cell. This result was equal to that obtained using the empirical method of Chang and Wilke (1955) in conjunction with literature values for *n*-octanol viscosity at 15°C and 30°C (Timmermans, 1950) and a linear extrapolation of calculated $\ln D_o$ against reciprocal absolute temperature. Because of this excellent agreement, the empirical approach was therefore employed to determine

TABLE 1

OCTANOL/WATER PARTITION COEFFICIENTS[1] AND AQUEOUS AND OCTANOL DIFFUSION COEFFICIENTS[2] FOR METHYL NICOTINATE AT 5 TEMPERATUES

T (°C)	K	$10^9 \cdot D_{aq}$ ($m^2 \cdot s^{-1}$)	$10^9 \cdot D_o$ ($m^2 \cdot s^{-1}$)
5	7.04	0.48	0.17
10	7.46	0.57	0.22
15	8.26	0.66	0.27
20	8.62	0.77	0.33
25	9.90	0.88	0.41

[1] Values are the mean of at least 3 determinations; the maximum S.D. was ±3%.

[2] The accuracy of these parameters is to within ±4–5% (Cadman et al., 1981; Kreevoy and Wewerka, 1967).

the remaining D_o values at the other temperatures.

The numerical determinations and estimations of K, D_{aq} and D_o for methyl nicotinate at the 5 temperatures studied are collected in Table 1.

Methyl nicotinate, *n*-octanol and potassium chloride were obtained at least 99% pure from Sigma Chemical, St. Louis, and were considered satisfactory for use without further purification. Aqueous solutions were prepared with water distilled from an all glass apparatus and were saturated with octanol before use.

Theory

Solute transport measurements in the RDC experiments of this study yield an overall permeability coefficient (P) given by Eqn. 1 (Albery et al., 1976):

$$\frac{1}{P} = \frac{2(0.643\nu^{1/6}\omega^{-1/2})}{D_{aq}^{2/3}} + \frac{h}{\alpha K D_o} + \frac{2}{\alpha k_{ao}} \qquad (1)$$

The first term is the resistance to substrate movement through aqueous diffusion layers on either side of the rotating filter; ν is the kinematic viscosity of the aqueous phase and ω is the cell rotation speed. $h/\alpha K D_o$ is the barrier to diffusion through the octanol-impregnated filter of thickness h and porosity α; $h = 150\ \mu m$ and $\alpha = 0.75$ for the filters used in this investigation (Albery and Fisk, 1981). The third contribution to 1/P is the interfacial transfer resistance; k_{oa} is the aqueous–octanol transport rate constant and is related to the kinetics in the opposite direction (k_{oa}) by the partition coefficient ($K = k_{ao}/k_{oa}$).

Experimentally 1/P is measured as a function of $\omega^{-1/2}$; the theoretical slope is forced through the data to obtain the intercept ($h/\alpha K D_o + 2/\alpha k_{ao}$) in which the only unknown is k_{ao}. Hence interfacial transfer coefficients in both directions may be obtained since K is determined independently.

Results and Discussions

RDC experiments were first performed with methyl nicotinate transferring from a 1 M aqueous potassium chloride solution across an isopropyl myristate (IPM)-impregnated filter into 1 M KCl (aq.) at 25°C. The results are shown in Fig. 1 and are compared to previously published values (Fleming et al., 1983; Guy et al., 1982a), which were obtained using water alone as the aqueous inner and outer compartment solvent. The two sets of data are indistinguishable and lie very close to the theoretical slope calculated from Eqn. 1. Agreement between the two studies shows that the presence of KCl in the inner and outer aqueous phases is not altering the transport properties of this substrate across the filter region at a level that is detectable using the diffusion cell.

Fig. 2 presents the data (as plots of $1/P$ against $\omega^{-1/2}$) for methyl nicotinate transport, at 5 temperatures, across the RDC filter filled with *n*-octanol. The theoretically calculated slopes (determined using Eqn. 1 with appropriate kinematic viscosities and the D_{aq} values in Table 1) are forced through the experimental data to obtain the 1/P-axis intercepts. Good agreement is found between the predicted gradients and the observed results. Table 2 summarises the slope and intercept parameters for each temperature.

The intercepts in Fig. 2 correspond to the flux of substrate that would be observed at infinitely fast rotation speeds ($\omega^{-1/2} \rightarrow 0$) when the first term in Eqn. 1 is zero. The two intercept terms ($h/\alpha KD_o + 2/\alpha k_{ao}$) are separated in Table 3 using

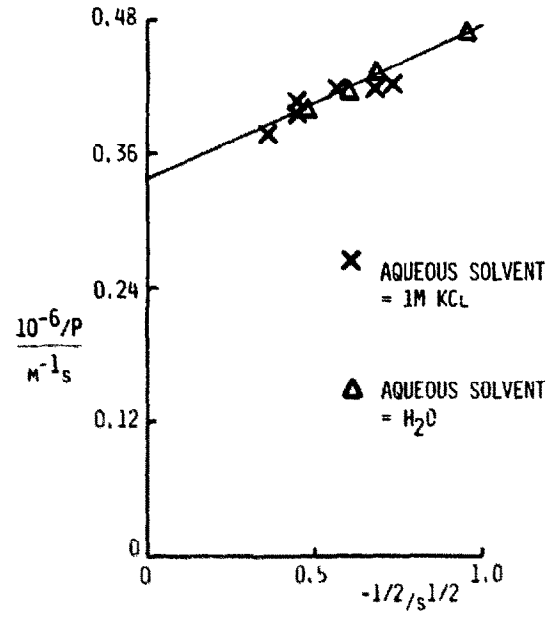

Fig. 1. RDC results for methyl nicotinate crossing an isopropyl myristate-impregnated filter. Previously published data (triangles), for which the aqueous phase solvent was water, are compared with those (crosses) determined in this study, for which the aqueous phase solvent was 1 M potassium chloride. The theoretical gradient, calculated for water as the aqueous phase, is drawn through the experimental points.

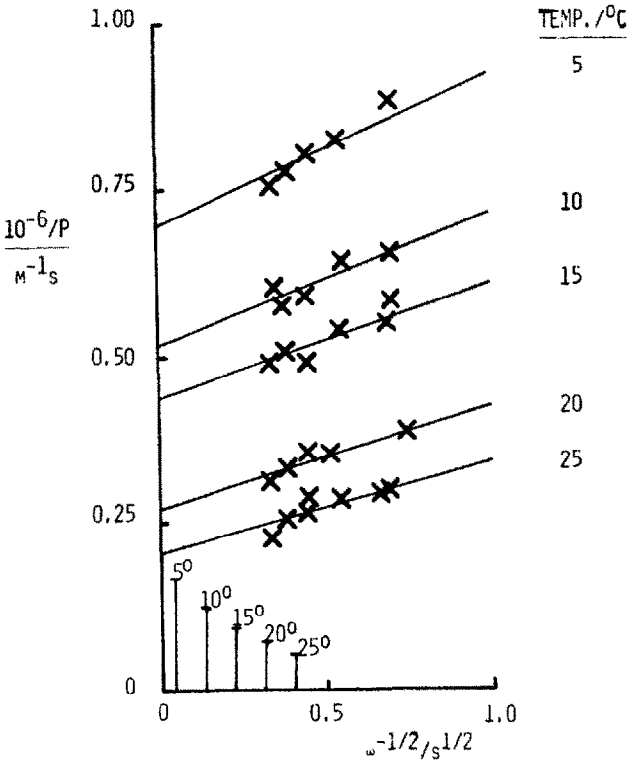

Fig. 2. RDC experimental results for methyl nicotinate transport across an octanol-impregnated filter. Each data point for 1/P is the mean of at least 8 separate determinations; the standard deviations were less than ±5%. The lines drawn through the data are the theoretical gradients listed in Table 2.

the quoted values for h, α, K and D_o, and the phase transfer rate constants k_{ao} and k_{oa} are evaluated. It is apparent that approximately 75% of each of the measured intercepts is the result of resistance to interfacial transport. This observation is also illustrated in Fig. 2 in which the filter diffusion part of the overall barrier is indicated at each temperature by vertical bars in the left-hand corner of the graph. Therefore, in this transport system, interfacial transfer is rate-determining.

Turning to the energetics of the interfacial kinetics measured, the free energy of activation ($\Delta G^{\ddagger}$) and its enthalpic ($\Delta H^{\ddagger}$) and entropic ($\Delta S^{\ddagger}$) contributions are

TABLE 2

NUMERICAL VALUES OF THE THEORETICAL GRADIENTS AND DERIVED INTERCEPTS OF THE LINES DRAWN THROUGH THE EXPERIMENTAL DATA PRESENTED IN FIG. 2

T (°C)	10^{-6} gradient ($m^{-1} \cdot s^{1/2}$)	10^{-6} intercept ($m^{-1} \cdot s$)
5	0.225	0.696
10	0.196	0.528
15	0.173	0.438
20	0.153	0.273
25	0.137	0.206

TABLE 3

SEPARATION OF INTERCEPT VALUES INTO FILTER DIFFUSION AND INTERFACIAL TRANSFER COMPONENTS. INTERFACIAL TRANSFER RATE CONSTANTS FOR METHYL NICOTINATE CROSSING AN AQUEOUS PHASE–OCTANOL BOUNDARY ARE FOUND FROM THE INTERFACIAL RESISTANCE AND THE PARTITION COEFFICIENT (K)

T (°C)	$10^{-6} h/\alpha KD_o$ ($m^{-1} \cdot s$)	$10^{-6} \cdot 2/\alpha k_{ao}$ ($m^{-1} \cdot s$)	$10^6 k_{ao}$ ($m \cdot s^{-1}$)	$10^6 k_{oa}$ ($m \cdot s^{-1}$)
5	0.167	0.529	5.0	0.72
10	0.122	0.406	6.6	0.88
15	0.090	0.348	7.7	0.93
20	0.070	0.203	13	1.5
25	0.049	0.157	17	1.7

evaluated in the conventional fashion (Albery et al., 1976). The results at 20°C are given in Table 4. Thermodynamic information determined from the octanol–aqueous solution partition coefficients of methyl nicotinate is also given in Table 4.

ΔG for distribution of the solute implies that partitioning into octanol is favored. However, the ΔH and ΔS terms show that the driving force in this direction is entropic. This may reflect the fact that, in bulk octanol, there is less scope for hydrogen bonding between the substrate and solvent than in water. Decrease of this H-bonding capacity as the molecule is removed from bulk H_2O to octanol is likely, therefore, to lead to an increase in entropy as solute-bound H_2O molecules are freed into the aqueous solution. That solvation is more efficient in water than in the organic phase may contribute to the fact that ΔH is positive.

The interfacial transfer thermodynamics (Table 4) include, as has been reported for many other systems studied with the RDC (Albery et al., 1976; Fleming et al., 1983; Guy and Hadgraft, 1981; Guy et al., 1982a, c; Sagert et al., 1981), an appreciable free energy barrier for interphase movement in both directions. For transport aqueous → organic, the barrier is entirely enthalpic and the small positive entropy change of little significance. In the opposite direction, the positive enthalpy again contributes considerably to the barrier but now nearly 30% of $\Delta G^{\ddagger}$ results

TABLE 4

THERMODYNAMIC PARAMETERS FOR INTERFACIAL TRANSFER AT 20°C [1]

	Aqueous (interface) $\xrightarrow{k_{ao}}$ Octanol (interface)	Octanol (interface) $\xrightarrow{k_{oa}}$ Aqueous (interface)
$\Delta G^{\ddagger}$ ($kJ \cdot mol^{-1}$)	38.6	43.9
$\Delta H^{\ddagger}$ ($kJ \cdot mol^{-1}$)	43.0	30.9
$\Delta S^{\ddagger}$ ($J \cdot mol^{-1} \cdot K^{-1}$)	15	−44

[1] The thermodynamics determined from the bulk partition coefficients for the substrate between octanol and water are: $\Delta G = -5.3$ kJ·mol^{-1}, $\Delta H = 11.4$ kJ·mol^{-1} and $\Delta S = 57$ J·mol^{-1}·K^{-1}.

from an entropic source. It is possible that the latter is due to the increasing solvation of the substrate as it emerges from the organic side of the interface into the more polar aqueous environment.

By way of comparison, for the same substrate traversing an aqueous phase–IPM interface, large positive ΔS values (> 100 $J \cdot mol^{-1} \cdot K^{-1}$) have been observed (Fleming et al., 1983; Guy et al., 1982c) and interpreted in terms of the disruption of an ordered interfacial structure. The results for the system reported in this paper imply that such an arrangement cannot be maintained at the interface between water and octanol. It may be suggested, therefore, that in this regard, octanol provides a poorer biomembrane model than IPM since some degree of water molecule ordering at the head group region of a phospholipid bilayer is generally acknowledged to exist.

In summary, the results presented in this paper show that there is significant resistance to transport at an octanol–water interface and that an assumption of instantaneous equilibration may be erroneous in situations for which diffusional barriers are low (e.g. when diffusion path lengths are small). Such circumstances can arise in passive membrane transport and in biphasic drug delivery systems; thus, more detailed probing of the phenomenon of solute transfer kinetics across liquid–liquid phase boundaries seems warranted.

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