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# A Novel Method to study the Permeability of a Phospholipid Barrier

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*Summary* The development of a new filter system is described which enables the permeability of a phospholipid barrier to be determined.

In the rotating diffusion cell (RDC) of Albery *et al.*[1] species diffuse through a rotating disc of filter paper. In many experiments two interfaces are established with the aqueous phase above and below the filter which is itself impregnated with the organic phase. We describe here a system whereby a solid phospholipid barrier is supported in the filter and its permeability to methyl nicotinate is followed.

The use of a filter to support phospholipid has been previously described[2,3] and an attempt was made to set up a lipid barrier using this method. This involves dropping a solution of lipid in chloroform on to the filter and allowing the solvent to evaporate. In this way the pores of the filter are filled with lipid and the surface of the filter is also covered by a very thin layer. However, on rotation of the filter, lipid is gradually thrown off and out of the filter into the outer compartment of the RDC. This problem has been overcome by the use of a second non-impregnated filter and the complete procedure is now outlined.

A 47 mm diameter Millipore cellulose ester MF type filter (0·22 $\mu$m pore size) is first mounted on the rotatable Perspex cylinder of the RDC 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% hexane, 33% dichloroethane, and 1% water) to leave a white, opaque, permeable centre, *ca.* 1 cm in diameter, surrounded by the transparent, impermeable, collapsed material. A solution of DL-$\beta\gamma$-dipalmitoyl-$\alpha$-lecithin (DPPC)† in $CHCl_3$ is then dropped on to the permeable region of the filter and the solvent allowed to evaporate (typically *ca.* 1 mg of lipid is deposited per cm$^2$ of filter). Centrally on top of the first filter is placed a 25 mm Millipore filter to the periphery of which collapsing solution is applied so that a firm seal with the first filter is achieved. The DPPC on and in the pores of the first filter is now effectively trapped in position and the barrier retains its efficacy throughout the course of a normal experiment.

The filter system separates the inner compartment of the RDC, which initially contains 40 cm$^3$ of 0·1 mol l$^{-1}$ methyl nicotinate in water, from the outer, containing a known volume of distilled water. The flux of the nicotinate across the filter is followed at 37 °C by periodically sampling the outer compartment and analysing the diffusant concentration spectrophotometrically at 262 nm.

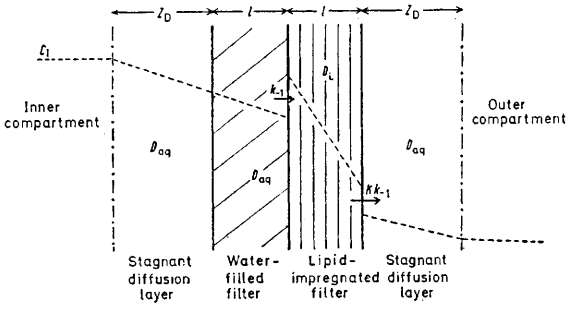


Figure 1. Diagrammatic representation of the filter system across which the diffusion of methyl nicotinate is followed. The predicted concentration profile is shown by the broken lines. It should be noted that the diffusion length through the lipid is taken to be that of the impregnated filter ($l = 1{\cdot}5 \times 10^{-4}$ m); in this preliminary account we have ignored the very thin layer of phospholipid on the surface of this filter.

† This phospholipid $C_{40}H_{80}NO_8P$ was supplied 99·9% pure by Fluka A.G.

In Figure 1 we represent diagrammatically the system through which the nicotinate must diffuse. The rate of transfer ($J$/mol s$^{-1}$) of the diffusing species from the inner compartment to the outer is given by equation (1), where $A$

$$J = kAC_I \quad (1)$$

is the area of the filter, $C_I$ is the bulk concentration in the inner compartment, and $k$ can be expressed as in equation (2). $2Z_D/D_{aq}$ describes diffusion through the aqueous

$$\frac{1}{k} = \frac{2Z_D}{D_{aq}} + \frac{l}{aD_{aq}} + \frac{Kl}{aD_L} + \frac{2}{ak_{-1}} \quad (2)$$

stagnant diffusion layers on either side of the filter system where $Z_D$, the thickness of these layers, is given by the Levich equation[4] and $D_{aq}$ is the aqueous diffusion coefficient of the diffusant. $l/aD_{aq}$ describes diffusion through the non-impregnated filter of length $l$, where $a$ is the area of the pores of the filter divided by $A$. $Kl/aD_L$ describes diffusion through the lipid-filled filter, where $K$ is the partition coefficient (aqueous/organic) for methyl nicotinate between water and phospholipid and $D_L$ is its diffusion coefficient in the lipid. The final term on the right-hand side of equation (2) describes the interfacial transfer of methyl nicotinate from the aqueous phase to the lipid phase, where $k_{-1}$ is the heterogeneous rate constant for the process.

The Levich equation predicts that $Z_D$ is proportional to $W^{-\frac{1}{2}}$, where $W$ is the rotation speed, and in Figure 2 we plot $k^{-1}$ against $W^{-\frac{1}{2}}$ for methyl nicotinate crossing the new filter. The slope of the line is calculable from the Levich equation and is found to be within 5% of the theoretical value. From the intercept we may deduce equation (3).

$$\frac{Kl}{D_L} + \frac{2}{k_{-1}} = 0{\cdot}904 \times 10^6 \text{ m}^{-1} \text{ s} \quad (3)$$

Then, taking a typical value[1] for $k_{-1}$ of $10^{-5}$ m s$^{-1}$ we may calculate the permeability coefficient ($P$) for the lipid barrier from the remaining term [equation (4)] and hence,

$$P = \frac{D_L}{Kl} = 1{\cdot}42 \times 10^{-6} \text{ m s}^{-1} \quad (4)$$

the expression in equation (5). This ratio compares very

$$\frac{D_L}{K} = 2{\cdot}13 \times 10^{-10} \text{ m}^2 \text{ s}^{-1} \quad (5)$$

favourably with a recently determined[5] value of $1{\cdot}62 \times 10^{-10}$ m$^2$ s$^{-1}$ (corresponding to $P = 0{\cdot}48 \times 10^{-6}$ m s$^{-1}$)

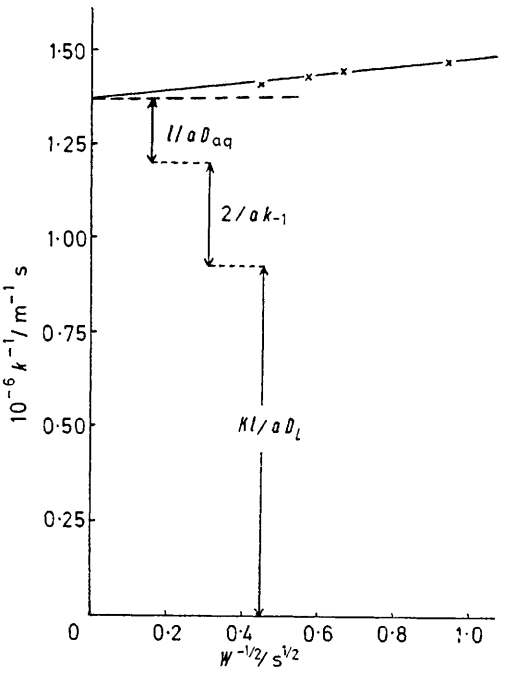


FIGURE 2. Plot of $k^{-1}$ ($= AC_I/J$) against $W^{-\frac{1}{2}}$ for methyl nicotinate diffusing through the new filter system. The slope predicted by the Levich equation is $1{\cdot}065 \times 10^5$ m$^{-1}$ s$^{\frac{1}{2}}$, the measured slope is $0{\cdot}978 \times 10^5$ m$^{-1}$ s$^{\frac{1}{2}}$. The contributions to the intercept of each of the last three terms of equation (2) are indicated.

for methyl nicotinate across the human stratum corneum where it was deduced that the route of penetration through the skin was *via* the lipid-filled channels in between the keratinised cells. Furthermore, under the conditions of our experiment, we expect the predominant structual arrangement of the lipid to be lamellar consisting of stacked bilayers[6] (DPPC being below its crystal-to-liquid crystal phase transition at 37 °C) which suggests that future results might be usefully compared with the permeability of multilamellar liposomes.

The new filter system, therefore, may well provide an easily constructed and hydrodynamically well controlled method for studying some aspects of transport in biological systems.

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