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LONG-TIME SOLUTION OF THE EQUATIONS DESCRIBING THE FLOW OF $^{22}Na^+$ IN A FINITE COMPOSITE SYSTEM CONTAINING A SYNTHETIC PHOSPHOLIPID–PROTEIN MEMBRANE: CALCULATION OF PERMEABILITY COEFFICIENT

RICHARD H. GUY and ROBERT FLEMING

Department of Pharmaceutical Chemistry, The School of Pharmacy, University of London, 29/39 Brunswick Square, London, WC1N 1AX (England)

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SUMMARY

Flow equations are developed using Laplace transform theory to describe the diffusion of $^{22}Na^+$ through a composite system consisting of a protein solution and a phospholipid sol with a membrane at the interface. An approximation, valid at long times ($t > 1.5 \times 10^5$ s), is then introduced enabling calculation of an unambiguous limiting value for the permeability coefficient of the membrane.

INTRODUCTION

In two previous publications Fleming and Castleden (1970; 1972) presented the flow equations describing the diffusion of $^{22}Na^+$ in both: (i) a semi-infinite composite system containing a membrane; and (ii) a finite composite system containing a membrane. The composite system considered, which was contained in a length of precision-bore capillary tubing, consisted of a layer of phospholipid sol and a layer of bovine plasma albumin solution. When 10^{-3} M $CaCl_2$ and cholesterol is added to each layer a membrane is formed at the interface (Saunders, 1963a, b) and produces a resistance to diffusional flow. The system was studied by a method resembling but not equivalent to a self-diffusion technique and has been reported by the same authors (Castleden and Fleming, 1966, 1968, 1970). Solutions to the diffusion equations were obtained using Laplace transform theory and inversion of the resulting expressions required somewhat complex mathematical techniques. Furthermore, in order to obtain information about the permeability coefficient of the membrane, it was then necessary to employ iterative computer programs.

In this paper, we again solve the flow equations using Laplace transform theory but are able to proceed directly to a permeability coefficient by use of an approximation valid at long times ($t > 1.5 \times 10^5$ s) (Castleden and Fleming, 1970). In the earlier treatment of the finite composite system (Castleden and Fleming, 1970), a long-time approximation was also used but the results indicated that the membrane permeability was decreasing to a limiting value. We show that the approximation presented here can lead unambiguously to a permeability coefficient very close to this limiting value, i.e. when the barrier effect of the membrane is most efficient.

The experimental data used in this paper is taken from the finite composite system publication of Castleden and Fleming (1970) (experiments 1 and 2) and a set of unpublished results (Castleden, 1967) (experiment 3). As previously, the notation used for the transformed equations is that of Carslaw and Jaeger (1959).

THEORY

The composite finite system with a membrane can be diagrammatically represented as shown below

$x = 0$ (open end)

$x = \ell_1$ (membrane)

$x = \ell = \ell_1 + \ell_2$

Applying Fick's second law of diffusion to each layer gives

$$\left(\frac{\partial c_1(x,t)}{\partial t}\right)_x = D_1\left(\frac{\partial^2 c_1(x,t)}{\partial x^2}\right)_t \tag{1}$$

$$\left(\frac{\partial c_2(x,t)}{\partial t}\right)_x = D_2\left(\frac{\partial^2 c_2(x,t)}{\partial x^2}\right)_t \tag{2}$$

where D_1 and $c_1(x, t)$, D_2 and $c_2(x, t)$ are the diffusion coefficient and activity of $^{22}Na^+$ in layers I and II respectively.

As previously described (Castleden and Fleming, 1970), Eqns. 1 and 2 are solved to comply with the initial condition

$$c_1(x,t) = c_2(x,t) = c(x,0) \quad \ell > x > 0, \quad t = 0$$

and the following boundary conditions

$$c_1(x,t) = 0 \text{ at } x = 0, \quad t > 0$$

$$D_1\left(\frac{\partial c_1(x,t)}{\partial x}\right)_t = D_2\left(\frac{\partial c_2(x,t)}{\partial x}\right)_t \quad \text{at } x = \ell_1, \quad t > 0$$

$$D_1\left(\frac{\partial c_1(x,t)}{\partial x}\right)_t = H(c_2(x,t) - c_1t)) \quad \text{at } x = \ell_1, \quad t > 0$$

where H is the permeability coefficient

$$\left(\frac{\partial c_2(x,t)}{\partial x}\right)_t = 0 \quad \text{at } x = \ell, \quad t > 0$$

The Laplace transforms of Eqns. 1 and 2 are

$$\frac{d^2\bar{c}_1}{dx^2} - q_1^2\bar{c}_1 + \frac{c(x,0)}{D_1} = 0 \tag{3}$$

$$\frac{d^2\bar{c}_2}{dx^2} - q_2^2\bar{c}_2 + \frac{c(x,0)}{D_2} = 0 \tag{4}$$

where $\bar{c}_1 = c_1(x,t)/p, \bar{c}_2 = c_2(x,t)/p, q_1 = (p/D_1)^{1/2}$ and $q_2 = (p/D_2)^{1/2}$

The transformed boundary conditions become

$$\bar{c}_1 = 0, \quad x = 0 \tag{5a}$$

$$D_1\frac{d\bar{c}_1}{dx} = D_2\frac{d\bar{c}_2}{dx} \text{ at } x = \ell_1 \tag{5b}$$

$$D_1\frac{d\bar{c}_1}{dx} = H(\bar{c}_2 - \bar{c}_1) \text{ at } x = \ell_1 \tag{5c}$$

$$\frac{d\bar{c}_2}{dx} = 0 \text{ at } x = \ell \tag{5d}$$

Solving the differential Eqns. 3 and 4 with the boundary conditions (5a–d) gives the following expressions for $\bar{c}_1$ and $\bar{c}_2$:

$$\bar{c}_1 = \frac{c(x,0)}{p} - \frac{c(x,0)}{p} \times \left\{\frac{D_1q_1 \sinh q_2\ell_2 \cosh q_1(\ell_1 - x) + Hk \cosh q_2\ell_2 \cosh q_1(\ell_1 - x) + H \sinh q_2\ell_2 \sinh q_1(\ell_1 - x)}{D_1q_1 \cosh q_1\ell_1 \sinh q_2\ell_2 + Hk \cosh q_1\ell_1 \cosh q_2\ell_2 + H \sinh q_1\ell_1 \sinh q_2\ell_2}\right\} \tag{6}$$

$$\bar{c}_2 = \frac{c(x,0)}{p} - \frac{c(x,0)}{p} \times \left\{\frac{Hk \cosh q_2(\ell_1 + \ell_2 - x)}{D_1q_1 \cosh q_1\ell_1 \sinh q_2\ell_2 + Hk \cosh q_1\ell_1 \cosh q_2\ell_2 + H \sinh q_1\ell_1 \sinh q_2\ell_2}\right\} \tag{7}$$

where $k = (D_1/D_2)^{1/2}$.

The long-time approximation is now introduced before the inversion of Eqns. 6 and 7. For large values of t, p will be very small and the hyperbolic functions may be approximated (Abramovitz and Stegun, 1970).

$$\left.\begin{array}{l}\sinh z \to z\\ \cosh z \to 1\end{array}\right\} z \ll 1$$

Eqn. 6 can now be reduced to

$$\bar{c}_1 = \frac{c(x,0)}{p} - \frac{c(x,0)}{p}\left\{\frac{D_1 q_1 q_2 \ell_2 + Hk + Hq_2 \ell_2 q_1 (\ell_1 - x)}{D_1 q_1 q_2 \ell_2 + Hk + Hq_2 \ell_2 q_1 \ell_1}\right\}$$

After substitution for q_1, q_2 and k, on inversion we obtain

$$c_1(x,t) = c(x,0)\,\frac{Hx}{D_1 + H\ell_1}\exp\left[\frac{-D_1 H}{\ell_2(D_1 + H\ell_1)}\,t\right] \qquad (8)$$

Integration of Eqn. 8 with respect to x, from 0 to ℓ_1, gives the total activity in layer I at time t

$$c_1 = \int_0^{\ell_1} c_1(x,t)\,dx = c_0\,\frac{H\ell_1^2}{2\ell(D_1 + H\ell_1)}\exp\left[\frac{-D_1 H}{\ell_2(D_1 + H\ell_1)}\,t\right]$$

Applying the same approximation to Eqn. 7

$$\bar{c}_2 = \frac{c(x,0)}{p} - \frac{c(x,0)}{p}\left\{\frac{Hk}{D_1 q_1 q_2 \ell_2 + Hk + Hq_1 q_2 \ell_1 \ell_2}\right\}$$

Inverting as before and then integrating with respect to x over the length ℓ_1 to ℓ_2, we obtain the total activity in layer II at time t

$$c_2 = c_0\,\frac{\ell_2}{\ell}\exp\left[\frac{-D_1 H}{\ell_2(D_1 + H\ell_1)}\,t\right]$$

It follows, therefore, that the long-time approximation predicts that the total activity in the tube at time t will be

$$c = c_1 + c_2 = \frac{c_0}{\ell}\left\{\frac{H\ell_1^2}{2(D_1 + H\ell_1)} + \ell_2\right\}\exp\left[\frac{-D_1 H}{\ell_2(D_1 + H\ell_1)}\,t\right] \qquad (9)$$

RESULTS AND DISCUSSION

The long-time approximate solution, Eqn. 9, indicates that the natural logarithm of the total activity in the capillary is directly proportional to the time of the experiment

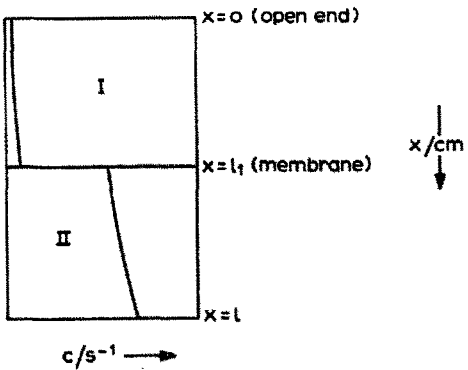

Fig. 1. Diagrammatic representation of the concentration profile in the capillary after the experiment has been allowed to run for a prolonged period.

and that the only unknown in the proportionality constant is the permeability coefficient, H, of the membrane at the interface. The linearity of an lnc against t graph will therefore give an indication of the applicability of the model. Consideration of the proportionality constant reveals that the activity remaining in the capillary at long-time does not depend on D_2, the diffusion coefficient of $^{22}Na^+$ in the lower layer. This agrees entirely with Fig. 1 which is a diagrammatic representation of the concentration profile in the capillary after the experiment has been allowed to run for a long period. There is a very small concentration gradient in layer I which implies that the amount of activity remaining in the capillary depends upon the size of the barrier at the interface (given by H), the diffusion coefficient of $^{22}Na^+$ in layer I (D_1) and the length of the capillary (given by ℓ_1 and ℓ_2), i.e. of the 3 steps involved in reducing the activity in the capillary (a) diffusion in layer II; (b) permeation through the membrane; and (c) diffusion in layer I – only the last two are rate-determining at long times.

We cannot, however, make use of the pre-exponential factor in Eqn. 9 which should

TABLE 1

CAPILLARY ACTIVITY–TIME DATA FROM 3 EXPERIMENTS

Expt. 1 [a]		Expt. 2 [a]		Expt. 3 [b]	
t/s	c/s^{-1}	t/s	c/s^{-1}	t/s	c/s^{-1}
150093	310.7	165040	380.2	169482	709.8
162470	297.4	175808	370.4	177535	693.9
175549	284.5	188383	358.8	186852	675.2
236921	231.5	204149	344.4	206811	634.2
252742	219.3	252104	300.2	255141	554.9
265101	211.6	265197	293.8	266385	537.0
		271438	290.3	279311	518.3

[a] From Castleden and Fleming (1970).
[b] From Castleden (1967).

TABLE 2

SUPPLEMENTARY DATA AND CALCULATED PERMEABILITY COEFFICIENTS

Expt. no.	$10^5 D_l/\mathrm{cm}^2\ \mathrm{s}^{-1}$ [a]	ℓ_1/cm [a]	ℓ_2/cm [a]	$10^6 H/\mathrm{cm} \cdot \mathrm{s}^{-1}$ [b]
1	0.9396	0.6600	1.4865	7.67
2	0.7010	0.8500	1.2965	5.72
3	0.7010	1.1000	0.9713	4.96

[a] From Castleden and Fleming (1970) and Castleden (1967).

[b] Calculated from the gradients of the lnc against t graphs in Fig. 1 using Eqn. 9.

give the value of c at t = 0. To do so would be conceptually incorrect since the derivation given in the theory involves specific assumptions valid only at long times. We therefore concentrate attention on the gradients of the graphs of lnc against time.

The experimental data is collected in Tables 1 and 2 and shown graphically in Fig. 2. It is immediately apparent that the correlation coefficients for the plots of lnc against t indicate good linearity. The permeability coefficients calculated from the gradients are reasonably consistent; agreement between experiments 2 and 3 is particularly good as these experiments were performed with the same batch of lecithin, different from that used in experiment 1 (this also accounts for the differences in D_l).

The previously calculated values of H (Castleden and Fleming, 1970) were found to decrease with increasing time as shown graphically in Fig. 3 for experiments 1 and 2. Also

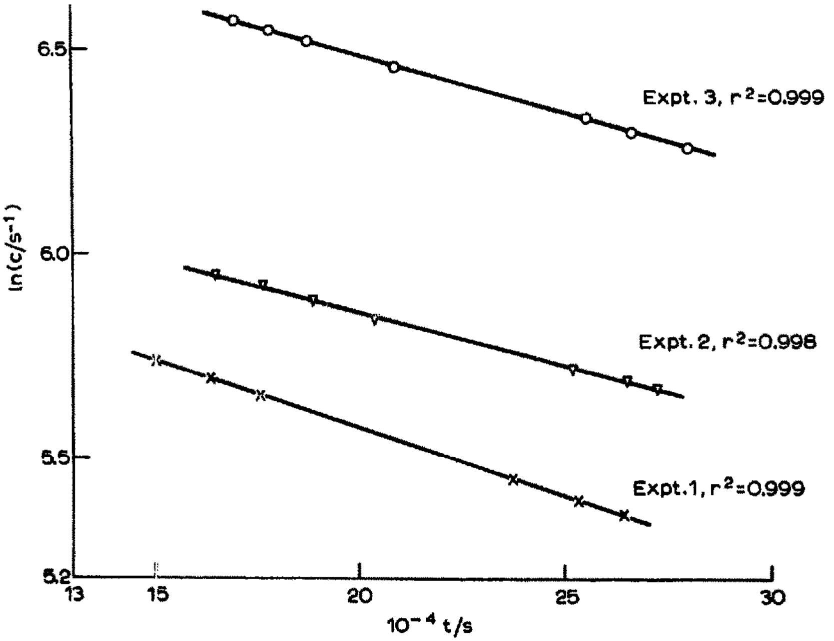

Fig. 2. Plots of $\ln(c/\mathrm{s}^{-1})$ against t from the data in Table 1. The values of the permeability coefficient given in Table 2 are calculated from the gradients of the lines using Eqn. 9.

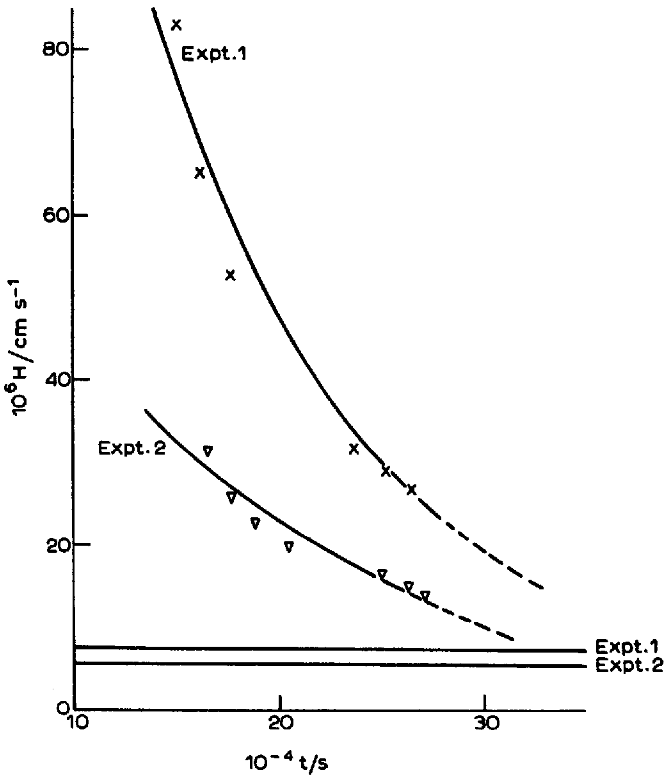

Fig. 3. Previously calculated (Castleden and Fleming, 1970) values of H plotted against time for experiments 1 and 2. The horizontal lines represent the single values of H calculated using the long-time approximation presented in this paper.

indicated in the figure are the single values of H as calculated in this paper for the same two sets of data. It does not seem unreasonable to suggest that our values for the permeability coefficient are good estimates of the limit which the previously calculated values seem to be approaching.

We have shown, therefore, how the relatively complex equations of flow through a composite system containing a membrane may be solved easily by restricting attention to data obtained at long times. The permeability coefficient of a synthetic phospholipid–protein membrane is estimated to be of the order of 6×10^{-6} cm · s^{-1} once the membrane reaches its maximum barrier efficiency.

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