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Some studies of the diffusion of sodium ions through protein solutions and phospholipid sols

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Diffusion of sodium-22 in solutions containing sodium chloride, calcium chloride, bovine plasma albumin, lecithin and cholesterol has been examined by two methods which were modifications of the open-end capillary technique of Anderson & Saddington (1949) and the continuous monitoring technique of Mills & Godbole (1958). Preliminary investigations of semi-infinite systems consisting of lecithin sols layered on albumin solutions have been made. The activity in the capillary at various times during diffusion has been compared with the theoretical activity and good agreement has been found.

THE diffusion of components present in aqueous solutions of large molecules has received but little attention so far. Wang (1954) found that the self-diffusion coefficient of water in ovalbumin solutions was less than in water itself and he was able to obtain information about the shape and hydration of the protein molecules. Other authors (Brady & Salley, 1948; Huinzenga, Grieger & Wall, 1950; Dux & Steigman, 1958, 1959; Clifford & Pethica, 1964) have used radioisotope techniques to study the diffusion of ions in detergents and synthetic ion-exchange resins. Using a conductimetric method, Saunders (1963) has investigated the diffusion of electrolytes (NaCl and $CaCl_2$) in aqueous phospholipid sols containing cholesterol.

In the present paper tracer methods for studying diffusion in aqueous albumin solutions and lecithin sols are described. The effect of concentration of solute and of the addition of calcium chloride and cholesterol is reported. Preliminary investigations of composite systems consisting of lecithin sols layered on albumin solutions are also discussed. By postulating that the bovine plasma albumin layer is semi-infinite and the phospholipid layer finite, and by analogy with conduction of heat in solids, equations have been derived from which it is possible to calculate the concentration of radioisotope in the layers at any given time during the diffusion experiment.

Two experimental techniques have been used: (i) the open-end capillary method of Anderson & Saddington (1949) and (ii) a modification of the continuous monitoring method of Mills (Mills & Godbole, 1958).

Theory

The diffusion of a particle in a system where there is no chemical potential gradient and in which the movement of the particle is due to random molecular motion, is termed self-diffusion. The usual equations describing self-diffusion have been used and modified to satisfy the boundary conditions. Some of our experiments did not strictly follow the conditions for self-diffusion and this is pointed out where applicable.

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The active material was contained initially in a short length of capillary tube, sealed at one end. Diffusion out of the tube took place at the open end into a stirred bath of inactive solution of the same ionic concentration as that present in the tube. The stirring ensured that the activity at the open end of the capillary was zero throughout the experiment, in accordance with the boundary condition.

The transport of material by diffusion is defined by Fick's law:

$$\left(\frac{\partial^2 C}{\partial x^2}\right)_t = \frac{1}{D}\left(\frac{\partial C}{\partial t}\right)_x \quad .. \quad .. \quad .. \quad (1)$$

where C = concentration of solute in any plane, x, from the boundary, t = time during which diffusion has been taking place, D = diffusion coefficient of the solute.

The partial differential equation (1) was solved to satisfy the particular initial and boundary conditions under which the experiment was conducted. An expression was found for the activity, C(x,t), at a plane, x, in the tube after a given time. Integration over the whole length of the capillary yielded an equation for the total activity, $C_{(tot)}$ remaining in the capillary at time, t.

Various mathematical techniques were applied in the derivation of the equations given below and reference was made to Crank (1956), Carslaw & Jaeger (1959) and Jost (1960). The Laplace transformation method was of particular use in the solution of equations applicable to the more complicated systems, e.g., composite semi-infinite media.

1. *Solution of equation* (1) *for a semi-infinite medium*

Initial conditions: $C(x,t) = C(x,0),\ x>0,\ t = 0.$

Boundary conditions: $C(x,t) = C(x,0),\ x = \infty$; $C(x,t) = 0,\ x = 0$ } $t > 0.$

The solution for the activity, C(x,t), complying with the initial and boundary conditions was:

$$C(x,t) = C(x,0)\ erf\ \frac{x}{2\sqrt{Dt}} \quad .. \quad .. \quad (2)$$

Integration of equation (2) over the length of the capillary, i.e.,

$$\int_{x=0}^{x=} C(x,t).dx$$

gave

$$C_{(tot)} = C_0 - \frac{2C_0\sqrt{Dt}}{l\sqrt{\pi}} + \frac{C_0\sqrt{Dt}}{l}\,2\ ierfc\ \frac{l}{2\sqrt{Dt}} \quad .. \quad (3)$$

where C_0 = total initial activity in the capillary of length, *l*.

2. *Solutions for the finite medium*

Initial conditions: $C(x,t) = C(x,0),\ 0<x<l,\ t = 0.$

$$\text{Boundary conditions: } \left.\begin{aligned} C(x,t) &= 0, \; x = 0 \\ \frac{\partial c}{\partial x} &= 0, \; x = l \end{aligned}\right\} \; t > 0.$$

Solving the partial differential equation (1) for the above conditions gave equations (4) and (5) for the activity at plane, x, in the tube. The final equations (6) and (7) were obtained by integration of (4) and (5) respectively.

$$C(x,t) = C(x,0) - C(x,0)\sum_{n=0}^{\infty}(-1)^n\left\{erfc\,\frac{(2nl+x)}{2\sqrt{Dt}} + erfc\,\frac{2(n+1)l-x}{2\sqrt{Dt}}\right\} \quad .. \quad .. \quad .. \quad (4)$$

$$C(x,t) = \frac{4C(x,0)}{\pi}\sum_{n=0}^{\infty}\frac{(-1)^n}{2n+1}\exp\left[-\frac{D(2n+1)^2\pi^2 t}{4l^2}\right]\sin\frac{(2n+1)\pi x}{2l} \quad .. \quad .. \quad .. \quad (5)$$

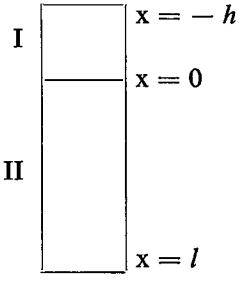
$$C_{(tot)} = C_0 - \frac{2C_0\sqrt{Dt}}{l}\left\{\pi^{-1/2} - \sum_{n=0}^{\infty}(-1)^n\, 2\, ierfc\,\frac{nl}{\sqrt{Dt}}\right\} \quad .. \quad (6)$$

$$C_{(tot)} = \frac{8C_0}{\pi^2}\sum_{n=0}^{\infty}\frac{1}{(2n+1)^2}\exp\left[-\frac{D(2n+1)^2\pi^2 t}{4l^2}\right] \quad .. \quad .. \quad (7)$$

The series converge satisfactorily if equation (6) is used for calculations in which the time of diffusion, t, is small and equation (7) for moderate and long times.

3. *Semi-infinite composite medium*

The composite system discussed in this paper consisted of two layers (I and II). The upper one (I) was treated as a finite medium while the lower one (II) was considered semi-infinite.



The differential equations to be solved were

$$\left(\frac{\partial^2 C_1}{\partial x^2}\right)_t - \frac{1}{D_1}\left(\frac{\partial C_1}{\partial t}\right)_x = 0, \quad -h<x<0, \quad t>0, \quad .. \quad (8)$$

$$\left(\frac{\partial^2 C_2}{\partial x^2}\right)_t - \frac{1}{D_2}\left(\frac{\partial C_2}{\partial t}\right)_x = 0, \quad l>x>0, \quad t>0, \quad .. \quad (9)$$

where C_1 and C_2 are the concentrations at a plane, x; D_1 and D_2 are the diffusion coefficient, of sodium-22 in layers I and II respectively.

Equations (8) and (9) were solved to conform with the following conditions:

Initial condition C_1 and $C_2 = C(x,0)$, $\quad -h<x<\infty \quad t = 0$

and assuming that there is no resistance to diffusion at the interface, $x = 0$, the boundary conditions are:

$$\left.\begin{array}{lll} C_1(0,t) = C_2(0,t) & & \\ D_1 \dfrac{\partial C_1}{\partial x} = D_2 \dfrac{\partial C_2}{\partial x} & \Big\} & x = 0, \\ C_1(x,t) = 0, & & x = -h, \\ C_2(x,t) = C(x,0), & & x = \infty, \end{array}\right\} \; t>0$$

By applying the Laplace transformation method the following solutions were obtained:

$$C_1(x,t) = C(x,0) - C(x,0)\sum_{n=0}^{\infty} \alpha^n \left\{ erfc \frac{(2n+1)h + x}{2\sqrt{D_1 t}} - \alpha\, erfc \frac{(2n+1)h - x}{2\sqrt{D_1 t}} \right\} \quad .. \quad (10)$$

$$C_2(x,t) = C(x,0) - \frac{2C(x,0)}{1+\sigma} \sum_{n=0}^{\infty} \alpha^n\, erfc \frac{(2n+1)h + kx}{2\sqrt{D_1 t}} \quad .. \quad (11)$$

where $k = \left(\dfrac{D_1}{D_2}\right)^{1/2}$, $\sigma = \left(\dfrac{D_2}{D_1}\right)^{1/2}$, $\alpha = \dfrac{\sigma - 1}{\sigma + 1}$

Expressions for total activity in the capillary after time, t, were obtained by integrating the Laplace transforms of equations (10) and (11) and then applying the Inversion Theorem.

$$C_{1(tot)} = {}^{I}C_0 + \frac{{}^{I}C_0\sqrt{Dt}}{h} \sum_{n=0}^{\infty} \alpha^n \left\{ 2\, ierfc \frac{(2n+1)h}{2\sqrt{D_1 t}} + \alpha\, 2\, ierfc \frac{(2n+1)h}{2\sqrt{D_1 t}} - 2\, ierfc \frac{nh}{\sqrt{D_1 t}} - \alpha\, 2\, ierfc \frac{(n+1)h}{\sqrt{D_1 t}} \right\} \quad .. \quad .. \quad (12)$$

$$C_{2\,(tot)} = {}^{II}C_0 - \frac{{}^{II}C_0\sqrt{D_2 t}}{l(1+\sigma)} \sum_{n=0}^{\infty} \alpha^n \left\{ 2\, ierfc \frac{(2n+1)h}{2\sqrt{D_1 t}} - 2\, ierfc \frac{(2n+1)h + kl}{2\sqrt{D_1 t}} \right\} \quad .. \quad .. \quad (13)$$

$$C_{(tot)} = C_{1\,(tot)} + C_{2\,(tot)} \quad .. \quad .. \quad (14)$$

where l = length of infinite layer, $C_{1\,(tot)}$ and $C_{2\,(tot)}$ = activity in layers I and II respectively after time, t, ${}^{I}C_0$ and ${}^{II}C_0$ = initial total activity in layers I and II respectively, i.e., $C_0 = {}^{I}C_0 + {}^{II}C_0$.

Alternative solutions of equations (8) and (9) can be obtained by contour integration. These contain an integral which must be evaluated numerically. This method is particularly useful for systems which are more complicated than that described in this section, e.g. systems with a resistance to diffusion at the interface or a system consisting of three layers. In these cases the expansions of the exponential functions of the Laplace transforms become more difficult to handle so that it is not easy to obtain error-function solutions. Work is proceeding on equations describing the flow of sodium-22 in these systems.

Experimental

METHOD (*a*)

A modification of the open-end capillary technique of Anderson & Saddington (1949); (Wang, 1951; Wang & Miller, 1952; Mills & Kennedy, 1953; Mills & Adamson, 1955). A three-necked flask of one litre capacity held the inactive solution and was immersed in a thermostat bath at 25° ± 0·01° (see Fig. 1). The centre neck of the flask was used to accommodate a mercury-seal stirrer and the capillaries, containing the active solution were placed in the flask on small perspex holders attached to rods through the side necks. The capillaries were made from precision bore Pyrex glass tubing (trade name "Uniform," and distributed by Jencons, Hemel Hempstead, Herts), and were 2–4 cm long with an

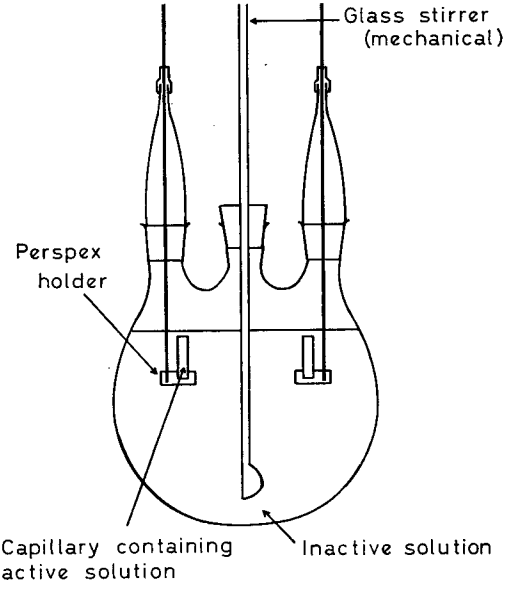


FIG. 1. Apparatus for open-end capillary technique. Method (*a*).

internal diameter of 0·08 cm. One end of the capillary was sealed with a small globule of glass, and the other end was ground flat to promote streamline flow of liquid across the top of the capillary, thus reducing the scooping out of active solution by inactive solution as it flows over the surface. Also to reduce this error, excess active liquid was placed on top of the capillary before immersion in the inactive solution. The capillary internally and externally was coated with a 2% w/w solution of dimethyldichlorosilane in carbon tetrachloride to reduce to a minimum the adsorption of radioactive sodium (Mills & Kennedy, 1953) onto the surface of the capillary. As recommended by other workers (loc. cit.) the stirring rate was approximately 60 rpm. The rate of flow across the open end of the capillary is critical and should be just sufficient to keep the concentration of active ion zero at this point in accordance with the requirements of the mathematical theory. For a more detailed discussion of errors reference should be made to the literature (Wang, 1951; Mills & Kennedy, 1953; Berne & Berggren, 1960).

The lengths of the capillaries were measured with a metal plunger attached to a micrometer: this was the most precise way of obtaining these measurements ($\pm$0·0005 cm).

The solutions were prepared as described below and sufficient radioactive sodium added to give initial counts of approximately 500–1500 cps. The volume of active solution added was taken as negligible and the solutions were not degassed. Capillaries were filled by means of a fine glass pipette and the initial activity determined in a scintillation counter using a sodium iodide well crystal. Counts were accurate to $\pm$0·1% ($P = 0{\cdot}68$). When thermal equilibrium had been established the capillary was immersed completely in the solution in the flask so that the open end was a few millimetres below the surface of the inactive solution. Diffusion took place for a time sufficiently long for the condition

$$\frac{Dt}{l^2} > 0{\cdot}24$$

to be obeyed thus ensuring the validity of equation (7). At the end of the run the capillary was withdrawn and the remaining activity determined.

METHOD (*b*)

Apparatus for continuous monitoring (Mills & Godbole, 1958, 1959). The apparatus was made of brass protected by a non-corrosive paint and held 5½ litres of inactive solution. It was surrounded by a thermostat bath which was maintained at 25° $\pm$ 0·01°. The capillary was placed in a perspex holder in the centre of the sodium iodide well crystal (see Fig. 2). The crystal was shielded against radiation from external sources and from the bath solution by a ring of lead 2 cm thick. To improve the flow of solution over the open end of the capillary a separate compartment containing the stirrer was constructed and the solution circulated through holes in the vertical and horizontal partitions. The rate of stirring was approximately 70 rpm and was controlled by a constant speed device. The capillary was made as in method (*a*) approximately

2 cm long and 0·08 cm diameter and was filled as described previously. In most experiments the initial activity, C_0, was determined because this quantity was used in some of the later calculations. The capillary was then immersed in the inactive solution and the activity remaining in the capillary was counted at intervals; the middle of the count was taken as the corresponding time, t, at which the measurement was made. Diffusion was allowed to proceed for about five days, the capillary was then removed and the background determined: that this was constant throughout the runs was checked by removing the capillary at definite time intervals and counting the residual activity in the bath. After an initial sharp increase the background counting rate was practically constant, an observation in agreement with Mills & Godbole (1958, 1959).

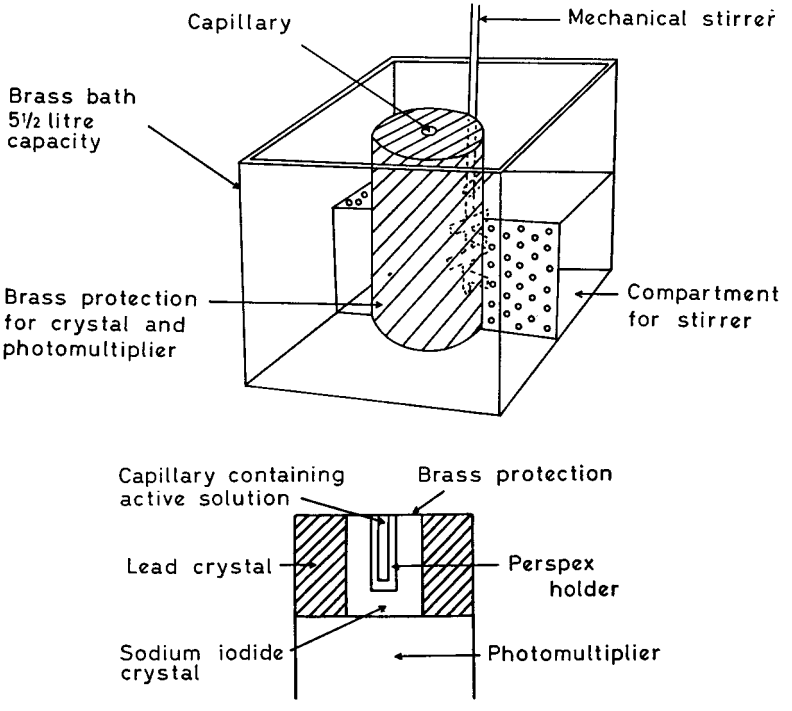


FIG. 2. Apparatus for continuous monitoring. Method (*b*). The lower diagram shows a section through the capillary holder.

PREPARATION OF ALBUMIN SOLUTIONS

A weighed amount of bovine plasma albumin (Fraction V from Bovine Plasma prepared by Armour Pharmaceutical Company Ltd.) was dissolved in boiled and cooled distilled water. Ion-exchange resin (Amberlite Monobed Resin MB-1) kept in methanol (approximately 500 mg) was added and shaken in the albumin solution for 15 min, by which time removal of small electrolytes was assumed to be complete; the resin was

then removed by centrifuging. The required quantities of sodium chloride and calcium chloride were added and the solution made up to weight with water.

PREPARATION OF LECITHIN SOL

Commercial material (A. Merck, Darmstadt) was purified as described by Attwood (1965) and was stored as a solution in chloroform. A suitable quantity of this was evaporated *in vacuo* to constant weight and dissolved in ether together with cholesterol when required. A known volume of boiled, cooled distilled water was added; the ether was removed *in vacuo*, warming when necessary. Nitrogen was bubbled (10 min) through the sol, which was then subjected to ultrasonic irradiation (20 kilocycles/sec for 1–3 hr). The vessel containing the sol was surrounded by ice and water. Ion-exchange resin was added (as for the albumin solutions) and shaken in the sol. The sol was centrifuged to remove the resin together with any titanium deposited in the solution from the probe of the ultrasonic irradiator. The required amounts of calcium chloride, sodium chloride and water were added. Since this caused coagulation of the lecithin-cholesterol micelles the sol was again subjected to ultrasonic irradiation for about 30 min and centrifuged once more.

CALCULATIONS

Diffusion coefficients were calculated from equation (7) which became: (for $t > 140{,}000$ sec).

$$D = \frac{9{\cdot}212\, l^2}{\pi^2 t} \log \left[\frac{8C_0}{\pi^2 C_{(tot)}} \right] \qquad .. \quad .. \quad (15)$$

For method (*a*) this involved a direct substitution of experimental values and a short calculation. To obtain the diffusion coefficients by method (*b*) a plot of log $C_{(tot)}$ against time, t, was made, and the slope of the straight line was calculated by a least squaring procedure. D was found using the equation

$$\text{Slope} = \frac{D\pi^2}{9{\cdot}212\, l^2} \qquad .. \quad .. \quad .. \quad (16)$$

When applying the equations containing the error function terms (equations 3, 12, 13 and 14) it was simpler to calculate $C_{(tot)}$ and to compare this with the experimental result at a given time, t, using values for the diffusion coefficient obtained by equations (15) and (16).

Results

Diffusion of sodium-22 was investigated in solutions containing sodium chloride, calcium chloride, albumin, lecithin and cholesterol. In the composite systems the lower layer (II) consisted of an albumin solution and the upper layer (I) of a lecithin-cholesterol sol.

Tracer diffusion coefficients for sodium-22 in solutions of sodium chloride are listed in Table 1 and for other systems in Table 2. Each result is the mean of at least six measurements except where indicated by the figures in brackets.

Calculated and experimental values for $C_{(tot)}$ are compared in Table 3–5. The percentage difference was evaluated by the following equation:

$$\% \text{ difference} = \frac{\text{difference between calculated and experimental values for } C_{(tot)}}{\text{experimental value for } C_{(tot)}} \times 100 \quad .. \quad (17)$$

TABLE 1. TRACER DIFFUSION COEFFICIENTS FOR SODIUM-22 IN 0·1M AND 0·01M SODIUM CHLORIDE SOLUTIONS

Concentration NaCl mole litre^{-1}	Method	D × 10^5 cm^2 sec^{-1} ± standard deviation (P = 0·68)	
		with stirring	without stirring
0·1	a	$1{\cdot}25_6 \pm 0{\cdot}03$	$1{\cdot}24_9 \pm 0{\cdot}01$
0·1	b	$1{\cdot}27_9 \pm 0{\cdot}01$	—
0·01	a	$1{\cdot}28_3 \pm 0{\cdot}07$ (2)	$1{\cdot}27_6 \pm 0{\cdot}03$
0·01	b	1·306 (1)	—

TABLE 2. TRACER DIFFUSION COEFFICIENTS OF SODIUM-22 IN VARIOUS SYSTEMS

Experiment No.	Method	Composition of solutions		D × 10^5 cm^2 sec^{-1} ± standard deviation (P = 0·68)
		in capillary	in bath	
1	a	0·1M NaCl 10% BPA	0·1M NaCl 10% BPA	$1{\cdot}11_7 \pm 0{\cdot}08$
2	a	0·1M NaCl 0·001M $CaCl_2$ 10% BPA	0·1M NaCl 0·001M $CaCl_2$ 10% BPA	$1{\cdot}13_5 \pm 0{\cdot}08$
3	a	0·1M NaCl 0·001M $CaCl_2$	0·1M NaCl 0·001M $CaCl_2$	$1{\cdot}20_6 \pm 0{\cdot}04$
4	b	0·1M NaCl 2·5% BPA	0·1M NaCl	$1{\cdot}24_4$ (1)
5	b	0·1M NaCl 5% BPA	0·1M NaCl	$1{\cdot}23_8 \pm 0{\cdot}09$
6	b	0·1M NaCl 7·5% BPA	0·1M NaCl	$1{\cdot}20_3$ (1)
7	b	0·1M NaCl 10% BPA	0·1M NaCl	$1{\cdot}07_0$ (1)
8	a	0·1M NaCl 10% BPA	0·1M NaCl	$1{\cdot}13_3 \pm 0{\cdot}05$
	a	0·1M NaCl 0·001M $CaCl_2$ 10% BPA	0·1M NaCl 0·001M $CaCl_2$	$1{\cdot}10_8 \pm 0{\cdot}07$
10	b	0·1M NaCl 0·001M $CaCl_2$ 10% BPA	0·1M NaCl 0·001M $CaCl_2$	$1{\cdot}11_8 \pm 0{\cdot}01$ (2)
11	a	0·1M NaCl 5% lecithin	0·1M NaCl	$1{\cdot}17_5 \pm 0{\cdot}05$
12	b	0·1M NaCl 5% lecithin	0·1M NaCl	$1{\cdot}20_8 \pm 0{\cdot}05$
13	a	0·1M NaCl 0·001M $CaCl_2$ 10% lecithin 5% cholesterol	0·1M NaCl 0·001M $CaCl_2$	$0{\cdot}70_1 \pm 0{\cdot}03$
14	a	,,	,,	$0{\cdot}92_5 \pm 0{\cdot}01$
15	b	,,	,,	$0{\cdot}94_0 \pm 0{\cdot}04$

All solutions of bovine plasma albumin (BPA), lecithin and cholesterol were % w/w
Results (13) and (14) different batches of lecithin.

Discussion

CALIBRATION OF THE APPARATUS AND COMPARISON OF METHODS (*a*) AND (*b*)

To ensure that the correct stirring speeds were being used in both types of apparatus (*a*) and (*b*), diffusion runs were made with sodium-22 in solutions of sodium chloride at various strengths. The self-diffusion coefficients thus obtained were compared with those in the literature.

TABLE 3. COMPARISON OF EXPERIMENTAL AND CALCULATED VALUES OF $C_{(tot)}$ FOR DIFFUSION OF SODIUM-22 IN 0·1M SODIUM CHLORIDE AQUEOUS SOLUTIONS WHEN THE SYSTEM IS SEMI-INFINITE
[Experiments 1, 2 and 3 by method (*a*). Experiment 4 by method (*b*)]

Experiment No.	Time of diffusion (sec)	C(tot) (experimental) ±0·1%	C(tot) (calculated)	Difference %
1	19,680	471·0	478·4	1·6
2	63,720	331·8	342·7	3·7
3	68,640	707·1	711·0	0·6
4	3,652	981·5	985·9	0·4
,,	6,670	944·4	941·8	0·2
,,	10,648	906·5	897·6	1·0
,,	15,045	874·7	857·2	2·0
,,	17,698	852·4	836·7	1·8
,,	73,786	559·3	577·4	3·2

This is shown graphically in Fig. 3 where D is plotted against $\sqrt{C}$ together with the results from other authors (Nielson, Adamson & Cobble, 1952; Wang & Miller, 1952; Mills & Adamson, 1955; Mills & Godbole, 1960). The deviation about the mean is indicated for our results and for those from the literature which were measured at the same concentrations.

TABLE 4. COMPARISON OF EXPERIMENTAL AND CALCULATED VALUES OF $C_{(tot)}$ FOR DIFFUSION OF SODIUM-22 IN 0·1M SODIUM CHLORIDE AND 0·001M CALCIUM CHLORIDE AQUEOUS SOLUTIONS CONTAINING (1) 10% W/W ALBUMIN OR (2 AND 3) 10% W/W LECITHIN AND 5% W/W CHOLESTEROL WHEN THE SYSTEM IS SEMI-INFINITE USING CONTINUOUS MONITORING METHOD (*b*)
[Results (2) and (3) from different sols]

Experiment No.	Time of diffusion (sec)	C(tot) (experimental)	C(tot) (calculated)	Difference %
	3,010	883·8	882·6	0·1
1	60,740	596·7	579·6	2·9
	75,238	545·0	531·9	2·4
	4,251	567·7	561·2	1·1
2	58,467	406·8	391·7	3·7
	85,802	355·0	347·1	2·2
	2,454	597·0	590·5	1·0
	5,631	575·3	563·7	2·0
3	75,495	370·7	365·1	1·2
	86,210	351·6	350·1	0·4
	93,518	339·6	234·7	1·5

TABLE 5. COMPARISON OF EXPERIMENTAL AND CALCULATED VALUES OF $C_{(tot)}$ FOR THE DIFFUSION OF SODIUM-22 IN SEMI-INFINITE COMPOSITE SYSTEMS FROM TWO EXPERIMENTS

Experiment No.	Time of diffusion (sec)	C(tot) (experimental)	C(tot) (calculated)	Difference %
	4,670	605·3	603·6	0·39
	7,864	594·1	582·7	1·45
1	12,748	575·6	562·2	2·10
	14,373	571·6	555·7	2·94
	20,997	554·0	532·5	3·88
	3,241	1,316	1,332	1·23
	6,796	1,262	1,269	6·55
2	10,587	1,224	1,227	0·21
	16,422	1,175	1,174	0·94
	25,742	1,115	1,107	0·72

Using method (*b*) (continuous monitor) our results are in good agreement with those of Mills & Godbole (1958), who found that the self-diffusion coefficient of the sodium ion in 0·1M sodium chloride was $1{\cdot}277 \pm 0{\cdot}002_3 \times 10^{-5}$ cm^2sec^{-1}. The standard deviation of the measurements reported in this paper at the same concentration was slightly larger. The correct stirring speed is important when using method (*b*) and when this was reduced from 72 rpm to 50 rpm the diffusion coefficient fell from 1·28 to $1{\cdot}26 \times 10^5$ cm^2sec^{-1} at 0·1M sodium chloride.

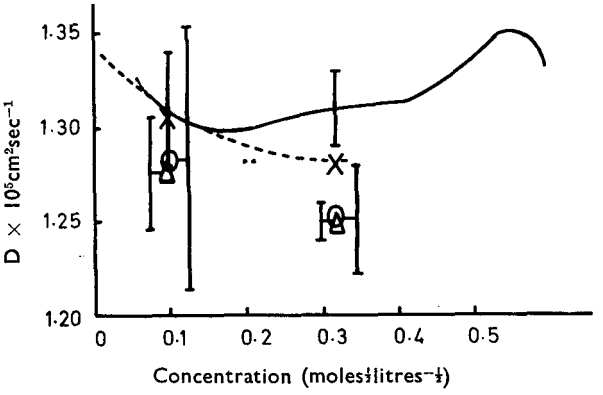


FIG. 3. Graph of $D \times 10^5$ cm^2 sec^{-1} of sodium-22 against concentration (moles½ $litre^{-½}$) for solutions of sodium chloride. The accuracy of the results from method (a) is indicated. 1. Open-end capillary (Wang & Miller 1952). 2. Continuous monitoring (Mills & Godbole 1960). 3. × no stirring; ○ stirring; △ continuous monitoring. See Table 1.

All the results obtained by method (*a*) were slightly lower than those quoted in the literature although all likely sources of error had been reduced to a minimum. There is a wide variation in the results reported by different authors and no completely satisfactory explanation has been given. Maintaining the correct stirring speed does not appear to be critical with method (*a*) and this is in accordance with the conclusion reached by Clifford & Pethica (1964).

Method (*a*) is relatively simple and the time of a diffusion experiment is shorter than in method (*b*). In addition, several tubes can be immersed in the inactive solution at the same time, thus enabling a number of simultaneous results to be obtained. It is less accurate (±2·0%) than method (*b*) but can yield much useful information.

The chief disadvantage of the continuous monitoring technique, method (*b*), is that only one capillary can be placed in the apparatus, and as each diffusion run may take a week, it takes a long time to obtain a series of results. However, it is more accurate (±0·5%) than method (*a*) and it is also possible to follow changes in the diffusion rate throughout the experiment. This is advantageous in experiments with composite systems, where for example there is a change of interfacial resistance with time.

DIFFUSION OF SODIUM-22 IN ALBUMIN SOLUTIONS AND LECITHIN SOLS

The boundary conditions imposed for the solution of equation (1) require that the concentration of the radioactive ions at the open end of the capillary be zero, and to comply with this it is necessary to have a large volume of inactive solution surrounding the capillary. This is clearly impracticable when using costly materials like lecithin and albumin. The differential diffusion coefficient for albumin is approximately $6{\cdot}7 \times 10^{-7}$ cm^{2}sec^{-1} (Baldwin, Gosting, Williams & Alberty, 1955; Chatterjee, 1964) and the self-diffusion coefficient of ovalbumin in 10% ovalbumin solution is $3{\cdot}32 \times 10^{-7}$ cm^{2}sec^{-1} (Wang, Anfinsen & Polestra, 1954), almost a hundred times slower than that of the sodium ion. Thus it seemed reasonable to assume that the rate of the diffusion of the albumin out of the capillary was negligible in comparison with that of the sodium ion, and that the slowly diffusing albumin would not markedly alter the self-diffusion coefficient of the sodium ion.

To investigate whether or not this assumption was justified, some measurements were made of the self-diffusion coefficients of sodium ions in solutions containing albumin (i.e. albumin in the capillary and in the bath). About 50 ml of inactive solution was used, but because of the impossibility of obtaining the correct flow conditions in such a small volume the solution was not stirred: it has been shown that the effect of stirring is small (Table 1). The results in Table 2 (Nos 1 and 2) are for two solutions; both contained 0·1M sodium chloride and 10% albumin, but with 0·001M calcium chloride added to the second. In both cases the diffusion coefficient agreed within experimental error for inactive solutions containing no albumin (Table 2, Nos 8 and 9) and it was therefore assumed in the present experiments that the absence of albumin in the inactive solution was not important. A similar conclusion was reached for the diffusion of sodium ions in lecithin sols where the micelles probably diffuse one hundred times slower than the sodium ion (Saunders, 1953; Thomas & Saunders, 1959). The molecular weight of micelles treated with ultrasonic irradiation is of the order of 10^6 (Attwood, 1965) and one would expect them to diffuse slowly.

DIFFUSION IN SYSTEMS CONTAINING MACROMOLECULES

The diffusion coefficients of sodium in albumin solutions and lecithin sols each containing 0·1M sodium chloride are lower than the corresponding value in 0·1M sodium chloride alone. This decrease could be due to the increased diffusion path owing to the presence of large molecules (obstruction effect) or it could be due to the adsorption of sodium ions, or a combination of both factors. Similar decreases have been found in other systems: sodium ion diffusion in solutions of sodium dodecylsulphate (Clifford & Pethica, 1964), and in solutions containing ion-exchange resins (Brady & Salley, 1948; Huinzenga & others, 1950; Dux & Steigman, 1958, 1959), and for the diffusion of water in ovalbumin solutions (Wang & others, 1954).

In experiments with ion-exchange resins two types of ions have been considered; bound ions which are those adsorbed on to the molecule,

and the rest which are classed as "free" ions. An estimate can be obtained of the fraction, F, of total ions bound to the macromolecules using the relationship:

$$F = \frac{C_b}{C} = \frac{D - D'}{D - D_m} \quad .. \quad .. \quad .. \quad (18)$$

where C_b = concentration of bound ions, C = total concentration of ions, D_m = diffusion coefficient of macromolecule, D′ = observed diffusion coefficient of ions, D = diffusion coefficient of ions in medium, containing no macromolecules.

Assuming such a relationship is valid for the systems that we are studying, when the fraction of bound sodium ions is plotted against the albumin concentration, F increases with increasing concentration and then appears to tend to a limiting value. This suggests that F is not solely controlled by the albumin concentration.

It has also been assumed that this relationship (18) is valid for lecithin sols since the critical micelle concentration is negligibly small; values of the fraction bound are given in Table 6. More sodium ions appear to be bound in lecithin sols than in albumin solutions at the same concentration.

TABLE 6. FRACTION OF BOUND SODIUM IONS IN VARIOUS LECITHIN SOLS

Experiment No.	Method	Composition of solution		Fraction bound
		in capillary	in bath	
11	a	0·1M NaCl 5% w/w lecithin	0·1M NaCl	0·064
12	b	,,	,,	0·056
14	a	0·1M NaCl 0·001M $CaCl_2$ 10% w/w lecithin 5% w/w cholesterol	0·1M NaCl 0·001M $CaCl_2$	0·267
15	b	,,	,,	0·265

A linear relationship has been suggested by Wang between the apparent diffusion coefficient of water and the concentration of ovalbumin in solution. The albumin systems used in the present work behave less simply, except at low concentrations. This is to be expected because the structure of the solutions will be more complicated than Wang's model due to the presence of sodium chloride.

When calcium chloride was added to a 10% albumin solution it caused an increase in the diffusion rate. This is probably caused by a displacement by the calcium ions of sodium ions adsorbed on to the albumin molecules. Thus less sodium would diffuse with the protein molecule.

The addition of cholesterol and of calcium chloride to a 10% lecithin sol resulted in a large reduction in the diffusion coefficient. This could be caused partly by the increase in the size of the micelles due to the incorporated cholesterol and partly by the greater number of micelles due to the increased concentration of lecithin.

SEMI-INFINITE SYSTEMS

Table 3 illustrates the application of equation (3) to systems where active 0·1M sodium chloride is contained in the capillary and 0·1M sodium chloride in the bath (inactive solution); Table 4 shows the result of applying equation (3) to systems in which either lecithin or albumin with 0·1M sodium chloride (active) is contained in the capillary and inactive sodium chloride in the bath. The results in both Tables show good agreement between experimental and calculated activities. The application of the equations described in section 3 to semi-infinite composite systems consisting of lecithin sols layered on top of albumin solutions is depicted in Table 5. By substituting the total length of the albumin layer (the semi-infinite medium II) for x in equation (11), it was found that there was no change of C(x,t) at the closed end of the tube until t was approximately 6 hr. From this it was concluded that the system was semi-infinite up to 6 hr after the start of the experiment; thereafter the system became finite. It appears that there is no resistance to or facilitation of diffusion at the boundary between the albumin and lecithin layers during this time. However other results, not published here, indicate that under certain conditions a resistance to diffusion at the boundary may exist.

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