85. *Diffusion Studies with Lysolecithin.*

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A method for preparing crystalline lysolecithin is described. This material gave clear sols in water and so diffusion coefficients could be determined by the Gouy method. Measurements showed that the diffusion coefficient (D) was independent of sol concentration in the range studied. The low value of D and the regularity of the interference patterns indicated the presence of large, uniform micelles in the sols. The presence of calcium chloride did not affect either D or the viscosity of a sol.

The phosphatides are important constituents of cell membranes and studies of the physical properties of their sols in water should contribute to our knowledge of formation of cell membranes. We now give details of a method for preparing crystalline lysolecithin. Diffusion and viscosity measurements have been carried out with its sols over a limited concentration range. The effect of calcium chloride on diffusion and viscosity of a lysolecithin sol has also been studied, since this salt has an important effect on the stability of lecithin sols.

The viscosity of the lysolecithin sols varied with the viscometer used, indicating that the sols exhibited non-Newtonian flow. Changes in viscosity were followed by observing the times required for a fixed volume of the sols to pass through a particular viscometer.

Experimental

The diffusion was measured across a free boundary by using a Gouy diffusiometer described previously.[1,2]

Materials.—Lysolecithin. This was prepared by a method based on that of Hanahan, Rodbell, and Turner.[3] The yolks of 24 eggs were broken and stirred for a few minutes with 1 l. of acetone. The solution was then filtered, and the residue washed with fresh acetone. This process was repeated once. To the residue were added 300 ml. of warm ethyl alcohol. After being stirred, the alcoholic solution was decanted off and this process was repeated four times. The alcoholic solution was then evaporated under reduced pressure to dryness, the temperature being kept below 55°. The dry extract was dissolved in the minimum quantity of warm ether and the solution was added, with stirring, to a sufficient quantity of acetone to precipitate all the phosphatides. The precipitate was centrifuged off and dissolved in 500 ml. of ethyl alcohol. Alumina was added to the solution until it gave no coloration on heating with ninhydrin solution, indicating the complete removal of cephalins and other phosphatides containing primary amino-groups. The alcoholic solution was filtered and evaporated to dryness to give a residue of lecithin containing some lysolecithin. This was dissolved in ether so as to give a 1% solution, which was not completely clear owing to the insolubility of lysolecithin in ether. To the ethereal solution was added 10 mg. of Russell viper venom, which had been dissolved in 10 ml. of water. The mixture was shaken and kept for 1 hr. at room temperature. Most of the supernatant liquid was decanted off, leaving a gelatinous precipitate of lysolecithin: treatment of this with acetone caused flocculation, so that the remaining supernatant liquid was easily decanted off. The solid was then dissolved in the minimum amount of warm chloroform, and thence precipitated by addition of excess of ether. The precipitate was separated by decantation, and the process repeated three times. The lysolecithin was then dissolved in warm ethyl alcohol so as to give about a 20% solution. The warm solution was centrifuged to remove the residue from the venom present as a suspended solid and the lysolecithin was then allowed to crystallise from the alcoholic solution at 0°. This crystallisation was repeated three times (Found: N, 2·8; P, 5·7%).

Calcium chloride. " AnalaR " reagent was used.

Preparation of Sols.—Lysolecithin sol. The lysolecithin was dissolved in a small volume of distilled water, with the aid of very gentle warming. The sol was then passed over a bed of

[1] Saunders, *J.*, 1953, 519.
[2] Brudney and Saunders, *J.*, 1955, 2916.
[3] Hanahan, Rodbell, and Turner, *J. Biol. Chem.*, 1954, **206**, 431.

mixed Amberlite ion-exchange resins IR-120(H) and IRA-400(OH) to remove any traces of non-colloidal electrolyte present. The resins were then washed with small successive portions of distilled water and the sol was finally made up to volume. The specific conductivity of a 1% sol was 160,000 mhos/cm. Concentrations are expressed as % (w/v).

Lysolecithin sol containing calcium chloride. A sol of lysolecithin in water was prepared as above, except that the concentration of the sol was twice that required in the final sol. To this was added an equal volume of calcium chloride solution of twice the concentration required in the final sol.

Results.—In Table 1 are given the results obtained from three Gouy patterns observed when a solution of 1·1% lysolecithin diffused into water. These show the consistency of C_t.

Effect of Temperature on D.—The integral diffusion coefficient for a 1·4% sol of lysolecithin at 30° was $6{\cdot}72_2 \times 10^{-7}$ cm.^{2} sec.$^{-1}$.

Effect of Calcium Chloride.—Sols were prepared containing 1·2% of lysolecithin and varying concentrations of calcium chloride as shown in Table 3. Boundaries were then formed in the diffusion cell, between these sols and solutions containing the same concentrations of calcium chloride as the sols but without the lysolecithin.

TABLE 1. *Integral diffusion coefficient for a 1·1% lysolecithin sol.*

$j_m = 77{\cdot}0$ (the symbols are defined in *J.*, 1953, 519).

	t = 19,140 sec.		t = 24,600 sec.		t = 27,540 sec.	
j	γ	C_t	γ	C_t	γ	C_t
1	1·3507	1·496	1·1924	1·320	1·1262	1·247
2	1·3003	1·495	1·1472	1·319	1·0842	1·246
3	1·2561	1·492	1·1097	1·318	1·0485	1·245
4	1·2178	1·494	1·0737	1·317	1·0146	1·245
5	1·1799	1·492	1·0414	1·317	0·9850	1·245
6	1·1462	1·494	1·0105	1·317	0·9553	1·243
7	1·1130	1·494	0·9820	1·318	0·9286	1·246
8	1·0811	1·493	0·9548	1·319	0·9024	1·246
9	1·0508	1·493	0·9287	1·319	0·8772	1·246
10	1·0222	1·492	0·9037	1·319	0·8531	1·245
s		0·0014		0·0010		0·0014
10^7D		$6{\cdot}57_3$		$6{\cdot}56_4$		$6{\cdot}57_0$

D (Average for 5 patterns) $= 6{\cdot}57 \times 10^{-7}$.

(s is the standard deviation of each column of C_t values. D is the diffusion coefficient in cm.^{2} sec.$^{-1}$.)

DISCUSSION

The low value obtained for the diffusion coefficient of lysolecithin indicates that in water it exists in the form of large micelles. The regular nature of the Gouy patterns obtained shows that only one diffusing component is present, *i.e.*, the micelles must be of fairly uniform size.

The micelles being assumed spherical, the volume of a single micelle can be calculated by using the Stokes–Einstein equation from which the molar volume of a micelle was found to be $1{\cdot}289 \times 10^5$ c.c. corresponding to a volume of $2{\cdot}15 \times 10^5$ Å^3 per molecule.

In order to estimate the molar volume of monomeric lysolecithin, the density of the solid was determined by a flotation technique with di-iodomethane–benzene. This gave a value of 1·095 g. ml.$^{-1}$, corresponding to a molar volume of 472 ml. Division of the micellar volume by this gave a value of 273 as the mean number of molecules in a micelle.

Statistical Analysis of Results.—When the results of Table 2 are plotted as a graph showing the variation of diffusion coefficient with concentration, some scatter is observed as is expected with a colloid. A summary of a statistical analysis of these results and those of Table 3 is given below.

(1) *Data of Table* 2. In order to assess the variation of the diffusion coefficient D with concentration c, the correlation coefficient r was calculated. In Table 2 the values of D given are in fact the means of four or more Gouy patterns analysed for each concentration. In the statistical calculations all the individual values of D for each Gouy pattern have been

included, except for the first two results in the table, where an extrapolation procedure had to be adopted; in these cases the single extrapolated values of D were taken, thus giving these two results less weight than the others. There were 39 degrees in the

TABLE 2. *Diffusion coefficients and viscosities of lysolecithin sols.*

Concn. of lysolecithin (w/v %)	Integral diffusion coeff. D at 25° (cm.^{2} sec.$^{-1}$)	Time (sec.) required for solution to pass through viscometer at 25°
0·88	$6{\cdot}55_5$ *	1123
0·94	$6{\cdot}55_0$ *	1125
1·02	$6{\cdot}62_8$	1127
1·10	$6{\cdot}57_3$	1131
1·20	$6{\cdot}50_1$	1132
1·30	$6{\cdot}59_7$	1134
1·40	$6{\cdot}41_8$	1137
1·58	$6{\cdot}36_0$	1143
1·72	$6{\cdot}55_1$	1147
1·87	$6{\cdot}57_5$	1152

* Indicates an extrapolated value. In these experiments it was found that D decreased with time, and the true diffusion coefficient was obtained by plotting D against $1/t$ and extrapolating to $1/t = 0$.[1]

No results could be obtained with sols of concentration below 0·88% since the density differences between the sols and water were too small for stable boundaries to be formed.

TABLE 3.

$CaCl_2$ concn. (M)	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
10^7D	$6{\cdot}42_3$	$6{\cdot}58_0$	$6{\cdot}45_0$	$6{\cdot}59_7$	$6{\cdot}37_1$	$6{\cdot}34_0$
Viscosity relative to pure lysolecithin sol	1·00	0·99	0·99	0·99	0·99	1·00

calculation of the correlation coefficient of D with c, and its value was $r = -0{\cdot}26$. The theoretical value of r for the 0·95 probability level is 0·35,[4] and therefore, since the calculated value is appreciably less in magnitude than the theoretical one, the correlation is not significant, *i.e.*, there is no real variation of D with c.

This result being accepted, the variance of the diffusion coefficient values about their mean was estimated as $6{\cdot}464 \times 10^{-17}$ with 39 degrees of freedom. This corresponded to a standard deviation of $0{\cdot}08 \times 10^{-7}$. From this, limits of error of a diffusion coefficient of lysolecithin determined from five Gouy patterns were estimated as $\pm 0{\cdot}097 \times 10^{-7}$ at the 0·99 probability level. The mean value of D was $6{\cdot}544 \times 10^{-7}$ and so these limits as a percentage of the mean are $\pm 1{\cdot}48$.

(2) *Data of Table* 3. A correlation coefficient for the variation of D with calcium chloride concentration was calculated as −0·117, with five degrees of freedom. The theoretical value at the 0·95 probability level is 0·754.[4] Since the observed value is much smaller in magnitude than this it is concluded that the diffusion coefficient is independent of calcium chloride concentration. A t-test showed that the mean value of D in the presence of calcium chloride does not differ significantly from the mean value for the pure sols.

The conclusion that both D and the relative viscosity of a lysolecithin sol are unaffected by the presence of calcium chloride was unexpected in view of the marked effect of calcium salts on lecithin sols.[5] It had been expected that bonding between the calcium ions and the phosphate groups of the lysolecithin would have caused a reduction in the rate of diffusion and an increase in relative viscosity.

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[4] Saunders and Fleming, "Mathematics and Statistics," p. 162, Pharmaceutical Press, London, 1957.
[5] Elworthy and Saunders, *J.*, 1957, 330.