

FLOCCULATION AND CONDUCTIVITY OF PHOSPHATIDE SOLS

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Results obtained in flocculation and conductivity studies with sols of lecithin and lysolecithin and solutions of calcium and potassium chlorides are reported. Calcium chloride interacts with lecithin, the extent of interaction depending upon the method of preparation of the lecithin sol. Potassium chloride does not interact with lecithin. These results may explain why potassium ions can be transported across a cell membrane whilst calcium ions cannot.

DANIELLI and Stein (1956) have summarised the information that exists about the nature of a red cell membrane. It contains roughly equal weights of lipid molecules including phosphatides and of complex protein molecules. The lipid content in a membrane is just sufficient to constitute a layer two molecules in thickness and the molecules are thought to be arranged in the form of a bimolecular leaflet, with the non-polar parts directed inwards and with the polar groups on the exterior. Proteins are adsorbed on both surfaces of the leaflet.

Why certain salts diffuse through a cell membrane whilst other salts do not is still a mystery. It is possible that phosphatides play a part in the functioning of a membrane by interacting with certain salts and preventing their passage across the membrane, whilst other salts which do not interact with phosphatides may be those which are transferred across it. The interactions between the phosphatides lecithin and lysolecithin, and calcium and potassium chloride solutions have been studied by means of flocculation and conductivity experiments.

Apparatus

EXPERIMENTAL

The conductivity measurements were made using a Pye conductance bridge (Cat. No. 11700). A Cambridge conductivity cell (Ref. No. 43932/2), having a cell constant of 0.1 and bright platinum electrodes, was used. Constant results could not be obtained with a cell the electrodes of which had been covered with platinum black owing to the difficulty of removing impurities from the surface of the electrodes.

Materials

The methods for preparing lecithin and lysolecithin have been described previously (Saunders, 1957). The substances had the following characteristics:

	Lecithin	Lysolecithin
Nitrogen	1.83 per cent	2.73 per cent
Phosphorus	3.92 per cent	5.86 per cent
Specific rotation	$[\alpha]_D^{25} + 7.17$ (5 per cent w/v solution in ethanol)	$[\alpha]_D^{25} + 1.60$ (5 per cent w/v solution in ethanol)
Iodine number	74.3	3.0
Mean molecular weight (from N and P contents) ..	778	521

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Preparation of Sols

Lysolecithin sol. Lysolecithin was dissolved in water with warming. The sol was passed down a column containing mixed ion-exchange resins (Amberlite IR-120(H) and IRA-400(OH)). The resins were then washed with small successive quantities of distilled water and the sol was finally made up to volume.

Lecithin sol. Lecithin was dissolved in 5 ml. of ether. About 20 ml. of water was added with intermittent shaking. The ether was removed by displacement with nitrogen which in turn was removed under reduced pressure. The solution was then passed down a column containing mixed resins and finally made up to volume.

Mixed sols. Method A. Sols of lecithin and lysolecithin prepared by the above methods were mixed in the required proportions. *Method B.* Weighed quantities of lecithin and lysolecithin were dissolved in ethanol. The ethanol was removed under vacuum leaving a film of intimately mixed phosphatides. Water was added and the flask shaken for 5 hr. at 55°. The sol was then passed down a column containing mixed resins and finally made up to volume.

All sols were aged for 24 hr. before being used, since viscosity studies have shown that changes occur in the sols with time (Thomas and Saunders, 1958).

Flocculation Studies

1 ml. of a sol containing 0.5 g. of phosphatide in 100 ml., prepared by method A, was placed in a small sample tube and 0.2 ml. of a salt solution was added by means of an Agla micrometer syringe. After stirring the sols, the tubes were placed in an incubator at 25° ($\pm 1^\circ$).

TABLE I
EFFECT OF CALCIUM CHLORIDE ON THE STABILITY OF LECITHIN SOLS

Concentration of CaCl ₂ in moles/litre ($\times 10^3$)	After standing for 24 hr.		After standing for 72 hr.	
	0.42 per cent lecithin	0.21 per cent lecithin	0.42 per cent lecithin	0.21 per cent lecithin
1.25	—	—	—	—
3.00	—	—	—	—
6.00	+	—	+	+
125	+	—	+	+
300	—	—	—	—
600	—	—	—	—

+ = Flocculation.
— = No flocculation.

Effect of total phosphatide concentration on flocculation produced by salt solutions. Two series of experiments were made, one with a 0.42 per cent lecithin sol, and the other with a 0.21 per cent sol. To them was added 0.2 ml. of various concentrations of calcium chloride and potassium chloride solutions, with the results given in Tables I and II.

The results given in Tables I and II show that with a sol containing 0.42 per cent (w/v) of lecithin flocculation occurs much more rapidly

than with a sol containing 0.21 per cent. However, after allowing the mixtures to stand for three days identical results were obtained with both sols. Calcium chloride concentrations from 6×10^{-5} moles per litre to 1.25×10^{-3} , caused flocculation of the sols. With potassium chloride concentrations above 3×10^{-3} moles per litre caused flocculation.

TABLE II
EFFECT OF POTASSIUM CHLORIDE ON THE STABILITY OF LECITHIN SOLS

Concentration of potassium chloride in moles/litre ($\times 10^3$)	After standing for 24 hr.		After standing for 72 hr.	
	0.42 per cent lecithin	0.21 per cent lecithin	0.42 per cent lecithin	0.21 per cent lecithin
1.25	—	—	—	—
3.00	+	—	+	—
6.00	+	—	+	+
8.33	+	—	+	+

Effect of lysolecithin on the flocculation of lecithin sols by salt solutions. The effect of the presence of varying amounts of lysolecithin on the stability of lecithin sols towards various concentrations of calcium chloride and potassium chloride was studied. The rate of flocculation was slower with the mixed sols than with lecithin sols, and the mixtures were allowed to stand until constant results were obtained. The results obtained with calcium chloride are given in Table III. With a sol having a weight

TABLE III
EFFECT OF CALCIUM CHLORIDE ON THE STABILITY OF MIXED PHOSPHATIDE SOLS
(Total phosphatide concentration in each mixture = 0.42 per cent (w/v).

Calcium chloride in moles/litre ($\times 10^3$)	Weight fraction of lecithin				
	1.0	0.9	0.8	0.7	0.6
1.25	—	—	—	—	—
3.00	—	—	—	—	—
6.00	+	—	—	—	—
8.33	++	+	+	—	—
10.0	++++	+++	++	—	—
12.5	++++	+++	++	+	—
30.0	+++++	+++	++	—	—
60.0	++++	++	+	—	—
83.3	+++	+	—	—	—
125.0	++	—	—	—	—
300.0	—	—	—	—	—
600.0	—	—	—	—	—

++++ = Large precipitate.
+ = Small precipitate.
— = No precipitate.

fraction of lecithin of 0.9, concentrations of potassium chloride of 1.25×10^{-2} M and above were required to cause flocculation. When the weight fraction of lecithin was 0.8, concentrations of 8.33×10^{-2} M and above were required to cause flocculation, and when the weight fraction was 0.7 or less no flocculation occurred with any concentrations below 1.25×10^{-1} M.

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Conductivity Studies

Effect of concentration of phosphatide sol. Two per cent sols of lecithin and lysolecithin were prepared by the methods described previously and their conductivities measured. These sols were then diluted with successive quantities of conductivity water and the conductivities of the diluted sols were measured. That due to the phosphatide was calculated by subtracting the conductivity of the water from the specific conductivity of the sol. The results obtained are given in Table IV.

TABLE IV

THE VARIATION OF SPECIFIC AND EQUIVALENT CONDUCTIVITIES OF LECITHIN AND LYSOLECITHIN SOLS WITH CONCENTRATION

Concentration in per cent (w/v)	Lysolecithin		Lecithin	
	Specific conductivity mhos cm. ⁻¹ (× 10 ⁶)	Equivalent conductivity	Specific conductivity mhos cm. ⁻¹ (× 10 ⁶)	Equivalent conductivity
2.0	1.472	0.383	0.580	0.225
1.8	1.352	0.391	0.528	0.228
1.6	1.213	0.395	0.478	0.232
1.4	1.096	0.407	0.430	0.239
1.2	0.952	0.413	0.372	0.241
1.0	0.806	0.419	0.312	0.242
0.8	0.687	0.447	0.254	0.247
0.6	0.540	0.468	0.194	0.251
0.4	0.435	0.566	0.155	0.301
0.3	0.340	0.590	0.142	0.368
0.2	0.232	0.604	0.097	0.377
0.1	0.142	0.739	0.080	0.622

Effect of phosphatide sols on the conductivities of salt solutions. The specific conductivities of calcium chloride and potassium chloride in the presence of phosphatides were compared with the specific conductivities of the salt solutions of the same concentration in water.

The reproducibility of the specific conductivities obtained on adding certain volumes of calcium chloride or potassium chloride solutions to water were within ± 1 per cent. Therefore changes in the specific conductivity less than this were not considered to be significant.

In each experiment 20 ml. of the phosphatide sol was placed in the conductivity cell which was then placed in a thermostat bath at 25° for 15 min. to attain constant temperature. The specific conductivity of the sol did not vary with time once temperature equilibrium had been attained. Small volumes (1 ml.) of salt solutions were added to the sol successively by means of a pipette.

The specific conductivity of the salt in water was calculated by subtracting the specific conductivity of the water from that of the salt solution. The specific conductivity of salt in the presence of a phosphatide sol was calculated by subtracting the specific conductivity of the phosphatide sol (after making a small correction to allow for the small dilution of the sol by the salt solution), from the specific conductivity of the sol containing salt.

The effects of varying the proportions of lecithin to lysolecithin in the sols on the conductivity of calcium chloride are given in Tables V and VI.

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TABLE V

EFFECT OF PHOSPHATIDE SOLS (PREPARED BY METHOD A) ON THE SPECIFIC CONDUCTIVITIES OF CALCIUM CHLORIDE SOLUTIONS

Molar concentration of CaCl_2 ($\times 10^3$)	Specific conductivity due to CaCl_2 ($\times 10^3$)	Weight fraction of lecithin in the mixtures							
		0	0.3	0.5	0.6	0.7	0.8	0.9	1.0
		Change per cent in specific conductivity of CaCl_2							
4.762	1.313	-0.53	+0.76	+0.30	-2.13	-2.22	-2.83	-3.12	-4.95
9.091	2.498	-0.88	+0.48	+0.28	-2.80	-3.12	-3.60	-3.96	-5.08
13.04	3.567	-0.73	+0.36	-0.39	-2.45	-2.38	-3.28	-3.28	-4.85
20.00	5.407	+0.64	+0.61	+0.11	-1.42	-1.98	-1.98	-2.68	-4.34
57.69	15.33	-0.26	-0.19	-0.20	-1.63	-1.74	-1.89	-1.83	-2.74
92.59	24.28	-0.04	-0.37	+0.33	-1.06	-1.12	-1.32	-1.28	-1.73
125.0	32.42	-0.55	-0.74	-0.77	-0.74	-0.24	-1.06	-1.17	-1.28
155.2	39.81	-0.15	+0.05	+0.70	-0.78	-0.18	-0.60	-0.48	-0.68
183.3	47.07	-0.44	-0.28	+0.06	-0.87	-0.25	-0.60	-0.17	-0.79
500.0	123.0	-0.27	+0.49	-0.32	-0.37	-0.65	-0.66	-0.24	-0.18

TABLE VI

PERCENTAGE CHANGES IN THE SPECIFIC CONDUCTIVITIES OF CALCIUM CHLORIDE SOLUTIONS IN THE PRESENCE OF PHOSPHATIDE SOLS (PREPARED BY METHOD B)

Concentration of CaCl_2 in moles/litre ($\times 10^3$)	Weight fraction of lecithin in mixture					
	0.3	0.5	0.6	0.8*	0.9*	1.0*
4.762	-7.10	-11.33	-13.14	-9.92	-11.63	-12.01
9.091	-6.00	-9.66	-11.37	-10.8	-10.97	-9.98
13.04	-6.61	-9.16	-11.18	-10.98	-10.90	-10.07
20.00	-3.72	-6.69	-7.99	-9.84	-8.18	-6.88
57.69	-2.19	-3.73	-4.96	-6.25	-5.86	-5.08
92.59	-1.10	-2.57	-3.47	-4.94	-4.57	-3.47
125.0	-1.94	-2.88	-3.73	-4.29	-4.15	-4.03
155.2	-1.52	-2.56	-3.02	-3.59	-3.63	-3.27
183.3	-1.32	-1.78	-2.82	-3.61	-4.03	-3.24
500.0	-0.93	-1.49	-1.72	-1.30	-1.24	-1.32

* Sols were opaque.

The effects of lecithin and lysolecithin on the conductivities of potassium chloride solutions are given in Table VII.

TABLE VII

PERCENTAGE CHANGES IN THE SPECIFIC CONDUCTIVITIES OF POTASSIUM CHLORIDE SOLUTIONS IN LECITHIN OR LYSOLECITHIN SOLS (0.5 PER CENT W/V)

Concentration of KCl in moles/litre ($\times 10^3$)	Lecithin		Lysolecithin	
0.4762	+0.29	-0.73		
0.9091	-0.82	-0.97		
5.217	-0.73	-0.80		
9.167	-0.23	-0.46		
48.80	+0.86	+0.54		
85.38	+0.10	0		

DISCUSSION

From the results it can be seen that calcium chloride is much more effective in causing flocculation of lecithin sols than is potassium chloride. Flocculation starts at $6 \times 10^{-5}\text{M}$ with calcium chloride, whilst with potassium chloride a concentration of $3 \times 10^{-3}\text{M}$ is required. These results are in agreement with earlier studies which showed that the higher

the valency of the ions in the added salt the more effective was the coagulating effect on lecithin sols (DeJong and Teunissen, 1935; Remesov, 1930; Saunders, 1957).

Malquori (1932), Rona and Deutsche (1926) and Saunders and Elworthy (unpublished), have reported that divalent metal chlorides give two precipitation zones, with a region of peptisation between them. The results given in Table III show one region of precipitation and the beginning of the region of peptisation. If the effect of much higher concentrations of calcium chloride had been studied another region of precipitation would have been expected.

Conductivity studies indicate that some interaction occurs between lecithin and calcium chloride. It is possible that the calcium ions interact with the negatively charged phosphate group of the lecithin molecules, forming either an insoluble complex or cross-links between lecithin molecules in different micelles causing aggregation of the micelles to form a precipitate. Also it is possible that some calcium ions are adsorbed on to the micelles and neutralise their small negative charge and so cause them to aggregate. At higher concentrations of calcium chloride more calcium ions are probably adsorbed and the charge on the micelles may be reversed and consequently no precipitation occurs. This is supported by the fact that at higher concentrations of calcium chloride, conductivity studies indicate that the extent of interaction between lecithin and calcium chloride is increased. The narrowing of the range of concentration of calcium chloride over which flocculation occurs on increasing the proportion of lysolecithin in the sol, may be because lysolecithin solubilises or protects the "complex" formed between lecithin and calcium chloride and thus prevents its precipitation.

The precipitating action of potassium chloride is probably a salting out effect. The results given in Table VII show that there is no significant interaction between potassium chloride and lecithin or lysolecithin. The relatively high concentrations required to cause precipitations probably remove the water of hydration around the particles, thus facilitating their aggregation. Lecithin sols are protected by lysolecithin against the precipitating action of potassium chloride, probably because the hydration layer is much more difficult to remove from lysolecithin than from lecithin.

Lysolecithin was much more effective in protecting lecithin sols against potassium chloride than it was against calcium chloride. This is further evidence that the mechanisms of precipitation are different for the two salts. The physical appearance of the precipitates also differed. The precipitates caused by calcium chloride were bulky, whilst those caused by potassium chloride were much less so and more granular.

The very low conductivities exhibited by lecithin and lysolecithin sols indicate that the phosphatides exist as large, slightly negatively charged micelles in aqueous solution. The equivalent conductivity of lysolecithin sols is higher than that for lecithin sols and thus suggests that the size of the micelles is smaller in lysolecithin sols. This is in agreement with diffusion and light-scattering studies (Robinson and Saunders, 1959, 1960; Saunders and Thomas, 1958; Thomas and Saunders, 1959).

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From Table V it can be seen that lysolecithin sols do not have a significant effect upon the conductivity of calcium chloride solutions. This finding is in agreement with the fact that calcium chloride does not have any effect upon the viscosity or diffusion rate of lysolecithin sols (Saunders and Thomas, 1958).

The effect of mixed sols on the conductivity of calcium chloride has been found to be dependent upon the method of preparation. The sols prepared by method A were clear if the weight fraction of lecithin present was 0.6 or below, and translucent if the weight fraction of lecithin was 0.7 or above. Only sols having a weight fraction of lecithin of 0.6 or above caused a significant fall in the conductivity of calcium chloride solutions, although an interaction probably occurred with sols having a weight fraction below 0.6, but the interaction was not large enough to cause a significant fall in the conductivity.

TABLE VIII

TABLE SHOWING THE MOLAR RATIO OF LECITHIN/CaCl₂ REACTED ($\times 10^{-2}$) FOR SOLS PREPARED BY METHOD A

Molar concentration of CaCl ₂ ($\times 10^3$)	Weight fraction of lecithin				
	0.6	0.7	0.8	0.9	1.0
4.762	38.0	42.6	38.2	38.9	27.3
9.091	15.2	15.9	15.7	16.1	13.9
13.04	12.1	14.5	12.0	13.5	10.2
20.00	13.6	11.4	13.0	10.8	7.40
57.69	4.10	4.48	4.72	5.48	4.07
92.59	3.93	4.34	4.21	4.88	4.01
125.0	—	—	3.88	3.95	4.02

TABLE IX

TABLE SHOWING THE MOLAR RATIO OF LECITHIN/CaCl₂ REACTED ($\times 10^{-2}$) FOR SOLS PREPARED BY METHOD B

Molar concentration of CaCl ₂ ($\times 10^3$)	Weight fraction of lecithin					
	0.3	0.5	0.6	0.8*	0.9*	1.0*
4.762	5.70	5.96	6.16	10.9	10.4	11.2
9.091	3.53	3.66	3.73	5.24	5.80	7.09
13.04	2.24	2.69	2.64	3.59	4.07	4.89
20.00	2.59	2.40	2.41	2.61	3.54	4.67
57.69	1.53	1.49	1.35	1.42	1.71	2.19
92.59	1.89	1.35	1.20	1.12	1.37	2.00
125.0	0.795	0.893	0.827	0.959	1.11	1.28
155.2	0.815	0.810	0.822	0.923	1.03	1.27
183.3	0.798	0.986	0.745	0.777	0.784	1.08
500.0	0.416	0.431	0.448	0.791	0.934	0.973

* Sols were opaque.

When the sols were prepared by method B, clear sols were again obtained when the weight fraction of lecithin present was 0.6 or below, whilst opaque sols were obtained with higher weight fractions of lecithin. Sols prepared by this method, contrary to expectations, caused larger changes in the conductivity of calcium chloride than did sols prepared by method A, and significant falls occurred with sols having a weight fraction of lecithin of less than 0.6.

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The extent of interaction between the lecithin molecules and the calcium chloride can be estimated from the molar ratio obtained by dividing the number of moles of lecithin present by the number of moles of calcium chloride which have reacted with the lecithin. These ratios are given in Tables VIII and IX.

The results in Table VIII show that for a given concentration of calcium chloride the extent of interaction between the lecithin and calcium chloride is constant and the presence of lysolecithin does not appear to affect it. As the concentration of calcium chloride is increased, so the interaction is increased.

The results given in Table IX show that the interaction between lecithin and calcium chloride is far greater when the sols are prepared by method B. Even the sols having a weight fraction of lecithin of 0.7 or above, which were opaque and had very large particles present in them reacted to a greater extent with calcium chloride than did sols prepared by method A. With sols prepared by method B, the interaction seems to increase with increasing amounts of lysolecithin present. This is probably explained by the fact that the greater the amount of lysolecithin present in the sol, the greater will be the amount of lecithin which is completely solubilised, and consequently the greater the interaction between the lecithin and the calcium chloride. If all the lecithin had been completely solubilised, the extent of interaction would probably have been independent of the amount of lysolecithin present, since with clear sols having a weight fraction of lecithin of 0.6 or below the extent of interaction is independent of the lysolecithin content of the sols.

Calcium chloride interacts with lecithin, whilst potassium chloride does not. This may have a bearing on the different permeabilities of a cell membrane towards the two cations. The binding of calcium ions by lecithin may be the reason for the relative impermeability of a cell membrane to calcium ions, whilst the lack of such binding between potassium chloride and lecithin may be the reason for the permeability of a cell membrane to potassium ions.

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