58. *Surface Force and Stability of Lecithin Sols.*

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The stability of lecithin sols in the presence of electrolytes depends on the method of preparation of the lecithin; lecithin which has been subjected to harsh chemical treatment gives very unstable sols. Small quantities of soaps in the lecithin greatly increase the stability.

Surface-force determinations on sols of lecithin prepared by chromatography show that both uni- and bi-valent metal chlorides give surface forces at the sol–water interface. The production of surface force is governed by the degree of instability of the sols.

The lecithin sol–water interface and the surfaces of some simple cells are similar in many respects.

The effects of various uni- and bi-valent metal chlorides on the surface force * of the lecithin sol–water interface have already been reported.[1] The lecithin used was purified by a method which involved precipitation with cadmium chloride.[2]

Some samples of lecithin prepared in this way were contaminated with traces of cadmium chloride (detected by use of diphenylcarbazone [3]), even after passage of an alcoholic solution over mixed weak ion-exchange resins. Although concentrations of metal were only 10^{-4}—10^{-5}M in the sol, these amounts can have an appreciable effect on the surface force. When an alcoholic solution was passed over mixed strong ion-exchange resins, damage to the lecithin molecules resulted, giving a material of low specific rotation and iodine number. It was therefore necessary to use a method of purifying lecithin which did not involve precipitation with metal salts; Lea and Rhodes's chromatographic method was adopted.[4]

Various lecithin preparations have been made, and in the presence of electrolytes the stability of the sols differed according to the method used. Very small concentrations of soaps greatly increased this stability.

The effects of some simple salts and soaps on the surface force have been studied, and a correlation has been made between the stability of the sols and the surface force.

Experimental

Materials.—Chromatographic purification of lecithin. Mixed egg-yolk phosphatides were prepared by separating the yolks of twelve eggs, extracting them repeatedly with acetone until a white powder resulted, and extracting the acetone-free powder with successive portions

* The authors have used the term " surface force " to denote the quantity γ which occurs in the expression $2\pi\gamma d$ for the force required to pull a circular ring of diameter d through the sol–water boundary since, in their opinion, this quantity is more akin to the force operating in a cell membrane than to the true interfacial tension at a boundary between two liquids.

[1] Elworthy and Saunders, *J.*, 1955, 1166.
[2] Pangborn, *J. Biol. Chem.*, 1951, **188**, 471.
[3] Feigl, " Spot Tests," Elsevier, London, 1954, Vol. I, p. 94.
[4] Lea and Rhodes, *Biochem. J.*, 1954, **57**, xxiii.

(100, 300, 100 ml.) of absolute alcohol. The alcoholic solution was passed over a weak cation-exchange resin; the mixed phosphatides obtained on evaporation of the extract were recrystallised from acetone–ethyl methyl ketone (4 : 1).

The chromatographic solvent was 25% methanol in chloroform; mixed phosphatides (5 g.) dissolved in this solvent were run on Mallinckrodt silica gel (75 g.) mixed with acid-washed " Celite " (20 g.), an automatic fraction collector being used. The first fraction gave a colour reaction with ninhydrin, and the second contained lecithin (2·05 g. from 5 g.). The lecithin fraction was evaporated to dryness, dissolved in dry alcohol, and passed through alumina (5 × 1 cm.) to remove traces of ninhydrin-reactive substances. After evaporation of the alcohol, the lecithin was recrystallised from the above ketone mixture. The crystals were washed with acetone, dried in a vacuum, dissolved in dry alcohol, and stored under nitrogen at −5°. Analytical figures are given in Table 1. Lecithin prepared by chromatography is called " lecithin-A " below.

Lecithin treated in alcohol with ion-exchange resins. Lecithin purified through the cadmium chloride complex [1] was dissolved in dry alcohol, and passed three times over mixed strong ion-exchange resins. Analytical figures are given in Table 1. This sample of lecithin is called " lecithin-B."

Sodium dodecyl sulphate, lauric acid, and cholic acid. These substances were the gift of Dr. N. Brudney. Analytical figures (quoted from Brudney [5]) were: sodium dodecyl sulphate, S, 11·1% (Calc. for $C_{12}H_{25}O_4SNa$: S, 11·2%); cholic acid, $[\alpha]_D^{20}$ +34·91° (lit., 35° ± 0·5°), m. p. 198·9° (lit., 200°), equiv., 411·9 (Calc. for $C_{24}H_{40}O_5$: equiv., 408·6) lauric acid, m. p. 42—43° (lit., 44°), equiv., 200·7 (Calc. for $C_{12}H_{24}O_2$: equiv., 200).

Sodium cholate and potassium laurate were prepared by shaking the theoretical quantity of acid and alkali together, warming the mixture, and making it up to volume when the fatty acid had dissolved.

Other materials. Inorganic salts were " AnalaR " materials. Water was prepared by distillation in a seasoned all-glass still.

Preparation of Sols.—0·5% Sols were made by evaporating a sample of the stock solution of lecithin to dryness, dissolving the known quantity of lecithin in ether, adding distilled water, and evaporating off the ether with a stream of nitrogen. The sol was freed from included air by means of a filter pump, passed down a small column of mixed strong ion-exchange resins (this treatment did not significantly affect the iodine number of the lecithin, which was 72·0 before and 71·6 after passing through the column), and finally made up to volume. This method of preparation gave a stable sol which did not settle overnight.

Stability or Coagulation Tests.—Qualitative tests. The sol was distributed in 2 ml. portions in a series of sample tubes, and the substances whose coagulating effects were to be investigated were added from an Agla microsyringe, and the mixture stirred and left overnight. Arbitrary figures 0—4 were used to represent the degree of settling; 0 indicated no settling.

Quantitative tests. 16 ml. portions of sol were used, the substances under investigation being added as before. Next morning a 5 ml. portion of the supernatant fluid was removed by pipette and drained into a small Buchner flask, and the water evaporated off at 110°. The amount of lecithin present in the sample was determined by weight.

Surface-force Determinations.—These were carried out as described previously.[1] To ensure drainage of the sol from the ring, paraffin wax was found to be a suitable alternative to Silicone varnish. For experiments of short duration either of these ring coatings could be used, but when the ring was left in the sol overnight considerable adhesion of the sol to the ring resulted. Cheesman [6] found that a layer of carbon deposited from a benzene flame prevented variation of the contact angle at the xylene–water interface when the plate-pull method of measuring interfacial tension was used. In the present work, too, this coating prevented adhesion of sol to the ring even during long periods. All experiments were done at 25° ± 0·05°.

Results

Strong ion-exchange treatment of the lecithin dissolved in alcohol lowered the iodine number and specific rotation; strong ion-exchange treatment of lecithin dispersed in water did not have this effect, because in water the lecithin is in a macromolecular form which will not penetrate the resins.

[5] Brudney, Thesis, London, 1955, p. 52.
[6] Cheesman, *Arkiv Kemi, Min., Geol.*, 1946, **22**, *B*, No. 1.

The two samples (see Table 1) of lecithin-A have reasonably consistent analytical figures.

Effects of electrolytes and of soaps on the stability of 0·5% sols are shown in Tables 2 and 3. Sols of lecithin-B are more unstable than sols of lecithin-A in the presence of potassium chloride.

From Table 3 it can be seen that the addition of very small quantities of soaps to lecithin sols greatly increases their stability to electrolytes. Sols of lecithin-B require larger amounts of soap to stabilise them to 0·01M-potassium chloride than do sols of lecithin-A.

TABLE 1. *Analytical figures for various lecithin samples.*

Type of lecithin	N (%)	P (%)	$[\alpha]_D^{20}$	I no.	Spec. resistance of a 0·5% sol (megohms cm.$^{-1}$)
Purified through $CdCl_2$ complex	1·9	4·1	7·54	66·7	0·75
Purified through $CdCl_2$ complex and treated with ion-exchange resins (lecithin-B)	2·0	4·0	6·05	60·4	—
Chromatographic (lecithin A)	1·83	3·73	7·94	72·0	0·72
Chromatographic (lecithin A)	1·83	3·79	8·00	71·6	—

TABLE 2. *Effect of electrolytes on the stability of lecithin sols.*

Lecithin-A						Lecithin-B	
KCl concn. (10^{-5}M)	Amount of settling	$CaCl_2$ concn. (10^{-5}M)	Amount of settling	$CdCl_2$ concn. (10^{-5}M)	Amount of settling	KCl concn. (10^{-5}M)	Lecithin not pptd. (%)
150	0	3·99	0	1·00	0	18·8	80·9
200	1	4·99	1	1·99	1	28·1	69·3
250	2	5·98	3	2·99	2	31·3	66·0
300	3	6·98	1	3·99—19·9	4	34·4	15·6
400	3	7·98	2	29·9	3	37·5	6·8
500	4	8·98—40·2	4	39·9	2	100	7·2
		50·2	3	49·9	1		

TABLE 3. *Effect of soaps on the stability of lecithin sols.*

Lecithin-A in the presence of 0·01M-potassium chloride

K laurate		Na cholate		Na dodecyl sulphate	
Concn. (10^{-5}M)	Amount of settling	Concn. (10^{-5}M)	Amount of settling	Concn. (10^{-5}M)	Amount of settling
0·25	3	2·32	4	0·088	4
0·50	2	3·46	4	0·175	2
1·00	1	4·63	3	0·350	1
2·00	1	5·79	2	0·700	1
3·00	0	6·95	0	1·05	0
4·00	0	8·11	0	1·40	0

Lecithin-A in the presence of 0·10M-KCl

Na cholate		Na dodecyl sulphate	
Concn. (10^{-5}M)	Amount of settling	Concn. (10^{-5}M)	Amount of settling
12·4	4	1·40	4
18·6	3	1·58	4
24·7	2	1·75	3
30·9	2	2·28	3
37·1	1	2·80	2
43·3	0	3·50	1
49·5	0	4·06	1
55·7	0	4·55	0

Lecithin-B in the presence of 0·01M-KCl

K laurate	
Concn. (10^{-5}M)	Lecithin not pptd. %
0·99	7·2
1·99	6·4
2·99	21·2
3·79	32·4
5·19	75·3
6·39	78·0
10·18	80·0

The results of the surface-force experiments are shown in Figs. 1—4. 0·5% Sols of lecithin-A were used in all experiments, and were weighted with 0·01M-potassium chloride unless otherwise stated. Soaps were placed in the sols, while calcium salts were dissolved in the water layer only, as their presence in the sols gave rapid coagulation.

Figs. 1 and 2 show that 0·1M- and 0·01M-potassium chloride produced surface forces of the order of 0—0·24 dyne/cm., and these surface forces could be reduced by adding a soap. With

a standard sol, consisting of 0·5% lecithin, 0·01M-potassium chloride, and 0·0000926M-sodium cholate, increasing the calcium chloride concentration in the upper liquid gave two maxima of surface force (Fig. 3). This plot has the same general form as that previously reported [1] for the effect of calcium chloride on the surface force of sols of lecithin purified by means of the cadmium chloride complex. The surface forces produced by calcium chloride can be decreased by adding a soap to the sols (Fig. 4).

In addition to these results it was found that calcium nitrate produced smaller surface forces than those given by equal concentrations of calcium chloride. At 0·0003M-concentration, calcium chloride gave 0·229 dyne/cm. and calcium nitrate gave 0·117 dyne/cm. At 0·0005M-concentration, calcium chloride gave 0·237 dyne/cm. and calcium nitrate gave 0·103 dyne/cm.

Discussion

As small concentrations of bivalent metal salts have a large effect on the surface force, it is desirable to use a method of purifying lecithin which does not involve the addition of salts. The chromatographic method gives lecithin samples whose analytical figures are reasonably consistent.

Fig. 1. *Effect of varying the sodium cholate concentration on the surface force in the presence of* 0·10M-*potassium chloride.*

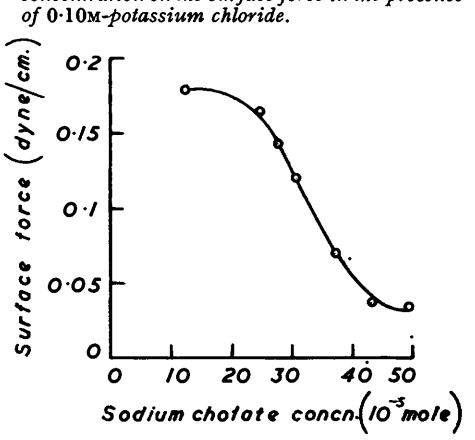
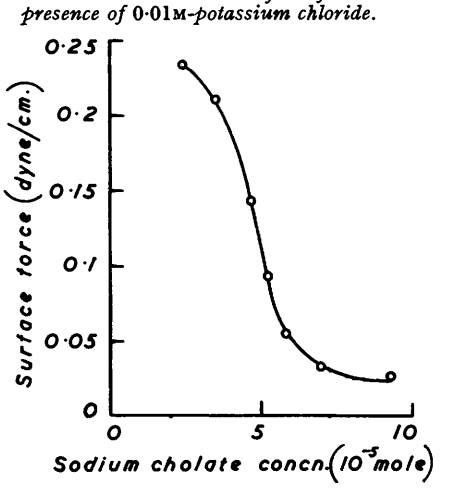


Fig. 2. *Effect of varying the sodium cholate concentration on the surface force in the presence of* 0·01M-*potassium chloride.*



Attempts to remove cadmium from samples prepared by means of the cadmium chloride complex result in the lecithin's having low iodine numbers and specific rotations, which are possibly due to the loss of fatty acids from the molecule (lecithin-B). Also, sols of lecithin-B are more unstable in the presence of potassium chloride than those of lecithin-A, and they require larger amounts of potassium laurate to stabilise them. The lecithin normally produced by means of the cadmium chloride complex, which has *not* been subject to strong ion-exchange treatment, gives more stable sols than those of lecithin-A. No instability to low concentrations of potassium chloride was observed in the work previously reported,[1] in which sols of lecithin purified by means of the cadmium chloride method were used. Faure [7] has stated that co-solubilisation phenomena can occur between lecithin and allied substances. It is possible that some soap-like materials, such as the lysolecithins which increase the stability of lecithin sols, are not removed in the purification by cadmium chloride complex but are removed in the chromatographic procedure. To give stability towards 0·01M-potassium chloride, only 0·0006 g. of sodium dodecyl sulphate is required for every gram of lecithin-A.

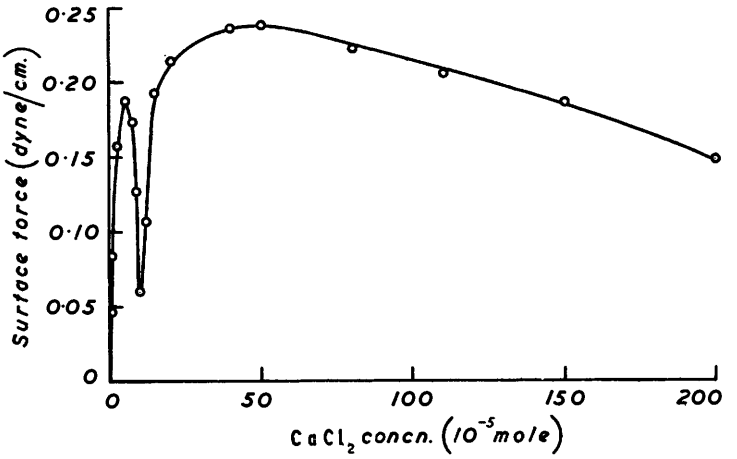
Stability tests are useful as routine tests for the purity of lecithin. They can show

[7] Faure, *Bull. Soc. Chim. biol.*, 1950, **32**, 503.

when molecular damage has occurred (lecithin-B), or when small quantities of soaps are present which would not be revealed by analytical determinations. Samples of lecithin produced by different methods can be characterised by the behaviour of their sols in the presence of electrolytes.

Sodium dodecyl sulphate is active in smaller concentrations than either potassium laurate or sodium cholate in preventing the coagulation of lecithin-A sols by 0·01M-potassium chloride; this is probably because it is not hydrolysed in very dilute solution.

FIG. 3. *Effect of varying the calcium chloride concentration on the surface force of a standard sol*



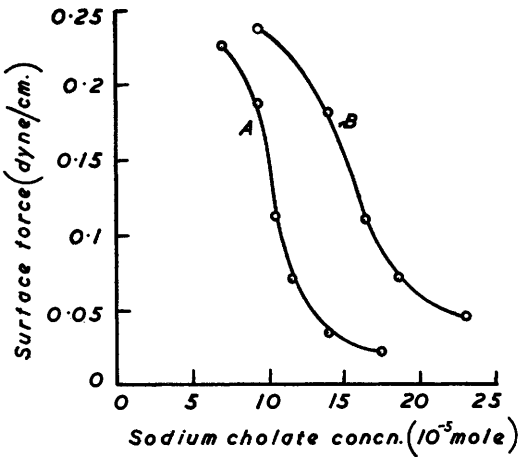


FIG. 4. *Effect of varying the sodium cholate concentration on the surface force in the presence of* (*A*) 0·00005M-*calcium chloride, and* (*B*) 0·0005M-*calcium chloride.*

At the point where sols of lecithin-A are completely stable to 0·01M-potassium chloride the molar ratios of lecithin (monomer) : soap are, 615 : 1 for sodium dodecyl sulphate, 214 : 1 for potassium laurate, and 93 : 1 for sodium cholate. The soaps are presumably adsorbed by the lecithin micelles and affect their electrical properties.

Surface Forces of Lecithin-A Sols.—In previous work [1] on sols of lecithin prepared by means of the cadmium chloride complex, potassium chloride produced only small surface forces, of the order of 0·01 dyne/cm. With sols of lecithin-A the same potassium chloride concentration gave large surface forces (Figs. 1 and 2), provided only small soap concentrations were present. As the soap concentration was increased, the surface force decreased. By comparing the data in Figs. 1 and 2 with figures for the stability of sols of lecithin-A and sodium cholate in the presence of 0·01M- and 0·1M-potassium chloride

(Table 3) it can be seen that surface force is a function of the stability of the sols, and the more unstable they become, the higher are their surface forces.

A comparison of Table 2 and the data in Fig. 3 shows that the concentrations of calcium chloride which produce instability of the sols also give surface forces. Fig. 4 shows that addition of sodium cholate to the sols at the two peaks on the surface force–calcium chloride concentration curve brings about a decrease in the surface force. $13{\cdot}8 \times 10^{-5}$M-Sodium cholate reduces the surface force by 0·193 dyne/cm. at 0·0005M-calcium chloride, and a $6{\cdot}8 \times 10^{-5}$M-solution reduces it by 0·191 dyne/cm. at 0·00005M-calcium chloride. The difference in the quantities of soap needed to cause almost the same reduction in surface force suggests that the structure of the interfacial film is different at the two calcium chloride concentrations.

Calcium nitrate gives surface forces of roughly half those produced by equal calcium chloride concentrations: so the anion also appears to be a factor in production of surface force.

Instability of the sols seems to be the prime cause of surface force at the sol–water interface. The instability is believed to be brought about by the effect of electrolytes on the charges on the sol particles, and the maximum instability probably occurs when the sol particles are least charged.

Correlation of the Present Work with Some Properties of Biological Membranes.—When stable boundaries are formed between an aqueous phosphatide sol and water, the structure of the interfacial film is believed to be that of an extended bimolecular leaflet, with polar groups on its outside surface. An organised surface of this type fits in with Danielli and Davson's conception of the general structure of a simple cell membrane.[8] The lecithin sol–water interface shows a resemblance to cells which are believed to have a naked lipoid surface, *e.g.*, *Amoebae*, red blood cells.

The greatest similarities are apparent in the magnitude of the surface forces. By changing the ionic content of the sols and the upper liquid, the surface force of the interface can be varied from 0 to 0·24 dyne/cm. Surface forces of this order are possessed by several simple cells, *e.g.*, *Arbacia punctulata* 0·135—0·2 dyne/cm., red blood cells 0·25 dyne/cm., *Amoeba dubia*.1 dyne/cm.[9]

Ionic effects can be compared. Both magnesium and calcium chlorides toughen the surfaces of *Amoebae* [10,11] and of *Fundulus* eggs,[12] and they have the same effect at the lecithin sol–water interface. With some organisms potassium chloride has a dispersing effect on the cell membrane; with sols of lecithin prepared by means of the cadmium chloride complex and containing certain amounts of calcium chloride, the surface force can be diminished by adding potassium chloride.[1] In other cases this salt toughens the membrane,[12] and similar effects take place with sols of lecithin-A.

It is hoped that studies of the lecithin sol–water interface may help in the elucidation of the structure and function of the surfaces of simple cells.

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[8] Danielli and Davson, *J. Cell. Comp. Physiol.*, 1935, **5**, 495.
[9] Norris, *J. Phys. Chem.*, 1942, **46**, 1111.
[10] Reznikoff, *J. Gen. Physiol.*, 1928, **11**, 221.
[11] Chambers and Reznikoff, *ibid.*, 1926, **8**, 369.
[12] Loeb, *ibid.*, 1922, **5**, 231.