THE PHYSICAL PROPERTIES OF LYSOLECITHIN AND ITS SOLS

PART I.—SOLUBILITIES, SURFACE AND INTERFACIAL TENSIONS

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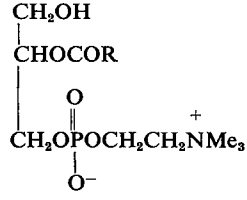
The solubilities of lysolecithin in water and some organic solvents have been determined and the effect of lysolecithin at air-water and chloroform-water interfaces examined. The results indicate that lysolecithin has marked surface-active properties and that a critical micelle concentration occurs in the aqueous sols in the range 1 to 2×10^{-3} per cent weight/volume. The effects of acid, alkali and mono- and di-valent cations on the lowering of the interfacial tensions was found to be small. Lysolecithin had little or no lowering effect on the surface tensions of ethanol and chloroform.

THIS work is a continuation of the study of the colloidal properties of lecithin and lysolecithin[1–5], in relation to the structure of cell membranes. A molecule of lysolecithin can be regarded as having two distinctly different regions, a long hydrocarbon chain of non-polar character which is lipophilic and a phosphoric acid-choline radical, polar in nature, which is hydrophilic.



Zwitter-ion structure of lysolecithin
(R is a saturated hydrocarbon chain, principally C_{13} to C_{17})

The molecule, therefore, possesses an amphipathic character (Hartley[6]) and can be expected to show surface-active properties comparable with soaps. The surface activity of lysolecithin was investigated at air-water and chloroform-water interfaces and the work extended to examine the effects of acid and alkali and mono- and di-valent salts on these interfaces. The effects of lysolecithin on the surface tension of ethanol and chloroform were also determined.

The surface tension measurements were carried out using the ring (dynamic)[7] and Wilhelmy plate (static)[8] methods. The ring method only was used for the experiments on interfacial tension.

Some measurements were made to obtain information on the solubility of lysolecithin in water and various organic solvents.

EXPERIMENTAL

Preparation of Lysolecithin

Mixed phosphatides were prepared by separating the yolks of twelve eggs, extracting them repeatedly with acetone until a white powder was

obtained, and this was further extracted with successive portions (100, 300, 100 ml.) of ethanol. The ethanolic solution of the crude lecithin was then shaken with successive portions of alumina powder until the solution was free from amino-nitrogen phosphatides. About 6 g. of lecithin was obtained at this stage and stored under nitrogen-saturated absolute ethanol at − 5°.

Lysolecithin was prepared from a 5 g. sample of lecithin by Saunders modification[5] of the method of Hanahan[9] using Russell viper venom.

TABLE I

SOLUBILITY OF LYSOLECTHIN IN SOME ORGANIC SOLVENTS

Solvent	Solubility in g./100 ml. solution 25° C.	40° C.
Chloroform	27·830	32·400
Methanol	14·600	19·130
Ethanol	3·870	10·690
Pyridine	0·330	1·030
Acetone	0·022	0·085
Diethyl ether	0·002	0·076
Light petroleum 60°–80°	0·023	0·062
Benzene	0·012	0·057

The yield was about 2 g. of a white solid which had a nitrogen content of 2·72 per cent, a phosphorus content of 5·98 per cent, an iodine value of 5·5 and $[\alpha]_D^{21} = + 2{\cdot}26°$ (5·457 g. in 100 g. ethanol). The mean molecular weight calculated from the nitrogen and phosphorus contents was 516. Calculated values for nitrogen and phosphorus in hydrated lysolecithin when the fatty acid is palmitic, molecular weight 513, are 2·72 and 6·04

TABLE II

VARIATION OF SURFACE TENSION OF LYSOLECITHIN SOLS WITH TIME

	Static method		Dynamic method
Time	Concn 0·0104 per cent w/v lysolecithin $\gamma 20°$	Concn 0·00104 per cent w/v lysolecithin $\gamma 20°$	Concn 1·076 per cent w/v lysolecithin $\gamma 20°$
0 min.	39·67	43·95	37·93
2 "	39·63	43·94	37·50
5 "	39·62	no change	37·26
10 "	no change	"	37·17
20 "	"	"	37·01
30 "	"	"	36·88
60 "	"	"	36·86
120 "	"	"	36·68
Agitation	—	—	37·91
140 "	—	—	37·86
150 "	—	—	37·57
15 hours	39·60	43·55	—

respectively. The low iodine value obtained with the highly specific catalyst lecithinase-A indicates the almost complete absence of unsaturated fatty acid groups in the lysolecithin.

In some work on the hydrolysis of lecithin, cobra venom preserved in a cork-stoppered bottle for 30 years was found to possess some activity, 1·25 g. of lysolecithin being obtained from 5 g. of lecithin by a method similar to the above[5]. With this catalyst the hydrolysate formed a gel and not the white precipitate given by the Russell viper venom.

Preparation of Sols

Pure aqueous sols of lysolecithin were prepared in distilled water and passed through an ion exchange column containing a mixture of Amberlite resins IR-120 and IRA-400. Electrical conductivity measurements showed that any traces of ions present as impurities had been removed. The column was then washed with distilled water and the combined

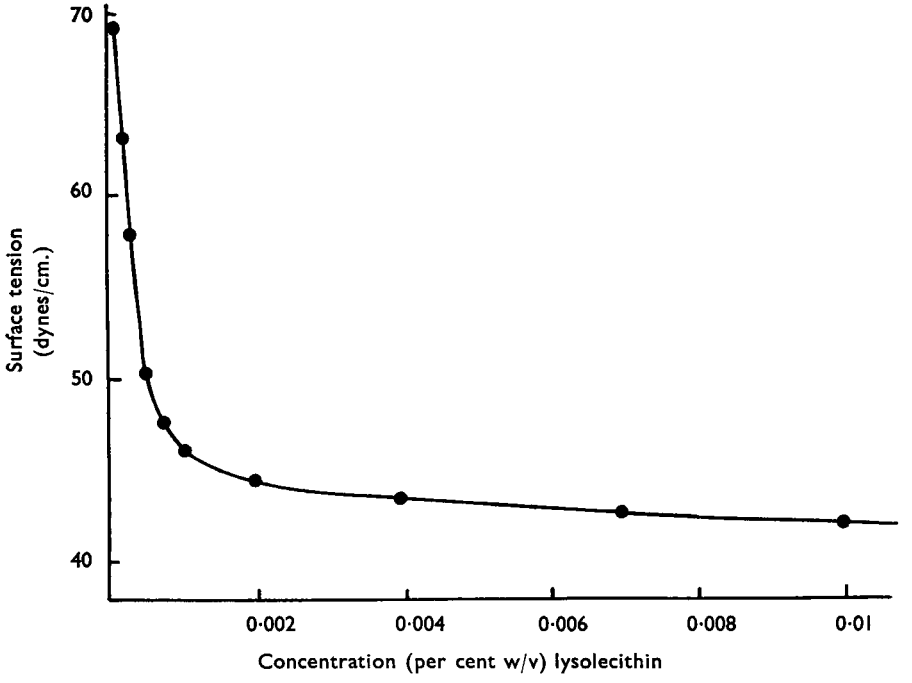


FIG. 1. Variation of surface tension of water (dynamic method) with concentration of lysolecithin. Temperature 20°.

effluent made up to volume to give a concentration of approximately 1·0 per cent w/v. Measured quantities of this concentration were diluted as required.

Solutions of the electrolytes were made with Analar materials and measured quantities mixed with 0·1 per cent w/v lysolecithin sol, shaken and made up to volume. All the solutions were optically clear.

Specific Rotation

Specific rotations of lysolecithin in absolute ethanol were made with a Bellingham and Stanley polarimeter.

Solubility Determination

The solubility of lysolecithin in a number of solvents was determined at 25° and 40°. The approach to equilibrium was accomplished from undersaturation by adding the solute to the solvent at the temperature

of the experiment. Equilibrium from a supersaturated solution was obtained by shaking the solute and solvent at a slightly higher temperature and allowing to cool to the required temperature. The dissolution was made in a Microid Flask shaking machine operating at a rate sufficient to ensure solution without the production of foam, for a period of four hours. 50 ml. pyrex glass tubes, fitted with ground-glass stoppers, each containing 25 ml. of solution were then allowed to stand upright overnight in a water thermostat, controlled to $\pm$ 0·05°, to enable excess solid to settle. Four tubes were taken for each solvent, two used for undersaturation and two for supersaturation.

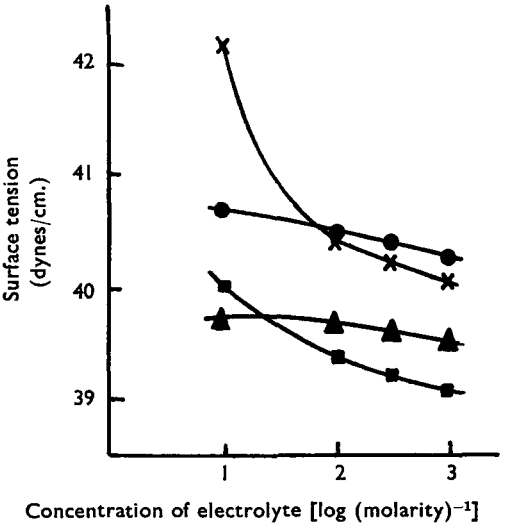


FIG. 2. Effect of various electrolytes on the surface tension of lysolecithin sols. Temperature 20°.
Concn. 0·0152 per cent w/v lysolecithin.
▲ HCl; ■ NaOH.
Concn. 0·00985 per cent w/v lysolecithin.
● NaCl; × $CaCl_2$.

For analysis of the saturated solutions, separation was by rapid transfer to the weighing vessels, with an Agla microsyringe preheated to 2° or 3° above the temperature of the solutions. These weighing vessels were 1 ml. rimless beakers with small watch glass covers. Samples of 0·5 ml. were analysed by slow evaporation of the volatile solvents, drying *in vacuo* for 12 hours at 30° and weighing the residue to constant weight. A possible error to be overcome in the determination of solubility was the taking up of moisture by the hygroscopic lysolecithin. Using a pre-set balance no difficulty was found in obtaining for each solvent eight residues which showed close agreement.

All organic solvents were purified according to Vogel[10].

Surface and Interfacial Tension Apparatus

The solutions for surface tension measurements were contained in a circular pyrex dish 15 cm. diameter and 3 cm. deep, immersed in a water thermostat controlled to $\pm$ 0·05°. The plate and ring suspensions were enclosed within a glass cylinder to prevent disturbances due to the movements of air.

In both the Wilhelmy plate method and the ring method a Webb chainomatic balance reading accurately to 10^{-4} g. adapted to the general assembly was used; this was constructed on the same pattern as that of Harkins and Jordan[7]. It stood on a platform which could be moved up and down

slowly and smoothly by means of a rack and pinion mechanism. In the Wilhelmy method a platinum plate was used in preference to glass since it was found to give reproducible results and could be more easily cleaned. The plate, of dimensions 3·500 × 2·602 × 0·0342 cm., was supported from the balance arm by thin chrome-nickel wire. In the ring method a platinum ring of mean diameter 1·765 cm. was suspended by nylon thread.

TABLE III

EFFECTS OF LYSOLECITHIN ON THE SURFACE TENSION OF ETHANOL AND CHLOROFORM

Ethanol		Chloroform	
Concn per cent w/v lysolecithin	$\gamma 20°$	Concn per cent w/v lysolecithin	$\gamma 20°$
0·0	22·26	0·0	27·19
0·985	22·17	0·985	27·19
0·0985	22·18	0·0985	no change
0·00985	22·19	0·00985	no change
0·000985	22·26		

To ensure that the plate and ring hung horizontally, they were levelled above a metal table which was mirror finished on top.

Chloroform was chosen for the work on interfacial tension.

RESULTS

Solubility

Lysolecithin dissolved in water to give a perfectly clear solution. At a concentration of 36 per cent by weight the solution became slightly viscous, the viscosity increasing with concentration until a thick fluid, still quite clear, was obtained at 50 per cent. At no time throughout the concentration range was a saturation point reached. The solubility of lysolecithin in some organic solvents is shown in Table I.

Surface Tension

The effects of lysolecithin on the surface tension of aqueous solutions are shown in Figures 1, 2 and Table II; of non-aqueous solutions in Table III.

Interfacial Tension

The interfacial tension effects of lysolecithin on a chloroform-water system are shown in Figures 3 and 4.

DISCUSSION

Surface Tension

The surface tension of water decreased with increasing concentration of lysolecithin and indicated positive adsorption at the surface. A considerable lowering effect was found at concentrations of less than 0·001 per cent w/v and at this value the surface tension was lowered from 72·67 dyne/cm. to 45·35 dyne/cm., while increasing the concentration of lysolecithin to 1 per cent lowered the surface tension of water to 37·6 dyne/cm.

It is probable that at low concentrations the lysolecithin molecules are present in water as single molecules or small micelles to which the surface activity can be attributed. When the concentration of lysolecithin reached the range 1 to 2 $\times$ 10^{-3} per cent w/v its lowering effect on the surface tension of water became noticeably less and it seems likely that this change in behaviour is due to the commencement of formation of

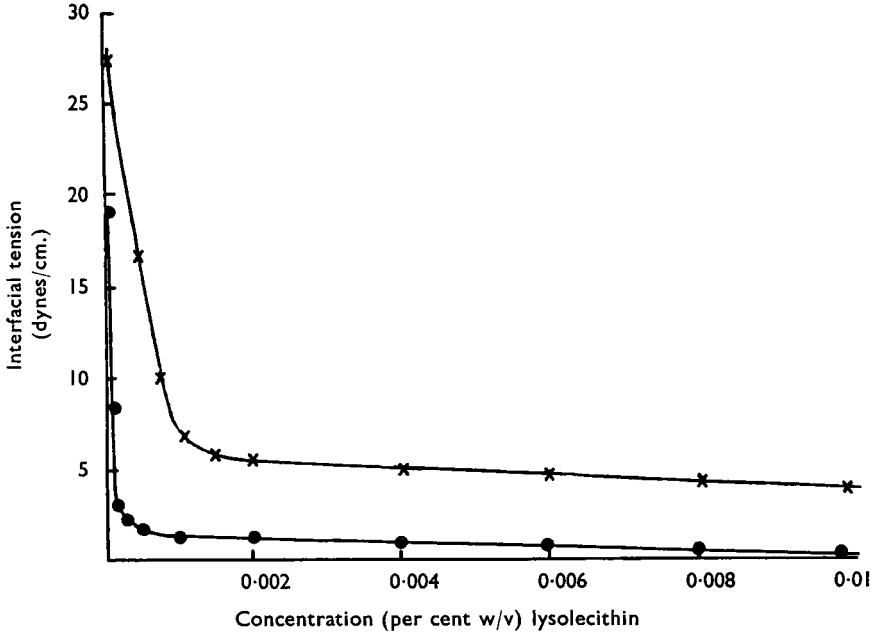


FIG. 3. Variation of interfacial tension of a chloroform-water system with concentration of lysolecithin. Temperature 20°.
$\times$ Lysolecithin in water layer; ● Lysolecithin in chloroform layer.

much larger micelles within the solution. Saunders and Thomas[11] have shown in their work on the diffusion of lysolecithin in aqueous media that such larger micelles do exist at higher concentrations (1 to 2 per cent w/v), consisting of aggregates of about 270 single molecules.

The change in surface tension with time at the two concentrations measured by the static method (Table II) showed an initial slight lowering within 5 minutes perhaps due to diffusion of molecules and orientation at the surface layer. Thereafter a further 15 hours showed a very small change whilst equilibrium was being established between the body of the sol and the surface layers. This could be regarded as further evidence for the presence of large particles which are very soluble and remain in the bulk of the sol showing no tendency to migrate to the surface.

Within its limitations the dynamic method gave surface tension-time results (Table II) which showed more clearly the progress towards equilibrium between the surface layer and the bulk of the sol. The surface tension decreased by 0·67 dyne/cm. in the first 5 minutes, followed

by a less rapid fall of 0·58 dyne/cm. in the next 115 minutes. Agitation of the sol then caused restoration of equality of concentration within the system and the surface tension regained its original value, which was again followed by a decrease with time following a similar pattern.

The effects of acid, alkali, sodium and calcium chlorides on the surface tension of a sol of concentration 0·00985 per cent w/v were quite small (Fig. 2). A reduction in the surface activity of the lysolecithin with increasing alkalinity was shown, but in acid conditions there was no change beyond an initial fall. A 0·1 molar calcium chloride solution reduced the surface tension lowering by approximately 7·5 per cent. The stability of lysolecithin sols to salts has been examined by Saunders[5] who found that sodium and calcium chlorides had no precipitating effect on the sols within the concentration range used by the present authors. The effect of these salts may, therefore, have caused interionic forces to predominate at the surface resulting in desorption of lysolecithin.

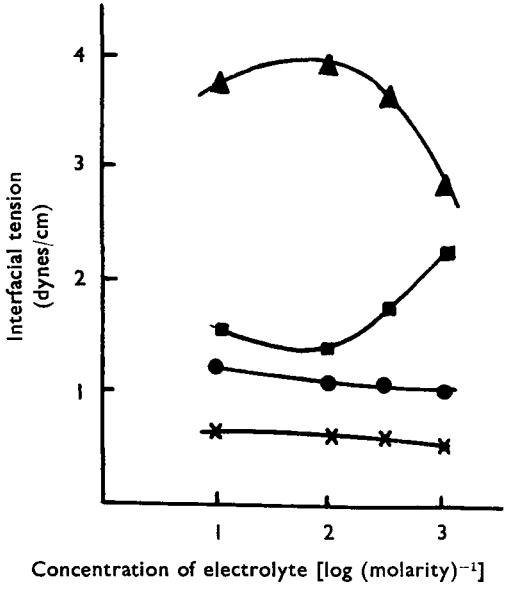


FIG. 4. Effect of various electrolytes on the chloroform (containing 0·01 per cent w/v lysolecithin)–water interface of tension 0·6 dyne/cm. Temperature 20°.
▲ HCl; ■ NaOH; ● NaCl; × $CaCl_2$.

No lowering of the surface tension of chloroform by lysolecithin within the concentration range 0·1 per cent to 0·001 per cent w/v took place. The surface tension lowering of ethanol by a 0·1 per cent w/v lysolecithin solution was only 0·09 dyne/cm. Pure lysolecithin is quite soluble in chloroform and ethanol and there appears to be no tendency to repel either polar or non-polar regions of the molecule from the solvents, and consequently no surface layer is formed.

Interfacial Tension

The general pattern of the lowering of the interfacial tension of the chloroform-water system with increasing concentration of lysolecithin (Fig. 3) is similar to that at the air-water interface (Fig. 1). This is not necessarily to be expected, since both hydrophilic and lipophilic characteristics come into play at the chloroform-water interface.

The greatest lowering effect took place with a concentration of 10^{-4} per cent w/v lysolecithin dissolved in the chloroform layer when the

interfacial tension of 32·73 dyne/cm. decreased to 2·91 dyne/cm. (Fig. 3). This was further lowered to less than 1 dyne/cm. by increasing the concentration to 10^{-2} per cent w/v which enabled the two liquids to be readily emulsified with the slightest mixing. The final lowering to 0·35 dyne/cm. at a concentration of 0·1 per cent w/v lysolecithin in chloroform was 3 dyne/cm. more than when lysolecithin was dissolved in the aqueous phase.

The greater surface activity shown when lysolecithin was dissolved in the chloroform layer may have been due to the presence of smaller micelles than those in the aqueous layer. When lysolecithin was dissolved in the aqueous layer the interfacial tension between the concentration range 1 to 2×10^{-3} per cent w/v became noticeably less in a way similar to that shown at the air-water interface. Aggregation of single molecules or small micelles is again probably taking place in the aqueous phase within this concentration range.

The effects of acid, alkali and sodium chloride in the aqueous phase (Fig. 4) were small and comparable to those at the air-water interface; the effect of calcium chloride, however, was less marked at the chloroform-water interface.

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