

THE PHYSICAL PROPERTIES OF LYSOLECITHIN AND ITS SOLS

PART II. REFRACTIVE INDICES AND DENSITIES OF SOLS. MICELLE FORMATION

BY N. ROBINSON AND L. SAUNDERS

From the Physical Chemistry Department, School of Pharmacy, University of London

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The refractive index difference ($n_{\text{sol}} - n_{\text{water}}$) and density difference ($\rho_{\text{sol}} - \rho_{\text{water}}$) of lysolecithin sols indicate a change in the properties of the sols in the region of 0.02 per cent w/v, above this concentration the refractive index difference:concentration relation and the density difference:concentration relation are linear. Values for the partial specific volumes of lysolecithin in water, calculated from density measurements, are given. Surface tension:concentration measurements at four different temperatures indicate that the critical micelle concentration increases with an increase in temperature. Values for the change in heat content in the molecular aggregation process, calculated from the temperature coefficient of the critical micelle concentration are small and negative, increasing numerically from 3432 calories at 20° to 6100 calories at 40°. The associated entropy changes in this process are small and show a slight increase negatively with an increase in temperature.

STUDIES on the surface activity of lysolecithin previously reported^{1,2} have been extended to include other properties of the sols of this biologically important compound. Experimental work reported here was made on the well-known β -(acyl)lysolecithin with the exception of a comparison with the α -(acyl)lysolecithin by refractive index measurements.

Refractive index measurements were made to find a relation between the refractive index difference ($\Delta n = n_{\text{sol}} - n_{\text{water}}$) and concentration of the lysolecithin sols to obtain a criterion for the determination of concentration of these sols. By comparing the measurements of an α -(acyl)lysolecithin with those of the β compound, the effect of the structural differences on the refractive index difference (Δn) could be examined.

The effect of concentration on the density difference ($\Delta \rho = \rho_{\text{sol}} - \rho_{\text{water}}$) of lysolecithin sols was examined at three different temperatures and values for partial specific volumes obtained.

We have shown elsewhere¹ that micelles commence to form in lysolecithin sols at a concentration of 0.001 per cent w/v. The variation of surface tension with concentration in this region has been investigated at four different temperatures to measure the effect of temperature on the molecular aggregation process and to obtain values for the change in heat content for the equilibrium: single molecule in solution \rightleftharpoons single molecule in aggregate. Values for the associated entropy change (ΔS) in this equilibrium have been calculated.

EXPERIMENTAL

Preparation of Lysolecithins

β -(Acyl)lysolecithin was prepared by treating lecithin obtained from egg yolks with Russell viper venom according to the method described by

Saunders³. A sample of α -(acyl)lysolecithin was kindly given by Dr. G. M. Gray, Lister Institute of Preventive Medicine. Analysis of the α compound (made by the Lister Institute) and the β compound (the authors') gave the following:—

		α	β
Nitrogen (per cent on dry wt.)	..	2.76	2.77
Phosphorus (per cent on dry wt.)	..	5.9	5.95
N:P ratio	1:0.97	1:1.03
Iodine value	109	4.5

Preparation of Aqueous Sols

Sols of each lysolecithin compound were prepared according to the method previously described¹.

Apparatus

Refractive index. A Rayleigh interference refractometer (Hilger model M154) in combination with a 150 c.p. Pointolight light source was used to obtain difference readings (Δn) between pure water and the lysolecithin

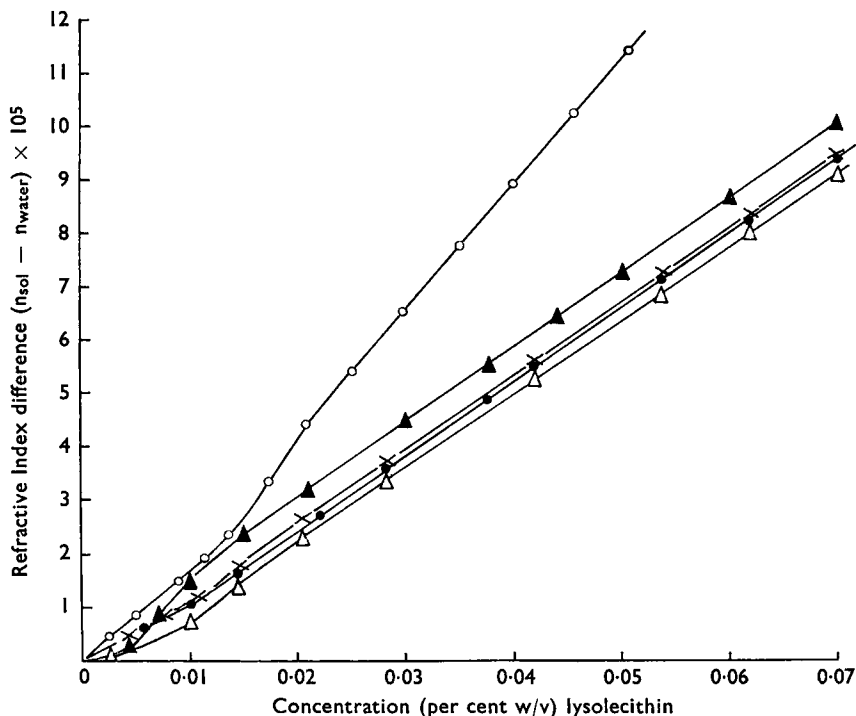


FIG. 1. Variation of refractive index difference ($n_{sol} - n_{water}$) with concentration of lysolecithin.

\circ —25° } α -(acyl) lysolecithin \times —20° } β -(acyl) lysolecithin
 \blacktriangle —40° } α -(acyl) lysolecithin \bullet —25° } β -(acyl) lysolecithin
 \triangle —40° } β -(acyl) lysolecithin

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sols. The liquids were contained in 1 cm. and 10 cm. fused silica cells enclosed in a thermostat controlled to $\pm 0.01^\circ$. The instrument was calibrated using a 1 per cent solution of maltose hydrate⁴.

Density. Measurements to examine density differences (Δd) between pure water and lysolecithin sols were obtained by the sinker method described by Wirth⁵. The solutions were contained in a cylindrical pyrex glass vessel with a hemispherical base into which a sinker of similar shape and material was placed, allowing for a clearance of 0.5 cm. between sinker and container. The sinker contained sufficient mercury to give a pull of approximately 1 g. weight when completely submerged in water. It was suspended by a fine platinum wire, coated with platinum black where the wire passed through the liquid surface, and supported on one arm of a chainomatic balance assembly previously described¹. A second sinker of similar pattern was used to enable duplicate readings to be taken successively. Volumes of the sinkers were about 220 ml. and weighings were determined to within ± 0.1 mg. Temperature control to $\pm 0.001^\circ$ was obtained using a lagged water bath and a Beckmann thermometer.

Surface tension. Surface tension measurements were made at 20° , 25° , 32.5° and 40° using the ring (dynamic) method described elsewhere¹. Sols of concentrations up to 0.005 per cent w/v lysolecithin were examined.

RESULTS

The refractometer used was the differential type which measured the refractive index difference between the sol and pure water. Measurements of α - and β -(acyl)lysolecithin sols below a concentration of 0.1 per cent w/v are shown in Figure 1. Values for sols of β -(acyl)lysolecithin at higher concentrations are given in Table I.

TABLE I
VALUES OF REFRACTIVE INDEX DIFFERENCES ($n_{\text{sol}} - n_{\text{water}}$) FOR INCREASING CONCENTRATIONS OF β -(ACYL) LYSOLECITHIN

Concentration (per cent w/v) lysolecithin	Refractive index difference ($n_{\text{sol}} - n_{\text{water}}$)		
	20°	25°	40°
5.00	0.0069626	0.0069677	0.0068357
4.60	0.006372	0.006341	0.006287
4.17	0.005743	0.005735	0.005673
3.70	0.005126	0.005121	0.005041
3.30	0.004458	0.004466	0.004477
2.50	0.003372	0.003369	0.003367
2.00	0.002753	0.002750	0.002691
1.40	0.001926	0.001923	0.001874
0.91	0.001251	0.001212	0.001214
0.50	0.000722	0.000708	0.000710
0.25	0.000332	0.000331	0.000313
0.10	0.000139	0.000137	0.000131

Variations of density difference between β -(acyl)lysolecithin sols and pure water for increasing concentrations of the sols are shown in Figure 2. The values for the partial specific volume of lysolecithin, calculated from density measurements, are 0.99827, 0.99836 and 0.99885 ml.g.⁻¹ at 20° , 25° and 40° respectively.

The variation of surface tension with concentration of lysolecithin at 20° , 25° , 32.5° and 40° is shown in Figure 3. The changes in heat content

associated with the molecular aggregation process were calculated using the equation developed by Stainsby and Alexander⁶ (an approximate form of the equation was used taking the activity coefficient as unity). The entropy change (ΔS) associated with the equilibrium between lysolecithin molecules in solution and in the micelles was obtained from the equation $\Delta H = T\Delta S$. The curve relating $\log C_m$ and $1/T$ (where C_m is the

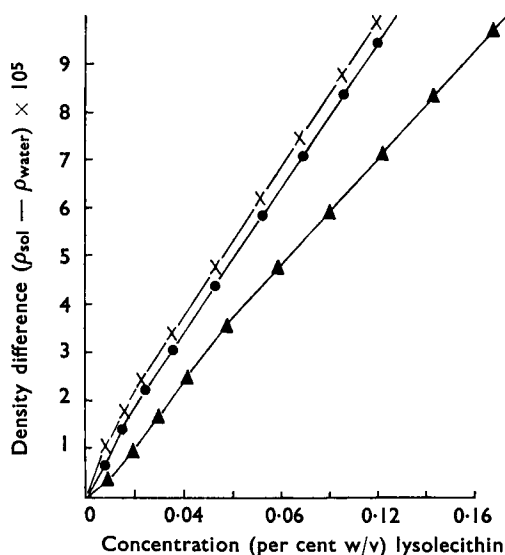


FIG. 2. Variation of density difference ($\rho_{sol} - \rho_{water}$) with concentration of lysolecithin. X—20°; ●—25°; ▲—40°.

critical micelle concentration at a given temperature) is shown in Figure 4. Values for the critical micelle concentration, the changes in heat content (ΔH) and entropy (ΔS) are shown in Table II.

DISCUSSION

Refractive Index

Above a concentration of 0.02 per cent w/v the refractive index difference bears a linear relationship to concentration for α - and β -(acyl)lysolecithins at the three temperatures of the experiment. The presence of double bonds in the α -(acyl)lysolecithin gives a higher specific refraction

increment $\left(\frac{n_{sol} - n_{water}}{C}\right)$ than the saturated β compound. The rate of increase in the refractive index difference with concentration was less for β -(acyl)lysolecithin than for the α compound, an increase in temperature having the effect of lowering this rate of increase for both compounds.

The apparent physical change of the sols at a concentration of 0.02 per cent w/v is supported by studies on the surface activity of lysolecithin¹. There it was shown that the rate of change of surface tension with concentration, greatest at 0.001 per cent w/v, rapidly diminished to almost zero at 0.02 per cent w/v where it was thought that the bulk phase consisted almost entirely of large micelles.

The break in the refractive index difference/concentration curves for each lysolecithin sol occurred at a slightly higher concentration with increasing temperature, a behaviour to be expected since the kinetic energy of the molecules is increased and hence the instability of the micelles is also increased.

Density

Measurements on the density difference between lysolecithin sols and pure water showed that below approximately 0.02 per cent w/v the

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increase in density difference with increasing concentration does not obey a linear relationship; above this concentration, however, linearity becomes established at 20° and 25° although at 40° it is reached at 0.065 per cent w/v lysolecithin.

TABLE II

VALUES OF CHANGE IN HEAT CONTENT AND ASSOCIATED ENTROPY CHANGES OBTAINED FOR LYSOLECITHIN FROM THE SURFACE TENSION/CONCENTRATION RELATIONSHIPS

Temperature °C.	Range of critical micelle concentration	Mean value of critical micelle concentration		ΔH Calories	ΔS Calories/mole deg.
		Per cent w/v	Moles/l. $\times 10^4$		
20	0.00045 to 0.00158	0.00101	1.95	-3432	-11.71
25	0.00044 to 0.00180	0.00112	2.168	-4233	-14.20
32.5	0.00021 to 0.00272	0.00136	2.628	-4955	-16.22
40	0.00012 to 0.00325	0.00168	3.256	-6100	-19.49

Below approximately 0.02 per cent w/v the rate of increase in density with increasing concentration appears to be greater than above this value. It is probable that the lysolecithin is present as small aggregates which are subjected to high compression by the strongly cohesive field of the water

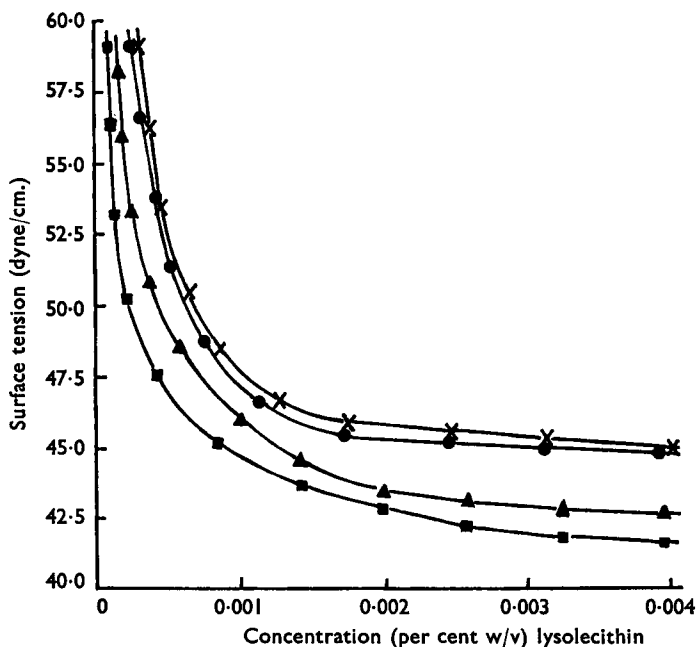


FIG. 3. Variation of surface tension with concentration of lysolecithin. X—20°; ●—25°; ▲—32.5°; ■—40°.

molecules. Above 0.02 per cent w/v it is likely that large aggregates of lysolecithin molecules are present and the presence of these large particles reduces the cohesion between the water molecules surrounding the aggregates, resulting in less compression on the aggregates.

The effect of temperature on the point at which a linear relationship between density difference and concentration is established shows a

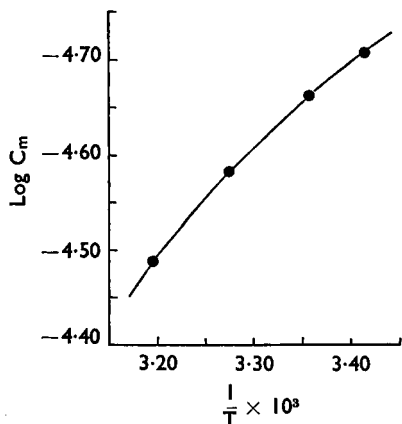


FIG. 4. Variation of logarithm critical micelle concentration ($\log C_m$) with the reciprocal of absolute temperature ($\frac{1}{T} \times 10^3$).

relation, showed that the increase in concentration at which aggregation takes place is greater at higher temperatures.

Values for the change in heat content in the equilibrium: single molecule in solution \rightleftharpoons single molecule in aggregate, calculated from the temperature coefficient of the critical micelle concentration, increased numerically from 3432 calories per mole at 20° to 6100 calories per mole at 40° and were negative, that is, the heat liberated in the equilibrium increases with temperature. These figures are valid if the following main considerations are taken into account. Firstly, a critical concentration for micelle formation midway along the concentration range shown by the surface tension/concentration relationship was used in the calculation. Secondly, in their work on colloidal electrolytes, Stainsby and Alexander have shown that ions have little effect on the change in heat content and this has been an assumption we have made in obtaining values for lysolecithin sols which are essentially non-electrolytic in character. Thirdly, it has been assumed that the activity coefficient is unity since the values of C_m (critical micelle concentration) are quite small. Fourthly, the micelles formed by lysolecithin are approximately spherical, a probability substantiated by the low relative viscosity of the sols³.

The changes in heat content for the lysolecithin system are smaller than the values obtained by Stainsby and Alexander for some fatty acids and larger than those for some typical soaps.

a similar pattern to the temperature effect on the refractive index difference/concentration relationship.

The increase in density difference was considerably less for increasing concentrations at 40° than at 20° and 25°, which is probably due to a reduced strength in the cohesive field of the water molecules.

The value obtained for the partial specific volumes of lysolecithin indicate a slight increase in volume of the solute molecules for an increase in temperature.

Micelle Formation

The concentration region in which small aggregates are formed increases with an increase in temperature. This, a non-linear

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The entropy change in the aggregation process is small, showing a slight increase negatively, that is, a loss in entropy, as the temperature rises.

REFERENCES

1. Robinson and Saunders, *J. Pharm. Pharmacol.*, 1958, **6**, 384.
2. Robinson and Saunders, *ibid.*, 1958, **10**, 227 T.
3. Saunders, *ibid.*, 1957, **9**, 834.
4. McDonald, *U.S. J. of Research of National Bureau of Standards*, 1951, **46**, 165.
5. Wirth, *J. Amer. chem. Soc.*, 1937, **59**, 2549.
6. Stainsby and Alexander, *Trans. Far. Soc.*, 1950, **46**, 587.