Viscosity of phospholipid sols

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The viscosities of lysolecithin sols in water were found to be Newtonian at the concentrations investigated. A Couette viscometer was used. In the mixed sols containing both lysolecithin and lecithin, thixotropy was absent at all ratios and only the sol with a high lecithin fraction showed some non-Newtonian behaviour. The axial ratio of the mixed micelles calculated by the Simha method varies with the lecithin/lysolecithin ratio, rising to a maximum when the phospholipid mixture is of one mole lecithin to two moles lysolecithin. This phenomenon is probably due to the packing of the various hydrophobic groups in the micelle.

IN the work reported here the apparatus, techniques, and interpretation of results and preparation of lecithin were those described previously (Perrin & Saunders, 1966).

PREPARATION OF LYSOLECITHIN

Lysolecithin was prepared by a modification (Saunders, 1957) of Hanahan's method (Hanahan, Rodbell & Turner, 1954). The procedure described for lecithin (Perrin & Saunders, 1966) was followed as far as the ion-exchange treatment. The solution from the Dowex 1 × 4 ion exchange resin was evaporated to dryness and the residue taken up in 1 litre of ether. Water (10 ml) containing 5 mg of Russell viper venom was added and the flask shaken gently for 1 min. The flask was then allowed to stand for 2–3 hr until precipitation of the lysolecithin was complete. The supernatant ethereal solution was decanted and the lysolecithin was washed with three portions of 500 ml ether and finally with 500 ml acetone. The lysolecithin was dissolved in a small quantity of chloroform and precipitated with 6 volumes of ether. This procedure was repeated four times. The lysolecithin was dissolved in 200 ml ethanol and centrifuged. The solution was evaporated to dryness, the residue being washed with acetone. This purified lysolecithin was again taken up in 200 ml absolute ethanol and the solution centrifuged. The lysolecithin was stored at 0° in this clear alcoholic solution, when crystals of lysolecithin appeared on standing. The yield was 2 g from 12 eggs.

Typical analysis. Total phosphorus, 6·02; total nitrogen, 2·75; sugar, 0·0; choline, 22·8; ester, 52·5; iodine number, 0. Ratio

$$\frac{nitrogen}{phosphorus} = 1·01 \text{ (theoretical} = 1·00).$$

Preparation of sol. Lysolecithin was dried at the vacuum pump and dissolved in ion-free water by shaking. The optically clear sol was then freed of dust by spinning at 6000 rev/min in the laboratory centrifuge.

Viscosity measurements. All sols examined behaved in a Newtonian manner and data derived from the measurements are shown in Table 1. The density of lysolecithin was taken as the 1·095 g/ml found by Thomas (1958).

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DISCUSSION

Lysolecithin sols have very low relative viscosities in water, and for the application of Simha's equations (1940) for ellipsoidal particles, measurements must be made on very dilute solutions. However, the

TABLE 1. VISCOSITIES OF LYSOLECITHIN SOLS

conc.	$\phi \times 10^2$	η_{rel}	η_{sp}	η_{sp}/ϕ
7·88	7·71	1·38	0·383	5·33
7·27	7·12	1·32	0·321	4·84
6·72	6·58	1·26	0·258	4·20
6·21	6·08	1·24	0·244	4·31
5·52	5·41	1·19	0·190	3·75
4·99	4·88	1·15	0·154	3·38
4·41	4·32	1·15	0·150	3·73
3·92	3·86	1·13	0·128	3·57
3·39	3·32	1·12	0·124	4·00
2·98	2·92	1·09	0·086	3·16
2·65	2·59	1·07	0·075	3·10

Couette viscometer is not suitable for the measurement of relative viscosities less than 1·10, and so in the present work measurements were made on sols more concentrated than is desirable for Simha's interpretation. The results indicate that the value of η_{sp}/ϕ is close to the 2·5 (Einstein, 1906) value required for spherical particles. Such particles are stable to shearing and orientation effects do not occur, so accounting for the lack of thixotropy and variation of viscosity with low shear rates. Robinson (1961) and Thomas (1958) found η_{sp}/ϕ to be slightly greater than 2·5 in capillary viscometers, the discrepancy being due to hydration of the particles. Correlation of viscosity data with data from diffusion, light scattering and ultracentrifugation have been reported elsewhere by these authors (Perrin & Saunders, 1964) and give an anhydrous micellar weight of 95,000 for lysolecithin in aqueous solution.

MIXED SOLS CONTAINING LECITHIN AND LYSOLECITHIN

Preparation of mixed sols

The two phospholipids were prepared as described above. A volume of alcoholic lecithin was evaporated almost to dryness in a weighed flask warmed to 35° on a bath using a vacuum pump. The lecithin was precipitated from the syrupy liquid with acetone and then evaporated to complete dryness overnight. After weighing, the procedure was repeated after the addition of alcoholic lysolecithin solution, but in this instance the drying was completed in under 2 hr. The preparation of the sol from the dried mixed phospholipid depended on the percentage of lysolecithin in the mixed phospholipid. All concentrations are expressed as weight in weight.

(a) *Sol containing less than 40% lysolecithin in the mixed phospholipid.* The mixed phospholipid was dispersed in a small quantity of ether (lecithin is soluble in ether, lysolecithin is ether insoluble). Ion-free water was then added and the ether gently removed by warming, bubbling with nitrogen, and shaking. The nitrogen was only used when small quantities of lysolecithin were present, in order to avoid the formation of a stable foam. After about an hour the flask was reweighed and the

sol aged for 24 hr in accordance with the findings of Thomas & Saunders (1958).

(*b*) *Sol containing more than* 40% *lysolecithin of mixed phopholipid.* The phospholipids were dried as above and were dispersed in the ion-free water by shaking and warming for an hour. After reweighing the sol was aged for 24 hr.

Viscosity measurements

All measurements were made in the Couette viscometer as previously described (Perrin & Saunders, 1966). Ten major sols were prepared as above, and their viscosities measured after testing for thixotropy. All these sols were sheared at about 9 rev/min (shear rate of approximately 9/sec) and no fall in deflection was noted after shearing for 1 hr, so demonstrating a lack of thixotropy at these slow shear rates. This absence of thixotropy enabled all revolutions to be timed against a stop watch. The viscosities of dilutions of the major sols were measured to enable the viscosity factor γ ($\eta_{sp/\phi}$ at zero concentration) to be extrapolated.
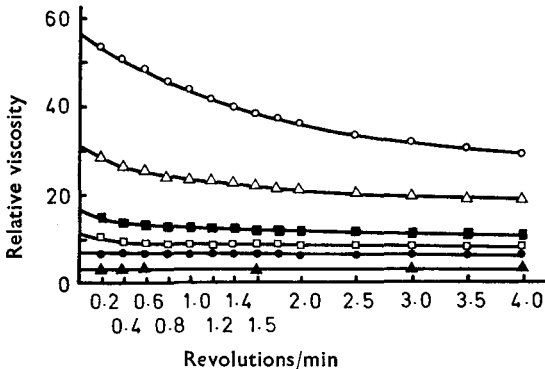

FIG. 1. Change of relative viscosity with shear rate for a sol containing phospholipid mixed in the ratio 87·4% lecithin and 12·6% lysolecithin. Concentrations are total phospholipid weight in weight in the sol. O = 4·925, △ = 4·471, ■ = 3·953, □ = 3·607, ● = 3·304, ▲ = 2·3022.

Non-Newtonian behaviour

In some instances the major sols showed very slight non-Newtonian behaviour (especially the more viscous ones), but only with the sol containing 87·4% lecithin of the mixed phospholipid was the phenomenon very marked. Fig. 1 shows the plot of relative viscosity against shear rate for this sol containing mainly lecithin in the phospholipid fraction, and the values of η_{rel} at zero shear obtained from the graph are:

Sol conc.	Relative viscosity at zero shear
4·925	56·7
4·471	31·5
3·953	16·7
3·607	11·5
3·304	6·9

On further dilution the viscous behaviour became Newtonian.

Newtonian behaviour

Most major sols exhibited Newtonian or near Newtonian behaviour as shown in Fig. 2. The more viscous sols had to be much diluted so that
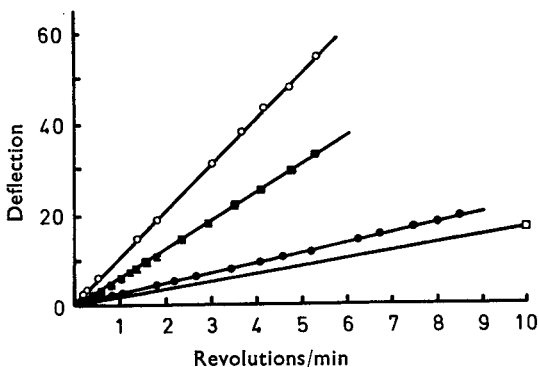

FIG. 2. Flow curves of a sol containing phospholipid in the ratio 77·6% lecithin and 22·4% lysolecithin. O = 5·96% phosphatide, $\blacksquare$ = 4·99, $\bullet$ = 1·94; $\square$ is pure water.

measurements could be taken with a capillary viscometer. Sols were diluted until measurable relative viscosities (less than 15) were obtained. Values of $\eta_{sp/\phi}$ were obtained and plotted against ϕ so that a viscosity factor γ was found by extrapolation to zero concentration as shown in Fig. 3.
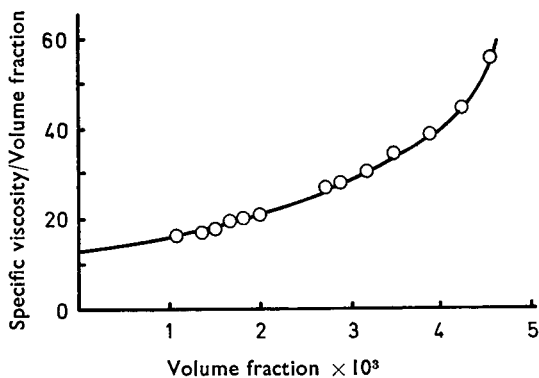

FIG. 3. Graph to obtain the value of specific viscosity/volume fraction at zero volume fraction for a sol containing phospholipid in the ratio 77·6% lecithin and 22·4% lysolecithin.

Table 2 shows the data obtained from the 10 major sols and gives the viscosity factors obtained and the axial ratios computed using Simha's equations, derived assuming ellipsoidal particles.

Fig. 4 shows a plot of viscosity factor against percentage weight in weight lecithin in the mixed phosphatide.

TABLE 2. VISCOSITIES OF THE MIXED SOLS

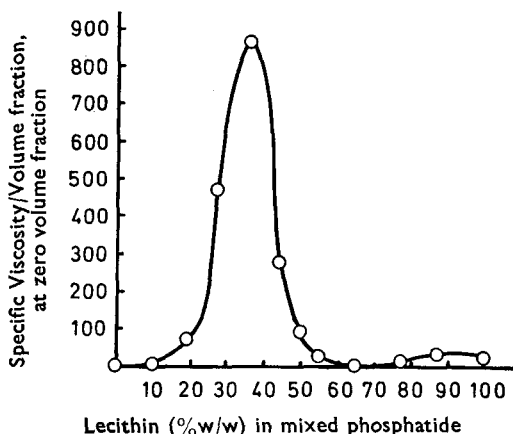| Lecithin in mixed phospholipid	ε_{sp}/ϕ $\phi{\to}0$)	Axial ratios	
		Prolate	Oblate
87·4	36·0	19·0	52
77·6	11·7	9·0	15·0
65·0	4·0	3·5	4·0
55·4	26·0	15·5	37
50·3	89·5	33	120
44·5	280	66	412
36·7	870	125	1280
27·7	475	87	700
19·4	72	29	104
10·0	3·0	2·25	2·3

FIG. 4. Effect of phosphatide mixture on specific viscosity/volume fraction at zero volume fraction.

DISCUSSION

Thixotropy is completely absent from the mixed sols at these very low shear rates (less than 10/sec), whereas Thomas & Saunders (1958) found considerable thixotropy in mixed sols sheared at between 20 and 20,000/sec in a Ferranti-Shirley cone and plate viscometer. The addition of 12% lysolecithin solubilizes lecithin sufficiently to prevent breakdown of the micelles on shearing. The non-Newtonian characteristics are also diminished or lost on addition of lysolecithin, the only mixed sol showing change of η_{rel} with shear rate contained a high proportion of lecithin (87·4%) in the mixed phospholipid. The Table of axial ratios calculated from the viscosity factor by the Simha method for ellipsoids does not take into account hydration, and particularly in the region 50–20% lecithin of mixed phospholipid the sols are gels and probably much solvent binding has occurred. The change of viscosity factor with phosphatide composition in Fig. 4 suggests variation from almost spherical particles to asymmetric discs or rods. The curve shows a minimum in the region of 65% lecithin of total phospholipid and this minimum corresponds to axial ratios of 3·5 for a rod or 4·0 for a disc. This can be compared to an equimolar mixture of lecithin and lysolecithin which has a lecithin fraction of 60–62%. After this minimum the viscosity factor increases

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with decreasing lecithin percentage until a maximum is reached in the region of 40% lecithin, giving axial ratios of 125 for a rod and over 1200 for a disc. A phospholipid mixture of 1 mole lecithin and 2 moles of lysolecithin would have a lecithin percentage of between 40 and 44. After this maximum the viscosity factor falls sharply until at 10% lecithin content axial ratios of approximately 2·3 are obtained assuming either rods or discs.

Thomas (1958) studied the diffusion of mixed phospholipids containing up to 30% lecithin and found that sols containing up to 15% lecithin had diffusion coefficients only slightly lower than that of lysolecithin. These lower diffusion coefficients suggest asymmetry or hydration causing an increased micellar weight and are in agreement with the viscosity findings. Robinson (1961) examined a mixed micelle containing approximately 14% lecithin by the light-scattering technique and he quotes a dissymmetry of 2·27 for the mixed phosphatide compared with values of 1·44 for lecithin and 1·08 for lysolecithin. The expected value of viscosity factor for a sol of this composition would be nearly 30, a value only slightly greater than that of lecithin. With sols containing a bigger percentage of lecithin in the mixed phosphatide, Thomas was unable to obtain diffusion coefficients because of difficulty in forming boundaries and anomalous flow properties. Robinson did not investigate sols containing a bigger fraction of lecithin than 14·3% because the sols were no longer optically clear. Thomas & Saunders (1958) studied the complete range of mixed micelles in Cannon-Fenske viscometers and obtained a similar plot of viscosity against lecithin content of mixed phosphatide. However, they obtained no minimum at 65% lecithin and their maximum occurred at approximately 45% lecithin instead of at 40% as reported here. The values of $\eta_{sp/\phi}$ given by Thomas are different from those quoted here, probably because of (a) variation in the preparation of the sols, (b) higher and varying shear rates are used in viscosity measurements in the capillary viscometers. Thomas found his sols to be thixotropic and so the viscosity would depend upon their previous history.

The addition of lecithin (two fatty acid chains) to lysolecithin (one fatty acid chain) would alter the packing of the molecules in the micelle because the bulkier hydrophobic groups of the lecithin molecule would interfere with the arrangement of the wedge-shaped lysolecithin molecules so causing the production of asymmetric micelles, the degree of asymmetry varying with the molar ratio of lecithin to lysolecithin. This effect is fully discussed by Saunders (1966).

References

Einstein, A. (1906). *Annln Phys.*, **19**, 289.
Hanahan, D. J., Rodbell, M. & Turner, L. D. (1954). *J. biol. Chem.*, **206**, 431–441.
Perrin, J. H. & Saunders, L. (1964). *Biochim. biophys. Acta*, **84**, 216–217.
Perrin, J. H. & Saunders, L. (1966). *J. Pharm. Pharmac.*, **18**, 271–277.
Robinson, N. (1961). *Ibid.*, **13**, 53–57.
Saunders, L. (1957). *Ibid.*, **9**, 834.
Saunders, L. (1966). *Biochim. biophys. Acta*, **125**, 70–74.
Simha, R. (1940). *J. phys. Chem., Ithaca*, **44**, 25–35.
Thomas, I. (1958). Ph.D. Thesis, University of London.
Thomas, I. & Saunders, L. (1958). *J. Pharm. Pharmac.*, **10**, *Suppl.*, 182*T*–185*T*.