

Viscosity of phosphatidylcholine (lecithin)

J. H. PERRIN* AND L. SAUNDERS

The viscosities of egg lecithin dispersed in water have been measured by means of a Couette viscometer. The more concentrated dispersions are thixotropic, but below 1.8% w/w of lecithin the sols become Newtonian. From the extrapolated ratio of specific viscosity to volume fraction, the dispersed particles are considered to be of an elongated rod-like shape. When the coarse dispersions of lecithin are irradiated ultrasonically, an irreversible clearing of the sols occurs, accompanied by a big decrease in viscosity. None of the irradiated sols examined was thixotropic and the extrapolated viscosity ratio indicated a low asymmetry and degree of hydration for the dispersed particles. The effect of ultrasonic irradiation is not only to reduce size and asymmetry of the aggregates but also to expel water from them.

PREVIOUSLY, Saunders (1957) and Thomas & Saunders (1958), using capillary viscometers and a Ferranti-Shirley cone and plate viscometer, showed that sols containing lecithin alone and mixtures of lecithin and lysolecithin exhibit anomalous flow behaviour. They also showed that, in the mixed sols, the intrinsic viscosity depends on the ratio of the two phospholipids. A Couette viscometer has been constructed to investigate these effects in more detail at known low shear rates. This paper gives the results obtained for lecithin sols.

Experimental

THE COUETTE VISCOMETER

The viscometer was based on published designs (Lawrence, Needham & Shen, 1944; Ogston & Stanier, 1953; Perrin, 1962).

An electronic speed control was used, with a 0.08 h.p. electric motor designed to give infinitely variable control over speeds from 0 to 86 rpm. The unit was modified by replacing the 50,000 ohm variable resistance with a helical potentiometer of 50,000 ohm resistance and fitted with a ten turns counting dial. This modification gave a much closer control over the speed of the motor, but a variation of up to 2% in speed was noticed during the day with each potentiometer setting. All revolutions were therefore timed against a stop-watch for Newtonian systems; a previous calibration of potentiometer setting against rev min^{-1} was used only with thixotropic sols.

The outer cylinder of the viscometer was made of stainless steel and had a length of 13 cm and a bore of 2.1 cm. A perspex window was cemented into the bottom end of the cylinder and was scribed with a concentric cylinder of 8 mm diameter. The inner surface of the outer rotating cylinder was highly polished. The inner cylinder or bob was made of titanium, chosen because of its lightness, high corrosion resistance and its ability to take a high polish. Two bobs of 1.9 cm diameter were made. The larger bob was 9 cm long and weighed approximately 125 g with fittings, whilst the small bob was 7 cm in length and weighed approximately

From the Physical Chemistry Laboratories, School of Pharmacy, University of London, Brunswick Square, London, W.C.1.

* Present address, Pharmacy Department, University of Wisconsin, Madison, Wisconsin, U.S.A.

100 g. To minimise end effects, the lower ends of the bob were made concave to trap an air bubble.

A beryllium-copper alloy wire, hardened at 300° for 4 hr, was used to suspend the bobs; using 0.13 cm diameter wire in lengths of approximately 30 cm to suspend the small bob, deflections of 14–17 scale divisions were obtained with water at 10 rev min⁻¹. The heavier bob was suspended by 0.14 cm diameter wire of approximately 30 cm length, giving deflections of 9–11 scale divisions at 10 rev min⁻¹ with water. The suspension wire was attached to the bob by means of a brass rod. Vanes attached to the bob suspension rod rotated freely in an annular trough containing silicone oil. This damping served to minimise the effects of draughts without appreciably affecting the sensitivity of the apparatus.

To measure the deflections of the bob, a 0.6 cm diameter concave aluminised mirror was attached to the bob suspension rod. This mirror projected an image of a vertical hair line onto a circular scale at its focal distance (1 metre), the 200 cm long perspex scale being graduated in 1 mm divisions. Water was pumped through the thermostat jacket surrounding the housing for the rotating cylinder by the external pumping circuit of a thermostat water-bath maintaining a temperature of 25.0° ± 0.1° within the rotating cylinder.

Method of operation. The instrument was supported on a balance table, and the axis of rotation was made accurately vertical by using two spirit levels at right angles to one another, placed on the base plates containing the cylinder housing. 10 ml of water was placed in the outer cylinder and the bob was lowered into the cylinder until it just touched the bottom; it was then raised about 0.5 cm from the bottom of the inner cylinder so permitting free movement. The bob was manipulated to trap an air bubble of similar diameter to the circle scribed on the perspex base of the outer cylinder and was then centred, its position being observed by an inclined mirror placed on the balance table underneath the perspex base of the outer cylinder. Fifteen min were allowed for temperature equilibrium and then the zero deflection on the scale was noted. Deflection readings were noted at various speeds of the outer cylinder (timed against a stop watch). If the material under study was thixotropic, previous calibration of the speed control setting against a stop watch was used. Using this procedure a 20% w/w solution of sucrose gave a relative viscosity of 1.81 ± 0.01 compared with a value of 1.79 computed from published figures (National Bureau of Standards, Circular 440). Errors with phospholipid sols were probably greater than with the sucrose solution due to increased difficulty in centering the bob and filtering the solutions, and at higher relative viscosities (above 6) the deflection became increasingly difficult to read, possibly because of slight variation in rotational speed.

PREPARATION OF LECITHIN

Egg yolks were extracted with ethanol-ether (4:1) and the extract was taken up in ether and precipitated with acetone four times. The precipitate was dissolved in absolute ethanol and freed of ninhydrin reacting

VISCOSITY OF PHOSPHATIDYLCHOLINE (LECITHIN)

material by passing through a Dowex 1 \times 2 50–100 mesh ion-exchange column in the bicarbonate form (Perrin & Saunders, 1960). The choline-containing phospholipids were then freed from lysolecithin by silicic acid chromatography. The product was recrystallised from methyl ethyl ketone-acetone (1 : 3) and then stored at 0° in ethanol under nitrogen. A typical analysis was as follows (%): phosphorus 3.82; quaternary nitrogen 1.71; total nitrogen 1.71; plasmologen 0.3; sugar (galactose) 0.1; iodine number 72; ester bond/phosphorus = 2.00 (theoretical = 2.00); quaternary nitrogen/phosphorus = 0.993 (theoretical = 1.00).

PREPARATION OF LECITHIN SOLS

A measured quantity of the ethanolic stock solution of lecithin in a weighed flask was evaporated to dryness in a vacuum oven overnight, and the flask was then reweighed. The lecithin was dissolved in as small a quantity of ether as possible, the requisite amount of de-ionised water was then added and the ether removed by bubbling nitrogen through the dispersion, warmed to 30°. This bubbling was continued for 30 min and the flask was then gently shaken for 1 hr to remove any last traces of ether. The flask was reweighed and the dispersion aged for 24 hr. All sol concentrations reported are percentage weight in weight.

MEASUREMENT OF VISCOSITY

Measurements were extremely difficult with the turbid lecithin sols since the dispersions above 2% could not be spun on the laboratory centrifuge because they sedimented giving clear and turbid liquid layers, and the air bubble and the inner cylinder could not be seen.

To overcome the first difficulty, filtered water was used and care taken to keep the sol dust free, and the solution was de-gassed by means of a vacuum pump. Using water, it was found that if the inner cylinder was put into position by the same technique every time then the size of the air bubble was reproducible; a similar technique was therefore used with the lecithin sols. A circuit containing a milliammeter was used to ensure that the bob did not touch the outer cylinder. In this circuit the bob was insulated from the rest of the viscometer and so with a solution in the outer cylinder and the bob accurately centered no reading was observed on the ammeter. On the other hand if the bob touched the wall of the outer cylinder a large deflection was observed. Before any experiment with lecithin sols was attempted, the bob was accurately centred using water, and then the water was replaced by the sol. Preliminary experiments showed the lecithin sols to be thixotropic, and to check whether or not the flow curves passed through the origin the apparatus was geared to give speeds as low as 0.2 rev min⁻¹ (shear rate of 0.2 sec⁻¹).

Results

A plot of deflection against rev min⁻¹ for a Newtonian liquid is a straight line passing through the origin. Water and sols containing less than 1.83% lecithin behaved in a Newtonian manner at the shear rates

used. In such instances relative viscosity = η_{rel} = viscosity of sol/viscosity of solvent, η_{rel} = slope of line with sol/slope of line with water; specific viscosity = η_{sp} = $\eta_{rel} - 1$.

Simha's equations (below) assume ellipsoidal particles in dilute Newtonian systems, correlating a viscosity factor ν with the axial ratio of the ellipsoids; ν is the ratio of specific viscosity to volume fraction of solute ϕ , extrapolated to zero volume fraction, and f is the axial ratio.

$$(a) \nu = \frac{f^2}{15 (\log 2f - 1.5)} + \frac{f^2}{5 (\log 2f - 0.5)} + \frac{14}{15}, \text{ for rods}$$

$$(b) \nu = \frac{16f}{15 \tan^{-1} f} \text{ for discs}$$

Where f is the axial ratio.

$$\nu = [\eta_{sp}/\phi]_{\phi \rightarrow 0}$$

$\phi = \frac{\text{volume of particles}}{\text{volume of solution}} = \frac{c}{100\rho}$ to a close approximation where c = concentration expressed as percentage weight in weight and ρ = density of lecithin = 1.016 g/ml (Elworthy, 1959).

Values of axial ratios for various viscosity factors have been computed using the above equations (Scheraga, 1955).

VISCOSITY AT VARIOUS CONCENTRATIONS

Less concentrated sols were prepared by weight in weight dilution with de-ionised water. If the sols exhibited thixotropy, the rate of shear was found from the previous calibration of the potentiometer setting and a period of 1½ hr was allowed between dilutions for the sols to recover. The degree of thixotropy was diminished on dilution until at 1.83% no thixotropy was exhibited.

The values of η_{rel} shown were obtained from the usual plots of deflection against rev min⁻¹. The η_{rel} at a given shear rate (rev min⁻¹) being taken

TABLE 1. DEFLECTION READINGS FROM THE COUETTE VISCOMETER AND VALUES OF η_{rel} AT ZERO SHEAR FOR NON-NEWTONIAN SOLS

Deflection readings 3.2% sol			Relative viscosities of lecithin dispersions at zero shear	
Revs min ⁻¹	A	B	Sol. conc. w/w %	η_{rel} at zero shear
0.22	26.2	5.5	3.81	190
0.35	32.8	8.5	3.55	177
0.43	35.8	9.5	3.33	149
0.62	44.8	12.3	2.95	118
0.84	53.5	14.7	2.78	88
1.08	61.6	16.8	2.62	60
1.26	67.5	19.1	2.29	49
1.43	73.4	21.8	2.12	9.7
1.72	78.0	23.8	1.95	2.2
1.97	80.5	26.5		
2.53	88.8	31.7		
3.11	99.0	37.3		
3.78	106.5	42.2		
4.25	112.2	46.5		
4.88	118.2	53.0		
5.45	126.8	57.3		

VISCOSITY OF PHOSPHATIDYLCHOLINE (LECITHIN)

as deflection with sol at a given shear rate/deflection with water at same shear rate.

Column A in Table 1 gives the deflection readings at the rates of rotation shown, for a 3.2% lecithin sol, aged after each reading; column B shows results obtained when the sol had been sheared at 5.45 rev min⁻¹ to a constant deflection and then the speed had been dropped to the figure shown. For B, Table 1, all the readings were taken within 10 sec of reducing speed.

Some values of η_{rel} at zero shear for non-Newtonian sols are also shown in Table 1; in Table 2 the viscosities for more dilute dispersions which showed Newtonian behaviour, are given.

TABLE 2. VISCOSITIES OF DILUTE DISPERSIONS OF LECITHIN SHOWING NEWTONIAN BEHAVIOUR

Sol conc. w/w %	Volume fraction, $\phi \times 10^2$	η_{rel}	η_{sp}	η_{sp}/ϕ
1.833	18.04	1.562	0.562	31.2
1.727	16.99	1.486	0.486	28.6
1.621	15.96	1.421	0.421	26.4
1.531	15.06	1.394	0.394	26.2
1.430	14.07	1.378	0.378	26.9
1.339	13.18	1.336	0.336	25.5
1.255	12.35	1.300	0.300	24.3
1.165	11.46	1.286	0.286	25.0
0.938	9.23	1.258	0.258	28.0
0.849	8.36	1.218	0.218	26.1
0.783	7.71	1.194	0.194	25.2
0.687	6.77	1.181	0.181	26.8
0.628	6.19	1.176	0.176	28.5
0.535	5.27	1.118	0.118	22.4

The values of η_{sp}/ϕ in Table 2 appear to fluctuate about a mean value of 26.5. Taking this value as the viscosity factor gives an axial ratio of 15.5 for a prolate ellipsoid (rod) or 37 for an oblate ellipsoid (disc).

SOLS DISPERSED BY ULTRASONICS

Lecithin was dispersed in ion-free water using a 60 W Mullard ultrasonic generator, to drive a titanium ultrasonic drill head. The generator was

TABLE 3. VISCOSITIES OF LECITHIN SOLS DISPERSED BY ULTRASONICS

Sol conc.	ϕ	η_{rel}	η_{sp}	η_{sp}/ϕ
(a) Couette viscometer				
4.998	0.0492	1.206	0.206	4.1
4.653	0.0458	1.184	0.184	4.0
4.321	0.0425	1.162	0.162	3.8
3.941	0.0383	1.136	0.136	3.5
3.517	0.346	1.113	0.113	3.3
3.083	0.0303	1.089	0.089	2.9
(b) Cannon-Fenske viscometer No. 25 viscometer				
1.302	0.0128	1.057	0.057	4.5
1.205	0.0119	1.049	0.049	4.1
1.143	0.0113	1.045	0.045	3.9
1.091	0.0107	1.040	0.040	3.7
1.044	0.0103	1.037	0.037	3.6
0.995	0.0098	1.036	0.036	3.7
0.948	0.0093	1.034	0.034	3.6
0.906	0.0089	1.033	0.033	3.7

tuned to give maximum cavitation in the liquid. All irradiations were carried out in an atmosphere of nitrogen and the vessels containing the sols were surrounded by ice cold water. The clear sols were irradiated until there was no change in opacity, they were then mixed with Amberlite Monobed MBI ion-exchange resin and spun on the centrifuge at 6,000 rpm. Measurements in the Couette viscometer showed that even a 5% ultrasonically irradiated sol behaved in a Newtonian manner and had a low relative viscosity. The results are given in Table 3.

Averaging the last five of the capillary viscometer results in Table 3 gives a viscosity factor of 3.7. When substituted in Simha's equation this gives an axial ratio of 3.0 for a prolate ellipsoid and 3.5 for an oblate ellipsoid.

Discussion

In water, phosphatidylcholine or lecithin prepared from egg yolk or brain, exists as large molecular aggregates with the polar phosphorylcholine groups orientated outwards; X-ray diffraction studies of concentrated sols indicate that bimolecular leaflets are present. In the more concentrated sols these particles are probably associated to form larger structures which are easily broken on shearing. The structure present in the more concentrated sols is shown by the large values of η_{rel} at zero shear; however, this association is weak enough to be broken down at low shear rates. During the recovery time the smaller particles re-associate by Brownian motion. In more dilute sols the tendency to association is less, and the thixotropy is lost on dilution.

Simha's equations are derived assuming low shear rates, low concentrations and Newtonian behaviour, the viscosity factor of 26.5 compares with a value of 27.9 calculated from previous investigations (Thomas & Saunders, 1958) using a Cannon-Fenske viscometer.

Coarse lecithin dispersions by light-scattering (Robinson, 1960) gave an axial ratio of 13.2 for disc shaped particles and a molecular weight of 27.2×10^6 using data obtained in the concentration range of 1 to 20×10^{-5} g ml.⁻¹ Taking a rod as a model and using the above particle weight, Robinson suggested a triple-layer laminated structure. This unlikely structure would give an axial ratio of approximately 6 for a rod-shaped aggregate. These values compare with the axial ratio of 15.5 for a rod and 37 for a disc reported above (Table 3). The discrepancies between the viscosity and light-scattering results may be due to hydration, to the different concentration ranges used in the two investigations, and to the fact that the scattering of the sols that have not been dispersed by ultrasonics, is above the level at which reasonably accurate calculations of size and shape can be made.

At the extremely high shear rates obtained by ultrasonic irradiation, the sols become optically clear and stable. These ultrasonic irradiated sols are of much lower viscosity than the untreated sols and give a viscosity factor of 3.7 corresponding to an axial ratio of 3.5 for a rod and 3.0 for a disc. Diffusion and sedimentation coefficients (Gammack, Saunders, &

VISCOSITY OF PHOSPHATIDYLCHOLINE (LECITHIN)

Perrin, 1964) have been obtained for these optically clear sols and correlation with the above viscosity data suggests a micellar weight in the region between 2 and 7×10^6 . A more exact estimate, based on light scattering, shows that prolonged ultrasonic irradiation reduces the aggregate size to 2×10^6 ; the low axial ratio estimated from the viscosity results is confirmed by light scattering (Attwood & Saunders, 1965).

The absence of thixotropy and the low value of ν for the ultrasonic dispersions suggest that the amounts of water bound in these sols is comparatively small. In addition to the reduction in size of the dispersed particles, the effect of ultrasonics appears to be to expel water from the aggregates present in the coarse dispersions. It is this latter effect, which is irreversible, that causes a change of configuration of the aggregates.

Fuller accounts of the effect of ultrasonics on lecithin sols have been reported (Perrin, 1962; Saunders, Perrin & Gammack, 1962; Attwood & Saunders, 1965).

References

- Attwood, D. & Saunders, L. (1965). *Biochim. biophys. Acta*, **98**, 344–350.
Elworthy, P. H. (1959). *J. chem. Soc.*, 1951–1956.
Gammack, D. B., Saunders, L. & Perrin, J. H. (1964). *Biochim. biophys. Acta*, **84**, 576–586.
Lawrence, A. S. C., Needham, J. & Shen, S. C. (1944). *J. gen. Physiol.*, **27**, 201–232.
Ogston, A. & Stanier, J. E. (1953). *Biochem. J.*, **53**, 4–7.
Perrin, J. H. (1962). Ph.D. Thesis, University of London.
Perrin, J. H. & Saunders, L. (1960). *J. Pharm. Pharmac.*, **12**, 257T–259T.
Robinson, N. (1960). *Trans. Faraday Soc.*, **56**, 1260–1264.
Saunders, L. (1957). *J. Pharm. Pharmac.*, **9**, 834–839.
Saunders, L., Perrin, J. H. & Gammack, D. B. (1962). *Ibid.*, **14**, 567–572.
Scheraga, H. A. (1955). *J. chem. Phys.*, **23**, 1526–1532.
Simha, R. (1940). *J. phys. Chem., Ithaca*, **44**, 25–34.
Supplement to the National Bureau of Standards Circular 440.
Thomas, I. L. & Saunders, L. (1958). *J. Pharm. Pharmac.*, **10**, 182T–185T.