ULTRASONIC IRRADIATION OF SOME PHOSPHOLIPID SOLS

By L. Saunders,* J. Perrin* and D. Gammack†

*From *the Department of Chemistry, School of Pharmacy, University of London, Brunswick Square, London, W.C.1, and the †Institute of Psychiatry, Maudsley Hospital, Denmark Hill, London, S.E.5*

Received June 13, 1962

Ultrasonic irradiation of turbid sols of egg lecithin and of highly unsaturated synthetic lecithins, breaks down the large particles in the sols into micelles of micellar weight of about 5×10^6. The irradiated sols are clear and do not show any great increase of turbidity over a period of several days; they are capable of solubilising large quantities of cholesterol. The size and shape of micelles in the irradiated sols has been studied by means of viscosity and diffusion measurements and by determination of sedimentation coefficients in the ultracentrifuge.

Lecithin sols of concentration above 5 per cent readily separate into two layers on centrifugation at low speeds (Saunders, 1960). The lower layer contains all the phospholipid, has a concentration of about 15 per cent (w/w) and has the appearance of a thick emulsion. Microscopic examination of these concentrated sols shows that they contain large structures, as shown in Fig. 1. Attempts to clarify the 15 per cent emulsion by the addition of surface-active agents were not successful, but we have found that ultrasonic irradiation at 20 kc/sec. breaks down the structures and gives optically clear sols containing slightly asymmetric micelles of molecular weight about 10^7. Providing oxygen is excluded, the lecithin does not undergo any drastic chemical change.

The clarified sols are not flocculated by electrolytes and they are able to solubilise concentrations of lipids such as cholesterol, to give clear sols. This effect has been noted by Fleischer and Brierley (1961) who have pointed out the potential value of these sols in biochemistry. They may also be of use in preparing injection solutions of water insoluble lipids.

Experimental

Ultrasonic dispersion was done with a 60 watt Mullard ultrasonic generator using titanium probes. The tip of the probe was immersed to 2 mm. in the surface of the liquid contained in a glass tube of diameter about 1 cm. greater than that of the probe and cooled in an ice bath. The generator was tuned to give maximum cavitation which is mainly effective in breaking down structures in the liquid (Thomas, 1959; Littlewood, 1962). The irradiation was made in an atmosphere of nitrogen.

Materials. Egg lecithin was prepared and purified by chromatography and crystallisation, as described in previous papers (Saunders, 1957; Perrin and Saunders, 1960). The synthetic phospholipids were the gift of Professor van Deenan and Dr. de Haas of Utrecht University.

Preparation of sols. Egg lecithin sols were formed by adding water to an ethereal solution, evaporating the ether under reduced pressure until

only a small volume of liquid remained, then adding water to give the final required concentration. The concentrations are in weight per cent.

Optical density measurements. Measurements were made with a Spekker absorptiometer using a 0·25 cm. cell and a mercury vapour lamp with a neutral density filter.

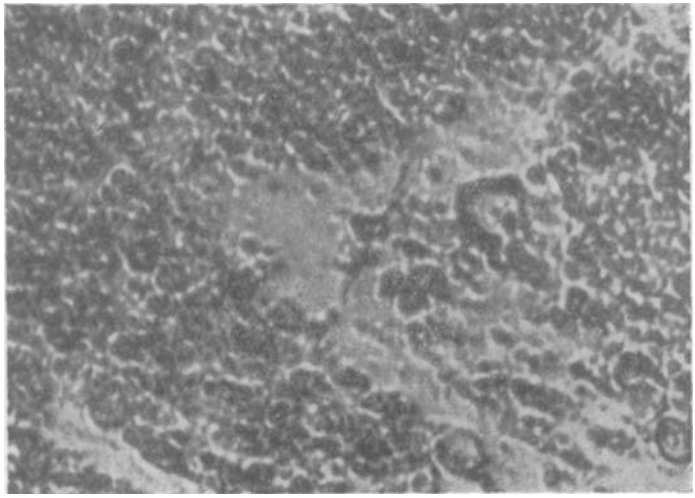

FIG. 1. A concentrated lecithin sol before irradiation (x 700).

TABLE I

VARIATION OF OPTICAL DENSITY WITH TIME FOR A 5 PER CENT EGG LECITHIN SOL AFTER ULTRASONIC IRRADIATION

Measurements made using a mercury light source, a neutral density filter and a 0·25 cm. cell

Time (hr.)	Optical density
0	0·097
1	0·098
2	0·098
4	0·100
6	0·101
17	0·101
43	0·099
67	0·105
77	0·112

Twenty min. of ultrasonic irradiation of lecithin sols gave a constant low optical density. On stopping the irradiation, the optical density decreased further and then remained constant over a period of several days showing that there is no spontaneous reversion of the sol to its original form; the initial drop in optical density could be eliminated by centifuging the sol. Results for a 5 per cent dispersion are given in Table I. The original dispersion could only be regained by evaporating to dryness and redispersing by the ether and water method; the residue on evaporation was completely soluble in ether showing that little lysolecithin was formed in the ultrasonic treatment.

A study of the effect of dilution on the clarified sol indicated that the optical density of a diluted sol usually was slightly higher than that of a sol of the same concentration irradiated directly, but this effect was small and the diluted sols showed no change of density with time.

Viscosity measurements. Concentrated dispersions of egg lecithin in water are thixotropic and give ratios of specific viscosity to volume fraction (zero concentration and shear) of 26·5 at 25° indicating highly asymmetric particles (cf. Robinson, 1960). The ultrasonically dispersed sols, however, showed no variation of viscosity with shear rate and no fall in deflection was noted on shearing a 5 per cent sol at 7·1 rev./sec. in a Couette viscometer, also the plot of deflection against shear rate was linear. In view of the Newtonian behaviour of these sols, the intrinsic viscosity was determined by measurements in a Cannon-Fenske capillary viscometer. The results gave a ratio of specific viscosity to volume fraction, extrapolated to zero volume fraction, of 3·7. This differs from the theoretical value for spheres sufficiently to suggest that the micelles in these sols are asymmetric.

Light scattering. Some preliminary measurements indicate that the scattering of very dilute ultrasonically irradiated sols is of the same order as the very dilute sols dispersed by normal methods (Robinson, 1960).

Sedimentation studies. These were carried out using a Spinco model E ultracentrifuge fitted with a schlieren phase plate. Sedimentation coefficients (S) were computed from plots of ln(x) against time at constant speed, where x is the distance of the sedimenting boundary from the centre of rotation. The observed sedimentation coefficients were corrected to water at 25°.

The sedimentation of a 5 per cent sol of egg lecithin which had not been treated ultrasonically, was first examined. This emulsion gave a clear separation into two phases at low speeds and when a centrifugal field of up to 250,000 *g* was applied only a slight compression of the lower layer occurred. At 30,000 *g* the concentration of lecithin in the lower layer was 12 per cent, at 250,000 *g* it was increased to 18 per cent. There was no evidence of any phospholipid remaining in the upper layer.

Three separately prepared, ultrasonically irradiated sols were next examined. These were all of a concentration of 1 per cent of egg lecithin in water. The schlieren patterns were all similar to those shown in Fig. 2. No splitting of the peaks was observed but they are clearly asymmetric with a trail away from the centre of rotation, indicating some degree of polydispersity. The rate of sedimentation was low but this is due to the small density difference between lecithin and water. To obtain a mean micellar weight estimate from the sedimentation data the partial specific volume of the phospholipid must be measured with great accuracy. This was done by measuring densities of the sols and gave a value for egg lecithin of 0·9833 ml./g. (cf. Elworthy (1959), density of dry lecithin 1·016 g./ml.).

The values of S corrected to water at 25° obtained for the three samples were 3·12, 2·60 and 3·45.

Diffusion. Rates of diffusion of the ultrasonically dispersed lecithin micelles into water and into more dilute sols were measured by the Gouy

interference method (Brudney and Saunders, 1955; Thomas and Saunders, 1959) at 25°. The patterns obtained indicated solute heterogeneity and the mean diffusion coefficient which was very low, decreased with time. Results obtained for diffusion from a 1·4 per cent sol into a 0·4 per cent sol gave more regular patterns than the diffusion into water and gave a diffusion coefficient of $1{\cdot}8 \times 10^{-7}$ cm.^{2}/sec. which fell after 24 hr. to a value of 8×10^{-8} remaining constant over a four day period. It is clear from these results that a range of micellar sizes is present in the ultrasonically dispersed sols.

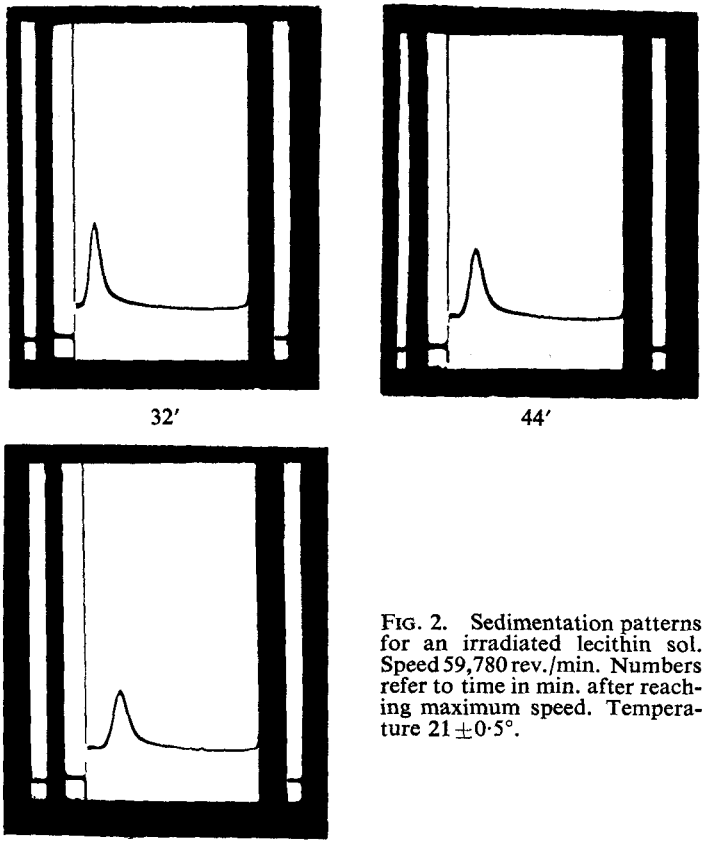

FIG. 2. Sedimentation patterns for an irradiated lecithin sol. Speed 59,780 rev./min. Numbers refer to time in min. after reaching maximum speed. Temperature $21 \pm 0{\cdot}5°$.

Synthetic phospholipids. Synthetic lecithins containing only long chain saturated fatty acids cannot be dispersed in water (Saunders, 1957) (dilauryl)lecithin being the longest chain lecithin which gives stable aqueous dispersion. The work of de Haas and van Deenan (1961) has led to the synthesis of lecithins containing unsaturated acyl groups and also to the preparation of mixed acid lecithins containing both saturated and unsaturated fatty acids; these should show properties similar to the natural substances.

We have attempted to disperse (stearoyl-oleyl)- and (di-oleyl)lecithin in water. Some difficulty was found in obtaining the initial dispersion; this was finally made by adding water to an ethanol solution of the phospholipid, evaporating to small bulk under vacuum adding water and re-evaporating and then finally making up to weight with water. Turbid 0·5 per cent sols were obtained, the (di-oleyl)lecithin sol was cleared by sonic irradiation, but unlike the natural material this clearing was reversible and after 1 hr. the sol was again turbid. Attempts to disperse (di-oleyl)cephalin in water were unsuccessful. (Di-linoleyl)lecithin did, however, give dispersions in water which could be cleared irreversibly by the ultrasonic irradiation and which were capable of solubilising cholesterol.

Solubilisation of cholesterol. When cholesterol is dissolved in an ethereal solution of egg lecithin, the lipids can be dispersed together in water. If, after evaporating off the ether, the sol is irradiated ultrasonically, an optically clear dispersion can be obtained. A concentration of up to 10 per cent of cholesterol in water can be achieved in the presence of 20 per cent lecithin, and on standing this concentrated sol forms a clear gel. At a slightly lower concentration (16 per cent lecithin, 8 per cent cholesterol) the sol remains liquid and is stable over periods of a week or more. The lecithin cholesterol sols are precipitated by salts, unlike the irradiated sols containing lecithin alone.

Discussion

Ultrasonic irradiation of concentrated egg lecithin and highly unsaturated synthetic lecithin sols breaks down the microscopically visible structures present, into micelles of molecular weight of about 10^7. The viscosity results indicate some asymmetry and if the micelles are considered as disc shaped entities they would correspond to oblate ellipsoids of axial ratio about 3·5. The diffusion and viscosity results together indicate a range of micellar weights of from 4 to 50×10^6, the sedimentation and diffusion results give a range of 2 to 7×10^6.

If the oblate ellipsoid representing the micelle is considered to have a thickness equal to that of a bimolecular lecithin layer, then the theoretical molecular weight assuming the above axial ratio would be 2×10^6, a doubled bimolecular layer would give a micellar weight of 16×10^6.

The ultrasonic irradiation method permits the preparation of phospholipid sols containing relatively high concentrations of lipids such as cholesterol. It therefore offers a new approach to the study of dispersions of bio-colloids from which membranes, resembling cell membranes, can be precipitated.

Acknowledgement. We are grateful to the Medical Research Council for providing the Spinco Analytical Ultracentrifuge.

References

Brudney, N. and Saunders, L. (1955). *J. Pharm. Pharmacol.*, 7, 1012–1021.
de Haas, G. H. and van Deenan, L. L. M. (1961). *Rec. Trav. Chim. Pays-Bas*, **T80**, 951–970.
Elworthy, P. H. (1959). *J. chem. Soc.*, 1951–1956.

Fleischer, S. and Brierley, G. (1961). *Biochem. Biophys. Research Communications*, **5**, 367–373.
Littlewood, K. (1962). *Royal Inst. Chem. J.*, **86**, 78–86.
Perrin, J. and Saunders, L. (1960). *J. Pharm. Pharmacol.*, **12**, 253.
Robinson, N. (1960). *Trans. Farad. Soc.*, **56**, 1260–1264.
Saunders, L. (1957). *Proceedings of the Second International Congress of Surface Activity*, p. 302. London: Butterworths.
Saunders, L. (1957). *J. Pharm. Pharmacol.*, **9**, 834–840.
Saunders, L. (1960). *Ibid.*, **12**, Suppl. 253*T*–256*T*.
Thomas, I. L. and Saunders, L. (1959). *J. chem. Soc.*, 2731–2734.
Thomas, J. R. (1959). *J. phys. Chem.*, **63**, 1725–1729.