MICELLE FORMATION BY LECITHIN IN SOME ALIPHATIC ALCOHOLS

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Lecithin has been studied in methanol, ethanol, butanol, and hexanol using light scattering and viscosity techniques. A new type of light scattering cell was employed. Micelles were present in all solvents, the micellar weights being 2,300; 7,100; 18,000, and 22,000 in the four alcohols respectively. It appeared that as the polarity of the solvent decreased, the micellar size increased. Dissymmetry measurements showed that no dimension of the micelles exceeded 270Å ($\lambda/20$), but high observed depolarisations showed that the particles were anisotropic. Viscosity measurements also gave an idea of the shape of the micelles, giving intercepts of plots of specific viscosity/solute volume fraction against solute volume fraction of 4·29, 4·26, 4·26, and 3·56 for the four alcohols respectively. Using the dimensions of the lecithin molecule, and the number of monomers present in the micelle, calculations of the theoretical viscosity intercept have been made for each solvent, and compared with values obtained experimentally.

MICELLE formation by lecithin in benzene has been studied (Elworthy, 1959) using osmotic pressure, diffusion, and viscosity measurements. The micelles had a laminar structure with the phosphorylcholine head groups situated in the centre of the micelle, and the hydrocarbon chains extending outwards into the solvent. The micellar weight was 57,000. There are presumably repulsive forces between the polar head groups and the solvent, giving a reversal of the type of micelle structure found in water, in which the hydrocarbon chains form the interior of the micelle, and the polar groups are on the outside.

As a continuation of the study of micelle formation by lecithin in organic solvents, it was decided to study lecithin in solvents which should provide intermediate conditions between water and benzene. A series of aliphatic alcohols was chosen, varying in dielectric constant from methanol (33·6) to hexanol (14·3). Price and Lewis (1929) have reported that monomers were present in ethanol, using ebullioscopic measurements.

The light scattering method was used for determining micellar weight, and to give an idea of particle shape. Viscosity measurements also gave an idea of shape.

EXPERIMENTAL

Materials

Lecithin was prepared from fresh chickens' egg yolks by treatment with alumina to remove ninhydrin reacting materials, followed by chromatography on silica to remove lysolecithin (Elworthy and Saunders, 1957). Two samples were used in this work; sample one had N, 1·8; P, 3·8 per cent; I No. 73; sample two had N, 1·8 per cent; P, 3·8 per cent; I No. 60.

Analar benzene was fractionally crystallised, dried, fractionally distilled, stored over sodium, and had n_D^{25} 1·4979 (1·4981). Analar toluene was dried, fractionally distilled, stored over sodium, and had n_D^{25} 1·4940 (1·4941). The alcohols were dried (Vogel, 1950), fractionally distilled, and stored over anhydrous calcium sulphate. Methanol had n_D^{15} 1·3315 (1·3306), ethanol had n_D^{20} 1·3619 (1·3614), butanol had n_D^{15} 1·4020 (1·4012), and hexanol had n_D^{20} 1·4181 (1·4179). Figures given in brackets are literature values of refractive index (Timmermans, 1950, Hodgmen, 1957).

Viscosity Measurements

Viscosities of solutions relative to solvent were measured in a suspended level dilution viscometer.

Light Scattering Measurements

The light scattering photometer previously described (Elworthy, 1960) has been considerably modified. Light from a stabilised 250 W mercury vapour lamp was made parallel by a lens system, and the green line
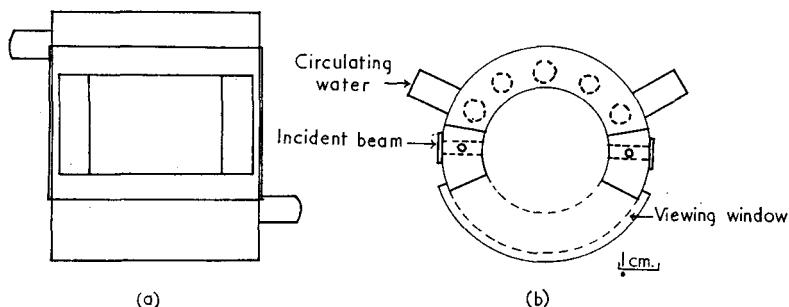

FIG. 1. Light scattering cell. (a) Front view; (b) from above.

(5461Å) isolated by means of an interference filter together with a neodymium glass to remove the last traces of the sodium lines. Final collimation of the beam was achieved by passing it through two 2 mm. wide slits placed 32 cm. apart; the beam passing through the cell measured 2 × 26 mm.

The light scattering cell was of a new design (Fig. 1). It was cylindrical with two parallel flattened faces to accommodate the windows through which the incident beam passed. The scattered light was viewed through a curved glass window, allowing observation between 45 and 135° to the incident beam. A constant temperature was achieved by circulating water through a series of tubes cut in the back wall of the cell. The two principal factors found to reduce stray light were, firstly, complete blackening of the inside of the cell by the Relonol process, and secondly, making the channels inside the cell, through which the incident beam entered and left, sufficiently long so that the actual points where the beam struck the glass windows could not be seen through the curved viewing window.

The eleven-stage photomultiplier receiving the scattered light was mounted on a broad tufnol arm which rested on a tufnol plate. The arm was pivoted beneath the centre of the cell, giving a very smooth movement of the photomultiplier around the cell. The signal was amplified (Ottewill and Parreira, 1958) and read on a galvanometer.

A polished perspex block was used as a light scattering standard, being calibrated from Ludox solutions using Maron and Lou's (1954) procedure. As a check on the calibration, the Rayleigh's ratios of benzene and toluene were determined, giving $16 \cdot 0 \times 10^{-6}$ and $17 \cdot 9 \times 10^{-6} cm.^{-1}$ respectively, which agreed with literature values (Ottewill and Parreira,

TABLE I

LIGHT SCATTERING AND VISCOSITY DATA

Solvent	Methanol	Ethanol	Butanol	Hexanol
dn/dc, ml. g.$^{-1}$	0·135	0·118	0·087	0·071
Micellar weight	2,300	7,100	18,000	22,000
Monomers in micelle	3	9	24	28
Depolarisation	0·345	0·476	0·552	0·509
Dissymmetry Z_{45}	1·01	1·00	1·00	1·00
$\left(\dfrac{\eta_{sp}}{\varnothing}\right)_{\phi \,=\, 0}$	$4 \cdot 2_9$	$4 \cdot 2_6$	$4 \cdot 2_4$	$3 \cdot 5_6$

1958). The molecular weight of a National Chemical Laboratory's sample of polystyrene was found to be 370,000 as against 390,000 quoted.

All solutions were clarified by filtering through No. 5 sintered glass filters until dust free.

Specific Refractive Index Increments (dn/dc)

These were determined with a Hilger-Rayleigh interference refractometer using Bauer's (1945) technique for monochromatic light. The instrument was checked using sodium chloride solutions as standards.

Depolarisations

Measurements were made in the usual way with a polaroid disc (Stacey, 1956). As high depolarisations are reported in this paper, particular care was taken in checking the technique. A comparison of the sensitivity of the photomultiplier to light of horizontal and vertical polarisation was made, and corrections applied for its greater sensitivity to the horizontal component. The contribution of the solvent to both components was subtracted from the values for solutions. The depolarisation of dilute Ludox solutions was found to be 0·01, benzene gave $\rho = 0 \cdot 41$, and toluene $\rho = 0 \cdot 42$, in good agreement with literature figures (Ottewill and Parreira, 1958). All solutions were tested for fluorescence as described by Brice, Nutting, and Halwer (1953). No fluorescence was observed in any of the systems studied.

RESULTS

The results of the light scattering measurements are given in Fig. 2 and Table I. T is the observed turbidity, c is the concentration in g. ml.$^{-1}$, and $H = 32\pi^3 n_0^2 \, (dn/dc)^2/3\lambda \,^4 N$, where n_0 is the refractive index of the solvent,
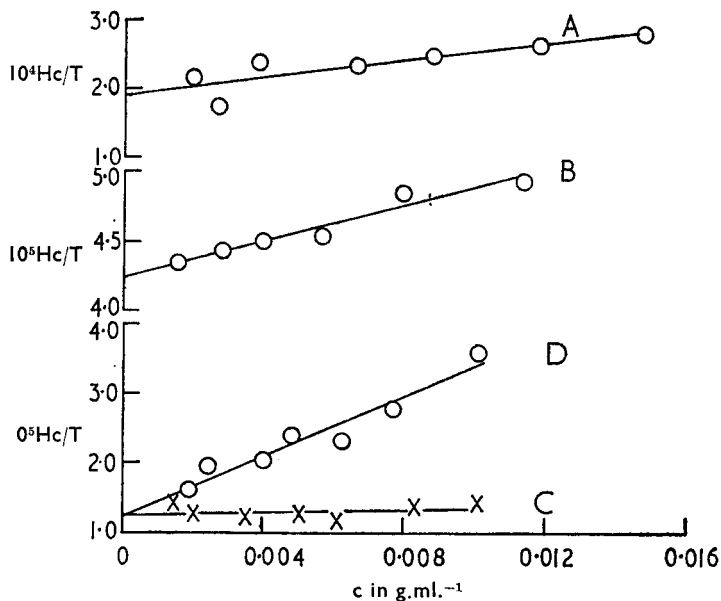
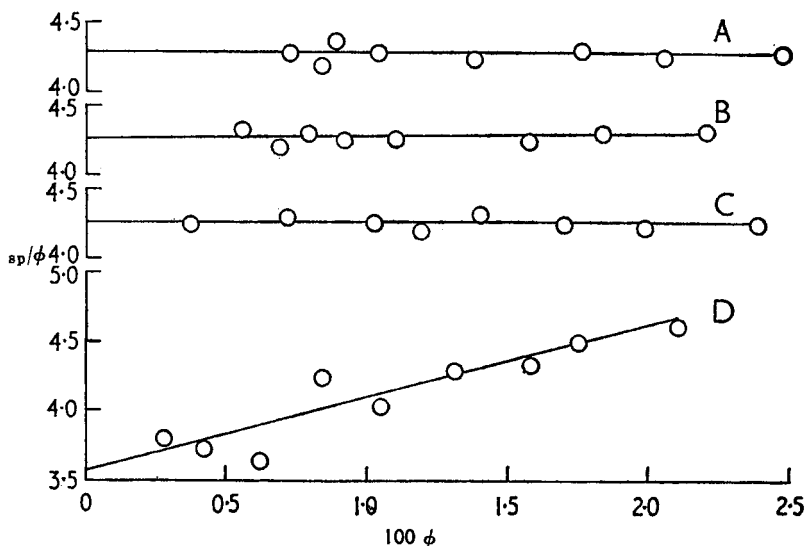FIG. 2. Graphs of Hc/T against c. A, methanol; B, ethanol; C, butanol; D, hexanol.

FIG. 3. Graphs of η_{sp}/ϕ against ϕ. A, methanol; B, ethanol; C, butanol; D, hexanol.

and λ the wavelength of the light used. For the calculation of micellar weight, the Cabannes factor, calculated from the depolarisation, was applied to the Hc/T value at zero concentration. All measurements were made at 20°.

There was very little slope of the Hc/T against c plots for methanol, ethanol, and butanol, but a moderate slope for hexanol. This may indicate a greater interaction between solute and solvent in hexanol than in the three lower alcohols. Graphs of concentration against turbidity gave straight lines passing through the origin (after subtraction of the solvent turbidity from the turbidity of each solution). There were none of the breaks associated with critical micelle concentrations. If these are present, they are at concentrations too small to be measured accurately by the present technique.

The viscosity results are given in Fig. 3 as graphs of η_{sp}/ϕ against ϕ, where η_{sp} is the specific viscosity and ϕ is the volume fraction of solute
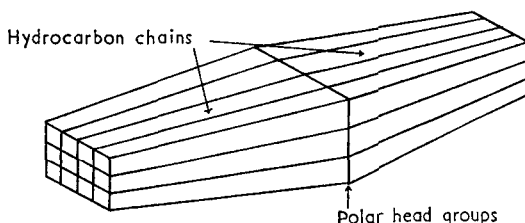

FIG. 4. Model of lecithin micelle.

The slope of the graphs for the three lowest alcohols is very small, but the hexanol system shows a definite slope.

No differences between the properties of the two lecithin samples used were observed.

DISCUSSION

It can be seen from Table I that the micelle size increases on passing from methanol to hexanol, that is, increasing as the dielectric constant of the solvent decreases. As the solvent series is ascended, there are probably increased repulsive forces between the solvent and the polar head group of the lecithin molecule which tend to aid the formation of larger micelles. As there is a small aggregate of monomers present in methanol, it appears that there is only a small lack of affinity between this solvent and the polar head groups. Ethanol appears to have still less affinity, causing the polar heads to be tucked inside the micelle. This type of structure was observed in benzene (Elworthy, 1959), which is a much less polar solvent than the alcohols; the observed micellar weight of 57,000 emphasises how the micelles grow in passing into a non-polar solvent. In water the type of micellar structure described is reversed, the hydrocarbon chains being inside the micelles. The lecithin-methanol system would appear to represent a half way point between the type of micelles formed in aqueous systems, and those present in the higher alcohols and benzene.

The micellar size in benzene decreased from 57,000 at 25° to 43,000 at 40°. This trend may explain why in ethanol at 20° we have found small micelles, while Price and Lewis (1929) reported that monomers were present at the boiling point.

The observed dissymmetries (intensity of light scattered at 45°/intensity scattered at 135°) were close to unity, indicating that no dimension of any of the micelles studied exceeded 270Å (λ/20). However, from the high observed depolarisations, some asymmetry is indicated. Anisotropic particles composed of a uniform material have been treated as ellipsoids (Rayleigh, 1918), having different degrees of polarisability along their different axes, which leads to cases where this type of particle gives higher depolarisations than small isotropic ones.

The large observed depolarisations may also be a consequence of the arrangement of monomers in the micelle, which will have a general structure, except for those present in methanol, as in Fig. 4. It is tentatively suggested that the high depolarisations may be due to the hydrocarbon chains being roughly parallel to one another, and allowing a much greater polarisation to occur along the axis of the bundle of hydrocarbon chains. It is not suggested that the bimolecular leaflet structure occurs in methanol, as there is present only a small aggregation of monomers.

Large Cabannes' factors had to be applied to the Hc/T intercepts in the calculation of the micellar weights. Measurements in benzene (Elworthy and McIntosh unpublished results) also gave a large depolarisation ($\rho =$ 0·265), giving M = 50,000 with dn/dc = 0·038. In view of the smallness of dn/dc, this result agrees reasonably well with that found by diffusion (57,000). Diffusion measurements in methanol gave 2,000 for the micellar weight (Elworthy, unpublished results).

From the viscosity experiments, values of $(\eta_{sp}/\phi)_{\phi = 0}$ (abbreviated to ν) were obtained. Values of this function should be 2·5 if the particles are unsolvated and spherical (Einstein, 1906, 1911). From the micellar weights we can calculate ν for certain model structures. The simplest model to choose at the present stage of the work is a rod-like structure as shown in Fig. 4. The length of each lecithin molecule was taken as 35Å, and the head group area as 55Å², from molecular models. In methanol we assume that the monomers lie side by side, and in the other solvents that a bimolecular leaflet is present. The ratio of the length to the breadth of this rod-like structure can be related to ν (Mehl, Oncley, and Simha, 1940). This treatment gave ν = 3·6 for methanol, 4·4 for ethanol, 3·4 for butanol, and 3·1 for hexanol. The calculated values of ν are of the correct order, but generally lower than the observed values. This may be due to deformation of the particles on flow, yielding rather higher viscosities than predicted on a volume fraction and asymmetry basis, or to solvation of the particles. No solvation was apparent in benzene, where ν was 2·8. Viscosity studies will be continued using a Coutte viscometer.

REFERENCES

Bauer, N. (1945). In *Physical Methods of Organic Chemistry*, editor, Weissberger, A. Vol. 1, p. 729, New York: Interscience.
Brice, B. A., Nutting, S. C., and Halwer, M. (1953). *J. Amer. chem. Soc.*, 75, 824–828.

Einstein, A. (1906). *Ann. Physik.*, **19**, 289–306.

Einstein, A. (1911). *Ibid.*, **34**, 591–592.

Elworthy, P. H. (1959). *J. chem. Soc.*, 813–817, and 1951–1956.

Elworthy, P. H., and Saunders, L. (1957). *Ibid.*, 330–335.

Elworthy, P. H. (1960). *J. Pharm. Pharmacol.*, **12**, *Suppl.* 260–266*T*.

Hodgman, C. D. (1957). *Handbook of Chemistry and Physics*, ed. 39, p. 976. Cleveland, Ohio: Chemical Rubber Publishing Co.

Maron, S. H., and Lou, R. L. H. (1954). *J. Polymer Sci.*, **14**, 29–36.

Mehl, J. W., Oncley, J. L., and Simha, R. (1940). *Science*, **92**, 132–133.

Ottewill, R. H., and Parreira, M. C. (1958). *J. phys. Chem.*, **62**, 912–916.

Price, H. I., and Lewis, W. C. M. (1929). *Biochem. J.*, **23**, 1030–1043.

Rayleigh, Lord (1918). *Phil. Mag.*, **35**, 373–381.

Stacey, K. A. (1956). *Light Scattering in Physical Chemistry*, p. 96. London: Butterworths.

Timmermans, J. (1950). *Physico-chemical Constants of Pure Organic Compounds.* London: Elsevier.

Vogel, A. I. (1950). *Textbook of Practical Organic Chemistry*, ed. 2, p. 166–169. London: Longmans.