390. *The Structure of Lecithin Micelles in Benzene Solution.*

By P. H. Elworthy.

Diffusion coefficients of lecithin in benzene were determined by using the Gouy diffusiometer. Integral measurements yielded patterns which were characteristic of two-component diffusion, from which the mean molecular weight of the small micelles was determined. In semi-differential diffusion measurements the interference patterns complied with the theory for diffusion of a single solute, and the molecular weight of the large micelles was determined from these measurements. Viscosity studies showed that the large micelles were asymmetric, and treatment as ellipsoids gave axial ratios of about 2 : 1. The final molecular weights for the large and the small micelles agreed with those obtained from osmotic data. The properties of solutions of model structures were calculated and compared with the observed properties; this indicated that the micelles had a laminar structure.

Previously,[1] osmotic studies of the lecithin–benzene system at 25° showed that small micelles were present below a concentration of 0·73 g. $l.^{-1}$; above this concentration (the critical micelle concentration) aggregation to large micelles occurred. The molecular weight of the large micelles could not be obtained directly, but only by fitting a law of mass-action equation to the experimental results, when one of the constants of the equation could be used to calculate the molecular weight. In this paper the molecular weight of the large micelles has been obtained from diffusion measurements, and the shape of the micelles from viscosity measurements. By comparison of experimental results with those calculated from the properties of model structures, it appears that the micelles have a laminar structure.

Experimental

Materials.—The physical properties of the lecithin and benzene used have been reported.[1]

Apparatus.—The apparatus was based on the Gouy diffusiometer described by Gosting *et al.*[2] and by Saunders.[3] All components were mounted on a 2 m. optical bench which rested on a vibration-free girder. Green light (λ = 5461 Å) from a mercury vapour lamp was isolated by the appropriate interference filter, and illuminated a horizontal slit 12·5 μ in width. The image of the slit was focused through the diffusion cell on a Kodak P2000 photographic plate.

A new diffusion cell, suitable for use with non-aqueous liquids, was designed for the rapid formation and sharpening of boundaries (Fig. 1). The cell had the general form of a U-tube; the two upright arms were 2 × 2 cm. in area, and were made from a single brass block. Part of the face of channel *B* was milled to allow optically flat (λ/2) cell windows *C* to be fitted. Gaskets for the cell windows were made of a rubber resistant to benzene, and were well extracted before use. A mask *G* fitted over the cell window on the plate side of the cell. The two 1 × 1 mm. square holes cut in the mask were separated by a vertical distance of 5 mm., and were so placed that the boundary was equidistant between them. Photographs of the interference patterns produced by the square holes, first with the solution in the cell, and secondly with the boundary present, provided a means of determining fractional *jm*;[3] *jm* is the difference in optical path length between the two liquids in the cell in wavelengths of light. Photographs for determining fractional *jm* were taken with the triangular aperture of *G* masked. The shape of the aperture helps to cut down the intensity of the undeviated slit image and inner fringes of the pattern, which are usually more intense than the outer fringes.

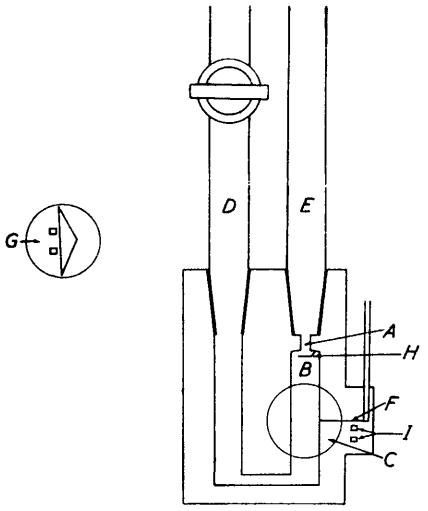
A constriction *A*, diameter 2 mm., length 5 mm., was drilled at the top of *B*. Two glass reservoirs, *D* and *E*, were made to fit into the top of the brass block, and the glass–metal joints were carefully ground and polished until a liquid-tight seal was achieved. The solution-containing reservoir, *D*, had a good quality vacuum tap fused to it. The boundary was sharpened by flow through a 50 μ slit in the cell wall (*F*). The interference pattern from two

[1] Elworthy, *J.*, 1959, 813.
[2] Gosting, Hanson, Kegeles, and Morris, *Rev. Sci. Instr.*, 1949, **20**, 209.
[3] Saunders, *J.*, 1953, 519.

square holes, *I*, cut in the side of the cell, was deflected downwards by an inclined plate of optical glass, and provided a reference trace on the photographic plate from which all distances were measured. The cell was clamped rigidly in a bath fitted with optically flat ($\lambda/2$) windows, and supplied with water from a thermostat regulated at 25° ± 0·05°.

Measurement of Diffusion Coefficients.—The reservoir *D* and U-tube of the cell were filled with solution, the tap on *D* closed, and the solution brought to the middle of the constriction by flow through the slit. The reservoir *E* was inserted, and the cell clamped in position in its bath on the optical bench, and allowed to come to temperature equilibrium. A series of exposures of the reference trace and undeviated slit image were taken. The solvent, or more dilute solution in the case of a semi-differential diffusion, was warmed to a few degrees above 25°, and run slowly into *E* by pipette. The constriction prevented the two liquids from mixing to any extent. Flow through the slit *F* lowered the boundary to slit level, *i.e.*, to the middle of the cell windows. The heights of the liquids in the two reservoirs were equalised, and the tap on reservoir *D* opened. Flow was continued through the slit so that both liquids were flowing out from the cell, and the diffuse region produced by displacement of the boundary downwards from the constriction was swept away. The purpose of the plate *H* (5 × 5 mm.) was to prevent fast-moving liquid emerging from the narrow constriction and impinging directly on the boundary.

FIG. 1.



Flow through the slit was maintained at 1 ml. min.$^{-1}$ for 25 min.; the boundary was finally sharpened by four minutes' flow at 4 ml. min.$^{-1}$. Flow was stopped abruptly by closing a brass clip which constricted the tube carrying the outflow from the cell; diffusion commenced when flow was stopped. Immediately after the start of diffusion the cell was masked so that only the square holes in the cell mask and the reference trace were illuminated. Photographs of the interference patterns were used in the determination of fractional *jm*.

The interference patterns produced by diffusion (the square holes in the cell mask were covered during these exposures) were photographed at timed intervals after the beginning of the experiment; time was measured with an accurate chronometer-watch. Distances on the plate were measured on a Cambridge Universal Measuring Machine to within 0·0002 cm.

Extremely sharp boundaries were produced by this technique, and no correction for Δt[4] (the time taken for an infinitely sharp boundary to reach the state of the existing boundary when diffusion commenced) was necessary, as the diffusion coefficients varied only randomly with time.

Density of Solid Lecithin.—Finely-divided dry lecithin was kept in a stoppered density bottle which was half full of dry acetone for several days, with pumping at intervals to remove air. The density was determined by the usual displacement technique. After the measurement the amount of lecithin used was determined by drying and weighing. Two repeat measurements gave 1·016 and 1·015 g.ml.$^{-1}$.

Viscosity Measurements.—Viscosities relative to benzene were determined in a No. 0 Ostwald capillary viscometer.

Osmotic Pressures.—These were measured as previously described.[1]

RESULTS

The terminology used in describing the diffusion measurements is that of Stigter *et al.*[5] The results of a series of integral diffusion experiments (diffusion into pure solvent) are shown in Table 1.

[4] Longsworth, *J. Amer. Chem. Soc.*, 1947, **69**, 2510.
[5] Stigter, Williams, and Mysels, *J. Phys. Chem.*, 1955, **59**, 330.

TABLE 1. *Integral diffusion coefficients of lecithin in benzene.*

Concn. (g. l.$^{-1}$)	5·00	8·76	15·00	17·45	19·54	20·12	22·83	31·77
$10^6D'$ (cm.^{2} sec.$^{-1}$)	2·27	2·07	$1·79_4$	$1·62_5$	$1·42_1$	$1·58_7$	$1·50_7$	$1·36_9$
jm	6·97	10·60	16·61	19·13	20·92	21·72	24·26	33·15
$\Lambda_{max.}$	0·125	0·089	0·054	0·043	0·033	0·031	0·026	0·008

The patterns obtained in these experiments were characteristic of two-component diffusion. Ct, the ratio of the observed to the theoretical displacement of a fringe above the undeviated slit image, was not constant, but increased as the fringe number decreased. (The outermost fringe in the pattern was numbered zero.) For two-component diffusion the Gaussian distribution curve suffers kurtosis, and the results were best expressed by a weight average diffusion coefficient D', which was calculated by Akeley and Gosting's method.[6] D' was obtained by examining the relative fringe deviation of the patterns; maximum values of the relative fringe deviation, $\Lambda_{max.}$ are shown in Table 1.

Semi-differential diffusion experiments (diffusion from a concentrated into a dilute solution) gave patterns which fitted the theory for single-solute diffusion.

TABLE 2. *Semi-differential diffusion coefficients of lecithin in benzene.*

$\bar{C}$	6·00	15·00	25·00	8·50	12·50	17·78	25·00	32·77
ΔC	10·00	10·00	10·00	15·00	15·00	15·00	15·00	15·00
10^6Da (cm.^{2} sec.$^{-1}$)	1·269	1·281	1·284	1·268	1·272	1·282	1·285	1·294
jm	10·39	10·17	10·18	14·35	14·30	14·15	14·18	14·10

$\bar{C}$ = mean concentration of the two solutions used in an experiment in g. l.$^{-1}$. ΔC = concentration difference between the two solutions in g. l.$^{-1}$.

TABLE 3. *Viscosities of solutions of lecithin in benzene.*

$10^3\phi_1$	$10^3\phi_2$	$\eta_{sp.}$	Correction to $\eta_{sp.}$	ν
0·72	—	0·004	—	6
0·91	1·85	0·010	−0·005	2·7
0·96	4·57	0·021	−0·005	3·5
1·01	10·03	0·048	−0·006	4·2
1·02	12·91	0·066	−0·006	$4·6_5$
1·04	17·37	0·090	−0·006	5·4
1·04	19·83	0·118	−0·006	$5·6_5$
1·05	26·24	0·170	−0·006	$6·2_5$

$\eta_{sp.}$ = observed specific viscosity. $\nu = \eta_{sp.}$ (corrected) ϕ_2^{-1}. A plot of ν against ϕ_2 gave a straight line with an intercept at $\phi_2 = 0$ of $2·7_8$.

Ct was constant, and the height-area average diffusion coefficient, Da, was calculated from Longworth's equation:[4]

$$Da = (b^2 jm^2\lambda^2)/(4\pi Ct^2 . t) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

where b is the optical distance from the centre of the cell to the photographic plate in cm., λ is the wavelength of the light in cm., and t is the time in seconds at which the photograph was taken.

All patterns were formed above the undeviated slit image, since the refractive indices of lecithin solutions were less than those of solvent or more dilute solutions.

The viscosity results are shown in Table 3. There was a small relative viscosity at 0·73 g. l.$^{-1}$, the critical micelle concentration, where the solute was present mainly as small micelles. To obtain the relative viscosity due to the large micelles, the results were corrected by subtracting the relative viscosity due to the small micelles (obtained from a knowledge of the relative viscosity at 0·73 g. l.$^{-1}$, and the concentrations of small micelles present in the solution) from the total observed relative viscosity. The volume fraction of the small micelles, ϕ_1, and the large micelles, ϕ_2, were calculated from the concentrations of the two species in a solution, which were obtained from the mass-action equation.[1]

To check that the mass-action equation held at concentrations greater than 10 g. l.$^{-1}$, additional osmotic pressure measurements were made (Table 4). There was reasonable agreement between the observed osmotic pressures and those calculated from the mass-action equation.

[6] Akeley and Gosting, *J. Amer. Chem. Soc.*, 1953, **75**, 5685.

TABLE 4. *Extension of osmotic pressure measurements.*

Concn. (g. l.$^{-1}$)	10·31	15·21	25·04	30·02
$10^2\pi_{obs.}$ (atm.)	1·18	1·47	1·81	2·08
$10^2\pi_{calc.}$ (atm.)	1·18	1·41	1·85	2·07

DISCUSSION

The weight-average diffusion coefficient, D', obtained from integral diffusion measurements, decreases as concentration increases (Table 1). This is expected as the concentration of small micelles, C_1, increases little above the critical micelle concentration, while the concentration of large micelles, C_2, increases rapidly (see Table 5). At high concentration C_2 is twenty to thirty times larger than C_1, and values of D' approach those for the diffusion of large micelles alone. As concentration decreases, the amounts of large and small micelles become more equal, and D' increases, as the diffusion coefficient of the small micelles has more effect on the averaging process which produces D'. Also, as concentration decreases, $\Lambda_{max.}$ becomes larger, indicating a larger deviation from a normal Gaussian distribution curve.

The concentration region below 5 g. l.$^{-1}$ could not be explored with the present apparatus, as there was insufficient refractive index difference between the solvent and the solutions to give a reasonable value of jm. For this reason the diffusion coefficient of the small micelles could not be measured directly, but below 20 g. l.$^{-1}$ a plot of $1/D'$ against concentration gave a straight line which could be extrapolated to $D' = 2{\cdot}67 \times 10^{-6}$ cm.^{2} sec.$^{-1}$ at the critical micelle concentration, where the solution consisted almost entirely of small micelles. This value was taken as the diffusion coefficient of the small micelles.

The mass-action equation [1] was used to calculate C_1 and C_2 in a solution of total concentration $C_T = C_1 + C_2$, and from a knowledge of the refractive index increment per unit concentration of the large micelles, α (obtained from the experimental values of jm), D' was calculated from

$$D' = \alpha D_2 + (1 - \alpha)D_1 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$

where D_2 was the diffusion coefficient of the large micelles, and D_1 was the diffusion coefficient of the small micelles.

TABLE 5. *Comparison of calculated and observed diffusion coefficients for the integral diffusion of lecithin in benzene.*

C_T	5·00	8·76	15·00	17·45	19·54	20·12	22·83	31·77
C_1	0·97	1·00	1·04	1·05	1·05	1·06	1·06	1·09
C_2	4·03	7·75	13·96	16·39	18·49	19·06	21·77	30·68
$10^6D'_{obs.}$ (cm.^{2} sec.$^{-1}$)	2·3	2·1	1·8	1·6	1·4	1·6	1·5	1·4
$10^6D'_{calc.}$ (cm.^{2} sec.$^{-1}$)	2·0	1·8	1·6	1·6	1·5	1·5	1·5	1·4

There is general agreement between the observed and calculated diffusion coefficients. Large errors occurring in the graphical integrations used in finding $D'_{obs.}$ would account for some of the discrepancies. The agreement confirms the general applicability of the mass-action equation to this system.

The two sets of semi-differential diffusion coefficients (Table 2) at $C = 10{\cdot}00$ and 15·00 g. l.$^{-1}$, show little variation of diffusion coefficient with ΔC, as would be expected in non-polar solvent where charge effects should be almost absent. The pairs of solutions used in each experiment contain almost the same concentrations of small micelles, a consequence of the mass-action law; they differ mainly in their concentrations of large micelles, which is the species diffusing. A plot of Da against concentration yields a straight line, giving $Da = 1{\cdot}264 \times 10^{-6}$ cm.^{2} sec.$^{-1}$ at $C = 0$. This value is taken as the diffusion coefficient of the large micelles.

Size and Shape of the Micelles.—The value of ν when $\phi_2 = 0$ from the viscosity experiments was $2{\cdot}7_8$. A system obeying the postulates on which Einstein's [7] relationship is

[7] Einstein, *Ann. Physik*, 1906, **19**, 289; 1911, **34**, 591.

based would give $\nu = 2{\cdot}5$ when $\phi_2 = 0$. Higher values of ν than 2·5 are attributed either to asymmetry or to solvation of the particles. The large micelles being treated as ellipsoids, and it being assumed that the divergence of ν from 2·5 is due to asymmetry, Mehl, Oncley, and Simha's [8] tables give values for the axial ratios a/b of 1·79 (prolate) or b/a of 1·88 (oblate). These axial ratios can be used to find the ratio of the frictional coefficient of the ellipsoid to that of a sphere of the same molecular weight $(f/f_0)[f/f_0 = 1{\cdot}030$ (prolate) and 1·035 (oblate)].[9]

As $f/f_0 = D_0/D$, where D is the diffusion coefficient of the ellipsoid and D_0 is the diffusion coefficient of a sphere of the same molecular weight, values of D_0 can be calculated from the experimental values of D. For a prolate ellipsoid $D_0 = 1{\cdot}302 \times 10^{-6}$ cm.^{2} sec.$^{-1}$, and for an oblate $D_0 = 1{\cdot}308 \times 10^{-6}$ cm.^{2} sec.$^{-1}$. The molecular weights are 55,200 and 54,400 respectively, calculated from the Stokes–Einstein relationship, the viscosity of benzene being taken as 0·006028 poise.[10]

FIG. 2.

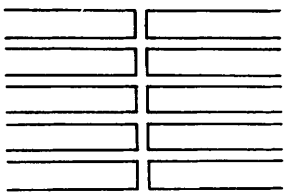
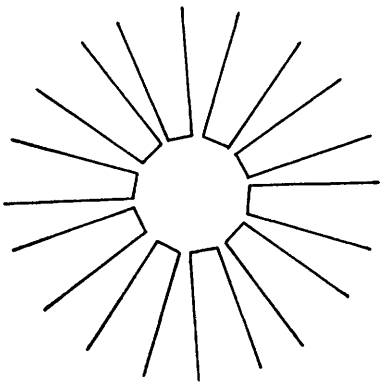


FIG. 3.



The data for the small micelles are not so exact as for the large ones. Only an approximate value of ν can be assigned, owing to the small observed relative viscosity. ν is taken from the result at 0·73 g. l.$^{-1}$ and is approximately 6. A similar procedure to that described above being used, $a/b = 5{\cdot}1$ (prolate), $b/a = 6{\cdot}9$ (oblate), $f/f_0 = 1{\cdot}26$ (prolate), $f/f_0 = 1{\cdot}32$ (oblate), $D_0 = 3{\cdot}4 \times 10^{-6}$ (prolate), and $D_0 = 3{\cdot}5 \times 10^{-6}$ cm.^{2} sec.$^{-1}$ (oblate). The molecular weights were 3100 (prolate) and 2800 (oblate).

Comparison with Model Structures.—It is probable that the polar head groups of the large micelles are turned inwards remote from the benzene, and the hydrocarbon chains of the fatty acids protrude outwards. There are two general possibilities for arrangement of monomers in the micelle. First, as a laminar micelle in which the head groups are arranged as in a sandwich (Fig. 2). Secondly, as a spherical micelle where the head groups are arranged to cover the surface of a sphere (Fig. 3). From a molecular model the overall length of the monomer is 35 Å with fully extended hydrocarbon chains, and the area of the polar head group is 50 Å^2. The length of the monomer is based on the average fatty acid composition of lecithin.

The following calculation shows the spherical arrangement to be unlikely. A micelle containing seventy monomers being taken, arranged as in Fig. 3, it is possible to calculate the radius of the " hole " in the micelle interior. The total radius of the micelle can then be calculated (52 Å) and hence the diffusion coefficient ($7{\cdot}0 \times 10^{-7}$ cm.^{2} sec.$^{-1}$) ; this is roughly half the observed diffusion coefficient.

[8] Mehl, Oncley, and Simha, *Science*, 1940, **92**, 132.
[9] Svedberg and Pederson, " The Ultracentrifuge," Clarendon Press, Oxford, 1940, p. 41.
[10] Weissberger and Proskauer, " Organic Solvents," Interscience, New York, 1955, p. 72.

Calculations of the properties of the following models have been made, based on the laminar arrangement and allowance of a 2 Å gap between the two halves of the sandwich: (*a*) Prolate and oblate ellipsoids with a molecular weight of 57,000 (molecular weight from osmotic-pressure measurements). (*b*) Prolate ellipsoids with molecular weight of 55,200 (molecular weight based on diffusion and viscosity data). (*c*) Oblate ellipsoid with a molecular weight of 54,400 (molecular weight based on diffusion and viscosity data). The results of the calculations, as well as the experimental data, are shown in Table 6.

TABLE 6. *Comparison of results from experiment and from models.*

	Experimental		Osmotic		Diffusion–viscosity	
	Prolate	Oblate	Prolate	Oblate	Prolate	Oblate
M	55,200	54,400	57,000		55,200	54,400
n	70·4	69·4	72·7		70·4	69·4
10^6D (cm.^{2} sec.$^{-1}$)	1·264	1·264	1·269	1·219	1·282	1·229
10^6D_0 (cm.^{2} sec.$^{-1}$)	1·302	1·308	1·288		1·302	1·308
f/f_0	1·030	1·035	1·015	1·057	1·016	1·064
ν	2·78		2·63	2·97	2·64	3·04
a/b or b/a	1·79	1·88	1·50	2·24	1·52	2·35
$10^{-6}\beta$	2·15		2·12	2·12	2·12	2·12

M = molecular weight; n = number of monomers in micelle; for β see text.

All figures calculated from models have a moderate agreement with experimental data, indicating that the laminar arrangement of monomers is likely. In general, diffusion coefficients calculated for oblate models diverge rather more from the experimental values than those calculated for prolate models. No absolute choice between the prolate and oblate form can be made owing to the divergence of the actual micelle from perfect ellipsoidal shape, and to the combined errors of the experimental results.

β is a quantity defined by Scheraga and Mandelkern [11] and should depend only on the axial ratio of the ellipsoid. For an increase of b/a from 1 to 300 for oblate ellipsoids, β increases from $2{\cdot}12 \times 10^6$ to $2{\cdot}15 \times 10^6$ and for a prolate ellipsoid variation of a/b from 1 to 300 causes β to increase from $2{\cdot}12 \times 10^6$ to $3{\cdot}60 \times 10^6$. In the present case the choice between oblate and prolate ellipsoids cannot be made as β is insensitive at small axial ratios; it is noteworthy that $\beta = 2{\cdot}13 \times 10^6$ (prolate) and $2{\cdot}12 \times 10^6$ (oblate) for axial ratios of 2 : 1. The value of β calculated from the experimental results is thus in the correct region.

The agreement between the molecular weight from osmotic pressure measurements and that from diffusion–viscosity experiments is good, the molecular weight from the latter methods being lower. If the micelles were much solvated it would be expected that the diffusion–viscosity molecular weight would be larger than that obtained from osmotic pressure, owing to the solvating molecules' travelling with the micelle, and affecting the diffusion coefficient to a large extent.

Consideration of models of the small micelles is based on osmotic data alone, owing to the uncertainties in the determination of the diffusion coefficient and ν. The osmotic molecular weight was 3180, corresponding to four monomers (3140). If these micelles have the same structure as the large ones, the overall length will be 72 Å, and the cross-sectional area at the head groups will be 100 Å^2. In this case the small micelles can only approximate to a prolate ellipsoid. The four-monomer model will have a/b of approximately 7, which is in agreement with the experimental value. The molecular weight obtained from diffusion–viscosity data was 3100. The agreement with the osmotic result is perhaps fortuitous.

I thank Dr. L. Saunders for discussions, and Mr. P. C. Barden for making the cell.

SCHOOL OF PHARMACY, UNIVERSITY OF LONDON,
BRUNSWICK SQUARE, LONDON, W.C.1. [*Received, January 27th*, 1959.]

[11] Shiraga and Mandelkern, *J. Amer. Chem. Soc.*, 1953, **75**, 179.