Our attention has been particularly attracted by the fact that this equilibrium between first and second black films is extremely sensitive to the nature of the counterions present. This is in marked contrast to the phenomena discussed heretofore, which are quite unspecific both with respect to the surfactant ion and with respect to the counterion. The second black film, on the other hand, exhibits the same kind of sensitivity to the specific nature of the counterion as so many colloidal and other phenomena, but this sensitivity seems to be greater by orders of magnitude. This makes it easy to discern the same kinds of influences that are apparent for example in the effect of counterions on the critical micelle concentration in bulk solutions of surfactants,[37] namely the role of the hydrated size of the ions and that of specific binding by hydrophobic bonds.

Thus here, as in many of the aspects which we have briefly reviewed, soap films offer an effective and unique tool to approach a problem in colloid and surface chemistry in addition to their intrinsic interest and aesthetic appeal.

(37) P. Mukerjee and K. J. Mysels, paper presented at the 131st National Meeting of the American Chemical Society, Miami, Fla., April, 1957, and unpublished results.

The Interaction of Water with Lecithin Micelles in Benzene

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When water was introduced into the lecithin–benzene system, it was solubilized by the solute. Light scattering, viscosity, and diffusion measurements on the solubilized systems indicated that the number of monomers in the micelle was independent of solubilizate concentration between zero and 0.25 g. of water/g. of lecithin. The viscosity results indicated that as the water content was increased from zero to 0.055 g. of water/g. of lecithin, micellar asymmetry developed; the addition of more water caused an alteration in micellar structure, and spherical micelles were present at high water contents. A maximum solubilization of 0.33 g. of water/g. of lecithin was recorded.

The critical micelle concentration (c.m.c.) of lecithin in benzene at 25° has been reported[1] to be 33×10^{-5} %; above the c.m.c. small micelles containing four or five monomers are formed. A second association limit is present at 0.073%, where the small micelles aggregate into large ones.[2] The large micelles have the structure of bimolecular leaflets, with the polar head groups in the interior of the micelles and the hydrocarbon chains directed outward into the solvent[3]; at 25° the micellar weight was 55,000 to 57,000; at 40° it was 43,000. It was felt[4] that these large micelles might provide a biologically significant model of part of nerve myelin, as Finean[5] has shown that a similar bimolecular leaflet structure occurred in nerve myelin. Studies[6,7] of the effect of dielectric constant on micellization by lecithin have suggested a potential mechanism of cell wall permeability.

Although some solubilization studies[1,4] on the dry

(1) I. Blei and R. E. Lee, *J. Phys. Chem.*, **67**, 2085 (1963).
(2) P. H. Elworthy, *J. Chem. Soc.*, 813 (1959).
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(5) J. B. Finean, *Experientia*, **9**, 17 (1953).
(6) P. H. Elworthy and D. S. McIntosh, *J. Pharm. Pharmacol.*, **13**, 663 (1961).
(7) P. H. Elworthy and D. S. McIntosh, *Kolloid-Z.*, **195**, 27 (1964).

lecithin–benzene system have been made, it was felt that the large micelles would provide a more realistic biological model if water were present in the head group region of the bimolecular leaflets. The solubilization of water by lecithin has been studied by Demchenko,[8] who found the maximum quantity of water solubilized to be 0.33 g./g. of lecithin and to be independent of the solvents used (benzene, toluene, and xylene).

Experimental

Materials. Lecithin was prepared from chickens' egg yolks[9] by treatment with alumina to remove ninhydrin-reacting materials, followed by chromatography on silica to remove lysolecithin. It was deionized by dissolving in chloroform and stirring with mixed strong ion-exchange resins (biodeminrolit, B.D.H., previously well extracted with chloroform) for 2–3 hr. After several precipitations from dry ether into dry acetone, it was stored under dry acetone. The sample had N, 1.87%, P, 4.13%, I_2 no. 62. (Molecular weight calculated from N and P analysis figures = 750). Analar benzene was fractionally frozen and fractionally distilled, being stored over sodium, and had $n^{25}D$ 1.4979 (1.4981 Timmermans[10]). Tropaeolin 000 (Orange II) was recrystallized twice from water.

Light Scattering Measurements. The apparatus of Elworthy and McIntosh[6] was used. Solutions were made dust-free by filtering through sintered glass disks (mean pore size, 1 μ), concentrations being checked interferometrically after filtration. Solutions containing more than 0.15 g. of water/g. of lecithin could not be satisfactorily clarified either by filtering or by centrifuging. Specific refractive index increments (dn/dc) were measured on a Hilger–Rayleigh interference refractometer using Bauer's[11] technique for monochromatic light. All measurements were made at 20° using the 5461-Å. green line.

Diffusion Measurements. The modified interference refractometer was used as previously described[7]; Harned and Nuttall's treatment[12] of restricted diffusion was applied to measurements of concentrations at levels in the diffusion column $^1/_6$ and $^5/_6$ the column length. Differential diffusion coefficients were obtained, the concentration at the end of an experiment being determined from the interferometer reading after mixing the cell contents.

Viscosity Measurements. A suspended level dilution viscometer was used, the error in the relative viscosity being ±0.2%. A few representative solutions were checked for Newtonian flow in a Couette viscometer. Each intrinsic viscosity reported was obtained from eight to ten individual measurements as a function of concentration.

Solubilization of Tropaeolin 000. Powdered dye, the solution of lecithin in benzene, and a glass marble were placed in flasks whose stoppers were sealed in with parafilm. The flasks were rocked in a thermostat bath, samples being withdrawn occasionally. Optical densities were measured at 483 mμ in 1-cm. cells against benzene using a Hilger and Watts Uvispek. Uptake of dye was complete in 3 days. Negligible amounts of dye were taken up in dry benzene (optical density in 1-cm. cell = 0.006).

Solubilization of Water. Water was incorporated by either shaking the lecithin solution with a weighed quantity of water in a sealed flask or allowing dry lecithin to adsorb the required amount of water vapor and dissolve in dry benzene. No differences in behavior were observed between solutions made by the two methods. To give a value of the maximum water uptake, optical densities of 1% lecithin solutions containing various amounts of water were measured at 546 mμ (Fig. 2). Vapor phase equilibrium experiments between lecithin solutions and benzene with added water were carried out by connecting two flasks containing the solutions with a U-shaped adapter.

Results and Discussion

Figure 1 shows the results for the solubilization of Tropaeolin 000 by lecithin in benzene. The c.m.c. is 26×10^{-5}% at 20°. Taking this value with Blei and Lee's 33×10^{-5}% at 25° and mean value of 86×10^{-5} at 40° and using the standard relationships[13] derived for the two-phase model of micelle formation, $\Delta H_m = -10$ kcal. mole^{-1} and $\Delta S_m = -35$ cal. deg.$^{-1}$ mole^{-1} for the heat and entropy of micellization, respectively. These results must be treated tentatively because of the smallness of these micelles and the limitations of the pseudo-phase model of micellization.

The second association limit occurs at 0.075%, in reasonable agreement with the value found at 25° (0.073%). As we are interested in the large micelles, this higher transition concentration was used for cor-

(8) P. A. Demchenko, *Colloid J. USSR*, **22**, 309 (1960).

(9) P. H. Elworthy and L. Saunders, *J. Chem. Soc.*, 330 (1957).

(10) J. Timmermans, "Physico-Chemical Constants of Pure Organic Compounds," Elsevier Publishing Co., New York, N. Y., 1950, p. 147.

(11) N. Bauer, K. Fajans, and S. Z. Lewin, "Physical Methods of Organic Chemistry," A. Weissberger, Ed., Vol. I, 3rd Ed., Interscience Publishers, Ltd., New York, N. Y., 1960, Part 2, p. 1139.

(12) H. S. Harned and R. L. Nuttall, *J. Am. Chem. Soc.*, **69**, 736 (1947).

(13) K. Shinoda, "Colloidal Surfactants," Academic Press, London, 1963, p. 30.

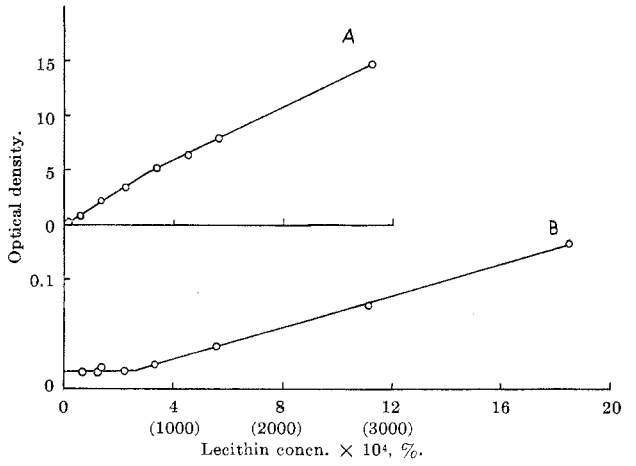

Figure 1. Solubilization of Tropaeolin 000 by lecithin in benzene in the absence of water. Determination of c.m.c. (B) and second association limit (A). Optical densities of solutions in 1-cm. cells. Values in parentheses are for the upper scale.

recting viscosity results for the presence of the small micelles, in the manner previously described[3]; the corrections are small. As irreproducible results for dye solubilization were obtained when water was present in the micelles, the value obtained in the dry condition was used in the correction procedure. The irreproducibility may be due to the effect of water on the nature of the micelles.

It is necessary to gain some idea of the location of the water in the lecithin–benzene system, as there is the possibility of a significant partition between the micelles and the surrounding benzene. Lecithin is intensely hygroscopic, making it seem likely that nearly all the water is associated with the solute; as the interpretation of the viscosity results depends to some extent on knowing the location of the water, an attempt was made to determine its position. Benzene saturated with water was equilibrated through the vapor phase with a 3% solution of lecithin in dry benzene. After 6–8 days, the presence of water in the benzene was no longer detectable, while it could be detected in the lecithin–benzene solution. Other tests indicated that there was no loss of water from the system, *i.e.*, transfer between water saturated and dry benzene gave the correct equilibrium distribution. Further tests on benzene saturated with water in vapor phase contact with a solution containing 0.15 g. of water/g. of lecithin also showed that an undetectable amount of water remained in the benzene at equilibrium.

If the partition of water favored the benzene rather than the micellar solute in the benzene, a significant amount of water would be expected to remain in the benzene in vapor contact with this system. There must be some equilibrium between the water in the micelles and in the surrounding benzene, and since the amount of water remaining in the benzene was too small to be detected, it seems reasonable to assume that almost all the water present is associated with the solute.

The maximum amount of water that could be solubilized by 1% solutions of lecithin in benzene was found to be 0.33 g. of water/g. of lecithin (Fig. 2), which agrees well with the value of Demchenko.[8]

The light scattering results are given in Fig. 3 as plots of $H(c - c_1)/(T - T_1)$ against $(c - c_1)$ where

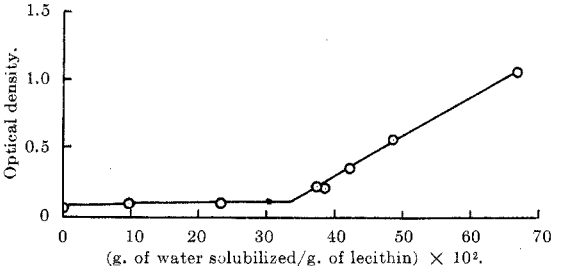

Figure 2. Optical densities (in 1-cm. cells) of 1% lecithin solutions in benzene as a function of the water content of the lecithin.

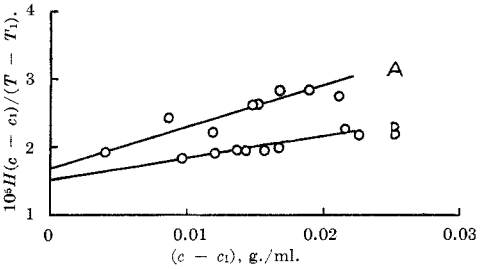

Figure 3. Light scattering results for lecithin in benzene: A, lecithin containing 0.053 g. of water/g. of lecithin; B, containing 0.105 g. of water/g. of lecithin.

$H = 32\pi^3 n_0^2 (dn/dc)^2/3\lambda^4 N$; c is the total concentration of lecithin plus solubilized water in g./ml., c_1 is the second association concentration, and T_1 is the turbidity at this concentration. Z_{45} values of close to unity were obtained (Table I) indicating that no dimensions of the micelles exceeded $\lambda/20$. The results have been corrected for depolarization. Micellar weights were calculated from the usual two-component theory

$$H(c - c_1)/(T - T_1) = 1/M + 2B(c - c_1) \quad (1)$$

Provided that the solubilizate is completely associated with the micelles, it seems reasonable to treat the

Table I

% (w/w) H_2O in lecithin	10^6D^a	M l.s.[b]	M_{ob}	M_{pro}	M_{solv}	M_{calcd}	dn/dc	Z_{45}[c]	ρ
5.3	...	59	..	..	..	63	−0.052	1.00	0.028
5.4	0.97_6	..	64	67	63	63	...	..	...
10.5	...	65	..	..	..	66	−0.058	1.01	0.050
25.5	0.96_2	..	71	73	71	75	...	..	...

[a] D = diffusion coefficient at c.m.c. in cm.^{2} sec.$^{-1}$. [b] All micellar weights $\times 10^{-3}$, M_{calcd} = 60,000 + amount of water solubilized/micelle. [c] Z_{45} = dissymmetry at 45°.

system as essentially a two-component one. This treatment has been used elsewhere for the light scattering of solubilized systems.[14] Because of the smallness of the specific refractive index increments, the solutions possessed only small turbidities, and this, together with the ±7–10% error in the determination of molecular weights by light scattering,[15,16] make further checks on the micellar weight necessary. A combination of diffusion coefficients (after extrapolation to the second association concentration) (Fig. 4) and intrinsic viscosities (Fig. 5) give the micellar weights reported in Table I, which are calculated for three alternative interpretations of the deviation of the viscosity intercept from the Einsteinian value, *e.g.*, for oblate (M_{ob}), prolate (M_{pro}), ellipsoids of revolution, and for spherical particles solvated with solubilized water (M_{solv}).

The micellar weight grows as the amount of water solubilized increases. Under dry conditions,[7] M = 60,000, and the increase of micellar weight reported here, within experimental error, corresponds to 60,000 plus solubilized water. This indicates that the number of monomers per micelle remains reasonably constant, as the concentration of water solubilized increases.

The intercept from the viscosity runs (the same ratio of water to lecithin being maintained in each run) (Fig. 5) shows an increase from $[\eta] = 2.84$ at zero water content to a peak value of $[\eta] = 3.94$ at 0.055 g. of water/g. of lecithin, followed by a decrease when further water is incorporated. At 25°, under anhydrous conditions, $[\eta] = 2.82$, close to the value found here at 20°. Using

$$[\eta] = \nu[\bar{V}_2 + W_1V_1^0] \qquad (2)$$

where ν = Simha's shape factor,[17] $\bar{V}_2$ = specific volume solute, W_1 = g. of solvating solvent per g. of solute, and V_1^0 = specific volume solvent; the viscosity intercepts for spherical particles using W = g. of water solubilized/g. of solute were calculated (Fig. 5B). This treatment does not account for the observed behavior of the viscosity intercepts at low water contents, indicating that changes in micellar shape occur in this region.

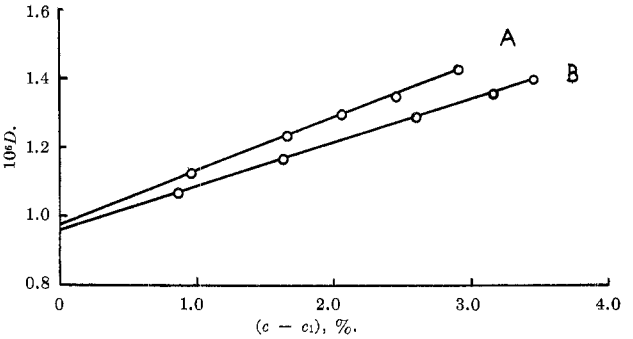

Figure 4. Diffusion coefficients (cm.^{2} sec.$^{-1}$) of lecithin in benzene: A, lecithin containing 0.054 g. of water/g. of lecithin; B, containing 0.255 g. of water/g. of lecithin.

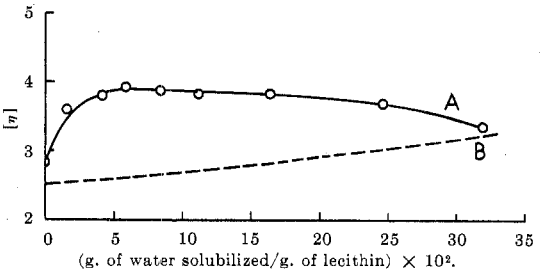

Figure 5. Intrinsic viscosities of lecithin–benzene systems as a function of the amount of water solubilized by lecithin: A, experimental; B, calculated for spherical micelles containing solubilized water.

A short extrapolation of Fig. 5 to 0.33 g. of water/g. of lecithin gives $[\eta] = 3.28$, yielding $W = 0.327$ g. of water/g. of lecithin from eq. 2, assuming that the particles are spherical. The agreement between this value and the actual amount of water present indicates

(14) T. Nakagawa, K. Kuriyama, and H. Inouye, *J. Colloid Sci.*, **15**, 268 (1960).

(15) N. Robinson and L. Saunders, *J. Pharm. Pharmacol.*, 11, 115T (1959).

(16) P. H. Elworthy and C. B. Macfarlane, *J. Chem. Soc.*, 537 (1962).

(17) R. Simha, *J. Phys. Chem.*, **44**, 25 (1940).

that the micelles do become spherical at this water content. A spherical cavity of radius 19.8 Å. in the micelle center would be filled by 0.327 g. of water/g. of lecithin.

Assuming, as in the calculations above, that no electrostriction of solubilized water takes place and that the micelles are unsolvated with respect to benzene (which seems reasonable from preceding work[3]), some idea of the changes of micellar shape occurring on addition of water may be gained using models of the lecithin micelles. Molecular models show that the polar head group can be represented as a block 7 × 8 Å., and, using the length of half the bimolecular leaflet (36 Å.), it is possible to calculate $[\eta]$ for a micelle of 80 monomers arranged to form prolate or oblate ellipsoids.

When water is absent, the short semiaxis for the prolate model is $b = (56 \times 40/\pi)^{1/2}$ enabling a/b to be found (a = 36 Å.), and this gives $[\eta]$ = 2.54, rather lower than the experimental result. In the presence of water, we shall attempt to calculate $[\eta]$ at 0.055 g. of water/g. of lecithin, *i.e.*, at the peak of the intrinsic viscosity–water content curve. There are two principal ways of incorporating this amount of water. Firstly, it may lie in the gap between the two sheets of polar groups, and its incorporation would increase the a dimension; 0.055 g. of water/g. of lecithin corresponds to 2.3 water molecules per monomer, and even allowing a row of six water molecules between the two halves of the leaflet gives $[\eta]$ = 2.87, which is much lower than the experimental value of 3.94 at this water content. Secondly, water may be inserted between the polar heads in each sheet, giving an increase in the cross-sectional micellar area, *i.e.*, an increase in b, the summation of the areas of polar heads plus the areas of water molecules giving $[\eta]$ = 2.62. The third possibility is that both types of micellar expansion may occur, but these changes are likely to balance one another as far as asymmetry is concerned.

From water vapor sorption studies on lecithins[18,19] it was shown that 2.5 water molecules/molecule of lecithin represented the completion of first-layer sorption; this figure agrees reasonably well with the water content giving the peak value of $[\eta]$ in Fig. It was also shown, in interpreting the transport properties of lysolecithin, that this first layer might fit into cavities in the polar head group, with little increase in the effective monomer length.

A consideration of oblate ellipsoids is more difficult as decisions have to be made regarding the geometry of the sheet of polar head groups. Solubilization studies[4] indicated that the sheet of polar head groups was three times as long as it was wide. If the shorter dimension is taken as 32 Å. ($= 2a = 4 \times 8$ Å.), then b^1 = 44.6 Å., using the mean of 44.6 and 36 Å. to give an average value of b; $[\eta]$ = 3.09 in the absence of water. Incorporation of six rows of water molecules between the two polar sheets gives $[\eta]$ = 3.5, while using the water to increase the area of the sheets gives $[\eta]$ = 3.6. These calculations for the oblate model are tentative, being sensitive to the exact geometry of the lecithin head group, and having the drawback that the model does not exactly fit the oblate shape. Nevertheless, they do provide a guide to the development of micellar asymmetry.

Over-all, it appears that the micelles resemble oblate ellipsoids and that the addition of water initially increases their asymmetry. Above 0.055 g. of water/g. of lecithin, the micelles tend toward a more spherical shape as $[\eta]$ decreases with increases of solubilizate concentration. This change may represent a rearrangement of micellar structure. To prevent contact between the water-treated polar heads and benzene, as the volume of water in the micelle center grows, the micelle shape tends to become spherical and finally reaches this condition at 33% water.

This system, with water incorporated between two polar sheets of phosphatide materials, resembles part of the structures found in Finean's[5,20] X-ray studies of nerve myelin and has promise as a model of part of this structure.

Acknowledgment. We thank Miss M. Buchanan for the microanalyses.

(18) P. H. Elworthy, *J. Chem. Soc.*, 5385 (1961).

(19) P. H. Elworthy, *ibid.*, 4897 (1962).

(20) J. B. Finean and J. D. Robertson, *Brit. Med. Bull.*; **14**, 267 (1958).