163. *Micelle Formation by Lecithin in Benzene.*

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The osmotic pressures of solutions of lecithin in benzene have been measured at 25° and 40°. Small micelles appear even at very low concentrations, and there is a critical micelle concentration (0·73 g. $l.^{-1}$) at which aggregation of small into large micelles begins. The equilibrium between the two types of micelle is investigated by means of the law of mass action. Certain thermodynamic functions for the system are reported.

In spite of fairly extensive studies on aqueous sols, little of the physical chemistry of lecithin in non-aqueous solutions has been investigated. It has been shown, from ebullioscopic and dialysis experiments, that in alcoholic solution monomers are present, while in benzene a form with higher molecular weight exists.[1,2] In the present work osmotic-pressure measurements show that two types of micelle are present in benzene solutions, with a sharply defined critical micelle concentration at which the smaller micelles begin to aggregate to large micelles.

Experimental

A modified Schultz [3] and Wagner [4] osmometer was used (Fig. 1). The cell consisted of a B24 joint (*A*) whose foot was ground perfectly flat and polished; this rested on the membrane (*B*) which was supported by filter paper (*C*) upon a brass plate (*D*). The brass plate

[1] Price and Lewis, *Biochem. J.*, 1929, **23**, 1030.
[2] Faure and Legault-Demare, *Bull. Soc. Chim. biol.*, 1950, **32**, 509.
[3] Schulz, *Z. phys. Chem.*, 1936, *A*, **176**, 317.
[4] Wagner, *Ind. Eng. Chem. Analyt.*, 1944, **16**, 520.

was perforated with $\frac{1}{16}$" diameter holes. The cell was held firmly in place upon the membrane by a collar and screwed rods (*E*) (two of the three rods are shown in the Figure). A B7 socket (*F*) was fused to the upper end of the B24 joint, and received a B7 cone fused to the end of a capillary tube (*G*) of uniform bore (0·5 mm.). A tube (*H*) was joined to the top of the B24 joint, and was filled with mercury which prevented leakage of liquids from the cell. A mirror scale for reading the osmotic height was attached to the capillary tube.

The whole osmometer rested inside a large flat-bottomed tube (5 × 55 cm.) which contained the solvent. It was held upright inside the tube by a wire attached to the top of the capillary. The assembly was placed in a thermostat regulated at 25° ± 0·05° or at 40° ± 0·05°.

Membranes.—Circular membranes, 3 cm. in diameter, were cut from non-waterproofed Cellophane No. 600. They were soaked in 25% alcohol for 2 hr. to remove impurities, then in 50% alcohol for 24 hr. to swell them to a suitable porosity for osmotic measurements in the

FIG. 1. *Section of the osmometer.*

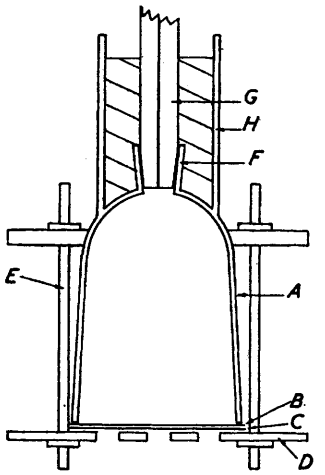
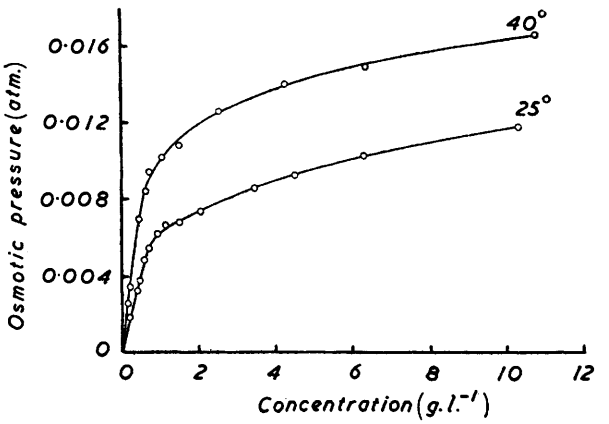


FIG. 2. *Relation of osmotic pressure to concentration at 25° and 40°.*



molecular-weight range 3000—1,000,000.[5] They were transferred through solutions of increasing alcohol content, soaked in absolute alcohol for 24 hr., and transferred to dry benzene. Tests for specific membrane effects with solvent in the cell showed no rise or fall greater than 1 mm.

Materials.—Lecithin from fresh egg yolks was prepared as previously described [6] by treatment with alumina to remove ninhydrin-reacting materials, followed by chromatography on silica gel to remove lysolecithin. After crystallisation from butan-2-one–acetone (1 : 4), the lecithin was washed with acetone and stored under dry acetone. The product (Found: N, 1·8; P, 3·8%) had $[\alpha]_D^{20}$ +7·87° (10% solution in absolute alcohol) and I no. 71.

Thiophen-free benzene, fractionally crystallised, fractionally distilled from phosphoric oxide, and stored over sodium, had b. p. 80·1°, n_D^{25} 1·4979, d_4^{25} 0·87360. Timmermans [8] gives b. p. 80·1°, n_D^{25} 1·4981, d_4^{25} 0·87368.

Method.—Solutions were prepared by thoroughly drying the lecithin in a vacuum and dissolving it in dry benzene. After assembly of the cell, during which the membrane was kept wet with solvent, the cell was washed out three times with solution, and filled so as to exclude air bubbles. It was placed in a beaker containing solvent at 25° (or 40°) and allowed to come to temperature equilibrium. This avoided, to a large extent, any thermometer effects when the assembled osmometer was placed in the thermostat.

The capillary tube was half-filled with solution, inserted into the B7 socket, and solution allowed to flow from the unseated joint until only a few cm. remained in the capillary tube;

[5] Carter and Record, *J.*, 1939, 660.
[6] Elworthy and Saunders, *J.*, 1957, 330.
[7] Elworthy, unpublished work.
[8] Timmermans, "Physico-Chemical Constants of Pure Organic Compounds," Elsevier, London, 1950, pp. 145—147.

the joint was then firmly seated. The outside of the cell was washed with solvent, and mercury poured into tube (*H*). The osmometer was lowered into solvent already at the correct temperature in the large tube, and moved up and down with this tube held at an angle of 45° to remove air bubbles from below the membrane. After the osmometer had been placed perfectly upright, the large tube was stoppered.

The osmotic head was read from the mirror scale. Equilibrium was often obtained in 1 hr., and the osmometer was left overnight before final readings were taken; the osmotic head was constant for this time. After removal from the large tube, the outside of the osmometer was dried, the capillary tube and mercury were removed, and the density and concentration (by drying a portion of the solution) were determined. The capillary correction was found by using some of the solution from the cell and a piece of capillary tube identical with that of the osmometer. Very little variation of capillary rise with concentration was noted.

Results and Discussion

Fig. 2 shows the osmotic pressure at 25° and at 40° plotted against concentration. At both temperatures there is a large initial rise of osmotic pressure with concentration,

Fig. 3. *Relation of π/c to concentration at 25° and 40°.*

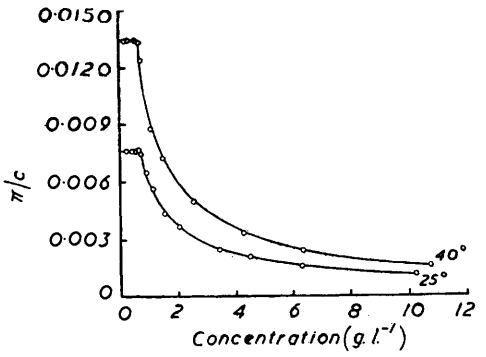
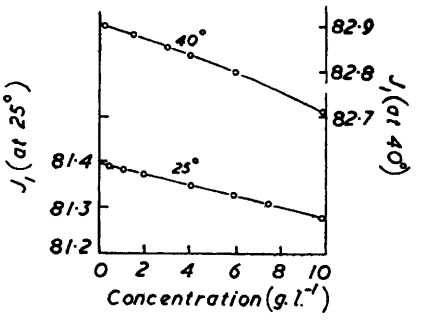


Fig. 4. *Relation of partial molar volumes to concentration at 25° and 40°.*



followed by a slower rise. Fig. 3 shows that between 0·2 and 0·73 g. l.$^{-1}$ at 25°, and between 0·2 and 0·68 g. l.$^{-1}$ at 40°, there is a constant value of π/c (where c = concentration). This indicates that, within experimental error, the molecular weight of the solute is constant in this concentration region; the average values of π/c, 0·0077 at 25° and 0·0134 at 40°, correspond to molecular weights of 3180 and 1830 respectively. Even in very dilute solutions it appears that lecithin molecules are aggregated to small micelles. Although the molecular weight at 40° was small, there was no observed decrease of the equilibrium head with time, indicating that the micelles were retained by the membrane.

The abrupt change in the properties of the curves in Figs. 2 and 3 is considered to be due to the aggregation of the small micelles into large ones, and the slow rise of osmotic pressure with concentration after the critical micelle concentration is a consequence of the formation of large micelles.

An approximate idea of the degree of aggregation of small into large micelles can be obtained by assuming that a mass-action law, without activity coefficients, applies to the aggregation:

$$\log c_2 - n \log c_1 = K \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

where c_2 is the concentration of large micelles, c_1 is the concentration of small micelles, n is the number of small micelles aggregating into one large one, and K is a constant. The fraction x of small micelles aggregated will equal $c_2/(c_1 + c_2)$. The constants n and K are derived by successive approximation, which is illustrated for the results at 25°. At

10·299 g. l.$^{-1}$ $\pi = 0{\cdot}0118$ atm. A value of n is chosen, and also a value of x. The osmotic pressure due to the small micelles will be given by $\pi_1 = c_1RT/M_1 = 0{\cdot}0077c_1$, where M_1 is the molecular weight of the small micelles; that due to the large micelles is given by $\pi_2 = c_2RT/M_2$, where M_2 is the molecular weight of the large micelles. The total osmotic pressure, $\pi = \pi_1 + \pi_2$. M_2 is obtained from the assumed value of n, and x is adjusted till the sum of π_1 and π_2 equals the observed osmotic pressure. Values of c_1, c_2, and n are substituted into equation 1, and K is calculated. The values of n and K are used to provide the value of c_1 when $c_2 = 1$ and $\log c_2 = 0$. The total osmotic pressure at this point is calculated, and compared with the observed value. The process is repeated until the mass-action equation predicts an osmotic pressure which exactly equals the observed value at $c_2 = 1$ g. l.$^{-1}$.

TABLE 1. *Association of small into large micelles at 25° and 40°. (Concns. in g. l.$^{-1}$; π in atm.).*

At 25°						*At 40°*					
c_T	c_1	c_2	x	$10^2\pi$ (calc.)	$10^2\pi$ (obs.)	c_T	c_1	c_2	x	$10^2\pi$ (calc.)	$10^2\pi$ (obs.)
0·712	0·700	0·012	0·017	0·54	0·54	0·654	0·650	0·004	0·006	0·87	0·87
0·740	0·720	0·020	0·027	0·55	0·56	0·691	0·680	0·011	0·016	0·91	0·91
0·837	0·770	0·067	0·080	0·60	0·59	0·789	0·730	0·059	0·075	0·98	0·94
0·933	0·800	0·133	0·142	0·62	0·62	0·916	0·760	0·156	0·170	1·02	0·98
1·113	0·834	0·279	0·251	0·65	0·66	1·309	0·800	0·508	0·389	1·10	1·08
1·464	0·870	0·594	0·405	0·69	0·69	1·823	0·823	1·000	0·588	1·16	1·16
1·896	0·896	1·000	0·531	0·73	0·73	2·962	0·850	2·112	0·713	1·26	1·30
2·537	0·920	1·617	0·637	0·78	0·79	4·517	0·870	3·647	0·807	1·37	1·41
3·817	0·950	2·867	0·751	0·85	0·89	6·357	0·885	5·472	0·861	1·50	1·50
6·987	0·990	5·997	0·858	1·02	1·05	8·979	0·900	8·079	0·900	1·66	1·59
8·190	1·000	7·190	0·878	1·08	1·10	10·105	0·905	9·200	0·910	1·73	1·73
10·299	1·015	9·284	0·902	1·18	1·18						

The final equation at 25° was: $\log c_2 - 17{\cdot}9 \log c_1 = 0{\cdot}8567$; at 40°, $\log c_2 - 23{\cdot}5 \log c_1 = 1{\cdot}9837$.

Table 1 sets out the values of c_T (the total concentration), c_1, c_2, x, and the observed and calculated osmotic pressures. The observed values of π agree with the calculated values fairly well over the whole concentration range studied. The mass-action treatment indicates that small concentrations of large micelles are present below the critical micelle concentration. Above it, c_2 increases rapidly for a small change in c_1, and at the highest concentrations studied most of the solute is present as large micelles.

The molecular weights of the large micelles are obtained from the values of n, the number of small micelles aggregating into one large micelle, used in the mass-action equation which fits the experimental results. At 25° $M_2 = 57{,}000$, and at 40° $M_2 = 43{,}000$. The molecular weight of the lecithin monomer can be calculated as 784, from the nitrogen and phosphorus analyses; the large micelles contain 73 monomers at 25° and 55 monomers at 40°. Both the small and the large micelles present in the solutions have, it is suggested, structure with the hydrocarbon chains directed outwards into the benzene, and the polar heads facing into the interior of the micelle. This is a reversal of the micellar structure in water. It is likely that polar materials can be rendered soluble in the interior of the micelle in benzene, while non-polar substances can be rendered soluble in water.[7]

Some Thermodynamic Functions of the Solvent in the Lecithin–Benzene System.—Density measurements on lecithin solutions were made in a stoppered pycnometer, and the results used to provide partial molar volumes, which are shown in Fig. 4. The partial molar free energies, heat contents, and entropies of the solvent were calculated from the osmotic-pressure results by using the standard thermodynamic relation. The partial molar heat content of the solvent (ΔH_1) was calculated from the temperature coefficient of osmotic pressure, and $\Delta\overline{H}_1$ was assumed to be linear over the temperature range 25—40° (see Table 2). All the thermodynamic properties conform to the same general pattern: there

is a relatively large increase for a unit change of concentration below the critical micelle concentration, and above it only a small increase. $\Delta\bar{H}_1$ and $\Delta\bar{S}_1$ reach a roughly constant value in the concentration range 2·0—10·0 g. l.$^{-1}$.

TABLE 2. *Thermodynamic functions of the solvent in the lecithin–benzene system. (Concns. in g. l.$^{-1}$; thermodynamic functions in 10^2 cal. mole^{-1}).*

Concn.	0·2	0·4	0·6	0·7	0·8	1·0	1·5	2·0	4·0	6·0	8·0	10·0
$-\Delta\bar{G}_1$ (25°)	0·30	0·61	0·91	1·05	1·14	1·25	1·37	1·47	1·75	1·99	2·16	2·30
$-\Delta\bar{G}_1$ (40°)	0·53	1·07	1·61	1·83	1·90	2·01	2·22	2·38	2·77	2·97	3·12	3·26
$\Delta\bar{H}_1$ (25°)	4·04	8·22	12·4	13·6	13·2	13·1	14·8	15·9	17·5	16·7	16·2	16·0
$\Delta\bar{S}_1$ (25°)	0·015	0·030	0·045	0·049	0·048	0·048	0·054	0·058	0·065	0·063	0·062	0·061

An ideal solution would contain monomers of lecithin, so any aggregation into micelles represents a deviation from ideality, the solvent having smaller $-\Delta\bar{G}_1$ values than ideal. The larger the degree of aggregation, *i.e.*, the larger the micelles, the smaller is the contribution which is made to $-\Delta\bar{G}_1$. The lecithin–benzene system represents an extreme

TABLE 3. *Osmotic coefficient (g) for the lecithin–benzene system at* 25°.

Concn. (g. l.$^{-1}$)	0·2	0·4	0·6	0·7	0·8	1·0	1·5	2·0	4·0	6·0	8·0	10·0
g	0·247	0·246	0·246	0·245	0·232	0·204	0·146	0·119	0·071	0·054	0·044	0·037

case of non-ideality, for even at very small concentrations there is aggregation into micelles. This is illustrated by an osmotic coefficient (g), the ratio of the observed osmotic pressure to the value calculated for a solution of monomers (see Table 3). g is small even in the region below 0·73 g. l.$^{-1}$ when small micelles are present, and decreases sharply in the region where aggregation into large micelles takes place.

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