263. *Boundaries Between Some Aqueous Fatty Sols and Water.*

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Sharp boundaries formed between clear aqueous sols of some fatty materials solubilized by polyethylene oxide esters of stearic acid, and water, have been studied with the Gouy diffusion apparatus. The esters had small mean diffusion coefficients (D) of the same order as those of serum albumens; solubilized oleic acid increased D, but solubilized stearic acid and cholesterol decreased D slightly. No stable sol–water interfaces were formed with these materials.

A lecithin sol, clarified with one of the esters, had a small mean value of D which decreased markedly with time; a lecithin sol clarified with a small amount of bile salt gave still lower results for D which decreased after 3 days to a remarkably small value, the clarified sol–water boundary then being still clearly visible, though diffuse.

Some measurements have been made of the pull exerted on a platinum ring when it was drawn slowly up through a stable, turbid lecithin sol–water interface. A mean pull of 0·2 dyne/cm. was found for the lecithin sol alone. When a small quantity of a serum albumen sol was injected just above the interface and the boundary was allowed to mature for 20 hours before the ring was drawn through it, the pull was found to have increased to 0·8 dyne/cm., an effect which is attributed to a tanning of the lecithin film by an unfolded protein film. These pulls are of the same order of magnitude as the experimentally determined surface forces of living cells.

STUDIES have been made of the changes with time of sharp boundaries formed between some aqueous sols and water. The apparatus and methods used to form and examine these interfaces have already been described (*J.*, 1953, 519). In order to follow changes at the boundaries by the Gouy interference method it is necessary to have optically clear sols; to achieve this, polyethylene oxide stearates have been used as solubilizing agents. In contrast to the ionic soaps, these esters have been found to have low diffusion coefficients in aqueous solution, of the same order as serum albumen, as would be expected if their solutions contain electrically uncharged micelles.

EXPERIMENTAL

Materials.—Polyethylene oxide stearate A (ester A) had a setting point, *i.e.*, the constant temperature reached after supercooling, of 42·4°; its mean molecular weight, determined cryoscopically in benzene, was 2101; its loss in weight on heating at 110° was 0·5%. Polyethylene oxide stearate B (ester B), containing a lower proportion of ethylene oxide than A, had a setting point of 30·7°, M 1001, and a loss on heating at 110° of 0·8%.

Redistilled oleic acid had n_D^{25} 1·458; purified stearic acid was fractionally frozen to give acid of m. p. <69°; cholesterol had m. p. 148·5°.

Diffusion Coefficients.—Sols were freshly prepared before each measurement by melting the ester, adding to it the insoluble fatty material, and dispersing the clear liquid so obtained in warm conductivity water.

The sol–water boundary was formed in an all-glass cell as previously described (*loc. cit.*); diffusion from the interface was followed by photographing Gouy interference patterns at suitable intervals. The patterns obtained with these slow-diffusing materials showed some anomalies in the earlier stages of diffusion, and long exposures were required to obtain clear records of the outer fringes. In the later stages of diffusion, regular patterns were obtained which could be interpreted quite accurately by Kegeles and Gostling's theory (*J. Amer. Chem. Soc.*, 1947, **69**, 2516). The mean diffusion coefficients calculated for slower-diffusing materials seem to be liable to a greater percentage error than those for more rapidly diffusing compounds,

such as glycine. In the following Table, the uncertainty in D, the mean diffusion coefficient in C.G.S. units, is of the order of $\pm 2\%$.

Mean diffusion coefficients for fatty sols into water at 25°.

Expt. no.	Solubilizing ester *	Solubilized material	$D \times 10^7$	Time interval, hours	Expt. no.	Solubilizing ester *	Solubilized material	$D \times 10^7$	Time interval, hours
1	A 2·41	—	$5{\cdot}4_6$	½—2	7	B 4·07	Oleic acid 0·88	10·3	½—2
2	A 4·79	—	$5{\cdot}6_0$	½—2	8	B 4·35	Stearic acid 0·79	$4{\cdot}1_5$	½—2
3	A 10·44	—	$5{\cdot}5_5$	½—2	9	B 5·02	Stearic acid 0·34	4·5	1—5
4	A 2·09	Oleic acid 0·52	11·6	½—2	10	B 4·36	Cholesterol 0·53	$3{\cdot}9_8$	½—2
5	B 3·92	—	$5{\cdot}8_6$	½—2	11	B 1·97	Lecithin 4·10	1·1	6
6	B 4·44	—	$5{\cdot}8_4$	1—4			,, ,,	0·2	70

* Numbers are g. per l. in sol.

After overnight storage, all the boundaries had an extremely diffuse appearance, except in Expt. 11.

The first three results in the Table show that the mean value of D for ester A does not vary very much with concentration and is comparatively low; if the ester is assumed to be associated into spherical micelles in the solution, this value of D corresponds to a micelle "molar volume" of $2{\cdot}1 \times 10^5$ ml. *i.e.*, each micelle contains about 105 ester molecules. Expt. 4 shows that oleic acid increases D very considerably, suggesting that the unsaturated acid molecules do not fit into the ester micelles without disrupting them.

Expts. 5 and 6 show that ester B diffuses a little more rapidly than A; D corresponds to a micelle "molar volume" of $1{\cdot}8 \times 10^5$, *i.e.*, a micelle of 185 molecules. Expt. 7 shows that the disruption of the micelles by oleic acid occurs also with ester B. Expts. 8 and 9 suggest that solubilized stearic acid enters into the ester micelles, increasing their size and lowering D. Expt. 10 shows that cholesterol behaves similarly. Expt. 11 records a measurement carried out with a lecithin sol clarified with a minimum amount of ester B. The lower fringes of the Gouy patterns were not recorded clearly until 6 hours after the start of diffusion, even when exposures of several minutes were made. The pattern at 6 hours conformed with Kegeles and Gostling's theory and gave a value of $D = 1{\cdot}1 \times 10^{-7}$ After 70 hours a well-spaced pattern still persisted, which on analysis gave $D = 2 \times 10^{-8}$.

A further measurement was made, in which a lecithin sol was clarified with a minimum amount of the most effective agent for this purpose, *viz.*, reprecipitated bile salt (Valette, *Bull. Soc. Chim. biol.*, 1937, **19**, 1676). The final sol consisted of 3·75 g./l. of lecithin and 0·25 g./l. of bile salt. After 3 days, a definite, but diffuse boundary was still visible, in line with the cell channel. Diffusion did, however, occur, as was revealed by the Gouy photographs. The individual patterns could again be fitted quite well by theory, but the values of D obtained from them decreased markedly with time; after 70 hours D was $7{\cdot}8 \times 10^{-9}$, a much smaller diffusion coefficient than those recorded for most colloidal materials. An idea of the size of the diffusing units implied by such a small coefficient is obtained by comparing it with values of D for other bio-colloids; *e.g.*, hæmocyanin with a diffusing unit "molecular weight" of 6,800,000 has $D_{20} = 1{\cdot}4 \times 10^{-7}$ (Alexander and Johnson, "Colloid Science," Vol. I, Oxford, 1949, p. 288), while a thromboplastic lung lipoprotein, where particles were shown by electron microscopy to consist of spheres of mean diameter 100 mμ, had $D_{20} = 3{\cdot}8 \times 10^{-8}$ (Chargaff, Moore, and Bendich, *J. Biol. Chem.*, 1942, **145**, 593).

Gouy patterns obtained with clarified lecithin sols.

$j_m = 8{\cdot}78$ $t = 1{\cdot}152 \times 10^4$ sec. $t = 2{\cdot}498 \times 10^5$ sec.

j	f(z)	e^{-z^2}	Y	C_t	Y	C_t
1	0·199	0·607	0·607	1·000	0·607	0·494
2	0·313	0·478	0·483	1·010	0·483	0·472
3	0·427	0·369	0·371	1·005	0·371	0·458
4	0·541	0·273	0·268	0·982	0·268	0·470
5	0·655	0·190	0·184	0·979	0·184	0·462
6	0·769	0·117	0·116	0·992	0·116	0·483

$\bar{C}_t = 0{\cdot}991$ $\bar{C}_t = 0{\cdot}473$

$t\bar{C}_t^2 = 1{\cdot}14 \times 10^4$ $t\bar{C}_t^2 = 5{\cdot}59 \times 10^4$

$D = 3{\cdot}8 \times 10^{-8}$ $D = 7{\cdot}8 \times 10^{-9}$

For the meaning of the symbols, see *J.*, 1953, 519.

This decrease of D with time may be due to the formation of a film structure at the interface, and this effect will be further investigated. One important feature of these slowly diffusing fatty sols, including the lecithin sols, is that their relative viscosities at 25° as determined by an Ostwald viscometer are all appreciably less than unity (*i.e.*, 0·7—0·85).

The results recorded here do not indicate any possibilities of forming stable fatty membranes between aqueous liquids, without the use of lecithin; only lecithin notably decreases the mean

diffusion coefficient of the ester. To complete this stage of the work, some measurements of the mechanical properties of lecithin films between aqueous liquids have been made.

Mechanical Properties of Lecithin Films.—These have been studied in a preliminary way by means of a ring-pull interfacial tension apparatus modelled on that decsribed by Harkins and Jordan (*J. Amer. Chem. Soc.*, 1930, **52**, 1756), a jack and a chainomatic balance being used to raise the ring.

The boundary was formed in the diffusion cell between a 0·5% turbid lecithin sol and conductivity water, with the ring freely suspended in the sol about 5 mm. below the interface. After the sol and water had been run in from pipettes, the boundary was sharpened by flow in the usual way. The flow was then stopped, and the ring raised very slowly through the boundary. While it passed through the sol, no pull on the ring was experienced (to within $\pm$ 0·2 mg.), but on reaching the sol–water boundary a very definite pull of the order of 2 mg. occurred. As the ring came through the boundary, the balance pointer swung sharply over just as in an ordinary interfacial-tension measurement. The interfacial pull was reproducible to within $\pm$0·2 mg. and was independent of the time, up to 20 hr., for which the boundary was allowed to stand after flow had stopped. Pulling the ring through the interface caused comparatively little disturbance, and on lowering the ring into the lecithin sol layer and drawing it up through the boundary again, the same value of the interfacial pull was obtained, suggesting that the film is rapidly re-formed after being broken.

The author (*loc. cit.*) has reported a visual observation that injection of a protein sol above a lecithin sol–water interface improves the mechanical properties of the boundary. This has been confirmed by the ring-pull method. The lecithin sol–water interface was prepared, as above, and 0·1 ml. of a 0·5% bovine serum albumen (Armour Laboratories, recrystallised material) sol was injected from an Agla microsyringe, a few mm. above the interface. When the ring was raised through the boundary, soon after the injection of protein, an unchanged interfacial pull of 2 mg. was obtained. In a subsequent experiment the boundary was allowed to mature for 20 hours, before the ring was pulled through it; the interfacial pull was then very considerably increased. Four repeat measurements gave values between 7·7 and 8·5 mg. When the ring was lowered back into the sol layer and then drawn through the boundary again, low results were obtained unless the boundary was left to settle for an hour of two, suggesting that the protein-reinforced film is not re-formed rapidly after rupture.

These interfacial pulls, like the interfacial " tensions " between a living cell and its environment, are measures of the mechanical properties of interfacial films rather than of true liquid–liquid interfacial tensions.

On the basis of the crude ring-pull theory, *viz.*, pull on ring = 2 $\times$ ring circumference $\times$ interfacial tension, " tensions " for the lecithin sol–water interface of 0·2 dyne/cm., and for the protein-reinforced interface of 0·8 dyne/cm., are obtained. It is noteworthy that these values are of the same order as those found experimentally for the surface forces of living cells, *e.g.*, 0·2 dyne/cm. for *Arbacia punctulata* (Harvey, *Trans. Faraday Soc.*, 1937, **33**, 943) and a maximum value of 1·4 dyne/cm. for nucleated erythrocytes (Norris, *J. Cellular Comp. Physiol.*, 1939, **14**, 117).

Further studies of these interfacial film " tensions " will be made with more sensitive apparatus.

Discussion

The formation of stable boundaries at the aqueous sol–water interface has not been observed with fatty sols other than lecithin. An explanation for the film-forming properties of lecithin can be found by examining its molecular structure. From a molecular model of an α-lecithin, it can be seen that this compound is able to assume a configuration in which the fatty chains are parallel and the negatively charged oxygen atom of the phosphate group lies directly below one fatty acid chain while the positively charged nitrogen atom of the choline group lies directly below the other. In this arrangement, the lecithin molecules will readily form stable laminar micelles in water, the hydrophobic chains forming the interior of the micelle leaving the hydrophilic, electrically charged parts of the molecules facing outwards towards the water on either side of the micelle. Further stabilization can be expected by interaction between the choline group of one molecule and the phosphate group of its neighbour.

The mechanical shearing effect produced by the flowing of the lecithin sol against water in the diffusion cell seems to play a necessary part in the production of a large stable film; the sol itself mixes quite freely with water under normal conditions.

The sheet of dipoles left facing the water will provide a surface which will readily

adsorb amphoteric materials with a similar zwitterionic structure. This may lead to the joining of several bimolecular sheets into a thick film. The enhanced mechanical strength of lecithin films after treatment with protein is probably due to a tanning process in which the zwitterionic lecithin sheet unfolds the amphoteric protein molecules into a surface-denatured form. This is likely to be a slow process, as is found experimentally.

The observation, reported previously (*loc. cit.*), that a stable boundary is not formed between a lecithin sol and a serum albumen sol, indicates that it is necessary to have the lecithin film already in existence before the protein can be adsorbed; the presence of protein inhibits formation of this film, possibly by combining to form a lecithin–protein complex in which the protein molecules are still in the folded form.

It is to be expected that synthetic zwitterionic compounds containing fatty acid groups and appropriately spaced charged groups will have film-forming properties similar to those of lecithin.

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