105. *Interfaces between Aqueous Liquids.*

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Interfaces formed when normally miscible, aqueous liquids flow together through a common channel have been studied optically. The apparatus has been so designed that it can be used to study diffusion processes in clear liquids by the Gouy interference method or it can be used to photograph an interface directly when there is turbidity at the boundary.

Interfaces formed between some colloid sols of biological interest have been examined in order to provide a background for more detailed physicochemical work with materials of better-defined constitution.

Very stable interfaces have been obtained with lecithin sols and this effect is considered to be due to the spreading of a film of fatty acid at the boundary. The possibility of forming a mixed lecithin–protein film at the boundary between two aqueous liquids has been investigated and there is some evidence that such a film, which would be a very useful model for the study of biological adsorption, can be produced.

LANGMUIR and WAUGH (*J. Gen. Physiol.*, 1938, **21**, 745) wrote: "For many biological purposes it would be desirable to produce a membrane of one or two layers of protein between two aqueous phases. Such a membrane would probably have some of the properties of a cell membrane." The study of the adsorption of biologically active compounds at stable or metastable interfaces formed between two aqueous liquids should be of considerable interest, since these interfaces will have a closer resemblance to natural cell membranes than the models hitherto used for this type of work, *e.g.*, protein or lipid films spread at air–water or oil–water interfaces.

The main problem to be solved is the production of stable surfaces of separation of two aqueous liquids, in an apparatus in which the electrical and optical properties of the surface can be studied. Langmuir and Waugh attempted to achieve this by mechanically transferring films of protein, formed at air–water or oil–water interfaces, into water so as to form membranes separating two aqueous columns. This technique was not very successful; pure protein films broke up on immersion but they were considerably strengthened by incorporation of lecithin. It would seem to be more profitable to produce stable films by the interaction of two aqueous liquids at a sharp boundary temporarily formed between them.

The work reported here describes some experiments carried out with an apparatus constructed for the optical study of interfaces formed between normally miscible liquids. The production of sharp boundaries between such liquids can be effected by several methods which have been developed in connection with electrophoretic and diffusion studies of colloid sols. The three principal techniques are: (1) sliding a section of a tube containing one liquid over a stationary section containing the other (Tiselius, *Trans. Faraday Soc.*, 1937, **33**, 524; Neurath, *Science*, 1941, **93**, 431); (2) withdrawing a plate separating two liquids (Claesson modification of the Lamm cell, *Nature*, 1946, **158**, 834); (3) causing the liquids to flow together through a horizontal channel (Coulson, Cox, Ogston, and Philpot, *Proc. Roy. Soc.*, 1948, *A*, **192**, 382). Of these, the first two methods do not produce very sharp boundaries, and the third seemed preferable.

The recently revived Gouy interference method for studying diffusion (Longsworth, *J. Amer. Chem. Soc.*, 1947, **69**, 2510; Coulson *et al.*, *loc. cit.*) provides a sensitive method for studying changes at the sharp boundaries, soon after their formation.

Experimental

The apparatus used (Fig. 1) was modelled on that described by Coulson *et al.* (*loc. cit.*) and by Gosting, Hanson, Kegeles, and Morris (*Rev. Sci. Instr.*, 1949, **20**, 209). It consists of a 2-m. optical bench mounted on a steel beam supported by concrete pillars embedded in the earth beneath a basement laboratory floor. A light source (*A*, Fig. 1) consists of a mercury-vapour lamp cooled by a water jacket and fitted with appropriate filters to isolate the green line (5461 Å); the light from this is condensed on to a horizontal spectrometer slit (*B*) set at 5-μ aperture. A precision camera lens (*C*), of 13·5-cm. focal length, focuses an image of the slit through the thermostat (*D*) containing the cell on to a camera plate (*E*) at the far end of the bench. The thermostat is fitted with circular, optically flat ($\lambda/2$) windows, set accurately perpendicular to the optic axis of the bench (to within $\frac{1}{3}'$ of arc); it is supplied with constant-temperature water from a larger tank, the connections between them being so arranged as to avoid transmitting vibration to the bench.

Gosting *et al.* (*loc. cit.*) use a sliding-section type of cell in which the boundary is sharpened after formation by drawing the liquids off together through a pipette whose tip is placed at the boundary; Coulson *et al.* (*loc. cit.*) describe a Perspex cell fitted with a channel at the level of the boundary and optically flat glass windows. This fitted-channel type of cell seemed most suitable for this work, but since the use of Perspex very much restricts the temperature range in which it can be used, an all-glass cell has been constructed, Araldite cement being used to join the plate-glass pieces. Two 2 × 2 cm. windows were half wave-length optical flats, but the remainder of the cell was not of optical quality. This cell has now been replaced by an all-glass one (Fig. 2) constructed by Hilger and Watts Ltd. (The author is indebted to Mr. H. W. Yates for help in designing this cell.) The great advantage of this cell is that the optically important, lower section (*A*) consists entirely of adhering glass pieces; it contains no cement and the faces are very accurately set parallel to one another and are polished flat to a half wave-length. The upper part of the cell (*B*) consists of plate-glass pieces joined together by Araldite cement. The upper part is joined to the lower section by a cold-setting cement. The two aqueous liquids are placed on either side of the central glass partition (*C*) and the boundary between them is formed by drawing them out together through the channel (*D*); this is of 50-μ width and is set accurately perpendicular to the sides of the cell. The channel was formed by grinding out two blocks of glass which were subsequently made to adhere together. The tube (*E*) through which the liquids are withdrawn is cemented into the block of glass.

The cell is put on a platform in the thermostat so that the boundary is formed in the optic axis of the bench; this axis is adjusted to be accurately horizontal by ensuring that the image of

FIG. 1. *Diffusion apparatus.*

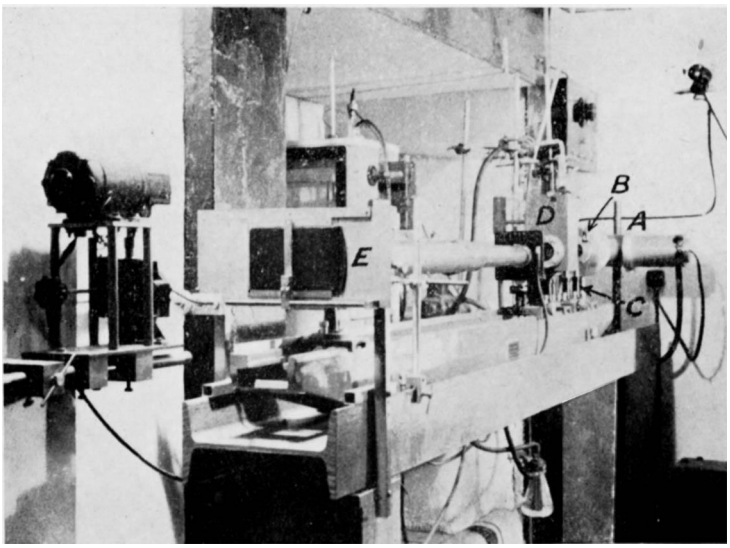

FIG. 2. *Stops and cell.*

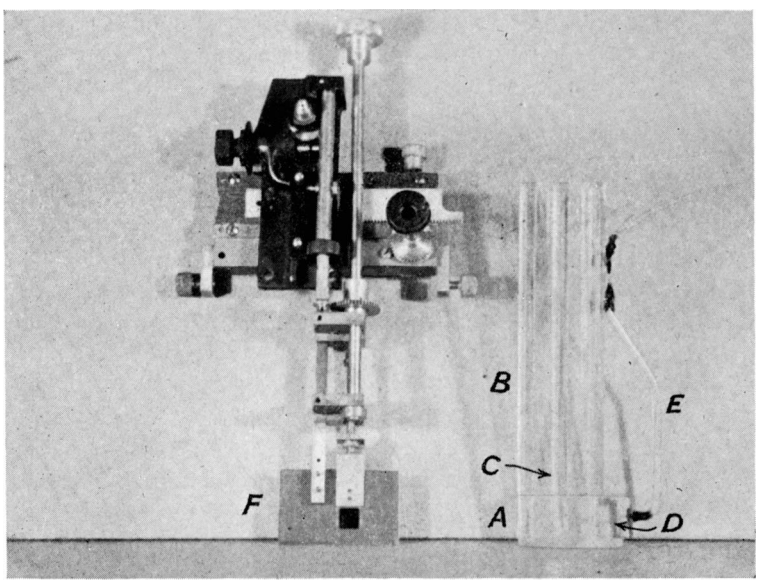

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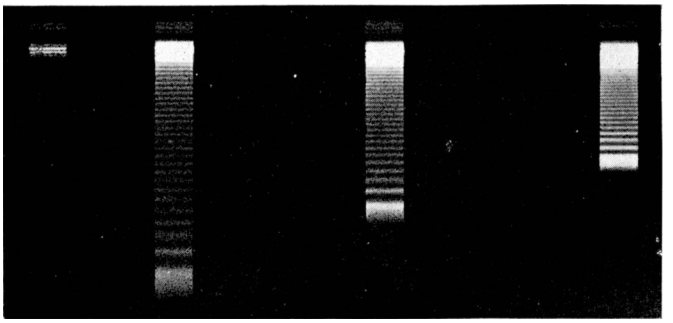
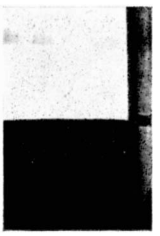
FIG. 3. *Reference and Gouy patterns (glycine). Intensity minima are dark.*

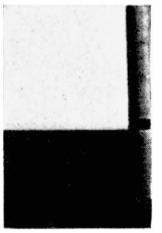


$t = 300$ 600 1200 *sec.*

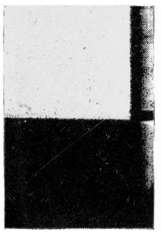
FIG. 4. *Lecithin sol (dark)/water (light) interfaces, showing the outflow channel of the cell as a reference point.*



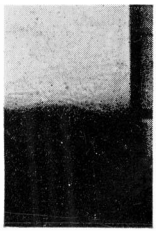
A. *While flowing.*



B. 2 *Hrs. after flow had stopped.*



C. 20 *Hrs. later.*



D. *Effect of tetradecylpyridinium bromide.*

the slit at the photographic plate is exactly the same height above the bench as the slit itself. After the flow of liquids through the cell channel has been stopped, the boundary normally spreads by diffusion and the refractive-index gradients produced cause a set of interference fringes to appear at the photographic plate, below the normal slit image. As diffusion proceeds, this Gouy pattern closes up towards the undeviated slit image, and by photographing it at different times and measuring the displacements of the intensity minima below the optic axis, the diffusion process can be quantitatively followed. The full theory of the interpretation of the patterns to yield values of diffusion coefficients is given by Kegeles and Gosting (*J. Amer. Chem. Soc.*, 1947, **69**, 2516). In this work the patterns have been measured to within a few microns, by means of a Cambridge measuring machine. A vertical slit placed immediately in front of the photographic plate enables a series of patterns to be recorded on a single plate. Normally ten seconds' exposure sufficed to give clear records of the patterns.

On the photographic plate side of the cell, fitting close up against the cell window, a set of stops is mounted (*F*, Fig. 2*a*) and these can be moved horizontally and vertically, from above the thermostat. The main aperture is 10 × 10 mm. and has serrated edges at top and bottom to break up the Fraunhofer patterns, which otherwise interfere with the Gouy pattern near the undeviated slit image; this aperture is centred on the boundary in the cell. To one side of the main aperture there are a pair of 1 × 1 mm. apertures arranged so that one is vertically above the

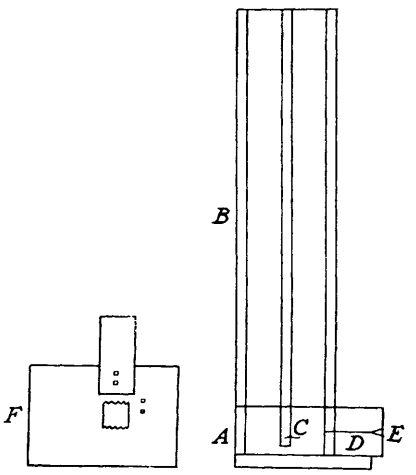


FIG. 2*a*. *Diagrams of stops and cell.*

other with a distance of about 3 mm. between them. These pass light which goes only through the glass block containing the cell channel and not through the liquids in the cell; the light from those two stops is displaced upwards by an inclined, optically flat glass plate, so producing a double slit interference pattern on the photographic plate, above the undeviated slit image; this serves as a reference trace to locate the optic axis of the apparatus, and all measurements of the patterns are referred to this trace. A similar pair of double stops can be lowered in front of the main aperture, by means of a screw.

To carry out a measurement of a diffusion coefficient, a photograph is first taken of the undeviated slit image and the reference trace, with a homogeneous liquid in the cell; the double stops are then lowered in front of the main aperture and a record of the two double slit patterns is made. From these, the mean distance between the centre of the reference trace and the optic axis of the apparatus is found. Provided the optic axis be horizontal, the two results agree very closely. The boundary is then formed between the two liquids and another photograph of the two double slit patterns is made, the double slit in front of the main aperture being arranged so that the boundary is between the two slits. This record is used to calculate the fractional part of j_m, the difference in optical path length between the two liquids in the cell, in wave-lengths of light; the integral part of j_m is found from the number of minima in the Gouy pattern. Finally, the double slit in front of the main aperture is raised and the flow of liquids through the cell channel is stopped. Photographs of the Gouy pattern and the reference trace are then taken at regular intervals after flow has stopped. A typical set of patterns, obtained in a measurement of this kind, is shown in Fig. 3. An abridged calculation of the diffusion coefficient of glycine from such a record is shown below.

The method finally adopted for introducing the two liquids into the cell while maintaining a sharp boundary between them was as follows. The liquids are contained in identical pipettes, the tips of which are bent through a right angle and drawn out to very fine jets. The pipettes are clamped above the cell with their tips on either side of the central partition, touching the cell walls immediately above the optically flat windows. The flow of liquids from the pipettes is controlled by rubber tubing, screw clips, and fine glass capillaries at their tops. The denser liquid is allowed to flow into the cell in the compartment farther from the channel, until it is above the channel level in both compartments, and liquid is then drawn through the channel. The denser liquid is delivered from the pipette at a very slow rate and the less dense liquid is allowed to flow into the cell compartment near the channel at a similar rate, both liquids being continuously drawn off through the channel. With care and practice, initial mixing of the liquids can be avoided. When the flow rates of the two liquids have been adjusted, the rate of withdrawal through the channel is reduced and columns of liquid are built up in the compartments of the cell. The inflow rates from the pipettes can be increased to about 0·5 ml./min. when the tips of the pipettes are covered. In later work, it was found necessary to build up large columns of liquids in the cell; provided great care be taken to avoid any initial mixing, about 10 ml. of each of them is sufficient. When sufficient quantities of the two liquids have been run into the cell, the pipettes are carefully removed and the slow rate of outflow through the channel is maintained for some time to ensure temperature equilibrium. The withdrawal rate is increased for a few minutes and then stopped completely at the start of the Gouy pattern measurements.

By these methods, the apparatus has been calibrated with " AnalaR " glycine as a standard substance. An abridged calculation is shown below.

Calculation of diffusion coefficient of glycine.—0·5000 G./100 ml. of solution diffusing into water.

Distance, centre-reference trace to optic axis	= 0·1651 cm.
Distance, centre-reference trace to centre of double slit pattern with boundary between slits	= 0·1853 cm.
Mean interband distance for double slit pattern	= 0·0252 cm.
Fractional part of $j_m = (0{\cdot}1853 - 0{\cdot}1651)/0{\cdot}0252$	= 0·80
Integral part of j_m	= 38

In the following table, t is the time (in seconds) after stoppage of flow at which the photograph is taken; j is the number of each interference pattern minimum, the outermost being numbered zero and the number increased as the undeviated slit image is approached; Y is the displacement of each minimum below the optic axis, in cm.; f(z) is equal to $(j + 3/4)/j_m$ and e^{-z^2} is the displacement of each fringe derived from the theory of the process and interpolated from the tables given by Kegeles and Gosting (*loc. cit.*); C_t is the ratio of observed displacement to theoretical displacement and should be constant for a given pattern. The apparent diffusion coefficient D' is calculated from Longworth's equation $D' = b^2 j_m^2 \lambda^2 / 4\pi C_t^2 t$, where b is the optical distance from the centre of the cell to the photo plate (135·5 cm. in this case), λ is the wave-length of the light in cm., and $t = 180$ sec.

j	Y	f(z)	e^{-z^2}	C_t	j	Y	f(z)	e^{-z^2}	C_t
2	1·388	0·0709	0·798	1·739	14	0·721	0·380	0·411	1·755
4	1·239	0·122	0·711	1·743	16	0·638	0·432	0·364	1·753
6	1·113	0·174	0·638	1·744	18	0·559	0·483	0·320	1·748
8	1·002	0·226	0·573	1·748	20	0·483	0·535	0·278	1·737
10	0·904	0·277	0·514	1·759	22	0·420	0·586	0·239	1·757
12	0·809	0·329	0·461	1·755	24	0·353	0·638	0·209	1·748

Mean $C_t = 1{\cdot}749$; standard deviation = 0·007, standard error = 0·002.

At other values of t, mean values of C_t were :

t	180	360	720	1440
C	1·749	1·278	0·909	0·652
$10^5 D'$	1·192	1·116	1·103	1·072

The decrease of D' with increasing t was noted by Longsworth (*loc. cit.*), who attributed it to the fact that the boundary between the liquids is not infmitely sharp. He corrected for this by introducing a quantity Δt representing the time which an infinitely sharp boundary would take to reach the state of the boundary existing when flow is stopped; D' is then plotted against $1/t$ and the straight line is extrapolated to $1/t = 0$ to give the true value D of the diffusion coefficient. The slope of the line gives a measure of Δt.

In this case extrapolation gave $10^5D = 1{\cdot}05_9$ (cf. Lyons and Thomas, *J. Amer. Chem. Soc.*, 1950, **72**, 4506, who give 1·057) and $\Delta t = 24$ seconds.

The value of Δt is very much a function of the rate of flow of liquids through the channel just before it is stopped. With more rapid flow, the Δt effect can be reduced to the same order as the experimental error in C_t; *e.g.*, in a measurement with glycine (0·5 g. in 100 ml.) in which great care was taken to avoid initial mixing and a high rate of flow through the channel was maintained, the following results were obtained:

T	600	1200	2400	
C	0·903	0·638	0·450	
$10^5D'$	1·058	1·059	1·063	Mean = 1·060

showing that very sharp boundaries can be obtained by this flow technique.

The Gouy interference-pattern method is suitable for studying changes at the boundaries between optically clear liquids which do not interact to form cloudy products, though the theory for interpreting the patterns will have to be simplified by approximations in order to yield a physical picture of changes occurring as the boundary thickens, when more than two components are present. If turbidity appears at the boundary or if one of the liquids is not optically clear, the Gouy method is less useful, and in such cases the optical system of the apparatus has been modified so as to photograph directly the turbid areas in the cell. To do this, the lens C in Fig. 1 is set so as to give parallel light from the spectrometer slit which is opened to 100-μ aperture, and a new lens is put between the thermostat and the photographic plate to focus a direct image of the cell contents on to the plate. With this and the Gouy method, a wide variety of boundaries can be studied; the only limitations are that one liquid should be optically clear and that any materials formed at the interface should be sufficiently fluid to pass through the outflow channel of the cell.

Results.—In order to provide a background for more detailed physicochemical studies with materials of defined chemical constitution, the boundaries formed with some colloid sols of biological interest have been examined.

(1) *Interfaces between oppositely charged colloid sols.* The possibility of forming stable interfaces between sols carrying opposite electrical charges has been investigated, gelatin (positive) and acacia (negative) sols at pH 4 being used. These interact at room temperature to give a flocculent precipitate; at higher temperatures a completely liquid coacervate is formed (Kruyt, "Colloid Science," Vol. II, London, 1949, p. 255).

The sols were prepared so as to contain minimum concentrations of micro-ions. 2% Gelatin sols were made by dissolving low-ash gelatin in warm, recently boiled conductivity water; the sol was passed through a mixed-bed ion-exchange column (H form of Zeo-Karb 215 plus OH form of Amberlite IRA-400; cf. Kenchington and Ward, *Research*, 1951, **4**, 247) from which it emerged with a pH of 5·2 (this was independent of the relative proportions of the two resins) and a specific electrical conductivity reduced from 6×10^{-4} to 7×10^{-6} mho/cm., indicating almost complete removal of micro-ions. The gelatin was then precipitated with ethanol in the cold, filtered off, washed with cold water, redissolved in warm water, and dialysed against water to remove traces of ethanol; the sol was adjusted to pH 4 by addition of a few drops of N-hydrochloric acid. Acacia sols were prepared by dissolving the gum in conductivity water and shaking the solution with mixed ion-exchange resins to remove free micro-ions; the sol was then shaken with the H form of Zeo-Karb 215 alone until sufficient of the bound micro-cations (Ca, Mg, and K) were replaced by hydrogen ion to give a pH of 4.

Diffusion coefficients of the sols were measured by the Gouy method; the patterns agreed moderately well with theory and gave values of diffusion coefficients at 36° of $2{\cdot}4_3 \times 10^{-6}$ c.g.s. units for a 2% gelatin sol and $2{\cdot}7_5 \times 10^{-6}$ for a 1% acacia sol.

When boundaries were formed between the two sols in the cell at 36°, a very sharp interface was obtained during flowing, provided initial mixing of the sols was avoided; on mixing, they gave a dense white coacervate which was difficult to sweep out of the cell by running large volumes of liquid out through the channel. When flow was stopped, the boundary thickened steadily as the sols diffused into one another; the upper and the lower edge of the region of interaction remained quite sharp for up to an hour, and by direct photography the thickness of this turbid region could be estimated at different times. This thickness was found to be approximately a linear function of $\sqrt{t}$.

Boundary thickness, cm. ...	0·077 ± 0·006	0·100 ± 0·011	0·126 ± 0·012	0·180 ± 0·014
Time, t, sec.	300	600	1200	2400

The effects of some surface-active materials on the rate of thickening of this boundary were studied by injecting their solutions into the interface just after flow had stopped, by means of an Agla micro-syringe. No significant effects were observed.

(2) *Lecithin sols.* The film-forming properties of phospholipids are well known (*Adv. Protein Chem.*, 1949, **5**, 434) and it seemed possible that a material such as lecithin might form a stable film between aqueous liquids at the flowing interface.

A purified preparation of lecithin was made from commercial egg lecithin by cadmium chloride precipitation and light petroleum–ethanol partition (Pangborn, *J. Biol. Chem.*, 1945, **161**, 71). The resulting waxy solid was stored in an atmosphere of dry nitrogen, in the dark. It was not easily dispersed in water and 0·5% sols were prepared by dissolving the solid in a minimum amount of ether and adding this slowly to conductivity water with vigorous shaking; the ether was removed by evaporation at 40°/15 mm. The sol was invariably turbid and was little clarified by centrifugation. The difficulty of obtaining clear sols from egg lecithin has been described by McFarlane (*Discuss. Faraday Soc.*, 1949, **6**, 79). No further attempts were made to clarify these sols, and boundaries formed with them were examined by direct photography.

An extremely sharp, bright interface was formed by running the 0·5% sol against water (Fig. 4A); two hours after flow was stopped the interface was still bright and sharp and had moved very slightly (Fig. 4B), and a Gouy photograph through the clear upper liquid showed no lines due to diffusion from the interface, and even 20 hours later, the boundary was still sharp and bright (Fig. 4C) and had not moved appreciably. It was not until several days later that it began to appear diffuse, probably owing to decomposition of the lecithin. This remarkably stable sharp interface between two phases consisting mainly of water appears to be due to the formation of a fatty acid film at the boundary which may be bimolecular (a type of laminar micelle across the boundary) or it may consist of a number of bimolecular layers. A similar stable sharp boundary between deoxyribonucleic acid sols and water has been noted by Butler and James (*Nature*, 1951, **167**, 844); this boundary moved continuously, however, and they attributed its formation to the existence of a gel network in the sols; with lecithin, however, there is little likelihood of this since its sols are very mobile.

When the cell containing a lecithin sol–water interface was moved, the boundary showed little mechanical strength, and on injection of a solution of a cytolytic agent (tetradecylpyridinium bromide) just above the boundary, visible disruption occurred (Fig. 4D).

(3) *Serum albumen and lecithin sols.* If lecithin can cause a relatively stable fatty acid film to spread at the flowing interface, it seems reasonable to suppose that a protein which is denatured at an oil–water interface might form a mixed surface layer with the lecithin and provide a backbone for the fatty acid film, giving it some mechanical strength. To test this idea, a boundary was formed between 0·5% lecithin and bovine serum albumen sols (made from recrystallised material supplied by Armour laboratories). A sharp boundary was obtained at first, but after 1 hour penetration of the clear albumen sol by the lecithin was evident. A mixed sol of lecithin and albumen was then caused to flow against water; a sharp turbid sol–clear liquid boundary was obtained, but after 2 hours a Gouy photograph taken through the clear liquid showed lines due to diffusion, and after 20 hours a second sharp boundary appeared above the original one.

On injection of an albumen sol above a lecithin sol–water interface, the protein appeared to spread over the boundary. After 20 hours, the boundary remained sharp, and when the cell was moved it showed some mechanical strength, settling back to its original position even after considerable movement of the cell. This suggests that a protein film is formed at the boundary, and this technique may provide the answer to the problem stated by Langmuir and Waugh.

Discussion

It appears that lecithin sols have the property of forming stable interfacial films between aqueous liquids, and there is evidence that these films can be strengthened by injection of a serum albumen sol at the interface. Mixed films of this type should provide a useful model for the study of biological adsorption of organic compounds, since they can be formed in apparatus in which their optical and electrical properties can be readily studied.

Lecithin is not a well-defined chemical entity, and it would be desirable to produce fatty acid films between aqueous liquids from materials of better known composition. The formation of these stable boundary layers is likely to be related to the production of myelin forms by lecithin in contact with water, and since the latter property is also shown by other fatty materials (Dervichian and Magnant, *Bull. Soc. Chim. biol.*, 1946, **28**, 419), it is likely

that other fatty sols or solutions will be capable of forming stable interfaces between aqueous liquids. Further work will be directed towards the study of diffusion and membrane formation at the flowing interface, with aqueous solutions of surface-active materials of known constitution.

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