Surface Forces of Lecithin Sols in the Presence of Some Inorganic Salts.

By P. H. Elworthy and L. Saunders.

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A sensitive apparatus has been devised for the measurement of surface forces at sharp boundaries formed between lecithin sols and water. The ring-pull technique for the measurement of surface force has been used and a new type of cell for making the boundaries is described.

The effects of various inorganic salts on the surface force have been studied. If the lecithin sol was first stripped of all small ions by treatment with exchange resins, only a very small surface force could be measured in the presence of potassium or sodium chloride. The addition of very low concentrations of bivalent metal chlorides caused a considerable increase in the surface force, the order of activity being $CdCl_2 > MgCl_2 > CaCl_2 > BaCl_2$.

The properties of lecithin films at sol–water boundaries are of interest in relation to biological membranes. In a previous paper (*J.*, 1953, 1310) preliminary measurements of small " interfacial tensions " or " surface forces," at sharp boundaries formed between lecithin sols and water, were reported. In this paper, a more sensitive apparatus for their measurement is described, and the results of some experiments on the effect of inorganic salts on these surface forces are recorded.

The surface forces, γ (dynes/cm.), have been calculated by the formula $mg = 2\pi\gamma d$ where m is the force in grams-weight required to pull a circular platinum ring of diameter d cm. through the sol–water boundary.

The extremely small density difference between the sols and water precludes the use of correction factors of the type proposed by Harkins and Jordan (*J. Amer. Chem. Soc.*, 1930, **52**, 1751). Also this surface is more nearly akin to the force of extension of a membrane than to the work of extension of a simple liquid–liquid interface.

Experimental

Apparatus.—The cell in which the boundaries were made (Fig. 1) consists of two parts: the male part of a 25-mm. ground-glass joint, the tubing above which was drawn to a construction (*A*), 0·3-cm. wide, and 1 cm. above the joint; and the female part of the joint, the tubing below which was closed at the bottom (*B*). Halfway up this lower tube was sealed a side-arm with a fine horizontal jet (*C*) inside the cell; near the bottom on the opposite side, was a filling tube (*D*). Rubber tubing joined the jet side-arm to a stopcock, by which the rate of flow of liquid from the cell could be controlled. The filling tube could be closed by a rubber tube and a clip.

The platinum ring hung inside the cell below the level of the jet (*C*), suspended from the arm of a precision torsion balance, by means of a fine Nylon thread. The cell was immersed in a water-thermostat, controlled to $\pm 0{\cdot}05°$. To prevent errors due to the Nylon suspension's touching the sides of the constriction, the clamp holding the cell was attached to a centering device by which the cell could be moved smoothly in two mutually perpendicular directions until the suspension was exactly in the centre of the constriction.

The balance had a capacity of 5 mg. and a mirror scale which could be read to 0·01 mg. It stood on a platform which was moved up and down a stout metal pillar by means of a rack and pinion. By operating the pinion, the balance, and therefore the platinum ring, could be raised or lowered slowly and smoothly.

The platinum ring was rendered completely planar and circular by careful tapping in a groove cut in a brass plate. To ensure that it was horizontal when suspended, it was hung over a mirror (Harkins and Jordan, *loc. cit.*). Its mean diameter (1·027 cm.) was measured by means of a Cambridge Universal Measuring Machine.

Materials.—Lecithin was prepared from a cold, ethanol extract of egg powder by formation and purification of the cadmium chloride complex (Pangborn, *J. Biol. Chem.*, 1949, **188**, 471).

The cadmium chloride was removed by adding methanolic ammonia to a solution of the purified complex in ammonia, and the lecithin was recrystallised twice from 4 : 1 acetone–ethyl methyl ketone. After being washed with acetone and dried in a vacuum, it was a crisp, cream-coloured solid which appeared uniformly crystalline under the polarising microscope, exhibiting parallel extinction. It was dissolved in dry ethanol and passed through a column of mixed weak anion- and cation-exchange resins to remove traces of cadmium- and amino-containing impurities. The lecithin gave, on analysis, N 1·9%, P 4·1%, I no. 66·7, $[\alpha]_D^{20}$ 7·54° (10% solution in EtOH). Lecithin of the mean fatty acid composition found by Riemenschneider, Ellis, and Titus (*ibid.*, 1938, **126**, 255) requires N 1·8%, P 4·0%, I no. 74.

Water was prepared by distillation in a seasoned all-glass still.

Inorganic salts were " AnalaR " materials.

Preparation of Sols.—The lecithin sols were made by evaporating to dryness a portion of alcoholic solution of lecithin of known concentration. The dry lecithin was then shaken with water until completely dispersed. The sol was freed from included air by means of a filter-pump and was passed through a column of mixed ion-exchange resins; here any small ions still present were completely removed, the specific electrical resistance at 20° of a 0·5% sol rising from 0·11 to 0·75 megohm cm., while the loss in weight of solids was less then 2%. The resulting sol had pH 7. By this method complete removal of the particularly persistent cadmium ion (Macpherson, *Nature*, 1954, **173**, 1195) was ensured. The salt under investigation was added to the sol after ion-exchange, and the whole then made up to the required volume. About 25 ml. of sol were required for each measurement.

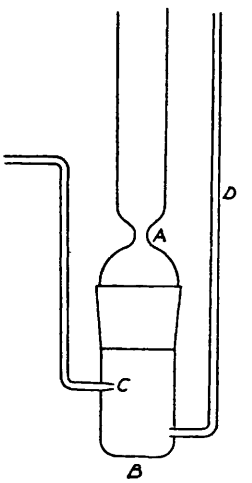


FIG. 1. *Cell for surface-force measurements.*

Boundary Formation.—Both the sol and the water or solution with which the boundary was to be formed were preheated to 35° to prevent formation of bubbles on the walls of the cell which might rise into the constriction and prevent free movement of the suspension. The sol was poured into the lower part of the cell with the jet (*C*) filled with water. The Nylon suspension, with ring attached, was passed through the constriction in the upper part of the cell, and the joint was fitted together so that the ring hung in the cell. Sol was then drawn in through the constriction by alternately applying and releasing pressure on the filling tube (*D*) until the cell was filled to a level above the constriction. The cell was placed in the thermostat and allowed to reach temperature equilibrium; liquid was then run out of the jet (*C*) until the sol level reached a point halfway up the constriction. The upper liquid was introduced carefully so as to fill the top part of the cell, the constriction preventing mixing of the two liquids and enabling an initial sharp boundary to be formed between them. The tap on the jet (*C*) was then opened to permit sol to flow out of the cell, causing the boundary to move downwards; despite its increased area the boundary remained sharp, providing the interior walls of the cell were treated with a " methyl silicone " (" Repelcoat "). When the boundary reached the level of the jet (*C*), flow-off was continued to give a final sharpening.

There is very little density difference between a lecithin sol and water and, although boundaries could be formed directly, there were many failures. To facilitate boundary formation a relatively inert weighting agent was added to the sols, 0·01M-potassium chloride being found most suitable.

Measurement of Surface Force.—After the final sharpening of the boundary, the ring was allowed to remain in the sol for 30 min. It was then raised either to the level of the boundary or just above it and allowed to hang there for 1·5 hr., to ensure complete drainage of sol from the ring. Just before the measurement was taken, the centering device was adjusted so that the suspension was clear of the sides of the constriction. The ring was then lowered into the sol by lowering the balance platform, and the balance pointer was adjusted to zero. The balance was then raised and when the ring reached the boundary the pointer was pulled downwards by the surface force.

Increasing tension was applied slowly and smoothly to the balance arm until the ring broke through the boundary, at this point the zero pointer of the balance moved sharply upwards as in an interfacial-tension measurement. After the break-through a film of lecithin could be seen, attached to the ring. The force required to achieve the break-through was read from the indicator arm of the balance and was converted into mg. by means of calibration graphs

obtained from measurements with standard weights. Repeated measurements could usually be obtained by re-sharpening the boundary by further flow from the jet (*C*).

Difficulties in the measurement of surface force. (1) If the ring was wetted by water before meeting the lecithin sol, as in the plate-pull type of cell, no surface forces could be measured. It was therefore necessary to devise a method of boundary formation such that the ring was initially wetted by the sol.

(2) If the ring was deeply immersed in the sol for 2 hr., then on raising it through the boundary only a very small surface force was found and the movement of the zero pointer of the balance was sluggish at the break-through point. This was due to adhesion of sol to the ring and its support; as it came through the boundary, it paid out this adhering sol and,

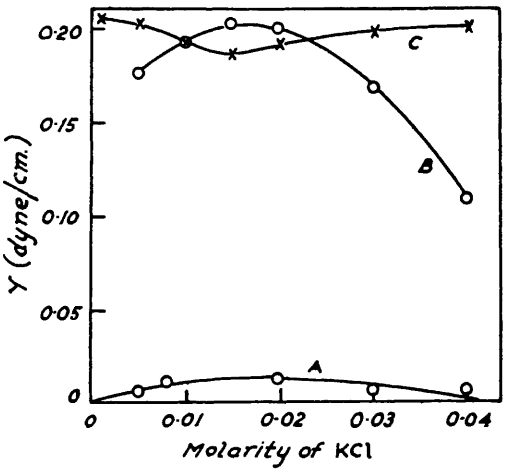


FIG. 2. *Effect of varying potassium chloride concentration on the surface force.*
A, *No bivalent metal present.*
B, 5·6 × 10^{-5}M-$CaCl_2$.
C, 7·2 × 10^{-5}M-$CaCl_2$.

although the ring could be seen to carry a curtain of sol up into the water layer, very little force was exerted on the ring. This difficulty was overcome by allowing a period of drainage for the ring before a measurement and also by coating the ring with a " Silicone " varnish.

(3) If the weight of the ring and suspension was too great, their inertia when set in motion upwards carried the ring through the boundary, giving low pulls with a sluggish break-through.

RESULTS

The results of the measurements of surface forces are summarised in Fig. 2—4. Equal concentrations of bivalent metal salts were present both in the sol and in the upper liquid, except where otherwise indicated. M/100-Potassium chloride was present in the sol to facilitate boundary formation. Except for those of Fig. 4, the sols all contained 0·5% w/v of lecithin. The measurements were carried out at 25° ± 0·05°.

In the absence of all small ions, which are removed by the ion-exchange treatment of the sol, the surface force at the sol–water boundary is negligible. On addition of potassium chloride to the sol a very small but measurable force of the order of 0·011 dyne/cm. is obtained (Fig. 2, *A*). A similar effect occurs with sodium chloride. Substantial surface forces are obtained in the presence of ions of bivalent metals; these ions produce forces of 0·1—0·25 dyne/cm. at concentrations of the order of 10^{-5}M.

In Fig. 3, the effect of calcium chloride on γ is shown, the graph shows two peaks: after the main peak, the value of γ falls off slowly as the salt concentration is increased. The lecithin molecule exists as a monomer only in polar organic solvents (Fauré and Legault-Demare, *Bull. Soc. Chim. biol.*, 1950, **32**, 509), and in water the sol consists of large micellar aggregates of molecules. Since the lecithin sol alone has no surface force at the boundary with water, it appears that the micelles in the boundary layer are only loosely connected together. When very low concentrations of calcium ions are introduced, a linking of micelles appears to take place as in flocculation at very low calcium concentrations, and this micellar linking results in a measurable surface force. The link is probably formed between terminal phosphate groups of adjacent micelles. Fig. 2, *C* shows

that varying potassium chloride concentration has little effect on γ at a calcium chloride concentration above that giving the main peak. Fig. 2, *B* shows that, at calcium chloride concentrations below this peak, variation of potassium chloride concentration does affect γ appreciably. In the first case (Fig. 2, *B*), the micelles are presumably completely cross-linked and further added electrolyte has little effect; in the other (Fig. 2, *C*) the cross-linking may not be complete and another electrolyte can affect the intramicellar structure.

In general form the $CaCl_2$ curve of Fig. 3 is similar to the curves published by Price and Lewis (*Biochem. J.*, 1929, **23**, 1030) to show the effect of calcium chloride on lecithin sol–air surface tensions. The decline in γ at higher calcium concentrations is in accord with Malquoris's observations (Wittcoff, "The Phosphatides," Reinhold Publ. Corp., New York, 1951, p. 74) that very low calcium chloride concentrations cause flocculation of lecithin sols, higher concentrations peptise the colloid, and still higher cause flocculation again.

In Fig. 3, the effects of the chlorides of several bivalent metals are shown. The heights of the maxima are in the order of increasing atomic volumes of the metals Cd,

FIG. 3. *Effect of bivalent metal chlorides on the surface force.*

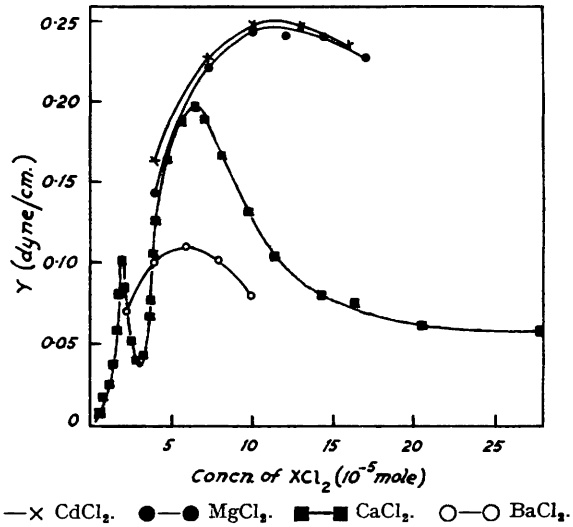
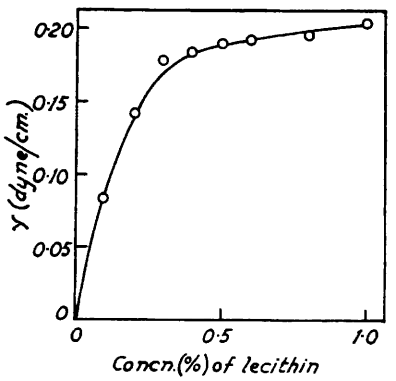


FIG. 4. *Effect of varying lecithin concentration at a fixed ratio, lecithin : XCl_2.*



Mg, Ca, Ba, but not in the same order as the ionic radii of the crystalline chlorides (Mg 0·65, Cd 0·97, Ca 0·99, Ba 1·35 Å; Pauling, "Nature of the Chemical Bond," Cornell Univ. Press, New York, 1945, p. 346). The large effect of cadmium on γ may be due to the fact that, in addition to the cross-linking of micelles through the phosphate groups, cross-linking could also occur by a type of covalent link between the fatty acid ester groups in the lecithin molecule and the bivalent metal. The tendency of cadmium to form covalent links is well known, and the covalent nature of the lecithin–cadmium chloride complex is illustrated by its solubility in solvents such as chloroform.

Fig. 4 shows the effect of varying the concentration of lecithin in the sol at a fixed lecithin–bivalent salt ratio. γ is at first a sensitive function of lecithin concentration until at 0·3% the curve shows an abrupt change of slope; by analogy with the B.E.T. isotherm it can be supposed that at this point the boundary layer becomes completely filled with micelles giving a close-packed film.

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SCHOOL OF PHARMACY, UNIVERSITY OF LONDON,
17 BLOOMSBURY SQUARE, LONDON, W.C.1.

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