

# CXI. THE PHYSICO-CHEMICAL BEHAVIOUR OF LECITHIN.

## I. THE CAPILLARY ACTIVITY OF LECITHIN AS A FUNCTION OF $p_H$ .

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OWING to the general biological significance of lecithin it was considered to be of importance to carry out a systematic physico-chemical investigation of its properties and behaviour. This is now rendered possible through the researches of MacLean and of Levene as a result of which the substance may be obtained in a high degree of purity. The present paper deals with one aspect of the behaviour of pure lecithin, namely its capillary activity when dispersed in aqueous solutions at various  $p_H$  values, and likewise in "solution" in alcohol-water mixtures also at different  $p_H$  values. The effects produced by the presence of certain salts in aqueous solution at different  $p_H$  values have likewise been determined.

### EXTRACTION AND PURIFICATION OF LECITHIN.

The lecithin was extracted from fresh egg yolks and purified by the method of Levene and Rolf [1927]. In order to minimise oxidation of the lecithin the material, wherever possible, was manipulated in an atmosphere of dry nitrogen. The yield of lecithin obtained varied with different batches of eggs but a rough average was about 15 g. of lecithin from ten dozen eggs.

The purity of the lecithin obtained was tested by hydrolysing a sample with hydrochloric acid and estimating the total amount of nitrogen present by the Kjeldahl method, and the percentage of amino-nitrogen present by the Van Slyke method. The percentage of amino-nitrogen gives the amount of kephalin mixed with the lecithin.

The samples tested gave the correct value for total nitrogen and contained no amino-nitrogen. Hence it was concluded that the samples were pure lecithin free from kephalin and other impurities.

*Analysis.*

1.720 g. lecithin was hydrolysed with 10 % hydrochloric acid and the filtrate made up to 25 cc.

Samples of this solution were analysed for amino- and for total nitrogen.

*Kjeldahl estimation.*

5 cc. required 4.55 cc. 0.097 *N* HCl

5 cc. required 4.50 cc. 0.097 *N* HCl

Mean 4.53 cc. 0.097 *N* HCl

Found: N = 1.79 %.

Calculated for lecithin: N = 1.80 %.

(5 cc. gave no nitrogen in Van Slyke's apparatus.)

## THE CAPILLARY METHOD EMPLOYED.

St Johnston [1927] has found that the surface tension of protein solutions varies with  $p_H$  in a manner such as to give a maximum value at the isoelectric point of the protein. He measured the surface tension by Sugden's [1922, 1924] maximum bubble pressure method. This is a dynamic method and consequently gives the value of the surface tension of a freshly formed surface. It is thus sharply distinguished in principle from the results which would be given by a static method in the case of colloidal and dispersed materials which would be likely to show surface aggregation and precipitation.

The surface tensions (measured by Sugden's method), as recorded in this paper, are reproducible within the limit of the experimental error, namely  $\pm 0.5$  % of the value recorded. Some of the dispersions have been purposely kept over considerable periods of time (up to 36 hours) and on measuring the dynamic surface tension the same values have been obtained. Again, after measuring the surface tension at a given  $p_H$ , the  $p_H$  has been altered and ultimately brought back to the initial value. The surface tension has likewise been found to return to the value obtained for a freshly prepared dispersion of lecithin having the same lecithin content. Further, the time taken to form the bubble has been varied between 1 second and 4 seconds; this variation has not produced any change in the value for the surface tension. In view of these observations it is felt that the surface tension values recorded possess a real significance and serve to characterise the surface activity of lecithin dispersions and solutions in respect of their dynamic (but not necessarily their static) behaviour.

The method consists essentially in measuring the pressures required to draw bubbles of air through the solution under investigation, from two tubes of different diameters. The difference between these pressures is substituted in an empirical expression from which the surface tension of the solution is calculated.

As the longest time taken by a bubble to form and break is 4 seconds, any alteration in the surface tension value as a consequence of an electric charge acquired by the air bubble as it is forced through the liquid, is negligible; Currie and Alty [1929] have shown that the time required by an air bubble to acquire its full charge, even in pure water, is not less than 200 seconds.

The apparatus was calibrated with pure benzene and tested against distilled water and found to be accurate to within 0.5 %.

The  $p_H$  values were measured, at 25°, by means of a gold-quinhydrone electrode [Corran and Lewis, 1924]. The surface tension measurements were carried out in a thermostat at 25° ( $\pm 0.05^\circ$ ).

*Surface tension of lecithin dispersed in water.*

Surface tension measurements were carried out on 0.5 % and 0.1 % dispersions of lecithin in water, at various  $p_H$  values. A stock 1 % dispersion, prepared by shaking lecithin with water, was diluted to the requisite concentration with hydrochloric acid or sodium hydroxide solution. The density of each of several of these dispersions was determined and found to deviate very little from unity and consequently the density of the dispersions was taken as unity in calculating the surface tension. Each reading of the pressure difference, used to calculate the surface tension, was the mean of five readings taken at different rates of bubbling from 1 bubble per second to 1 bubble per 4 seconds. As a preliminary a table is given for the surface tension of water and aqueous solutions of hydrochloric acid and sodium hydroxide covering the range of  $p_H$  employed in the case of the lecithin dispersions.

Table I. *Showing the variation of the surface tension with  $p_H$  for acid-alkaline solutions in water at 25°.*

$p_H$	Surface tension (dynes/cm.)	$p_H$	Surface tension (dynes/cm.)
0.01	70.7	3.95	71.6
0.26	70.7	5.80	71.5
1.12	71.3	7.00	71.7
1.39	71.5	7.85	71.7
2.27	71.5		

The values given above form one of the curves of Fig. 1.

Table II. *Showing the variation of the surface tension with  $p_H$  when using 0.5 % aqueous dispersions of lecithin at 25°.*

$p_H$	Surface tension (dynes/cm.)	$p_H$	Surface tension (dynes/cm.)
0.15	66.4	3.07	65.2
0.36	63.9	3.28	62.2
0.80	46.6	3.50	56.2
0.97	42.9	3.80	55.5
1.20	40.6	4.26	64.6
1.46	45.1	4.36	66.4
1.69	56.5	4.63	66.5
1.70	56.6	6.00	66.5
2.14	66.8	6.89	66.5
2.26	67.4	7.80	66.7
2.78	68.6	8.10	66.4
3.00	66.2		

Table III. *Showing the variation of the surface tension with  $p_H$  when using 0.1 % aqueous dispersions of lecithin at 25°.*

$p_H$	Surface tension (dynes/cm.)	$p_H$	Surface tension (dynes/cm.)
0.00	68.5	2.55	71.2
0.40	68.9	2.70	70.8
0.63	68.9	2.82	70.0
0.74	69.0	3.10	67.5
1.14	67.0	3.32	66.1
1.38	58.4	3.51	67.0
1.84	62.3	3.83	68.2
2.00	64.9	4.73	69.6
2.07	65.0	6.85	70.2
2.21	68.8	7.54	70.8
2.34	70.3		

The curves obtained by plotting the surface tension values against the  $p_H$  values for the 0.5 % and the 0.1 % dispersions are shown in the graphs, Fig. 1. It will be noticed that the maximum surface tension occurs at  $p_H$  2.6 in both curves. This value is therefore taken as the isoelectric point of lecithin and it will be seen to agree with the value obtained using alcoholic solutions of lecithin (described later) and with the value 2.7 obtained by Fugii [1923-24] from the distribution ratio of lecithin between water and ether at different  $p_H$  values.

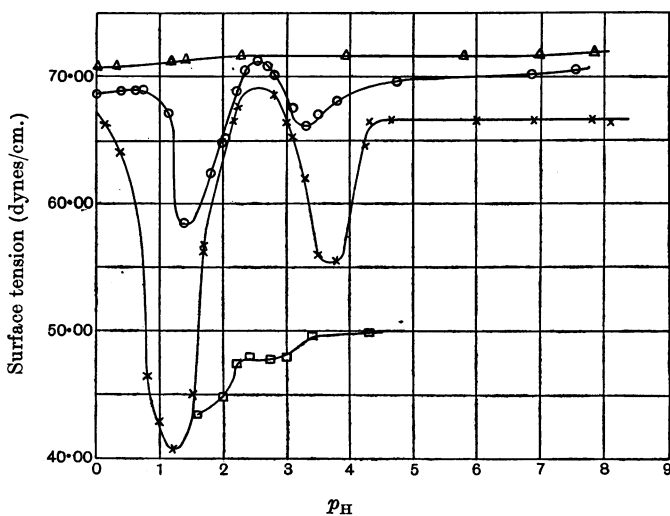


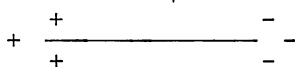
Fig. 1.

—x—x— 0.5 % pure lecithin.      —o—o— 0.1 % pure lecithin.  
 —□—□— 0.5 % commercial lecithin.      —Δ—Δ— Values for water (+ HCl or NaOH).

It is not known with certainty at the present time whether lecithin at the isoelectric point, that is, in the isoelectric condition, exists as electrically uncharged units or in the form of zwitterions [Bjerrum, 1923]. In view of the strength of the acidic grouping it is highly probable that the material exists in the zwitterion form. Owing to the equivalent amounts of positive and

negative electricity on each lecithin unit the surface tension will approximate to that of the solvent, namely 0.0025 *N* hydrochloric acid. Thus at the isoelectric point the surface tension would be expected to be a maximum. Comparison of the curves obtained by St Johnston for proteins shows considerable resemblance to the curve obtained for lecithin. The curves have a maximum surface tension at the isoelectric point with a "secondary maximum" on either side. Thus both curves should be amenable to the same sort of explanation.

If the zwitterion condition be assumed for lecithin at the isoelectric point the  $\gamma$ - $p_H$  curve (where  $\gamma$  stands for the surface tension) may be anticipated on the basis of simple orientation considerations as follows. A single lecithin unit in the isoelectric state may be represented thus:



A row of such units aligned in the surface layer in such a way as to "neutralise" their mutual charges may be represented diagrammatically as in *A*, Fig. 2. Owing to equivalent amounts of positive and negative electricity the surface tension approximates to that of the solvent. The several charges on each group in the figure are supposed to represent the existence of groups of molecules instead of a simple molecule. It is not intended that any significance attaches to the choice of three such charges. The horizontal line in each of the diagrams of the figure is intended to represent the median line of the surface layer.

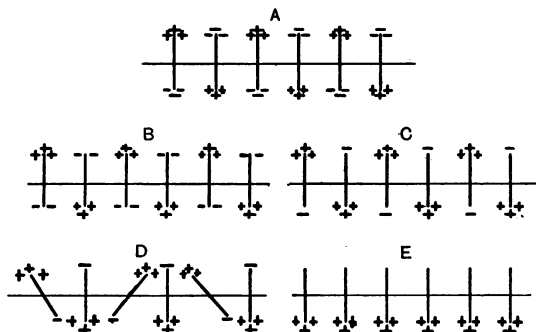


Fig. 2.

If we consider a point slightly on the acid side of the isoelectric point the surface layer has now more positive charges relative to negative than in Fig. 2 *A*. This condition is shown in Fig. 2 *B*. The surface tension is therefore reduced owing to electrical repulsions.

As we go still further on the acid side of the isoelectric point a region is reached where the positive charge largely predominates thus lowering the surface tension to a minimum as shown in Fig. 2 *C*.

Naturally a purely static arrangement is unstable and thermal agitation would bring about such changes as are represented by Fig. 2 *D*.

General experience has shown that ions and electrically charged groupings usually become solvated. There will therefore be a tendency for the state represented in Fig. 2 *D* to alter in the sense that the most highly charged portions will tend to immerse themselves. Thus in the case considered the positively charged groups tend to point downwards into the solution. As the solution becomes more acid the acidic hydroxyl groups in the lecithin unit become practically un-ionised and the configuration approaches that represented in Fig. 2 *E*. All the positively charged groups now point downwards into the solution and the surface tension is affected only by the un-ionised (uncharged) acidic groups. Hence in the attainment of the configuration *E*, the surface tension will rise towards that of the acid solution itself.

In this manner it is suggested that the surface tension first falls and then rises as we pass from the isoelectric point to the more acid region. Exactly analogous considerations may be applied to the  $p_H$  region on the alkaline side of the isoelectric point.

Thus on theoretical grounds one would expect a maximum value of the surface tension at the isoelectric point with a "secondary maximum" on either side. This is what is found in the case of egg-albumin by St Johnston and by the writers in the case of lecithin. In the curve for lecithin, the fact that the minimum values of the surface tension at  $p_H$  1.20 and at  $p_H$  3.70 are not the same is possibly due to the different combining capacities of the acidic and the basic groups in the lecithin molecule.

For the sake of comparison the  $\gamma$ - $p_H$  curve for commercial egg-lecithin from a reputable source has been likewise included in Fig. 1. The nature of the latter emphasises the importance of working with the pure material.

#### *Surface tension of aqueous-alcoholic solutions of lecithin.*

To compare the behaviour of the relatively coarse dispersion of lecithin in water with the finest dispersion possible, experiments were carried out with lecithin dissolved in water rich in alcohol.

Lecithin dissolves in absolute alcohol very readily and as will be shown later it forms a true solution in this solvent. It also dissolves in aqueous-alcoholic solutions (above 65 % alcohol) giving a solution which is water clear. It is probable that the aqueous solutions of lecithin are true solutions, that is, dispersion of the solute has proceeded to the molecular limit.

A stock 2 % solution of pure lecithin in absolute alcohol was prepared and diluted, in the one case to 1.4 % in 70 % alcohol and in another case to 1 % in 75 % alcohol, by adding hydrochloric acid solution.

All surface tension measurements were made in a thermostat at 25° and all  $p_H$  measurements were made at the same temperature using a gold-quinhydrone electrode. However, owing to the varying solubility of quinhydrone in alcohol-water mixtures it was necessary to use a different value of  $E_0$  (in the expression for  $p_H$  in terms of the E.M.F.) from that used in the

case of aqueous solutions. The value of  $E_0$  used was taken from a table of values of  $E_0$  for alcohol-water mixtures given by Millet [1927].

Table IV. *Showing the variation of surface tension with  $p_H$  when using a 1.4 % solution of lecithin in 70 % alcohol at 25°.*

$p_H$	Surface tension (dynes/cm.)	$p_H$	Surface tension (dynes/cm.)
2.11	26.0	3.08	26.9
2.15	26.2	3.14	26.3
2.34	26.6	3.27	26.2
2.57	27.3	3.44	26.1
2.83	26.8	4.03	26.2
2.91	26.2		

Table V. *Showing the variation of surface tension with  $p_H$  when using a 1 % solution of lecithin in 75 % alcohol at 25°.*

$p_H$	Surface tension (dynes/cm.)	$p_H$	Surface tension (dynes/cm.)
2.35	25.6	3.16	25.6
2.49	25.9	3.19	25.4
2.68	25.4	3.58	25.1
2.90	25.5	4.38	25.6
3.07	25.9		

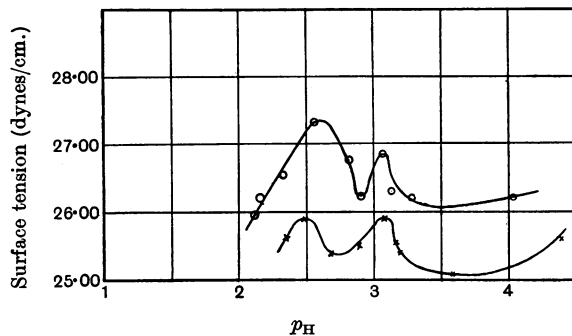


Fig. 3.

—○—○— 1.4 % lecithin in 70 % alcohol. —×—×— 1.0 % lecithin in 75 % alcohol.

The results obtained on plotting surface tension against  $p_H$  values, are shown in the graph, Fig. 3. It will be seen that the maximum surface tension in both curves is at  $p_H$  2.5–2.6, and taking this as the isoelectric point of lecithin it agrees very well with the value obtained with lecithin dispersed in water and with the value 2.7 obtained by Fugii by an entirely different method. It will be observed at the same time that in the presence of alcohol the total variation in the absolute value of the surface tension is very small compared with that found for the dispersions in water; in fact the variations in the case of alcohol are just outside the experimental error of the method employed.

The curves obtained for the variation of the surface tension of lecithin with the  $p_H$  seem at first sight to be contradictory to the behaviour of substances such as the proteins. St Johnston and Peard [1925] found that for surface

tension- $p_{\text{H}}$  curves at 25° the effect of decreasing the concentration of gelatin was to increase the surface tension. In the curves for lecithin the value obtained for the surface tension of the 1.4 % solution was always greater than that obtained for the 1 % solution at the same  $p_{\text{H}}$  value. That this anomaly is only apparent can be understood when it is remembered that the 1.4 % solution was in 70 % alcohol while the 1 % solution was in 75 % alcohol. Firth [1920] and Bircumshaw [1922] using the "drop weight" method and, working on the surface tension of alcohol-water mixtures, found that between 10 % alcohol and 90 % alcohol the surface tension of the mixture decreases progressively as the percentage of alcohol increases. Thus the difference between the surface tension values caused by the differing concentration of lecithin is more than counterbalanced by the differing concentration of the alcohol. Hence the surface tension of the 1.4 % solution of lecithin is greater than that of the 1 % solution at the same  $p_{\text{H}}$  value.

As already mentioned the variation of the surface tension with  $p_{\text{H}}$  in the aqueous-alcoholic media is very limited in range, *i.e.* of the order of 1 dyne. A much wider range was obtained for aqueous dispersions. It is not unlikely that this is due to the fact that in alcoholic solutions the tendency to ionise at all is largely suppressed so that lecithin resembles more closely a non-electrolyte at all  $p_{\text{H}}$  values. It may be concluded however that the type of capillary activity exhibited by lecithin dispersions in water is essentially a quality of the individual molecules composing the aggregates.

*The influence of neutral salts on the surface tension of suspensions of lecithin in water.*

This section of the investigation may be divided into two parts:

- (a) the variation of the surface tension of a 0.5 % dispersion of lecithin with  $p_{\text{H}}$ , in the presence of a constant concentration of salt;
- (b) the variation of the surface tension of a 0.5 % dispersion of lecithin with salt concentration, at constant  $p_{\text{H}}$ .

The surface tensions and  $p_{\text{H}}$  values were measured as before, at 25°.

Table VI. *Showing the effect of 0.1 N sodium chloride on the surface tension of 0.5 % aqueous dispersions of lecithin at various  $p_{\text{H}}$  values.*

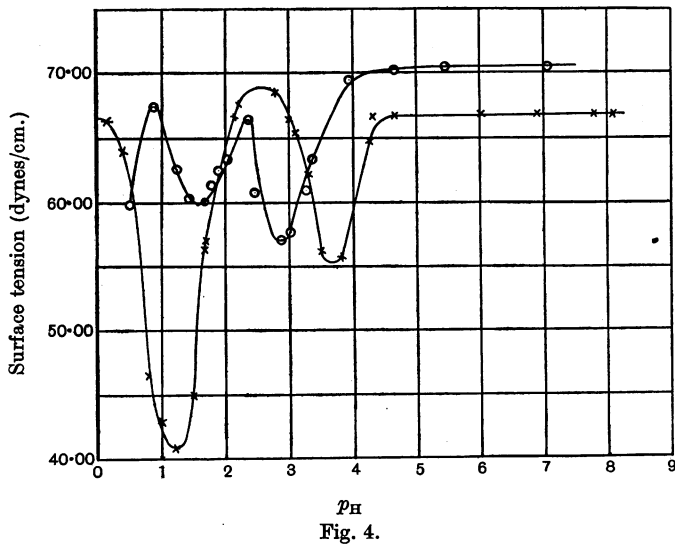
$p_{\text{H}}$	Surface tension (dynes/cm.)	$p_{\text{H}}$	Surface tension (dynes/cm.)
0.51	59.8	2.45	60.6
0.88	67.4	2.88	56.8
1.25	62.6	3.02	57.5
1.45	60.3	3.28	60.8
1.69	60.0	3.36	63.3
1.78	61.3	3.92	69.3
1.92	62.5	4.65	70.0
2.06	63.3	5.45	70.3
2.37	66.4	7.02	70.2

It will be noticed (Fig. 4) that the maximum value corresponding to the isoelectric point in the "salt-free" curve has been moved slightly to the acid side in the presence of salt.



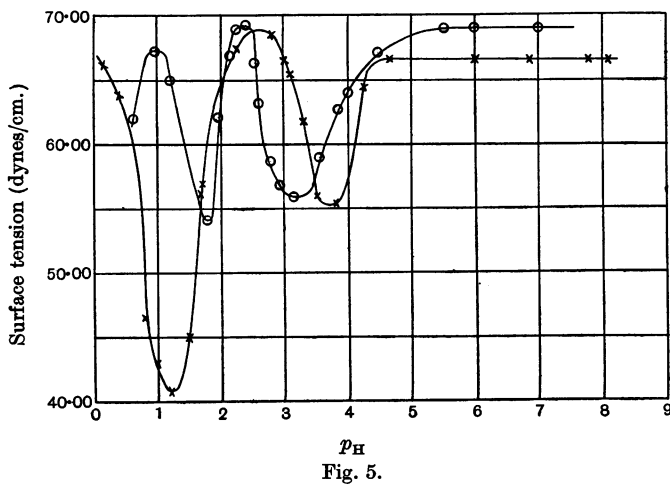
Table VII. *Showing the effect of 0.1 N calcium chloride on the surface tension of 0.5 % aqueous dispersions of lecithin at various  $p_H$  values.*

$p_H$	Surface tension (dynes/cm.)	$p_H$	Surface tension (dynes/cm.)
0.65	62.0	2.74	58.8
0.98	67.3	2.90	56.9
1.22	65.0	3.14	56.0
1.79	54.2	3.56	59.0
1.86	62.8	3.86	62.8
1.95	57.0	4.00	63.7
2.14	66.8	4.50	67.0
2.24	69.0	5.50	69.0
2.41	69.3	6.00	69.0
2.55	66.4	7.01	69.0



—x—x— 0.5 % salt-free lecithin.

—o—o— 0.5 % lecithin + 0.1 N NaCl.



—x—x— 0.5 % salt-free lecithin.

—o—o— 0.5 % lecithin + 0.1 N CaCl<sub>2</sub>.

As in the case of sodium chloride the maximum on the curve indicating the isoelectric point is shifted slightly to the acid side. The magnitude of the shift is the same as that in the case of sodium chloride. This is probably due to the concentration of the chloride ion being the same in both cases. The shift of the maximum is in agreement with the results of other investigators. Cohn [1922], working with the proteins serum-globulin, caseinogen and tuberin, found that "multivalent ions even in low concentration shift the point of maximum precipitation to a hydrogen ion concentration other than the isoelectric point of the protein." He also found that "even uniunivalent salts of the type of sodium chloride in relatively low concentrations may shift the maximum precipitation and therefore the apparent isoelectric point of tuberin and serum-globulin to a slight degree." Schröder [1928], investigating the influence of inorganic ions on dispersions of egg-albumin and haemoglobin at different hydrogen ion concentrations, noted that anions shift the maximum flocculation toward the acid side. Whether, in the case of lecithin, the alteration in the isoelectric point is real or only apparent it is not possible to determine on the evidence thus far available. The results of Tables VI and VII are given in Figs. 4 and 5. For the sake of comparison the  $p_H$  curve in the absence of salt is reproduced in both.

Table VIII. *Showing the effect of calcium chloride on the surface tension of 0.5 % lecithin dispersions at  $p_H$  1.66.*

Salt concentration	Surface tension (dynes/cm.)	Salt concentration	Surface tension (dynes/cm.)
0.000 N	55.0	0.060 N	58.0
0.010	69.3	0.070	58.4
0.026	61.3	0.080	57.2
0.030	60.9	0.090	57.6
0.040	59.5	0.100	57.0

The results are plotted on the graph, Fig. 6.

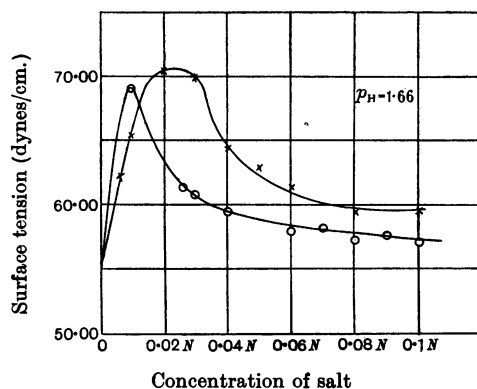


Fig. 6.

—○—○— 0.5 % lecithin + CaCl<sub>2</sub>.

—×—×— 0.5 % lecithin + NaCl.

Table IX. *Showing the effect of sodium chloride on the surface tension of 0.5 % lecithin dispersions at  $p_H$  1.66.*

Salt concentration	Surface tension (dynes/cm.)	Salt concentration	Surface tension (dynes/cm.)
0.000 N	55.0	0.040 N	64.5
0.006	62.3	0.050	63.0
0.010	65.3	0.060	61.4
0.020	70.3	0.080	59.4
0.030	69.9	0.100	59.8

These values are likewise plotted on the graph, Fig. 6.

Table X. *Showing the effect of calcium chloride on the surface tension of 0.5 % lecithin dispersions at  $p_H$  2.40.*

Salt concentration	Surface tension (dynes/cm.)	Salt concentration	Surface tension (dynes/cm.)
0.000 N	68.5	0.050 N	62.8
0.010	69.6	0.060	61.7
0.020	71.4	0.070	64.8
0.030	67.8	0.080	69.0
0.040	69.7	0.100	69.3

The above results were plotted and the curve shown in Fig. 7 obtained.

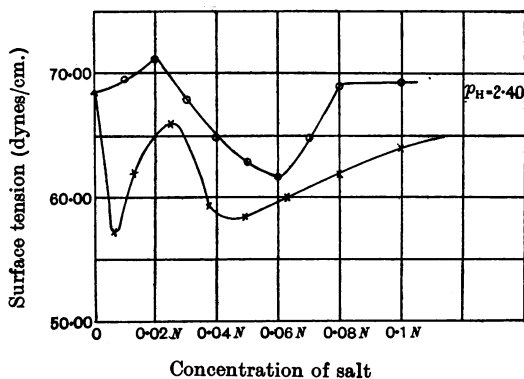


Fig. 7.

—○—○— 0.5 % lecithin + CaCl<sub>2</sub>.      —×—×— 0.5 % lecithin + NaCl

Table XI. *Showing the effect of sodium chloride on the surface tension of 0.5 % lecithin dispersions at  $p_H$  2.40.*

Salt concentration	Surface tension (dynes/cm.)	Salt concentration	Surface tension (dynes/cm.)
0.000 N	68.5	0.050 N	58.6
0.006	57.3	0.063	60.2
0.013	62.0	0.080	61.8
0.025	66.0	0.100	64.0
0.037	59.3		

These values are likewise plotted on the graph, Fig. 7.

Table XII. *Showing the effect of calcium chloride on the surface tension of 0.5 % lecithin dispersions at  $p_H$  4.50.*

Salt concentration	Surface tension (dynes/cm.)	Salt concentration	Surface tension (dynes/cm.)
0.000 N	66.5	0.060 N	68.9
0.010	61.3	0.070	66.3
0.020	62.3	0.080	64.0
0.040	63.9	0.100	67.0
0.050	66.4		

These results, when plotted, gave the curve shown in Fig. 8.

 Table XIII. *Showing the effect of sodium chloride on the surface tension of 0.5 % lecithin dispersions at  $p_H$  4.50.*

Salt concentration	Surface tension (dynes/cm.)	Salt concentration	Surface tension (dynes/cm.)
0.000 N	66.5	0.060 N	60.0
0.010	68.2	0.066	59.0
0.020	69.4	0.080	62.8
0.040	68.8	0.100	69.9
0.050	64.2		

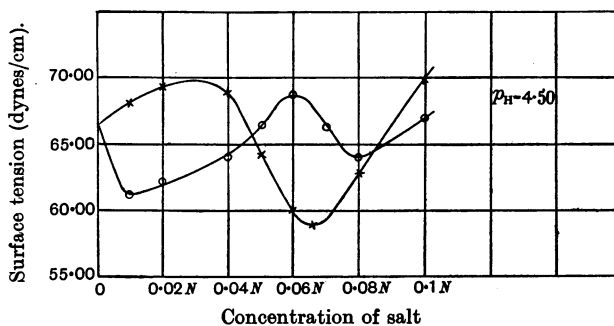


Fig. 8.

—○—○— 0.5 % lecithin +  $\text{CaCl}_2$ .

—×—×— 0.5 % lecithin +  $\text{NaCl}$ .

These values are likewise plotted on the graph, Fig. 8.

It is evident that the effects produced by simple salts upon the surface tension of lecithin suspensions is far from negligible and at the same time is of a much more complex character than might have been anticipated. It would be altogether premature at the present stage of the work to attempt an explanation of the effects. Doubtless they are associated with the "chemical" change of the lecithin from the state of cation, through zwitterion, to anion, and at the same time with "physical" changes in the structure and hydration of the micelles. On comparing Figs. 6, 7 and 8 it will be seen that on the acid side of the isoelectric point the curves for sodium chloride and calcium chloride resemble one another to a certain extent. This might be expected in view of the fact that at this region the lecithin is positively charged and therefore the anion of the salt, namely the chloride ion in both cases, is probably operative.

On the other hand, on the alkaline side of the isoelectric point lecithin is negatively charged and therefore the salt cations are likely to be involved. The antagonistic effect of  $\text{Ca}^{++}$  and  $\text{Na}^+$  is indicated in Fig. 8.

*The molecular weight of lecithin.* Applying Menzies's [1921] differential thermometer to ebullioscopic measurements after the manner of Menzies and Wright [1921], the molecular weight of pure lecithin has been determined in ethyl alcohol and in benzene. The molecular weight of commercial lecithin was also determined in both solvents. It may be mentioned that the manipulation of lecithin for the molecular weight determinations presents certain difficulties. It was found convenient to weigh the substance in tubes of very thin glass, open at both ends to facilitate dissolution in the solvent.

In ethyl alcohol the molecular weight of pure lecithin was found to be 797, that of commercial lecithin 766. Scott [1916], using commercial lecithin and the ordinary rise-in-boiling-point method, found the molecular weight in ethyl alcohol to be consistently 1300, and he speaks of polymerisation in view of his results with glucose.

In benzene the molecular weight of pure lecithin was found to be 3388 while the value for commercial lecithin was 2994, showing polymerisation in this solvent.

The value of 797 for the molecular weight of lecithin in ethyl alcohol lends support to the simpler of the two possible molecular structures suggested by Levene and Rolf [1921].

#### SUMMARY.

1. Pure egg-lecithin has been prepared by the method of Levene and Rolf.
2. The capillary behaviour of egg-lecithin has been studied and the surface tension- $p_{\text{H}}$  curves plotted for two concentrations in the case of aqueous dispersions and for two concentrations in the case of the aqueous-alcoholic solutions.
3. From the surface tension- $p_{\text{H}}$  curves the isoelectric point of lecithin has been determined and the value obtained when using aqueous dispersions is similar to that obtained when using aqueous-alcoholic solutions, namely 2.6. This value also agrees fairly well with Fugii's value of 2.7 for the isoelectric point of lecithin.
4. An explanation of the surface tension- $p_{\text{H}}$  curves, on the basis of simple orientation considerations has been offered.
5. The effects produced by the presence of salts such as sodium chloride and calcium chloride upon the surface tension- $p_{\text{H}}$  relation for dispersions of lecithin have been measured. The effects are of an unexpectedly complex character.
6. The molecular weight of egg-lecithin has been found to be 797 in ethyl alcohol and 3388 in benzene. The value in alcohol serves to discriminate between the two possible structures suggested by Levene and Rolf.

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