XLIII. THE SURFACE TENSION OF GELATIN SOLUTIONS.

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IN the course of some experiments on a vegetable protein, a marked variation of the surface tension of its solution with changing hydrion concentration was observed. Reference to the literature showed that beyond the well-known surface tension lowering effect of proteins and other colloids, the few observations that have been made have usually taken no account of the hydrion concentration.

It was therefore decided to investigate the effect of the p_H on the surface tension of gelatin solutions, in order to see if the surface tension varied in a similar manner to the other physical properties with change of p_H . Gelatin was used as it can be easily obtained in a relatively pure state and its other physical properties are well known.

Reynolds [1921] gave the value 71.1 dynes/cm. for a fresh 0.5 % gelatin solution as determined by the capillary rise method; this fell to 65.5 dynes/cm. on standing 24 hours, which he attributed to increased surface concentration; he also determined the interfacial tension of gelatin solution against benzene by the drop method.

Mardles [1924] determined the surface tension of gelatin in water and other solvents at various temperatures. The only investigation taking account of the p_H that we can find is a recent paper by Davis, Salisbury and Harvey [1924], who studied various commercial gelatins and gelatin rendered ash-free by Loeb's method, using the drop number method of determining surface tension. Different curves were obtained for various gelatins, the most marked variations with p_H being shown with the ash-free gelatin. This gave a maximum surface tension at about p_H 3.4. They also studied the effects of concentration, time and temperature at constant p_H . No explanation was advanced by them of the changes observed.

The surface tension between the particle and the medium, as an important factor in the stability of colloids, is considered in the theories of Bredig [1901], Donnan [1901], Freundlich [1922], von Weimarn [1911] and others; further references to the literature are given in the *Fifth Report on Colloid Chemistry* [1923].

At the isoelectric point the charge on the particle is a minimum, hence,

according to the Lippman effect, the surface tension should be a maximum. Burton and Wiegand [1912] have, however, shown that an electrical charge does not affect the surface tension *per se*, but in general acts so as to oppose the effect of the surface tension.

Loeb [1922] has shown that Donnan's theory of membrane equilibrium explains the colloidal properties of gelatin, and in particular the membrane potentials. Thus we should expect to find some relationship between the P.D.- p_H curves and the surface tension- p_H curves for gelatin.

EXPERIMENTAL.

The measurement of the surface tension was made by Sugden's maximum bubble pressure method [1922, 1924]. This was found to be convenient, rapid and reasonably accurate, besides being unaffected by vibration.

Reynolds [1921] has shown that a dynamic method is desirable for colloids, otherwise the surface tension changes with age owing to increasing concentration in the surface layer; hence the surface tension by a static method falls on standing.

The apparatus was calibrated with pure benzene and was accurate to within 0.3 % for pure liquids, but probably less accurate for colloidal solutions.

When not in use the apparatus was kept filled with either nitric or chromic acid; it was washed with tap water and distilled water before use, and between each determination it was washed with warm nitric acid, and subsequently with tap and distilled water. Similarly all vessels used were pickled in chromic acid, then washed with tap and distilled water and drained dry. These precautions were necessary to ensure freedom from grease.

The gelatin used was Nelson's Gold Label powdered, of an original ash content of 1.5 % and p_H about 5-6. To render it ash-free a modification of Loeb's method of washing was adopted as being more convenient. 15 g. of powdered gelatin were soaked for 24 hours with one change in 500 cc. of a sodium acetate-acetic acid buffer mixture of p_H 4.7 and concentration $N/50$, then for a further 24 hours with distilled water with one change. The temperature was kept below 5° during the soaking. The gelatin was finally washed by decantation and at the pump on a Buchner funnel with 2-3 litres of distilled water, keeping the temperature as low as possible, usually below 8°. The washed gelatin was dissolved in about 300 cc. of distilled water by immersing the beaker in a water-bath kept at 52° for half-an-hour exactly, in which time the gelatin completely dissolved and the temperature of its solution rose to 50°. The warm solution was filtered to remove any undissolved particles, and the concentration was determined by evaporating a portion in a platinum dish in a steam oven for 12 hours by which time the weight had become constant. The dried gelatin in the dish was ashed. The ash content by this method was found to be between 0.08 and 0.13 % on the dry gelatin, which is equivalent to a salt concentration of about $M/30,000$, calculating as $Ca_3(PO_4)_2$, in a 1 % solution of gelatin..

The gelatin solution was just remelted in the 52° water-bath and diluted to make the 2 % stock solution, about 500 cc. being obtained. This also set to a gel and each time before use it was just melted in the water-bath, then rapidly cooled to 25° and the required amount withdrawn in a pipette calibrated for that temperature. 15 cc. of the 2 % gelatin solution were added to 15 cc. of solution containing the requisite amount of *N*/10 acid or alkali and distilled water, contained in a conical flask fitted with a rubber bung washed free of grease with spirit. A drop of toluene was added as a preservative, as this was found to have no effect on the surface tension. The solutions were kept for 24 hours in an oven at 25° before determination, in order to allow complete equilibrium to be established. The surface tension was then determined at 25°. In the case of the determinations at 40°, the solutions were only kept an hour at that temperature before determination.

In view of the effect of pretreatment of the gelatin on its physical properties, the above routine was strictly adhered to, although it was later found that the surface tension does not vary with time to an appreciable extent once equilibrium has been established, except in the case of isoelectric gelatin.

Fig. 1. Titration curve of 0.4 % and 1.0 % gelatin.

THE EFFECT OF HYDROGEN-ION CONCENTRATION.

The hydrogen-ion concentrations were determined colorimetrically, using Walpole and Sørensen's standards and a Cole and Onslow's comparator, and were accurate to 0.1 p_H . The titration curve, cc. *N*/10 acid or alkali in 30 cc. against p_H , was plotted as in Fig. 1. The results above p_H 7.5 were only approximate.

In the determination of the surface tension by Sugden's bubbler, small jets giving a pressure difference of between 7 and 13 cm. alcohol were used, and the bubble rate was usually one bubble per 2–3 seconds on the small jet. The mean of 5 or 6 readings at various bubble rates of about that order was taken as the required value. According to Sugden too slow a rate would give lower values as well as allowing minor leaks in the apparatus to take effect. Too fast a rate (greater than 1 per second) led to excessive frothing and too high values. The determination of the surface tension of the isoelectric gelatin was found the most troublesome. As will be seen from the time curves in Fig. 4, 1 % gelatin p_H 4.7 gradually flocculated in a varying time with different samples, no doubt owing to different ash content or absorbed CO_2 or NH_3 .

from the air; the surface tension therefore was not determined within 24 hours but after flocculation had occurred, usually in 36–48 hours, though sometimes longer. Owing to the presence of solid particles, a permanent froth was formed which added to the difficulty of determination, and also the values fell on prolonged bubbling, again possibly owing to absorption of CO_2 or NH_3 from the air passing through. From the sharpness of the curves at p_{H} 4.7 in Fig. 2 this fall of surface tension is easily understood.

The results for 1 % gelatin in presence of HCl , H_2SO_4 or NaOH at 25° and for gelatin in presence of HCl or NaOH at 40° are shown in Fig. 2, all showing a uniform sharp variation of surface tension with p_{H} , particularly on the acid side.

In view of recent work by Knaggs, Manning and Schryver [1923] on the further purification of gelatin by electrolysis and recrystallisation, a sample of gelatin of ash content 0.08 % was electrolysed according to their method.

Fig. 2. Variation of surface tension with p_{H} of 0.4 % and 1.0 % gelatin solutions at 25° and 40° .

$\times$	0.4 %	gelatin in presence of HCl or NaOH	at 25°
$\bullet$	1.0 %	" " " "	25°
$\circ$	1.0 %	" " " "	40°
$\triangle$	1.0 %	" " H_2SO_4	at 25°

We are indebted to Mr R. W. W. Sanderson, of the General Electric Co., for carrying out this electrolysis. The resulting gel was ash-free and an attempt was made to recrystallise it from a 1 % solution, but possibly owing to absorption of CO_2 or NH_3 from the air, it did not flocculate completely and the recrystallised gelatin was difficult to separate either by centrifuging or filtering. The separated gelatin was redissolved, giving a 0.8 % stock solution. The surface tension of the resulting 0.4 % gelatin in presence of HCl or NaOH at 25° is also shown in Fig. 2.

As electrical facilities were not available on the spot, it was not possible to flocculate electrically according to the latest method of Knaggs and Schryver [1924].

THE EFFECT OF NEUTRAL SALTS AND NON-ELECTROLYTES.

As typical salts of uni- and bi-valent acids NaNO_3 and Na_2SO_4 were used, and as a typical non-electrolyte glucose (99 % pure). These determinations were carried out at p_{H} 3.3, and the solutions were prepared either by directly

weighing out the required amount of salt or sugar and dissolving in 4 cc. $N/10$ HCl + 11 cc. distilled water, or else by making up a solution and diluting so that 11 cc. of it together with 4 cc. HCl gave the final required concentration in 30 cc. The surface tension at p_H 3.3 with no additions was the mean of three separate determinations. The p_H of the solutions was found to fall gradually from p_H 3.3 to p_H 3.15 with increasing salt concentration up to $M/16$.

As will be seen from Fig. 3 the surface tension falls with increasing salt concentration and more for Na_2SO_4 than for $NaNO_3$. The effect of the fall of p_H would tend to raise the surface tension, as can be seen by consideration of the curve for gelatin in HCl in Fig. 2. The non-electrolyte glucose had very little effect even in concentration $M/4$ —the slight lowering being probably due to the ash content of the glucose.

Fig. 3. Effect of neutral salts and of glucose on surface tension of 1 % gelatin in presence of HCl at p_H 3.3 and 25° .

THE EFFECT OF TIME.

Although the results in Figs. 2 and 3 were all obtained under uniform conditions, the effect of time on the surface tension was studied in one or two cases. The determinations were started immediately the gelatin had been dissolved and filtered.

Equilibrium was apparently reached at p_H 3.3 in an hour or so, and then remained constant within experimental limits for the several days over which it was observed. With isoelectric gelatin, the surface tension rose gradually from a low value of about 66 dynes/cm. up to 71.5 in 72 hours, *i.e.* approaching that of water, as flocculation proceeded. In other cases where no previous readings of the surface tension were taken it had risen very nearly to 72 in 36–48 hours, when complete flocculation had occurred. The results are shown in Fig. 4.

DISCUSSION OF RESULTS.

Considering first the surface tension- p_H curves at 25° in Fig. 2, it will be seen that the effect of decreasing the concentration of gelatin from 1 % to 0.4 % is to increase the surface tension as would be expected. All the curves at 25° show a maximum at p_H 4.7, as was expected, values approaching that of water being obtained. This corresponds to a very low P.D. and solubility

and a maximum of neutral un-ionised particles at the isoelectric point, but presumably there is some dissociation there, causing a very slight lowering of surface tension. The curve at 40° differs from those at 25° in that the surface tension at the isoelectric point is considerably lower than that of water at 40° and that there is no sharp maximum. There is evidently considerably more gelatin in solution at that temperature, and presumably more dissociation into electrically equal amounts of positive and negative ions. Pauli [1921] has shown from mathematical considerations that this would be expected.

The 40° curve shows a marked flattening between p_H 4.6 and 6.0, probably due to the above increased dissociation; the dissociation constants of positive and negative dissociation may increase at different rates with temperature rise, and from the expression $C_H = \sqrt{\frac{K_a K_b}{K_w}}$ for the hydron concentration of the isoelectric point, where K_a , K_b and K_w are the dissociation constants of positive and negative dissociation of the protein and of water respectively,

Fig. 4. Effect of time on surface tension of gelatin at 25°.

we should expect a small change in the p_H of the isoelectric point with temperature. Our results are not accurate enough to determine this, beyond the marked flattening, except that, whereas at 25° the surface tension of isoelectric gelatin was found to be practically independent of concentration, as is to be expected theoretically from the calculations of Pauli, Michaelis and Sørensen, at 40° the surface tension at p_H 4.7 was found to vary with the concentration values:

1 % gelatin,	surface tension = 66.1 dynes/cm.	
2 " " "	" " " " = 64.4 " "	
6 " " "	" " " " = 62.3 " "	

This may be an indication that the isoelectric point at 40° is not exactly at p_H 4.7.

All the curves for gelatin in presence of HCl show a sharp point of minimum surface tension at p_H 3.8, and for gelatin in presence of H_2SO_4 at p_H 3.9–4.0. This agrees well with Loeb's calculation and determination of the maximum P.D. of 1 % gelatin at p_H 3.8–4.0. The P.D. of gelatin in presence of H_2SO_4 is only two-thirds that of gelatin in presence of HCl, though no difference in the

lowering of surface tension was found by us, and, as will be seen later from the effect of neutral salts, the surface tension lowering cannot be considered as entirely dependent on the P.D. Loeb further shows that the P.D. decreases steadily from this maximum value at p_H 3.8–4.0 down to p_H 1. We, however, find a well-marked maximum value again for the surface tension at about p_H 3.0, after which the surface tension again falls rapidly with increased acid concentration. Davis, Salisbury and Harvey show a similar maximum about p_H 3.4. This may be due to a change in the gelatin molecule with formation of "acid protein." This conclusion is supported by the titration curves shown in Fig. 1, which agree with those of other workers in showing a sharp change of direction about p_H 2.5, indicating increased combination of acid. Henderson, in Osborne's *The Vegetable Proteins* (1923), shows that at p_H 1–2, and again at p_H above 10, the titration curves of proteins no longer agree with those required by theory and attributes this to changes in the molecule in more concentrated acid or alkali. Pauli finds that other proteins with strong acids are most sensitive to coagulating agents such as alcohol at a p_H of about 3.0. According to Bredig, Burton [1923] and others, a high surface tension would be conducive to precipitation. Surface tension may show this change in the molecule before the titration curves do, as it is obviously very sensitive to small changes; hence our maximum occurs earlier, about 2.8–3.0. On the other hand, the drop in surface tension after this maximum may be due to increased dispersion brought about by peptisation. Further experiments are necessary to find out what is occurring in this region.

On the alkaline side a minimum surface tension is observed at a p_H of about 9–10, which agrees with Loeb's observed and calculated values for maximum P.D. and also for swelling of gelatin in NaOH solutions.

The results obtained by the addition of neutral salts to gelatin in presence of HCl are entirely unexpected. The Donnan theory of membrane equilibrium demands that such addition should depress the P.D. of the gelatin and the more so the greater the concentration of added salt. Moreover, the depression of the P.D. caused by a salt of a univalent acid, such as sodium nitrate, should be only two-thirds that produced by equimolar amounts of a salt of a bivalent acid, such as sodium sulphate. If surface tension is influenced by the P.D., depression of the latter should lead to a rise in surface tension. We find, however, as will be seen from Fig. 3, that the addition of a neutral salt produces a depression of the surface tension very nearly proportional to the concentration of the added salt. Curiously enough the depression produced by a salt of a univalent acid is approximately two-thirds of that produced by a salt of a bivalent acid when the concentration of the added salt exceeds $M/256$. Berczeller [1917] found that the addition of salts lowered the surface tension of protein solutions. Davis, Salisbury and Harvey similarly found that the surface tension of ash-free gelatin was much higher than that of a commercial gelatin containing mineral impurities.

The addition of a non-electrolyte, such as glucose, does not alter the

surface tension of the sol except for a slight depression due to the mineral impurities of the glucose. Since it can play no part in the Donnan equilibrium, this is to be expected.

Recent work by Manning [1924] on electrically flocculated isoelectric gelatin has shown that this may be peptised or dispersed to a stable gel by very small concentrations of acid and alkali, whilst relatively larger concentrations of neutral salts will produce the same effect. Evidently the addition of neutral salts tends to increase dispersion of the gelatin, making relatively more gelatin in the sol form. This affects the surface tension, causing a lowering.

The surface tension-time curves in Fig. 4 show that the rapidly cooled gelatin solution has a low surface tension, which at p_H 3.3 rises within an hour or so to its equilibrium value, which it then maintains within experimental limits over the period observed. Gelatin at the isoelectric point similarly starts at a low value, which rises gradually as the solution changes over to the gel form and flocculates, when a maximum surface tension near that of water is reached. Smith [1919] has shown by following the mutarotation that there is an equilibrium between the sol and gel forms of gelatin between the temperatures of 15° and 35° depending on the temperature.

Evidently then anything which increases the amount of the sol form in the solution, whether it be rise of temperature, presence of acid or alkali, or neutral salts, will cause an increased lowering of surface tension. Donnan's membrane equilibrium theory apparently accounts quantitatively for the ionisation of ash-free gelatin and for the membrane potentials observed by Loeb, but as far as surface tension is concerned, the other factors, such as peptisation of the neutral gelatin particles by neutral salts, and strong acids in higher concentration, and the physical state of the gelatin as influenced by temperature and time must also be taken into account. Further, the effect of strong acids at concentrations giving a p_H less than 3 is more marked on the surface tension than on any other property, though as to what actually occurs further investigation is required. It will be seen then that it is not possible in our present state of knowledge of the relationship of the surface tension of solutions to the many factors affecting it, to make any generalisations, except that lowering of surface tension is coincident with increased dispersion and stability of the colloid, and that a high surface tension tends to coagulation. As a means of determining the isoelectric points of proteins whose other properties are difficult of determination, the method may be useful, provided distinction is possible between the true isoelectric point and the maximum produced at a higher acid concentration.

Further work with other proteins is being undertaken in this direction.

SUMMARY.

1. No direct quantitative relationship could be traced between the surface tension of a colloidal solution and the potential difference between the particles and the medium.

2. The surface tension of gelatin solutions is found to be a maximum at the isoelectric point (p_H 4.7); a minimum at p_H 3.8–4.0 on the acid side, and in the neighbourhood of p_H 9 on the alkaline side. A second maximum is found at p_H 2.8–3.0, after which the surface tension decreases steadily. It is suggested that this second maximum marks the point at which the formation of "acid-gelatin" begins.

3. Increase of temperature and addition of electrolytes both cause a lowering of the surface tension of gelatin solutions. This indicates that increased solubility or peptisation of the gelatin lowers the surface tension.

4. The surface tension of gelatin solutions is little affected by time after equilibrium is attained, which occurs in a few hours at 25°, except in the case of isoelectric gelatin, whose surface tension slowly rises nearly to that of water as flocculation proceeds.

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