CIII. THE SURFACE TENSION OF GELATIN SOLUTIONS. (PART II.)

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(Received July 2nd, 1926.)

IN a previous communication [1925] it was shown that the surface tension of ash-free gelatin solutions varied with the p_H of the solution, giving a maximum at the isoelectric point and another maximum about p_H 3.0. Further work was undertaken in an endeavour to find the cause of the fall of surface tension in the more acid solutions, which was not expected from consideration of the other physical properties of gelatin, or from Donnan's membrane equilibrium theory.

de Caro [1925] has confirmed the existence of this second maximum of surface tension at p_H 3.0 in an investigation of the effect of p_H on the drop numbers of 2 % gelatin at 30° as determined with the Traube stalagmometer, but contrary to our results he found the surface tension a minimum at the isoelectric point, and therefrom draws the conclusion that the surface tension is most lowered by the undissociated protein molecule. That this cannot be the case we have previously shown both theoretically and practically and many subsequent determinations have confirmed that the surface tension of isoelectric gelatin solutions rises from an initial low value very nearly to that of water as flocculation proceeds, so that it seems probable that de Caro did not allow sufficient time for flocculation to occur before determining the drop number. This effect of time is less marked at other values of p_H , but all our determinations were made after the solutions had stood at 25° for 24 hours, or when completely flocculated in the case of isoelectric gelatin.

The values for the p_H of our gelatin solutions and also those of de Caro were determined colorimetrically. On checking these values electrically with the quinhydrone and hydrogen electrodes we find the colorimetric method is affected by a larger protein error than was anticipated, and there is a considerable discrepancy, varying with the p_H , between the methods. Consequently it has been necessary to correct our previous surface tension- p_H results to some extent as regards p_H .

The existence of a "protein error" in determining the p_H of protein solutions by means of indicators has been pointed out by Sørensen [1909, 1912], Kolthoff [1921], Clark [1922] and others. Although we find the magnitude

of the difference between the electrical and colorimetric values of the p_H to vary with the state of dissociation of the protein in apparently a regular manner, in view of the complexity of the problem due to other possible sources of error, such as the use of two-colour indicators in the standards, we consider it best merely to refer our results to the experimental values determined electrically, as advised by Clark [1922], for the particular protein and indicators investigated.

As a result of the electrical determination of p_H , it will be seen that the second maximum of surface tension is at a point more acid than originally found, namely about p_H 2.2 instead of about p_H 3.0. This seemed to encourage the idea that the further fall of surface tension in solutions of higher acidity might be due to some effect of the strong acid on the protein, such as hydrolysis or denaturation. An attempt was therefore made to detect hydrolysis by means of formaldehyde titrations for amino-acid, but definite evidence that it was due to hydrolysis of the protein with formation of amino-acids was not obtained.

EXPERIMENTAL.

The gelatin, Nelson's Gold Label powdered, was rendered ash-free as previously described [1925] and in all cases contained less than 0.1 % ash in the dry gelatin.

The surface tension was determined by Sugden's maximum bubble pressure method [1922, 1924]. The colorimetric determinations of p_H were carried out as previously described and the standard buffer solutions checked electrically.

The electrical determinations of p_H were made with the quinhydrone electrode at 20° below p_H 7.5 and above that with the hydrogen electrode. We are indebted to Professor A. R. Ling for the use of the hydrogen electrode.

The discrepancy between the electrometric and colorimetric determinations of the p_H of gelatin solutions.

Solutions of 1 % and 0.4 % gelatin with varying amounts of $N/10$ HCl or $N/10$ NaOH were prepared from a 2 % stock solution of isoelectric gelatin and the p_H determined by both methods after allowing to stand at 25° for 24 hours to ensure equilibrium being attained.

Electrometric determinations were repeatable within 0.02 p_H but colorimetric determinations of the apparent p_H were only readable to 0.05 and varied with the observer owing to the difficulty of colour matching, hence the given values are the mean of several independent observations. Owing to the absence of electrolytes it was difficult to obtain a good reading electrometrically for the isoelectric point. The results for 1.0 % and 0.4 % gelatin are given in Table I. Fig. 1 shows the discrepancy, or "protein error," plotted against the p_H as determined electrometrically; the error is very nearly the same in the case of the 0.4 % as in the 1.0 % solution when bromophenol blue is the indicator, but differs somewhat with the concentration for thymol blue.

Table I. *The p_H of gelatin solutions determined colorimetrically and electrometrically.*

A. 1 % gelatin solution.

cc. $N/10$ HCl in 100 cc.	Indicator	Colorimetric p_H	Mean	Electro- metric p_H	Discrepancy (colorimetric - electrometric)
0	Bromophenol blue	$\begin{Bmatrix} 4.7 \\ 4.75 \\ 4.8 \end{Bmatrix}$	4.75	4.86	-0.11
1.7	"	$\begin{Bmatrix} 4.4 \\ 4.4 \\ 4.45 \end{Bmatrix}$	4.42	4.33	+0.09
3.3	"	$\begin{Bmatrix} 4.2 \\ 4.3 \end{Bmatrix}$	4.25	4.03	+0.22
5.0	"	4.2	4.2	3.65	+0.55
6.7	"	$\begin{Bmatrix} 4.00 \\ 3.95 \\ 4.05 \\ 3.95 \\ 4.05 \end{Bmatrix}$	4.0	3.27	+0.73
10.0	"	$\begin{Bmatrix} 3.6 \\ 3.7 \\ 3.65 \\ 3.55 \end{Bmatrix}$	3.6	$\begin{Bmatrix} 2.74 \\ 2.74 \end{Bmatrix}$	+0.86
13.3	"	$\begin{Bmatrix} 3.3 \\ 3.25 \\ 3.2 \\ 3.25 \end{Bmatrix}$	3.25	$\begin{Bmatrix} 2.36 \\ 2.38 \end{Bmatrix}$	+0.88
16.7	"	$\begin{Bmatrix} 3.0 \\ 2.9 \\ 2.8 \\ 3.0 \\ 3.0 \end{Bmatrix}$	2.95	$\begin{Bmatrix} 2.11 \\ 2.12 \end{Bmatrix}$	+0.83
"	Thymol blue	2.75	2.75	"	+0.63
23.3	"	$\begin{Bmatrix} 2.3 \\ 2.3 \end{Bmatrix}$	2.3	1.87	+0.43
33.3	"	$\begin{Bmatrix} 2.0 \\ 1.9 \end{Bmatrix}$	1.95	1.64	+0.31
50.0	"	$\begin{Bmatrix} 1.75 \\ 1.7 \\ 1.7 \end{Bmatrix}$	1.71	1.43	+0.28
cc. $N/10$ NaOH in 100 cc.					
0.8	Bromocresol purple	$\begin{Bmatrix} 5.7 \\ 5.6 \end{Bmatrix}$	5.65	5.49	+0.16
1.7	$\begin{Bmatrix} \text{"} \\ \text{Bromothymol blue} \end{Bmatrix}$	$\begin{Bmatrix} 6.3 \\ 6.1 \end{Bmatrix}$	6.2	6.15	+0.05

B. 0.4 % gelatin solution.

cc. $N/10$ HCl in 100 cc.	Indicator	Colorimetric p_H	Mean	Electro- metric p_H	Discrepancy (colorimetric - electrometric)
0	Bromophenol blue	$\begin{Bmatrix} 4.8 \\ 4.7 \end{Bmatrix}$	4.75	4.86	-0.11
0.8	"	$\begin{Bmatrix} 4.45 \\ 4.40 \end{Bmatrix}$	4.425	4.21	+0.215
1.7	"	$\begin{Bmatrix} 4.25 \\ 4.2 \end{Bmatrix}$	4.225	3.82	+0.405
3.3	"	$\begin{Bmatrix} 4.0 \\ 3.75 \end{Bmatrix}$	3.875	3.22	+0.655

Table I (*continued*).

cc. $N/10$ HCl in 100 cc.	Indicator	Colorimetric p_H	Mean	Electro- metric p_H	Discrepancy (colorimetric - electrometric)
6.7	Bromophenol blue	$\begin{Bmatrix} 3.5 \\ 3.5 \end{Bmatrix}$	3.5	2.76	+0.74
8.3	"	$\begin{Bmatrix} 3.3 \\ 3.25 \end{Bmatrix}$	3.275	2.38	+0.895
10.0	"	$\begin{Bmatrix} 3.0 \\ 2.9 \end{Bmatrix}$	2.95	2.20	+0.75
"	Thymol blue	2.8	2.8	"	+0.60
13.3	"	$\begin{Bmatrix} 2.5 \\ 2.3 \end{Bmatrix}$	2.4	2.06	+0.34
16.7	"	$\begin{Bmatrix} 2.2 \\ 2.0 \end{Bmatrix}$	2.1	1.93	+0.17
20.0	"	$\begin{Bmatrix} 2.05 \\ 1.9 \end{Bmatrix}$	1.975	1.81	+0.165
23.3	"	$\begin{Bmatrix} 1.9 \\ 1.8 \end{Bmatrix}$	1.85	1.76	+0.09
33.3	"	1.6	1.6	1.57	+0.03
cc. $N/10$ NaOH in 100 cc.					
0.3	$\begin{Bmatrix} \text{Bromothymol blue} \\ \text{Bromocresol purple} \end{Bmatrix}$	$\begin{Bmatrix} 5.9 \\ 5.7 \end{Bmatrix}$	5.8	5.38	+0.42
0.6	"	$\begin{Bmatrix} 6.8 \\ 6.9 \end{Bmatrix}$	6.85	6.70	+0.15

In the colorimetric determinations, the matching of the gelatin solutions with the standards was rendered difficult by the difference in quality of the light transmitted through the gelatin solution and indicator from that transmitted through the standard and indicator, although the latter was compensated with another tube of the gelatin solution without indicator placed in front of it. The use of compensating tubes helped to overcome in part the difficulty with a dichroic indicator such as bromophenol blue due to the different absorption of the two colours by the solution, which thus alters the quality of the transmitted light according to the thickness of solution traversed. In the case of a turbid solution this is particularly noticeable as light which has entered from the side of the tube is reflected from the particles, thus all the transmitted light has not traversed the same thickness of solution. Except in the case of isoelectric gelatin, the solutions of both 0.4% and 1.0% gelatin were apparently optically clear, so any such reflection should be small and would be greatest near the isoelectric point. Such changes of shade certainly seemed most noticeable with bromophenol blue near the isoelectric point, but the colour change of the indicator with p_H is very marked about there, which considerably aided the matching. Reference to Fig. 1 will show that the discrepancy is least about the isoelectric point.

The use of a colour screen to exclude one of the principal colours, as advocated by Clark [1922], did not seem to improve the accuracy of the matching as it considerably lessened the colour change with p_H of the indicator. As the gelatin solutions are free of neutral salt, there may be a difference in apparent p_H between them and buffer solutions containing the

same indicator, the latter being standardised against the electrical determination for a definite concentration of neutral salt usually $M/10$ or $M/15$. Lepper and Martin [1926] have, however, shown that the magnitude of this difference does not exceed ± 0.2 between $M/1$ and $M/1000$ for phenol red indicator, but Kolthoff [1921] finds that bromophenol blue is particularly influenced by neutral salt action. The error obviously depends on the indicator used, as shown by Sørensen [1909], *e.g.* we found that bromocresol purple and bromothymol blue are little affected, but consideration of Fig. 1 shows that it depends to

Fig. 1. Variation of the "protein error" in the colorimetric determination of p_H with the p_H as determined electrometrically.

○ Bromophenol blue and 1.0 % gelatin. ● Thymol blue and 1.0 % gelatin.
+ " " " 0.4 % " " × " " " 0.4 % "

a great extent on the state of dissociation of the protein for bromophenol blue and thymol blue. The error is a minimum about the isoelectric point, and a maximum about $p_H 2.4$ (electrometric). Actually $p_H 2.4$ is outside the range of bromophenol blue (2.8–4.6), although the solution appears to have the $p_H 3.25$ and is alkaline to thymol blue (1.2–2.8); hence the error due to this cause is probably increased since indicators work most accurately near their point of 50 % dissociation, which is at $p_H 4.1$ for bromophenol blue. Evidently the dissociation of the protein affects the indicator by combination or by adsorption to a greater extent when dissociated than when undissociated, thereby upsetting the normal ionic equilibrium of the indicator and hence changing its colour.

The effect of p_H on the surface tension of gelatin solutions.

In Fig. 2 is given the effect of p_H on the surface tension of gelatin solutions, the p_H being determined electrometrically. It will be seen that the maximum at the isoelectric point remains unaltered whilst the minimum previously

found at p_H 3.8–4.0 is now at p_H 3.5, and the second point of maximum surface tension is considerably more acid than formerly, namely, p_H 2.0–2.2 instead of p_H 2.8–3.0. The curve of 1 % gelatin in presence of H_2SO_4 does not agree so closely with that of 1 % gelatin in presence of HCl as formerly observed, but the point of minimum surface tension is at exactly the same p_H for each acid. Previously recorded experiments [1925] on the effect of time and of neutral salts on the surface tension of 1 % gelatin in presence of HCl at p_H 3.3 as found colorimetrically were thus actually carried out at an original p_H of 2.37, excluding the effect of neutral salts on the p_H . This alteration, due to the error in estimating p_H , does not affect our previous conclusions, except that the point of minimum surface tension at p_H 3.5–3.6 does not agree so well with the point of maximum potential difference found by Loeb [1922] at p_H 3.8–4.0, though it agrees well with the maxima of osmotic pressure and viscosity.

Fig. 2. Variation of surface tension with p_H of 0.4 % and 1.0 % gelatin solutions at 25° and 40°.

× 0.4 % gelatin in presence of HCl or NaOH at 25°.
 ● 1.0 % " " " " " 25°.
 ○ 1.0 % " " " " " 40°.
 △ 1.0 % " " " " H_2SO_4 at 25°.

The possible hydrolysis of gelatin in acid solutions of p_H less than 2.2–2.0.

It will be seen that on the corrected surface tension- p_H curve of gelatin solutions the second maximum occurs at p_H 2.2–2.0, which is at a point considerably more acid than we previously found. Hence it is still more likely that the fall of surface tension in the most acid solutions after the second maximum is due to the hydrolysis of the gelatin by the strong acid. An attempt was made therefore to investigate this by following the change of surface tension and of the amount of nitrogen estimable by formaldehyde titration with time. It was hoped that it would be possible to correlate a decrease of surface tension with an increased formaldehyde titration and that the effect would be found only in solutions of p_H less than 2.2–2.0.

A quantity of gelatin was prepared in the usual manner and found to have an ash content of 0.1 % on the dry substance. The solutions contained 0.5 % gelatin and were brought to p_H 2.4, 2.2, 1.9 by addition of the appropriate quantities of HCl, and a fourth solution was made up at the isoelectric point,

p_H 4.7. All the solutions contained 2 cc. saturated solution of thymol and a little toluene per 500 cc. as a preservative. The two solutions at p_H 4.7 and 2.4 are both alkaline to the maximum and hence should not show an appreciable alteration of formaldehyde-N or surface tension with time; the solution at p_H 2.2 is at the maximum, and that at p_H 1.9 is acid to the maximum. The solutions were kept in a constant temperature oven at 25°.

The total N of each of the 0.5 % solutions was found by Kjeldahl's method to be 0.0896 g. per 100 cc., from which the value for the concentration is calculated to be 0.498 % (taking gelatin to contain 18 % N). The formaldehyde-N is expressed as a percentage of the total N.

Only 50 cc. of gelatin could be spared for each estimation, so that small titres were obtained on which the error was 5-7 %.

At stated intervals a sample was withdrawn from the solution in the oven for the surface tension and formaldehyde-N determinations and any portion of the sample remaining was thrown away.

The results are shown in Table II.

Table II. 0.5 % gelatin solutions.

Days	p_H 4.7		p_H 2.4		p_H 2.2		p_H 1.9	
	% form- aldehyde- N	Surface tension (dynes/cm.)	% form- aldehyde- N	Surface tension (dynes/cm.)	% form- aldehyde- N	Surface tension (dynes/cm.)	% form- aldehyde- N	Surface tension (dynes/cm.)
2	2.5	67.5	2.8	66.9	2.8	69.6	3.5	68.5
8	2.8	—	2.8	66.6	3.1	68.4	4.4	—
10	—	67.0	—	—	—	—	—	64.8
16	2.8	—	2.8	66.9	2.8	67.8	3.1	66.8
23	—	—	—	—	—	68.5	—	68.0
27	2.5	68.0	2.8	66.8	2.5	—	3.1	—
42	3.1	66.2	3.1	66.8	2.8	65.4	3.7	66.7

The figures for formaldehyde-N at the commencement of the experiment were all higher than expected, especially when no acid was present, and, as the surface tensions were lower than we had previously found them, it was thought that the gelatin, which was from an old stock and had developed a slightly "gluey" odour, might be at fault. Further, the isoelectric solution never flocculated properly and the centrifuged filtrate from it contained 46 mg. N per 100 cc., a quantity much in excess of the 15-20 mg. per 100 cc. found by Knaggs, Manning and Schryver [1923]. The experiment was, therefore, repeated with some fresh gelatin from the same source—Nelson's Gold Label. After washing in the usual manner it was found to contain 0.06 % ash on the dry substance. A 1 % isoelectric solution flocculated completely in 2 days, giving a surface tension of 72.3 dynes per cm. The solutions were made up to a final concentration of 1 % and the p_H brought to 2.5, 2.1, 1.8, 1.4 by the addition of the appropriate amounts of HCl. The total N of each solution was found to be 0.179 %, of which 2.6 % was found to be formaldehyde-N, *i.e.* the same percentage as was found in the previous experiments with a

supposedly deteriorated gelatin. Toluene only was used as a preservative, as it was possible that the thymol had lowered the surface tension of the 0.5 % solutions. In this experiment the values of the surface tensions agree with earlier determinations, but owing to the abolition of the thymol slight mould contamination was found in the two less acid solutions after 4 months. The solutions were kept at 25°.

The results are shown in Table III.

Table III. 1 % gelatin solutions.

Days	p_H 2.5		p_H 2.1		p_H 1.8		p_H 1.4	
	% form- aldehyde- N	Surface tension (dynes/cm.)	% Form- aldehyde- N	Surface tension (dynes/cm.)	% form- aldehyde- N	Surface tension (dynes/cm.)	% form- aldehyde- N	Surface tension (dynes/cm.)
1	2.5	69.0	2.6	70.7	2.6	67.7	2.8	—
4	—	69.0	—	68.2	—	67.3	—	66.9
11	3.7	—	3.8	66.9	3.6	67.3	3.4	67.6
18	3.4	67.3	3.5	64.8	3.7	66.4	3.9	65.7
22	—	66.4	—	—	—	—	—	—
25	3.4	—	3.8	—	3.4	—	3.6	64.4
114	3.4	64.7	3.9	64.6	4.6	63.2	6.2	63.6

There is a gradual fall of surface tension coupled with a rise in the amount of formaldehyde-N present, and both these effects increase in a fairly regular manner as the acidity of the solution is increased, indicating a slow hydrolysis taking place at all acidities, even at the isoelectric point, and increasing with the acidity (the most acid solutions in each case gave the most formaldehyde-N on standing).

The amount of formaldehyde-N in the 0.5 % solution increased very slightly in 6 weeks, though the solution of p_H 1.9 showed a sudden increase in the first 2 days, after which it behaved like the other solutions. In the 1 % solutions all rose in 10 days, but after 4 months only the two most acid ones had risen further. The surface tensions however show a continuous fall over the whole period of the experiment in each case. The p_H were found unaltered at the end of the experiment.

There is no evidence that hydrolysis of the gelatin molecule commences suddenly at the p_H of the second maximum of surface tension. Hence we are unable to ascribe the decrease in surface tension which we find in solutions of p_H less than 2.2–2.0 to hydrolysis with the formation of a less complex "acid protein" titratable in presence of formaldehyde.

Recent experiments by Sørensen [1925] on the coagulation of egg-albumin suggest another possible explanation. Sørensen found that the first process of coagulation is denaturation, which probably consists of the splitting-off of water from the protein, and that decomposition with formation of substances titratable in presence of formaldehyde is only a secondary process occurring on heating the denatured protein. The denaturation takes place more rapidly and at a lower temperature the higher the concentration of H or OH ions,

whilst the subsequent coagulation of the denatured protein, which behaves like a typical suspensoid in the case of egg-albumin, is most rapid at the isoelectric point. A similar denaturation is possibly occurring in the case of gelatin, the increase of formaldehyde-N in the most acid solutions on long standing at 25° being due to the secondary decomposition of the denatured gelatin.

Owing to the high solubility and non-coagulability on heating of gelatin, no definite evidence of denaturation of gelatin was obtained experimentally, but work now in progress on other proteins shows a relationship between the fall of surface tension with increasing acidity and denaturation of the protein.

SUMMARY.

1. The "protein error" of certain indicators with gelatin solutions has been investigated. Bromophenol blue gives a minimum error at the isoelectric point and a maximum error about p_H 2.4.

2. The surface tension- p_H curves of gelatin have been corrected as regards p_H for the "protein error" in the colorimetric estimation of p_H .

3. The possibility of acid hydrolysis of gelatin in solutions acid to p_H 2.0 has been investigated by means of formaldehyde titration. A certain amount of hydrolysis occurred on long standing, but there is no evidence of an immediate hydrolysis which would account for the sudden fall of surface tension below p_H 2.0.

We are indebted to the Directors of Bass, Ratcliff and Gretton, Ltd., for permission to publish this work.

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