pared by the action of metallic sodium on trimethylstannic chloride in liquid ammonia solution.

The molecular weight of the stannide, as calculated from vapor density determinations, was found to be 165.53, compared with the theoretical value of 164.78.

Trimethylstannane reacts with sodium in liquid ammonia solution to form sodiumtrimethylstannide, with evolution of hydrogen. Treated with hydrochloric acid in aqueous solution, trimethylstannane yields trimethylstannic chloride and hydrogen.

The boiling point of trimethylstannane is 60°.

WORCESTER, MASSACHUSETTS

THE TWO FORMS OF GELATIN AND THEIR ISO-ELECTRIC POINTS[1]

BY JOHN ARTHUR WILSON AND ERWIN J. KERN

In an investigation of the degree of "plumping" of calf skin as a function of hydrogen-ion concentration, Wilson and Gallun[2] observed two points of minimum, one at P_H 5.1 and the other at 7.6. In view of the close relation existing between collagen and gelatin, this rather surprising discovery raised the question as to whether similar points of minimum might not occur also in the swelling of gelatin, especially since we found no mention in the literature of measurements of swelling at short intervals of change in hydrogen-ion concentration between P_H 5 and 8.

Procedure and Results

A series of buffer solutions was prepared, each member of which had a final concentration of 0.1 M phosphoric acid plus the amount of sodium hydroxide required to give the desired hydrogen-ion concentration as determined by the hydrogen electrode at 20°. The Sörensen values ranged from 3 to 12; 200 cc. of each solution was put into a stoppered bottle and kept in a thermostat refrigerator at 7°. After each solution had reached 7°, a small strip of high-grade gelatin of known weight was put into it. All strips were taken as nearly alike as possible and were kept in the solutions at 7° for 4 days, after which each strip was quickly blotted off and weighed. The experiment was repeated on another occasion with fresh solutions and the results were practically identical with those of the first experiment. In Table I are given the gains in weight

[1] Presented before the Leather Division at the 64th Meeting of the American Chemical Society, Pittsburgh, Penna., September 5 to 9, 1922.

[2] Points of Minimum Plumping of Calf Skin, *read at same meeting.*

per gram of dry gelatin and the initial and final Sörensen values of the buffer solutions.

TABLE I

RELATION OF DEGREE OF SWELLING OF GELATIN TO HYDROGEN-ION CONCENTRATION

P_H value of buffer solution at 20°		Increase in wt. of 1 g. of dry gelatin	P_H value of buffer solution at 20°		Increase in wt. of 1 g. of dry gelatin
Initial	Final	G.	Initial	Final	G.
2.90	2.92	13.20	6.96	6.94	8.31
3.50	3.50	9.49	7.08	7.10	8.25
3.96	4.01	7.72	7.41	7.37	8.03
4.14	4.17	6.91	7.68	7.62	7.62
4.47	4.59	6.68	7.97	7.89	8.39
4.78	4.86	6.20	8.42	8.36	8.59
5.08	5.12	7.02	8.56	8.48	8.60
5.29	5.38	7.13	9.03	8.96	8.78
5.57	5.61	7.22	9.57	9.51	8.91
5.78	5.80	7.56	10.00	9.96	8.98
6.04	6.08	7.80	10.47	10.41	9.24
6.29	6.29	7.83	11.06	10.98	9.55
6.48	6.49	8.02	11.52	11.48	9.95
6.69	6.70	8.29	12.00	11.95	10.73

The curve in Fig. 1, representing the degree of swelling of gelatin as a function of Sörensen value, is similar to that obtained by Wilson and Gallun for the plumping of calf skin.
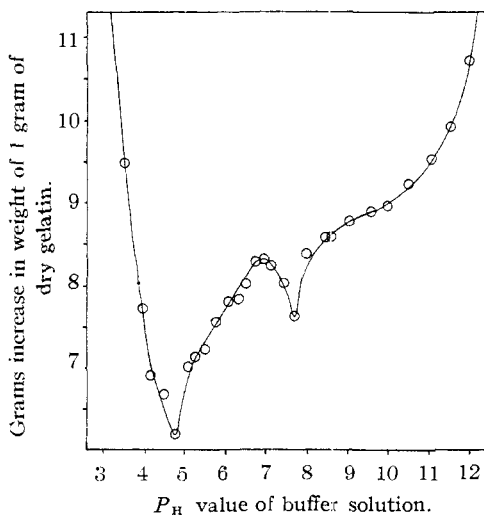

Fig. 1.—The two points of minimum swelling of gelatin.

Theoretical Discussion

The discovery of two points of minimum, for both the plumping of calf skin and the swelling of gelatin, although unexpected, actually correlates and explains a number of puzzling data in the literature. Experiments upon the mutarotation of gelatin led Smith[3] to suggest that gelatin exists in two forms, a sol form having a specific rotation of $[\alpha]_D = -141$ and being stable at temperatures above 35°, and a gel form, with a specific rotation of $[\alpha]_D = -313$ and stable under 15°, a condition of equilibrium existing between the two forms at intermediate

[3] Smith, THIS JOURNAL, 41, 135 (1919).

temperatures. The terms gel form and sol form, as used in this paper, have no significance other than to indicate the form of gelatin which is capable of setting to a jelly and the form which is not. The sol-gel equilibrium has recently been discussed by Bogue.[4]

Davis and Oakes[5] measured the viscosities of 1% gelatin solutions at 40° at different concentrations of hydrogen ion and found a minimum at P_H 8, but none at 4.7, the iso-electric point of gelatin determined by Loeb.[6] They commented upon this as follows: "There may be considerable difficulty in reconciling this minimum viscosity at P_H about 8 with the iso-electric point at P_H 4.7." But Davis and Oakes really measured the point of minimum viscosity of the sol form, since their determinations were made at 40°, whereas Loeb determined the iso-electric point of the gel form.

Lloyd[7] found that gelatin in alkaline solution changes from the gel to sol form. Gelatin recovered from acid solution, by neutralization and precipitation with ammonium sulfate, dissolved readily in hot water and set to a jelly upon cooling. Gelatin recovered from alkaline solution in a similar manner was soluble in cold water and would not set to a jelly even when evaporated to small bulk. Lloyd suggested that gelatin changes from a keto form to an enol form in alkaline solution and that the change is irreversible. However, when hydrochloric acid was added to gelatin dissolved in a hot solution of sodium hydroxide until the Sörensen value, as determined by the hydrogen electrode, was reduced to 4.7 and the solution then allowed to cool, it set to a firm jelly, indicating that the change is reversible. Lloyd's experiment showed that it is not readily reversed by the addition of only the quantity of hydrochloric acid equivalent to that of the sodium hydroxide originally employed.

Loeb[8] has shown in numerous experiments that the iso-electric point of the gel form of gelatin lies at $P_H = 4.7$. Thomas and Kelly,[9] as well as Porter,[10] have shown that the iso-electric point of collagen, or hide protein, has approximately the same value as that for gelatin, at room temperature. But the work of Davis and Oakes indicates an iso-electric point for the sol form of gelatin at about P_H 8. In experiments upon the bating of calf skin at 40°, with varying hydrogen-ion concentration, Wilson and Daub[11] observed that a point of minimum plumping occurred

[4] Bogue, THIS JOURNAL, **44**, 1313 (1922).

[5] Davis and Oakes, *ibid.*, 464 (1922).

[6] Loeb, *J. Gen. Physiol.*, **1**, 39 (1918).

[7] Lloyd, *Biochem. J.*, **14**, 147 (1920).

[8] Loeb, "Proteins and the Theory of Colloidal Behavior," McGraw-Hill Book Co., 1922.

[9] Thomas and Kelly, THIS JOURNAL, **44**, 195 (1922).

[10] Porter, *J. Soc. Leather Trades Chem.*, **5**, 259 (1921); **6**, 83 (1922).

[11] Wilson and Daub, *J. Ind. Eng. Chem.*, **13**, 1137 (1921).

in the region of P_H 8, but not at 4.7. The work of Smith and Lloyd suggests that the change from the gel to the sol form takes place both with rise of temperature and with rise of Sörensen value.

Since our experiments were performed at 7°, we were dealing with the gel form of gelatin in the acid solution and it is significant that a point of minimum swelling was observed at a Sörensen value of about 4.7, the iso-electric point of the gel form. The appearance of a second point of minimum at P_H 7.7 seems to indicate that between 4.7 and 7.7 the gelatin passes from the gel to the sol form and that the second point of minimum occurs at the iso-electric point of the sol form. It was only by working at a temperature as low as 7° that we were able to prevent the gelatin from passing into solution at the higher Sörensen values.

The recent work of Kelly[12] on the rate of tanning as a function of hydrogen-ion concentration has an important bearing upon this problem. It was found that the rate of combination of hide protein and tannin decreases to a minimum at P_H 5, increases to a maximum at about 8, and then decreases rapidly towards zero, with increasing Sörensen values. Since tannin is negatively charged, this work would seem to indicate that collagen carries a positive charge between the iso-electric points of its two forms. Between the iso-electric points of the two forms of any protein, any of the gel form present would have a negative charge and any of the sol form a positive charge, the net charge of the system being determined by the preponderance of one of the forms.

The similarity of behavior of collagen and gelatin indicates the possibility of a wider use of data on gelatin in tannery problems. The experiments also suggest that only the sol form of collagen is convertible into gelatin and that the sol forms of the two proteins may actually be identical. The fact that the transition from gel to sol form occurs near the neutral point makes the work of great importance in biological problems.

Summary

Gelatin, like collagen, shows two points of minimum swelling with change of hydrogen-ion concentration, one at P_H 4.7 and the other at 7.7. It is suggested that the two points of minimum represent the iso-electric points of the gel and sol forms of gelatin, respectively.

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[12] The Hydrogen-Ion and Time Factors in the Fixation of Tannins by Hide Substance. Margaret W. Kelly. Read before the Leather Division at the 64th meeting of the American Chemical Society, Pittsburgh, Sept. 6, 1922.