[CONTRIBUTION FROM THE DEPARTMENT OF LEATHER RESEARCH, INSTITUTE OF SCIENTIFIC RESEARCH, UNIVERSITY OF CINCINNATI]

The Isoelectric Point of Collagen

BY JOHN H. HIGHBERGER

Although the isoelectric point of collagen is usually given as *p*H 4.7, a second critical point in the behavior of collagen and gelatin has frequently been noted at *p*H 7.7. Wilson and Gallun[1] found two points of minimum plumping of limed calf skin, at *p*H 5.1 and 7.6, and Wilson and Kern[2] located similar points at *p*H values of 4.7 and 7.7 in studying the swelling of gelatin. Davis and Oakes[3] found a minimum in the viscosity of 1% gelatin sols at about *p*H 8, when measured at 40°, but none at 4.7. Just the opposite result was obtained by Hitchcock,[4] whose curves show a minimum viscosity at 4.7, but not at 8. Hitchcock attributed the difference between his results and those of Davis and Oakes to the practice of the latter workers of heating their sols to 75° in the course of their preparation. Higley and Mathews[5] studied the absorption of light by gelatin sols as a function of *p*H, and found two sharply defined minima in the wave lengths absorbed, at *p*H values of 4.7 and 7.7. Kraemer and Dexter,[6] on the other hand, found a maximum in the light scattering of gelatin at *p*H 8 only in the case of pig skin gelatin, while for other gelatins it was found close to *p*H 5. Electrophoretic studies[7] have established the isolectric point of commercial calf skin gelatin as close to *p*H 4.7 to 4.8, the exact location depending upon the nature and ionic strength of the buffer salts present.

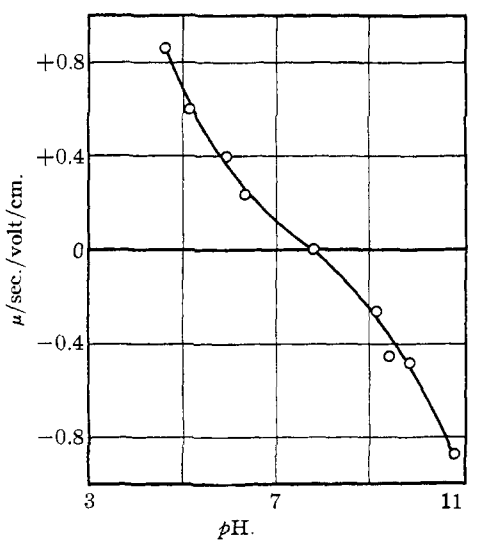

Fig. 1.—*p*H–mobility curve of collagen.

Experimental

A study of the electrophoresis of the collagen of animal skin, using the technique described by Moyer,[8] has been in progress in this Laboratory for some time. Several lots of collagen prepared from fresh steer hide by a process previously described by the writer,[9] and varying in ash content from 0.05 to 0.08%, have been used in this work. Powdered material which had passed a 100-mesh sieve was used to make suspensions in acetate, phosphate, and borate buffers of ionic strength 0.005, and covering the *p*H range from 4 to 11. Relatively dilute buffer systems were found necessary on account of the unusually small slope of the mobility curve over a considerable range of *p*H.

All measurements were made in the vertical microelectrophoresis cell of Abramson, Moyer and Voet,[10] which was found to be very satisfactory for use with these rather rapidly settling suspensions. At least ten particles were timed at each of the stationary cell levels 0.2 and 0.8, and the results averaged. The particles measured ranged in size from about 15 to about 75 μ. No temperature correction was made. Room temperature varied between 23 and 26° during the observations. Hydrogen electrode measurements were made in duplicate, using Clark rocking electrode vessels and a saturated calomel electrode. The reference standard was "standard acetate," of *p*H 4.65.[11]

The *p*H–mobility curve thus obtained is given in Fig. 1, from which it will be seen that this collagen is isoelectric at *p*H 7.8 under the experimental conditions mentioned. This observation is in accord with swelling data already reported[12] for similar collagen preparations, which showed a pronounced minimum zone between *p*H 7 and 10 in unbuffered solutions. An experiment by Beek[13] indicates that the isoionic point of this collagen is also close to *p*H 7.7.

Preliminary observations on a mixed gelatin made by extracting a similar collagen preparation several times with distilled water at 100° indicate that this gelatin is isoelectric at *p*H 6.1, when adsorbed on collodion particles and observed in buffers of ionic strength 0.005. Qualita-

(1) Wilson and Gallun, *Ind. Eng. Chem.*, **15**, 71 (1923).
(2) Wilson and Kern, THIS JOURNAL, **44**, 2633 (1922); **45**, 3139 (1923).
(3) Davis and Oakes, *ibid.*, **44**, 464 (1922).
(4) Hitchcock, *J. Gen. Physiol.*, **6**, 457 (1924).
(5) Higley and Mathews, THIS JOURNAL, **46**, 852 (1924).
(6) Kraemer and Dexter, *J. Phys. Chem.*, **31**, 764 (1927).
(7) Hitchcock, *J. Gen. Physiol.*, **14**, 685 (1931); Abramson, *ibid.*, **15**, 575 (1932).
(8) Moyer, *J. Bact.*, **31**, 531 (1936).
(9) Highberger, *J. Am. Leather Chem. Assoc.*, **31**, 93 (1936).
(10) Abramson, Moyer and Voet, THIS JOURNAL, **58**, 2362 (1936).
(11) Hitchcock and Taylor, *ibid.*, **59**, 1812 (1937).
(12) Highberger, *J. Am. Leather Chem. Assoc.*, **31**, 345 (1936).
(13) Beek, *J. Research Natl. Bur. Standards*, **21**, 117 (1938).

tive observations on a sample of standard hide powder[14] showed it to be isoelectric in the neighborhood of *p*H 5.

Discussion

Wilson has interpreted the two critical points, at *p*H values of 4.7 and 7.7, as the respective isoelectric points of the gel and the sol forms of collagen and gelatin.[15] The fact that the collagen used in the present work received only a mild alkaline treatment with half-saturated calcium hydroxide solution in the course of its preparation, while commercial hide powder and gelatin are given a much more drastic alkaline treatment, suggests that the isoelectric point of native collagen is near *p*H 7.8, and that treatment with alkaline solutions causes some structural change the effect of which is to lower the isoelectric point of the collagen and of the gelatin made from it.[16] The work of Briefer,[17] who found that gelatin made from alkaline pretreated collagen possesses minimum jelly consistency and maximum turbidity at about *p*H 5, while that made from acid pretreated collagen has similar properties at about *p*H 8, is recalled in this connection.

In addition to its unusually small slope, the curve of Fig. 1 displays a further feature of interest when compared to similar curves for other proteins. The curvature in such cases, while slight, is usually convex in the region of positive mobilities and concave in the negative. Just the opposite relations are found in the curve of Fig. 1. No explanation of this difference in shape, which is undoubtedly related to the mode of linkage of the polar groups of the protein, will be attempted at this time. Further work on the cause of the shift in the isoelectric points of collagen and gelatin is in progress in this Laboratory.

The author wishes to acknowledge the valuable assistance of Dr. H.J. Kersten in mounting the vertical microelectrophoresis cell. The cell itself was made by Mr. J. D. Graham of Haddonfield, New Jersey, to whom the author's thanks are also due.

Summary

The isoelectric point of collagen prepared from steer hide with a minimum alkaline treatment has been shown, by measurements of electrophoretic mobility, to be at *p*H 7.8 in buffers of ionic strength 0.005. It is suggested that the shift of the isoelectric point to *p*H 4.7 in the case of commercial gelatin and hide powder is due to some structural change in the protein caused by the alkaline treatment given these materials.

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(14) Kindly furnished by Mr. Louis Cuthbert of The Standard Hide Powder Mfg. Co., Ridgway, Penna.

(15) For further discussion and references see J. A. Wilson, "The Chemistry of Leather Manufacture," Vol. I, The Chemical Catalog Company, New York, N. Y., 1928.

(16) After the completion of this work a paper on the electrophoresis of collagen was presented by Beek and Sookne at the meeting of The American Leather Chemists Association at Shawnee-on-Delaware, Penna., June 7, 1939. Using a horizontal cell and buffers of ionic strength 0.02, they located the isoelectric point of collagen similar to that used in the present work at about *p*H 7, and attributed the shift to a more acid value in the case of commercial gelatins to the hydrolysis of the amide groups during the liming treatment.

(17) Briefer, *Ind. Eng. Chem.*, **21**, 266 (1929).

[CONTRIBUTION FROM CALIFORNIA FRUIT GROWERS EXCHANGE RESEARCH DEPARTMENT]

A Study of the Boric Acid Color Reaction of Flavone Derivatives

BY C. W. WILSON[1]

It was observed in this Laboratory that lemon juice dried in the presence of boric acid produced a brilliant yellow color. Later the color reactive substance was identified as a constituent of Szent-Györgyi's citrin.[2]

This color reaction is sufficiently sensitive to detect 0.004 mg. of citrin or 0.002 mg. of quercitrin in 0.5 ml. of solution. The work here reported is an attempt to determine the specificity of the boric acid color reaction.

Experimental

Several representative flavones[3] and flavanones were purified by recrystallization from dilute alcohol at least three times. Table I shows the structural formulas of some of these substances and their boric acid color reactions.

The hydroxychalcones shown in Table I were prepared by alkaline treatment of naringin and hesperidin. A suspension of 2 g. of naringin or hesperidin with 25 ml. of water was placed in a 50-ml. flask equipped with a stopcock, the flask

(1) Corona Laboratory of California Fruit Growers Exchange, Research Department.

(2) Armentano, Bentsath Beres, St. Rusznyak and Szent-Györgyi, *Deut. med. Wochschr.*, **62**, 1326 (1936).

(3) The flavones used in this work were obtained by Mr. A. J. Lorenz from Dr. C. E. Sando of the U. S. Bureau of Chemistry and Soils.