previously suggested by Higley.[13] Some significance, therefore, may be attached to the fact that those esters, ethyl acetate and ethyl propionate, which are the most sluggish in their reaction with sodium in the inert solvents, react through the acetoacetic ester condensation when an excess of the ester is the solvent.

Summary

1. The reaction of ethyl acetate, propionate, butyrate, isobutyrate and trimethylacetate with sodium has been studied under various conditions.

2. The mechanisms which have been proposed for the formation of acyloins are discussed on the basis of the experimental results obtained.

MADISON, WISCONSIN

[COMMUNICATION NO. 449 FROM THE KODAK RESEARCH LABORATORIES]

A HEAT COAGULABLE PROTEIN FROM GELATIN

BY S. E. SHEPPARD, J. H. HUDSON AND R. C. HOUCK

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In a report on the specification of a standard gelatin[1] attention was called to the desirability of specifying the permissible amount of heat coagulable protein in a standard gelatin. The presence of albuminous substances in gelatin has been frequently indicated, especially in articles dealing with its manufacture and purification.[2]

The following is an account of some preliminary work on the estimation, separation, and analysis of the heat coagulable protein from a number of different gelatins.

The results are insufficient to decide whether the substance is a single homogeneous protein or a mixture. They do, however, definitely establish its nature, in gelatin, as a foreign impurity of different constitution and composition.

Experimental

In separating the coagulable protein from gelatin it is found necessary first to adjust the P_H of the gelatin, with acetic acid, to near the isoelectric point of gelatin. Fifty grams of a gelatin with a P_H range of 5.9 to 6 dissolved in 800 cc. of water will require about 5 cc. of glacial acetic acid to bring the P_H to 4.7. Heating overnight on a steam-bath at a temperature of 80 to 100° is sufficient to hydrolyze the gelatin and allow the protein to

[13] Higley, *Am. Chem. J.*, **37**, 302 (1907).

[1] Hudson and Sheppard, *Ind. Eng. Chem.*, **21**, 263 (1929), "A Contribution to the Preparation of Standard Gelatin," Communication 364. Davis, Sheppard and Briefer, *ibid.*, *Anal. Ed.*, **1**, 56 (1929), "Specifications for Gelatin Standards."

[2] Bogue, "Chemistry and Technology of Gelatin and Glue," McGraw-Hill Book Co., Inc., New York, **1922**, 1st ed.; Sheppard, "Gelatin in Photography," Monograph No. 1 from Kodak Research Laboratories, Rochester, New York, **1923**, p. 131.

coagulate. Fifty grams of a de-ashed gelatin (P_H 4.9) in 800 cc. of water is treated with only 0.5 cc. glacial acetic acid and heated to effect coagulation.

The clear gelatin solution is decanted off through a coarse filter paper and the coagulum is broken up in the beaker by a stream of hot water, after which it is washed into the filter. Washing with hot water is continued until all the gelatin solution is removed from the coagulated protein. The protein is finally collected in the cone of the filter and then transferred, with a stream of water, to a weighing bottle. The water is evaporated off and drying completed in the oven at 105 to 110°. The amounts of coagulable protein were determined in various gelatins and the protein was analyzed for nitrogen.

TABLE I

ASH AND WATER FREE BASIS

Gelatin	Protein, %	Percentage N on protein	Origin
X1	0.37	14.6	Domestic calfskin, de-ashed
X2	.63	14.6	Same
Y1	.27	12.0	Foreign calfskin, not de-ashed
X3	.62	14.6	Domestic calfskin, not de-ashed
W1	.40	13.7	Domestic bone, not de-ashed
X4	.22	11.0	Domestic hide, not de-ashed

For comparison, the whites of eggs were beaten up in water, filtered and analyzed.

TABLE II

ANALYTICAL DATA

	Egg whites	Coagulable protein from X2 gelatin
Ash, %	3.7	0.04
Inorganic S, %	0.24	0.00
Total S, %	1.54	.80
Labile S, %	0.49	.00063
Nitrogen, %	14.42	14.6

Isoelectric Point of Coagulated Proteins.—The material is obtained in coarse flocks, which are insoluble in water and in weakly acid and alkaline solutions. Methods of determining the isoelectric point by desolvation are not, therefore, applicable. Neither was the form of the material suitable for cataphoretic determinations, although it is believed that we have now available a method for rendering it suitable, and this will be tried in later work. The method used was that of electro-endosmose, the inverse of electrophoresis. In this process, liquid is moved under the influence of a potential instead of the particle. A porous plug is formed of the material and brought between the two electrodes. Solutions of known P_H are put in contact with the plug and the direction of flow of liquid through the plug is observed under an applied potential difference. The plug assumes a charge on coming into contact with the solution, the nature of the charge,

positive or negative, depending on the P_H of the solution and the isoelectric point of the material. The liquid in contact becomes oppositely charged, an electrical double layer being set up. By setting up a potential across the plug this layer is disturbed and liquid flows in one direction or the other; if on the alkaline side of the isoelectric point it flows toward the cathode, and if on the acid side toward the anode.

The apparatus employed was similar to that described by Briggs, Bennett and Pierson.[3] It is shown diagrammatically in Fig. 1, but it differs from that of Briggs in that the part which contains the plug is separable from the rest of the apparatus. This was found to be necessary because of the nature of the material used, since a satisfactory plug could not be obtained by filtering, as was done by Briggs and his co-workers. It was also desired to remove any doubt which might be cast on the results as being due to the presence of cotton or glass wool supporting the plug.

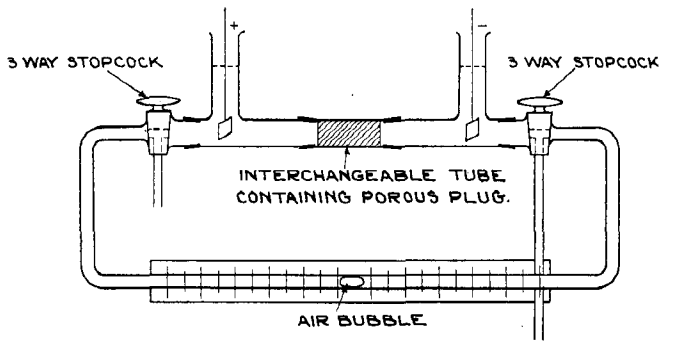

Fig. 1.—Electro-endosmose apparatus.

Accordingly the center portion of the apparatus was made an interchangeable unit in a centrifuge tube; the plug was formed by centrifuging, and then the plug along with the supporting tube was placed in the endosmose apparatus. Satisfactory plugs were obtained in this manner. The material was stirred up with the buffer to be used before centrifuging.

The plug having been formed and placed in position, the buffer of known P_H, with which the material had been stirred before centrifuging, was poured into the apparatus and all air bubbles were removed by tilting the apparatus. A loose plug was then tested for by closing both stopcocks and pouring in more buffer on one side and watching the levels in the anode and cathode compartments. If the levels remained stationary the experiment was continued. If not, a new plug was formed. The stopcocks were opened and after the levels had become stationary the current was turned on. In most of the experiments a potential of 110 volts was applied across the plug. The direction of flow was observed and in cases where possible

[3] T. R. Briggs, H. S. Bennett and H. L. Pierson, *J. Phys. Chem.*, **22**, 256 (1928).

the rate of flow determined. The direction of the current was then reversed and direction of flow and rate checked.

TABLE III

ISOELECTRIC POINT OF EGG ALBUMEN COAGULATED BY HEAT

*P*H of buffer	Direction of flow through plug	Rate of flow, cm./min.
5.4	Cathodic	0.74
5.0	Cathodic	.10
4.8	Cathodic	.007
4.6	Anodic	.006
4.2	Anodic	.30
3.8	Anodic	.51

Clark and Lubs buffers, potassium acid phthalate with varying amounts of hydrochloric acid or sodium hydroxide, were employed. The *P*H values of a number of these were checked electrometrically and found to be satisfactory.

Egg Albumen.—The results obtained with egg albumen denatured by heat are shown by Table III and Figure 2.

The egg albumen was precipitated by heating from a 4% solution of dialyzed material.

The results indicate a value of *P*H 4.7 as the isoelectric point of egg albumen denatured by heat. The values of the isoelectric point of genuine egg albumen vary: from a *P*H of 4.7 as found by Loeb[4] and by Reinders and Bendien,[5] to a *P*H of 4.8 as found by Svedberg and Tiselius,[6] and to a *P*H of 4.95 as found by Abramson[7] and by Prideaux and Howitt.[4]

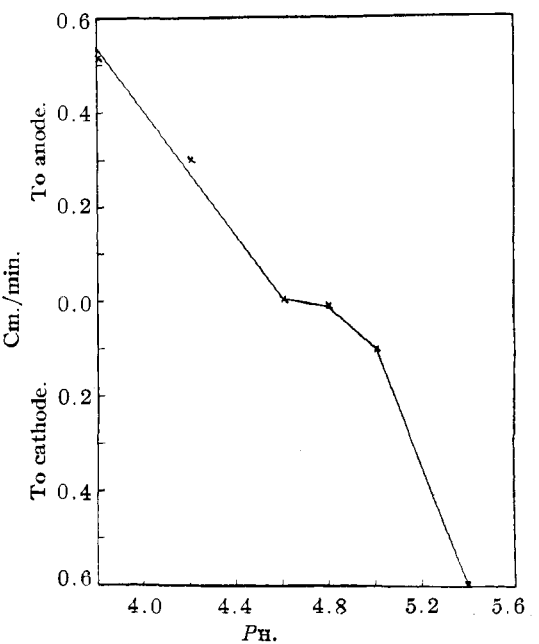

Fig. 2.—Isoelectric point of egg albumen denatured by heat.

[4] J. Loeb, "Proteins and the Theory of Colloidal Behavior," Chapt. 8, "Membrane Potentials," McGraw-Hill Book Co., New York, **1922**, pp. 120–150. Prideaux and Howitt, *Proc. Roy. Soc.* (London), **126**, 126 (1929). (Calculated from Loeb's figures of membrane potentials.)

[5] W. Reinders and W. M. Bendien, *Rec. trav. chim.*, **47**, 977 (1928).

[6] T. Svedberg and A. Tiselius, THIS JOURNAL, **48**, 2272 (1926).

[7] H. A. Abramson, *ibid.*, **50**, 390 (1928).

Thus, it seems fairly safe to say that the isoelectric point of egg albumen is not appreciably changed by being denatured by heat.

Protein Coagulated from Gelatin Sols by Heat.—The isoelectric point of the material coagulated from gelatin by heat was determined in a similar manner. The results are not quite as good as with egg albumen, as it seemed impossible to obtain rates of flow. The results obtained showed the *isoelectric point of this material to be at a* PH *of 3.9 to 4.2*, depending on the gelatin employed. The experimental results are shown by Tables IV and V.

TABLE IV

ISOELECTRIC POINT OF MATERIAL COAGULATED BY HEAT FROM GELATIN NO. 37

PH	Direction of movement
3.2	Anodic
3.4	Anodic
3.6	Anodic
3.85	Anodic
4.0	Cathodic
4.4	Cathodic
5.4	Cathodic

TABLE V

ISOELECTRIC POINT OF MATERIAL COAGULATED FROM GELATIN NO. 43

PH	Direction of flow of liquid
3.6	Anodic
3.85	Anodic
4.2	Anodic (weak)
4.4	Cathodic (strongly)
4.2 (repeated)	First one way then the other
4.4	Cathodic
5.0	Cathodic

The isoelectric point lies between PH 3.85 and 4.0.

Discussion

The analyses of the material for nitrogen, although not concordant for different gelatins, were in good agreement for the same make. The nitrogen content is in every case much lower than that of gelatin itself (*ca.* 18%) and approaches that of albumins. On the other hand, the total sulfur and labile sulfur contents definitely distinguish it from serum albumin and egg albumen. The lower isoelectric point is in agreement with the lower nitrogen content, although there is no necessity of this following, since it depends, *e. g.*, upon proportion of diamino to monamino acids.

In some properties the material resembles keratose, as described by Wilson, but the low sulfur content is in disagreement with this. More complete analyses are required before the constitution can be established.

Relation to Fractionation of Gelatin.—A so-called "insoluble gelatin fraction" was separated by Knaggs, Manning and Schryver,[8] and their work was extended by Kunitz and Northrop.[9]

The coagulable protein from gelatins appears to be identical with the "insoluble fraction of gelatin" found by Kunitz and Northrop.[9] In their procedure the isoelectric gelatin is dissolved to a 2% solution and kept at a constant temperature of 23° for five or six days. The gelatin solution turns milky, thickens and finally breaks up into clots and precipitates.

[8] J. Knaggs, A. B. Manning and S. B. Schryver, *Biochem. J.*, **17**, 473 (1923).

[9] M Kunitz and J. H. Northrop, *J. Gen. Physiol.*, **12**, 379 (1929).

After separating the coagulum from the solution it is redissolved and the procedure repeated twelve or fifteen times. A water-insoluble fraction is obtained at the end amounting to only about 1% of the original amount.

A similar substance was obtained by Kunitz and Northrop when they hydrolyzed a 5% solution of gelatin in $M/10$ hydrochloric acid at 90°.

If the "insoluble gelatin fraction" separated by Kunitz and Northrop is actually no constituent of gelatin at all, but a protein impurity, it seems unlikely that it is essential to the swelling mechanism, as suppose by them. We have obtained a gelatin which had merely a trace of this material, but which showed all the usual swelling properties of gelatin, and equal jelly strength and viscosity. We are continuing investigations on this foreign protein and its removal from gelatin.

Summary

It is found that gelatin prepared from calf-skins usually contains a small amount—less than 1%—of a heat coagulable protein as an impurity. The protein approaches albumin (egg albumen and serum) in nitrogen content (around 14%). Its organic sulfur content is considerably higher than that of gelatin (0.8 compared to 0.24%). Its isoelectric point lies at about P_H 4.0. It is suggested that the so-called "insoluble" gelatin of various investigators is identical with this protein impurity.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF WASHINGTON]

THE CONSTITUTION OF HOMOMESITYL OXIDE[1]

BY S. G. POWELL AND C. H. SECOY

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The homomesityl oxide derived from 2-butanone has been prepared by numerous investigators.[2] Earlier workers made no attempt to determine its structure, while later investigations produced some evidence that it has the formula $C_2H_5(CH_3)C{=}CHCOC_2H_5$.

Since 2-butanone reacts with aldehydes to give compounds of the type $RCH{=}C(CH_3)COCH_3$, one would expect the structure $C_2H_5(CH_3)C{=}C(CH_3)COCH_3$, and as the evidence in favor of the other formula was not conclusive and involved some contradictions, this investigation was undertaken to determine definitely the constitution of the homomesityl oxide.

[1] This paper is an abstract of a thesis offered by C. H. Secoy in partial fulfilment of the requirements for the degree of Master of Science in the University of Washington.

[2] (a) Pawlow, *Ann.*, **188**, 138 (1877); (b) Schramm, *Ber.*, **16**, 1581 (1883); (c) Descudé, *Ann. chim.*, [7] **29**, 494 (1903); (d) Barbier and Leser, *Bull. soc. chim.*, **31**, 278 (1904); (e) Bodroux and Taboury, *ibid.*, [4] **3**, 831 (1908); *ibid.*, [4] **5**, 950 (1909); (f) Pariselle and Simon, *Compt. rend.*, **173**, 86 (1921); (g) Becker and Thorpe, *J. Chem. Soc.*, **121**, 1303 (1922); (h) Ekeley and Howe, THIS JOURNAL, **45**, 1917 (1923); (i) Franke and Köhler, *Ann.*, **433**, 314 (1923).