THE ISOELECTRIC POINT OF GELATIN AT 40°C.

By DAVID I. HITCHCOCK.

(From the Laboratories of The Rockefeller Institute for Medical Research.)

(Received for publication, January 18, 1924.)

In order to explain certain experiments on the swelling of gelatin, together with other data collected from the literature, Wilson and Kern\(^1\) suggested that gelatin exists in two forms having different isoelectric points. At temperatures above 35°C. they assumed that gelatin exists only in a "sol form" having its isoelectric point at pH 7.7, while at lower temperatures it exists as mixtures of this with the "gel form" having its isoelectric point at pH 4.7. The change to the "sol form" was supposed to be favored by increase in alkalinity or in temperature. In order to test the validity of their idea, the following experiments have been carried out.

Measurements were made of the osmotic pressure of 1 per cent gelatin solutions at 40°C., by a method similar to that used by Loeb.\(^2\) The gelatin was purified by washing at the isoelectric point as described by Loeb.\(^3\) Solutions containing 1 per cent of gelatin and varying amounts of sodium hydroxide or hydrochloric acid were placed in collodion test-tubes holding about 15 cc. Each tube was fitted with a rubber stopper and a glass manometer tube, and was submerged in 250 cc. of distilled water in a wide-mouthed Erlenmeyer flask closed by a two-hole rubber stopper. The outside air was excluded by connecting the top of the manometer tube with the air space in the flask by means of rubber and glass tubing. The flasks were put in a water bath at 40° ± 0.05°, and left about 17 hours for the attainment of equilibrium. The osmotic pressure was measured

---


\(^3\) Loeb, p. 35.
in terms of millimeters of the solution in the tubes, and the pH of the inside and outside solutions was measured with hydrogen electrodes at 25°C, or, in some cases, colorimetrically. The results are given in Table I and Fig. 1.

Fig. 1 shows that near pH 4.7, the accepted isoelectric point of gelatin, the osmotic pressure is at a minimum, while near pH 7.7 it has high values. Moreover, in Table I the pH values of the inside solutions are higher than those of the corresponding outside solutions only when the inside solutions are more acid than pH 4.7, the reverse being true on the alkaline side of pH 4.7. According to the theory of membrane equilibria this change in sign of the difference in pH between the inside and outside solutions must occur at the isoelectric point of the non-diffusible ampholyte. Hence these results are incompatible with the idea that gelatin at 40° exists in a form isoelectric at pH 7.7.

Since the pH measurements had been made at 25°, while the osmotic pressure had been measured at 40°, a comparison was made of the titration curves of gelatin with sodium hydroxide at these two temperatures. Hydrogen electrode measurements were made in

\[
\begin{array}{|c|c|c|}
\hline
\text{Osmotic pressure} & \text{pH inside} & \text{pH outside} \\
\hline
164 & 4.4 & 4.1^* \\
72 & 4.6 & 4.2^* \\
29 & 5.0 & 5.3^* \\
147 & 5.5 & 6.6^* \\
283 & 6.2 & 6.5^* \\
324 & 6.4 & 7.2^* \\
376 & 7.6 & 8.7 \\
379 & 8.3 & 9.0 \\
410 & 9.0 & 9.4 \\
403 & 9.0 & 9.8 \\
402 & 9.2 & 10.0 \\
\hline
\end{array}
\]

* Colorimetric measurement.

\[^4\text{Loeb, p. 137.}\]
the titrating vessel described by Bovie, with the results shown in Fig. 2. The points fall practically on one curve below pH 8.

![Graph of osmotic pressure vs pH](image)

**Fig. 1. Effect of pH on osmotic pressure of 1 per cent gelatin solution at 40°C.**

Measurements were also made at 40° of the viscosity of 1 per cent solutions of gelatin containing varying amounts of hydrochloric acid or sodium hydroxide. The solutions were prepared by diluting a stock solution of isoelectric gelatin, melted by heating with warm

---

water not above 45°C., and after mixing were kept about 2 hours in the thermostat at 40°C. before measurements were made. The viscosity was determined with Ostwald viscometers, and is expressed as the ratio of the time of flow to that of distilled water. This neg-

Fig. 2. Titration of 1 per cent gelatin solutions with NaOH at 25° and at 40°C.

lects the difference in density between water and a 1 per cent gelatin solution, which, according to Davis and Oakes,\(^6\) amounts to less than 3 parts in 1,000, and is affected but little by the pH or the character of the gelatin.

The results of the viscosity measurements are represented in Fig. 3. In the more alkaline solutions the viscosity was slowly decreasing, the change in about 1 hour being represented in the upper curve by the distance between two points at the same pH. The lower curve represents a separate experiment made with a different lot of isoelectric gelatin. In each case the minimum viscosity was found to be near pH 4.7, and the curves are similar in shape to those obtained by Loeb\(^7\) at 24°C. A curve of quite different shape was obtained by Davis and Oakes,\(^6\) and reproduced by Wilson\(^8\) in support of his idea that gelatin at 40°C had an isoelectric point at pH 7.7. Possibly

\(^7\) Loeb, J., *J. Gen. Physiol.*, 1920–21, iii, 104.

the different shape of their curve may be due to their method of preparing the solutions, which included heating the gelatin to 75°C.

While the present experiments do not exclude the possibility of additional secondary minima in osmotic pressure and viscosity, such as that observed by Wilson and Kern in the case of swelling in phosphate buffers, they do indicate that at 40°C the principal minimum in each of the properties studied is near pH 4.7, the established isoelectric point of gelatin. Some additional experiments on the osmotic pressure of gelatin solutions in sodium hydroxide at 24°C gave no indication of a second minimum near pH 7.7, although the results were rendered somewhat uncertain by the great difficulty of obtaining constant readings in the pH measurements. Between pH 7 and 8 gelatin possesses almost no buffer value, as is indicated by the shape of the titration curves in Fig. 2.

SUMMARY.

Measurements have been made at 40°C of the osmotic pressure and viscosity of 1 per cent gelatin solutions containing varying amounts of hydrochloric acid or sodium hydroxide. Each property was found to exhibit a decided minimum near pH 4.7. In the osmotic pressure experiments the pH of the inside solutions was greater than that of the outside solutions at pH values below 4.7, while it was less than that of the outside solutions at values above pH 4.7. These results indicate that gelatin at 40°C retains its isoelectric point at about pH 4.7.

The writer wishes to acknowledge his indebtedness to Dr. Jacques Loeb for suggesting this work.