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Fig. 1

Figure 1: Fig. 1

Abstract

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MUTAROTATION AND STRUCTURE FORMATION IN GELATIN SOLUTIONS

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The increase in specific rotation during the setting of gelatin (mutarotation) was associated in early works directly with the formation of a three-dimensional network structure containing elements of order (crystallinity) (1-4). The present work is devoted to studying the interrelation between optical activity and structure formation in gelatin solutions. The experimental data obtained in this work are discussed on the basis of modern concepts of the structure and conformation of polypeptide chains (5), as well as recently established regularities that make it possible to determine unambiguously the conformation of polypeptide chains by measuring the rotation of the plane of polarization of light (6-8, 10).

Fig. 1

Experiments were carried out on "Photo" grade gelatin, which was purified and brought to the isoelectric state by Loeb's method (9). The degree of structuring of the gels, or the structure-forming ability of gelatin solutions, was determined by measuring the melting temperature of a gel obtained under standard conditions; for this purpose a special method was developed, the essence of which is explained by Fig. 1. A gelatin solution (gel) is heated to 50° and introduced into a glass tube (Fig. 1a) through its beveled end up to the mark present on the tube. The gelatin is then cooled for 2 minutes in partially frozen benzene (Fig. 1b). Next, the tube with the set gelatin is placed in a vertically mounted test tube (Fig. 1c), and an inertia-free thermocouple is introduced into the middle of the gelatin-gel column. The test tube is heated further by immersing it in water heated to 50°. The melting temperature of the gel was taken to be the temperature corresponding to the beginning of formation of a drop of gelatin solution on the beveled end of the tube. Experiments

showed that melting of gels occurs in a narrow interval, which does not exceed 1° and is readily reproducible.

Measurements of optical rotation were made on an ordinary polarimeter with

Fig. 2

Figure 2: Fig. 2

Fig. 3

Figure 3: Fig. 3

a reading accuracy of 0.05°. A thermocouple, kept continuously in the cuvette, made it possible to monitor the temperature and select the required value.

Fig. 2. Change in the specific rotation of an 8% gelatin solution during its cooling from 40° and subsequent storage at 20°.

At first, the change in the specific rotation of a gelatin solution during its natural cooling from 40 to 20° and its subsequent storage at the same temperature was investigated. The results are presented as the curve in Fig. 2, which consists of two linear portions, passing one into the other at a temperature of about 20°. Over the first, steeper portion of the curve, cooling of the gelatin and formation of a gel take place.

The initial portion of the curve in Fig. 2 shows no special features in the region of gelation, which indicates the absence of a visible connection between optical rotation and structure formation.

At 20° the curve undergoes a break and passes into a second rectilinear portion, showing a considerably slower increase in specific rotation with time, which becomes constant after 24 hours. It is known that gelatin gels are capable of aging. This is manifested in the fact that, at constant temperature (20°), the elasticity of a gelatin gel continuously increases with time. In one study (11), the equilibrium value of the elasticity of a gelatin gel was not reached even after 50 days. Therefore, the change in specific rotation with time at 20° might, as it were, be explained by the continuing structuring of the gelatin. However, for optical rotation we obtain its constant value already after 1-2 days, whereas the elasticity of the gel will continue to increase. The increase in specific rotation with time at constant temperature (20°) is explained rather by a slow conformational transformation coil → helix (Helix–Coil Transitions), proceeding independently of the structuring process. It is possible that the structuring process, for steric reasons, even has a retarding effect on the process of conformational transformation.

Fig. 3. Dependence of specific rotation on temperature for 5% gelatin solutions having different gel-melting temperatures. 1 –before thermolysis, m.p. 30—31°C; 2 –thermolysis 1.5 hours, m.p. 22—23°; 3 –thermolysis 5.5 hours, m.p. 14—15°. On the abscissa the gel-melting temperatures are marked by arrows.

In the following experiment the dependence of specific rotation on temperature was investigated for three grades of gelatin having different tempera-

Fig. 4. Schematic picture of conformational transformations of gelatin molecules in dilute and concentrated solutions at different temperatures

Figure 4: Fig. 4. Schematic picture of conformational transformations of gelatin molecules in dilute and concentrated solutions at different temperatures

the gel melting temperature, i.e., a different capacity for structure formation. The change in the capacity of gelatin for structure formation was achieved by heating a 5% gelatin solution in a flask with a reflux condenser, immersed in boiling water for various periods of time. In applying the thermolysis method, we proceeded from the established fact that, in this process, only the intermolecular bonds are destroyed, while hydrolysis of the polypeptide chains along the main valences does not occur (12–15).

Fig. 4. Schematic picture of conformational transformations of gelatin molecules in dilute and concentrated solutions at different temperatures

Measurements of the specific rotation were carried out during the cooling of gelatin solutions that had been preliminarily heated to 50°. The results are given in Fig. 3 and, in general, indicate the absence of a relationship between the capacity for structure formation and optical rotation. Indeed, on the curves shown there, no features are observed at the points corresponding to the melting temperatures of the gels. Moreover, the sharp rise of all the curves begins at one and the same temperature, 25°, which is not associated with any structural-mechanical properties of gelatin solutions.

The decrease in specific rotations (Fig. 3) as a result of heating cannot be explained by deep hydrolysis of gelatin, which would have led to a decrease in the concentration of optically active molecules. If this were the case, the values of $[\alpha]$ should have decreased also in the range 25–45°, but this is not observed. The more gradual course of curves 2 and 3 in Fig. 3 indicates that heating at 100° caused irreversible conformational changes in some of the gelatin molecules, as a result of which they acquired the optical stability characteristic of gelatin at 40° and ceased to participate in mutarotation phenomena. We believe that such denaturation is quite possible first of all for molecules with the greatest molecular weight, and represents an elementary act of the well-known phenomenon of thermal denaturation (cooking) of collagen.

Thus, the gelatin macromolecule, being a fragment of the structure of native collagen, under normal conditions has the form of a rod-like helical spiral, the turns of which are held together by hydrogen bonds. When the temperature is raised, the hydrogen bonds are destroyed and the helix melts, first turning into a flexible thread and then coiling into a disordered globule, i.e., assuming a conformation with a minimal reserve

free energy. A long flexible chain that has formed a coil together with hydrated and mechanically entrapped water may be regarded as a "liquid macromolecule" *.

In Fig. 4 the conformational transformations of gelatin molecules as a function of temperature are shown schematically.

From a comparison of the schemes for concentrated and dilute (non-gelling) solutions, it is easy to understand why the change in specific optical rotation (mutarotation) is not a consequence of structuring of the system.

In the upper left quadrant of Fig. 4 the formation is shown of aggregates (bundles) from helical molecules, which arise when a dilute gelatin solution is cooled from 50 to 10°. It was shown[16] that gelatin in this conformation can be isolated in the dry state, and its properties differ greatly from those of ordinary gelatin. Thus, for example, it dissolves in water at 10° and, upon heating, forms a gel; it shows a decrease in viscosity after tanning, etc., i.e., properties directly opposite to those of ordinary gelatin.

The schemes we have presented for conformational transformations of gelatin molecules, of course, do not reflect the entire diversity of the properties inherent in this remarkable substance, but they may be regarded as an attempt to reveal the true molecular nature of gelatin solutions, which becomes unambiguous through the application of the method of measuring the rotation of the plane of polarization of polarized light.

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REFERENCES CITED

[1] C. R. Smith, J. Am. Chem. Soc., **41**, 135 (1915).
[2] H. Bogue, T. Connell, J. Am. Chem. Soc., **47**, 1694 (1935).
[3] E. Kraemer, J. Fanslow, J. Phys. Chem., **29**, 1169 (1925); **32**, 834 (1928).
[4] I. R. Katz, J. Winehoven, Rec. trav. chim., Pays-Bas., **52**, 36 (1933).
[5] L. Pauling, R. Corey, H. Bronson, Proc. Nat. Acad. Sci. U. S. A., **37**, 205 (1951).
[6] P. Doty, A. Holtzer et al., J. Am. Chem. Soc., **76**, 4493 (1954).
[7] C. Cohen, J. Biophys. and Biochem. Citol., **1**, No. 3 (1955).
[8] P. Urnes, P. Doty, Advances in Protein Chem., **16**, 401 (1961).
[9] J. Loeb, *Proteins and the Theory of Colloidal Phenomena*, 1933, p. 52.
[10] W. Harrington, P. Hippel, Advances in Protein Chem., **16**, 1 (1961).
[11] L. V. Ivanova-Chumakova, P. A. Rebinder, G. I. Krus, *Colloid Journal*, **18**, No. 6, 682 (1956).
[12] O. Gerngross, Collegium, **262**, 1922; **388**, (1930), Ber., **63**, 1603 (1930).
[13] W. Simon, Chim. Ztg., **61**, 887 (1937).
[14] I. I. Sokolov, *Colloid Journal*, **4**, 533 (1938); **5**, 111 (1939); Dissertation, 1938.
[15] S. I. Sokolov, Dissertation, 1947.

[16] P. I. Zubov, Z. N. Zhurkina, V. A. Kargin, DAN, **67**, No. 4, 159 (1949).

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* The liquid-like state of gelatin molecules at 40–50° is apparently the principal cause of the formation of simple coacervates under the action on gelatin of sodium sulfate or alcohol. As a result of partial dehydration the "liquid" molecules coalesce, first forming "molecular" droplets, which continue to grow to submicroscopic and microscopic dimensions, and finally the coalescence of the latter leads to the formation of a macrophase in the form of a continuous layer of coacervate.

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