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Abstract

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ON SOME REGULARITIES OF SOLUBILIZATION IN PROTEIN SYSTEMS

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Solubilization* in aqueous solutions of soaps and other surface-active substances has been systematically studied in works ([1–7]) and others, and at the present time the basic regularities of this phenomenon have been established.

Protein substances, differing essentially in their chemical nature from ordinary soaps and being high-molecular compounds, have one property in common with soaps—they possess surface activity, and consequently solubilizing ability, which was first established experimentally by D. L. Talmud ([8]). Subsequently only isolated works appeared in this direction ([9]). Meanwhile, the phenomenon of solubilization in protein systems is of great interest both from the standpoint of the theory of protein structure and in relation to the study of biologically important processes of transport and metabolism in the living organism, and therefore requires systematic investigation.

The purpose of the present work was to clarify the connection between the state (form) of protein macromolecules in solution and their ability to solubilize substances sparingly soluble in water, in particular hydrocarbons.

Gelatin was chosen as the protein component. Gelatin is a typical polyampholyte, readily soluble in water and possessing pronounced surface activity. In addition, under certain conditions gelatin is capable of reversibly passing from the fibrillar form into the globular form ([10], [17]).

The gelatin was preliminarily purified by high-voltage multicompartment electrodialysis according to the method developed by V. A. Kargin and T. A. Matveeva ([11]). Purification of a 1% gelatin solution at a voltage of 1500 V in the middle chamber and 400 V in the auxiliary chambers lasted on average 30–40 hours. The purity of the product obtained was monitored by the current strength in the apparatus. At the end of purification the current strength was approximately 8–10 mA, which corresponds to the electrical conductivity of bidistillate. In addition, the purity of the product was checked by determining the ash content. After purification the ash content was zero in the fourth decimal place. Semiquantitative spectral analysis after purification showed only traces of sodium.

Benzene was chosen as the substance to be dissolved; it was carefully purified according to the generally accepted procedure ([12]). Twice-distilled water was

Fig. 1

Figure 1: Fig. 1

always used for preparing the protein solutions.

Quantitative measurement of the solubilization effect was carried out by the refractometric method proposed and developed by A. I. Yurzhenko ([13]). This method is based on the fact that, upon introducing a hydrocarbon into a solution of a surface-active substance, the refractive index of the solution usually increases up to saturation of the solution with the hydrocarbon and then becomes

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* "Solubilization" is the term customarily used for the phenomenon consisting in the spontaneous increase in the solubility of slightly soluble or practically insoluble substances in a given solvent under the influence of additions of surface-active substances.

constant. Subsequently S. S. Voyutskii (7) showed that the solubility of liquids can be determined on the basis of the additivity rule for specific refraction. The presence of droplets of emulsified excess hydrocarbon had no effect on the experimental results. Abbe and Pulfrich refractometers were used to determine the refractive index.

The state of protein molecules in solution depends primarily on the pH of the medium; therefore this factor was studied first of all. The pH of the medium was varied by adding HCl or NaOH in the range from pH 2 to pH 11.

The isoelectric point of the dialyzed gelatin was determined from the maximum turbidity and the minimum viscosity at 20°. These data showed that the isoelectric point of the dialyzed gelatin lies at pH 5.2, which agrees with the data of G. Weber (14).

Fig. 1. Dependence of the solubilization of benzene on the pH of the medium in gelatin solutions of different concentration C.
1—$C = 0$; 2—$C = 0.11\%$; 3—$C = 0.21\%$; 4—$C = 0.43\%$; 5—$C = 0.86\%$.
$t = 20°$.

Determinations of benzene solubilization were carried out as follows. First the refractive indices of gelatin solutions with different pH values and with different gelatin concentrations were determined. Then benzene was added in excess and, after equilibrium had been established ($\sim$ 48 hours), the refractive indices of the aqueous layer were measured again. After the appropriate calculations, the value of the solubility of benzene was obtained in grams per 100 ml of gelatin solution. The results of these measurements at 20° are presented in Fig. 1. From these data it is seen that, for all the concentrations studied, the greatest

Fig. 2

Figure 2: Fig. 2

Fig. 3. Dependence of the solubilization (1) and reduced solubilization (2) of benzene on the concentration of gelatin solutions

Figure 3: Fig. 3. Dependence of the solubilization (1) and reduced solubilization (2) of benzene on the concentration of gelatin solutions

solubilization of benzene is observed at the isoelectric point. In the acid and alkaline regions the solubilities are considerably smaller than at the isoelectric point, but nevertheless greater than in pure water.

Fig. 2. Dependence of the structural viscosity η on the shear stress P for a 0.43% gelatin solution at different pH values of the medium.
1 —before solubilization;
2 —after solubilization of benzene;

The maximum solubilizing action in the isoelectric point that we have found is a new fact characterizing the isoelectric state of the protein. The appearance of the maximum may be explained by the coiling of the branched gelatin chains at the isoelectric point, leading to the formation of globules, a characteristic feature of which, as in globular proteins and in soap micelles, is the presence of a hydrophobic core,

causing the solubilization effect. In strongly acidic and alkaline media, the mutual repulsion of like-charged functional groups leads to the straightening of the chains and solubilization decreases. The fact that solubilization does not disappear completely is probably explained by sorption of benzene molecules on the nonpolar regions (side chains) of the gelatin macromolecules.

In connection with the considerations set forth above, it was of interest to determine the change in the viscosity of gelatin solutions after benzene had been dissolved in them. The viscosity was measured with a rotational elastoviscosimeter of a design developed by one of us jointly with S. P. Alekhin (15).

Fig. 3. Dependence of the solubilization (1) and reduced solubilization (2) of benzene on the concentration of gelatin solutions

In Fig. 2 are presented viscosity data for a 0.43% gelatin solution at 20°.

As can be seen from Fig. 2, at pH 5.2 the structural viscosity at the isoelectric point shows a slight increase after solubilization. Apparently this is due to the fact that, at the isoelectric point, the gelatin globules have a sufficiently loose structure, and benzene, entering inside the globule, increases its dimensions only slightly.

In the acidic and alkaline regions away from the isoelectric point (see Fig. 2, pH 2.5 and 10.6), after dissolution of benzene a sharp decrease in structural

viscosity is observed. It may be assumed that the "coarsening" of hydrophobic regions due to sorbed benzene leads to partial coiling of the molecules (as a result of mutual attraction of the nonpolar portions of the chains by van der Waals forces (16)).

Figure 3 shows the dependence of benzene solubilization on the concentration of gelatin in solution. From Fig. 3, 1, it is seen that up to a 0.5% gelatin solution there is an approximately linear increase in solubilization; then, at higher concentrations, benzene solubilization reaches a constant value. Curve 2 shows that the amount of benzene bound by 1 g of gelatin decreases with increasing gelatin concentration in solution. Attention is drawn to the fact that the observed solubilization limit at a concentration of 0.5% coincides with the onset of structuring of the system (gel formation) (10). On the other hand, the increase in the reduced amount of bound benzene with decreasing concentration is a consequence of the facilitation of the globulization process due to the removal of gelatin macromolecules from one another.

We also investigated the solubility of benzene in gelatin solutions at 30° as a function of concentration and of the pH of the medium. It turned out that, in this case, the characteristic maximum of solubilization at the isoelectric point disappears, although the solubility of benzene in gelatin solutions is still somewhat greater than in water. The disappearance of the maximum and the decrease in the magnitude of solubilization are explained by the absence of globules in the gelatin solution at elevated temperatures, as indicated in the literature (10).

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Note: Figure translations are in progress. See original paper for figures.