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# CONCENTRATION DEPENDENCES OF MACROMOLECULAR SIZES IN AQUEOUS SOLUTIONS OF ALBUMINS[1]

*On the basis of experimental data for the shear viscosity in the aqueous solutions of ovine serum albumin and using the cellular model describing the viscosity in aqueous solutions, the concentration dependences of the effective radius of ovine serum albumin macromolecules in the aqueous solutions within a concentration interval of 3.65–25.8 wt% and a temperature interval of 278–318 K at the constant pH = 7.05 are calculated. The concentration and temperature dependences of the effective radii of ovine, bovine, and human serum albumin macromolecules are compared. It is shown that they are partially similar for the solutions of ovine and human serum albumins within concentration intervals of 0.12–0.49 vol% and 0.18–0.48 vol%, respectively, provided an identical acid-base balance (pH) in those solutions. The following conclusions are drawn: (i) the concentration dependences of the effective radii of structurally similar macromolecules of various albumins are similar, but provided an identical pH, and (ii) the dependence of the volume concentration of aqueous albumin solutions on the temperature at the constant radius of a macromolecule confirms the hypothesis about the existence of a dynamic phase transition in aqueous solutions at a temperature of 42 °C, at which the thermal motion of water molecules significantly changes.*

*K e y w o r d s*: ovine serum albumin, aqueous solution, effective macromolecular radius, cellular model.

## 1. Introduction

Serum albumins are globular proteins, each consisting of a sequence of amino acid residues that are linked into a single-stranded macromolecule with a rather complicated spatial structure [1]. The functional properties of albumin macromolecules depend on their spatial structures at the secondary and tertiary levels, which are determined by the specific sequence of amino acids in the molecular chain [1, 2]. The secondary structure of an albumin macromolecule consists of alpha-helices and beta-folds stabilized by hydrogen bonds, and a disordered part of the macromolecular chain. The tertiary structure of an albumin macromolecule is represented by its domain composition, with hydrophobic interactions between the domains being responsible for the globular structure of the protein [1–3].

Serum albumins are responsible for the osmotic blood pressure and provide the transport of hormones, fatty acids, metabolites, toxins, drugs, metal cations ($Na^+$, $K^+$, $Ca^+$), and so forth [1, 2]. The

[1] This article is dedicated to the 75th anniversary of Academician L.A. Bulavin

human serum albumin (albumin-I) and the bovine serum albumin (albumin-II), because of their presence as basic materials, have gained the widest application in medicine, pharmacology, molecular biology, and biochemistry [2–5].

The structure of albumin macromolecules in a solution is substantially affected by the temperature, concentration, acid-base balance, and the presence of salt ions and denaturants. One of the sensitive indicators of internal structural transformations occurring in a macromolecule in a solution is its hydrodynamic radius. The application of the modern cellular model [6–8] allows the concentration dependences of the hydrodynamic radius of macromolecules, as well as their variations with the temperature, acid-base balance, and ionic composition of the aqueous solution, to be calculated.

The aim of the work was to study the concentration dependences of the effective radius of ovine serum albumin (albumin-III) macromolecules in aqueous solutions at various temperatures in order to determine the peculiarities in the internal reconstruction of macromolecules in aqueous solutions in the course of their clustering.

## 2. Experimental Data

The experimental data were taken from work [9], where the shear viscosity of aqueous solutions of ovine serum albumin was studied in a concentration interval of 3.65–39.3 wt% and a temperature interval of 278–318 K at the constant pH = 7.05. The measurements were performed with the help of the capillary viscosimetry method. In the cited work, crystalline non-defatted ovine serum albumin (Sigma Chemical Company) was applied. It was dissolved in distilled water, so that the solutions did not contain salt ions.

## 3. Theoretical Part

The current stage of modeling the shear viscosity in macromolecular solutions is associated with the application of the cellular approach [6–8]. In the framework of this approach, it is assumed that the perturbations of hydrodynamic flows by a macromolecule are mainly localized within a spherical cell that surrounds this macromolecule. At the cell boundary, the normal component of the perturbation velocity and the tangential stress are assumed to equal zero, which means the absence of friction at the outer cell surface [6–8]. When applying the cellular approach, it is necessary to change from the solution mass concentration $C$ to the volume concentration of macromolecules, $\varphi$, which are mutually related by the formula

$$\varphi = \frac{4\pi\rho C N_{\rm A} R^3}{3M}, \tag{1}$$

where $R$ is the radius of a macromolecule, $\rho$ the solution density, $N_{\rm A}$ the Avogadro constant, and $M$ the molecular weight of a macromolecule.

The effective radii of albumin macromolecules were calculated on the basis of the data on the shear viscosity of solutions in the bulk concentration interval $\varphi \leqslant 0.2$ with the help of Batchelor's formula [10]

$$\bar{\eta} = \eta_0(1 + 2.5\varphi + 5.2\varphi^2 + ...), \tag{2}$$

where $\eta_0$ is the solvent viscosity, and $\bar{\eta}$ the average viscosity of the solution. This formula makes it possible to determine the radii of macromolecules, whose shape is close to spherical, and the results obtained correlate well with the results of other physical methods aimed at studying the radii of macromolecules at the bulk concentrations $\varphi \leqslant 0.2$. A detailed analysis and a procedure of using Batchelor's formula to determine the size of macromolecules in dilute solutions can be found in works [11, 12].

In works [6–8], the following theoretically substantiated relation was applied to calculate the effective radii of macromolecules from the shear viscosity data for the solutions in the bulk concentration interval $0.2 \leqslant \varphi \leqslant 0.5$:

$$\bar{\eta} = \eta_0 \frac{\psi(1-\psi)}{\psi(1-\psi) + 1 - \sqrt{1 + 2\psi^2(1-\psi)}}, \tag{3}$$

where $\eta_0$ is the solvent viscosity, $\bar{\eta}$ the average viscosity of the solution, $\psi = (R/R_0)^3$, $R$ the radius of a macromolecule, and $R_0$ cell's radius. Therefore, the problem of determining the average viscosity in a solution of macromolecules is reduced to finding a relation between the cellular model parameter $\psi$ and the specific volume quantity $\varphi = V/V_0$, where $V$ is the total volume occupied by macromolecules, and $V_0$ the volume of the system. The quantity $\varphi$ has a sense of the volume concentration of macromolecules in the solution and can be measured experimentally.

Formula (3) was obtained in the framework of the cellular approach. The latter involves the rotational degrees of freedom of the particle and allows one to

describe the behavior of the viscosity in dilute solutions of macromolecules in the interval of volume particle concentrations $\varphi \leqslant 0.5$. In effect, the indicated upper limit corresponds to a solution concentration, at which all macromolecules are in contact with one another [6–8, 11, 12]. The algorithm of calculations, according to formula (3), was described in works [11, 12] in detail.

## 4. Discussion of Results

The spatial structures of albumins are sensitive to changes in the acid-base balance, and the tertiary structures of their macromolecules change considerably with a variation of pH of the solution. Note that the average physiological value of the acid-base balance in the sheep blood equals pH = 7.40 [13, 14]. At this pH value, an albumin-III macromolecule is folded into a compact conformation like a "heart-shaped medallion"[15, 16]. The experimental acid-base balance of the albumin-III solutions equals pH = 7.05, i.e. in the vicinity of physiological pH values. Therefore, with some caution, the albumin-III macromolecule can be considered as a sphere. This circumstance gives us grounds to apply formula (3) to calculate the effective radii of the particles participating in the viscous flow. Hence, when processing the experimental data [9], we used formula (3) in a concentration interval of 3.65–25.8 wt%, which corresponds to the interval of volume concentrations of albumin-III macromolecules $\varphi = 0.12 \div 0.48$.

The analysis of the obtained concentration dependences for the effective radius of albumin-III macromolecules in the examined temperature interval allows two concentration areas that are characterized by a similar behavior of the effective radii to be marked. In a concentration interval of 3.65–11.2 wt% ($\varphi = 0.12 \div 0.30$), the effective radius of albumin-III macromolecule decreases non-monotonically with the growing concentration and temperature. The maximum value of the effective radius, 45.50 Å, is reached at $\varphi = 0.13$ and $T = 283$ K. In a concentration interval of 15.8–25.8 wt% ($\varphi = 0.39 \div 0.49$), the dependences of the effective radius of albumin-III macromolecules are almost linear. In addition, the temperature effect on the effective radius of albumin-III macromolecules is weaker here than in the lower-concentration interval (see Fig. 1). Unfortunately, the absence of experimental data for concentrations lower than 3.65 wt% did not allow us to trace the behavior of the effective radius at albumin-III concentrations $\varphi \leqslant 0.12$.

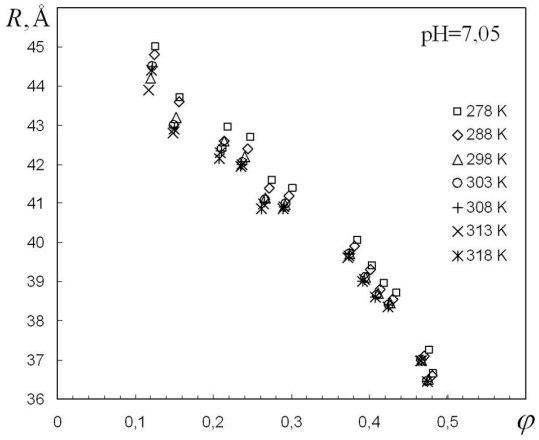


***Fig. 1.*** Concentration dependences of the effective radius of albumin-III macromolecules in the aqueous solution at pH = = 7.05 and various temperatures

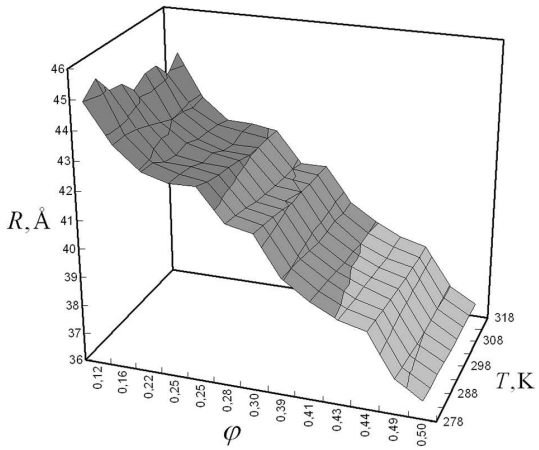


***Fig. 2.*** Dependence of the effective radius of albumin-III macromolecules in the aqueous solutions on the volume concentration and the temperature at pH = 7.05

Processing the experimental data according to formula (3) provided us with the concentration and temperature dependences of the effective radius of albumin-III macromolecules in the aqueous solutions in a temperature interval of 278–318 K at pH = 7.05 (see Fig. 2).

Let us analyze the temperature dependences of the albumin-III concentration in the aqueous solutions, at which the radius of albumin macromolecules remains constant. They give us characteristic curves that make it possible to trace changes in the size of

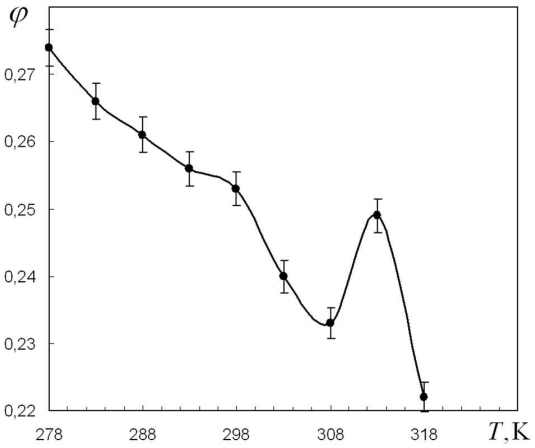


***Fig. 3.*** Dependence of the volume concentration in the aqueous solutions of albumin-III on the temperature at a constant radius of its macromolecules

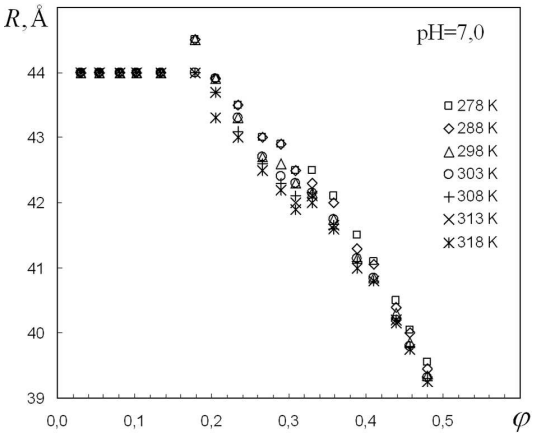


***Fig. 4.*** Concentration dependences of the effective radius of albumin-I macromolecules along the isotherms at pH = 7.0 [21]

albumin macromolecules induced by the water properties. Figure 3 demonstrates the characteristic curve $\varphi = f(T)\,|_{R=\text{const}}$ that corresponds to $R = 42.15 \pm \pm 0.05$ Å in a concentration interval of 7.3–9.3 wt% ($\varphi = 0.22 \div 0.27$). Note that these concentrations are somewhat higher than the percolation threshold concentration $\varphi \approx 0.23$ (about 7 wt% for albumin-III), at which the protein macromolecules form infinite percolation clusters in the solution [16]. This curve has a local minimum at the temperature $T_{\min} = 308 \pm 1$ K ($35 \pm 1$ °C) and a local maximum at the temperature $T_{\max} = 314 \pm 1$ K ($41 \pm 1$ °C). The presence of the maximum in the curve $\varphi = f(T)\,|_{R=\text{const}}$ testifies that a temperature of 42 °C is a peculiar one for the aqueous solutions of albumin-III. This fact confirms

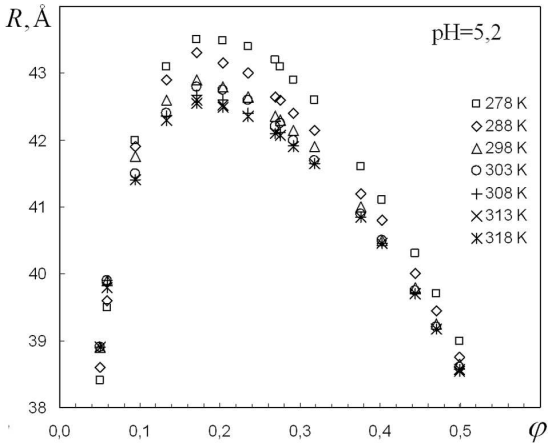


***Fig. 5.*** Concentration dependences of the effective radius of albumin-II macromolecules along the isotherms at pH = = 5.2 [22]

the hypothesis stated in works [17–19] about the existence of a dynamic phase transition in aqueous systems at $T = 42$ °C, at which the character of the thermal motion of water molecules substantially changes, and the redistribution of hydrogen bonds takes place.

Only a few publications dealing with the properties of albumin-III macromolecules in the aqueous solutions can be found in the literature. For instance, in work [15], using the dynamic light scattering method, the hydrodynamic radius $R_H = 36.0$ Å was obtained for the aqueous solution of albumin-III with a concentration of 0.9 wt% ($\varphi = 0.03$) at the temperature $T = 298$ K and pH = 7.4. In work [20], using the same method and under the same conditions ($T = 298$ K and pH = 7.4), the hydrodynamic radius $R_H = 38.0$ Å was obtained for the aqueous solution of albumin-III with a concentration of 0.1 wt% ($\varphi = 3.5 \times 10^{-3}$). Note that the indicated values were obtained from the self-diffusion coefficients of albumin-III macromolecules in dilute aqueous solutions using the Stokes–Einstein equation.

Let us compare the results obtained for albumin-III with the concentration and temperature dependences of the effective radii of the albumin-I [21] and albumin-II [22] macromolecules, which were studied by us earlier.

In particular, in work [21], the surface of the effective radius of albumin-I macromolecules as a function of the temperature (in an interval of 278–318 K) and the concentration in an interval of 0.82–18.2 wt% ($\varphi = 0.03 \div 0.48$) was plotted for

pH = 7.0. Three concentration areas were distinguished, where the behaviors of the effective radius of albumin-I macromolecules are different: (i) 0.82–3.65 wt% ($\varphi = 0.03 \div 0.14$), where the effective radius of albumin-I macromolecule is constant; (ii) 4.67–9.45 wt% ($\varphi = 4.67 \div 9.45$), where the effective radii of albumin macromolecules in the aqueous solution non-monotonically decrease; and (iii) concentrations above 10.2 wt% ($\varphi \geq 0.33$), where the effective radius of albumin-I macromolecules decreases almost linearly with the increasing concentration, and the slope of the descending dependences weakly depends on the temperature (see Fig. 4).

In work [22], on the basis of experimental data on the shear viscosity in aqueous albumin-II solutions, the surface for the effective radius of albumin-II macromolecules was plotted in a concentration interval of 2.0–20.0 wt% ($\varphi = 0.05 \div 0.49$) and a temperature interval of 278–318 K for the constant value pH = 5.2 corresponding to the isoelectric point of albumin-II. It was shown that, to concentrations of about 5 wt% ($\varphi \approx 0.17$), the effective radius of albumin-II macromolecules rapidly increases. At a concentration of 5 wt% ($\varphi \approx 0.17$), the effective radius of albumin-II macromolecules has a maximum, the position of which is almost independent of the temperature. This concentration corresponds to isolated albumin-II macromolecules because the concentration, at which they form infinite percolation clusters in the solution is equal to about 7 wt% ($\varphi \approx \approx 0.23$) [16]. In a concentration interval of 5–20 wt% ($\varphi = 0.17 \div 0.49$), the effective radius of albumin-II macromolecules in the solution decreases, and this behavior is almost linear at concentrations higher than 10 wt% ($\varphi > 0.32$) (see Fig. 5).

A partial similarity is observed between the concentration dependences of the effective radii of albumin-III macromolecules at $\varphi(\mathrm{III}) \geq 0.12$ and albumin-I macromolecules at $\varphi(\mathrm{I}) \geq 0.18$. Similar is the behavior of the effective radii of albumin-III macromolecules at $\varphi(\mathrm{III}) = 0.12 \div 0.30$ and albumin-I macromolecules at $\varphi(\mathrm{I}) = 0.18 \div 0.31$, where the effective radii of both macromolecules non-monotonically decrease with the increasing concentration and temperature; as well as in the concentration intervals of $\varphi(\mathrm{III}) = 0.39 \div 0.49$ and $\varphi(\mathrm{I}) = 0.33 \div 0.48$, where the both dependences are almost linear and weakly depend on the temperature. In our opinion, the similar character of the concentration dependences of the effective radii of the albumin-I and albumin-III macromolecules results from the similar spatial structures of those macromolecules under the condition of an identical pH in their aqueous solutions.

## 5. Conclusions

In this work, the experimental data on the shear viscosity in the aqueous solutions of ovine serum albumin have been processed in the framework of the cellular model. The surface of the effective radius of ovine serum albumin macromolecules is plotted within a concentration interval of 3.65–25.8 wt% ($\varphi = 0.12 \div 0.48$) and a temperature interval of 278–318 K at the constant pH = 7.05. The concentration and temperature dependences of the effective radius of ovine serum albumin macromolecules are compared with analogous results obtained earlier for the human and bovine serum albumins. It is shown that there exists a partial similarity between the concentration and temperature dependences of the effective radii for ovine and human serum albumin macromolecules within the volume concentration intervals 0.12–0.49 and 0.18–0.48, respectively, provided that the acid-base balances (pH) of the aqueous solutions of those albumins are identical within the measurement error.

Analyzing of the concentration dependences of the effective radii of albumin macromolecules, the following conclusions are made:

- the concentration dependences of the effective radii of structurally similar albumins are also similar, but provided identical pH values;
- the dependence of the volume concentration of the aqueous albumin solutions on the temperature at a constant macromolecular radius confirms the hypothesis [17–19] about the existence of a dynamic phase transition in aqueous solutions at the temperature $T = 42$ °C, at which the thermal motion of water molecules considerably changes.

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КОНЦЕНТРАЦІЙНА ЗАЛЕЖНІСТЬ РОЗМІРІВ МАКРОМОЛЕКУЛ АЛЬБУМІНІВ У ВОДНИХ РОЗЧИНАХ

Р е з ю м е

За допомогою сучасної коміркової моделі в'язкості водних розчинів із експериментальних даних зсувної в'язкості отримані концентраційні залежності ефективного радіуса макромолекул овечого сироваткового альбуміну у водних розчинах у концентраційному інтервалі (3,65–25,8) мас.% та інтервалі температур (278–318) K при сталому значенні pH = 7,05. Проведено порівняння концентраційних і температурних залежностей ефективних радіусів макромолекул овечого сироваткового альбуміну, бичачого сироваткового альбуміну та сироваткового альбуміну людини. Показано, що спостерігається часткова подібність концентраційних і температурних залежностей ефективних радіусів макромолекул овечого сироваткового альбуміну і макромолекул сироваткового альбуміну людини в інтервалах об'ємних концентрацій, відповідно, (0,12–0,49) і (0,18–0,48), причому кислотно-лужні баланси (pH) водних розчинів вказаних альбумінів однакові. Зроблено висновки про те, що: 1) концентраційні залежності ефективних радіусів структурно схожих альбумінів є подібними, але за умови однакових pH; 2) залежність об'ємної концентрації водних розчинів альбуміну від температури при сталому радіусі макромолекули підтверджує гіпотезу про існування у водних розчинах при температурі 42 °C динамічного фазового переходу, при якому має місце суттєва зміна характеру теплового руху молекул води.