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## CALCULATION OF THE MACROMOLECULAR SIZE OF BOVINE SERUM ALBUMIN FROM THE VISCOSITY OF ITS AQUEOUS SOLUTIONS

*On the basis of experimental data for the shear viscosity of aqueous bovine serum albumin (BSA) solutions and in the framework of the Malomuzh–Orlov cellular approach, the surface of effective radii of BSA macromolecules has been plotted for the constant  $\text{pH} = 5.2$  in the concentration interval of 2.0–27.2 wt% and the temperature interval 278–318 K. A rapid non-linear increase in the effective radii of BSA macromolecules is shown to take place up to BSA concentrations of about 5 wt% in the whole examined temperature interval. The maxima of the effective radii of BSA macromolecules are observed at a BSA concentration of 5 wt%, and their position is temperature-independent. In the concentration interval 5.0–27.2 wt%, the effective radii of BSA macromolecules decrease, and this reduction is linear at BSA concentrations higher than 10 wt%. A comparison of the calculation results with literature data on the self-diffusion coefficient of macromolecules in solutions testifies to the efficiency of the Malomuzh–Orlov formula for calculating the macromolecular radii of globular proteins on the basis of shear viscosity data for their aqueous solutions.*

*Keywords:* bovine serum albumin, aqueous solution, effective macromolecular radius, Malomuzh–Orlov theory.

### 1. Introduction

The conformation and the spatial structure of biomacromolecules in a solution govern, to a great extent, their functional properties in a living organism. Bovine serum albumin (BSA), which comprises about 70% of the total protein composition of blood plasma with a concentration of 35–55 g/l, is the best-studied and the most common protein in blood plasma [1]. Owing to the applicability of standard separation methods and the availability of initial material, BSA is widely used in the laboratory biochemical practice, in particular, as a standard molecular weight of proteins, in protein quantification methods, for the stabilization of some enzymes, and in immunohistochemistry [1, 2].

Bovine serum albumin consists of 583 amino acid residues. The latter are linked into a single-chain macromolecule with a molecular weight of 66.5 kDa and a rather complicated spatial structure, which is similar to that of human serum albumin (HSA) [1, 2]. One of the specific features in the BSA structure is the presence of two tryptophan residues (Trp135

and Trp214) in the macromolecular chain, in contrast to one residue (Trp214) in the HSA macromolecule [1]. At physiological pH values, the secondary structure of BSA macromolecule consists of alpha helices (50–68%) and beta folds (16–18%), which are stabilized by hydrogen bonds, as well as of a disordered part of macromolecular chain [1–3]. Seventeen disulfide bonds between cysteine residues of alpha helices are responsible for the formation of the tertiary BSA structure: there arise three domains, each of which being formed by subdomains consisting of three alpha helices, whereas hydrophobic interactions between the domains determine the globular protein structure [1, 2].

The spatial BSA structure is sensitive to changes in the acid-base balance: the tertiary structure can substantially, but reversibly, change with the variation in the pH of a solution. Conformational transitions occur at pH values equal to 2.7, 4.3, 8, and 10. At the physiological value  $\text{pH} = 7.4$ , the protein is folded into a compact conformation of an almost regular triangular prism of the heart-like shape (the so-called N-isoform); at  $\text{pH} = 3.5$ , the configuration of a biomacromolecule resembles an elongated ellip-

soid of rotation like a cigar (the F-isoform); finally, as pH decreases to 2.7, the protein denatures to a practically stretched macromolecular configuration (the E-isoform) [3–5]. Owing to the conformational N–F transition, the total protein charge changes from –16 at pH = 7.4 to +100 at pH = 3.5 [1, 5]. The isoelectric point, at which the total charge of the biomacromolecule equals zero, is passed at pH = 4.7 [1, 5].

One of the most important characteristics of BSA macromolecules in dilute solutions is their hydrodynamic radius. This parameter makes it possible to monitor changes in the internal structure of macromolecules in the solution with variations of the temperature, concentration, and acid-base balance (pH). Various physicochemical methods are applied in order to determine the hydrodynamic radius, such as fluorescence, photon correlation spectroscopy, X-ray diffraction, small-angle neutron scattering, NMR spectroscopy with a pulsed magnetic field gradient, and capillary viscosimetry. Since plenty of factors can affect the structure of protein macromolecules (these are the concentration, temperature, and pH of aqueous protein solutions, the presence of salts and denaturants, and so forth) and owing to differences among the physical approaches in the experimental research methods, there are mismatches in the determined values of the hydrodynamic macromolecular radius.

This work was aimed at a careful determination of the effective radius of BSA macromolecules in aqueous solutions and its dependence on the solution temperature and concentration. The effective radius was determined by analyzing the experimental data for the BSA shear viscosity using the Batchelor formula in the case of a rather dilute solution and the Malomuzh–Orlov one in the case of a concentrated solution. The results obtained are compared in detail with the values determined from the analysis of the self-diffusion coefficient of BSA macromolecules on the basis of the Stokes–Einstein formula. A meticulous study of the dependence of the effective macromolecular radii on the solution temperature, concentration, and acid-base balance is crucial for establishing the character of the internal restructuring of macromolecules and reproducing the oligomerization processes in the system.

## 2. Experimental Part

The required experimental data were taken from work [6], where the shear viscosity of aqueous bovine serum

albumin solutions was studied using the capillary viscosimetry method in a concentration interval of 1.76–36.34 wt% and a temperature interval of 278–318 K. The researches were carried out at the constant pH = 5.2, which corresponded to the vicinity of the BSA isoelectric point.

## 3. Theoretical Part

### 3.1. Application of the Batchelor formula to the shear viscosity data for solutions with the bulk concentrations $\varphi \leq 0.2$

Let us illustrate our method aimed at determining the effective radius of macromolecules by applying it to the simplest case where the ratio between the shear viscosities of the solution,  $\eta$ , and the solvent,  $\eta_0$ , satisfies the inequality  $\eta/\eta_0 < 1.5$ , which corresponds to the bulk concentrations  $\varphi \leq 0.2$ . This is the case where the results of accurate calculations by Batchelor [7] can be used. According to them,

$$\eta = \eta_0(1 + a_1\varphi + a_2\varphi^2 + \dots), \quad (1)$$

where

$$a_1 = 2.5, \quad a_2 = 5.2. \quad (2)$$

Rewriting Eq. (1) in the form

$$a_1\varphi + a_2\varphi^2 + \dots = \lambda, \quad (3)$$

where  $\lambda = \frac{\eta}{\eta_0} - 1$ , and assuming  $\lambda$  to be a small parameter ( $\lambda < 1$  or even  $\lambda \ll 1$ ), let us seek the solution  $\varphi$  of Eq. (3) in the form of an infinite power series in  $\lambda$ :

$$\varphi(\lambda) = b_1\lambda + b_2\lambda^2 + \dots \quad (4)$$

Substituting Eq. (4) into Eq. (3) and equating the coefficients in the terms with identical power exponents of  $\lambda$ , we obtain

$$b_1 = \frac{1}{a_1}, \quad b_2 = \frac{a_2}{a_1^3}. \quad (5)$$

In agreement with Eq. (2),

$$b_1 = 0.4, \quad b_2 = -0.3328. \quad (6)$$

The bulk concentration of macromolecular balls is related to the effective macromolecular radius  $R_{\text{eff}}$  by the relation

$$\varphi = \frac{4\pi\rho c_m N_A R_{\text{eff}}^3}{3M_w}, \quad (7)$$

where  $c_m$  is the mass concentration,  $\rho$  the solution density,  $N_A$  the Avogadro constant,  $M_w$  the average molecular weight, and  $R_{\text{eff}} = R_\eta$  or  $R_D$ . From whence, we have

$$R_\eta = \gamma\varphi^{1.3}, \quad \gamma = d(\rho c_m)^{-1/3}, \quad d = \sqrt[3]{\frac{3M_w}{4\pi N_A}}. \quad (8)$$

For albumin macromolecules,  $d_{\text{alb}} = 2.98 \times 10^{-8} \text{ kg}^{-1/3}$ . We will also take into account that

$$\rho = \rho_w(1 - c_m) + \rho_{\text{alb}}c_m, \quad (9)$$

where  $\rho_w$  is the water density, and  $\rho_{\text{alb}} = 0.07 \text{ g/cm}^3$  is the density of dry albumin [8]. By combining Eqs. (8) and (4), we obtain

$$R_\eta = \gamma(b_1\lambda)^{1/3}(1 + (b_1/b_2)\lambda + \dots)^{1/3} \Rightarrow \quad (10)$$

$$\Rightarrow \gamma(b_1\lambda)^{1/3} \left( 1 + \frac{b_2}{3b_1}\lambda - \frac{1}{9} \left( \frac{b_2}{b_1} \right)^2 \lambda^2 + \dots \right), \quad (11)$$

where

$$\frac{b_2}{3b_1} = -0.2773, \quad \frac{1}{9} \left( \frac{b_2}{b_1} \right)^2 = 0.0769.$$

The Batchelor formula makes it possible to determine the values for the radii of hard, compact, and almost spherical macromolecules that correlates well with the results of other physical methods in the bulk concentration interval  $\varphi \leq 0.2$ . However, it does not make allowance for the variation of the medium pH and the presence of salts, i.e. the factors to which the internal structure of the albumin macromolecule in a solution is extremely sensitive.

### 3.2. Application of the Malomuzh–Orlov formula to the shear viscosity data to solutions with the bulk concentrations $0.2 \leq \varphi \leq 0.5$

The further progress in finding the shear viscosity of more concentrated macromolecular solutions is associated with the use of cellular models. In the framework of them, the perturbations of hydrodynamic flows induced by particles in the suspension are assumed to be mainly localized within spherical cells around particles. It is also assumed that both the normal component of the perturbation velocity and the tangential stress equal zero at the cell boundary, which means no friction at the outer cell surface.

When calculating the effective radii of macromolecules on the basis of the data for the shear viscosity of solutions within the bulk concentration interval of  $0.2 \leq \varphi \leq 0.5$ , let us apply the Malomuzh–Orlov formula. Its theoretical substantiation was expounded in works [9, 10]. The Malomuzh–Orlov formula was obtained in the framework of the cellular approach. The latter considers the rotational degrees of particle freedom. The method of the Malomuzh–Orlov algorithm application was described in works [11, 12] in detail. This algorithm allows the viscosity behavior of dilute macromolecular solutions to be described within the interval of bulk particle concentrations  $\varphi \leq 0.5$ , i.e. up to concentrations that, in essence, coincide with the solution concentrations at which all macromolecules are in contact with one another [9–12].

### 3.3. Application of the Batchelor method while determining the self-diffusion coefficient of macromolecules in a solution

Note that, as was in the case of shear viscosity, the Batchelor method is applicable for bulk concentrations  $\varphi \leq 0.2$ . In works [13, 14], it was shown that the self-diffusion coefficient of hard spheres in a dilute solution is determined by the expression

$$D_S = D_0(1 + \alpha\varphi + O(\varphi^2)), \quad (12)$$

where  $\alpha = -2.1$ ,

$$D_0 = \frac{k_B T}{6\pi\eta_0 R_D} \quad (13)$$

is the Stokes–Einstein self-diffusion coefficient [13],  $k_B$  the Boltzmann constant,  $T$  the absolute temperature,  $\eta_0$  the solvent viscosity, and  $R_D$  the hydrodynamic radius of a macromolecule. Confining the consideration to the first order in the bulk concentration  $\varphi$ , we obtain the following expression for the self-diffusion coefficient [15]:

$$D_S = D_0(1 + \lambda_C\varphi + \dots), \quad (14)$$

where

$$\lambda_C = \lambda_V + \lambda_O + \lambda_D + \lambda_S + \lambda_A. \quad (15)$$

The terms in formula (15) are as follows:  $\lambda_V = 8x^3$  is the viral correction factor,  $\lambda_O = -6x^2$  is the Oseen contribution,  $\lambda_D = 1$  is the dipole contribution, and

$\lambda_S = 75/(256x^4) + O(x^{-5})$  and  $\lambda_A$  are contributions determined by hydrodynamic interactions. Here, the notation  $x = R_S/R_D$  was used, where  $x = R_S/R_D$  is the radius of the model hard sphere, and  $R_D$  is the hydrodynamic radius of the particle. In work [15], the nonlinearity of the dependence  $\lambda_C(x)$  was shown, and the coefficient  $\lambda_C$  in formula (14) was demonstrated to change from  $\lambda_C^{\min} = 1.454$  at  $x = 1.00$  to  $\lambda_C^{\max} = 7.251$  at  $x = 8.00$ .

In the linear approximation, the dependence of the self-diffusion coefficient of macromolecules on their bulk concentration  $\varphi$  looks like Eq. (14), where  $\lambda_C$  is a known proportionality coefficient. The bulk concentration is related to the effective macromolecular radius  $R_{\text{eff}}$  by the relation

$$\varphi = \frac{4\pi\rho_m c_m N_A}{3M_w} R_{\text{eff}}^3 \equiv P\rho_m c_m R_{\text{eff}}^3, \quad (16)$$

where  $c_m$  is the mass concentration of macromolecules in the solution,  $\rho_m$  is the density of the substance created by macromolecules, and  $R_{\text{eff}} = R_\eta$  or  $R_D$ . The self-diffusion coefficient  $D_0$  of macromolecules in the dilute solution is assumed to be determined by the Stokes–Einstein relation

$$D_0 = \frac{k_B T}{6\pi\eta_0 R_0} \quad (\varphi \rightarrow 0). \quad (17)$$

If the concentration of macromolecules is relatively low, their effective radius can be expressed in the form

$$R_D = R_0 + \delta R, \quad (18)$$

where  $R_0$  is the radius of a macromolecule in a dilute solution.

For weakly concentrated macromolecular solutions, the Stokes–Einstein formula (17) can be generalized in the following manner:

$$D_S = \frac{k_B T}{6\pi\eta_0 R_D} = D_0 \left( 1 - \frac{\delta R}{R_0} + \frac{\delta R^2}{R_0^2} + \dots \right). \quad (19)$$

Comparing this expression with Eq. (14), we obtain the equation

$$-\frac{\delta R}{R_0} + \frac{\delta R^2}{R_0^2} + \dots = \lambda\varphi. \quad (20)$$

It is obvious that

$$\varphi = P c_m (R_0 + \delta R)^3 \Rightarrow$$

$$\Rightarrow \varphi_0 \left( 1 + 3\frac{\delta R}{R_0} + 3\frac{\delta R^2}{R_0^2} + \dots \right), \quad \varphi_0 = P c_m R_0^3, \quad (21)$$

where  $\varphi_0 = P c_m R_0^3$ . From whence, we arrive at the following equation:

$$\frac{\delta R}{R_0} (1 + 3\lambda\varphi_0) - \frac{\delta R^2}{R_0^2} (1 - 3\lambda\varphi_0) + \dots = -\lambda\varphi_0. \quad (22)$$

In the linear approximation in  $\lambda\varphi_0$ , we have

$$\delta R = -\lambda\varphi_0 R_0 + 4\lambda^2 \varphi_0^2 R_0. \quad (23)$$

Recall that  $\lambda$  is negative for spherical macromolecules. Therefore, the radius of macromolecules increases with  $\varphi_0$ .

### 3.4. Self-diffusion coefficients of macromolecules in rather concentrated solutions

The literature database also contains more complicated expressions for the self-diffusion coefficient. In particular, in work [13], the following formula was proposed for the self-diffusion coefficient in rather concentrated solutions:

$$D_S = D_0(1 + \beta_1\varphi + \beta_2\varphi^2), \quad (24)$$

where

$$\beta_1 = -1.83, \quad \beta_2 = 0.91. \quad (25)$$

In work [13], it was pointed out that formula (24) correlates well with the data on the self-diffusion coefficient for the bulk concentrations  $\varphi \leq 0.1$ , whereas the self-diffusion coefficient at higher bulk concentrations is better approximated by a linear dependence, which is in contradiction with the theoretical Batchelor model. Neglecting the effects of changing the acid-base balance and the presence of salt ions introduces a significant error into the determined values of the radii of albumin macromolecules, the structure of which is sensitive to the changes of the mentioned solution parameters.

The hard-sphere model, the Medina-Noyola theory [16], and the mean-field approximation of Mazur and Geigenmüller [17] bring together to the following expression, which correlates well with experimental results and theoretical predictions and can be applied to describe the self-diffusion coefficient of spherical

macromolecules in the solution in the bulk concentration interval  $0.2 \leq \varphi \leq 0.5$  [18]:

$$D_S = D_0 \frac{(1 - \varphi)^3}{1 + 1.5\varphi + 2\varphi^2 + 3\varphi^3}. \quad (26)$$

For the determination of the self-diffusion coefficient of macromolecules and their sizes in solutions, the method of dynamic light scattering is widely applied. It is based on the analysis of the temporal autocorrelation function of the scattered radiation intensity fluctuations

$$G^{(2)}(\tau) = \left[ G^{(1)}(\tau) \right]^2 + 1 + \zeta(x), \quad (27)$$

where

$$G^{(1)}(\tau) = \int_0^{\infty} P(\Gamma) \exp(-\Gamma\tau) d\Gamma, \quad (28)$$

is the first-order correlation function,  $\zeta(x)$  the experimental noise,  $P(\Gamma)$  the distribution function of relaxation rates,  $\Gamma = 1/t_c = D_S q^2$ ,  $t_c$  is the correlation time, and  $q$  the wave vector of a concentration fluctuation [19].

The calculation of the macromolecular size on the basis of a temporal autocorrelation function has some advantages, but it is not free of disadvantages. Equation (27) is called the Siegert relation, and expression (28) is a Fredholm integral equation of the first kind. The latter is classified as an ill-posed problem, which means that if the function is given even with a small error, the solution may strongly differ from the true one and may not be a unique one. Therefore, within the experimental error limits, there can be an infinite number of different solutions, which would correspond to experimental data equally well. The more accurate the experimental data, the fewer the number of solutions will correspond to those data [19].

The determination procedure of the particle radius in a solution from the results of dynamic light scattering consists of several stages, namely,

- 1) the determination of the autocorrelation function values;
- 2) the approximation of the autocorrelation function using the Siegert relation;
- 3) the determination of the self-diffusion coefficient value;
- 4) the calculation of the particle radius using the Stokes–Einstein relation.

Thus, the determination of the macromolecular size in the framework of the dynamic light scattering method is a multistage process, each step of which requires the application of certain physical models with their own approximations and errors.

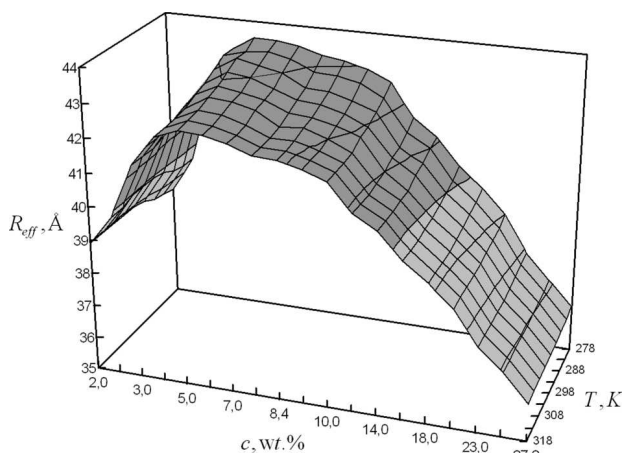
Hence, the determination of the sizes of macromolecules in a solution from the shear viscosity data using the Malomuzh–Orlov relation seems to be an experimentally simpler and more convenient method.

#### 4. Discussion of Results

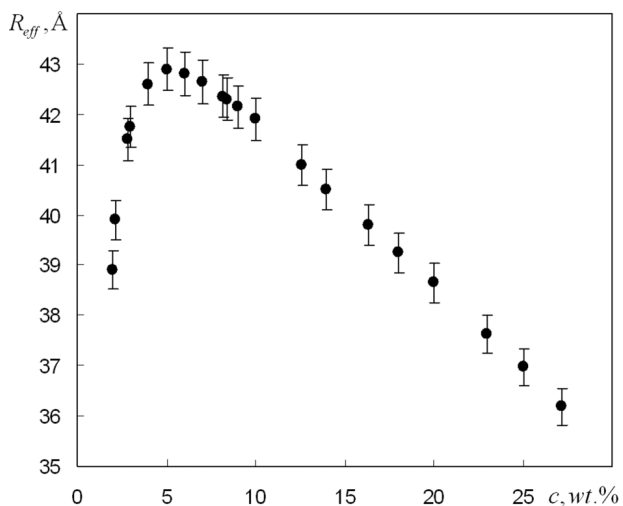
First of all, it should be noted that, at the given pH = 5.2, the BSA macromolecule curls into a compact heart-like conformation (the N-isoform). Therefore, the application of the Malomuzh–Orlov formula seems to be legitimate. This makes it possible to calculate the effective radii of a particle participating in the viscous flow. In order to use the Malomuzh–Orlov formula, it is necessary to change from the mass solution concentration  $c$  to the bulk concentration of macromolecules  $\varphi$ . The corresponding algorithm was applied to the concentration interval  $c = 2.0 \div 27.2$  wt%, which corresponded to the interval of bulk macromolecular concentrations  $\varphi = 0.04 \div 0.49$ .

By processing the experimental data using the Malomuzh–Orlov algorithm, the concentration dependences of the effective radii of BSA macromolecules in aqueous solutions along the isotherms are obtained (see Fig. 1). The analysis of the surface of effective radii in Fig. 1 testifies to a rapid nonlinear growth of the effective radii of BSA macromolecules up to concentrations of about 5 wt% in the whole temperature interval. At concentrations of about 5 wt%, the effective radii of BSA macromolecules have maxima, whose positions are independent of the temperature. With the temperature growth, the effective macromolecular radii insignificantly decrease: from 43.50 Å at 278 K to 42.55 Å at 318 K. In the concentration interval 5.0–27.2 wt%, a reduction in the effective radii of BSA macromolecules is observed. At concentrations higher than 10 wt%, the decreasing dependence can be approximated by a straight line. A typical concentration dependence of the effective BSA radii at a temperature of 298 K is exhibited in Fig. 2.

In our earlier work [12], we studied the effective radii of human serum albumin (HSA) macromolecules on the basis of the data on the shear viscosity of aque-



**Fig. 1.** Temperature-concentration dependence of the effective radii of bovine serum albumin macromolecules at pH = 5.2



**Fig. 2.** Concentration dependence of the effective radius of BSA macromolecules in the aqueous solution at  $T = 298$  K and pH = 5.2

ous HSA solutions at pH = 7.0 in a temperature interval of 278–318 K and a concentration interval of 0.82–36.9 wt%. It was found that the effective radii of HSA molecules remain constant up to a concentration of 3.7 wt% in the whole examined temperature interval, i.e., there is a “plateau” for the effective radii of HSA macromolecules at relatively low concentrations. At the same time, another behavior is observed for BSA macromolecules near the isoelectric point; namely, in dilute solutions, their effective radii increase with the concentration. A similar feature in the concentration-temperature dependences of the effective radii of BSA and HSA macromolecules consists

in that the effective radii of biomacromolecules remain almost temperature-independent at concentrations of 10 wt%. Owing to the similar spatial structure, the maximum effective radii of HSA (44 Å) and BSA (43.50 Å) macromolecules correlate well with each other.

Let us compare our results with literature data. It is worth noting that, despite a considerable number of works concerning the structure of albumin macromolecules in the solution, the available results depend on the physical approaches in the experimental research methods and produce a fragmentary, mosaic information about the dynamics of the albumin macromolecule structure in a solution, its dependence on the temperature, concentration, pH, the presence of salts, and the origin of buffer solutions. Taking the aforesaid into account, let us compare the sizes of macromolecules obtained from experimental methods based on the transfer phenomena, first of all, from the diffusion coefficients of macromolecules in a solution.

In work [20], using the method of dynamic light scattering in dilute aqueous BSA solutions ( $T = 25$  °C, pH = 5.0, an ionic strength of 0.15 M), the value  $D_S = (6.14 \pm 0.03) \times 10^{-11}$  m<sup>2</sup>/s for the self-diffusion coefficient was obtained. For this value, we obtain  $R_D = (39.92 \pm 0.03)$  Å. This result coincides with the value  $R_\eta = (39.90 \pm 0.05)$  Å calculated according to the Malomuzh–Orlov formula (with  $c = 2.14$  wt%,  $T = 25$  °C, and pH = 5.2). It is worth noting that the pH values of the solutions of BSA macromolecules whose radii are compared are located near the isoelectric point of BSA solutions.

In work [21], the method of dynamic light screening was applied to measure the diffusion coefficient of BSA macromolecules in the aqueous solution ( $c = 1$  wt%,  $T = 23$  °C, pH = 3–7, a phosphate buffer of 0.023 M). The results obtained made it possible to calculate the hydrodynamic radius of BSA macromolecule at pH = 5.0, which was found to equal  $R_D = 37.9$  Å. This value correlates well with the values presented in Fig. 2, if the curve is extrapolated to a concentration of about 1 wt%.

According to research [22] of the shear viscosity of aqueous BSA solutions in which the biomacromolecules are in the compact N-form at pH = 4–9, the effective length of BSA macromolecule was estimated to equal 83 Å, i.e. the effective radius amounted to 41.5 Å. In the same work, the method of dynamic

light scattering was applied to determine the diffusion coefficient. The corresponding value of the averaged hydrodynamic radius equaled 38.8 Å in the interval  $\text{pH} = 4\div 9$ . These results correlate well, in general, with the values obtained using the Malomuzh–Orlov formula (see Table).

In work [23], using the method of diffusion through porous diaphragms, the interdiffusion coefficient was obtained for dilute aqueous BSA solutions ( $c = 2.79$  wt%,  $T = 25$  °C,  $\text{pH} = 4.7$ , an ionic strength of 0.1 M). Considering corrections, the corresponding value of the hydrodynamic radius was found to equal  $R_D = 40.3$  Å. The value calculated from the viscosity of aqueous BSA solution ( $c = 2.79$  wt%,  $T = 25$  °C,  $\text{pH} = 5.2$ ) with the use of the Malomuzh–Orlov formula is slightly larger,  $R_\eta = 41.50 \pm 0.05$  Å, which can be connected with a certain mismatch of solution pH values and the presence of NaCl.

Thus, our results correlate well with the values calculated on the basis of literature data. Difficulties in the comparison of macromolecular radii are associated with the mismatch of the solution pH and the presence of salt ions to which the albumin structure is sensitive. However, there are also results that testify to somewhat smaller values of BSA macromolecular radii.

For instance, the analysis of shear viscosity data obtained for dilute aqueous BSA solutions at  $T = 25$  °C and  $\text{pH} = 5.0$  brought the authors of work [24] to a BSA radius of 33.7 Å, whereas the analysis of diffusion data brought them to a value of 34.8 Å. The authors of work [25] reported about the BSA macromolecular radius in the solution that was equal to 33.9 Å. By applying the hard-sphere model to BSA macromolecules in the normal saline solution, the authors of work [26] obtained a value of  $(34.2 \pm 1.4)$  Å for the hydrodynamic radius of a curled BSA macromolecule in dilute solutions (however, no information was provided about the solution temperature and pH). It is of interest that those results are similar to the results obtained using the molecular dynamics methods [5] for the N-isoform at  $\text{pH} = 7.4$ , when the distance between the centers of mass of domains 1 and 3 was estimated to equal  $(34.7 \pm 1.2)$  Å.

What is the origin of the indicated discrepancies among the effective radius values obtained using various physical research methods? In our opinion, those discrepancies can be explained by the following factors. First, the structure of the BSA macromolecule

that corresponds to a compact heart-like conformation deviates from the spherical shape and is better approximated by a somewhat elongated spheroid flattened at its poles. Such a form may affect the results of modeling the effective radii with the use of the Malomuzh–Orlov formula. Second, a protein macromolecule is inhomogeneously surrounded by a solvent layer (or layers), the molecules in which interact with the protein macromolecule by means of hydrogen bonds and, together with the macromolecule itself, participate in the process of viscous flow. Therefore, it seems reasonable that the hydrodynamic size of macromolecules should be slightly larger than that of macromolecules in the quasicrystalline state. Then, the following question arises: How many water molecules located near macromolecule's surface are involved into the process of macromolecular viscous flow?

Thus, the Malomuzh–Orlov formula turns out to be a rather simple efficient method for the determination of the macromolecular radii from the shear viscosity data for the macromolecular solutions. An experimental accessibility of the capillary viscosimetry method and an ability to quickly change the solution composition during the study make it possible to obtain the dependence of the macromolecular size on the temperature, concentration, pH, and ionic composition of the solution, which is important for understanding the complicated dynamics of biomacromolecules in the aqueous solution.

It is very important that the proposed method demonstrates a nonmonotonic variation of the effective radius of albumin macromolecules. This fact is especially important for establishing the origin of structural transformations in the albumin macromolecule.

When comparing our results with other literature data and analyzing the causes for the discrepancies

#### Comparison of values obtained for the effective radii of BSA macromolecules

$c$ , wt%	$R_\eta$ , Å	$R_D$ , Å	Remarks to $R_D$
1.00	35.6	37.9	23 °C, $\text{pH} = 5.0$ , ionic strength=0.023 M [21]
2.14	39.90	39.92	25 °C, $\text{pH} = 5.0$ , ionic strength=0.15 M [20]
2.79	41.5	40.3	25 °C, $\text{pH} = 4.7$ , ionic strength=0.1 M [22]

among the results obtained by various physical methods, it is evident that a comprehensive experimental study is required concerning the size change of albumin macromolecules with the variation of the pH and ionic strength of a solution. The results of corresponding researches will be reported in the following publications.

## 5. Conclusions

From experimental data on the shear viscosity in bovine serum albumin solutions and in the framework of the Malomuzh–Orlov cellular approach, the surface of effective radii of bovine serum albumin macromolecules is plotted in a concentration interval of 2.0–27.2 wt% and a temperature interval of 278–318 K at the constant pH = 5.2, which corresponds to a vicinity of the isoelectric point of bovine serum albumin.

It is shown that a rapid nonlinear growth of effective radii of bovine serum albumin macromolecules up to concentrations of about 5 wt% takes place in the whole examined temperature interval. At a concentration of 5 wt%, the effective radii of bovine serum albumin macromolecules have maxima, whose position is temperature-independent, whereas the effective radii insignificantly decrease with the temperature growth. In a concentration interval of 5.0–27.2 wt%, the effective radii of bovine serum albumin macromolecules decrease, and the decreasing dependence is linear at concentrations higher than 10 wt%. It is found that the effective radius of albumin macromolecules changes nonmonotonically; this fact is especially important for determining the character of structural transformations in the albumin macromolecule.

The concentration-temperature dependences of the effective radii of bovine serum albumin and human serum albumin macromolecules (the latter have been studied earlier) are analyzed. Accounting for similar spatial structures of albumin biomacromolecules, the similar features of the indicated dependences are as follows: the temperature independence of the effective radii of the biomacromolecules at concentrations higher than 10 wt% and practically identical maximum values of the effective radii of human serum albumin (44 Å) and bovine serum albumin (43.5 Å) macromolecules within the model error limits.

A comparison of the results obtained in this work on the basis of the self-diffusion coefficient of macro-

molecules in a solution with literature data testifies to the efficiency of the Malomuzh–Orlov formula for calculating the radii of globular protein macromolecules on the basis of shear viscosity data for their aqueous solutions. Assumptions are made which can explain discrepancies among the values of the macromolecular radii obtained in the framework of various physical research methods.

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ОБЧИСЛЕННЯ РОЗМІРІВ  
МАКРОМОЛЕКУЛ БИЧАЧОГО СИРОВАТКОВОГО  
АЛЬБУМІНУ ЗГІДНО ДАНИХ ІЗ В'ЯЗКОСТІ  
ЙОГО ВОДНИХ РОЗЧИНІВ

## Резюме

За допомогою коміркового підходу Маломужа–Орлова з експериментальних даних зсувної в'язкості розчинів бичачого сироваткового альбуміну побудована поверхня ефективних радіусів макромолекул бичачого сироваткового альбуміну у концентраційному інтервалі 2,0–27,2 мас.% та інтервалі температур 278–318 К при сталому значенні рН = 5,2. Показано, що у всьому температурному інтервалі до концентрацій ~5 мас.% відбувається стрімке нелінійне зростання ефективних радіусів макромолекул бичачого сироваткового альбуміну. При концентрації 5 мас.% спостерігаються максимуми ефективних радіусів макромолекул бичачого сироваткового альбуміну, положення яких виявляється незалежним від температури. У інтервалі концентрацій 5,0–27,2 мас.% простежується зменшення ефективних радіусів макромолекул бичачого сироваткового альбуміну, причому при концентраціях більших 10 мас.% спадає залежність носить лінійний характер. Проводиться порівняння результатів роботи із даними літературних джерел по коефіцієнту самодифузії макромолекул у розчині, яке вказує на ефективність формули Маломужа–Орлова для розрахунку радіусів макромолекул глобулярних білків із даних зсувної в'язкості їх водних розчинів.