https://doi.org/10.15407/ujpe64.4.287

O.V. KHOROLSKYI

Poltava V.G. Korolenko National Pedagogical University
(2, Ostrograds'kyi Str., Poltava 36000, Ukraine; e-mail: khorolskiy.alexey@gmail.com)

CALCULATION OF THE EFFECTIVE MACROMOLECULAR RADII OF HUMAN SERUM ALBUMIN FROM THE SHEAR VISCOSITY DATA FOR ITS AQUEOUS SOLUTIONS

The Malomuzh–Orlov theory is used to analyze the experimental shear viscosity data obtained for aqueous solutions of human serum albumin (HSA) at pH = 7.0 in wide temperature and concentration intervals, which allowed the effective radii of HSA macromolecules to be calculated. It is shown that three intervals of the effective molecular radius of HSA with different behaviors can be distinguished in a temperature interval of 278–318 K: 1) below the crossover concentration, the effective molecular radius of HSA remains constant; 2) in the interval from the crossover concentration to about 10 wt%, the effective molecular radius of HSA in the aqueous solution nonlinearly decreases; and 3) at concentrations of 10.2–23.8 wt%, the effective radius of HSA macromolecules linearly decreases, as the concentration grows. The assumption is made that the properties of water molecules in the solution bulk play a crucial role in the dynamics of HSA macromolecules at the vital concentrations of HSA in the solutions. The role of water near the surface of HSA macromolecules and the corresponding changes of its physical properties have been discussed.

K e y w o r d s: human serum albumin, aqueous solution, effective macromolecular radius, Malomuzh–Orlov theory.

1. Introduction

Albumins comprise about 2/3 of the total human blood plasma proteins. They have the smallest molecular weight among the blood plasma proteins (about 69000 Da) and insert the largest contribution to the colloid-osmotic blood pressure (75–80%) [1, 2]. The concentration of albumins in human blood serum amounts to 35–50 g/l [3]. Albumin has remarkable properties with respect to water. In particular, 1 g of albumin is capable of binding 18 ml of water [2]. Albumins participate in the transport of fatty acids, hormones, bilirubin, and medical drugs [1].

Human serum albumin (HSA) consists of 585 amino acid residues, which are combined into a single macromolecular chain with three α-helical domains [4]. In the crystalline state, a human serum albumin macromolecule is coiled into a compact conformation of a regular triangular heart-like prism with sides of about 80 Å and a height of about 30 Å [5]. The structure of an albumin macromolecule significantly changes in an aqueous solution owing to the thermal motion of water molecules and conformational changes in the macromolecular links.

One should expect that the structure and dynamics of a molecule of human serum albumin depend on the concentration, temperature, and pH of its aqueous solutions. It is worth noting that the blood pH is a rigid constant of the human body and varies within an interval of 7.36–7.44. The pH deviations modify the electrochemical properties of proteins and induce general metabolic disorders. The pH values exceeding the normal one by 0.4–0.5 invoke irreversible changes of homeostasis that are incompatible with life [1].

There is a large body of theoretical (see, e.g., works [6, 7]) and experimental (see, e.g., works [7–11]) researches dealing with the structure of the HSA molecule in aqueous solutions. However, a common picture of the relation between the structure of albumin macromolecule and the pH, concentration, and temperature of aqueous HSA solutions, as well as the presence of impurities in them, remains still absent. Moreover, the specific features in the dynam-

ics of water molecules near the surface of HSA macromolecule remain unclear. In particular, how many water molecules near the macromolecule surface are involved into the viscous flow process? Such issues require further systematic researches, and this work is one of them.

2. Experimental Part

Experimental data on the shear viscosity of aqueous HSA solutions in a temperature interval of 278–318 K, a concentration interval of 0.82–36.9 wt%, and at a constant pH value of 7.0 were taken from work [12]. Measurements in work [12] were carried out, by using the capillary viscometry method. The relative error did not exceed 2%.

3. Theoretical Part

Nowadays, the simulation of the shear viscosity in macromolecular solutions is connected with the application of cell models [13]. The latter take into account that the perturbation of hydrodynamic flows by particles in the solution is mainly localized in a spherical cell surrounding the particle. It is also assumed that the normal component of the perturbation velocity and the tangential component of the strain equal zero, which means the absence of friction at the external cell surface [13]. In particular, it was shown in works [13, 14] that the viscosity of dilute macromolecular solutions satisfies the Malomuzh–Orlov formula

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

where $\bar{\eta}$ is the average viscosity of the solution, η_0 the solvent viscosity, $\psi = (R_0/R)^3$, R_0 is the radius of a macromolecule, and R the cell radius. Thus, the problem of determining the average viscosity in a polymer solution is reduced to finding a relation between the model parameter $\psi = (R_0/R)^3$ and the specific volume $\varphi = V_0/V$, where V_0 is the total volume occupying by macromolecules, and V the total volume of the system. The parameter φ can be measured experimentally and means the bulk concentration of macromolecules in the solution. The Malomuzh–Orlov formula makes it possible to describe the behavior of the viscosity of dilute macromolecular solutions in the interval of bulk particle concentrations $\varphi \leqslant 0.5$. In effect, the upper limit coincides with the solution concentration, at which all macromolecules in the solution are in contact with one another [13, 14].

For the Malomuzh–Orlov formula to be useful, it is necessary to change from the mass solution fraction C to the bulk macromolecular concentration φ, which are connected by the relation

$$\varphi = \frac{4\pi R^3 \rho C N_{\mathrm{A}}}{3M_w}, \tag{2}$$

where R is the radius of a macromolecular coil, ρ the solution density, N_{A} the Avogadro constant, and M_w the average molecular mass of the macromolecular compound.

In order to determine the effective radius of a macromolecule, we use the following algorithm [15]:

1) according to Eq. (2), the effective radius of the macromolecule is determined by the formula

$$R_{\mathrm{eff}} = \left(\frac{3M_w}{4\pi\rho C N_{\mathrm{A}}}\varphi_{\mathrm{eff}}\right)^{1/3}, \tag{3}$$

2) φ_{eff} is the effective concentration of macromolecules in the solution bulk; this value is reached, when the shear viscosity of the solution η_{sol} is equal to the quantity $\eta_{\mathrm{MO}}[\varphi_{\mathrm{eff}}]$ obtained from the Malomuzh–Orlov formula,

$$\eta_{\mathrm{sol}} = \eta_{\mathrm{MO}}[\varphi_{\mathrm{eff}}]. \tag{4}$$

Note that φ_{eff} differs from the bulk concentration of macromolecules, φ, calculated by formula (2).

The experimental data were analyzed, by using the indicated algorithm and the Malomuzh–Orlov formula. The concentration dependences of the effective radii of HSA macromolecules were determined at the points along the aqueous HSA solution isotherms.

In the theory of dilute solutions of macromolecular compounds, the characteristic viscosity is used as a criterion for estimating the concentration mode of the solution:

$$[\eta] = \lim_{C\to 0}\left(\frac{\eta-\eta_0}{\eta_0 C}\right), \tag{5}$$

where η is the viscosity of the macromolecular compound solution, η_0 the solvent viscosity, and C the mass fraction of the solute in the solution. The solution is called dilute, if the volume occupied by the

macromolecules is much smaller than the total solution volume. As the concentration of the macromolecular compound increases, the solution structure changes from isolated macromolecules to aggregates and, when the critical concentration C^* of macromolecules, i.e. the beginning of the so-called crossover region, is reached, to a intermolecular network of links. The critical crossover concentration C^* can be determined experimentally by the viscometric method [16]:

$$[\eta]C^* = 1. \tag{6}$$

The calculation of the crossover concentration for aqueous HSA solutions in the whole temperature interval gave the average value $C^* = 3.7$ wt%.

4. Discussion of the Results Obtained

An analysis of the results obtained making use of the Malomuzh–Orlov algorithm testifies to a complicated temperature and concentration dependence of the effective radii of albumin macromolecules. In this dependence, three regions can be conditionally distinguished (Fig. 1). Up to and inclusive the crossover concentration $C^* = 3.7$ wt%, which determines the transition from dilute solutions to semidilute ones and which was calculated from the characteristic viscosity by formulas (5) and (6), the effective radii of albumin molecules remain invariant and equal to 44 Å within the whole temperature interval. A similar situation was described by us earlier in work [15] for the effective radii of polyvinyl alcohol (PVA) macromolecules in dilute aqueous solutions. In particular, a "plateau" of effective macromolecular radii was observed in the region of relatively low temperatures and concentrations, where the effective radii remained constant.

At concentrations of 4.65–9.45 wt%, the effective radii of albumin molecules in an aqueous solution diminish nonlinearly to values of about 42 Å. In a concentration interval of 10.2–23.8 wt% (the upper limit of this interval, corresponding to $\varphi \approx 0.47$, is the applicability limit of the Malomuzh–Orlov formula), the effective radii of albumin macromolecules decrease linearly with the concentration growth, with the slope angles of the decreasing dependences being weakly dependent on the temperature (Fig. 2).

An analysis of the data exhibited in Fig. 2 showed that the effective radius of an albumin macromolecule at concentrations of 0.82–3.65 wt% and 10.2–23.8 wt% remains almost temperature-independent. The albumin macromolecule becomes the most sensitive to temperature changes in a concentration interval of 4.65–9.45 wt%.

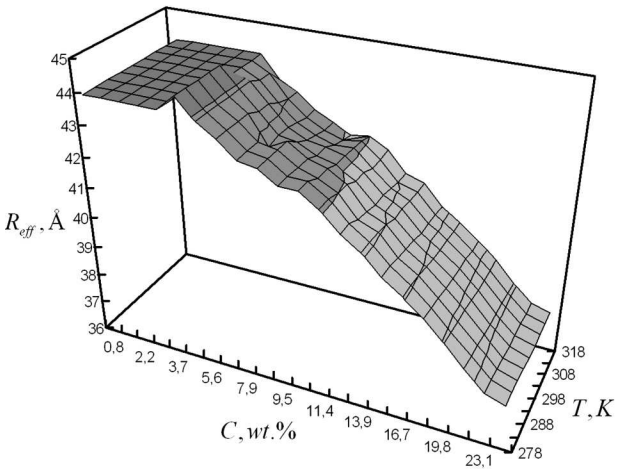

Fig. 1. Temperature and concentration dependence of the effective radii of human serum albumin macromolecules

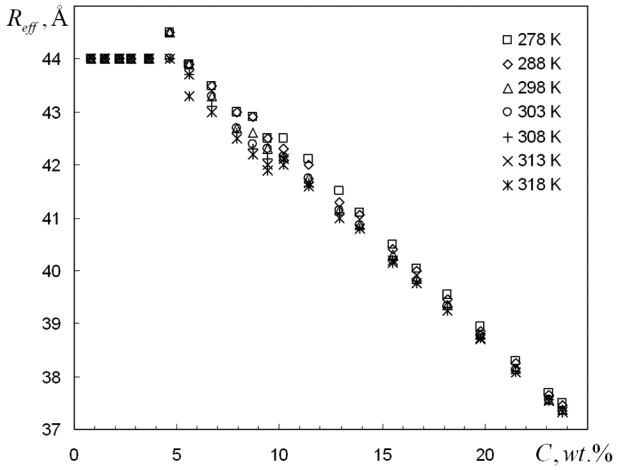

Fig. 2. Concentration dependences of the effective radii of human serum albumin macromolecules along isotherms

Hence, the effective radius of a human serum albumin macromolecule in the aqueous solution turns out much more sensitive to the changes in the protein concentration than to the changes of the solution temperature. From the viewpoint of the human body biochemistry, it is a deviation of the protein concentration in blood plasma from the normal value (homeostasis) that can result in the emergence of pathological states and diseases [1–3].

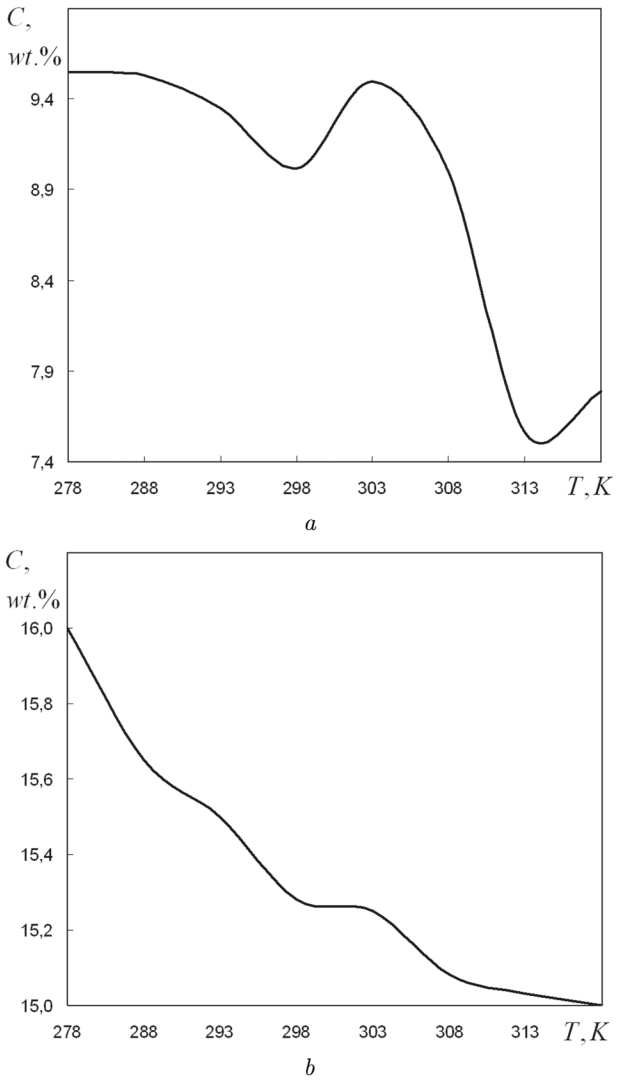

Fig. 3. Temperature dependences of the concentration of the aqueous albumin solutions corresponding to constant radii of macromolecular coils: 42.53 ± 0.05 Å (a) and 40.35 ± 0.05 Å (b)

In Fig. 3, the temperature dependences of the HSA concentration plotted for various constant radii of an albumin macromolecule, $C = f(T)|_{R=\text{const}}$, are depicted. These are characteristic curves that make it possible to detect changes in the size of an albumin macromolecule associated with the water properties in the solution bulk and near the macromolecule surface. It was shown earlier in work [15] that the characteristic curves for aqueous PVA solutions can be approximated by two straight lines with different slopes and intersecting each other at a temperature of (315 ± 2) K. A conclusion was drawn that this is a temperature, at which the microscopic properties of the liquid system PVA-water change, and these changes are induced by changes in the properties of the solvent, i.e. water. As was shown in work [17], water undergoes a dynamic phase transition at a temperature of 317 K (42 °C), at which the character of the thermal motion of water molecules significantly changes, and a redistribution of hydrogen bonds takes place.

Figure 3, a exhibits the characteristic curve $C = f(T)|_{R=\text{const}}$ corresponding to $R_{\text{eff}} = (42.53 \pm \pm 0.05)$ Å in a concentration interval of 7.0–10.0 wt%. This curve has two local minima at the temperatures $T_{\min 1} = (25 \pm 1)$ °C and $T_{\min 2} = (41 \pm 1)$ °C, as well as one local maximum at the temperature $T_{\max} = (30 \pm 1)$ °C. Note that the temperature interval between the local minima is an interval within which the living matter (more precisely, warm-blooded organisms) exists [18].

Figure 3, b demonstrates the characteristic curve $C = f(T)|_{R=\text{const}}$ corresponding to $R_{\text{eff}} = (40.35 \pm \pm 0.05)$ Å in a concentration interval of 14.5–16.0 wt%. As one can see, the indicated extrema disappear in this concentration interval. Let us try to interpret such a behavior.

In a concentration interval of 14.5–16.0 wt%, the role of water molecules located near the surface of albumin macromolecules increases [19]. Simultaneously, the disappearance of the extrema in the characteristic curve is observed. We may assume that this fact testifies to a change in the physical properties of water near the surface of an albumin macromolecule. Experimental studies of the aqueous solutions of biopolymers by the NMR method may be a certain evidence in favor of this assumption.

In particular, it was shown that a signal from "unfrozen" water is registered from aqueous solutions of biopolymer molecules frozen down to −35 °C, and the magnitude of this signal is proportional to the concentration of macromolecules [20, 21]. The cited authors concluded that 1) "unfrozen" water is in direct contact with the biopolymer macromolecule, and 2) "unfrozen" water is less mobile than in the water bulk [21]. The energy of interaction between a water molecule with the surface of a protein macromolecule is estimated as an energy required to break approximately one hydrogen bond [21]. But every water molecule can form four hydrogen bonds

with neighbor molecules. In the bulk, it is linked with neighbor molecules by about three hydrogen bonds [22, 23]. Therefore, in our opinion, it is incorrect to say about radical changes in the properties of water near the surface of an albumin macromolecule. Instead, we should consider a certain modification of water properties. This issue is to be considered in a separate article.

5. Conclusions

In this work, experimental data on the temperature and concentration dependences of the shear viscosity in aqueous solutions of human serum albumin are analyzed making use of the Malomuzh–Orlov formula. The latter allows the shear viscosity of the solutions of macromolecular coils to be modeled up to bulk concentrations $\varphi = 0.45$ and the effective radii of macromolecules to be calculated.

The dependence of the effective radii of the human serum albumin macromolecules as a function of both the temperature (within an interval of 278–318 K) and the concentration (within an interval of 0.82–23.8 wt%) at a constant pH value of 7.0 is plotted. It is shown that three concentration intervals can be distinguished in the examined temperature range, in which the behavior of the effective radius of HSA molecules changes.

1. At concentrations of 0.82–3.65 wt%, the effective radii of HSA molecules remain unchanged.

2. At concentrations of 4.67–9.45 wt%, the effective radii of albumin in its aqueous solution nonlinearly decrease.

3. At concentrations of 10.2–23.8 wt%, the effective radii of albumin macromolecules linearly decrease, as the concentration grows, with the slope of the decreasing dependences being weakly dependent on the temperature.

An assumption was made that, at vital concentrations of human serum albumin, the properties of water molecules located in the solution bulk play a crucial role in the macromolecular dynamics. As the HSA concentration increases, the physical properties of water located near the surface of albumin macromolecules undergo certain modifications, and its role changes.

The author is sincerely grateful to Academician L.A. Bulavin for his permanent attention and comprehensive support of our research dealing with the properties of macromolecules in solutions. The author also expresses deep gratitude to Prof. M.P. Malomuzh and Prof. O.P. Rudenko for a thorough discussion of the results obtained and their critical remarks to this paper.

1. L.M. Tarasenko, K.S. Neporada, V.K. Grygorenko. *Functional Biochemistry* (Poltava, 2000) (in Ukrainian).
2. L.O. Kovalkina, G.I. Moroz. Albumin as a polyfunctional drug. *Ukr. Zh. Extrem. Med.* **11**, 18 (2010) (in Ukrainian).
3. Yu.V. Khmelevskii, O.K. Usatenko. *Main Biochemical Constants of Human in Health and Disease* (Zdorov'ya, 1987) (in Russian).
4. T. Peters, jr. *All About Albumin: Biochemistry, Genetics, and Medical Applications* (Academic Press, 1996).
5. X.M. He, D.C. Carter. Atomic structure and chemistry of human serum albumin. *Nature* **3582**, No. 6383, 209 (1992).
6. B. Sjöberg, K. Mortensen. Interparticle interactions and structure in nonideal solutions of human serum albumin studied by small-angle neutron scattering and Monte Carlo simulation. *Biophys. Chemist.* **52**, 131 (1994).
7. M.L. Ferrer, R. Duchowicz, B. Carrasco, J.G. de la Torre, A.U. Acuña. The conformation of serum albumin in solution: A combined phosphorescence depolarization-hydrodynamic modeling study. *Biophys. J.* **80**, 2422 (2001).
8. P.C. Sontum, C. Christiansen. Photon correlation spectroscopy applied to characterization of denaturation and thermal stability of human albumin. *J. Pharm. Biomed. Anal.* **16**, 295 (1997).
9. A. Amoresano, A. Andolfo, R.A. Siciliano, R. Cozzolino, L. Minchiotti, M. Galliano, P. Pucci. Analysis of human serum albumin variants by mass spectrometric procedures. *Biochim. Biophys. Acta* **1384**, 79 (1998).
10. D. Pouliquen, Y. Gallois. Physicochemical properties of structured water in human albumin and gammaglobulin solutions. *Biochimie* **83**, 891 (2001).
11. J.K.A. Kamal, D.V. Behere. Spectroscopic studies on human serum albumin and methemalbumin: Optical, steady-state, and picosecond time–resolved fluorescence studies, and kinetics of substrate oxidation by methemalbumin. *J. Biol. Inorg. Chem.* **7**, 273 (2002).
12. K. Monkos. On the hydrodynamics and temperature dependence of the solution conformation of human serum albumin from viscometry approach. *Biochim. Biophys. Acta* **1700**, 27 (2004).
13. N.P. Malomuzh, E.V. Orlov. Static shear viscosity of a bimodal suspension. *Ukr. J. Phys.* **50**, 618 (2005).
14. E.V. Orlov. Shear viscosity of dispersions of particles with liquid shells. *Colloid J.* **72**, 820 (2010).
15. O.V. Khorolskyi. Effective radii of macromolecules in dilute polyvinyl alcohol solutions. *Ukr. J. Phys.* **63**, 144 (2018).
16. O.V. Khorolskyi. Viscometric research of concentration regimes for polyvinyl alcohol solutions. *Ukr. Fiz. Zh.* **60**, 882 (2015) (in Ukrainian).

17. L.A. Bulavin, N.P. Malomuzh. Dynamic phase transition in water as the most important factor in provoking protein denaturation in warm-blooded organisms. *Fiz. Zhivogo* **18**, 16 (2010) (in Russian)
18. A.I. Fisenko, N.P. Malomuzh. To what extent is water responsible for the maintenance of the life for warm-blooded organisms? *Int. J. Mol. Sci.* **10**, 2383 (2009)
19. Yu.I. Khurgin. Hydration of globular proteins. *Zh. Vsesoyuz. Khim. Obshsch.* **21**, 684 (1976) (in Russian)
20. A.B. Kurzaev, V.I. Kvlividze, V.F. Kiselev. Specific character of water phase transition on the surface of biological and inorganic dispersed bodies at low temperatures. *Svyaz. Voda Disp. Syst.* **4**, 156 (1977) (in Russian)
21. V.Ya. Volkov, B.V. Sakharov, L.A. Volkova. Radiospectroscopic methods in cryobiology. *Kriobiologiya* **4**, 3 (1985) (in Russian)
22. T.V. Lokotosh, N.P. Malomuzh, V.L. Zakharchenko. The relationship between the water structure and the anomalies of water density and dielectric constant. *Zh. Strukt. Khim.* **44**, 1085 (2003) (in Russian)
23. L.A. Bulavin, N.P. Malomuzh, K.N. Pankratov. Self-diffusion in water. *J. Struct. Chem.* **47**, Suppl. 1, S50 (2006).

Received 19.02.19.
Translated from Ukrainian by O.I. Voitenko

О.В. Хорольський

ЕФЕКТИВНІ РАДІУСИ МАКРОМОЛЕКУЛ АЛЬБУМІНУ ЛЮДИНИ ІЗ ДАНИХ ПО ЗСУВНІЙ В'ЯЗКОСТІ ЙОГО ВОДНИХ РОЗЧИНІВ

Р е з ю м е

Для обробки експериментальних даних зсувної в'язкості водних розчинів сироваткового альбуміну людини у широкому температурному та концентраційному інтервалах при сталому значенні pH = 7,0 використано формулу Маломужа–Орлова, яка дозволяє розрахувати ефективні радіуси макромолекул сироваткового альбуміну людини. Показано, що у температурному інтервалі 278–318 K можна виділити три області концентрацій з різною поведінкою ефективного радіуса сироваткового альбуміну людини: 1) до концентрації кросовера ефективні радіуси альбуміну залишаються незмінними; 2) від концентрації кросовера аж до концентрації ∼10 мас.% ефективні радіуси альбуміну у водному розчині нелінійно зменшуються; 3) при концентраціях 10,2–23,8 мас.% ефективні радіуси макромолекул альбуміну з ростом концентрації лінійно зменшуються. Висунуто припущення, що при вітальних концентраціях сироваткового альбуміну людини провідну роль у динаміці макромолекул відіграють властивості молекул води, які знаходяться у об'ємі. Обговорюється роль води біля поверхонь макромолекул альбуміну, фізичні властивості якої зазнають певних змін.