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## TEMPERATURE AND CONCENTRATION DEPENDENCES OF THE ZETA POTENTIAL OF ALBUMIN MACROMOLECULES IN THE AQUEOUS-SALT SOLUTION

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*Using the cellular model, the dependences of the zeta potential of human serum albumin on the salt concentration in aqueous NaCl solutions have been obtained for two temperatures, 300 and 318 K, and two values of albumin radius, 40 and 45 Å. It is found that the temperature variation within the considered interval does not significantly affect the examined parameter. An increase of the molecular radius by 5 Å leads to a noticeable reduction of the zeta potential from 3 to 10 units depending on the salt concentration. The obtained data can serve as a basis for interpreting the values of the albumin zeta potential under various pathological conditions.*

*Keywords:* aqueous solution, albumin, sodium chloride, zeta potential, pH, cellular model.

### 1. Introduction

This work is aimed at studying the electrophysical properties of macromolecules of human serum albumin in aqueous salt solutions. By serum albumin, we mean the globular protein that is one of the main components of blood plasma [1–3].

Albumin comprises the main fraction (63–65 wt%) of all proteins in blood plasma [1]. The others are

globulins (36.8 wt%) and fibrinogen (0.4 wt%). In addition, blood plasma also contains lipids, fatty acids, cholesterol, and so forth. Inorganic substances in blood plasma include such salts as sodium, potassium, and magnesium chlorides, as well as others; their total amount is about 10 g/l. Albumin is the main protein that is responsible for several important blood functions: transport, regulation, and accumulation [2].

According to work [1], human serum albumin is a globular protein consisting of a sequence of 585 amino acids arranged into one polypeptide chain 350 Å in length. The albumin molecule forms the secondary (the alpha-helix), tertiary, and quaternary structures. It consists of three domains, each of them consisting of two subdomains. The secondary structure of the albumin molecule arises due to the formation of hydrogen bonds between separate parts of the molec-

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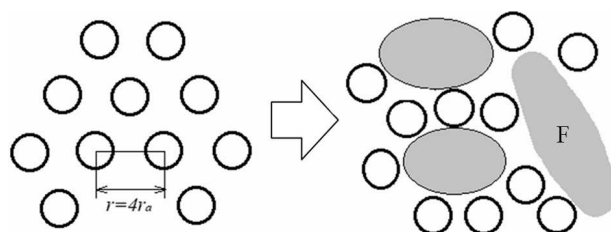
ular chain. The tertiary and quaternary structures arise due to the presence of hydrogen bonds, dispersion, and electrostatic interaction between separate parts of the molecule. In the condensed state, the macromolecule has an irregular heart-shaped form, which can be imagined, on average, as a triangular prism  $80 \times 80 \times 80 \times 30 \text{ \AA}^4$  in dimensions. Albumin is characterized by a good solubility in water and aqueous salt solutions.

When an albumin macromolecule is brought in an aqueous solution, the lateral parts of the amino acid residues of the macromolecule interact with water molecules, and the macromolecule picks up or loses hydrogen cations. Only those amino acid residues are taken into account that are located near the albumin macromolecule's surface. In this case, the aspartic and glutamic amino acids located at the surface of the albumin molecule lose hydrogen cations, whereas histidine, lysine, and arginine pick up them. These processes lead to the formation of a surface charge and (together with salt ions) a diffusion electric layer around the macromolecule. They also change the important property of the solution, the pH value [4–9].

When a molecule moves, the electric double layer becomes partially destroyed. The place, where the electric double layer is broken, is called the sliding plane. The zeta potential is the potential of a molecule located at the sliding plane. The zeta potential parameter evaluates the ability of a molecule to adjoin and transport molecules of medicinal substances, hormones, and so forth [6–14].

In dilute solutions, the potential distribution around an isolated macromolecule can be determined using the Debye approximation [15]. According to the result of work [14], the potential created by a molecule decreases exponentially with the distance from it. The effective thickness of the electrical layer around the albumin macromolecule is evaluated in terms of the Debye radius  $r_D$  [16, 17].

If the distance  $r$  between two albumin molecules is large,  $r > 2(r_a + r_D)$ , where  $r_a$  is the radius of the albumin molecule, their interaction can be neglected. At higher albumin concentrations, when the distance between the centers of albumin molecules satisfies the inequality  $r < 2(r_a + r_D)$ , the diffusion layers of neighbor macromolecules overlap, and the potential distribution, as well as the zeta potential value, substantially changes. The potential distribution also considerably changes owing to the in-



**Fig. 1.** Model representation of the arrangement of albumin macromolecules (circles) in an aqueous solution of albumin (left) and in blood plasma (right); the ellipses denote  $\gamma$ -globulin macromolecules, and the figure F is a fibrinogen macromolecule

fluence of all other proteins in plasma, because the total volume occupied by proteins (except albumin) is equal to the total fractional volume of albumin macromolecules. In this case, it is necessary to apply a new method for the calculation of the zeta potential, which is known in the literature as the cellular model [18, 19].

Let us briefly dwell on the concentration values for which the application of the Debye and cellular models is valid. In Western literature, it is generally accepted to use two terms: the mass density  $\rho$  and the number density  $n = \rho/m_0$ , where  $m_0$  is the mass of a molecule. We will use the definition of the number density  $n$  as the number of molecules per unit volume. It is not difficult to make sure that, at the maximum dense packing of albumin macromolecules, the molar fraction of albumin equals  $c_M \approx n_a/n_w \approx 6 \times 10^{-5}$ , where  $n_a = 3/(8\pi r_a^3)$  is the number density of albumin molecules at their maximum packing, and  $n_w$  is the number density of water molecules in the solution. Note that, hereafter, we use the approximate definition of the mole fraction, because  $n_a \ll n_w$ .

The average distance between the centers of albumin macromolecules in blood plasma is about  $\langle r_{12} \rangle \approx 4r_a$ . If blood contains only albumin macromolecules, the Debye approximation would be enough. However, macromolecules of  $\gamma$ -globulin and fibrinogen, whose volumes are substantially larger, violate the equidistant arrangement of albumin macromolecules and the conditions for the Debye approximation validity (see Fig. 1). In this case, a more adequate description can be achieved making use of the cellular model (see works [18, 19]).

An important issue in medicine and biophysics is the elucidation of the dependence of the macro-

molecule zeta potential on the solution pH, as well as the simultaneous dependences of the zeta potential and the pH on the salt concentration. The answer to the first question was obtained in work [20], where it is shown that the zeta potential takes positive values in the case  $\text{pH} < \text{pH}_e$ , where  $\text{pH}_e$  is the pH value at the isoelectric point, where the zeta potential changes its sign, and negative ones, if  $\text{pH} > \text{pH}_e$ . Unfortunately, the dependence of the zeta potential and the pH on the salt concentration has not been studied in detail. The first results were obtained in work [21].

This work had several purposes: 1) to construct a cellular model for the aqueous solution of human serum albumin, 2) to calculate theoretically the charge of the albumin molecule in the solution and its dependence on the pH, 3) to find the dependences of the zeta potential of the albumin macromolecule on the albumin and salt number concentrations, the temperature, and the pH, and 4) to analyze the theoretical concentration and temperature dependences of the zeta potential of albumin macromolecules.

## 2. Application of the Debye Approximation to Dilute Aqueous-Salt Solutions of Albumin

First, let us consider a dilute aqueous solution of albumin, when the influence of a molecule on another one is insignificant. To evaluate the zeta potential in this case, we apply the Debye equation with all standard approximations [16–19].

The field potential satisfies the Poisson equation

$$\Delta\varphi = -\frac{4\pi\rho}{\varepsilon}, \quad (1)$$

where  $\varepsilon$  is the real part of the effective dielectric constant of the electrolyte at zero frequency. The volume charge density is determined by the expression

$$\rho(r) = -\frac{\varphi}{4\pi r_D^2}. \quad (2)$$

The following boundary condition for the potential is obeyed at the surface of a spherical particle:

$$\left. \frac{\partial\varphi(r)}{\partial r} \right|_{r=r_p} = 4\pi\sigma, \quad (3)$$

where  $\sigma$  is the surface charge density. The other boundary condition is standard,

$$\varphi(r) \rightarrow 0, \quad r \rightarrow \infty. \quad (4)$$

The potential that satisfies Eq. (1) and the formulated boundary conditions can be presented in the form

$$\varphi(r) = \varphi_0(r_a)f(r), \quad (5)$$

where

$$\varphi_0(r_a) = \frac{4\pi\sigma r_a r_D}{\varepsilon(r_a + r_D)} \quad (6)$$

is the potential created by the charged surface in its vicinity, and the function

$$f(r) = \frac{r_a}{r} \exp\left(-\frac{r - r_a}{r_D}\right) \quad (7)$$

describes the potential decrease, as the distance from the albumin surface grows. If  $n_{Na}$  is the concentration of sodium ions, then the Debye radius corresponding to the effective thickness of the diffusion layer equals

$$r_D = \left(\frac{\varepsilon k_B T}{8\pi e^2 n_{Na}}\right)^{1/2}, \quad (8)$$

where  $e$  is the elementary charge,  $k_B$  the Boltzmann constant, and  $T$  the temperature.

The zeta potential is determined by the potential difference between the surface and an infinitely distant point. According to Eq. (5), it is equal to

$$\zeta = \varphi_0(r_a). \quad (9)$$

According to the results of zeta-potential studies in works [22–25], the surface charge of the albumin macromolecule is within the interval  $(15 \div 50)e$ . In the further calculations of the zeta potential, we will consider the surface charge of the albumin molecule in the solution to have a constant value of  $18e$ , which corresponds to the albumin charge at the physiological pH value of human blood [2, 22, 24].

In Fig. 2, the dependence of the zeta potential of albumin macromolecules on the ionic density in the solution calculated in the Debye approximation at  $T = 300$  K is shown. One can see that, as the concentration of ions in the solution increases, the zeta potential of the albumin molecule tends to zero, which corresponds to a complete screening of the macromolecule's charge.

## 3. Cellular Model

In order to determine the zeta potential of the albumin macromolecule, let us apply the method that was

proposed in works [18, 19] to describe the electrical properties of dust plasma. According to research [25], an albumin molecule in the solution acquires a shape that depends on the pH value. At  $\text{pH} > 6$ , its form can be approximated by an ellipsoid. As a result, we have an irregularly shaped molecule with a non-uniform charge distribution. Nevertheless, since an albumin molecule in the solution has a high rotational velocity, its shape can be considered as approximately spherical, so that the average surface charge density can be estimated (see Fig. 3).

When an albumin molecule finds itself in the solution, a redistribution of electric charges takes place over the molecule's surface and in the solution. A layered cloud of positive and negative charges, an electric double layer, emerges around the molecule. This layer completely screens the molecule's charge. Therefore, the field potential equals zero at some distance from the charged particle. Hence, the whole system can be divided into a set of identical regions, cells, with the electric field potential vanishing at their boundaries (see Fig. 4).

Let us determine the field potential in the cell as a function of the distance from the albumin molecule. The radius of the cell is determined from the condition that the cell volume is equal to the fractional volume per one particle of albumin,

$$r_D = \left( \frac{3}{4\pi n_p} \right)^{1/3}. \quad (10)$$

For this model, we use assumptions that are completely analogous to the Debye approximation (1)–(4). The first boundary condition is that the field potential equals zero at the cell boundary,

$$\varphi(r)|_{r=r_c} = 0. \quad (11)$$

At the molecule's surface, the field potential satisfies the condition

$$\left. \frac{\partial \varphi(r)}{\partial r} \right|_{r=r_p} = 4\pi\sigma, \quad (12)$$

where  $r_p$  is the radius of the albumin molecule in the solution.

The solution of the differential equation (1) with the boundary conditions (11) and (12) looks like

$$\varphi(r) = \varphi_0(r_a)f(r), \quad (13)$$

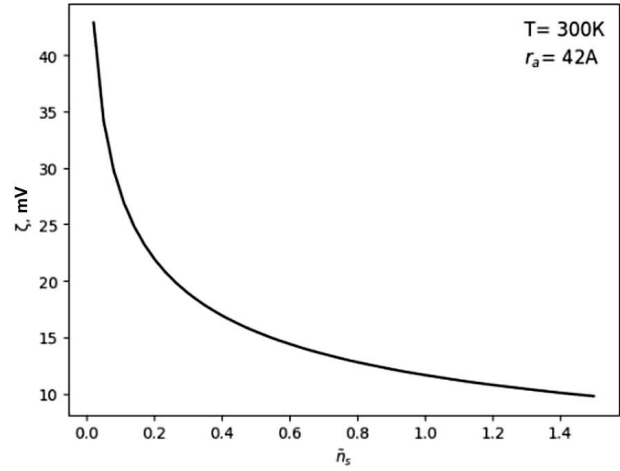


Fig. 2. Dependence of the zeta potential of albumin macromolecules on the normalized density of salt ions in the solution obtained in the Debye approximation at  $T = 300 \text{ K}$

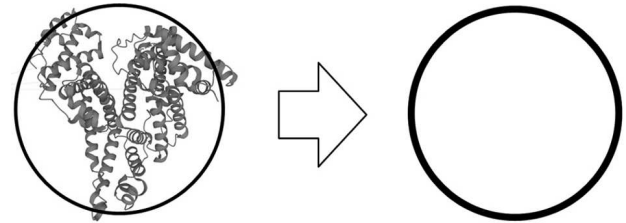


Fig. 3. Model representation of the albumin macromolecule as a sphere

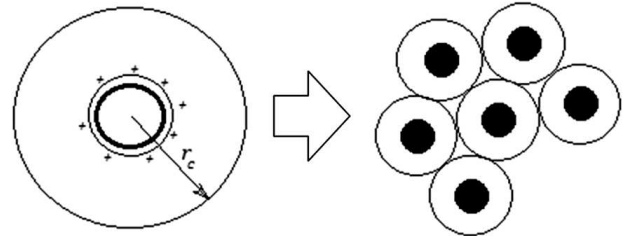


Fig. 4. Segmentation of the system into a set of identical cells

where

$$\varphi_0(r_a) = \frac{4\pi\sigma}{\varepsilon} \frac{r_a r_D \sinh \frac{r_c - r_a}{r_D}}{r_a \cosh \frac{r_a - r_c}{r_D} - r_D \sinh \frac{r_a - r_c}{r_D}}, \quad (14)$$

$$f(r) = \frac{r_a \sinh \frac{r_c - r}{r_D}}{r \sinh \frac{r_c - r_a}{r_D}}. \quad (15)$$

Since, according to the definition, the zeta potential is the difference

$$\zeta = \varphi(r_a) - \varphi(r_c) \quad (16)$$

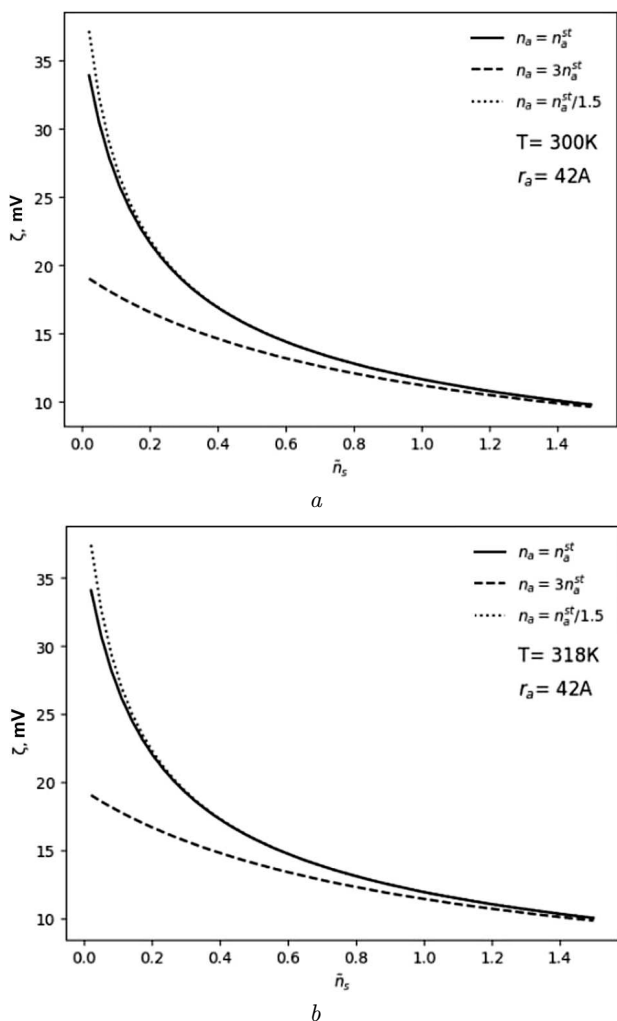


Fig. 5. Dependences of the zeta potentials of an aqueous solution of albumin molecules on the normalized density of salt ions at the temperatures  $T = 300$  (a) and  $318\text{ K}$  (b)

**Average values of volume concentrations of main protein groups in blood plasma**

Main protein groups in blood plasma	Volume concentration, $\omega_i$	Total volume concentration, $\omega$
Albumin	0.12	0.25
$\alpha_1$ -globulin	0.0068	
$\alpha_2$ -globulin	0.0156	
$\beta$ -globulin	0.0116	
$\gamma$ -globulin	0.0518	
Fibrinogen	0.034	

and  $\varphi(r_c) = 0$ , the zeta potential is equal to the potential at the surface of the albumin molecule,

$$\zeta = \varphi_0(r_a) = \frac{4\pi\sigma}{\varepsilon} \frac{r_a r_D \sinh \frac{r_c - r_a}{r_D}}{r_a \cosh \frac{r_a - r_c}{r_D} - r_D \sinh \frac{r_a - r_c}{r_D}}. \quad (17)$$

Hence, the zeta potential is a function of the same parameters that govern the behavior of the Debye radius, as well as the surface charge density of the particle and the dielectric constant of water.

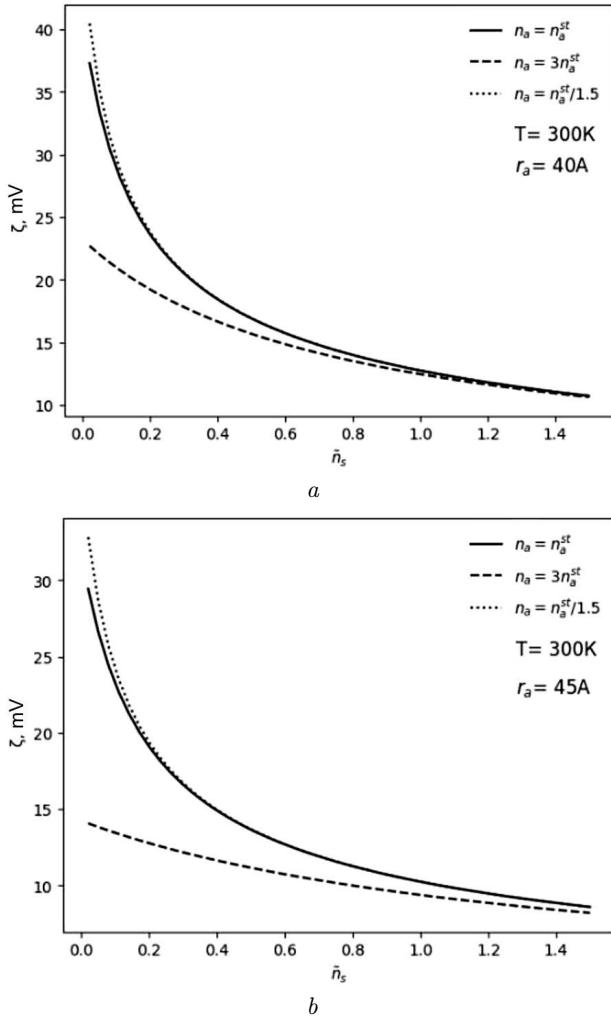
**4. Discussion of the Zeta-Potential Behavior in Aqueous-Salt Solutions of Albumin and Its Dependence on the Solution Parameters**

To illustrate the effect of the restrictions imposed by the cellular model, let us consider the dependence of the zeta potential on the albumin and salt concentrations. As is known [1, 2], albumin comprises the main fraction (63–65 wt%) of all proteins in blood plasma. The others are globulins (36.8 wt%) and fibrinogen (0.4 wt%). Globulins in blood plasma have been separated into three fractions:  $\alpha$ -,  $\beta$ -, and  $\gamma$ -globulins. The volume concentrations of plasma proteins are quoted in Table 1.

Blood plasma also includes the following salts: 1) NaCl with the molar fraction  $c_{NaCl} \approx n_{NaCl}/n_w = 2.6 \times 10^{-3}$ , where  $n_{NaCl}$  and  $n_w$  are the number densities of sodium chloride and water, respectively, molecules in the aqueous solution; 2) KCl with  $c_{KCl} = 9 \times 10^{-5}$ ; 3)  $CaCl_2$  with  $c_{CaCl_2} = 4.55 \times 10^{-5}$ ; 4)  $MgSO_4$  with  $c_{MgSO_4} = 1.82 \times 10^{-5}$ ; and 5)  $NaHCO_3$  with  $c_{NaHCO_3} = 5 \times 10^{-4}$ . As one can see, the influence of sodium chloride on the blood plasma properties dominates, because its content exceeds 80% of the total amount of blood plasma salts. Therefore, in our calculations, we confined attention to the amount of sodium chloride only.

Below, to describe the salt content in the solution, we use the normalized value of the number density, which is defined as the ratio between the salt density and its standard value in blood plasma,  $n_s = n_s/n_s^{(st)}$ , where  $n_s^{(st)} = 1.022 \times 10^{20} \text{ cm}^{-3}$  is the number density of salt corresponding to its content in the blood plasma of a healthy person. The albumin density is described similarly,  $\tilde{n}_a = n_a/n_a^{(st)}$ , where the standard value equals  $n_a^{(st)} = 4.5 \times 10^{17} \text{ cm}^{-3}$ .

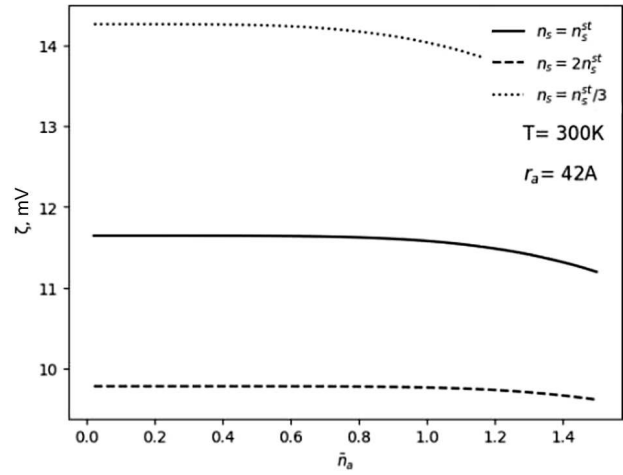
Figure 5 shows the dependences of the zeta-potential on the sodium chloride normalized den-



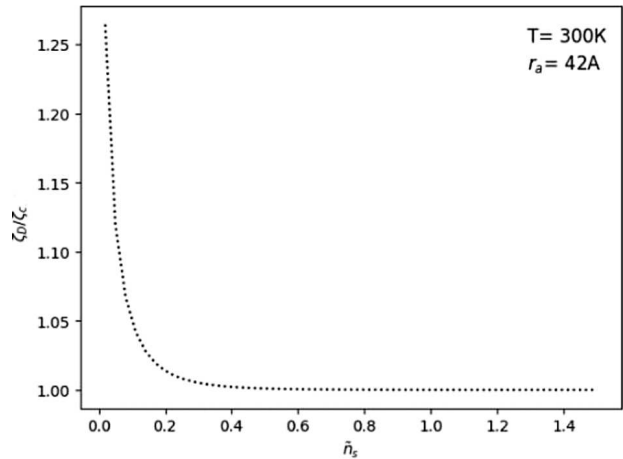
**Fig. 6.** Dependences of the zeta potential of albumin molecules on the normalized density of salt ions for an albumin radius of 40 Å (a) and 45 Å (b)

sity  $\tilde{n}_s$  for various number densities  $n_a$  of albumin: Fig. 5, a corresponds to the temperature  $T = 300\text{K}$ , and Fig. 5, b to  $T = 318\text{K}$ . From the obtained theoretical dependences, the conclusion follows that a temperature variation of 10–20 K has almost no effect on the concentration dependences of the zeta potential of albumin.

In works [26, 27], it was shown that the size of albumin molecules depends on the pH and concentration of albumin. The influence of the albumin macromolecule size is analyzed in Fig. 6: Fig. 6, a corresponds to an albumin radius of 40 Å, and Fig. 6, b to 45 Å. As one can see,



**Fig. 7.** Dependences of the zeta potential of albumin molecules on the normalized density of albumin for various number densities of salt ions



**Fig. 8.** Dependence of the ratio  $\zeta_D/\zeta_c$  on the normalized density of salt

1) the value of the zeta potential substantially increases, as the albumin density decreases (for  $r_a = 42\text{Å}$ ,  $\zeta = 16\text{mV}$  at  $\tilde{n}_a = 1.5$  and  $\zeta = 40\text{mV}$  at  $\tilde{n}_a = 1/3$ , i.e.,  $\Delta\zeta \approx 23 \div 24\text{mV}$ ; when the albumin radius increases by 2–3 Å, the difference  $\Delta\zeta$  remains the same);

2) the value of the zeta potential decreases with the growth of the sodium chloride density (from 25 to 12 mV at the standard concentration of albumin and  $r_a = 42\text{Å}$ ); notice that, as the albumin concentration increases, the salt effect on the zeta potential diminishes;

3) it follows from the analysis of Fig. 7 that the influence of the albumin density becomes less noticeable, as the salt density increases;

4) if the radius of albumin in the solution of albumin macromolecules increases (in our case, by 5 Å), the zeta potential decreases by 8–9 units at all albumin densities and  $\tilde{n}_s = 0.01$ ; as the salt density  $\tilde{n}_s$  increases, the variation of  $\zeta$  diminishes, as the radius decreases;

5) the value of the zeta potential is almost independent of the temperature: the magnitude of the zeta potential variation is of an order of  $10^{-1} \div 10^{-2}$  mV.

Finally, let us consider the ratio between the zeta potentials in the Debye,  $\zeta_D$ , and cellular,  $\zeta_c$ , models as a function of the normalized salt density  $\tilde{n}_s$ . It is assumed that  $\zeta_D$  and  $\zeta_c$  are determined by formulas (9) and (17), respectively. The plot of the dependence  $\zeta_D/\zeta_c = f(\tilde{n}_s)$  is shown in Fig. 8. For rather concentrated salt solutions, this ratio expectedly approaches unity. Substantial deviations from unity are observed only at the densities  $\tilde{n}_s < 0.4$ . This circumstance is very important for the interpretation of experimental data obtained for the zeta potential values.

## 5. Conclusions

The measurements of the zeta potential provide us with the information about a) the charge value at the surface of the albumin macromolecule and b) the parameters of the diffusion layer. That is why it is necessary to carefully study the dependence of the zeta potential on the albumin concentration and the concentration of salt ions, and to know their average values for a specific organism. A deviation of the zeta potential from the average value testifies to a change in the ion concentration and/or the albumin concentration.

It should be taken into account that experimental measurements of the zeta potential give us the information not only about albumin, but also about the distribution of charges over other plasma components, fibrinogens and globulins.

The study of the zeta potential gives some insight into the local characteristics of blood plasma, such as the pH value and the albumin concentration in the vessels.

The measurements of the zeta potential make it possible to detect the minimum concentration of those proteins that appear only in pathological states

of the organism (hematological diseases, Waldenström macroglobulinemia, leukemias, and autoimmune diseases). Those measurements can make a significant contribution to the early diagnosis of a number of abnormalities in the genetic apparatus.

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ТЕМПЕРАТУРНА І КОНЦЕНТРАЦІЙНА  
ЗАЛЕЖНІСТЬ ДЗЕТА-ПОТЕНЦІАЛУ  
МАКРОМОЛЕКУЛ АЛЬБУМІНУ  
У ВОДНО-СОЛЬОВОМУ РОЗЧИНІ  
ЗГІДНО З КОМІРКОВОЮ МОДЕЛЛЮ

За допомогою коміркової моделі побудовано концентраційні залежності дзета-потенціалу сироваткового альбуміну людини у водно-сольових розчинах; знайдено теоретичні залежності дзета-потенціалу водно-сольових розчинів сироваткового альбуміну людини від концентрації хлориду натрію для різних концентрацій альбуміну: а) за двох різних температур, 300 К та 318 К; б) за різних значень радіуса альбуміну, 40 Å та 45 Å. Виявлено, що зміна температури в розглянутих межах суттєво не впливає на дзета-потенціал водно-сольових розчинів альбуміну; збільшення радіуса молекули на 5 Å приводить до помітного зменшення дзета-потенціалу від 3 до 10 одиниць в залежності від концентрації солі. Отримані дані є базисом для інтерпретації значень дзета-потенціалу альбуміну за наявності різних патологічних станів.

*Ключові слова:* водний розчин, альбумін, хлорид натрію, дзета-потенціал, pH, коміркова модель.