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Formation of dipole complexes in protein solutions with low concentrations of heavy metal ions: diagnostics by the method of laser radiation scattering

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***Abstract*. The formation of macromolecular protein clusters is studied by the Rayleigh–Debye method of laser radiation scattering in solutions containing salts of toxic heavy elements, such as lead, copper, and cadmium, at low concentrations. It is found that the mass of clusters is maximal in the region of the isoelectric point of a protein and increases with increasing the ionic strength of the solution. It is shown that clusters decompose upon heating solutions up to the temperature about of 40 °C.**

Keywords: *heavy metals, proteins, light scattering, intermolecular interaction.*

1. Introduction

Metals play an important role in a human organism. Sodium, potassium, calcium, and magnesium constitute more than 5 % of the human body weight.

Such metals as iron, cobalt, copper, molybdenum, zinc, etc. exist in the human organism in the bound state (for example, iron is contained in hemoglobin, while most of the elements are bound with enzymes) and constitute less than 1 % of the human body weight [1]. An excess over the permissible concentration of metals in the environment is a serious hazard to human health. Heavy metals are especially hazardous. Even low concentrations of heavy metals in drinking water, blood, and other biological liquids are capable of distorting normal physiological processes proceeding in the human organism.

The toxicity of metals belonging to the zinc group (cadmium, mercury) drastically increases with their ordinal number. Cadmium is solvable in organic acids contained in food and forms salts, which are transformed to cadmium chloride under the action of a gastric juice. Even small amounts of cadmium can cause lethal toxic effects. Respiratory poisoning by cadmium vapour is especially hazardous.

Manganese, nickel, and lead are also toxic metals. The latter belongs to the group of the so-called heavy nonferrous metals and is probably the most widespread metal poison. Lead is used in industry for the preparation of paints and as additives to automobile fuel. Like mercury, lead is very toxic for a nervous system and kidney, its toxicity being dependent on the human age and the time of its action. A short-time action of lead can reduce the intellect and change the behaviour of children, whereas its prolonged action can damage brain cells and even can be lethal.

The basic physical mechanisms of the toxic action of heavy metal ions are their anomalous sorption on the lipoproteins surface, the anomalous molecular mobility of charged biopolymers, and the formation of dipole protein clusters.

In this paper, we study the influence of heavy metals, such as copper, lead, and cadmium, on the molecular parameters of proteins in aqueous solutions, which serve as models of biological liquids (for example, blood serum or lymph). The study was performed by the Rayleigh–Debye method [2] of laser radiation scattering in solutions observed upon changing the parameters of solutions such as pH, the ionic strength determined by the concentration of a salt solved and the solution temperature.

2. Theory of the method

The intensity of scattering of linearly polarised light in a solution of macromolecules is described by the expression

$$I_s = \frac{2\pi^2 n_0^2 (\mathrm{d}n/\mathrm{d}c)^2 cMI_0 Kv}{\lambda_0^4 N_A r^2} \sin^2\theta, \qquad (1)$$

where n_0 is the refractive index of a solvent; $\mathrm{d}n/\mathrm{d}c$ is the increment of the refractive index of the solution; c is the concentration of a dissolved substance; I_0 is the incident light intensity; v is a scattering volume; λ_0 is the wavelength of the incident light in vacuum; N_A is Avogadro's number; M is the molecular mass of a dissolved substance; r is the distance from the scattering volume to a detector of scattered radiation; θ is the angle of scattering; K is a coefficient determined by the optical anisotropy of scattering particles (Cabann factor).

Commonly, the scattering coefficient R is used, which is independent of the values of I_0, v, and r. For $\theta = 90°$, we have

$$R_{90} = \frac{I_{90} r^2}{I_0 v} = cHMK, \qquad (2)$$

where

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$$H = \frac{2\pi^2 n_0^2 (\mathrm{d}n/\mathrm{d}c)^2}{\lambda^4 N_A}$$

is the optical constant of the solution. One can see from (2) that the scattering coefficient is proportional to the concentration of scattering particles and to their mass. For this reason, the Rayleigh – Debye method was used recently for measuring the critical concentration for polymerisation [3] or quantitative analysis of various macromolecules in solutions [4].

Expression (2) is valid only if the positions of scattering particles are uncorrelated or the particles are separated by sufficiently large distances and the interaction between them can be neglected. In the case of finite concentrations, the intermolecular interaction always exists, and according to the Debye theory, the scattered light intensity can be expressed in terms of concentration fluctuations, which depend on the chemical potential. In this case, we have the relation

$$f(c) = \frac{cHK}{R_{90}} = \frac{1}{M} + 2Bc + \ldots, \qquad (3)$$

for diluted solutions of macromolecules, which allows us to represent the measurable quantity R_{90} in the form of a virial expansion in low concentrations.

The method considered here can be used for a direct determination of the molecular mass M by measuring R_{90} at several concentrations and extrapolating the obtained dependence $f(c)$ to the concentration $c = 0$. The slope of a straight line $f(c)$ equal to $2B$ gives the second virial coefficient B in the expansion for a free energy, which characterises the extent of deviation of the solution from a perfect one and characterises the intermolecular interaction in the solution.

Note that the coefficient K was assumed equal to unity in studies of scattering of light in protein solutions before the advent of lasers [5]. However, measurements of polarisation characteristics of scattered laser radiation showed [6] that the coefficient K differs from unity and depends on the protein properties.

According to Scatchard [7], the second virial coefficient, which describes pair interactions in solutions containing except a low-molecular solvent also the third component – a strong electrolyte, for example, NaCl, has the form

$$B = \frac{V_1}{M_2^2}\left(\frac{Z^2}{4m_3} + \frac{\beta_{22}}{2} - \frac{\beta_{23}^2 m_3}{4 + 2\beta_{33} m_3}\right), \qquad (4)$$

where V_1 is the specific volume of a solvent; Z and M_2 are the charge and mass of a macroscopic ion, respectively; and m_3 is the concentration of salt ions. The parameters β_{22}, β_{23} and β_{33} are related to the activity coefficients, which characterise the effective concentration of a given component. They characterise different interactions between ions in solution: the effect of an eliminated volume and the interaction between charges of different macroions (β_{22}), the interaction between macroions and salt ions (β_{23}), and the interaction only between salt ions (β_{33}).

According to expression (4), the coefficient of intermolecular interaction changes with increasing a total charge on a protein following a parabolic law (the Donnan effect), with a minimum at the isoelectric point (the isoelectric point of a protein is the value of pH at which the average charge at the protein macromolecule is $Z \sim 0$). The isoelectric points of some proteins are presented in Table 1. The values of pH in the protein solution and, hence, a charge at the protein molecule can be changed by adding an acid or a base to the solution, producing either excess or deficiency of protons compared to pure water. The dependence of the charge Z at a protein on pH is usually determined by titration or electrophoresis. The coefficient β_{22} is usually small compared to other terms in expression (4). At high salt concentrations in the solution, the term containing the coefficien β_{23} can substantially exceed (in modulus) the first two terms, and the parameter B can become negative.

Table 1.

Protein	M	p/D	Isoelectric point/pH
Human serum albumin	66000	500	4.8
Bovin serum albumin	68000	480	4.9
Oval albumin	50000	400	4.8

When the ionic strength of solution increases with increasing concentration of a dissolved salt, more complicated entities, containing Na^+ and Cl^- ions, are formed in the solution. A cloud of counter-ions appears around a charged molecule, which screens the Coulomb interaction. The value of B decreases with increasing the ionic strength μ; however, the parabolic dependence of B on pH is retained. The mass M of protein macromolecules in the solution remains virtually constant [5, 6, 8].

Light scattering studies [8 – 10] showed that, if protein solutions contained ions of heavy alkali metals such as cesium and rubidium, the mass of scattering particles did not remain constant. It increased for pH corresponding to the isoelectric point of a protein, and the type of intermolecular interaction changed. Instead of a parabolic dependence of the parameter B on the protein charge with a minimum at the isoelectric point, a nonlinear increase of B was observed with a maximum at $Z \sim 0$.

In this paper, we studied by the method of light scattering the aqueous solutions of serum and egg albumins in the presence of heavy metal salts (lead, copper, and cadmium) for various values of the surface charge of the protein and the ionic strength.

3. Experimental setup and materials

Protein macromolecules are complex polymer structures with the molecular mass up to several hundreds of thousands. The surface of a protein molecule can contain up to a few hundreds of charged groups, resulting in large dipole moments of protein molecules amounting to 1000 D (Table 1). Protein solutions represent polyelectrolytes. A total charge on the protein surface can vary in a broad range depending on the concentration of free protons. Table 1 presents the parameters of proteins studied in this paper (Sigma and Serva proteins). Table 2 lists salts studied in the experiments.

The scattering properties of aqueous solutions of proteins were studied using an optical setup containing a 632-nm He – Ne laser and a photodetector of scattered radiation (Fig. 1). A vertically polarised light beam was reflected by a prism (*4*) to a cylindrical glass cell (*8*) with a solution. The beam was modulated by an electromechanical chopper (*3*)

Table 2.

Heavy metals	Salts
Copper	Cuprous chloride $CuCl_2$
Lead	Lead acetate $Pb(CH_3COO)_2 \cdot 3H_2O$
Cadmium	Cadmium sulphate $CdSO_2$

at a frequency of ~ 70 Hz in order to separate a signal from a background light. The light scattered at an angle of 90° was detected with a photomultiplier (*11*), whose signal was fed to a resonance amplifier (*13*), then to a PC (*15*), and was digitised in an ADC (audio card). The signal was also observed visually on an oscilloscope (*14*). We used a system consisting of the Wollaston (*9*) and Glan–Thompson (*10*) prisms for separating the polarised and depolarised components of scattered radiation and measuring their intensities. With the help of a specially developed program, we extrapolated dependences $f(c)$ (3) by the method of least squares and calculated experimental errors.

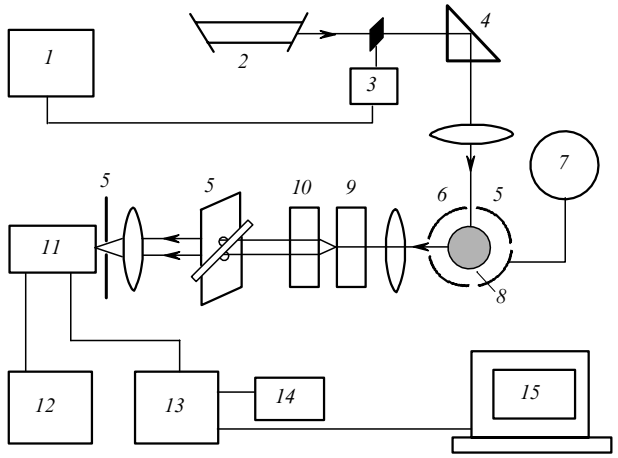

Figure 1. Scheme of the optical setup: (*1*) audio signal generator; (*2*) laser; (*3*) electromechanical modulator; (*4*) deflecting prism; (*5*) screen; (*6*) heater; (*7*) laboratory autotransformer; (*8*) cell with solution; (*9, 10*) Wollaston and Glan–Thompson prisms; (*11*) photomultiplier; (*12*) photomultiplier power supply; (*13*) resonance amplifier; (*14*) oscilloscope; (*15*) computer.

The molecular mass and the second virial coefficient were calculated from the relative intensities of radiation scattered at an angle of 90° for two mutually perpendicular polarisations, which allowed us to take into account the contribution from the optical anisotropy of macromolecules. The results were corrected taking into account the intensity of light scattered by pure water.

The intensity of scattered light was calibrated using benzene as a standard liquid ($R_{90} = 12.64 \times 10^{-6}$ cm^{-1} at $\lambda = 632$ nm) and taking into account the difference between the refractive indices of benzene and water. The measurement error R_{90} was 6 %.

An increment of the refractive index was measured with a Rayleigh interferometer (the measurement error was 2 %) in solutions with different pH (the measurement error was 3 %) and different protein concentrations. The concentration of free protons (pH) and the ionic strength μ were varied by adding small amounts of acid, base, and salt. The errors of measuring the coefficient B and the mass of scattering particles were 10 %.

4. Experimental results

Fig. 2 shows the dependences of the second virial coefficient B on pH (or Z) for a bovin serum albumin at different concentrations of lead acetate (different ionic strengths). One can see that, the dependence of B on pH drastically changes in the presence of a lead salt in the solution: the coefficient B becomes negative at low ionic strengths, although a parabolic dependence is retained; as μ is further increased, the value of B increases in the region of the isoelectric point, the maximum value of B being increased with increasing μ. In the region of large positive and negative charges on the protein, the coefficient B has close values for all μ.

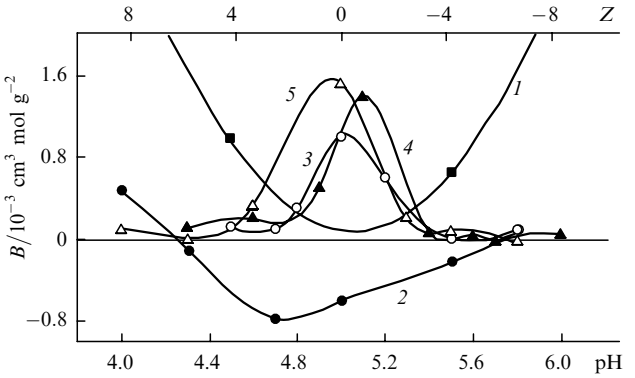

Figure 2. Coefficient of intermolecular interaction B as a function of pH (or the surface charge Z) for bovin serum albumin for ionic strengths $\mu = 0.00075$ (*2*), 0.00105 (*3*), 0.0012 (*4*), and 0.0015 mol L^{-1} (*5*). Curve (*1*) was obtained for albumin in pure water and corresponds to curve (*1*) in Fig. 5b.

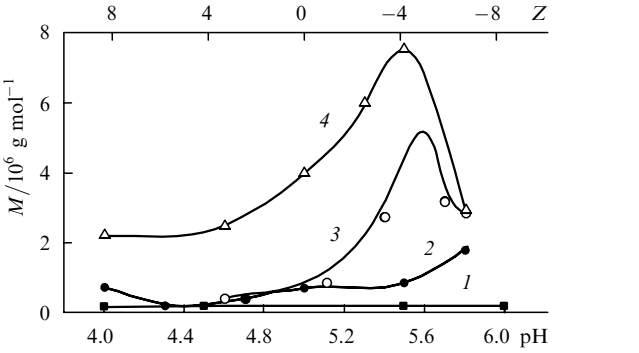

Figure 3. Effective mass M of scattering particles as a function of pH (or Z) for solutions of bovin serum albumin containing lead acetate for $\mu = 0.00075$ (*2*), 0.00105 (*3*), and 0.0015 mol L^{-1} (*4*). Curve (*1*) was obtained for albumin in pure water.

We calculated the effective masses of scattering particles by expression (3) using the scattering coefficients R_{90} measured in solutions of oval and serum albumins in the presence of lead salts (for different values of μ) (Figs 3, 4).

The dependences of the effective mass of scattering particles on pH also qualitatively change in the region of the isoelectric point. The mass of scattering particles in solutions with heavy metal salts does not remain constant, as in solutions with NaCl, but drastically increases near the zero total charge of the protein, the maximum value of M being increased with increasing the ionic strength μ.

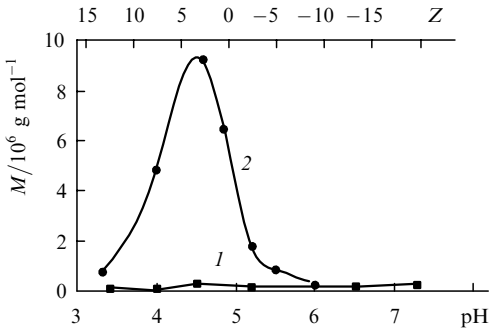

Figure 4. Dependences of the effective mass M of scattering particles on pH (or Z) for solution of oval albumin in pure water (*1*) and albumin solution containing lead acetate for $\mu = 0.00105$ mol L^{-1} (*2*).

We performed similar experiments with solutions of human serum albumin in the presence of cuprous chloride (Fig. 5) and solutions of bovin serum albumin containing a cadmium salt (Fig. 6).

5. Discussion of the experiment

Proteins in solutions represent particles, which interact with each other mainly due to the Coulomb repulsion. However, a comparison of the energy of electrostatic interaction between a charge ion and a dipole water molecule

$$E_{pq} = \frac{q^2 p_w^2}{12\pi\varepsilon r_0^4}\frac{1}{kT} \tag{5}$$

(where q is the ion charge; p_w is the dipole moment of a water molecule; $\varepsilon \sim 80$ is the dielectric constant of water; and r_0 is the ion radius) with the thermal energy [10] shows that metal ions having a larger mass and larger ionic radii (for example, the ionic radii of cadmium, copper, and lead Pb^{++} are $\sim 1.0, \sim 1.2$, and ~ 1.3 Å, respectively) hold the hydrate shell weaker than the sodium ion with $r_0 \sim 0.8$ Å. An ion with a larger value of r_0 is more tightly bound with a negatively charged group on a protein, and it can form the so-called Coulomb complex with a common hydrate shell on a protein macromolecule. In this case, ions completely compensate for the surface charge of the protein.

Due to a strong decrease in the surface charge, the dipole–dipole interaction can become the main interaction between macromolecules because proteins have very large dipole moments (Table 1).

Therefore, the type of interaction of protein macromolecules in such solutions is determined not by Coulomb but dipole–dipole forces. The energy of dipole–dipole interaction is

$$E_{pp} = \frac{p^4}{6\pi\varepsilon kTr^6}, \tag{6}$$

where p is the dipole moment of a protein. For the distance between the dipoles $r \sim 30$ Å, the energy E_{pp} can exceed

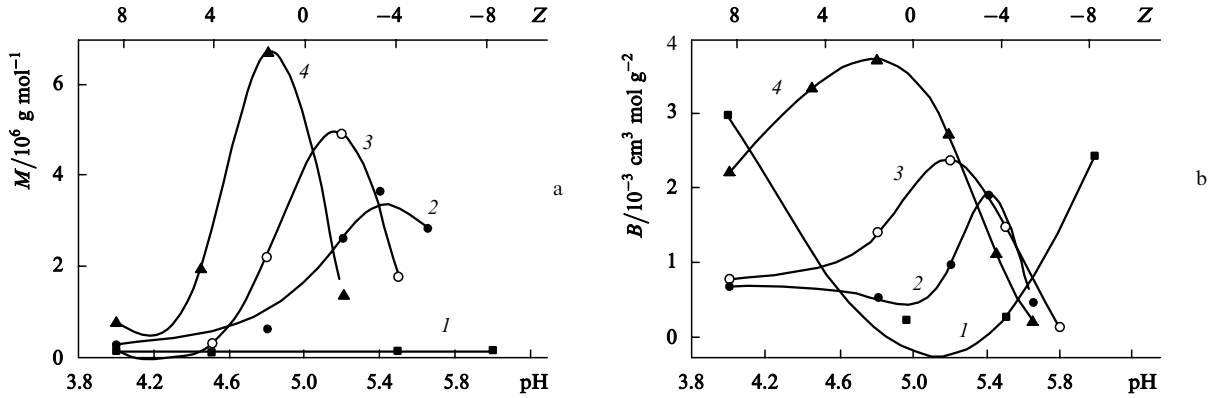

Figure 5. Dependences of the effective mass M of scattering particles (a) and the coefficient of intermolecular interaction B (b) on pH (or Z) for aqueous solutions of human serum albumin in the presence of $CuCl_2$ for $\mu = 0.008$ (*2*), 0.012 (*3*), and 0.02 M (*4*). Curves (*1*) were obtained for albumin in pure water.

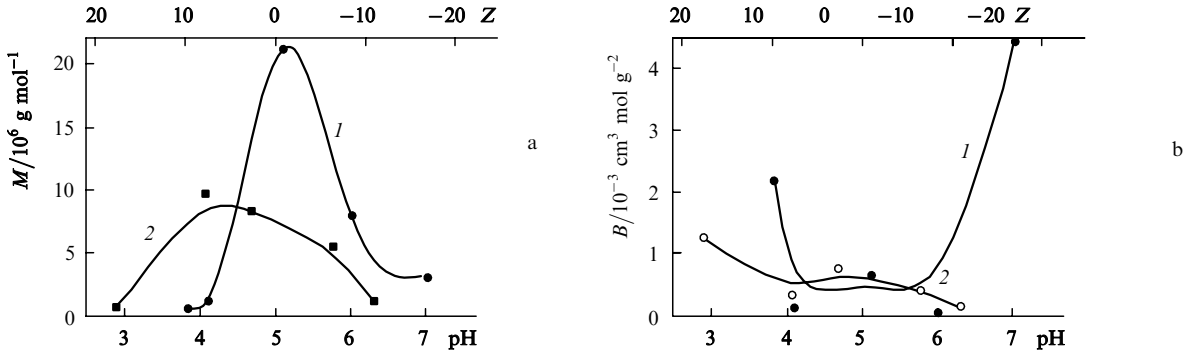

Figure 6. Dependences of the effective mass M of scattering particles (a) and the coefficient of intermolecular interaction B (b) on pH (or Z) for aqueous solutions of bovin serum albumin in the presence of $CdSO_4$ for $\mu = 0.0005$ (*1*), and 0.001 (*2*).

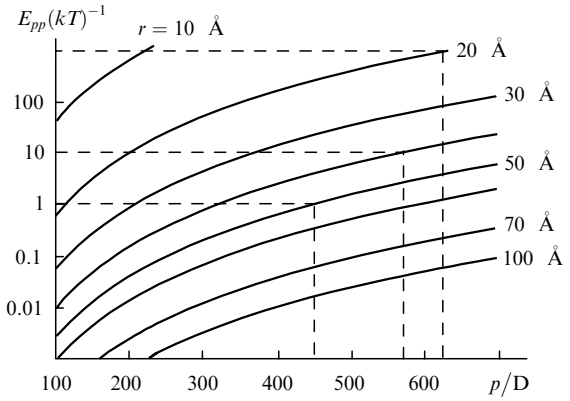

Figure 7. Dependences of the ratio of dipole–dipole interaction energy E_{pp} to the thermal energy kT on the dipole moment p of a molecule for different distances r between particles.

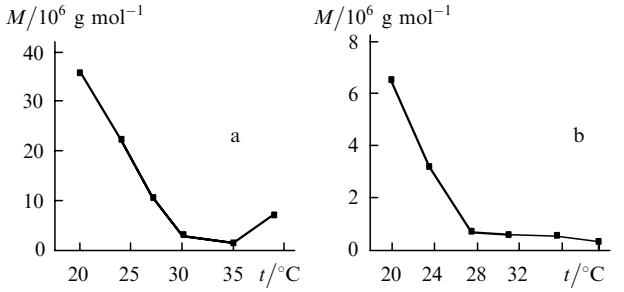

Figure 8. Variation in the effective mass M of scattering particles upon heating of solutions of bovin serum albumin (pH ~ 5) (a) and oval albumin (pH ~ 4.9) (b) containing lead acetate for $\mu = 0.00105$ mol L^{-1}.

the thermal energy kT almost by a factor of 100 (Fig. 7) [10]. For this reason, when the protein macromolecules are brought closer together, they can form a macromolecular complex – a dipole cluster.

In the case of charged molecules, the Coulomb repulsion energy is larger than kT. Albumin macromolecules, having, for neutral pH (~ 7), the charge q approximately equal to 10 esu do not come closer together because of the Coulomb repulsion with the energy

$$E_{qq} = \frac{q^2}{\varepsilon l}, \tag{7}$$

where l is the distance between charged molecules. As a total (negative or positive) charge on a protein molecule increases, the Coulomb repulsion forces also increase and clusters are destroyed, the effective mass of scattering particles approaching the molecular mass of the protein.

Because the ratio of the energy of dipole–dipole interaction to the thermal energy is close to unity for solutions under study, we can expect that even small variations in the environment temperature will result in a change in the solution parameters. We studied aqueous solutions of oval and bovin serum albumins containing lead ions at low concentrations ($\mu = 0.00105$ mol L^{-1}) in the temperature range from 20 to 45 °C in the region of their isoelectric points (Fig. 8). We found that the effective mass of scattering particles decreased approximately by an order of magnitude with increasing temperature. This can be caused by the destruction of macromolecular complexes – clusters. Our calculations showed that the minimum distance between dipole macromolecules in clusters increased more than twice with increasing the thermal energy only by 5 %.

6. Conclusions

We have found and studied in detail by the method of scattering of laser radiation the appearance of macromolecular aggregates (clusters) in protein solutions containing heavy metal ions. We have studied the influence of the ion concentration in solution and of the surface charge of protein molecules on the mass of macromolecular complexes and proposed the physical model explaining this effect by strong dipole–dipole interactions. We have also investigated the influence of temperature on dipole–dipole protein complexes.

The study of the interaction of protein macromolecules with heavy metal ions performed in this paper is important for establishing the molecular mechanisms of pathologic disorders in biological objects, for example, in blood serum caused by toxic ions. The results of this study can be used in ecology and medicine.

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