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Physical Mechanisms of "Poisoning" the Living Organism by Heavy Metals

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1. Introduction

The toxic affect of heavy metals over the living organisms is known to arouse from the alternation of the course for biological reactions in cells. One of such violations appears to be the process of supra-molecular structures formation, for example, the dipole protein nano-clusters in blood. This phenomenon can be well studied in the biological solutions, such as blood serum, or a widely adopted normal saline solution of albumin [1-3].

In the works devoted to problems of pollution of the surrounding environment and ecological monitoring, for today to heavy metals carry more than 40 metals of periodic system D.I. Mendeleyev with nuclear weight over 50 nuclear units: V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Mo, Cd, Sn, Hg, Pb, Bi, etc. Thus the important role in categorize heavy metals is played by following conditions: their high toxicity for live organisms in rather low concentration, and also ability to bioaccumulation. Almost all metals getting under this definition (except for lead, mercury, cadmium and the bismuth which biological role currently is not clear), actively participate in biological processes, are a part some many enzymes. On N.Rejmersa's classification, heavy it is necessary to consider metals with density more than 8 g/cm^3 .Thus, heavy metals concern Pb, Cu, Zn, Ni, Cd, Co, Sb, Sn, Bi, Hg.

The results of our investigations can to conclude that the process of cluster formation depends on the value of ionic radius metal.

In our works the interaction of some proteins – albumins, globulins, collagen, lisozym, collagenase, creatin cenase, pepsin with heavy metal ions like Cs, Rb, Cu, Cd, Pb in water solutions was studied.

Especial influence on some protein like albumins, globulins, collagen, lisozym and so on has the potassium. K^+ ions presence in the protein solutions also induced appearance of dipole protein nano-clusters.

The appearance of cluster cans disturbance metabolic processes in the cells, membranes, tissue.

The interaction of proteins with ions of alkaline heavy metals like Cs^+, Rb^+, and Cu^{2+}, Cd^{2+}, Pb^{2+} in aqueous solutions was studied in our earlier works [4-9] with the Rayleigh-Debye light scattering (RDLS), the photon correlation spectroscopy (PCS) and the fluorescence polarization (FP) methods. It should be noted, that the nano-sized cluster formation process was also registered in some proteins and enzymes solutions like albumin, globulin, collagen, lysozyme, collagenase and so on in the presence of K^+ ions which does not belong to the heavy metals group [8, 11].

Fig. 1. Metals and its ionic radii

2. Physical model

It was found earlier that cluster formation depends on metal ion radius [2–4]. Interaction of these ions with a protein surface involves, as a rule, their hydrated shells. In cases where protein solutions contain small ions like Na+ (the ion radius equals 0.87 Å), dipole clusters are not formed, because sodium ions are located near the protein surface surrounded by water molecules and cannot bind directly with the negative charges on the protein.

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

The energy of the ion and the water dipole molecule binding, determined by equation (q- is the charge of the ion, p_w – water molecule dipole moment, ε -is dielectrical permeability of water, r_0- the ionic radius), is inversely proportional to the fourth power of the ionic radius. In the heavy ions case, it may be of the same order or less than the heat energy kT, and the water shell cannot stay on ion surfaces. This is observed for ions with large radii, such as Cs^+, Rb^+, Cd^+, Ce^+, Pb^{2+}, and Eu^{3+}, as well as K^+. In interacting with the protein surface directly, a metal ion with a large radius is bound more strongly to negatively charged groups on the protein and can form a Coulomb complex on a protein macromolecule with a common hydrated shell. In this case, the metal ions compensate completely for the local surface charge of the protein molecule [5].

The effective decrease of the protein surface charge that takes place as a result of strong binding of metal ions with a large radius and the macromolecule can lead to a situation where the main type of interaction between the protein molecules is a dipole–dipole

attraction instead of Coulomb repulsion, because the proteins have abnormally high dipole moments (several hundred Debye uniques). So the protein molecules can go closely to each other and forming aggregates – dipole clusters (see fig 2)

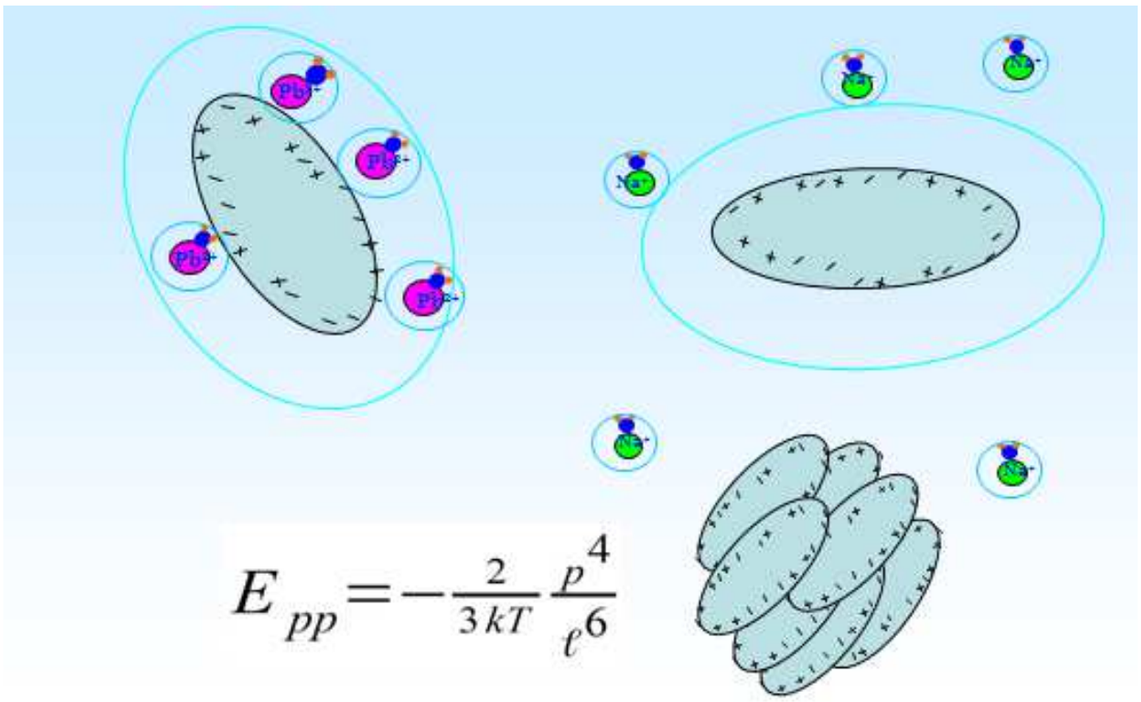

Fig. 2. The scheme of interaction processes small and big ions with protein surface.
(In the formulae , E_{pp} – the energy of dipole-dipole interaction, p is the protein molecule dipole moment, l- the distance between macromolecules)

3. Different optical methods of heavy metals interaction with biological macromolecules investigations

3.1 Rayleigh-Debye light scattering (RDLS) method

The RDLS method is reliable for the determination of static parameters of the solid compounds in the suspension. The way how the light is scattered in the solution bears the information on the effective mass and molecular interaction coefficient of the particles. In case of the diluted solutions the measured experimental value of the Rayleigh scattering coefficient R_{90} is related within Debye's theory to the mass of a macromolecule M, according to the virial expansion of the osmotic pressure by concentration c:

$$\frac{cH}{R_{90}} = \frac{1}{M} + 2Bc + \ldots, \qquad H = \frac{2\pi^2 n_0^2 \left(\frac{dn}{dc}\right)^2}{\lambda^4 N_A},$$

where λ_0 is the incident beam wavelength, n_0 and n are the refractive indexes of the dissolvent and the solution.

Fig. 3 presents data for pH-dependences of particle mass values obtained by RDLS method for the cases of the *Egg* albumin solution with Cesium (a), the bovine serum albumin (BSA) and the Gamma-globulin solutions with Potassium (b,c). All three graphs reveal the formation of large particles, one order heavier than the initial protein molecule. It should be noted that the maximum mass of nano-clusters in case of the K^+ ions in the solutions relates to the physiological pH values.

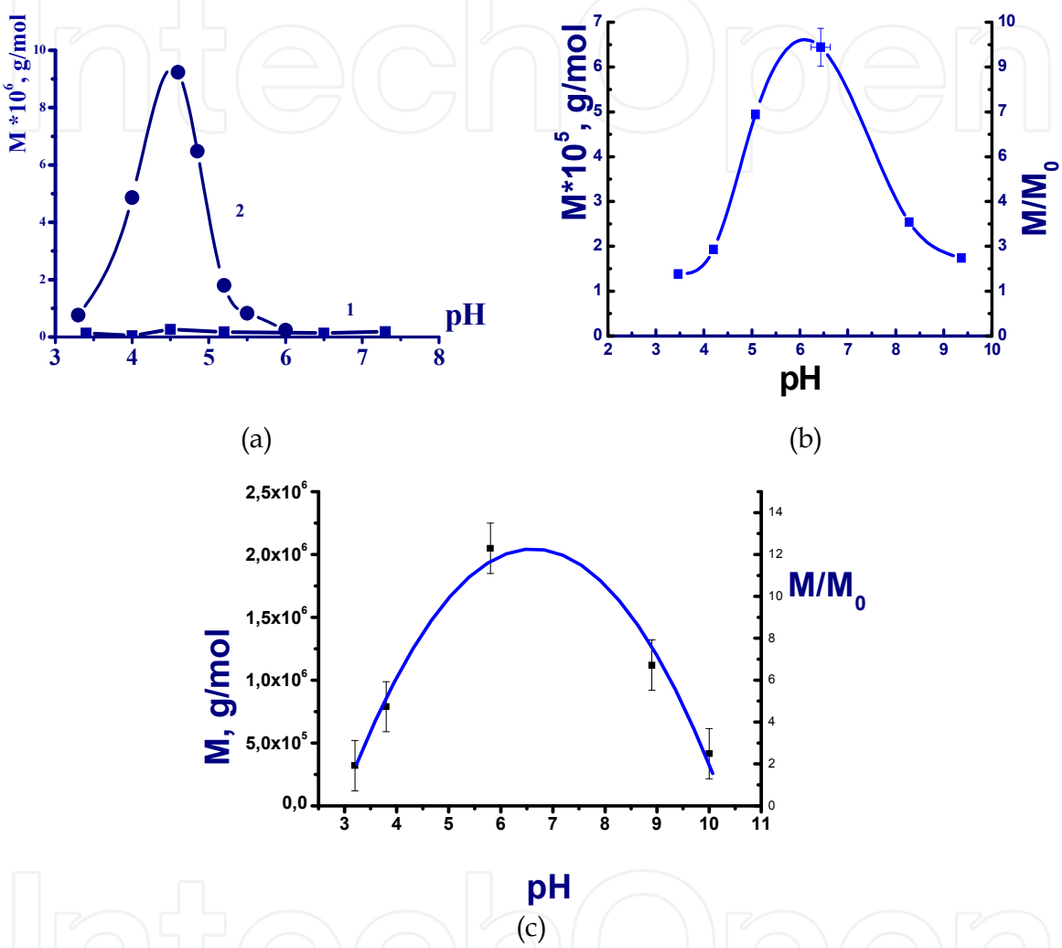

Fig. 3. (a) pH-dependencies of scattered particle mass for Egg albumin in water solution in presence of Cs ions (2) (μ = 0,00105 mol/l), (1) - Egg albumin in pure water solution. (b) pH-dependences of scattering particle mass for albumin, , containing ions K+. (c) pH-dependences of scattering particle mass for γ-globulin water solutions, containing ions K+.

3.2 Photon-correlation spectroscopy (PCS)

The PCS method was suggested to investigate the dynamic parameters of proteins in the aqueous solutions containing heavy metals [4, 5]. The translational diffusion coefficient D_t is described by the Stocks-Einstein-Debye formula as:

$$D_t = \frac{kT}{6\pi\eta r_h}$$

In this formulae η_h is viscosity, r_h - hydrodynamic radius of the particle. The normalized experimental autocorrelation function of the scattered light intensity relates to the translational diffusion coefficient D_t as:

$$g^{(1)}(\tau) = \exp(-D_t q^2 \tau),$$

where, q is wave-vector, τ - correlation time.

Fig. 4 shows the dependences of translation diffusion coefficient on pH for the pure gamma-globulin solution (a) and the one containing K^+ ions (b).

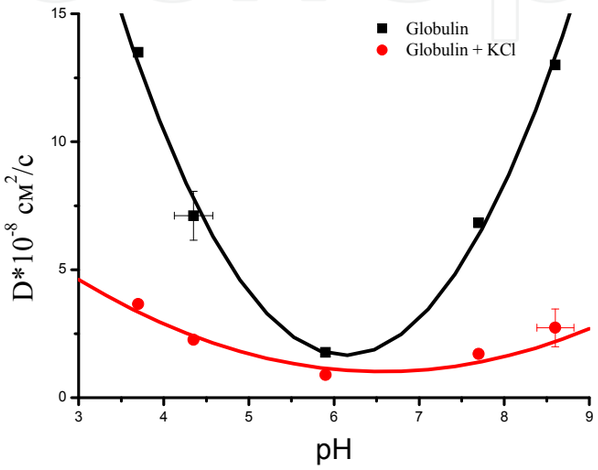

Fig. 4.Translation diffusion coefficient as function of pH for γ-Globulin water solutions with and without K^+ ions

The D_t value is twice less in the latter case when studied in the isoelectric point area of pH~6. It means that the mass of the particles in the solution with K^+ ions is one order greater than that of the gamma-globulin molecule:

$$\left(\frac{D_0}{D_K}\right)^3 = 11 \sim \frac{M_{cluster}}{M_{protein}},$$

where, $M_{protein}$ is the molecular mass of protein and $M_{cluster}$ - the mass of scattering particle.

3.3 Polarized fluorescence method

The fluorescence polarization (FP) method was used to determine the orientation correlation time t_{rot} of albumin in the solutions containing Pb^{2+} and Na^+ ions. This parameter is based on the fluorescence polarization experimental data [6] and is calculated according to the Levshin-Perrin relation [7]:

$$\frac{1}{P} = \frac{1}{P_0} + \left(\frac{1}{P_0} - \frac{1}{3}\right)\frac{t_{fl}}{t_{rot}}, \qquad t_{rot} = \frac{V\eta}{kT} = \frac{M\eta}{\rho kT},$$

where t_{fl} is the lifetime of the excited state. The latter proportion determines linear dependence of the t_{rot} on the mass M of the particle.

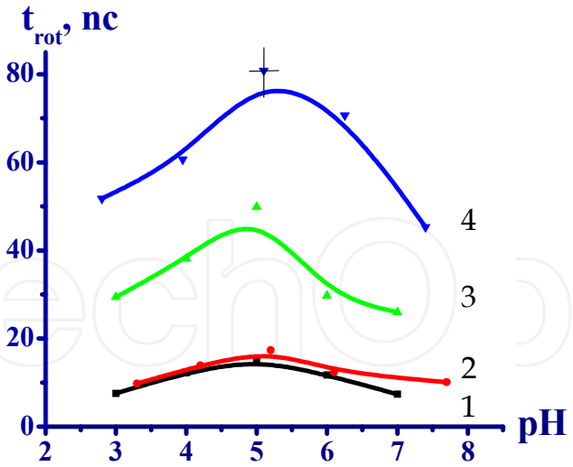

Fig. 5. pH-dependence of time rotation of albumin in the water solutions with Pb^{2+} and Na^{+} ions.
1. BSA 6,4 ·10^{-6}M + Na+ 5,6 ·10^{-3} M
2. BSA 6,4 ·10^{-6}M + Pb^{2+}8,3 ·10^{-10}M
3. BSA 6,4 ·10^{-6}M + Pb^{2+}1,7 ·10^{-7} M
4. BSA 6,4 ·10^{-6}M + Pb^{2+}6,3 ·10^{-5} M

As Fig. 5 shows the orientation correlation time increases along with the concentration of the heavy metal Pb^{2+} ions.

For comparison, fig 6 shows the plot of relative clusters mass depends on relative concentration - metal/protein for BSA solutions with potassium and lead ions.

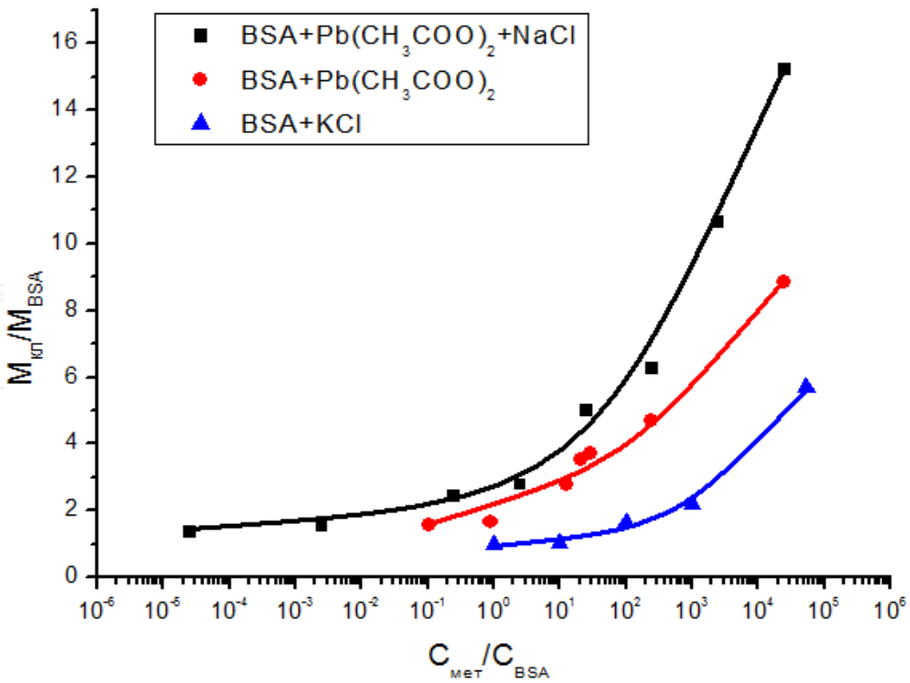

Fig. 6. Relative clusters mass dependences on relative concentration - metal/protein for BSA solutions with potassium and lead ions.

Thus, the FP method confirms the formation of the nano-sized clusters in the protein solutions with presence of heavy metal ions.

4. Sorption of the ions with various ionic radii on protein surface in the process of nano-clusters formation

In this part the sorption process of ions with various radii on the serum blood protein surface during the nano-clusters formation stage was study. A number of static parameters were achieved by Rayleigh-Debye light scattering, including effective masses and molecular interaction coefficient of the particles in the proteins aqueous solution containing ions of Na^+, K^+ and Pb^{2+} at different ionic strength. It was found that the nano-cluster formation process depends on the ionic radius of the metal.

4.1 Results and discussion

The following table represents the metal ions as studied in this investigation:

Metal	Mass, a. u.	Nuclear charge	Ionic radius, Å	Relative mass of cluster
Na^{11}_{23}	23	11	0,87	<2
K^{19}_{39}	39	19	1,33	20-35
Pb^{82}_{207}	207	82	1,2	>20

Table 1.

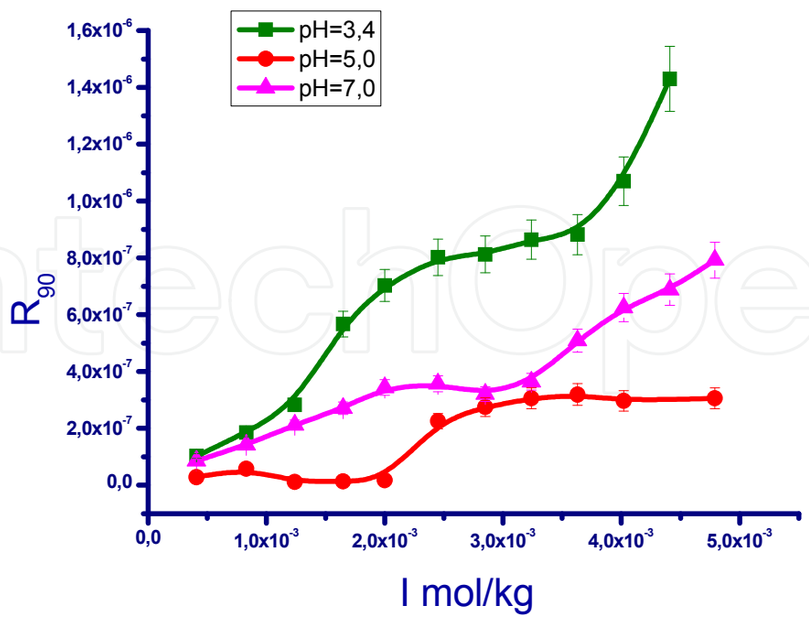

Fig. 7. Rayleigh scattering coefficient (R_{90}) as function of ionic strength of albumin water solution containing Na^+ ions.

The mentioned above metals were used to study the dependence of the Rayleigh scattering coefficient R_{90} on the value of the ionic strength I in the aqueous solutions of albumin produced by "Sigma Inc." (USA).
Fig. 7 shows the dependence of R_{90} on I for the solution with Na^+ ions, whereas Fig.8 shows the relative masses of scattering particles dependence for this solution at pH=7.0 on I, which is the concentration of Na^+ ions in this case.

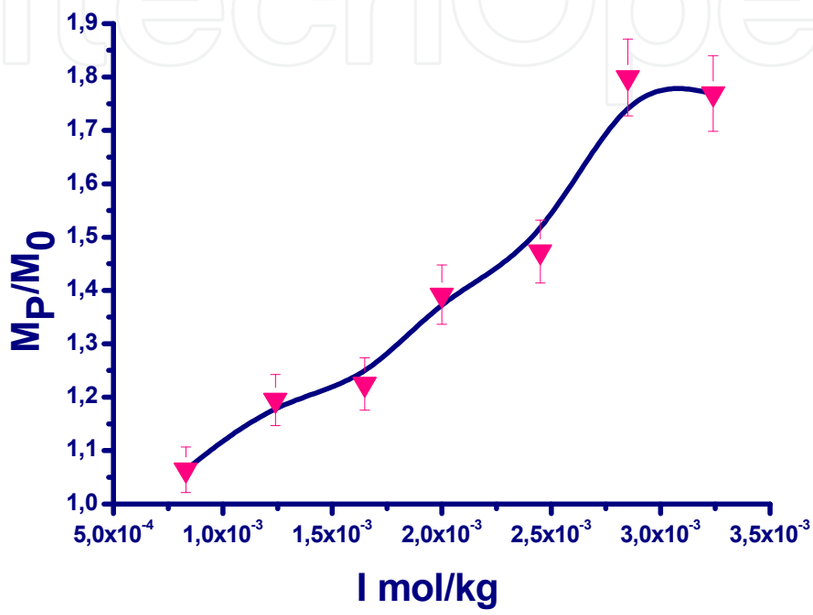

Fig. 8. Scattering particles (M_P) relative mass in albumin (M_0) solution as a function of ionic strength Na^+.

As follows from these graphs the presence of Na^+ ions in this solution at higher ionic strength slightly increases the masses of the scattering particles. Compared to the mass of the albumin molecule the masses of these particles are less than twice heavier, approx. ~ 1,8. Probably, a number of protein molecules in the albumin solution with Na^+ ions can form dimers.
Contrary to that the effect is absolutely different with K^+ and Pb^{2+} ions in the albumin solution.
Fig. 9 shows the dependences of R_{90} on ionic strength in the BSA solution, containing K^+ ions for a number of pH values. The dependence of relative masses of scattering particles for this solution at pH=7 is shown on Fig.10.
In this case the value of the relative mass $M_{cluster}/M_{protein}$,which represents the mass ratio of the nano-sized cluster to the albumin molecule, lies in the area of 20-35 for the ionic strength around 2-3 mmol/l.
The concentration variations of the Pb^{2+} ions in the albumin solution leads to a dramatic decrease of the molecular interaction coefficient, which is the second virial coefficient B upon the increase of the ionic strength.

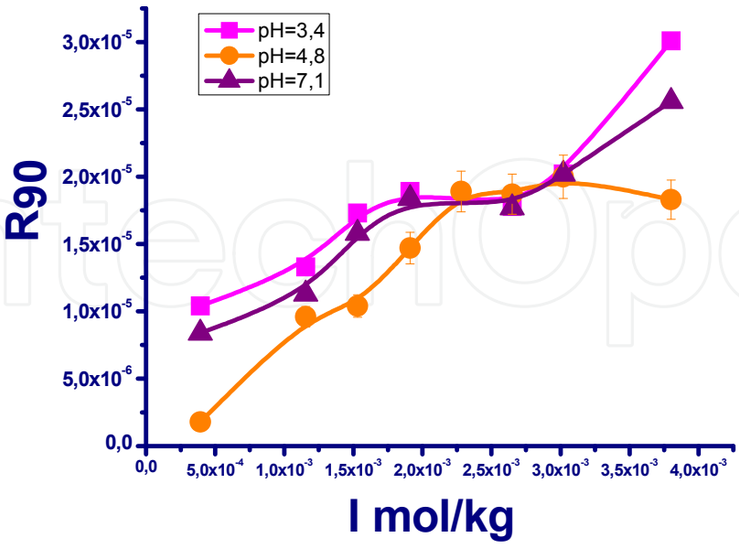

Fig. 9. R_{90} as the function of ionic strength in albumin water solution containing K^+ ions.

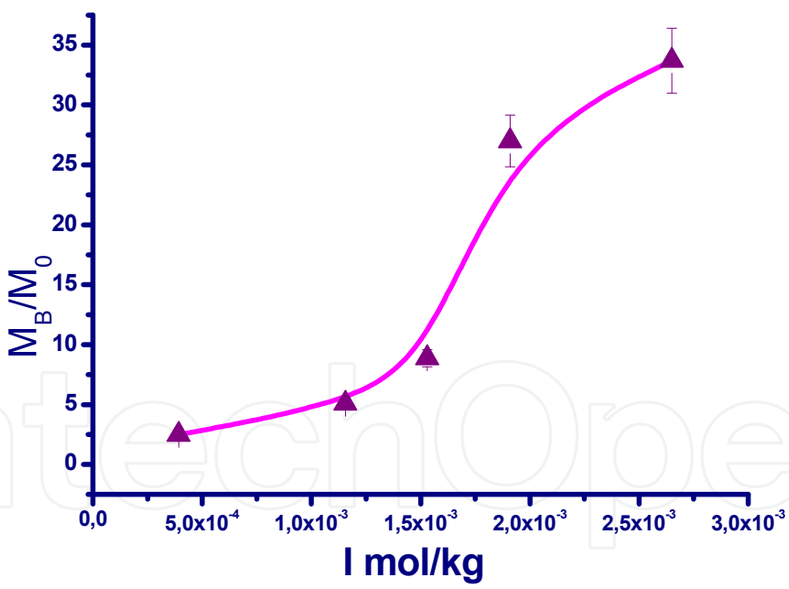

Fig. 10. Dependence of relative masses of scattering particles for BSA solution, containing K^+ at pH=7.

As Fig. 11 shows the former changes its sign and becomes negative when the latter reaches the values in the area of 10-15 mmol/l. This effect is due to the change in the type of molecular interaction which is caused by the increment of the Pb^{2+} ions concentration. In this case the Coulomb repulsion between protein macromolecules, when *B* is positive, diminishes, the pure dipole attraction takes over, and *B* descends below zero.

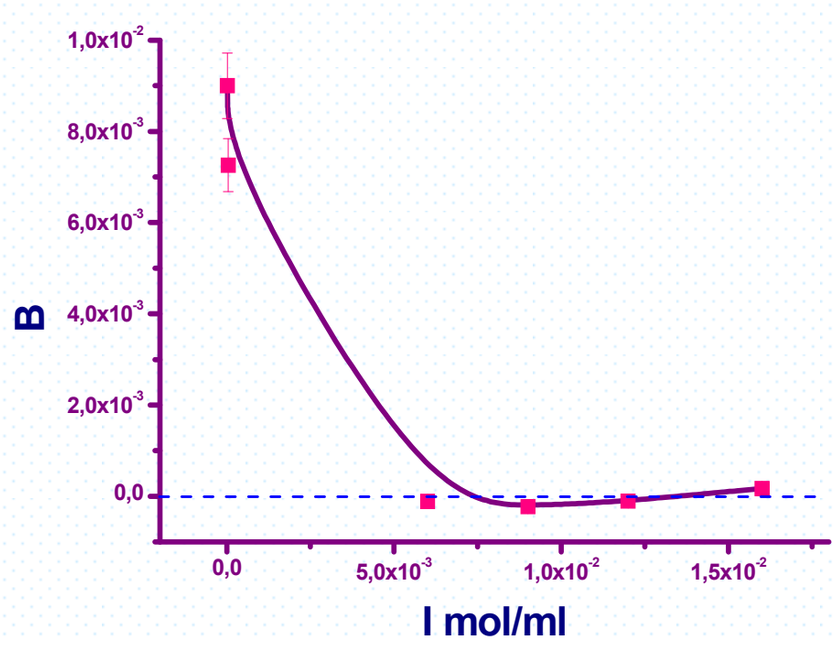

Fig. 11. Dependence of B (the second virial coefficient) from ionic strength in albumin solution with Pb^{++} ions.

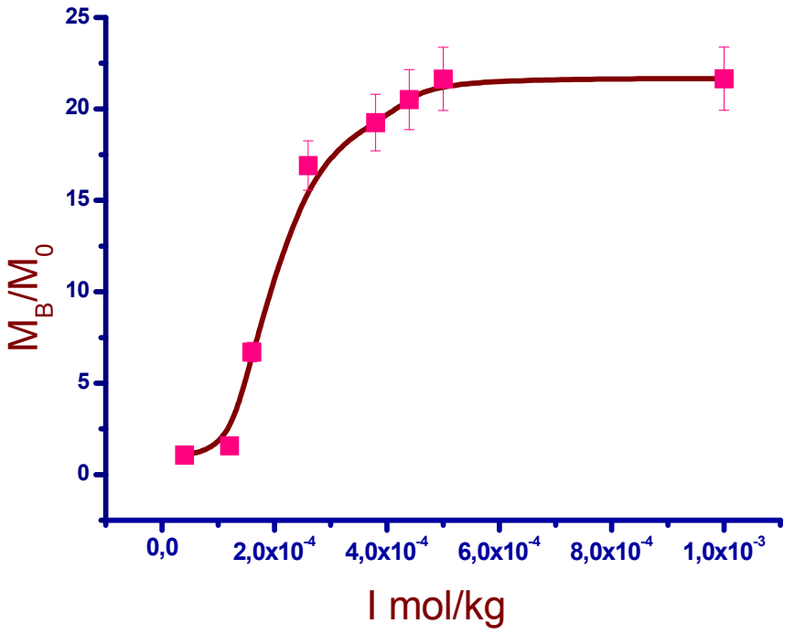

Fig. 12. Dependence of relative mass value from ionic strength of albumin solution with Pb^{++} ions (pH=7, 5)

Fig. 12 shows the dependence of the relative scattering particles mass on the ionic strength of the solution. The curve possesses a small slope rise of the relative mass. The ionic strength

values in the range from 0,05 mmol/kg to 0,17 mol/kg relate to the process of monolayer formation which takes place until the Langmuir saturation is achieved.
As graph data shows that the scattering particles masses are more than 20 times greater than the mass of the albumin molecule. It depicts the process of the formation of the larger particles which appear to be the nano-sized clusters generated by a number of the original macromolecules. With the presence of Pb^{2+} ions in the solution the cluster formation process occurs at the significantly smaller ionic strength values of 0,15 mmol/kg, as compared to the case of K^+ ions of 1,5 mmol/kg. Nonetheless, the cluster formation process runs faster in case of Pb^{2+} ions although the generated particles appear to be lighter than in the case with K^+ ions.

5. Conclusions

- The interaction of the metal ions with the charged surface of the protein in the solution is studied by the measurement of the light scattering coefficient along with the concentration variation of the former.
- The dependence of masses of the scattering particles on the ionic strength and pH of the solution shows the Langmuir sorption process which leads upon the monolayer saturation to the dipole cluster formation.
- The nano-sized clusters form as a result of the phase transition when the Coulomb repulsion forces diminish and the pure dipole attraction forces take over.
- The nano-cluster formation process in the protein solution depends on the ionic radii of metal. The clusters are formed in case of the solutions containing K^+ and Pb^{2+} ions, whereas the presence of Na^+ ions in the solution reveals no effect.
- Cluster formation process can explain toxic influence of heavy metal ions at the very small concentration on the living organisms.

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In memoriam of professor Yuriy M. Petrusevich (1935-2010).
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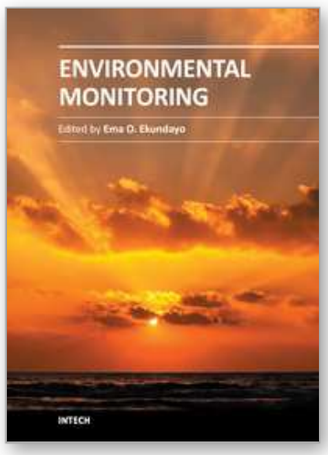

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