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ACOUSTICS AND MOLECULAR PHYSICS

PHYSICAL METHODS FOR STUDYING THE EFFECT OF LEAD IONS ON PROTEINS IN SOLUTIONS

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Using two independent physical methods (Rayleigh light scattering and electron paramagnetic resonance), the effect of lead, toxic heavy metal, on proteins in aqueous solutions (including blood serum proteins) was studied. A physical mechanism of interaction between heavy metal ions and charged biopolymers which is based on formation of strong bonds of metal ions with the surface of macromolecules is discussed. Based on the results obtained, a method to monitor natural liquid media pollution with heavy metals is proposed.

INTRODUCTION

Metals play an important part in a human organism. The amount of such metals as sodium, potassium, calcium, and magnesium in man's body is up to several per cent of its total mass (about 2 kg of 70 kg). Other metals, such as iron, cobalt, molybdenum and zinc, are contained in a human organism in very small concentrations being bound to enzymes: their content is less than 1% of body mass.

Concentrations of metals, particularly, heavy metals, exceeding the maximum allowable level in the organism are dangerous for man.

At present, it is of topical interest to study molecular mechanisms of the effect of heavy metal ions on biological fluids, for instance, blood, and to develop sufficiently simple and reliable physical methods for environmental monitoring of the occurrence of heavy metals in natural environments, in the first place, in water.

Table 1 lists the known chemical, biophysical and physical mechanisms of the effect of metals on the activity of cells and the entire living organism.

Comparatively recently we have discovered one of the physical mechanisms of a toxic action of metals presented in Table 1 in aqueous protein solutions. Aqueous protein solutions may be considered to be model solutions of blood serum. It was shown in [1, 2, 3] that if these solutions contain ions of the heavy alkali metals cesium and rubidium, interaction of these ions with protein macromolecules gives rise to supermolecular structures—clusters whose mass exceeds the mass of a protein molecule by one or more orders of magnitude.

Lead belongs to a group of so-called heavy nonferrous metals and is perhaps the most common "metal" poison. Lead is used for many industrial purposes—in making paints, as additives to automobile fuel, etc. Like mercury, lead is extremely toxic for the nervous system and kidneys, the gravity of injury depending on man's age and exposure time. In children, even low concentrations of lead cause lowering of the mental power and changes in behavior; prolonged exposure to lead may result in various kinds of cerebral lesion and even fatal outcomes.

Table 1

Basic Mechanisms of Toxic Effect of Heavy Metals

Chemical and Biophysical Mechanisms	Physical Mechanisms
1. Enzyme poisoning	1. Anomalous sorption at lipoprotein surface (oncology, aging)
2. Impaired membrane permeability	2. Anomalous molecular mobility of charged biopolymers (oncology, toxicosis)
3. Impaired electron transport	3. Formation of dipole protein clusters
4. Blocked nerve conduction	
5. Development of chain free-radical processes (oncology, aging, radiation injury)	

In this work we studied the effect of lead ions on proteins (albumins) in aqueous solutions.

An effective method for studying macromolecule solutions is Rayleigh light scattering [4, 5]. The known Debye equation helps relate the experimentally measured quantity—the light scattering coefficient (or turbidity) of a solution—to the solution concentration and the intermolecular interaction coefficient (the second virial coefficient in expansion of osmotic pressure in terms of low concentrations),

$$\frac{cHK}{R_{90}} = \frac{1}{RT}\frac{d\Pi}{dc} = \frac{1}{M} + 2Bc + \ldots, \quad H = \frac{2\pi^2 n_0^2 \left(\frac{dn}{dc}\right)^2}{\lambda^4 N_A}, \tag{1}$$

where c is the protein concentration; R_{90} is the Rayleigh coefficient of scattering at an angle of 90° to an incident beam; H is the so-called solution constant; K is the coefficient determining the optical anisotropy of scattering particles; R is the universal gas constant; T is the temperature; and Π is the osmotic pressure. The equation permits determination of the mass M of scattering particles and the intermolecular interaction parameter B.

In our study, scattering properties of aqueous protein solutions were investigated in an optical instrument (Fig. 1) using a helium–neon laser ($\lambda = 632$ nm) and a scattered radiation photoelectron detector. The polarization portion of the unit was composed of the two elements: a Wollaston prism and a Glan–Thompson prism. Two scattered rays with mutually perpendicular polarization directions were modulated at the same frequency (80 Hz) by means of a light chopper. A photomultiplier signal was sent to an amplifier tuned to the modulation frequency, and the amplified signal was measured by a digital voltmeter. The intensity of the laser beam was controlled by a photodiode.

The molecular weight and the second virial coefficient were calculated by measuring relative intensities of radiation scattered at 90° for two perpendicular directions of polarization. The measurements were made in solutions with different pH values and protein concentrations. The results were adjusted by taking into account the intensities of light scattered by a solvent (water).

To calibrate the intensity of scattered light, benzene was used as a standard liquid ($R_{90} = 12.64 \times 10^{-6}$ cm^{-1} for $\lambda = 632$ nm) with a correction for the difference in the refractive indices of benzene and water. The error of measuring R_{90} was 6%.

The refractive index increment was measured by a Raylegh interferometer (the measurement error was 2%). Concentration of free protons in the solution (pH) and ionic strength μ were varied by adding small amounts of acid, base and salt to the solution.

The effectiveness of this method can be illustrated by an experimental graph (Fig. 2) showing the relationship of the scattering parameter cH/R_{90} for a water hemoglobin solution and the concentration when a surface charge of protein (pH) is changed. It can be seen that with an increase of a positive protein charge to pH 3.5 a hemoglobin molecule disintegrates into two subunits which leads immediately to a relevant reduction of the solution scattering coefficient.

The mass of macromolecules in a solution can also be determined by another physical method, namely, electron paramagnetic resonance (EPR). A paramagnetic probe is commonly employed for the purpose.

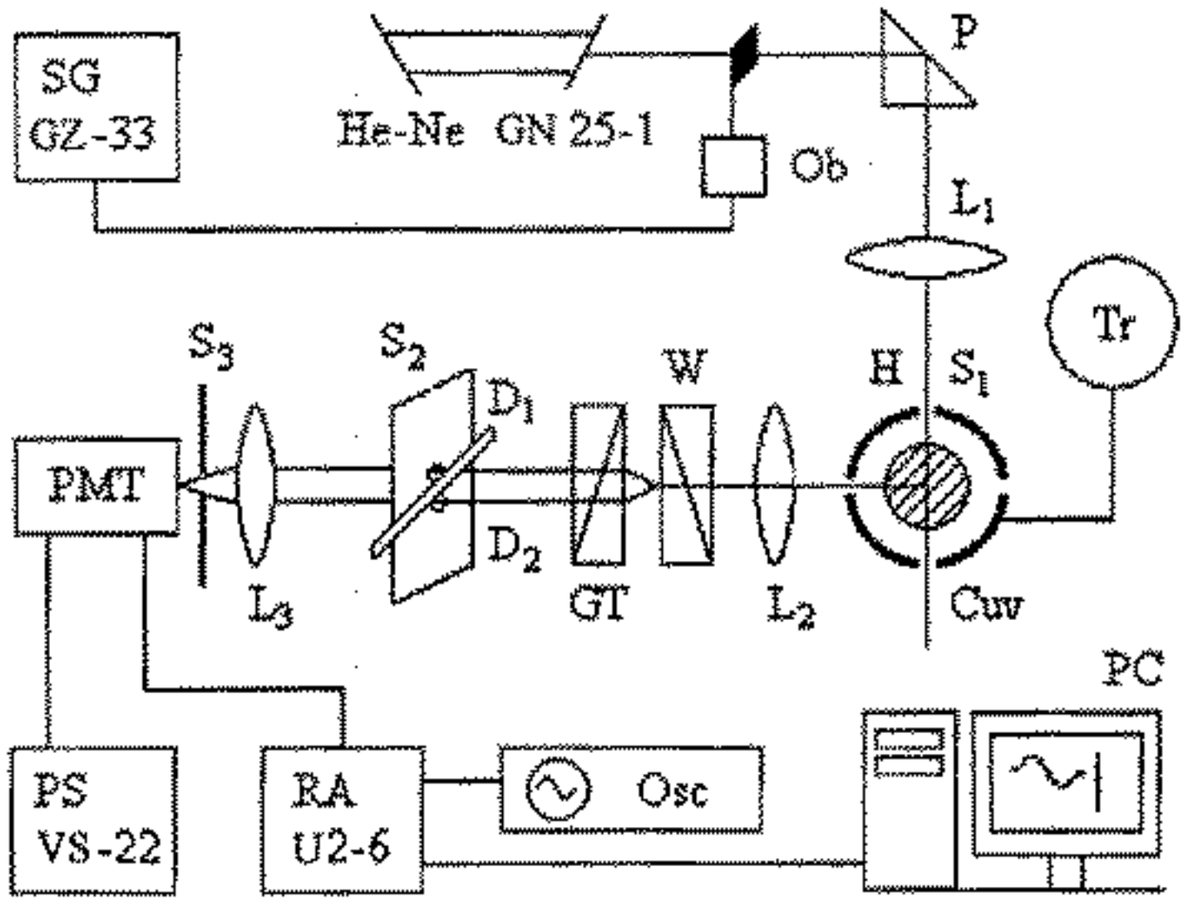

Fig. 1

Block diagram of the optical instrument: He–Ne GN 25-1—laser; SG—audio-frequency generator; Ob—modulator; P—deviating prism; L_{1-3}—lenses; S_{1-3}—screens; H—heating element; Tr—laboratory autotransformer; Cuv—cuvette with solution; W and GT—Wollaston and Glan–Thompson prisms (polaroid); $D_{1,2}$—diaphragms; PMT—photomultiplier; PS—photomultiplier power unit; RA—resonance amplifier; Osc—oscilloscope.

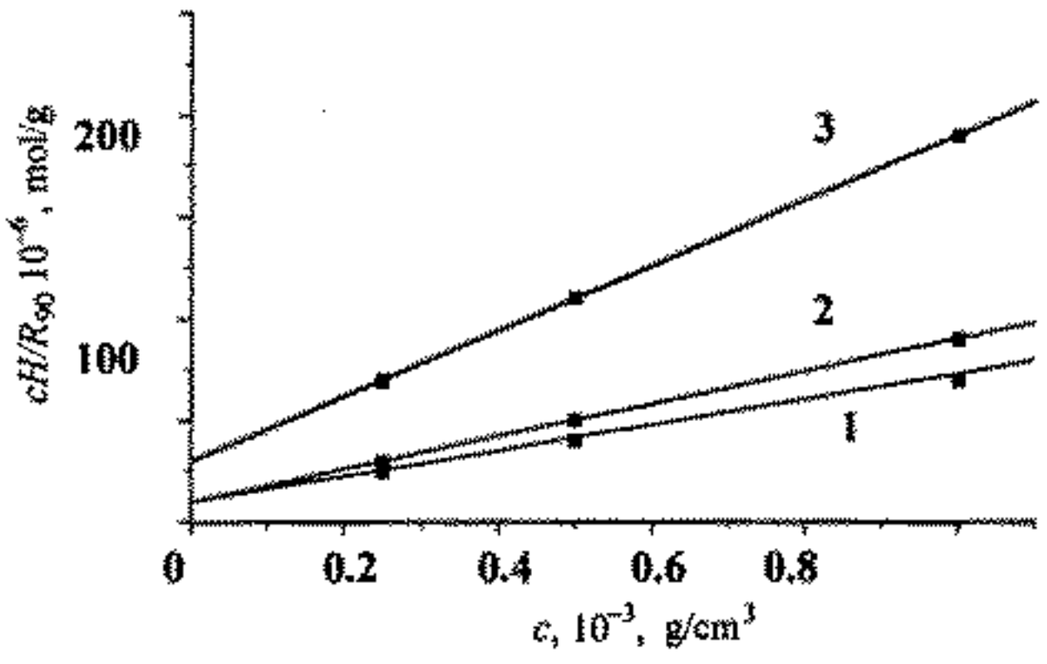

Fig. 2

Scattering parameter vs. concentration for water hemoglobin solution: (*1*) pH 5.2, (*2*) pH 6.5 ($M = 68\,500$), (*3*) pH 3.5 ($M = 39\,000$).

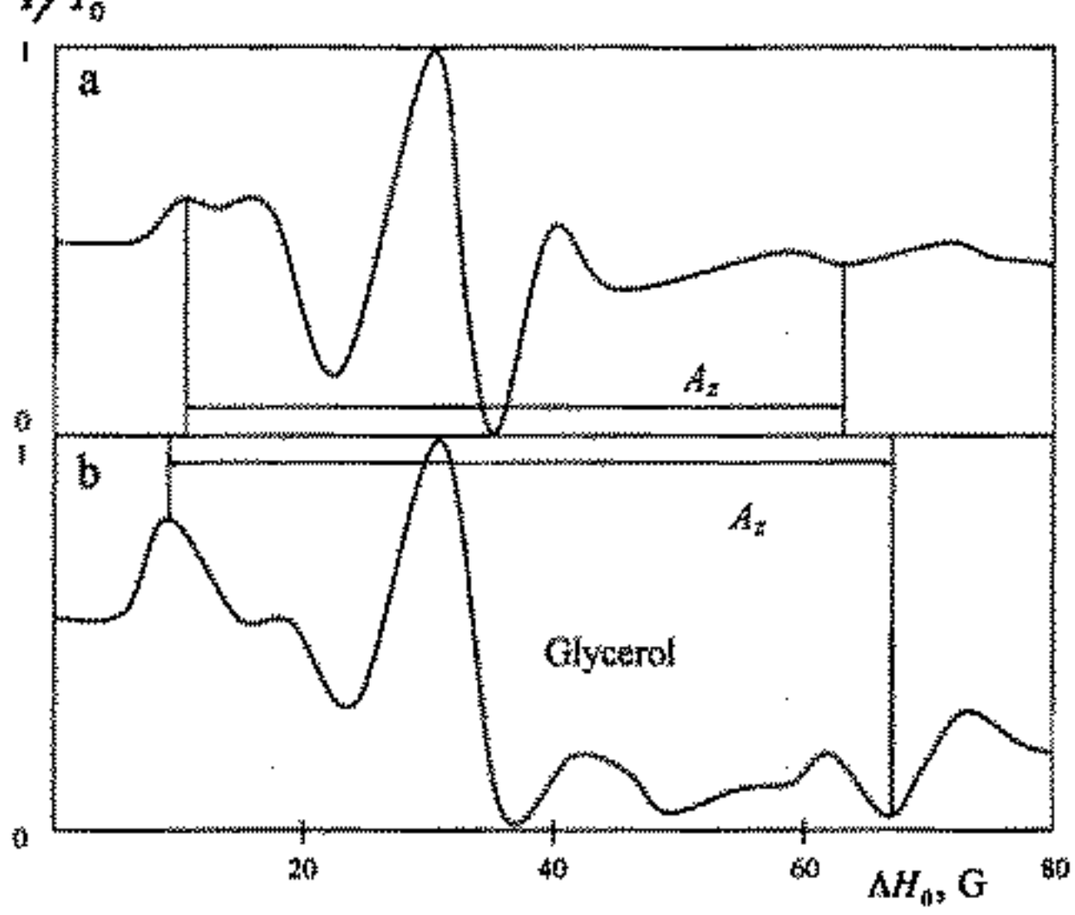

Fig. 3

EPR spectrum of protein: (*a*) in water solution, (*b*) in glycerol. ΔH_0 is the spectrometer constant field variation.

For slow motion of macromolecules the EPR spectrum of protein with an adsorbed nitroxyl radical has two distinctly separate peaks (Fig. 3*a* and *b*). According to the theory described in [6], such a spectrum can be interpreted using the parameter $S = A'_Z/A_Z$, where A_Z is the half-width of the distance between the high and the low peak of the albumin spectrum in an aqueous solution, and A'_Z is the same parameter but for a more viscous solution.

Table 2

Parameters of Proteins Used in Experiments

Protein	Molecular mass, a.m.u.	Dipole moment, D	Isoelectric point, pH
Bovine serum albumin	68 000	480	4.9
Egg albumin	50 000	400	4.8
Milk albumin	14 500	700	5
Hemoglobin	68 000	380	5.2

The authors of [6] propose the equation to calculate the correlation time τ in the time interval from 10^{-9} to 10^{-8} s,

$$\tau = a(1-S)^b, \tag{2}$$

where $b = -1.16$, $a = 8.5 \times 10^{-10}$, and the width of the central spectral line of nitroxyl radical $\Delta H_0 = 5$ G.

The mass of macromolecules can be found by the formula

$$\tau = \frac{V\eta}{kT} = \frac{M\eta}{\rho kT}, \tag{3}$$

where ρ is the density, and η is the viscosity of the solution.

In our case, a spin-labeled solution was prepared as follows: a weighed portion of each spin label was dissolved in ethanol and added to a protein solution, ethanol concentration being less than 1%. The EPR spectra were recorded by a PS-100X EPR spectrometer (ADANI model). Proteins of Serva Co. were used in the experiments (Table 2). The third component of the solution was lead acetate, $Pb(CH_3COO)_2 \cdot 3H_2O$, and lead nitrate, $Pb(NO_3)_2$.

RESULTS AND DISCUSSION

The study of aqueous solutions of some proteins using the Rayleigh light scattering method showed that the mass of a protein macromolecule remains virtually unchanged in conditions of varying solution pH and ionic strength determined by concentration of NaCl salt [5]. As an example, Fig. 4 illustrates the concentration dependence of the scattering parameter of the hemoglobin water solution at different ionic strengths and constant pH.

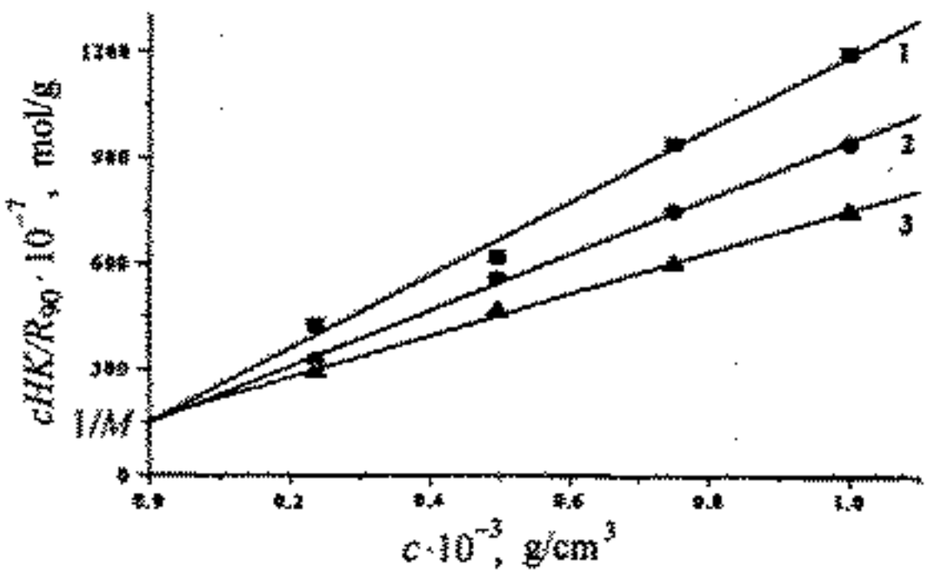

Fig. 4

Scattering parameter vs. concentration for water hemoglobin solution at different NaCl concentrations (μ), pH 5.5, $M = 65\,000$ g/mol: (*1*) $\mu = 0.001$ mol/l, (*2*) $\mu = 0.01$ mol/l, (*3*) $\mu = 0.05$ mol/l.

Light ions, like sodium, have comparatively small ionic radii and, therefore, retain securely a hydrate sheath at their surface. For heavy ions possessing large ionic radii, the processes of their adsorption at the surface of a protein macromolecule may take a different course [1].

If the energy of electrostatic interaction between an ion and a water molecule $E_{pq} < kT$, the hydrate sheath will not be retained at the ion surface, and the ions may form stable electrostatic pairs at the protein that compensate totally for its surface charge.

Figure 5 presents the relationships between the intermolecular interaction coefficient and pH(Z), where Z is the total charge at the protein surface, for aqueous solutions of bovine serum protein in the presence of a lead salt.

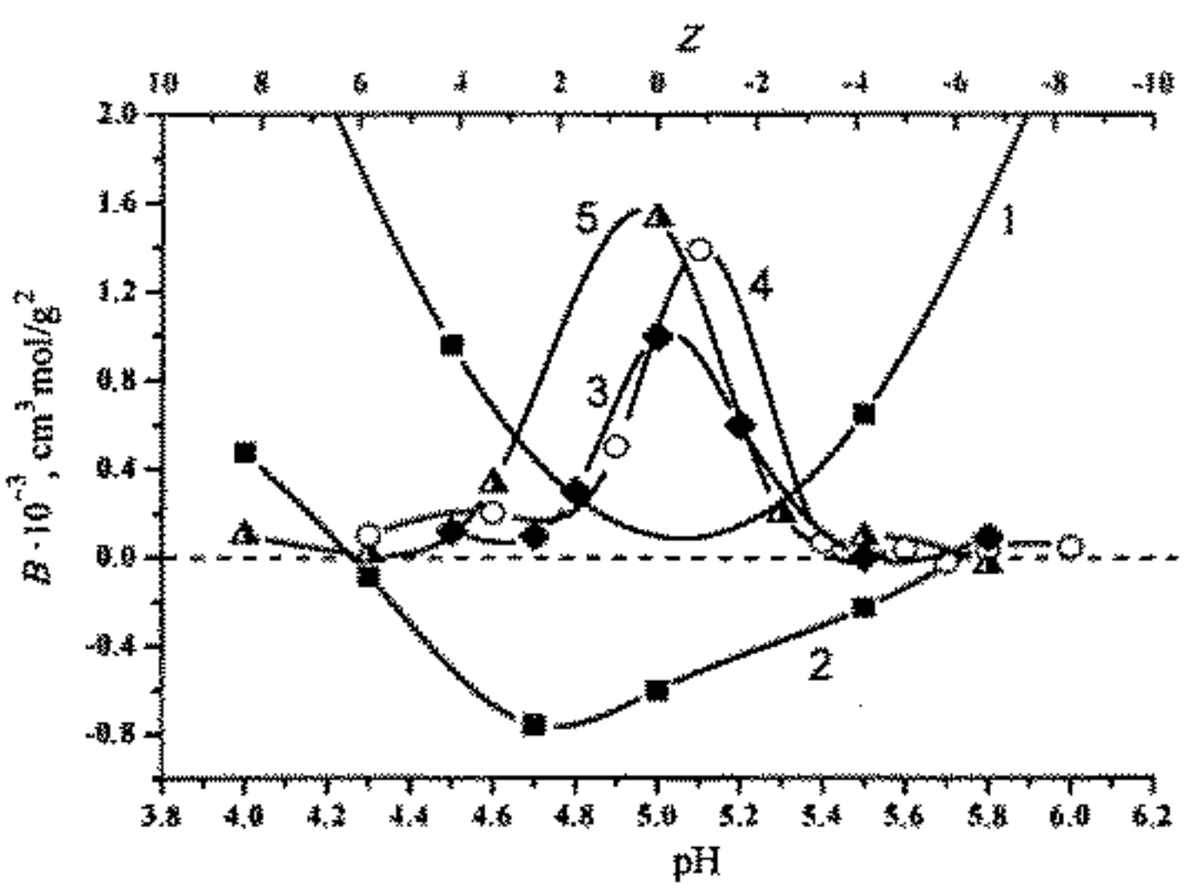

Fig. 5

Intermolecular interaction coefficient as a function of pH(Z) for serum albumin with added lead acetate $Pb(CH_3COO)_2$: (*1*) $\mu = 0$, (*2*) $\mu = 0.00075$ mol/l, (*3*) $\mu = 0.00105$ mol/l, (*4*) $\mu = 0.0012$ mol/l, (*5*) $\mu = 0.0015$ mol/l.

As can be seen from the curves in Fig. 5, the nature of intermolecular interaction in a solution containing lead salts is totally different from a pure protein solution (curve *1*). When the lead salt concentration is low, the intermolecular interaction coefficient assumes negative values (curve *2*), and with a further growth of ionic strength the interaction coefficient becomes maximum instead of minimum near the isoelectric point (curves *3*, *4*, *5*). Figure 6 shows pH dependences of the effective mass of scattering particles in the same solutions. The mass of protein clusters forming in the solution increases by more than one order of magnitude as compared to the mass of a macromolecule and reaches its maximum near the isoelectric point of protein.

The nature of interaction of protein (albumin) macromolecules in case when the solution contains ions with a large ionic radius depends largely on dipole–dipole forces. When dipoles are spaced at no more than 30–50 Å, the energy of dipole–dipole interaction may exceed thermal energy kT by almost 100 times [3]. Protein molecules may come extremely closely to one another to form a macromolecular complex—a dipole cluster.

As was shown in [3], in aqueous protein solutions in the presence of salts of heavy alkali metals (cesium and rubidium), the process of formation and decay of dipole complexes is reversible. Similar effects are observed in water solutions of bovine serum albumin in the presence of lead acetate (Fig. 6). In this case as well, with the growth of a charge (positive or negative) at the surface of a macromolecule the mass of scattering particles approaches the protein molecular mass.

Formation of large particles—supermolecular structures in aqueous solution can also be confirmed independently by electron paramagnetic resonance.

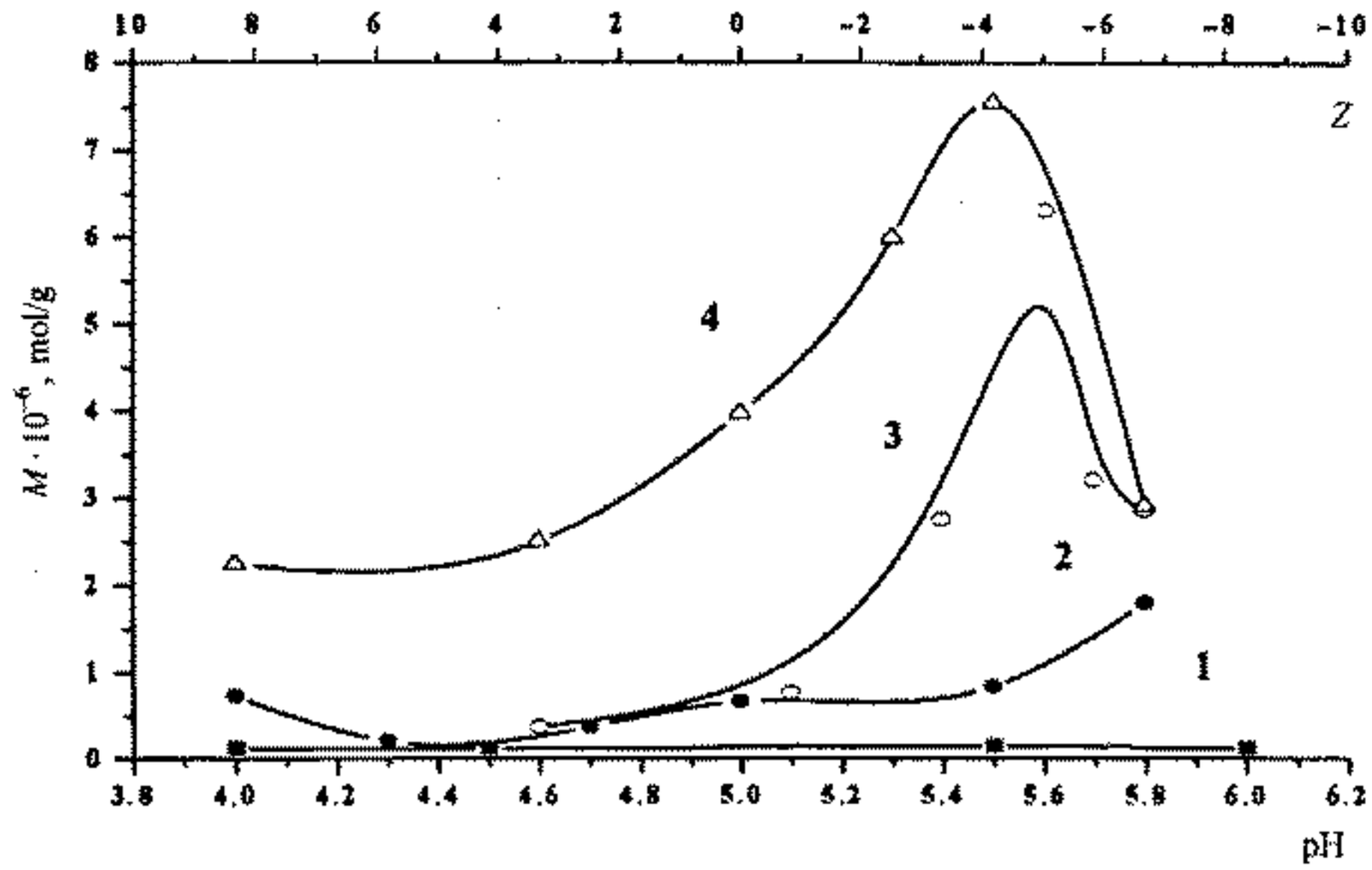

Fig. 6

Scattering particle mass vs. protein surface charge at different concentrations of lead ions: (*1*) $\mu = 0.00075$ mol/l, (*2*) $\mu = 0.00105$ mol/l, (*3*) $\mu = 0.0015$ mol/l.

Figure 7 presents EPR spectra in a water solution of pure protein (curve *1*) and in the case when lead acetate was added to the albumin solution (curve *2*). The paramagnetic label used was an imminoxyl radical. An almost two-fold increase of the resonance line width ΔH corresponds to a ten-fold growth of the mass of particles in the solution as follows from the calculation of rotational correlation time using formula (2).

Figure 8 illustrates the dependence of rotational correlation time and relative particle mass in a solution on lead salt concentration calculated from formula (2). It can be seen that rotational correlation time and

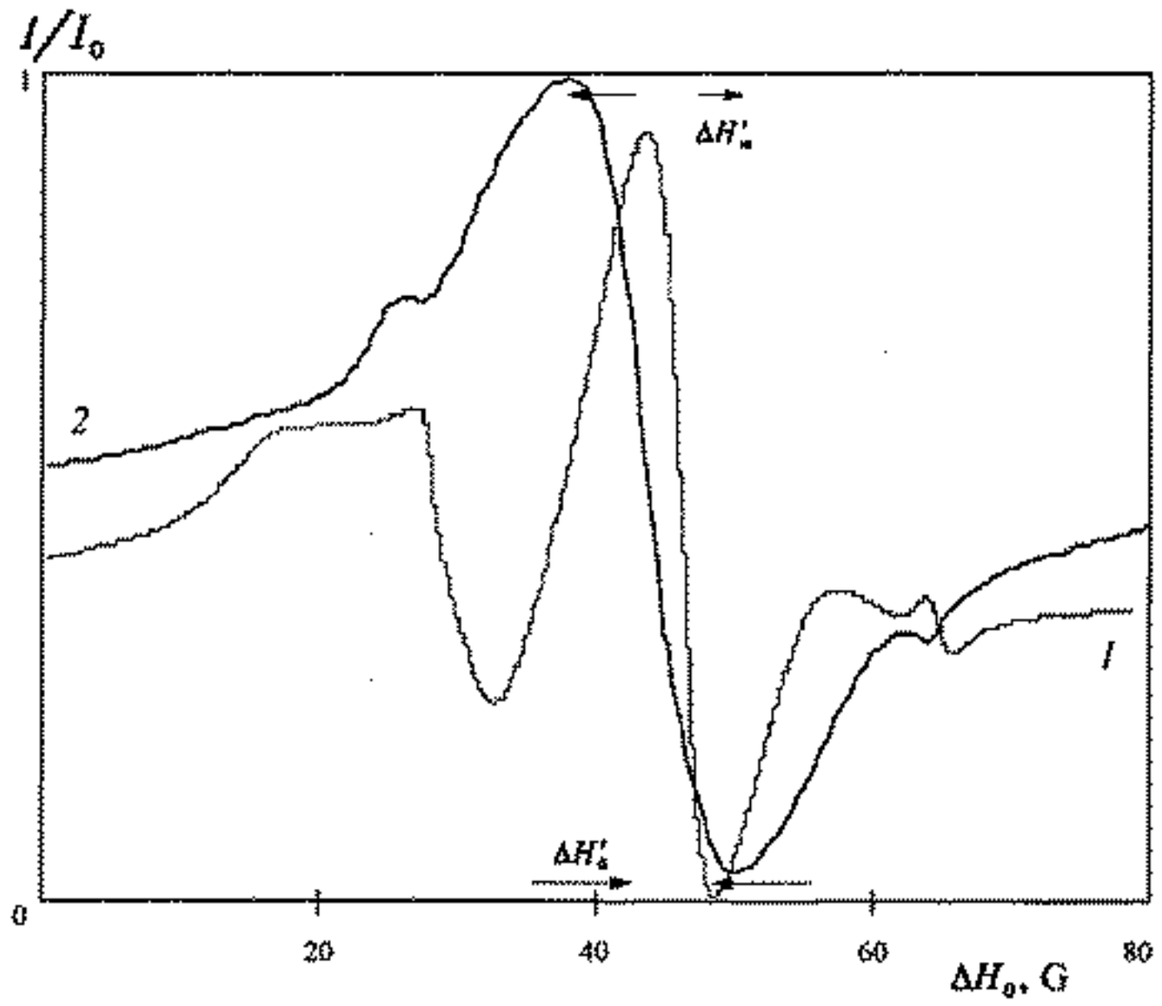

Fig. 7

EPR spectrum of a nitroxyl radical adsorbed on protein: (*a*) in a pure water solution, (*b*) in a water solution with addition of lead acetate.

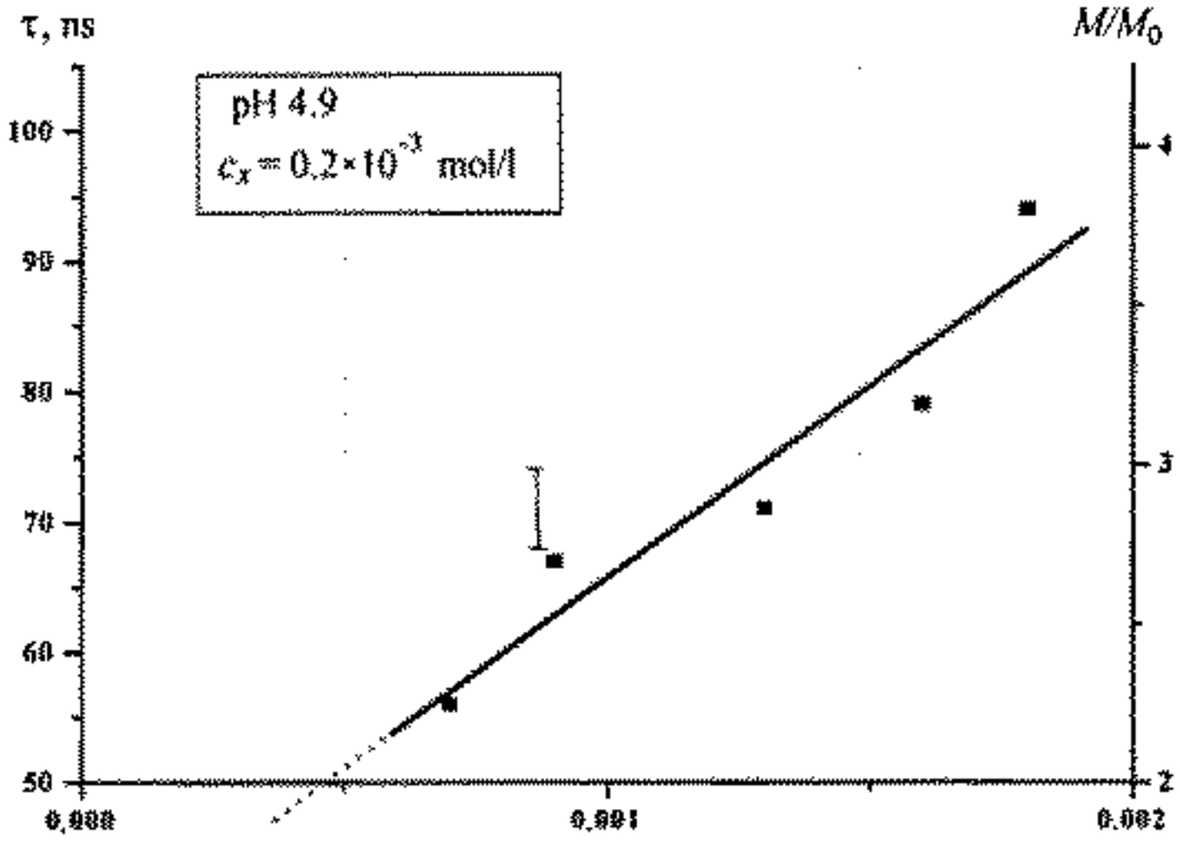

Fig. 8

Rotational correlation time τ and relative particle mass M/M_0 in solution vs. lead acetate concentration.

cluster mass increase linearly with the growth of ionic strength of the solution near the isolelectric point of protein. Thus, an independent physical method—EPR—confirms the formation of dipole protein clusters in solutions containing heavy metals.

The Rayleigh scattering and EPR techniques can be used to monitor pollution of natural environments, including potable water sources, with heavy metals, the light scattering method being the most simple and effective.

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