Light scattering in albumin solution under the influence of electric field in the regime of total internal reflection

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Abstract. In the paper the application of light scattering from biological macromolecules under the influence of electric fields in the regime of total internal reflection is discussed. An experimental setup on prism for the regime of total internal reflection was developed. The water solution of albumin with the concentration 4 % and pH of the medium is equal to 9.3 was used as an object of study. The obtained results demonstrate the applicability of method for study of biological macromolecules by light scattering in various fields of science.

1. Introduction
Biological macromolecules such as proteins, nucleic acids and polysaccharides have sizes from 1 nm to 1 µm. Particles with these sizes in liquids may be considered as colloidal systems and they carry a certain electrical charge due to electrical dissociation [1]. Each protein molecule contains many negatively and positively charged groups. Aggregation process, the environment and the pH of the medium have a great influence on charge of molecule. The structure and the properties of biological macromolecules change under the influence of various external factors. Optical methods, including light scattering, are widely used to control these processes. Light scattering is the most common technique used to measure the parameters of biological macromolecules in solution [2]. Analysis of laser light scattering under the influence of electric fields allows us to measure both the electrophoretic mobility and diffusion coefficients of charged biological macromolecules in solution [3].
The goal of our work was to study electrophoretic mobility of biological macromolecules by means of light scattering under the influence of the electric fields. We considered the theory of light propagation in media and light scattering from particles in the regime of total internal reflection. The research possibilities of the developed experimental setup in analysis of biological samples in the regime of total internal reflection are considered. Such characteristic as electrophoretic mobility of molecules under the influence of various values of electric field strength for albumin solution were studied.

2. Techniques
In the electric field a double electric layer appears on the surface of particles (on the particle-liquid interface) [4]. A layer of ions of a certain sign uniformly distributed over the surface and creates a surface charge (potential-determining ions) on it. Ions of the opposite signs (counter ions) are attracted from the liquid medium to this layer. Motion of the particles leads to destruction of double electric field in some area (called the slip plane). The slip plane lies on the boundary between the diffuse and adsorption layers or in the diffuse layer near this boundary. The potential on the slip plane is called the electrokinetic or zeta potential [4,5]. The zeta potential corresponds to the stability of the biological
macromolecules to aggregation. Molecules in electric fields move with the certain speed named electrophoretic mobility. Electrophoretic mobility relates to zeta potential. Electrophoretic mobility of the solution can be measured by the method of light scattering. The intensity of the scattered light is detected using an optical system containing a photodetector and a signal processing system that allows spectral or correlation analysis of the data [2].

In the case of a field application, the particles begin to move at a certain speed to the oppositely charged electrode. The influence of the electric field is taken into account in the autocorrelation function [6]:

$$g^{(1)}(\tau) = \int_0^\infty F(\Gamma)e^{-\Gamma\tau}e^{i\mu E \cos \phi}d\Gamma,$$

where $\Gamma$ is the diffuse spectrum broadening, $F(\Gamma)$ is the function contribution from the particles of one size into the total intensity of scattering, $E$ is a value of electric field, $\mu$ is an electrophoretic mobility. The expression (1) can be used for calculation in case when the reorientation of particles and chemical reactions are not considered. In an electric field, the correlation function will be modulated by a cosine function, the frequency of which is determined by the electrophoretic mobility, and the damping rate is determined by the diffusion coefficient.

3. Experimental setup

The scheme of experimental setup for measurement of electrophoretic mobility is presented in fig.1.

**Figure 1.** Experimental setup: 1 — laser, 2 — diaphragms, 3 — power supply, 4 — sample, 5 — electrodes, 6 — total internal reflection prism, 7 — optical fiber, 8 — photomultiplier, 9 — power supply of photomultiplier, 10 — ACD converter, 11 — computer

The area of investigation is located on prism (6). Two electrodes with sizes of 2*4 mm are placed on the top surface of the prism. The distance between the electrodes is 4 mm. A sample is located on the gap between the electrodes. The volume of the sample is 10 μl.
A focused light from a semiconductor laser with wavelength $\lambda = 655$ nm is directed on the side face of the total internal reflection prism at the angles greater than the critical angle. The value of the critical angle is determined by the formula (2)

$$\theta_c = \cos^{-1}\frac{n_2}{n_3},$$

(2)

where $n_1$, $n_2$ are the refractive indices of the medium. In this case, the value of the critical angle was $\theta_c = 28.2^\circ$.

The laser radiation incidents at angles greater than the critical angle. In this case radiation is reflected from the interface and is passed to the medium $n_1$. In optically medium with the smaller refractive indices $n_2$ value of electric field is limited. The penetration depth of radiation into the medium $n_2$ is defined as the distance at which the amplitude of the electromagnetic field in the optically less dense medium decreases in “$e$” times and depends on the incidence angle and the wavelength of the incident light. It is possible to change the incidence angle to study parameters of macromolecules in different depth. Such possibility increases the sensitivity and allows us to study the interaction of the sample with the surface.

The penetrated light is scattered from the sample and thus a speckle field is formed. The intensity is detected using a photoelectric multiplier placed at an angle of 5 degrees to the reflected light beam. Then the signal is passed to the ADC converter and to the computer for further processing the data.

4. Results

The water solution of albumin with the concentration 4 % and pH of the medium is equal to 9.3 was used as an object of study.

In the work we calculated autocorrelation functions for signals registered when different electric field were applied. Experimental results on albumin solution without external electric fields and in electric field $E = 20$ V/cm is shown in fig.2. In the experiments the electric field was changed in the range from 0 to 60 V/cm.

The electrophoretic mobility in the case of a monodisperse solution can be calculated according to the relation (3)

$$\Delta t = \frac{2\pi}{\mu E K \cos \varphi / 2},$$

(3)

where $\Delta t$ is the oscillation period of the autocorrelation function, the value of which can be found from
the obtained experimental dependences, $K$ is scattering vector, $\varphi$ is the angle at which scattered radiation is recorded. Dependence of electrophoretic mobility on the amplitude of the electric field of albumin solution in pH = 9.3 is shown in fig.3.

![Figure 3. Dependence of electrophoretic mobility of albumin solution on the electric field strength](image)

The electrophoretic mobility of the particles in the solution increases. The obtained results are in good correspondence with the theoretical data.

5. Conclusions
The results of experimental studies allow us to make a number of conclusions. Firstly, light scattering in the regime of total internal reflection makes it possible to study the dynamic characteristics of biological macromolecules in solution such as the electrophoretic mobility and diffusion coefficients. Secondly, the obtained autocorrelation functions allow us to calculate the electrophoretic mobility. An important advantage of the proposed method based on the regime of total internal reflection is possibility to analyze the parameters of small amount of the sample in express regime. In the measurement small values of electric field strength are used that has critical importance for the experiments with biological macromolecules.

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