Optoelectronic method for analysis of biomolecular interaction dynamics

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Abstract. Optoelectronic method of laser correlation spectroscopy for study of intermolecular interaction in biomolecular suspension is presented. The method of laser correlation spectroscopy is integrated with orthogonal laser light scattering and ultramicroscopy technique for visual control of biomolecular interactions. The capabilities of the method for analysis of biomolecular conglomerates dynamics are considered.

1. Introduction
A number of pathological processes in human body are accompanied by molecular parameters shift and changes in biological fluids [1-2]. For instance, autoimmune deceases cause changes in work of regulatory proteins of immune system and complement system, and in some cases the complement inhibitor introduction is necessary. So, express analysis of inhibitors and their interaction with complement system is important task in medicine [3-4].

Complement system should be tested in liquid medium with blood plasma that limits use of electron, tunnel and atomic-force microscopy. One of the effective optoelectronic methods of biological fluid media investigation is laser correlation spectroscopy. Recently the laser correlation spectroscopy method became a standard technique for measurement of such static characteristics as size, charge and diffusion constant of particles in range of 0.5-100 nm [5-6]. But resources of the method in investigation of biomolecular interaction dynamics are not studied enough [7].

The aim of our work is the development of laboratory model of small-sized portable laser correlation spectrometer with one of the measuring channels based on orthogonal light scattering with visual control for analysis of biomolecular dynamics in water suspensions. The light scattering was chosen as a complementary method of investigation due to its simplicity and measurement capabilities of evaluation of biomolecular interaction characteristics, affording solution-based measurements of dynamic equilibrium properties and kinetic phenomena [5].

2. Experimental setup and samples
2.1. Experimental setup
The procedure of measurements combined two methods – light scattering analysis (which includes laser correlation spectroscopy and orthogonal light scattering techniques) and ultramicroscopy technique. The scheme of the experimental setup is presented in figure 1.
Figure 1. Experimental setup for investigation of biomolecular dynamics in solution: 1 – laser power supply; 2 – junction laser; 3 – focusing lenses; 4 – optical cell with a solution; 5 – optical fiber; 6 – photomultiplier tube; 7 – power supply; 8 – oscilloscope; 9 – microscope lens; 10 – microscope eyepiece; 11 – CCD camera; 12 – computer.

Light from the junction laser (KLM-G650-13-5) (2) with wavelength 650 nanometers, emissive power 13 mW and diameter of a ray 5 mm passed through the focusing lens (3) with focal distance 1.4 mm. The light was focused on the optical cell with water suspension of biomolecules (4) and the beam with the maximum power density passed through the transparent cuvette completely filled with anolyte solution. The scattered light was registered by photomultiplier tube (H11706-01 Hamamatsu) (6) which may be positioned in different ways for registration of light scattered on angle degree in a range from 30° to 180°. The signal from photodetector was transmitted to the oscilloscope (8) and to the computer (12) with software for correlation and statistical analysis. The optical cell with solution was located directly under a lens of a microscope (9), the angle between the laser ray and optical axis of microscope was 90°. The scattered light image was visualized via microscope (10), registered by a CCD camera (11) and also transmitted to the computer (12) for further treatment.

Additional visualization of orthogonal scattered light allowed us to analyze character of conformation changes of biomolecular conglomerates structure and their relocations within the volume under the influence of external factors in real time. Such visual control gives additional information which can be very helpful in medical applications.
2.2. Samples
In our preliminary experiments the samples of distilled water suspensions of albumin, glycine and insulin at concentrations 0.001, 0.01, 0.05, 0.1 and 1 % were measured. The solution volume in the optical cell was equal to 0.3 ml. Measurements were carried out in standard conditions (room temperature was 23°C, relative humidity was 48 %, normal atmospheric pressure).

3. Results and discussion
Experimental results on measurement of scattered light characteristics of biomolecular suspensions in dependence on concentration, acidity of solution, ionic force of the solution, conformational structure and other factors were conducted. Albumin is one of the main blood and liquor proteins, so in this work we present results received on albumin solutions.

In figure 2 the dependence of average intensity of orthogonal scattered light on concentration of albumin in distilled water solution is presented. The received data revealed that average intensity of scattered light rises with increasing concentration which was expected [8].

![Figure 2](image_url)

**Figure 2.** Dependence of orthogonal scattered light intensity on concentration of albumin in solution.

For investigation of biomolecular dynamics/kinetics in peptide solutions the influence of media acidity and ionic force on conglomerate formation was studied. The medium acidity dependence was investigated for distilled water albumin solution (at concentration 1 %). In case the hydrochloric acid concentration reached the value 2 % the size of diffusers in solution (which was clearly seen due to visualization technique) and registered intensity of scattered light significantly increased. The rise of intensity of scattered light continued with acidity increasing till the acidity concentration was 4 %. Further increasing of solution acidity had no influence on scattered light intensity and we could observe the saturation region on the graph. Addition of electrolyte (NaCl) in initial solution caused the increase in the amount of scattered particles at lower acid concentrations (0.3 %), which was in agreement with work [9]. We measured solutions with ionic force \( \mu \) from 0.0025 to 0.85 mol/L. The received data revealed that increasing of solution ionic force affects the conglomeration process and protein aggregation ability. The dynamic of conglomerates formation was observed by CCD camera which allowed us to evaluate correspondence between intensity values and aggregation processes in solution.

More detailed numerical data were received by mathematical treatment of the results of laser correlation spectroscopy method – by registration of the signal via photomultiplier tube and
oscilloscope and further correlation analysis. Correlation function of the signal scattered on solution with different refractive indexes includes information about spectral structure of the signal, which is defined by the Doppler shifts caused by Brownian motion of particles. Spectral shift depends on the articles velocity, and according to Stokes's – Einstein equation (3), is directly connected with particle sizes. Thus, the evaluation of scattered light correlation function allows us to receive data about particle sizes and interaction level.

In the experiment polydisperse systems were investigated, and the correlation functions were on the form of a descending exponential curves superposition:

\[ G(\tau) = \int_0^\infty A(\Gamma) e^{-\Gamma \tau} d\Gamma, \]

where \( \tau \) is the time, \( A(\Gamma) \) is distribution of the rate of decay.

According to cumulants method we consider equation (1) as the Taylor series expansion of \( G(\tau) \) in small \( \tau \):

\[ G(\tau) = G(0) \exp \left[ - \sum_{n=1}^{\infty} K_n \tau^n \right], \]  

where \( K_n \) are cumulants which can be expressed through the central moments.

In the solution of these equations only the first cumulants are considered, and the minimal residual method is used. Taking into consideration that \( \Gamma = Dq^2 \) (where \( D \) is the diffusion coefficient) and Stokes-Einstein's formula:

\[ D = \frac{k_B T}{6\pi \eta R}, \]  

where \( T \) is the temperature, \( \eta \) is the viscosity, we can calculate the hydrodynamic radius of studied particles – \( R \).

In figure 3 the example of the received signal of dispersion on solution of the bull serumal albumine (BSA) in distilled water is presented.

![Figure 3. Example of the registered signal of scattered light from distilled water albumin solution (angle was 90°).](image)

Taking into consideration that the solution was a monodispersed, we calculated the value of diffusion coefficient of albumine molecules \( D = 7 \times 10^{-7} \text{ cm}^2/\text{s} \). Using Stokes-Einstein's formula we also calculated the average hydrodynamic radius of a particle \( R = 5 \text{ nm} \). According to tabular data the BSA
molecules has an ellipsoid form with half radiuses of 1.7 nm and 4.2 nm, which are in good agreement with the received results.

In the case the concentration of the hydrochloric acid in solution was 3% the diffusion coefficient was \( D = 4 \times 10^{-7} \text{ cm}^2/\text{s} \), and the average hydrodynamic radius of particles increased \( R = 7 \text{ nm} \).

Received data demonstrated that introduced method of measurements of scattered light characteristics allows investigating structure, interactions and kinetics of biomolecular systems in liquid mediums.

4. Conclusion
The utility of introduced optoelectronic method of investigation, which includes both laser correlation spectroscopy and visual control, was confirmed. Subsequently our approach will be realized as a model of small-sized portable laser correlation spectrometer with one of the measuring channels providing visual control. This spectrometer can be used for diagnostics of biomolecular solutions in diagnostics of such socially significant diseases as oncology, diabetes, cardio-vascular and autoimmune diseases and for assistance in therapy decision.

References
[1] Booth C S 2012 Chemical & Biomolecular Engineering Theses, Dissertations, & Student Research. 12 147
[2] Liu R, Li M, Zhi-Ping L, Wu J, Luonan C and Kazuyuki A 2012 Scientific Reports 2 813
[3] Hanlon A D, Larkin M I and Reddick R M 2010 Biophysical J. 98 297–304
[4] Le T M, Paul J S, Al-Nashash H, Tan A, Luft A R, Sheu F S and Ong S H 2007 IEEE transactions on medical imaging 26 833
[5] Some D 2013 J. Biophys. Rev. 5 147
[6] Minton A P 2007 Biophysical J. 93 1321
[7] Some D and Kenrick S 2012 Characterization of protein-protein interactions via static and dynamic light scattering Protein interactions ed J Cai (USA: Intech) chapter 20 pp 402–426
[8] Petrova G P, Petrysevich U M and Ten D I 2002 J. Quantum electronics 32 10
[9] Petrova G P, Petrysevich U M and Evseevicheva A N 1998 J. Vestnik of the Moscow university. Series 3. Physics. Astronomy 4