Spectrometry of molecular interactions in clusters

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Abstract. Molecular interactions in clusters have always been an important problem as to theoretical and experimental studies. In this paper, we describe a combination of spectrometric techniques for experimental analysis of molecular interactions and dynamics in clusters. We study different types of biological molecules (for instance, well-known albumin molecule) and metallic nanoparticles (for instance, Fe$_3$O$_4$) while their binding in solutions with help of laser correlation and absorption spectrometric techniques. Results of spectrometry of biomolecules interacting with metals show different degrees of association between different molecules and nanoparticles. This paper offers original data on spectrometry of such processes, which is useful both for medical and bioelectronics problems.

1. Introduction

The study of nanoparticles binding is extremely important for understanding the processes occurring in various molecular and biomolecular problems [1]. At the same time, methods that allow investigation of binding processes are mostly optical. In modern laboratories, the binding of nanoparticles is investigated by dynamic light scattering (laser correlation spectrometry), Fourier spectroscopy, Raman spectroscopy [2], etc. [3-5]. The methods of electron and atomic force microscopy used for similar tasks, as well as mass spectrometry, require complex sample preparation, are expensive to use, and do not allow observing the binding processes in dynamics [6].

In this paper, we paid attention to the study of possibilities of combining laser correlation spectrometry and absorption spectrometry to study the processes of particles binding into molecular clusters [7]. The use of these two techniques allows obtaining complex information about molecular interactions, the attraction and repulsion of particles in clusters and the dynamics of cluster formation under conditions of various external influences (such as applying a magnetic field to a solution of ferromagnetic Fe$_3$O$_4$ nanoparticles) [8].

Detection of the processes of molecular aggregation by determining their size makes it possible to investigate the interaction of molecules with nanoparticles and other biomolecules in complex biological solutions, such as blood serum [9]. The use of absorption spectrometry makes it possible to determine the conformation of biomolecules and to make assumptions about the molecular binding sites functionality, especially for carrier molecules, such as human serum albumin.
2. Materials and methods

2.1. Laser Correlation Spectrometry
Laser correlation spectrometry, also known as dynamic light scattering, is used to analyze the size and shape of nanoparticles [10], as well as zeta potentials (charges, in general, characterizing electrostatic interaction) of nanoparticles in solutions [11].

The modification of the laser correlation spectrometer and the method of processing [12] the experimental data, proposed by us for the analysis of multicomponent solutions, is described in [13, 14]. Figure 1 shows the general scheme of the laser correlation spectrometry experiment. We use a semiconductor laser with 650 nm wavelength, the absorption in the given wavelength range of the investigated solutions is minimal, as evidenced by the spectra obtained.

![Figure 1. Scheme of a laser correlation spectrometer.](image)

**Figure 1.** Scheme of a laser correlation spectrometer. 1 – semiconductor laser $\lambda = 650$ nm., 2 – a collecting lens, 3 – a cell with a sample (1 ml volume), 4 – a system of diaphragms, 5 – photomultiplier tube (Hamamatsu H10723-20), 6 – analog-digital board (L-Card E14-440), 7 – computer.

2.2. Absorption spectrometry
Absorption spectrometry in general makes it possible to determine the chemical composition of the solutions studied, but this method can also be used to assess the dynamics of the formation of aggregates in nanoparticle solutions.

![Figure 2. Scheme for measuring the absorption spectra of solutions.](image)

**Figure 2.** Scheme for measuring the absorption spectra of solutions. 1 – UV-VIS radiation source (deuterium + halogen lamps), 2 – broadband light guide, 3 – aperture system, 4 – cuvette with sample (1 ml volume), 5 – spectrum analyzer (Hamamatsu C10082SAN).

The absorption spectrometry in our work is implemented with Hamamatsu C10082SAN optical spectrum analyzer and UV-VIS illuminator Hamamatsu L10290 (deuterium + halogen lamps). The range of the spectra obtained is 300–850 nm, which makes it possible to estimate the absorption parameters of the solution in a wide frequency range from near ultraviolet to near infrared radiations.
3. Results and discussion
The absorption spectra and the size distribution of nanoparticles in solutions of biomolecules and metal nanoparticles, as well as their mixtures, were obtained. Figures 3–4 show the spectrum and size distribution of the human serum albumin molecules in a solution. As size in all measurements we calculated the hydrodynamic radii. The radii of the albumin molecule, according to literature, is around 6 nm [15]. That is in accordance to our calculations. The concentration of albumin in normal saline was 40 mg/ml, which is close to physical concentration [16].

A change in the size of the aggregates and absorption spectra can be observed with a change in the pH of the test solution or its temperature [17]. As is known, at the isoelectric point of albumin pH ~ 4.2, albumin molecules change their surface charge and tend to form agglomerates [18]. The dependence of the absorption spectra and the size of the resulting agglomerates on the pH of the solution can also be investigated with the help of two presented experimental facilities.

![Figure 3. Size distribution of human serum albumin molecules in a solution.](image)

![Figure 4. Absorbance spectrum for human serum albumin solution.](image)

Figures 5–6 show the spectrum and size distribution in a solution of a ferromagnetic fluid (Fe₃O₄ nanoparticles with concentration 1 mg/ml in water). The radius of single nanoparticles according to electron microscopy was around 5 nm, as can be seen from figure 5. Aggregation of nanoparticles in ferromagnetic fluids is observed when a magnetic field is applied to a sample [19–21]. Also particles
stick together with time. Depending on the concentration of magnetic nanoparticles and the used surfactant, the size and shape of the aggregates can vary significantly [22].

![Figure 5. Size distribution of magnetic fluid nanoparticles.](image)

Aggregation is also traced by changes in the characteristic absorption spectra and size distributions. Absorption peaks location depends not only on the size, but also on the shape of the formed aggregates and can be determined by the influence of the magnetic field on the nanoparticles [23, 24].

Comparing the absorbance spectra for albumin and magnetic fluid solutions one can notice, that albumin, as a biological molecule, has high absorbance in UV spectral region. Magnetic fluids in their turn have high absorbance in near infrared region. Around 560–710 nm spectral region both studied liquids have low absorbance. That determines our choice of laser light source for laser correlation spectrometry measurements.

![Figure 6. Absorbance spectrum for magnetic fluid solution.](image)

In the next series of measurements we mixed albumin and magnetic fluid solutions to detect aggregation processes. We prepared mixture with the following concentrations: 40 mg/ml albumin and 1 mg/ml magnetite nanoparticles in normal saline. From the figure 7 one can notice that sizes of aggregates in the mixture increased in comparison with pure magnetic fluid solution. That can stands for binding of albumin molecules and magnetic nanoparticles. Albumin molecule is a typical carrier in human body, so it has high binding ability to metal particles [25], which we have detected in laser correlation spectroscopy experiments. In the mixture we can still notice a small amount of non-aggregated nanoparticles.
Figure 7. Size distribution of human serum albumin molecules aggregated with magnetic fluid nanoparticles.

Figure 8. Absorbance spectrum for human serum albumin solution mixed with magnetic fluid.

If we analyze absorbance spectra for this mixture (figure 8), we can notice lower absorbance of albumin-magnetic nanoparticles aggregates in the region of 300–350 nm. It can be explained by blocking of tryptophan absorbance in albumin molecule by magnetic fluid nanoparticles, interacting with albumin binding sites. It is known that tryptophan is a fluorescence amino acid that absorb light in UV range of spectra.

4. Conclusions

In this paper, we compared the results of absorption spectrometry and laser correlation spectrometry of albumin and magnetic nanoparticles solutions. It has been shown that the combination of these methods provides more complete information on the nature of the interaction of nanoparticles in solutions, mainly on the types of clusters formed.

The spectra and size compositions of nanoparticles in solutions of albumin molecules and ferromagnetic nanoparticles are demonstrated. When the experimental conditions change, which suggests changes in the size pattern in the studied solutions, the absorption spectra also changed in a characteristic way.

We demonstrated binding of albumin molecules and magnetic fluid nanoparticles resulted in changes of UV light absorption. Apparently it can be caused by blocking the tryptophan absorbance of albumin molecule. Thus, it can be concluded that the binding of albumin and magnetic nanoparticles
violates the functionality of the molecule, blocking its binding center, which results in a decrease in absorption.

At the same time, the spectral picture depended not only on the size, but also on the shape of the formed clusters, which makes it possible to judge the nature of the interaction of particles within the clusters. The form of clusters also can be studied by presented methods and will be discussed in future works.

5. References
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