Use of electrophoretic light scattering for investigation the parameters of macromolecules

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Abstract. We present a new method for investigation of the electrophoretic mobility and radius of macromolecules based on electrophoresis in the regime of total internal reflection. The theory of dynamic light scattering and its application for the analysis of data obtained by the technique of capillary electrophoresis in the regime of total internal reflection is considered. Experimental setup for simultaneously separation and registration of macromolecules under study was developed. The results of studies of suspensions with latex microspheres are discussed.

1. Introduction

Macromolecules including proteins, nucleic acids, enzymes and ligands mediate biomolecular interactions which could be controlled by measurements of size and electrophoretic mobility [1]. The electrophoretic mobility is related to zeta potential. The degree of the mutual interaction of particles, as well as their interaction with the liquid medium, is determined by zeta potential. Such parameter of colloid dispersions as stability depends on the value of this electrokinetic potential [2]. Colloids with high electrokineic potential are electrically stabilized, while those with low electrokinetic potential are prone to coagulation because of the electrokineic interactions between the particles [2].

Investigation of light scattering parameters for the analysis, identification, and characterization of different macromolecules are widely used in various scientific areas [1]. The advantages of light scattering, such as, minimal sample preparation, rapid and simultaneous analysis, ease of quantitation and identification, make it an attractive technique for the analysis of biological analyte [3].

A variety of methods are used to characterize electrostatic properties of macromolecules. Capillary electrophoresis was developed into a strong analytical technique for separation and calculates electrophoretic mobility. Using this technique high efficiency can be obtained with short analysis times. One of the advantages of using capillary electrophoresis is that it can be coupled to a range of different techniques of detection signal [4]. In our work the theory of dynamic light scattering and capillary electrophoresis in the regime of total internal reflection is considered. The method which based on this theory is called electrophoretic light scattering.

2. Techniques

Electrophoretic light scattering (ELS) is a technique for measuring the electrophoretic mobility of
particles in multicomponent liquid systems with a recording of correlation characteristics of the light scattered by moving particles [5]. The ELS technique is based on the physical phenomenon known as electrophoresis, i.e., the motion of colloidal particles in an electric field. As the particles migrate, it begins to separate into its constituent components due to differences in their electrophoretic mobility [6]. This mobility is converted into zeta potential to enable comparison of molecular solution under different conditions. In the case of proteins, the measurement of protein mobility allows the calculation of protein charge, which in turn is related to factors such as activity and kinetics of chemical reaction [3].

There are two basic types of the electrophoresis cell that is used in modern electrophoretic light scattering measurements: the capillary and the parallel plate electrodes [7]. In this work the capillary type cell was used. This form of cell has the advantage that the electrodes can be large in size so reducing the current density at the surface and ensures that any gas bubbles produced [7].

3. Experimental setup

Separation and studies of physical parameters of the objects by the electrophoretic light scattering technique involved the regime of total internal reflection with the aim of increasing its sensitivity and reducing the volume of the sample and capillary. In order to carry out our studies, we developed an experimental setup presented in figure 1.

![Figure 1](Image)

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

A focused light from a semiconductor laser with wavelength $\lambda = 655$ nm and the power 2.5 mW was directed on the side face of the total internal reflection prism. The diaphragm was used to narrow the laser beam. The radiation incident at the angles greater than the critical angle was fully reflected from the internal sizes of the prism and returned back. The part of radiation penetrated into the object and
scattered from the particles. Scattering light was collected by optical fiber and registered by photomultiplier which can be located in different angles. Then the signal was digitized by ADC converter and recorded by the computer. The obtained data was processed in MS Excel and calculated autocorrelation functions were analyzed. The temperature was between 18 and 20°C.

4. Samples
The suspension with latex microspheres with radii of 1 μm and 320 nm were used as a test sample. The scheme of electrodes and sample in capillary is shown in figure 2. A sample was located in the capillary cell, which was placed on the top side of prism. The capillary cell consists of the two glass plates 2 cm in size with the gap between them of 90 μm and two electrodes 2*4 mm in size.

![Figure 2(a, b). The scheme of area of investigation (a) capillary cell; (b) scattering volume: 1 — the top side of the prism, 2 — electrodes, 3 — glass plates, 4 — gap between glass plates, 5 — laser beam, 6 — anode, 7 — cathode, 8 — particles.](image)

5. Results
The electrophoretic mobility in the case of a monodisperse solution can be calculated according to the relation (1)

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

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. The electrophoretic mobility of microspheres with a radius of 1 μm and 320 nm are 1.42 \(\times\) 10\(^{-5}\) cm\(^2\)/s⋅V and 2.59 \(\times\) 10\(^{-5}\) cm\(^2\)/s⋅V.

The autocorrelation functions for the measurements of mixtures of latex microspheres with radii of 1 μm and 320 nm without external electric field and in electric field with strength \(E = 1.25\) V/cm, 2.5 V/cm and 5 V/cm are presented in figure 3.
Figure 3(a - d). Autocorrelation functions of solution of latex microspheres with a radius of 1 μm and 310 nm in electric field (a) 0 V/cm; (b) 1.25 V/cm; (c) 2.5 V/cm; (d) 5 V/cm.

To obtain the separation of molecules in the solution the power spectrum of the photocurrent was calculated. The power spectrum of the photocurrent from suspensions of latex microspheres with radii of 1 μm and 310 nm in electric field 5 V/cm are presented in figure 4.
Figure 4. The power spectrum of the photocurrent from suspensions of latex microspheres with radii of 1 μm and 310 nm in electric field 5 V/cm.

According to the obtained power spectrum of photocurrent two components responsible for different sizes of particles in the polydisperse mixture are shown. The first component in the range from 0 to 100 Hz is responsible for the microspheres of 1 μm, the second in the range from 100 to 650 Hz is responsible for 320 nm. It is possible to obtain separation in the polydisperse system using this system. By analyzing calculated autocorrelation function (figure 3) we can obtain the distribution of electrophoretic mobilities in mixture of microspheres. For this purpose special processing program based on the Tikhonov regularization method is need to be used as a basis for the algorithm for the solution of the inverse problem of light scattering.

6. Conclusions
The first experimental results confirmed possibilities of the capillary ELS technique in the regime of total internal reflection to determine parameters of macromolecules (size, zeta-potential) and separate them by electrophoretic mobility in multicomponent liquid systems. The application of total internal reflection regime with small capillary allowed us to increase the sensitivity in comparison with standard methods of determine parameters of macromolecules and their separation.

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