Characterization of particles of the dispersed system based on low-concentrated aqueous solutions of L-cysteine and silver acetate

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Abstract. The authors of this work conducting a study of the precursor of reference materials of the zeta potential of particles in dispersed systems. The precursor is based on supramolecular solution of L-cysteine and silver acetate (CSSac). Particles and aggregates of CSSac were studied by dynamic light scattering (DLS), phase analysis of scattered radiation (PALS), inductively coupled plasma mass spectrometry (ICP-MS) and high-resolution transmission electron microscopy (HR-TEM). It was established that the diameter of the potential-determining aggregates of the CSSac is in the range from 20 nm to 100 nm. These aggregates consist of nanoparticles up to 10 nm in diameter.

1. Introduction
The measurement of the zeta potential by electrophoresis is used as the express-method to control dispersed systems. In the fundamental scientific research, the zeta-potential measurement is using to monitor the dynamic characteristics of biopolymers and synthetic nanoparticles. As an analytical method for controlling the quality of products in the chemical, biotechnological and food industries, the zeta-potential allows to determine stability in time of suspensions and emulsions [1]. Currently, the laboratory of metrological support for measurements of parameters of aerosols, suspensions and powdery materials of the Russian metrological institute of technical physics and radio engineering(VNIIFTRI) together with scientists of the Tver State University (TvSU) are developing reference materials of the zeta potential of particles of dispersed systems based on supramolecular systems as precursors [2].

It is known that nanoparticles with a stable zeta potential value are registered in supramolecular systems obtained from aqueous solutions of silver salts and the amino acid L-cysteine [3,4]. Depending on the silver salt forming the supramolecular system, the values of the zeta potential and the size of the charged nanoparticles change. Thus, in systems formed by silver nitrite, a stable value of the zeta potential of plus 35 with standard deviation is equal to 3 mV is observed for particles at their size of 50 nm [5]. In turn, when initiating the growth of nanoparticles with silver nitrate or silver acetate salts, a time-stable value of the zeta potential of plus 55 mV with standard deviation is equal to 5 mV and plus 69 mV with standard deviation is equal to 5 mV, respectively, is observed [2,6,7], but the distribution of particles in the systems is polydisperse.

To date, the matter of the structure of charged particles in supramolecular systems remains open. It is assumed that they have a complex structure of interconnected silver nanoparticles and chains of the...
form \([-(\text{Ag(LCys)}-\text{Ag(LCys)})^{-}]_n\) [7]. In order to characterize the size and structure of charged particles in supramolecular precursors, samples of the supramolecular systems based on L-cysteine and silver acetate (CSSac) with a concentration of 1.5 mM were used. Measurements of CSSac particle sizes were carried out by DLS and spICP-MS. PALS method was used to measure the zeta potential of particles and the method of HR-TEM was used as a control.

2. Experimental part

The chemical purity of the reagents supplied and indicated by the company Acros (Thermo Fisher) are L-cysteine with chemical purity is 99.0% and silver acetate with 99.8% chemical purity. All solutions were prepared on bidistilled water with an electrical conductivity value of at least 10 MO\(\text{cm}^{-1}\). For this purpose, the water obtained after distillation and deionization at the Millipore plant was additionally filtered with 0.2 \(\mu\text{M}\) filters. The following technique was used to prepare the CSSac solution: solutions of L-cysteine and silver acetate in concentrations of 10 mM each were prepared separately. The required amount of water was poured into an empty vessel, and then L-cysteine with silver acetate was dissolved in water in a molar amino acid/silver ratio equal to 1 to 1.25. The resulting solution was left for a day to mature, after which it became transparent with a slightly yellowish tinge (sample CSS1). To conduct experiments using the DLS method, a CSS2 sample was prepared by diluting the CSS1 sample with water in 10 times. In the case of using the spICP-MS method, a CSS3 sample was prepared by diluting the CSS1 sample with water to a concentration of 0.25 \(\mu\text{g/L}\) by amino acid.

Measurement of intensity of light scattering in the studied samples was carried out using analyzer Zetasizer Nano ZS (Malvern) with a He-Ne laser (633 nm), power of 4 mW. All measurements were carried out at 25°C in the backscattering configuration (173°), providing the highest sensitivity of the device. Mathematical processing of the results of the obtained cross-correlation functions of the diffuse light intensity fluctuations \(g^2\) was carried out in the program Zetasizer Software, where the solution of the obtained equation of the \(g^2\) dependence on the diffusion coefficient was performed by the cumulant method. The result of the solution was the function \(z(D)\). The hydrodynamic radiuses of the scattering particles were calculated from the diffusion coefficients by the Stokes-Einstein formula (1):

\[
D = \frac{kT}{6\pi\eta R}
\]

Where \(D\) is the diffusion coefficient, \(k\) is the Boltzmann constant, \(T\) is the absolute temperature, \(\eta\) is the viscosity of the medium, \(R\) is the radius of the scattering particles. Measurements of the electrophoretic mobility of aggregates in the samples were carried out in a U-shaped capillary cuvettes. Zeta potential distributions were calculated using the Henry equation (2):

\[
U_E = \frac{z\varepsilon z\cdot\varepsilon F K a}{3\eta}
\]

where \(U_E\) – electrophoretic mobility, \(z\) – zeta potential, \(\varepsilon\) – dielectric constant, \(\eta\) – viscosity, and \(f(Ka)\) – Henry’s function, \(f(Ka) = 1.0\) for systems with hydrodynamic diameter of particles less than 200 nm. The zeta potential of the particles in the samples was measured in Malvern DTS 1070 U-shaped capillary cuvettes.

The "PlasmaQuant MS" (Analytik Jena GmbH) mass spectrometer with inductive coupled plasma was used in the work. ICP-MS is equipped with Scott spray chamber with double passage. The mass spectrometer used argon gas (chemical purity of 99.993%). The sputtering efficiency was determined using a colloidal solution of \(\beta\)-cyclodextrin-stabilized Ag/CDx/W silver nanoparticles with a nominal particle size of 12 nm. The controlled isotope was \(^{107}\text{Ag}\). To quantify the \(^{107}\text{Ag}\) intensity in time-resolved analysis mode to take spICP-MS data on reconstituted Ag/CDx/W silver nanoparticles diluted to Ag mass fractions from 500 \(\mu\text{g/L}\) to 0.25 \(\mu\text{g/L}\). Solution of diluted Ag nanoparticles was
introduced via peristaltic pump into low-flow (0.7 mL/min) concentric nebulizer and impact bead
spray chamber cooled to 2°C. The delay time was set at 3 ms with typical data collection time of 60 s
for each measurement. The data processing was carry out in accordance with the calculation
methods presented in the papers of H.E. Pace et al. and Peters R. J. B. et al. [8, 9]

The microstructure and elemental mapping analysis of the samples were analyzed using a
transmission electron microscope JEM-2100 (JEOL Ltd.), equipped with the energy dispersive X-
ray spectrometer X-MAXN OXFORD instruments, with an accelerating voltage of up to 200 kV. To
get image signals of low-energy secondary electrons were detected, providing topographical contrast,
and high-energy back-up scattered (reflected) electrons that determine the composition and phase
contrast. Sample preparation consisted of spraying of the samples on the surface of a thin conductive
layer of platinum and drying in vacuum (10^-4 Pa).

3. Results and discussion

As a result of DLS studies, it was found that according to the distribution of particle sizes by intensity
(Figure 1.1a), the CSS1 sample under study is characterized by a wide distribution with a mode of 80
nm, and the presence of micro-dimensional structures is also observed. According to the particle size
distribution by count, the sample is characterized by a monomodal distribution with a mode of 30 nm
(Fig. 1.1b). According to the data obtained, it is possible to assume that the CSSac aggregates lying in
the submicron and micron size range consist of small nanoparticles. In order to confirm the
hypothesis, a sample of CSS1 with a concentration of 1.5 mM (pH=4) was diluted 10 times to a
concentration of 0.15 mM (pH=4.5) (CSS2). It was found that as a result of dilution, the width of the
particle size distribution in intensity decreased (Fig. 1.2.a), the distribution became bimodal with
modes of 20 nm and 80 nm. The distribution of particle sizes by count registers a monomodal
distribution with a mode of 18 nm (Fig. 1.2.b). As a result of measuring the zeta potential of the
particles, it was found that the value of the zeta potential decreased from plus 69 mV with standard
deviation is equal to 5 mV to plus 40 mV with standard deviation is equal to 4 mV. Thus, it can be
assumed that nanoparticles up to 30 nm in diameter and their aggregates are charge-determining, since
in early studies it was found that a change in pH in an upward direction leads to a decrease in the zeta
potential of CSSac particles due to a decrease in the charge on the amino acid [10].

![Figure 1. Particle size distributions obtained by the DLS method: 1 - Sample CSS1 with a
collection of 1.5 mM, 2 - Sample CSS2 with a concentration of 0.15 mM; A - size distribution
depending on the scattering intensity, B - size distribution depending on the countable number
of particles.](image-url)
reliable results by measuring the zeta potential. The results of measurements of the CSS3 sample by the spICP-MS method are shown in Fig. 2.a. According to the results obtained, the size distribution of stabilized silver nanoparticles lies in the range from 6 nm to 12 nm with a mode of 8 nm. The CSS3 sample was examined by the DLS method, according to the measurement results it was found that the particle sizes are in a wide range from 4 nm to 20 nm with a mode of 7 nm (Fig. 2.b).

Figure 2. Particle size distributions in the CSS3 sample obtained by the methods: A- spICP-MS, B - DLS.

Fig. 3 shows an analysis of the convergence of the results obtained using the comparison between 5 measurements of CSS3 sample. The relative expanded measurement uncertainty is 3.8% for the DLS method and 12.7% for the spICP-MS method. As a result of the analysis, it was found that both methods have partial convergence. As a control of the results obtained, micrographs of the CSS1 sample were obtained by the HR-TEM method. Fig. 4 shows the investigated aggregate with a total size of about 200 nm, formed, in turn, from particles with an average diameter of 30 nm. The microphotograph shows that all large particles are formed by association of tightly interconnected silver nanoparticles with a diameter of up to 10 nm. The presence of silver nanoparticles confirms the method of elemental analysis from the sample surface (Fig. 4.b). Microphotograph confirms the size distribution results obtained by the DLS method for CSS1 sample (Fig. 1.1.a). Taking into account the features of the supramolecular nature of the bond of nanoparticles in the studied samples, namely, that scattering and charged CSS1 particles are interconnected by weak bonds, the size of the observed nanoscale aggregate is associated with the enlargement of smaller particles under the action of freeze drying.
Figure 4.A - Microphotograph of the CSS1 sample obtained by HR-TEM; B - Results of elemental analysis from the CSS1 sample surface.

Thus, the HR-TEM method also established that the main size of nanoparticles in the CSS1 lies in the range up to 100 nm. Nanoparticles up to 100 nanometers are aggregates of interconnected amino acid stabilized silver nanoparticles with an average size of 10 nanometers. Analyzing the obtained results of DLS, spICP-MS and HR-TEM method, we establish that the sizes of potential-determining particles in the CSSac system lie in the size range from 20 nm to 100 nm. Comparison of results of SP-ICP-MS and HR-TEM methods confirms that all CSSac aggregates consist of silver nanoparticles. The presence of small silver nanoparticles in the CSSac was confirmed in early works by UV spectroscopy [10].

4. Conclusions
Using the DLS, PALS, SP-ICP-MS, HR-TEM methods, it was found that charged particles in a supramolecular system based on aqueous solutions of L-cysteine and silver acetate are nanoparticles up to 100 nm in diameter with a zeta potential value plus 69 mV with standard deviation is equal to 5 mV. It is established that the DLS and spICP-MS methods, which were used in this work, have partial convergence due to the expanded uncertainty. Comparison of the results of HR-TEM, elemental analysis and spICP-MS confirms that the charged aggregates in the supramolecular system under study are formed by stabilized silver nanoparticles.

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