

Effect of visible light onto self-assembly in L-cysteine-silver solution

A. N. Adamyan,^{*} A. I. Ivanova, M. D. Malyshev, S. D. Khizhnyak, and P. M. Pakhomov

Tver State University,
33 ul. Zhelyabova, 170100 Tver, Russian Federation.
Fax: +7 (482) 232 1274. E-mail: annadamyani@rambler.ru

Effects of irradiation with visible light on the process of self-assembly in an aqueous L-cysteine-silver solution (CSS) and hydrogels based on were investigated using a set of physico-chemical methods. It was found that the exposure to light of CSS and hydrogels based on L-cysteine and silver acetate colors them firstly into yellow and subsequently to brown, which is due to the plasmon resonance of free electrons at the surface of resulting silver nanoparticles (AgNPs). A mechanism involving participation of AgNPs was proposed for the self-assembly in CSS and hydrogel.

Key words: L-cysteine, silver acetate, aqueous L-cysteine-silver solution, visible light illumination, self-assembly processes, hydrogel, silver nanoparticles.

Currently, synthesis and investigations of properties of the hydrogels based on sulfur-containing amino acids and their derivatives are gaining especial attention of the researchers. Gels based on the molecules of L-cysteine, N-acetyl-L-cysteine, and glutathione are of great importance, since these sulfur-containing compounds play a special role in biochemical processes, thereby ensuring the vital activity of the human body.¹ The idea to design gel compositions based on these compounds is a direct consequence of their bioactive properties that can be employed for medical purposes, such as drug delivery, bioengineering, and the development of biosensors. One among the approaches to obtain hydrogels based on L-cysteine, N-acetyl-L-cysteine, and glutathione is the introduction of various salts of noble metals (Ag, Au, and Pt) into the system. The gel formation is possible due to the presence of an active thiol group in these organic compounds. Such gels belong to a separate class of metal-supramolecular gels.²

Silver is known as possessing highly pronounced bactericidal properties.³ In medicine, it can be administered as either a colloidal solution of nano- and microparticles of metallic silver^{4,5} or silver water containing silver ions.^{6,7} The obtaining and application of metal nanoparticles is of great importance in catalysis, electronics, and especially medicine and cosmetology.^{8–10} Silver nanoparticles (AgNPs) exhibit a wide range of antibacterial, antifungal, antiviral, anti-inflammatory, and antitumor activities, which have been demonstrated for human cells, animals, higher plants, algae, and bacteria.^{10,11} Hydrogels containing silver ions or its nanoparticles have also exhibited a high bactericidal activity.^{12,13}

We have for the first time synthesized supramolecular hydrogels (SMHG) based on low-concentration (~0.01%)

aqueous solutions of L-cysteine and silver nitrate (cysteine-silver solution, CSS).¹⁴ It has been found that CSS and SMHG exhibit the antimicrobial activity due to the presence of silver ions in their compositions.^{15,16} In addition to silver nitrate, its acetate¹⁷ and nitrite¹⁸ can be used to generate SMHG *via* self-assembly processes. The gel formation process proceeds in two steps in systems containing silver acetate or nitrate. The first step proceeds within 6–12 and is the maturation of CSS. It is monitored photometrically as the appearance and growth of the intensity of absorption bands at maxima of ~310 and 390 nm.^{15,16} The second step is the hydrogel formation and includes the introduction of a gelation initiator (electrolyte) into the CSS, whereas such initiators usually are sulfates or chlorides of various metals. Depending on the amount of the initiator introduced, the gel formation proceeds almost instantly or after some time, being accompanied by an increase in the viscosity and strength of the sample.^{15,16} Thus, molecules of silver mercaptide are obtained upon mixing aqueous solutions of L-cysteine and a silver salt, and they subsequently form supramolecular cluster chains during the maturation of CSS. The addition of an electrolyte to the CSS leads to the formation of a spatial network of the hydrogel.^{15,16}

In the case of aqueous solutions of L-cysteine and silver nitrite, hydrogels are prepared in one step without the addition of an electrolyte. In this case, both supramolecular chains of silver mercaptide molecules and AgNPs linked to these supramolecular chains are formed. The resulting SMHGs (of Ag nanoparticles) prepared from L-cysteine and silver nitrite exhibit a high cytotoxic activity against breast tumor cells (MCF-7).¹⁸

It has also been established that CSS is well compatible with polyvinyl alcohol (PVA). Experiments on the

self-assembly in aqueous solutions based on CSS and PVA have revealed^{19–21} that highly elastic porous films are formed upon their drying and can later be used as anti-septic band-aids. Tuning the viscosity of hydrogels is of importance for their end use, including both internal use and administration onto a surface of skin or mucous membrane.

Since CSS and especially SMHG based on it possess highly pronounced antibacterial properties and cytostatic activity, there are many opportunities for using nanostructured SMHGs in biomedicine as bactericidal preparations of various forms (solutions, sprays, gels, ointments, patches, *etc.*) and antitumor agents. Applications of CSS and hydrogels to impregnate a textile used for the fabrication of face masks may be of particular interest due to the spread of coronavirus infection.

It should be noted that many factors affect the self-assembly in CSS, *e.g.*, the ratio of concentrations of the initial components, pH of the medium, temperature, irradiation, *etc.*^{15,22,23} We have previously observed²³ the formation of AgNPs in CSS and hydrogels based on it upon their exposure to an electron beam during recording the micrographs by transmission electron microscopy (TEM).

Time-stable hydrogels possessing a high antibacterial activity were synthesized from CSS containing AgNPs.²⁴ Evaluations of the effect of pH on the synthesis of AgNPs in CSS have revealed that the size and stability of those NPs depend on the acidity of medium.²² At pH = 8.2, a monomodal distribution was achieved for AgNPs with the average size of 40 nm.

The present work was aimed at the estimation of effects caused by visible light onto processes of self-assembly and gel formation in CSS and hydrogels based on L-cysteine and silver acetate.

Results and Discussion

We noted that CSS and hydrogels based on it firstly become yellowish upon their exposure to visible light and subsequently, a brown color appears. At the same time, CSS and hydrogels based on L-cysteine and silver salts (silver nitrate, nitrite, and acetate) can preserve their properties for a long time (2 years or more) without any changes in color upon their storage in the dark. Figure 1 shows the effect of visible light on the color of CSS samples. A change in color of the solution from pale yellow to dark brown was observed upon increasing the duration of illumination, which may indicate the formation of metal NPs. The color of gels prepared from this CSS also changed in a similar manner upon their exposure to the light.

An analysis of the electronic absorption spectra of CSS and gels revealed that significant changes occur in the samples upon their exposure to the light (Fig. 2). The spectrum of CSS before its irradiation contains two absorp-

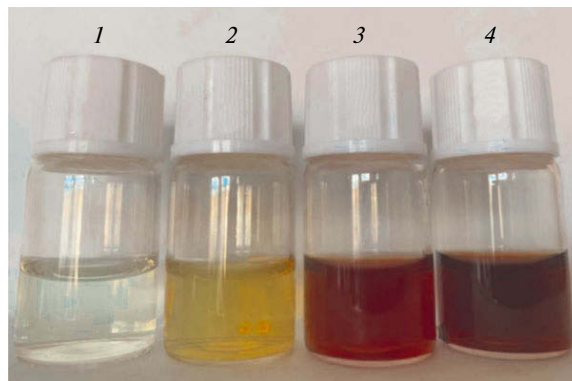


Fig. 1. Photo image of CSS samples before (1) and after exposure to daylight for one (2), two (3), and three (4) days.

tion bands with maxima at 314 and 394 nm (see Fig. 2, *a*, spectrum 1), which are characteristic of supramolecular chains of silver mercaptide molecules ($-\text{Ag}-\text{S}(\text{R})-\text{Ag}-\text{S}(\text{R})-$)_n that are formed due to non-covalent interac-

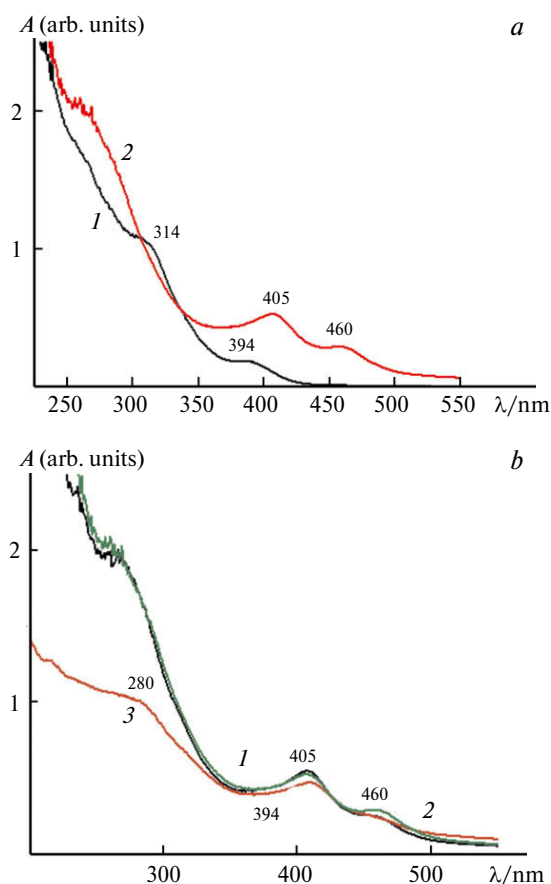
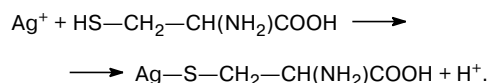


Fig. 2. (a) Electronic absorption spectra of the CSS before (1) and after three days of illumination (2); (b) electronic absorption spectra of samples of CSS (1) and hydrogels with NaCl (2) and Na₂SO₄ (3) after their exposure to visible light for 3 days. Concentrations (mmol L⁻¹) are 3.75 (AgOAc), 3.0 (L-cysteine), 0.95 (NaCl), and 0.95 (Na₂SO₄).

tion of molecules (R is the moiety of L-cysteine molecule).^{11,12} It has to be noted that the reaction of silver salts with a mercapto group is quite well known, which can be represented in the case of L-cysteine as the following reaction:^{15,16}



The spectrum of CSS exposed to the light for three days contains new bands with maxima at 280, 405, and 460 nm (see Fig. 2, *a*, spectrum 2). The absorption bands at 405 and 460 nm arose due to the plasmon resonance of free electrons at the surface of resulting silver NPs of various sizes.^{4,5,25}

The band observed at 280 nm is most likely characteristic of degraded supramolecular chains of silver mercaptide. It should be noted that longer illumination of the solution makes it cloudy with subsequent formation of a precipitate. The synthesis of nanoparticles of Ag and other metals upon the influence of various factors was considered in detail in a review.²⁶

The addition of an initiator salt (sodium chloride or sulfate) to the irradiated CSS launched the gel formation process. Silver nanoparticles formed in the CSS were incorporated into the spatial gel network in such a way that this did not lead to any change in the electronic absorption spectrum (see Fig. 2, *b*). Exposure of the hydrogel to visible light also caused a change in the color of samples to dark brown, while the illumination longer than three days resulted in the beginning of hydrogel destruction accompanied with some precipitation. The degradation of gel was especially fast in the case of using NaCl.

Sizes of clusters and AgNPs in CSSs matured in the dark and in daylight were estimated using dynamic light scattering (DLS) (Fig. 3). In the size distribution of scat-

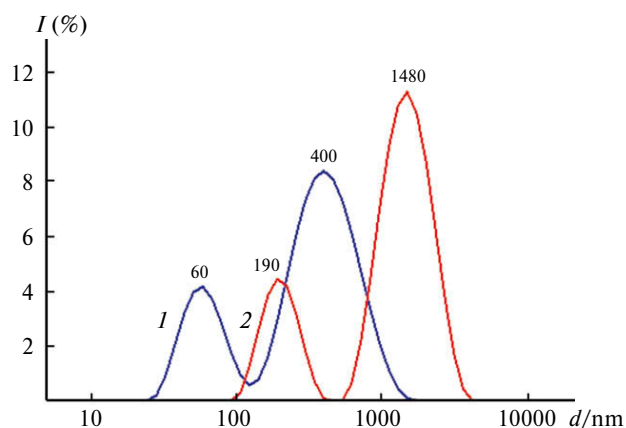


Fig. 3. Size distribution of scattering particles in CSS obtained by the DLS method after three days of either storage in the dark (1) or exposure to the light (2). The polydispersity index for distribution 1 was 0.566, and that for 2 was 0.639.

tering particles in the dark-matured CSS (spectrum 1), two types of particles can be distinguished by their average sizes of 60 and 400 nm. Scattering particles in this case are most likely clusters of supramolecular chains of silver mercaptide. In the case of CSS exposed to the light (spectrum 2), two types of particles with their average sizes of 190 and 1480 nm were formed. As was noted above, the light-induced destruction of supramolecular chains results in the transition of ionic form of silver into its metallic form to give Ag NPs, whereas small particles are AgNPs coated with molecules of silver mercaptide and with pieces of destroyed supramolecular chains, while large particles are agglomerates of those small particles linked to each other *via* hydrogen bonds. These bonds are formed by the amino and carboxyl groups of silver mercaptide located in the "crown" of AgNP.

The formation of large (400 and 1480 nm) scattering particles observed by the DLS method was also confirmed by micrographs recorded using scanning electron microscopy (SEM) and atomic force microscopy (AFM) (Fig. 4).

The SEM method revealed the structure differences between the CSS (see Fig. 4, *a*) and hydrogels based either on NaCl (see Fig. 4, *b*) or Na₂SO₄ (see Fig. 4, *c*). It turned out that, fragments of the spatial network were retained in the sample of Na₂SO₄-based hydrogel even after drying *in vacuo*, while there was no such a spatial network in the samples of NaCl-based hydrogel, regardless that AgNPs in the both cases adhered to each other, forming microparticles. This absence of a spatial network was probably due to the destruction of supramolecular chains via the formation of complexes with the chloride ion.¹¹

Analysis of micrographs of CSS (see Fig. 4, *a*) and hydrogel (see Fig. 4, *b* and *c*) samples, which were exposed to daylight for 3 days, indicates the presence of micron-sized (1–2 μm) scattering particles consisting of smaller spherical particles.

Elemental compositions of the samples were determined by X-ray spectral electron probe analysis (Fig. 5), which clearly indicated the quantitative differences between various scanned areas of the micrograph (see Fig. 4 and Table 1). Scanned area 1 captures microparticles with AgNPs in CSS (see Fig. 4, *a*) and shows the highest silver content as compared to scanned area 4, wherein such particles are absent. In the case of NaCl-based hydrogel, the scan of zone that does not contain AgNPs (see Fig. 4, *b*) demonstrates the lowest content of silver (scan 5), while scans 6–8 for microparticles with silver NPs show the highest content of this metal.

Elemental analysis of the Na₂SO₄-based hydrogel (see Fig. 4, *c*) exhibited similar results. Thus, scans 9–11 capturing the region of AgNPs show the silver content that is *ca.* 4–5 times higher than that for scan 12, wherein these particles are absent. Moreover, the analysis of the data listed in Table 1 revealed that in addition to silver, the microparticles also contain other atoms included in the

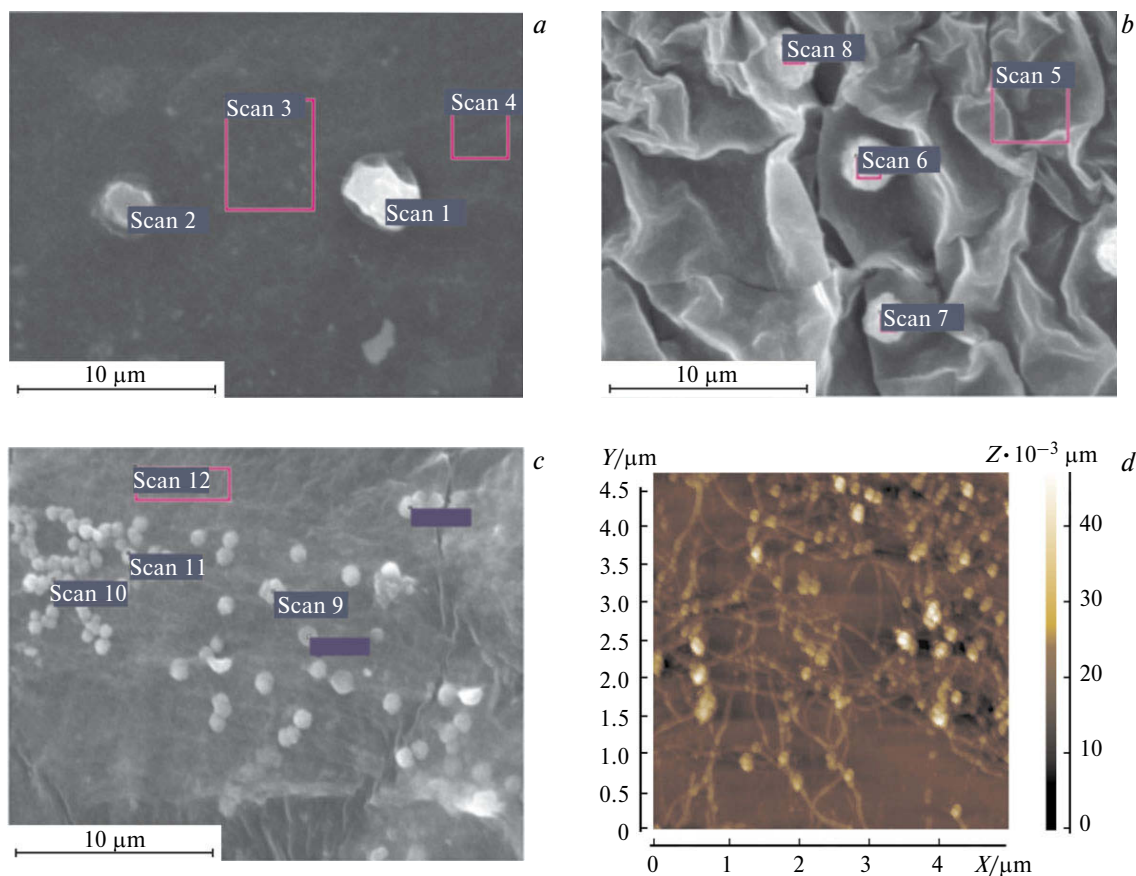


Fig. 4. Microphotographs of CSS (*a*), hydrogels with NaCl (*b*) or Na₂SO₄ (*c*), which were exposed to daylight for three days, as well as an AFM image of a hydrogel based on Na₂SO₄ (*d*). Concentrations (mmol L⁻¹) are 3.75 (AgOAc), 3.0 (L-cysteine), 0.95 (NaCl), and 0.95 (Na₂SO₄).

silver mercaptide molecule (see Fig. 4, *b*, scans 6 and 7). This means that small AgNPs are covered with a "crown" of mercaptide molecules.

Data acquired by TEM confirm the presence of AgNPs in the gel samples. In the image with micrograph of the Na₂SO₄-based gel (Fig. 6), one can observe supramo-

lecular chains forming a spatial network of the hydrogel and AgNPs (black particles). The electron diffraction pattern of the gel shows a system of diffraction rings and reflections, which exactly indicate the presence of AgNPs.

Based on the overall data acquired, a model was proposed for the processes of self-assembly and formation of

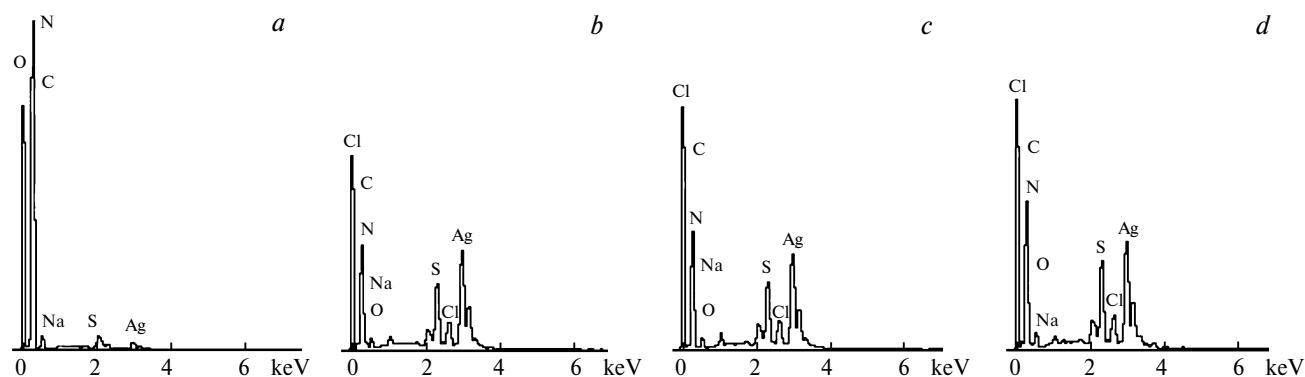


Fig. 5. Elemental composition of hydrogel based on CSS with NaCl after the exposure to daylight for three days, measured for different scanned areas. The full scale is 3806 for scans 5 (*a*), 7 (*c*), and 8 (*d*), and that for scan 6 (*b*) is 4757 pulse K keV.

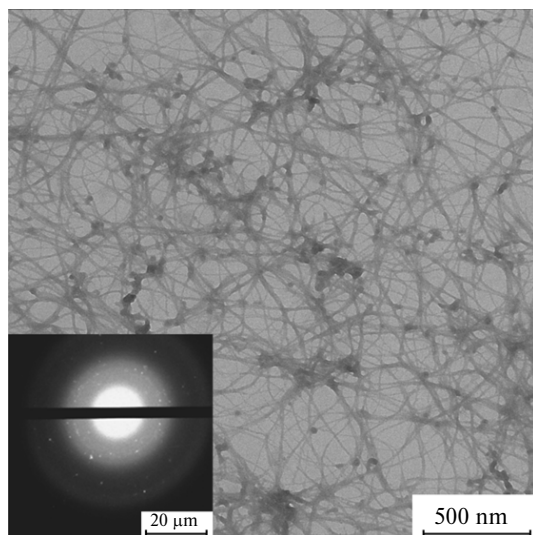


Fig. 6. TEM image and electron diffraction pattern (inset) of a hydrogel prepared from an L-cysteine-silver solution in the presence of Na_2SO_4 upon the exposure to visible light for one day (yellow color). Concentrations (mmol L^{-1}) are 3.75 (AgOAc), 3.0 (L-cysteine), 0.95 (NaCl), and 0.95 (Na_2SO_4).

microparticles from smaller AgNPs coated with a "crown" of fragments of supramolecular chains of silver mercaptide (Fig. 7). At the same time, silver atoms in the mercaptide molecule participate in the formation of AgNPs that are linked via hydrogen bonds between the amino and carboxyl groups.

In summary, this work revealed that upon exposure to visible light, the process of self-assembly in CSS proceeds with the formation of AgNPs from supramolecular mercaptide chains. At the same time, the formation of silver particles of various sizes, coated with silver mercaptide molecules, was also observed.

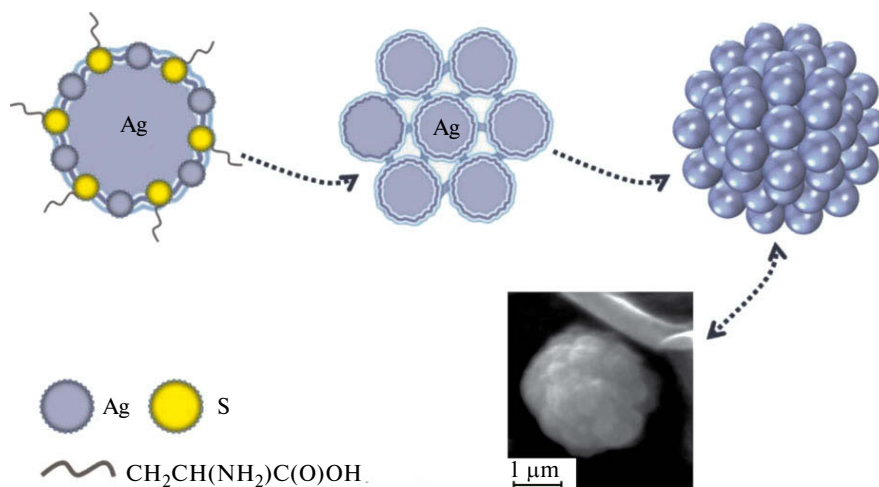


Fig. 7. Mechanism of self-assembly in CSS and hydrogel with the participation of AgNPs.

Table 1. Results of elemental analysis for CSS samples and hydrogels with NaCl or Na_2SO_4 after the exposure to daylight for three days

Scan ^a	Elements						
	C	N	O	Na	Cl	S	Ag
CSS							
1	55.87	13.62	24.80	—	—	1.43	4.27
2	61.04	12.41	22.21	—	—	1.10	2.99
3	78.32	5.35	13.50	—	—	0.54	2.28
4	83.08	2.01	13.33	—	—	0.40	1.19
CSS + NaCl							
5	80.70	4.51	10.16	0.11	0.06	0.72	3.69
6	34.59	11.77	5.33	0.93	2.94	6.52	37.91
7	38.53	6.94	6.43	1.39	3.07	6.77	36.87
8	39.29	8.16	7.46	0.76	2.98	6.88	34.47
CSS + Na_2SO_4							
9	61.02	6.52	17.52	1.11	2.77	—	10.50
10	67.39	6.77	14.23	0.71	2.37	—	7.97
11	61.54	9.83	15.21	1.29	2.80	—	8.81
12	75.50	5.29	15.91	0.48	0.80	—	2.02

^a The number of scan is shown in the Figure 4.

Experimental

Commercially available reagents were used: silver acetate (99%, Lancaster) and L-cysteine (99%, Acros). The studied samples were aqueous solutions of CSS and hydrogels with Na_2SO_4 and NaCl (of analytically pure grade, Russia).

Preparation of hydrogels. Hydrogel samples were prepared from aqueous solutions of L-cysteine and silver acetate at the concentration of 0.01 mol L^{-1} . Solution of L-cysteine (0.6 mL) was added to H_2O (0.65 mL), the mixture was stirred, and solution of silver acetate (0.75 mL) was added. Thus, a pale yellow opalescent solution (2 mL) was obtained, which gave transparent

CSS upon its storage in the dark for 6–12 h at room temperature. An aqueous electrolyte solution (Na_2SO_4 or NaCl ; 0.05 mL, 0.02 mol L^{-1}) was added to CSS (1 mL). Samples of CSS hydrogels were placed in a room protected from direct sunlight, where they were exposed to visible light (daylight) for 1–3 days.

Electronic absorption spectra were recorded on an Evolution Array UV spectrophotometer (Thermo Scientific, USA) in a quartz cuvette with a layer thickness of 1 mm.

Elemental compositions of the samples were determined by X-ray spectral electron probe analysis using an Oxford Instruments INCA Energy 350 energy-dispersive spectrometer (Great Britain) operating in a semi-contact scanning method and used as the analytical attachment to a JEOL JSM-6610LV scanning electron microscope (SEM) (Japan) and a SOLVER NEX atomic force microscope (AFM) (NT-MDT, Russia).

Structural and morphological characteristics of the samples were analyzed at the Transmission Electron Microscopy Center for Collective Use of the M. V. Lomonosov Moscow State University. The experiments were carried out using a Leo 912 AB OMEGA electron microscope (Carl Zeiss, Germany), which allowed simultaneous obtaining electron diffraction patterns of the samples. The L-cysteine-silver solution and hydrogels were applied as a drop onto a standard copper grid with a polyvinyl-formaldehyde polymer sample carrier (thickness of about 100 nm) and dried *in vacuo*, and then imaging was performed.

Light scattering intensity was measured by the DLS method for the studied solutions, using a Zetasizer Nano ZS laser particle analyzer (Malvern, Great Britain). The light source used was a He-Ne laser operating at a wavelength of 633 nm, and its power source was 4 mW. All the measurements were performed automatically at 25 °C in the backscattering configuration (173°), which provides the highest sensitivity of the instrument. Samples for the experiment were diluted 4 times. The distribution of particle sizes was calculated according to the Stokes–Einstein equation:

$$D = kT/(6\pi\eta R),$$

wherein D is the diffusion coefficient, k_B is the Boltzmann constant, T is the absolute temperature, η is the viscosity of medium, and R is the radius of scattering particles.

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