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PREPARATION OF BIOLOGICALLY ACTIVE COMPOSITIONS FROM AQUEOUS SOLUTIONS OF L-CYSTEINE, SILVER SALTS AND POLYVINYL ALCOHOL

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UV spectroscopy, viscosimetry, pH measurements, and scanning electron microscopy were used to study the behavior and morphology of hydrogels obtained from L•cysteine, silver nitrate, and polyvinyl alcohol. Good compatibility was found for aqueous solutions of cysteine and silver nitrate upon their mixing with a solution of polyvinyl alcohol (PVA). Increasing the concentration of PVA leads to an increase in viscosity indices of the hydrogels. The interaction between PVA macromolecules and supramolecules of the cysteine•silver solution occurs primarily through the formation of hydrogen bonds. The hydrogels were found to have macro• and superporous structure. Introduction of the polymer permits control of the porosity and favors encapsulation of the supramolecular structure in the polymer matrix.

Supramolecular gels derived from aqueous solutions of L•cysteine and silver salts possessing antimicrobial properties were first obtained in our laboratory at Tver State University [1, 2]. We should note that a supramolecular gel is formed with a very low content (0.01%) of the dissolved compounds. This system proved interesting since the precursors by themselves are biologically active compounds: silver has pronounced antibacterial properties, while cysteine plays an important role in metabolism and has radioprotective and antioxidant properties. A study of the antibacterial properties of this hydrogel showed that combined presence of cysteine and silver ions in its structure has a synergistic effect greater than for cysteine and silver nitrate separately.

A number of chemical fibers acquire antimicrobial properties upon impregnation of aqueous solutions or gels derived from L-cysteine and silver salts [5, 6]. It was also found that other therapeutic properties may be imparted to these supramolecular hydrogels by introducing suitable pharmaceutical preparations [2]. The hydrogels can also be used in tissue engineering.

Significant disadvantages of supramolecular hydrogels are their low viscosity and poor strength. A water-soluble polymer can be introduced to raise the viscosity of the hydrogel. However, it is not yet clear whether such supramolecular hydrogels will be compatible with the polymer solution, forming, for example, mutually permeable three-dimensional networks or whether the supramolecular system will be encapsulated within the polymer matrix. These questions hold both scientific and practical interest. The formation of mutually permeable networks would permit the formation of polymer fibers with a homogeneous distribution of the supramolecular hydrogels and, thus, pharmaceutical preparations in the polymer matrix. In the latter case, polymer capsules can be prepared containing pharmaceutical supramolecular hydrogels for the targeted delivery of drugs in living organisms. There are several variants for the preparation of such capsules. A supramolecular hydrogel can be encapsulated within the polymer hydrogel or a supramolecular hydrogel can be encapsulated with a xerogel (dried gel) of the polymer, more precisely, within its pores. Finally, a supramolecular hydrogel can be encapsulated within the xerogel of the polymer.

In our examination, special interest is found in the most available polymer hydrogels and the separate group of cryogels of polyvinyl alcohol (PVA) [7]. Hydrogels and cryogels of PVA have already been proposed for use as depot forms for the targeted delivery of biologically active molecules [8]. However, due to an excessively high concentration of the polymer (≥10%), such systems have relatively low porosity upon obtaining the gel. PVA by itself combines well with amino acids, improving the delivery of compounds to the heart and skeletal musculature, i.e., minimizes the rejection of implant materials [9]. Furthermore, PVA may be used as a plasma substitute in blood transfusions [10].

Thus, on one hand, the supramolecular structure derived from cysteine and silver nitrate has a number of useful properties but poor mechanical characteristics, while the morphology of such a system relative to the possibility of

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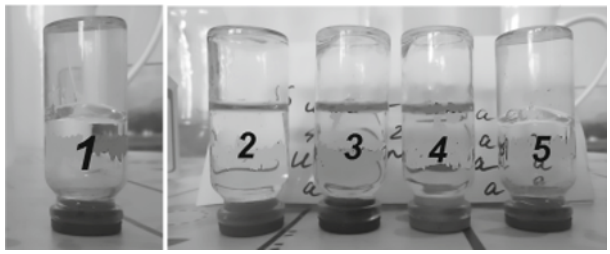


a *b*

Fig. 1. *a*) *1*) Photograph of hydrogels derived from 0.01% CSS + 2% PVA + 0.0001% Na_2SO_4, *b*) Photographs of hydrogels from 0.01% CSS + 0.0001% Na_2SO_4 (*2*, control), and 0.01% CSS + 0.02 (*3*), 1 (*4*) and 2% (*5*) PVA solution + 0.0001% Na_2SO_4.

forming porous structures has not yet been investigated. On the other hand, PVA as a macromolecular structure also has a number of useful properties but the drawback to its use lies in the low porosity of the hydrogels formed.

In the present work, we studied the self-assembly process of L-cysteine, silver nitrate, and PVA in mixed aqueous solutions and considered possible applications of these new systems. We again stress that both the supra- and macromolecular systems have biological activity, while being both nontoxic and biocompatible in organisms.

We used the following reagents: 99% L-cysteine and 99.8% silver nitrate from Acros, chemically-pure-grade sodium sulfate, and PVA with molecular mass $5 \cdot 10^4$ g/mole and less than 2% residual acetate groups (1.1% according to the label) from Azot (Nevinnomysek, Russia). All the solutions were prepared in distilled water.

The following procedure was used to prepare the cysteine-silver solution (CSS). Separate 10^{-2} M solutions of L-cysteine and silver nitrate were prepared. A given amount of water was initially introduced into a flask, followed by the L-cysteine solution and then the silver nitrate solution. The resultant opalescent solution was left for 24 h to age. A transparent yellowish solution of CSS was finally obtained. The aging of the CSS was monitored using UV spectroscopy and dynamic light scattering [2]. The hydrogels derived from the CSS mixture and an aqueous solution of PVA were obtained by adding the PVA solution to the CSS, followed by an aqueous solution of sodium sulfate electrolyte.

The UV spectra of these samples were taken on a Thermo Scientific Evolution Array spectrophotometer in a quartz cell with 1 mm path length.

The pH values of the solutions and gels were measured using a Mettler Toledo Seven Multi S70 pH-meter.

The viscosity of the samples was determined on an A&D SV-10 vibration viscosimeter, in which vibration of the sensor plates was at 30 Hz and constant amplitude ~1 mm. The viscosity of the solutions and gels was measured at 25°C.

The strength of the hydrogels was evaluated relative to a five-ball scale in accord with our previous recommendations (five balls is the top score) [2].

The morphology of the solutions and hydrogels was studied by scanning electron microscopy (SEM) using a JEOL JSM-6610 LV instrument with low accelerating voltage (5-7 keV) in a secondary electron regime at the Joint Use Center of Tver State University. The samples were placed as a thin layer onto a two-sided conducting carbon band providing for good electrical contact with the object table of the microscope. Drying was carried out in vacuum at 10^{-4} Pa.

Mixing and aged aqueous solution of CSS [Ag^+/cysteine = 1.27/1.00, 0.01 mass % in the final solution] with aqueous PVA solutions with concentrations of 0.002, 0.01, 0.02, 1, and 2 mass % (in the final solution) gives a homogeneous, optically transparent solution. The addition of 0.0001 mass % Na_2SO_4 electrolyte (in the final solution) leads after 30 min to structurization of CSS mixed with PVA and the formation of an optically transparent hydrogel (Fig. 1*a*). Since the hydrogel of cysteine and silver nitrate has thixotropic properties [2, 11], it was of interest to check if the hydrogels with added PVA would have the same properties. With daily destruction of the hydrogels during two months, all the gels showed thixotropic properties. However, their strength depended strongly on the PVA content in the gel. During this period, the system with 2% PVA showed 5 balls (upon turning over the bubble with the hydrogel, the system was in the gel state for 1 h and then destroyed). The other hydrogels (gel without PVA, 0.002, 0.01, 0.02, and 1% PVA) showed high strength (5 balls) in the first 7 days and then their strength dropped to 2 balls (Fig. 1*b*). Visual analysis showed that the PVA content in the hydrogel significantly affects its structurization. A detailed explanation of this effect will require a more complex investigation of this system. Furthermore, it is important to elucidate the nature of the interaction of PVA with the supramolecular structure of CSS.

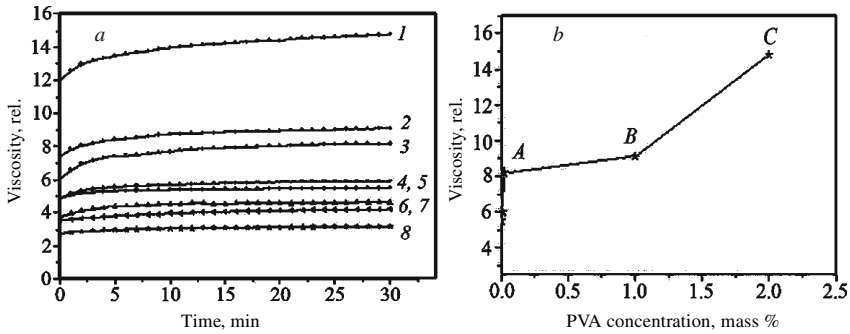


Fig. 2. *a*) Kinetic curves for the change in the viscosity of gel systems over time: *1*) CSS + 2% PVA + Na_2SO_4, *2*) CSS + 1% PVA + Na_2SO_4, *3*) CSS + 0.02% PVA + Na_2SO_4, *4*) CSS + 0.01% PVA + Na_2SO_4, *5*) CSS + 2% PVA, *6*) CSS + 0.002% PVA + Na_2SO_4, *7*) CSS + Na_2SO_4 (control), *8*) CSS, *b*) Dependence of the viscosity of the hydrogel on the PVA concentration. The viscosity was measured 24 h after preparation of the solution.

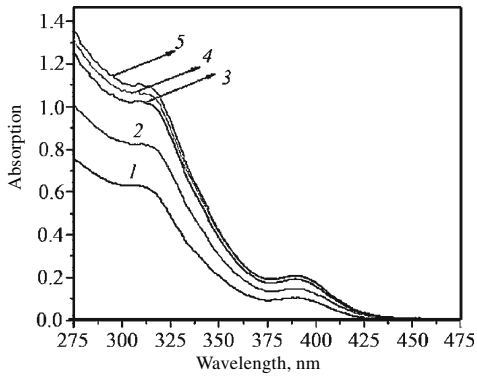


Fig. 3. UV absorption spectra of the hydrogels: *1*) CSS, *2*) 0.01% CSS + 0.0001% Na_2SO_4 (control), *3*, *4*, *5*) 0.01% CSS + PVA solution (1, 0.02, and 2%, respectively) + 0.0001% Na_2SO_4.

As expected, the rheological testing of these hydrogels showed the same features observed visually. Figure 2*a* gives kinetic curves for the change in the viscosity measurements of the gel systems over time. The curves are exponential in nature, gradually reaching saturation. Figure 2*b* shows the dependence of the hydrogel viscosity on the PVA concentration. A sharp increase in the hydrogel viscosity is seen upon the introduction of low concentrations of PVA (up to 0.02%). Further increase in the polymer concentration even 50-fold (up to 1%) relative to the CSS concentration hardly affects the viscosity in the system (segment AB), but in the case of a 100-fold increase in concentration (2% PVA), the polymer concentration has a significant effect on viscosity (segment BC). These hydrogels were mechanically destroyed and their viscosity was immediately measured. At PVA concentration comparable to the CSS concentration, the viscosity in the system is unchanged but it increases linearly with increasing polymer concentration by 50- and 100-fold. Thus, we may assume that the PVA macromolecules interact with the CSS supramolecules. What is the nature of this interaction?

The results of the UV spectral study of the hydrogels are given in Fig. 3. These spectra indicate the positions of the major absorption bands at 310 and 390 nm attributed to the CSS [2, 11] are not altered upon the addition of PVA, which indicates good compatibility of CSS and PVA. We should note that the addition of PVA enhances the intensity of the bands at 310 and 390 nm. The PVA macromolecules presumably either are spacers and connect supramolecular CSS chains or these macromolecules compete with CSS supramolecules for space, thereby extruding the supramolecules and concentrating them. Indeed, in obtaining the PVA cryogel, the formation of ice upon the freezing of water and extrusion of the PVA molecules into separate channels PVA are the driving force for rapid formation of the gel network [7], i.e., PVA is concentrated and a three-dimensional framework is formed.

Measurement of the pH of the hydrogels showed that increasing the PVA concentration to 2% leads to a shift of the pH toward alkaline values (from 2.6 without PVA to 3.1 for 2% PVA). This shift is predominantly due to the formation of hydrogen bonds between the PVA hydroxyl groups and the carboxyl group of the CSS supramolecule (Fig. 4). Furthermore, the interaction of the PVA macromolecules may also occur by dipole-dipole interactions of the polymer

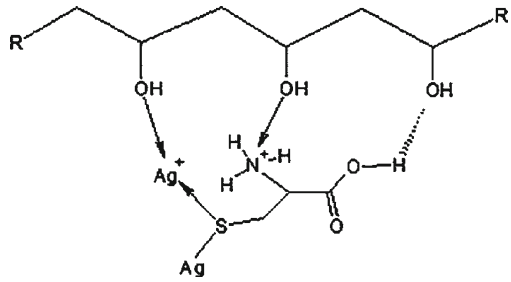


Fig. 4. Possible interactions of PVA macromolecules with CSS supramolecules.

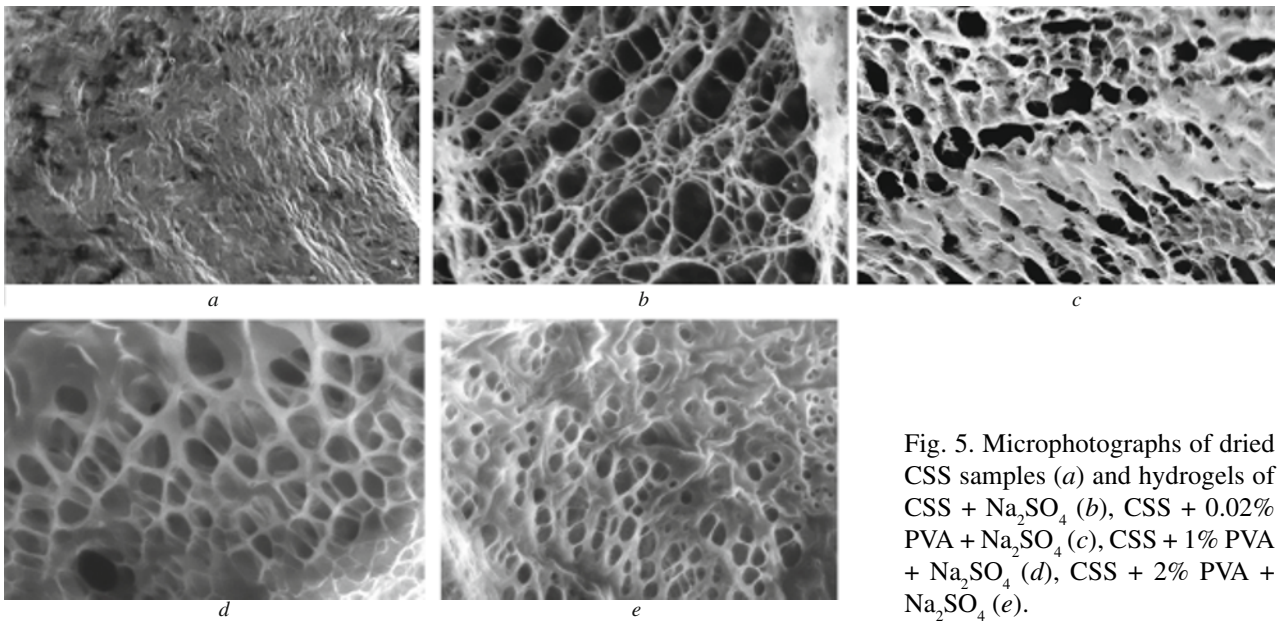


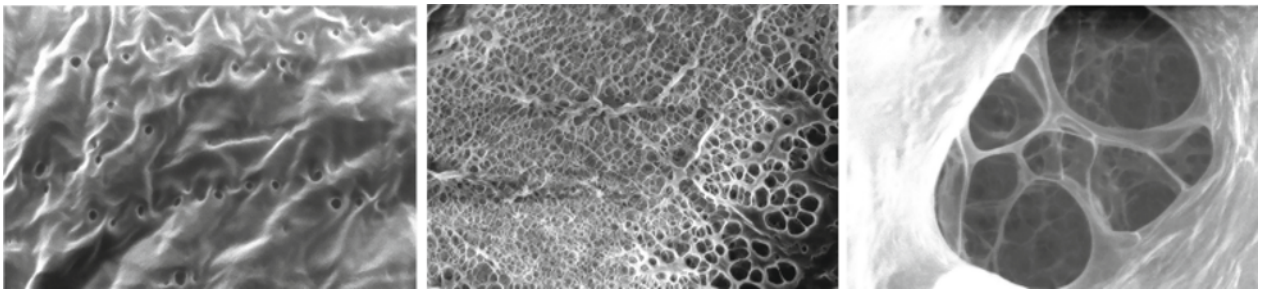
Fig. 5. Microphotographs of dried CSS samples (*a*) and hydrogels of CSS + Na_2SO_4 (*b*), CSS + 0.02% PVA + Na_2SO_4 (*c*), CSS + 1% PVA + Na_2SO_4 (*d*), CSS + 2% PVA + Na_2SO_4 (*e*).

hydroxyl group with the completely protonated positively-charged amino group of CSS or with the silver ions in the CSS structure [2, 11].

Hence, the introduction of PVA into a solution of CSS first leads to the formation of homogeneous, optically-transparent hydrogels, i. e., the polymer has good compatibility with CSS and, secondly, the introduction of PVA enhances the viscosity of the hydrogels.

Special interest is found in the study of the morphology of these hydrogels. SEM microphotographs of dried hydrogels (Fig. 5) show the presence of a spatial gel network. The gel without PVA (Fig.5*b*) shows a macroporous, quite regular structure: the mean pore diameter is ~5 μm, while the thickness of the network threads is ~7 μm. For comparison, Fig. 5*a* gives a microphotograph of the initial dried CSS, which, as expected, lacks a three-dimensional framework but has a dense chain structure previously observed for CSS by scanning electron microscopy [2, 11]. The addition of 0.02 mass % PVA into the system leads to a change in the pore shape from more spherical to more elliptical (Fig. 5*c*) along with enhanced thickness of the three-dimensional network threads to 2 μm. The mean pore diameter remains ~5 μm. The same features are found for the hydrogel with 1% PVA (Fig. 5*d*) but there is now evidence of a porous-laminar structure. Increasing the PVA to 2% (Fig. 5*e*) leads to thickening of the threads to 4 μm and a decrease in the mean pore diameter to 2 μm. The porous-laminar structure is retained.

Based on the results of complex investigations, we can assume that these xerogels are morphologically a three-dimensional network of supramolecular structure made from chains of silver mercaptide [2, 11] encapsulated within the PVA matrix, while water is found in the xerogel pores prior to drying. Indeed, increasing the polymer concentration leads to thickening of the three-dimensional network threads and decrease in pore size. In turn, an increase in the polymer concentration leads to enhanced viscosity of the solution, while the three-dimensional network of the hydrogel becomes denser. The supramolecular chains in the xerogel, as expected, are found in the PVA matrix. However,



a *b* *c*

Fig. 6. Microphotographs of a dried hydrogel of CSS + 2% PVA + Na_2SO_4 at different magnifications (superporous structure).

in solution, especially with 1-2% PVA concentration, the PVA matrix forms a network of channels, along which charged CSS particles can move.

Figure 6 shows the superporous structure of the xerogel for the system with CSS + 2% PVA + Na_2SO_4. This result proved very interesting both theoretically and practically. Indeed, when the PVA concentration is 2% ($5 \cdot 10^{-4}$ g/mole), the system approaches the features of a concentrated solution, in other words, the crossover concentration when the macromolecular clusters of PVA begin to attach to each other. It would have seemed that, in this case, we should not obtain such a highly-developed specific surface with a regular pore size distribution. Apparently, the system becomes thermodynamically stable and pore collapse does not occur upon mixing PVA with CSS due to their interaction.

Macroporous materials have found common use in technology and medicine [12, 13]. One of the obvious areas of application of our macroporous gels might lie in the encapsulation of various compounds both in the PVA matrix and in the pores of the hydrogel itself (or xerogel). A current trend is the use of such samples in tissue engineering. They are usually employed for the transfer of cells and/or growth factors, whose primary task is the support and development of healthy tissue as well as integration into surrounding tissues. In this regard, the bases selected should be biocompatible with the host tissues. Also, they should not release toxic substances or cause a discernible inflammatory response.

Furthermore, the macroporous structure of the hydrogel may be exploited for the targeted delivery of various biologically-active compounds to tissues. For example, various polyanions such as DNA and miRNA can be encapsulated by binding them to the positively-charged species of CSS, while the already formed structure will be encapsulated into the PVA matrix. In this case, the delivery of drugs to specific organs can be achieved by introducing magnetic particles into the pores of our samples.

Thus, we have created a thermodynamically-stable, compatible hydrogel system from cysteine, silver nitrate, and polyvinyl alcohol with improved rheological characteristics and enhanced porosity.

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