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ANTIMICROBIAL PREPARATIONS BASED ON *L*-CYSTEINE, SILVER ACETATE, AND PHMG-HC FOR IMPREGNATION OF CHEMICAL FIBERS AND TEXTILES

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Hydrogels prepared from L-cysteine, silver acetate, and polyhexamethyleneguanidine hydrochloride (PHMG-HC) were used in rheological, structural, and antibacterial studies. Aqueous solutions of L-cysteine and silver acetate were found to be very miscible with aqueous solutions of PHMG-HC to form transparent hydrogels. A model for the three-dimensional structure of the gel network was proposed. The hydrogel exhibited high antimicrobial activity against test cultures of pathogenic and conditionally pathogenic microorganisms.

The creation of highly effective disinfectants is a very challenging problem because of the significant increase in the number of microbial (bacterial, fungal, viral, etc.) infections. Also, various microorganisms are tending to show resistance to antibiotics and disinfectants [1].

Silver (Ag) is well-known to possess potent bactericidal properties [2]. Silver can be used in medicine as a colloidal solution of nano- and microparticles of metallic Ag [3, 4] or silver water in which Ag exists in the ionic form (Ag^+) [2]. Aqueous solutions of *L*-cysteine and Ag salts (nitrate, nitrite, and acetate), i.e., cysteine-silver solution (CSS) and supramolecular hydrogels based on CSS, exhibit potent bactericidal properties at low Ag^+ concentrations (~0.01%) [5, 6]. Gel formation in such systems usually occurs in two steps. The first step is CSS production; the second, gel formation by adding to CSS a gel-formation initiator salt. Hydrogels are formed in one step without adding an electrolyte to the system if aqueous solutions of *L*-cysteine and Ag nitrite are used [7]. Supramolecular chains of Ag mercaptide molecules form upon mixing aqueous solutions of *L*-cysteine and an Ag salt. Addition of an electrolyte, e.g., polyhexamethyleneguanidine hydrochloride (PHMG-HC), induces gel formation in CSS, i.e., formation of a three-dimensional network [5].

CSS and hydrogels based on it are interesting systems for use as bactericidal preparations (solutions, gels, ointments, sprays, etc.) and for formulating bactericidal materials (fibers and cloths impregnated with CSS). The use of CSS and hydrogels to soak cloth used as masks is especially timely because of the spread of coronavirus infections. Preliminary tests showed potent bactericidal properties of fibers and cloths impregnated with CSS and gels [8, 9].

On the other hand, PHMG-HC $(C_7H_{16}N_3Cl)_n$ is a cationic polyelectrolyte that possesses a unique combination of physicochemical and biocidal properties and enables this CSS to be used as a disinfectant. PHMG-HC is very soluble in H_2O and is widely used to prepare various bactericidal preparations with antibacterial, disinfectant, antimycotic, and antiviral properties [10-12].

The following questions seemed interesting. Are CSS and an aqueous solution of PHMG-HC compatible? Do they form hydrogels and to what extent do the gels manifest antibacterial properties? The aim of the present work was to answer these questions.

Solutions of Ag acetate (AgOAc, 99%; Lancaster); *L*-cysteine (99%, Acros); and PHMG-HC $(C_7H_{16}N_3Cl)_n$, where n = 4-50 and the molecular mass = 700-1000 amu, were used in the work. The studied samples were aqueous

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Table 1. Studied Samples and Their Concentrations

Sample No.	Studied sample	Concentration
1	$AgNO_3$	3.75 mM
2	CH_3COOAg	3.75 mM
3	*L*-cysteine	3.0 mM
4	CSS (*L*-cysteine/AgOAc)	1.0/1.25%
5	CSS + Na_2SO_4	$C(Na_2SO_4)$= 0.1 mM
6	CSS + NaCl	C(NaCL)= 0.1 mM
7	PHMG-HC	0.005%
8	CSS + PHMG-HC	$C_{PHMG-HC}$ = 0.005%

solutions of $AgNO_3$ (No. 1), AgOAc (No. 2), *L*-cysteine (No. 3), CSS (No. 4), CSS + Na_2SO_4 (No. 5), CSS + NaCl (No. 6), PHMG-HC (No. 7), and CSS + PHMG-HC (No. 8) (Table 1).

Hydrogels based on *L*-cysteine and AgOAc were prepared using aqueous solutions (0.01 M) of the starting components. CSS (2 mL) was obtained by pouring *L*-cysteine solution (0.6 mL) into H_2O (0.65 mL), stirring, and adding AgOAc solution (0.75 mL). This produced a pale-yellow opalescent solution that became transparent and yellow on standing for 6-12 h at room temperature in the dark. This was the first step of the CSS ripening stage [4]. A solution of an initiator salt, sodium chloride or sulfate, and PHMG-HC solution were added to produce a hydrogel. The initiator concentration in the samples varied in the range 0.05-0.375 M. The gel formation time and its strength depended on the type of electrolyte and its concentration and the temperature. Hydrogels with PHMG-HC were produced by preparing solutions with initial concentrations 0.1, 0.5, 1.0, 1.5, and 2.0% with the polymer volume varying from 0.01 to 0.05 mL. The final PHMG-HC concentration in the CSS varied from 0.001 to 0.1%. All solutions were prepared in distilled H_2O at 25ºC. The resulting hydrogels were observed for 15 d.

The strength of the gels was evaluated on a five-ball scale [5]. Their viscosity was measured on an SV-10 vibration viscometer (A&D Co., Japan) in which sensor plates vibrated at 30 Hz and a constant amplitude of ~1 mm. The gel strength and solution and gel viscosities were measured at 25ºC. Electronic spectra of the studied samples were recorded on an Evolution Array UV spectrophotometer (Thermo Scientific) in a 1-mm quartz cuvette. The morphology of samples was studied on a JEOL JSM-6610LV scanning electron microscope (SEM) (Japan).

The antibacterial activity of the material was determined by the agar-diffusion method in a test culture dish. *Bacillus subtilis* 6633, is a saprophytic microbe with a large bacterial cell that gives a Gram-positive stain and grows and multiplies in the presence of O_2. *Staphylococcus aureus* P209 ATCC 25923 is a conditionally pathogenic bacterium that inhabits various environmental objects and regions of the human body. The microbes colonize skin and membranes of internal organs. *E. coli* ATCC 25922 is a representative of the normal bacterial microflora of the human gastrointestinal tract. An increase or decrease in the number of *E. coli* from the norm is regarded as first degree dysbacteriosis. *Shigella sonnei III* No. 1908 is a Gram-negative, facultative anaerobic, non-motile, non-spore producing bacterium that causes dysenteric shigellosis. *Salmonella typhimurium* 5715 is a Gram-negative rod-shaped bacterium with flagella that provide it with a mode of motility. This bacterium is the main cause of food poisoning (salmonellosis) in people. *Pseudomonas aeruginosa* ATCC 27853 is a Gram-negative motile (monotrich) rod-shaped bacterium. It inhabits water and soil and is conditionally pathogenic for humans and a vector of nosocomial infections in people. *Candida albicans* ATCC 885-653 is a diploid fungus (white, a form of yeast-like fungi). It is capable of mating but not in the form of meiosis and causes opportunistic infections in humans.

The tested samples were placed using a 20-µL pipettor onto the surface of the optimal growth medium inoculated with a microorganism test culture and cultivated at 37ºC for 1 d. The antibacterial and fungicidal activity of the material was expressed in the diameter (mm) of growth inhibition zone.

The highest priority problem of the research was to answer the question about the compatibility of the CSS with the aqueous PHMG-HC solution. As it turned out, the CSS and the aqueous PHMG-HC were very miscible with each other and formed a transparent solution. It is also noteworthy that addition to the CSS of the aqueous PHMG-HC solution at a certain concentration ratio of the starting solutions could form rather strong hydrogels (Fig. 1). Previously, an electrolyte initiator was added to the CSS to start the gelation process [6]. However, gelation occurred in the CSS

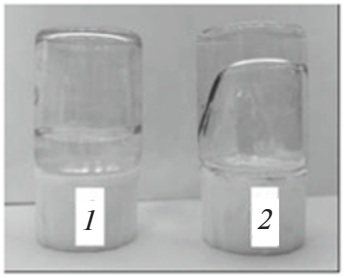


Fig. 1. Photographs of freshly prepared aqueous solutions of CSS + PHMG-HC immediately after mixing (1) and after 20 min (2).

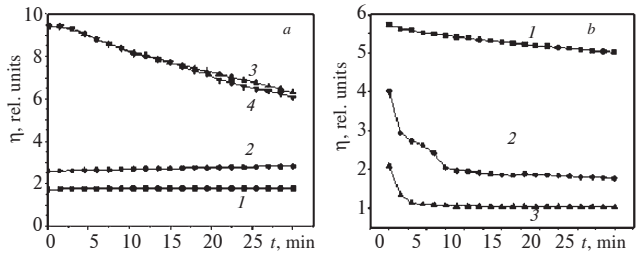


Fig. 2. Change of viscosity of aqueous solutions of CSS + PHMG-HC over time for various concentrations: initial CSS (*1*), 0.5 (*2*), 1.0 (*3*), and 1.5% PHMG-HC (*4*) 30 min (*a*) and 5 d after preparation (*b*).

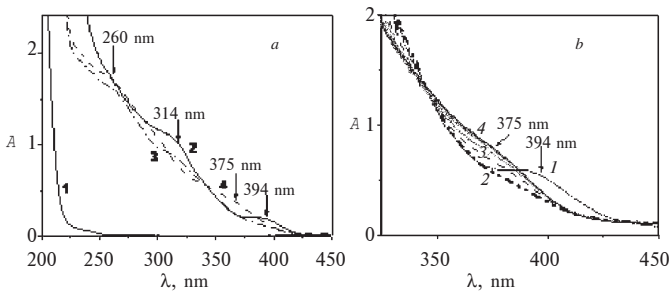


Fig. 3, Change of electronic spectra of solutions and gels: as a function of PHMG-HC concentration (samples studied 3 d after preparation): initial PHMG-HC solution (1); CSS + PHMG-HC ($C_{PHMG-HC}$ = 0.1%, $V_{PHMG-HC}$ = 0.025 mL per mL of CSS) (2); CSS + PHMG-HC ($C_{PHMG-HC}$ = 0.1%, $V_{PHMG-HC}$ = 0.0375 mL per mL of CSS) (3); CSS + PHMG-HC ($C_{PHMG-HC}$ = 0.1%, $V_{PHMG-HC}$ = 0.05 mL per mL of CSS) (4) (a); as a function of time after preparation: CSS + PHMG-HC immediately after mixing (1) and after 5 (2) and 10 min (3) ($C_{PHMG-HC}$ = 0.1%, $V_{PHMG-HC}$ = 0.05 mL per mL of CSS) (b).

solution with PHMG-HC without an electrolyte. The most persistent and strong hydrogels were obtained by adding PHMG-HC (0.5%, 0.025 mL). The final PHMG-HC concentration in the CSS was 0.0125%. In other instances, gelation was not observed because of a deficiency of the polymer at concentrations of 0.001-0.01% or gelation occurred but the hydrogels were destroyed. A precipitate formed with an excess of polymer at PHMG-HC concentrations of 0.02-0.10%.

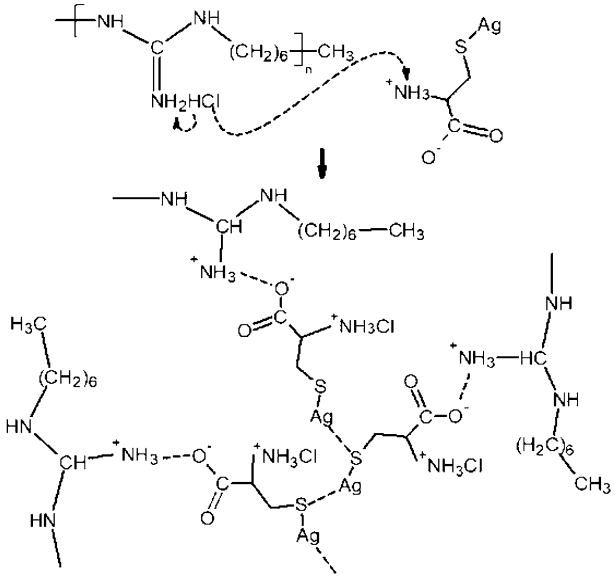
The viscosity of samples was measured 30 min after preparing the solutions. It was found that solutions with various PHMG-HC concentrations had different viscosities (Fig. 2). A hydrogel was prepared using aqueous PHMG-HC (0.025 mL) at a concentration of 0.5% (Fig. 2a, curve 2). However, even at this concentration the viscosity changed after 30 min by only 1 unit as compared with the viscosity of the starting CSS (curve 1). The viscosity increased sharply by four times (curve 3) upon doubling the PHMG-HC concentration (1.0%). The observed effect was indicative of rapid gel formation upon addition of 1.0% PHMG-HC. However, increasing the PHMG-HC concentration in the solution further to 1.5% did not give an even greater viscosity (Fig. 2a, curve 4). The viscosity measured for these samples after

5 d showed that the viscosity increased noticeably during storage of the sample with 0.5% PHMG-HC (Fig. 2b, curve 2). This indicated that a gel formed. Conversely, the viscosity decreased considerably for samples with 1.0-1.5% (curves 3 and 4) during their storage and rheological tests. This indicated that the gel was destroyed. Therefore, the optimum PHMG-HC concentration for gel formation was 0.5-1.0%.

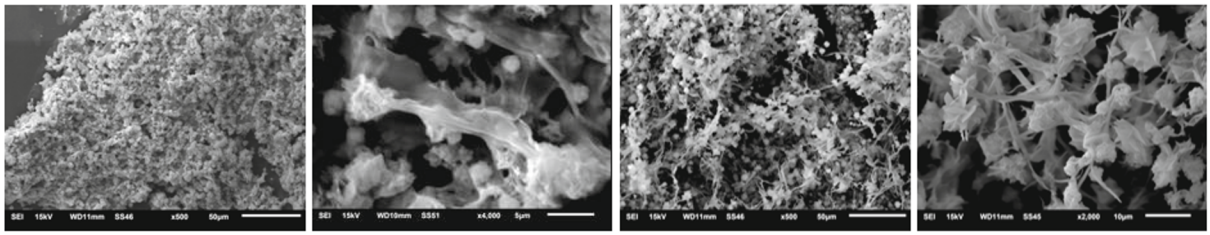
Electronic spectra of the solutions changed considerably upon adding a small amount of PHMG-HC to the CSS (Fig. 3). For example, absorption bands at 314 and 394 nm disappeared (Fig. 3a, spectra 2 and 3) upon adding 0.1% (0.0375 mL) of PHMG-HC to the CSS. New bands appeared at 260 and 375 nm (spectrum 4) upon adding 0.1% (0.05 mL). The PHMG-HC solution itself showed no absorption bands (Fig. 3a, spectrum 1). Kinetic studies of the change in the absorption bands in the CSS + PHMG-HC solution immediately after mixing showed that the absorption bands at 314 and 394 nm disappeared and new bands at 260 and 375 nm appeared already 10 min after mixing (Fig. 3b). Initiation of gelation by adding NaCl to the CSS solution gave an analogous effect [6]. Addition of PHMG-HC, like NaCl, to the CSS probably led to a considerable change in the electronic structure of the supramolecular chains of silver mercaptide molecules [1] that were responsible for the formation of the gel three-dimensional network because of the chloride ions in these solutions.

SEM images characterized the morphology of the hydrogels obtained using CSS and aqueous NaCl and PHMG-HC solutions. As it turned out, the structures of the studied hydrogels differed substantially (Fig. 4). The hydrogel with PHMG-HC had a looser structure than that obtained using NaCl. As a result, the gels with NaCl were stronger and more persistent than hydrogels with PHMG-HC.

A molecular model of the three-dimensional network structure of the CSS + PHMG-HC hydrogel was proposed based on the results.



The antibacterial properties of the hydrogels were an important problem in addition to the structural studies (Table 2). These studies found that samples Nos. 1 and 2 (aqueous solutions of Ag salts) had the greatest antimicrobial properties for all strains of bacteria and fungi. However, these solutions also exhibited high toxicity for living cells. Conversely, sample No. 3, which contained aqueous *L*-cysteine solution, did not possess antimicrobial properties because of the lack of Ag. Solutions and gels containing ionic Ag^+ (samples Nos. 4-6) possessed good antimicrobial properties. The CSS solution (No. 4) did not have antimicrobial activity against strains *S. aureus* ATCC 25923 and *S. sonnei III* No. 1908. The hydrogel based on CSS + Na_2SO_4 (No. 5) did not have antimicrobial properties only against *S. aureus* ATCC 25923. The CSS + NaCl hydrogel (No. 6) was inactive against two strains, i.e., *S. aureus* ATCC 25923 and *S. sonnei III* No. 1908. The aqueous PHMG-HC solution (No. 7) also had good antimicrobial properties except for the four strains *E. coli* ATCC 25922, *S. typhimurium* 5715, *S. sonnei III* No. 1908, and *P. aeruginosa* ATCC 27853. Finally, the CSS + PHMG-HC hydrogel had antimicrobial and antifungal properties against all bacteria strains.



a *b* *c* *d*

Fig. 4. SEM images of hydrogel based on CSS with NaCl (a, b) and based on CSS with PHMG-HC (c, d) at various magnifications ($C_{PHMG-HC}$ = 0.1%, $V_{PHMG-HC}$ = 0.05 mL per mL of CSS, C_{NaCl} = 0.5 mM).

Table 2. Antagonist Activity of Studied Samples Against Test Cultures of Pathogenic and Conditionally Pathogenic Microorganisms

Sample No.	Test culture growth inhibition zone, mm No. 1908						
	B.subtilis 6633	*S.aureus* ATCC 25923	*E.coli* ATCC 25922	*Salmonella typhimurium* 5715	*Sh.sonnei III* No. 1908	*P.aeruginosa* ATCC 27853	*C.albicans* ATCC 885-653
1	15	15	15	15	15	15	10
2	16	15	12	16	15	16	12
3	0	0	0	0	0	0	0
4	7	0	7	5	0	10	7
5	7	0	8	9	7	5	11
6	0	0	9	5	0	7	7
7	9	8	0	0	0	0	17
8	7	8	7	5	4	7	15

Thus, a hydrogel was formed in one step (without adding an initiator salt) and a synergistic effect increasing the antimicrobial properties against the studied bacteria and fungi strains was achieved by mixing aqueous CSS and PHMG-HC solutions.

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