Antimicrobial activity of silver—poly(1-vinyl-1,2,4-triazole) nanocomposites

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Novel water-soluble nanocomposites containing 1—26 nm silver nanoparticles stabilized with poly(1-vinyl-1,2,4-triazole) were prepared. They possess plasmon absorption with the maxima at 412 and 415 nm and exhibit the antimicrobial activity against Gram-negative reference and nosocomial strains, oxacillin/methicillin-susceptible staphylococci, micrococci, and streptococci, as well as fungi.

Key words: nanocomposites, silver nanoparticles, poly(1-vinyl-1,2,4-triazole), antimicrobial activity.

Nanocomposite materials containing silver nanoparticles possess unique properties and are promising for medicine, optoelectronics, and nanophotonics.[1—3] Polymeric nanocomposites containing metallic silver are efficient antimicrobial and antiviral agents.[4—11] High antibacterial activity of silver nanoparticles is caused by their developed surface providing effective contact with environment. In addition, they are sufficiently small and can penetrate through cell membranes and influence intracellular processes from the inside. In the formation of silver-containing nanocomposites, the nature and nano-stabilizing efficiency of the matrix are essential. Synthetic and natural polymers, *e.g.*, poly(vinylpyrrolidone), polyacrylamide, poly(vinyl alcohol), chitosan, arabinogalactan, are being studied intensively as the matrices.[1—3,8—14]

Poly(1-vinyl-1,2,4-triazole) is a biocompatible water-soluble non-toxic ($LD_{50} > 3000$ mg kg^{-1}) polymer having a controlled molecular weight (10^4—10^6), chemical stability, and thermal stability, as well as quaternization and complexation abilities.[15] The polymer is promising in design of modern biologically active products for medicine: materials for soft contact lenses,[16,17] biosynthetic activation of connective tissue cells,[18] sorbents for chromatographic purification of viral suspensions, latexes, and other colloidal systems, drug carriers, and blood plasma substitutes.[17] Effective flocculants for clarification and stabilization of juices and wines have been developed based on poly(1-vinyl-1,2,4-triazole)[17,19] and monomolecular layers and the Langmuir—Blodgett films have been produced from copolymers of 1-vinyl-1,2,4-triazole with styrene, methyl- and fluoroalkyl methacrylates.[20] High efficiency of poly(1-vinyl-1,2,4-triazole) as the polymeric stabilizing matrix of silver and gold nanoparticles has been shown.[21,22]

In the present work, we discuss the results of the synthesis of water-soluble nanocomposites containing silver nanoparticles stabilized with poly(1-vinyl-1,2,4-triazole), as well as the results of studies of their main physicochemical properties and antimicrobial activity.

Experimental

The nanocomposites were prepared by chemical dispersion of the silver metal particles into the polymeric matrix by reduction of silver ions (silver nitrate) with glucose or sodium borohydride in an aqueous medium in the presence of poly(1-vinyl-1,2,4-triazole). Poly(1-vinyl-1,2,4-triazole) (the molecular weight is 26000) was prepared by radical polymerization of 1-vinyl-1,2,4-triazole in the presence of azoisobutyronitrile (1%) at 60 °C in DMF.[15]

Synthesis of silver nanocomposite 1 (the reducing agent is glucose). An aqueous solution (5 mL) of $AgNO_3$ (0.17 g, 1.0 mmol) was added to poly(1-vinyl-1,2,4-triazole) (2.0 g, 21.0 mmol) in water (36 mL), the mixture was thoroughly stirred for 40 min at room temperature, and then an aqueous solution (54 mL) of glucose (0.27 g, 1.5 mmol) was added. The reaction mixture was adjusted to pH 9 by addition of a 1 *M* aqueous solution of NaOH (10 mL) and stirred for 12 h at room temperature. The composite was dialysized, precipitated with acetone, and washed with ethanol. The samples were dried *in vacuo* over $CaCl_2$ to obtain nanocomposite (1.96 g) as a dark-brown powder with the silver content of 5.0%.

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Synthesis of silver nanocomposite 2 (the reducing agent is $NaBH_4$).[21] An aqueous solution (4 mL) of $AgNO_3$ (0.17 g, 1.0 mmol) was added to poly(1-vinyl-1,2,4-triazole) (2.0 g, 21.0 mmol) in water (36 mL), the mixture was thoroughly stirred for 40 min at room temperature, $NaBH_4$ (0.04 g, 1.0 mmol) was added, and the mixture was stirred at room temperature for 12 h. The composite was dialysized, precipitated with an ethanol—acetone (1 : 2) mixture, dried *in vacuo* over $CaCl_2$ to obtain nanocomposite (1.35 g) as a dark-brown powder with the silver content of 7.8%.

Determination of antimicrobial activity. The study of antimicrobial activities of the nanocomposites obtained was performed by replications[23,24] on the reference strains, as well as on the strains of microorganisms isolated from the patients of the pyogenic septic center. The day-old strain cultures were prepared by tenfold serial dilutions with a sterile 0.9% saline and brought up to the concentration of 10^4—10^5 CFU mL^{-1}. The preparations under study (1 mL) were placed into sterile Petri dishes and the starting solvent (1 mL) was placed into the reference Petri dish. The melted and cooled beef-extract agar (BEA) and the Sabouraud medium (15 mL each) were added to the Petri dishes and stirred rapidly. When agar hardened, the dishes were dried a little to remove condensed moisture from the surface of the medium, to which the inoculi of test bacterial and fungal strains were applied in the form of a plaque using an inoculation loop. The dishes were incubated for 48 h at 32—34 °C (bacteria) and 3—5 days at 22—24 °C (fungi). Upon completion of incubation, *i.e.*, as soon as a typical growth pattern of the microorganisms under study appeared in the reference dishes without the preparation, the presence or absence of the growth of the bacteria and fungi was recorded in the media where different dilutions of the preparation were applied.

Instrumental methods. The IR spectra of the synthesized compounds were recorded on an FT-IR (RAM II) Bruker Vertex 70 spectrometer in KBr pellets. UV spectra were recorded on a Lambda 35 UV/VIS spectrophotometer (Perkin Elmer). The metal content in the nanocomposites was determined by atomic absorption analysis on an AAnalyst 200 spectrometer (Perkin Elmer). Microphotographs were obtained on a Leo 906E transmission electron microscope (Zeiss). X-ray analysis was performed on a D8 ADVANCE X-ray powder diffractometer (Cu radiation).

Results and Discussion

Reduction of silver nitrate with both sodium borohydride and glucose in an aqueous solution of poly(1-vinyl-1,2,4-triazole) was accompanied by the formation of colored sols, wherefrom samples **1** and **2** were isolated as dark-brown water-soluble powders. According to the data from elemental analysis and atomic absorption spectroscopy, the silver content in the samples was 5.0% (**1**) and 7.8% (**2**).

The electronic absorption spectra of the samples display the plasmon absorption bands with the maxima at 415 (**1**) and 412 (**2**) nm (Fig. 1), which suggests the formation of the silver particles in the nanosized zero-valent state.[1—3,25] Thus, polymeric nanocomposites are produced upon reduction of a solution of silver nitrate in the presence of poly(1-vinyl-1,2,4-triazole) followed by work-up.

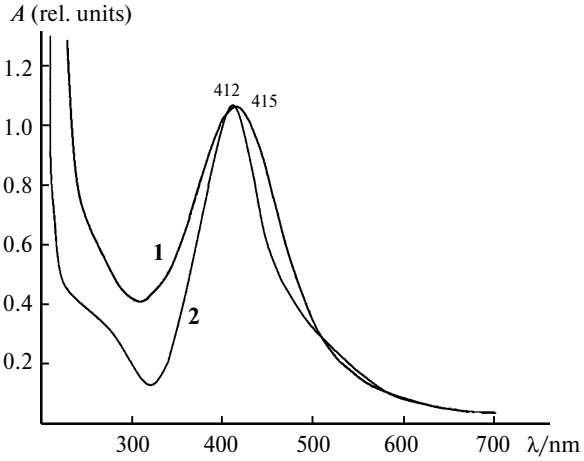
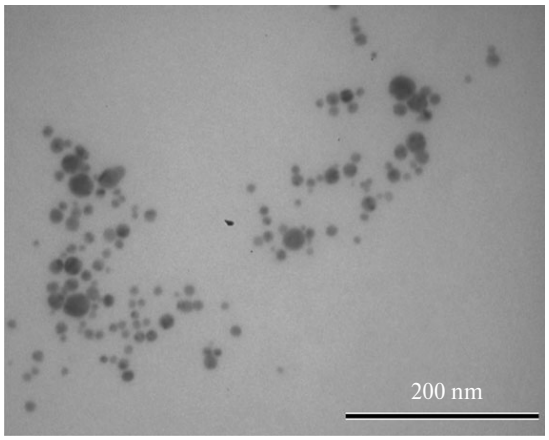
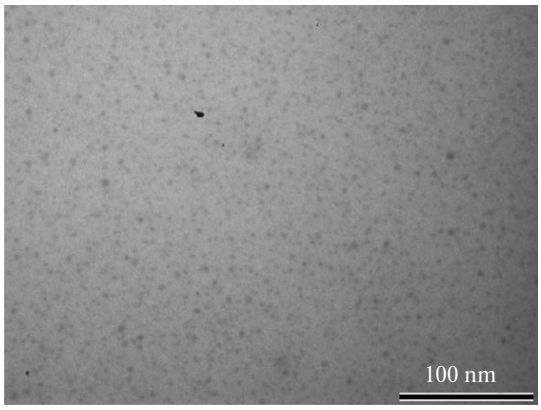


Fig. 1. Electronic absorption spectra of aqueous solutions of the silver nanocomposites **1** (5% Ag) and **2** (7.8% Ag).

The sizes and distribution pattern of the silver particles in the polymeric matrix depend on the nature of the re-



a



b

Fig. 2. Electronic microphotography of nanocomposites **1** (*a*) and **2** (*b*).

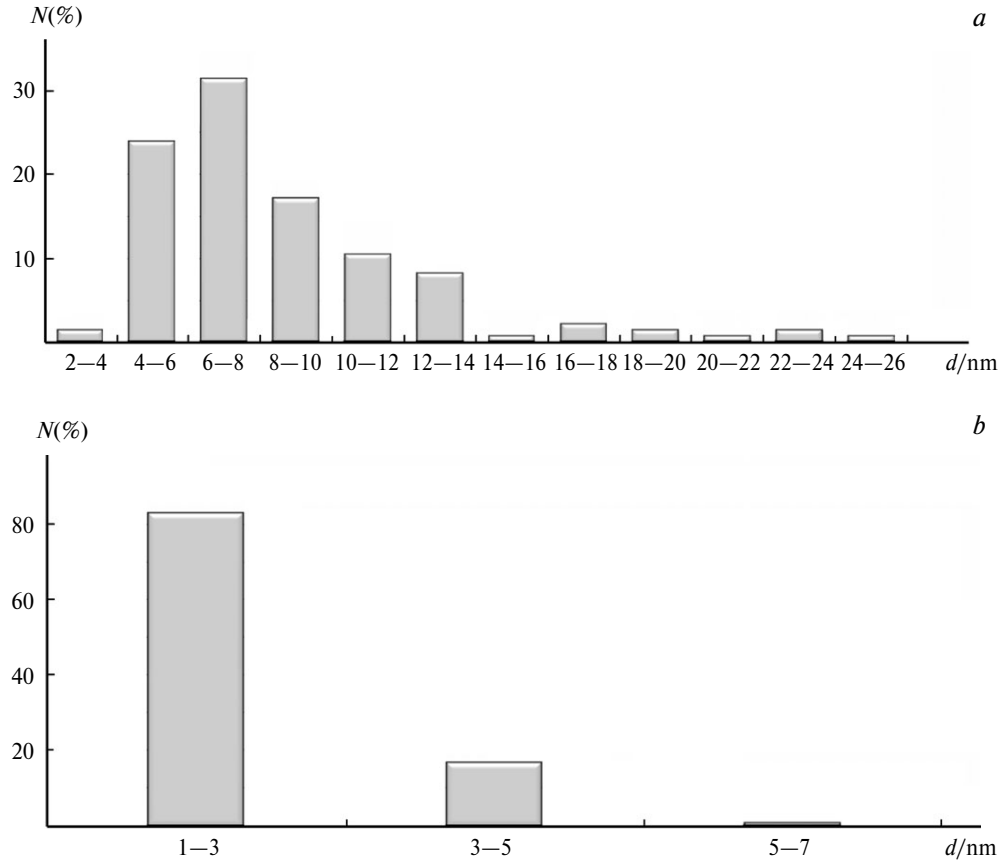


Fig. 3. Size distribution of the silver nanoparticles in nanocomposites **1** (*a*) and **2** (*b*).

ducing agent. According to the data from transmission electron microscopy, the silver nanoparticles in sample **1** (reduction with glucose) have sizes of 2—26 nm and represent moderately polydisperse crystalline formations in the poly(1-vinyl-1,2,4-triazole) matrix (Fig. 2, *a* and 3, *a*). When sodium borohydride was used for reduction of silver ions, nanocomposite **2** with the silver nanoparticles having sizes of 1—7 nm and uniformly distributed throughout the polymeric matrix (Fig. 2, *b* and 3, *b*) was obtained. The predominant amount of the silver nanoparticles have sizes of 2—10 nm (70%) in nanocomposite **1** and 1—3 nm (82%) in nanocomposite **2** (see Fig. 3).

The formation of the organic-inorganic nanocomposites **1** and **2**, *i.e.*, the presence of the amorphous polymeric phase and nanodispersed phase of metallic silver, was confirmed by the X-ray data. The diffraction patterns of the nanocomposites (Fig. 4) have an amorphous halo of an organic constituent and high-intensity lines typical of the planes of the silver(0) crystalline phase. The sizes of the metal particles assessed by the Scherrer formula[26] are 4—17 and 2—7 nm for nanocomposites **1** and **2**, respectively.

Determination of the antimicrobial activity was performed according to the guidelines[23,24] relating to various clinical isolates: Gram-positive microorganisms, such as *Staphylococcus aureus*, including oxacillin/methicillin-susceptible staphylococci, Gram-negative bacteria, such as *E.coli, Enterobacter*, and *Klebsiella*, nonfermenting bacteria, such as *Pseudomonas aeruginosa* and *Acinetobacter* isolated from the patients with the pyogenic infection, as well as fungi, such as *Candida*. A total of 39 microbial strains were studied and the obtained data on the antimicrobial activity are given in Table 1. The silver-containing nanocomposites **1** and **2** exhibit the virtually identical anti-

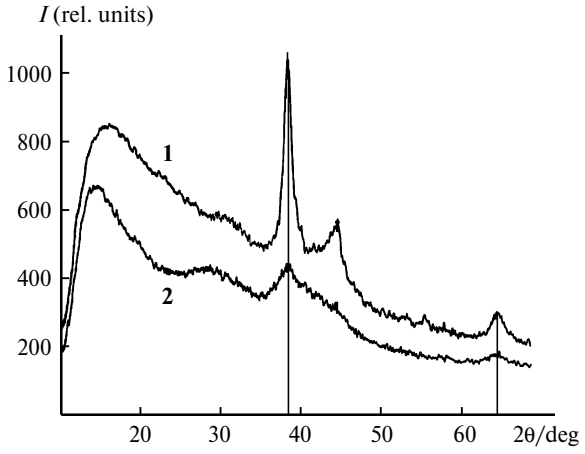


Fig. 4. Diffraction patterns of nanocomposites **1** and **2**.

Table 1. Antimicrobial activities (A) of the silver-containing nanocomposites **1** (5.0% Ag) and **2** (7.8% Ag)

Microorganisms	A/μg mL^{-1}	
	1	**2**
E.coli ATCC 25922	15	24
E.coli ESBL 1224	15	24
E.coli ESBL 2320	15	24
Shigella Zonnei	15	24
Shigella Flexsner 2a	15	24
Salmonella enteritidis	15	24
Salmonella typhimurium	15	24
Salmonella C1	15	24
Morganella morgannii	15	24
Serratia marcescens	15	24
Serratia marcescens ESBL 4601	15	24
Enterobacter cloacae	15	24
Yersinia pseudotuberculosis	15	24
Klebsiella pneumonia ESBL	15	24
Pseudomonas aeruginosa nosocomial strain	15	24
Pseudomonas aeruginosa ATCC 27853	15	24
*Acinetobacter baumanni*i	15	24
Acinetobacter baumannii nosocomial strain	15	24
Staphylococcus aureus ATCC 25923	15	24
Staphylococcus aureus MRSA 34R	94	48
Staphylococcus aureus MRSA 798	94	48
Staphylococcus aureus ATCC 29213	15	24
Staphylococcus aureus MRSA 67	94	48
Staphylococcus epidermidis MRSE	15	24
Staphylococcus epidermidis MSSE	15	24
Micrococcus luteus	15	24
Listeria monocytogenes	31	48
Enterococcus faecalis	94	146
Enterococcus faecium	94	146
Streptococcus pneumonia	15	24
Streptococcus pyogenes	15	24
Bacillus subtillis	94	48
Candida albicans	94	146
Candida glabrata	94	146
Candida tropicalis	94	146
Candida krusei	94	146
Candida krusei nosocomial strain	94	146
Cryptococcus neoformans	94	146

microbial effect against both Gram-negative and Gram-positive reference and nosocomial strains. The minimum limiting concentrations (MLC) suppressing the growth of the most of microorganisms are 15 and 24 μg mL^{-1}. According to the literature data, when the silver nanoparticles were stabilized with poly(vinylpyrrolidone), the nanocomposites exhibiting the antimicrobial activity against such microorganisms in the MLC range of 1.3—25 μg mL^{-1} (see Ref. 4) and 1.7—6.8 μg mL^{-1} (see Ref. 8) were obtained. The antimicrobial activity of nanocomposites **1** and **2** towards rods and bacteria are six-fold higher than that towards towards fungi and enterococci.

Thus, the use of water-soluble poly(1-vinyl-1,2,4-triazole) as a stabilizing matrix allowed us to obtain novel polymeric water-soluble silver-containing nanocomposites having antimicrobial activity, which are promising for medical application in the design of biocompatible antiseptic and antimicrobial materials.

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