Silver-containing nanocomposites based on galactomannan and carrageenan: synthesis, structure, and antimicrobial properties

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Water-soluble silver-containing nanocomposites based on biocompatible polysaccharides, galactomannan and carrageenan, were synthesized for the first time. A complex of physicochemical methods was employed to establish the phase composition, the average size of metal nanoparticles, and the morphology of new nanobiocomposites. The obtained nanomaterials possess high antimicrobial activity.

Key words: nanoparticles, nanocomposites, silver, polysaccharides, galactomannan, carrageenan.

Despite continuous sophistication of antibiotics, they are yet insufficiently selective (*i.e.*, have a wide range of action), toxic, and rapidly cause specific resistance in pathogenic and opportunistic microorganisms.[1] At the same time, preparations of highly dispersed silver are highly active antimicrobial agents known for many centuries.[2—5] The most important factor for the increasing biological activity of the silver preparations is the enhancement of their transmembrane permeability,[3] which makes it possible to substantially decrease the therapeutic dose and thus diminish the drug load on the organism. One of the promising methods of achieving transmembrane permeability can be the transformation of silver into the nanosized state with the simultaneous inclusion of silver nanoparticles into matrices of polysaccharides galactomannan and carrageenan, which impart water solubility and higher biocompatibility to the constructed materials. The galactomannan macromolecules contain the α-galactose residues performing membrane-transport functions due to the high affinity to asialoglycoprotein receptors of the cell membranes.[6—9] Carrageenan has another valuable property, *viz.*, the ability to form water-retaining hydrogels, which is very important for the development of materials for wound coatings.[10]

It is known that the choice of a stabilizer for thermodynamically unstable nanoparticles formed is very significant along with the choice of reactants in the syntheses of the nanocomposites.[11—17]. Compounds containing reductive and other polar groups (alcohols, aldehydes, saccharides, polysaccharides, and others), which can simultaneously act as reducing agents and stabilizers of formed nanoparticles, are widely used in practice for this purpose. For example, natural galactose-containing heteropolysaccharide, *viz.*, arabinogalactan, was successfully used[18—22] for the synthesis of related hybrid metal-containing nanocomposites.

The purpose of this work is the synthesis and study of the structure and antimicrobial properties of the hybrid nanocomposites based on biocompatible galactose-containing polysaccharides, *viz.*, galactomannan and carrageenan.

Results and Discussion

Water-soluble natural galactose-containing polysaccharides, galactomannan (2440 kDa) and carrageenan (1980 kDa), were used as stabilizing matrices for the preparation of new silver-containing nanocomposites. The basis of the galactomannan macromolecule **1** (see Ref. 23) is (1→4)-β-D-mannopyranan, and α-D-galactopyranose residues represent single side branches. The ratio Man : Gal

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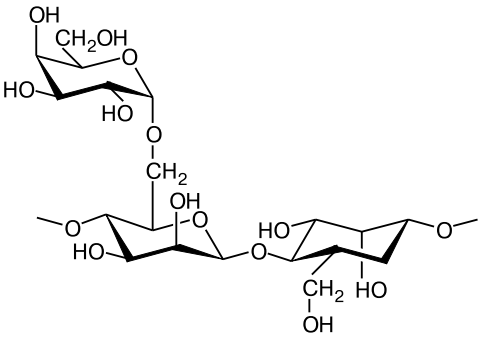
determined by the data from quantitative ^{13}C NMR spectroscopy is 1.6. Carrageenan **2** (see Ref. 24) is a natural sulfated polysaccharide, whose carbohydrate chain is built of regularly alternating residues of 3-*O*-substituted β-D-galactopyranose and 4-*O*-substituted 3,6-anhydro-α-D-galactopyranose. According to the elemental analysis data, the number of sulfate groups is 0.3 per hexose residue.



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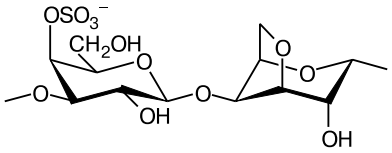

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In order to optimize the physicochemical properties (solubility, viscosity of aqueous solutions) of the starting polysaccharides, we carried out their alkaline destruction, which gave galactomannan and carrageenan samples with the molecular weights decreased to 1300 and 1100 kDa, respectively. The data from IR and ^{13}C NMR spectroscopy confirmed completely the retention of the structure and composition of the depolymerized polysaccharides. The estimation of the reducing ability of the initial and destructured polysaccharides from the number of carbonyl groups showed an insignificant change in its value.

The silver-containing nanocomposites based on galactomannan and carrageenan were synthesized using a procedure of nanoparticle formation in the matrix of the polysaccharide arabinogalactan developed by us earlier.[22] As a result of the reactions of silver nitrate with galactomannan and carrageenan in an aqueous-alkaline medium, we obtained a series of water-soluble nanocomposites, whose structures combine the above-mentioned polysaccharides and silver in the amount from 2.5 to 17.0%. It was established that rather stable nanocomposites (1—4 weeks) can be obtained using $AgNO_3$ in an amount of 0.04—0.33 and 0.04—0.30 g per 1 g of galactomannan and carrageenan, respectively.

The process of formation of the silver-containing nanocomposites of argentogalactomannan and argentocarrageenan is based on the redox reaction occurring in an aqueous-alkaline medium of silver ions with the hydroxyl groups of polysaccharides.[4,11] The simultaneous stabilization of the formed nanoparticles of reduced silver occurs, probably, due to cooperative, ionic, intermolecular, van der Waals, and other interactions between the nanoparticle surface and fragments of the polysaccharide macromolecules (hydroxyl and carbonyl groups, as well as sulfate groups in the case of carrageenan) to form the stable metallic nanophase.[11,22]

The substantial influence of the type of the polysaccharide matrix on the maximum content of silver in the stable nanocomposites was found. For instance, nanocomposites with the silver content up to 17.0% were obtained from galactomannan (branched polysaccharide). Macromolecules of linear carrageenan[24] provide the stabilization of up to 12.0% silver. When this upper limit is exceeded, the nanoparticles of reduced silver are aggregated and separated in the subsequent procedures of isolation and purification of the nanocomposites. This difference can be explained by steric factors characteristic of branched galactomannan macromolecules and involved in the mechanism of three-dimensional stabilization of metallic silver nanoparticles.[11,25]

The polysaccharides contain no chromophores absorbing in the ultraviolet and visible spectral regions, and the precursor used (silver nitrate) is characterized by a weak indistinct peak at 298—300 nm. However, the spectra of aqueous solutions of the prepared silver-containing nanocomposites have an intense absorption maximum caused by the collective excitation of silver conductivity electrons (plasmon resonance) at the wavelength 410 and 411 nm for argentogalactomannan and argentocarrageenan, respectively (Fig. 1). This effect reliably confirms that the obtained silver-containing nanocomposites contain silver in the zero-valent state.[26] The intensity of the plasmon absorption peak depends on the amount of silver in the nanocomposite composition. For example, for the galactomannan-based nanocomposite, with an increase in the silver content from 2.5 to 8.2%, the intensity of the plasmon absorption peak increases 2.3-fold (see Fig. 1, curves *1*—*4*).

The phase analysis of the nanocomposites was performed on the basis of the diffraction patterns, which show distinctly differentiated reflections of the metal component and the amorphous halo of the galactomannan and carrageenan matrices (Fig. 2). The absence of diffraction lines of silver ions confirms that silver was reduced completely. The unit cell parameters *a* equal to 4.0863(6) and 4.0882(5) Å were determined for the obtained samples of the nanocomposites with a silver content of 8.6%. The average sizes of crystallites, which are the most important characteristics of the metallic nanophase, of the formed silver particles[27] were calculated by the Debye—Scherrer formula. It was thus established that the nanocomposites based on silver and the polysaccharide matrices of galactomannan and carrageenan are formed as polysaccharide

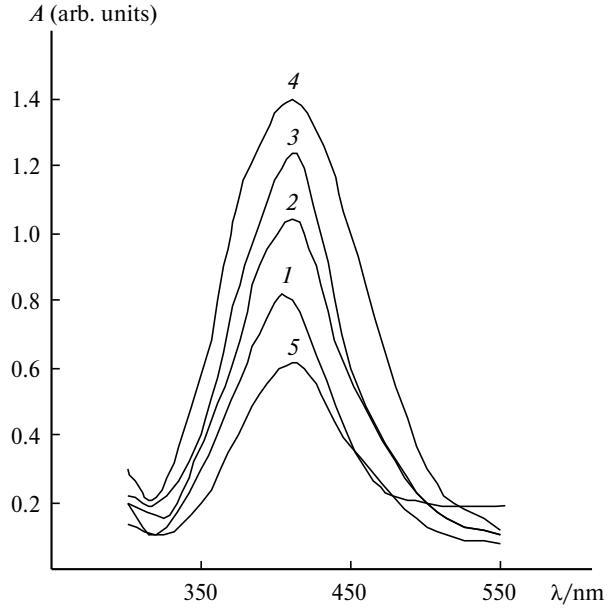

Fig. 1. Plots of the plasmon absorption intensity of 0.04% aqueous solutions of argentogalactomannan *vs* content of zero-valent silver (Ag^0): 2.5 (*1*), 5.0 (*2*), 7.1 (*3*), and 8.2% (*4*); and the absorption spectrum of argentocarrageenan containing 4.0% Ag^0 (*5*).

matrix-stabilized nanosized clusters of zero-valent silver with average sizes of 8.6 and 21.0 nm for galactomannan and carrageenan, respectively.

The difference in sizes of the silver nanoparticles can be explained by different stabilizing abilities of the polysaccharides due to the difference in the molecular weights and structures of the macromolecules and to the presence or absence of functional groups.

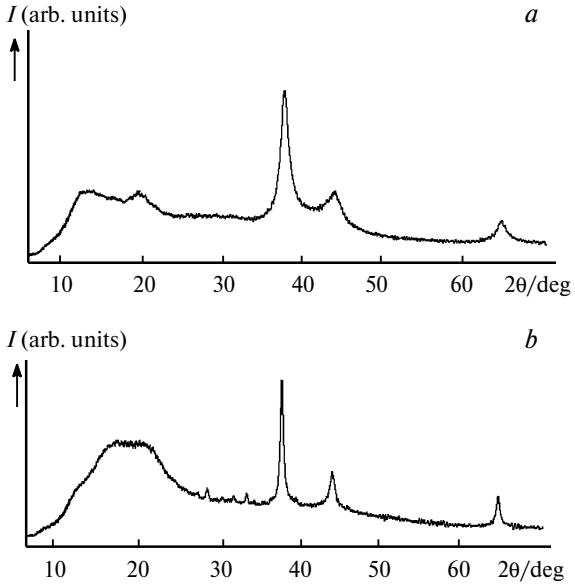

Fig. 2. Diffraction patterns of the argentogalactomannan (*a*) and argentocarrageenan (*b*) nanocomposites.

The morphology of the prepared silver-containing nanocomposites was studied by scanning electron microscopy. A substantial influence of the type of the polysaccharide matrix forming a shell around the silver nanoparticles on the morphological peculiarities of the nanocomposites was found. The galactomannan-based nanocomposites are formed as irregular particles, whose total size is 5 μm (Fig. 3, *a*). The nanocomposites based on linear polysaccharide carrageenan are organized as particles, whose size is about 8 μm (Fig. 3, *b*).

The introduction of nanodispersed silver particles into the biocompatible matrices of polysaccharides galactomannan and carrageenan assumes their potential biological activity. The antimicrobial activity of the silver-containing polysaccharide-based nanocomposites was determined on the reference strains and the strains of microorganisms isolated from patients of the purulent-septic center using the method of serial dilutions in a liquid medium.[28] The studies of argentocarrageenan containing 4.0% silver showed its high antimicrobial activity against a series of microorganisms: *Escherichia coli*, *Pseudomonas*

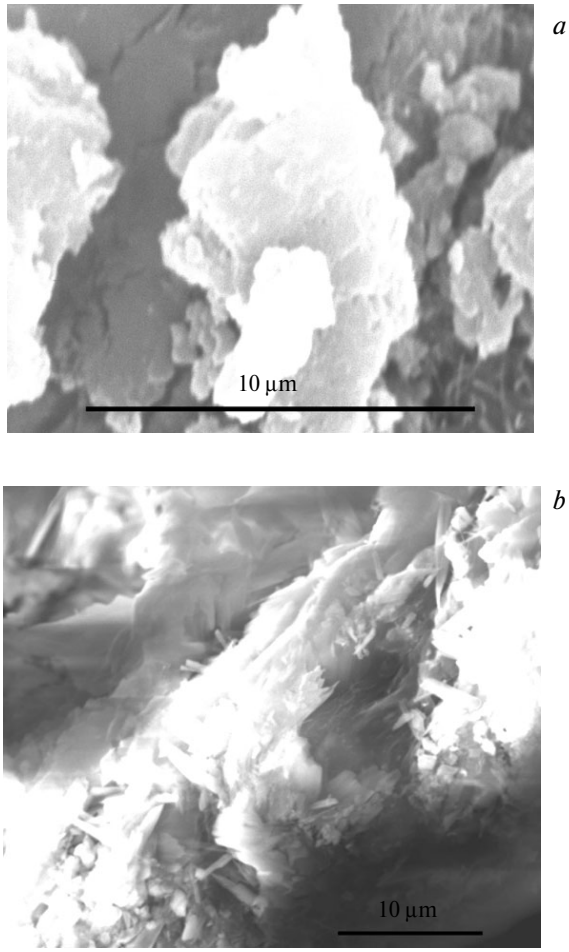

Fig. 3. Microphotographs of the argentogalactomannan (*a*) and argentocarrageenan (*b*) nanocomposites.

Table 1. Antimicrobial properties of the argento-carrageenan nanocomposite (4% Ag^0)

Microorganisms	MIC/μg mL^{-1}
E. coli ATCC 25922	10
E. coli (nosocomial strain)	15
P. aeruginosa ATCC 27853	25
S. aureus ATCC 25923	50
B. subtillis	50

aeruginosa, *Staphyllococcus aureus*, and *Bacillus subtillis*. The minimum inhibition concentrations (MIC) with respect to these microorganisms are listed in Table 1. Depending on the type and strain of the microorganism, the values of MIC range from 10 to 50 μg mL^{-1}, which is similar to the activity of the recently patented nanosilver preparations based on arabinogalactan[18,21] and polyvinylpyrrolidone.[3]

Galactomannan and carrageenan used in the work are the products designed for the use in food industry as gelling and moisture-retaining agents, their toxicities have been tested by the corresponding expert organizations; no limitations to the acceptable daily dose for oral consumption were established. The study of the toxicity of silver nanoparticles incorporated in various matrices is at its infancy; however, the new antimicrobial preparation Poviargolum[29] containing highly dispersed silver with a particle size of 1—6 nm was shown to be four to six times less toxic than earlier used Collargolum and Protargolum. Following successful clinical trials, Poviargolum actively enters the market of modern pharmacological preparations.[3,29] It is also known that the acute toxicity (LD_{50}) of the silver-containing argentoarabinogalactan nanocomposite, which is similar in composition and structure, is 11 600±4500 mg kg^{-1}, which allows one to assign this drug to the class IV of low dangerous substances and, hence, the nanocomposites obtained by us are expected to belong in their toxicity to the same class of danger.

Thus, the water-soluble nanocomposites consisting of silver nanoparticles and stabilized by the galactomannan and carrageenan polysaccharide matrices were prepared for the first time. The phase composition of argentogalactomannan and argentocarrageenan and the valence state and average size of the formed silver nanoparticles were established using X-ray phase analysis and UV spectroscopy. The morphology of the surface of the silver-containing nanocomposites was characterized, and the sizes of the formed particles were determined. The carrageenan-based nanocomposites were found to manifest the antimicrobial activity against a series of nosocomial microorganisms, which is the basis for the subsequent development of preparations of medical design from these nanocomposites.

Experimental

Galactomannan (LBG trade mark, 2300 kDa) and carrageenan (WR-78 trade mark, 1800 kDa) (CP Celko, Denmark) were used as received. The molecular weights were calculated by Dublie´s equation[30] using the values of intrinsic viscosity determined in a VPZh-type capillary viscosimeter with a capillary diameter of 0.56 mm by the Huggins equation.[31]

IR spectra were recorded in the frequency range 4000—400 cm^{-1} on a Bruker VERTEX 70 instrument in KBr pellets. ^{13}C NMR spectra were obtained on a Bruker DPX-400 instrument in D_2O at 25 °C. The chemical shifts coinciding with the literature data[23,30] are presented in the δ scale relative to Me_4Si used as an external standard. The quantitative spectra were detected with the addition of a relaxant (chromium acetylacetonate). The following abbreviations were used in the description of the NMR spectra: Gal is the galactopyranose residue, Man is the 4-*O*-substituted mannopyranose residue, Man$^+$ is the 4,6-*O*-disubstituted mannopyranose residue, anGal is 3,6-anhydrogalactopyranose, and Gal-SO_4 are the sulfated galactopyranose residues. The electronic absorption spectra of 0.04% aqueous solutions of the argentogalactomannan and argentocarrageenan nanocomposites were recorded relative to H_2O in the ultraviolet and visible spectral regions on a Perkin Elmer Lambda 35 spectrophotometer (path length 1 cm). The X-ray diffraction study was carried out on a Bruker D8 ADVANCE diffractometer equipped with a Hebbel mirror using Cu radiation in the Locked Coupled mode with an exposure of 1 s for phase analysis and 3 s for the calculation of the cell parameter and the size of the coherent scattering region (CSR) of silver. The cell parameter and the CSR size for each sample were averaged over three independent measurements. The crystalline phases were identified by comparing the obtained experimental values of interplanar distances and relative intensities with the reference values. The content of silver in the nanocomposites was determined by titrimetry with ammonium thiocyanate using Volhard´s method[32] after zero-valent silver has been transformed into the ionic form. The morphological structure of the nanocomposite samples was studied by scanning electron microscopy on a FEI Company Quanta 200 microscope.

Alkaline destruction of galactomannan and carrageenan. A 1 *M* solution of NaOH (13 mL) was slowly added to a solution of polysaccharide (1 g) in H_2O (30 mL) with vigorous stirring at 85 °C. After 1 h, the reaction mixture was neutralized with an equimolar amount of HCl and polysaccharides were precipitated with ethanol. The precipitate was filtered off, washed, and dried at room temperature under atmospheric pressure. The yields of the depolymerized polysaccharides were 65—79%. The reducing power (the number of carbonyl groups) of the initial and destructed polysaccharides was determined by the Somogyi—Nelson method,[33] and amounted to 0.6% for both carrageenan and galactomannan.

Galactomannan. IR, ν/cm^{-1}: 3434 (OH), 2925 (C—H), 1638 (H...OH), 1200—1000 (C—C, C—O), 870 and 811 (β-mannopyranose residues). ^{13}C NMR (Me_4Si), δ: 99.0 (C(1), Gal); 69.7 (C(2), Gal); 70.2 (C(3), Gal); 70.3 (C(4), Gal); 71.9 (C(5), Gal); 61.6 (C(6), Gal); 100.6 (C(1), Man); 99.9 (C(1), Man$^+$); 71.8 (C(2), Man); 71.7 (C(2), Man$^+$); 68.8 (C(3), Man); 68.1 (C(3), Man$^+$); 79.2 (C(4), Man); 79.3 (C(4), Man$^+$); 76.9 (C(5), Man); 75.7 (C(5), Man$^+$); 61.3 (C(6), Man); 61.5 (C(6), Man$^+$).

Carrageenan. IR, ν/cm^{-1}: 3560, 3422 (OH); 2970, 2942, 2913 (C—H); 1200—1000 (C—O); 910, 771 (β-glycosidic bonds of pyranose ring); 1638 (H...OH); 850 (SO_3). ^{13}C NMR, δ: 102.3 (C(1), Gal-SO_4); 70.7 (C(2), Gal-SO_4); 81.7 (C(3), Gal-SO_4); 73.7 (C(4), Gal-SO_4); 74.3 (C(5), Gal-SO_4); 61.2 (C(6), Gal-SO_4); 94.6 (C(1), anGal); 69.3 (C(2), anGal); 77.4 (C(3), anGal); 78.4 (C(4), anGal); 76.7 (C(5), anGal); 69.3 (C(6), anGal). For the original polysaccharide, found (%): C, 32.36; H, 5.23; K, 6.3; Na, 0.96; S, 5.04; for the depolymerized polysaccharide, found (%): C, 31.57; H, 4.93; K, 3.35; Na, 3.94; S, 5.24.

Synthesis of the argentogalactomannan nanocomposites. An aqueous solution of $AgNO_3$ (0.04—0.33 g of $AgNO_3$ in 10 mL of H_2O) (10 mL) was added dropwise to a solution of galactomannan (1 g) in H_2O (30 mL) at 90 °C, and then a 1 *M* solution of NaOH (3 mL) was added. The reaction time was 20 min, temperature was 90 °C, and the nanocomposites were precipitated by the addition of fourfold excess of ethanol. The precipitate was filtered off and dried at room temperature under atmospheric pressure. The yield was 72—94%. The quantitative content of silver in the obtained samples was 2.5—17.0%.

Synthesis of the argentocarrageenan nanocomposites was carried out similarly. The quantitative content of silver in the obtained samples was 2.5—12.0%.

Determination of the antimicrobial activity of argentocarrageenan containing 4.0% silver nanoparticles was carried out by the method of serial dilutions[28] using reference cultures of *Escherichia coli ATCC 25922*, *Pseudomonas aeruginosa ATCC 27853*, *Staphyllococcus aureus ATCC 25923*, and *Bacillus subtilis* and a nosocomial strain of *E. coli*. A suspension (0.1 mL) of the studied test cultures (1000 cells in 1 mL), the thioethylene glycol or the Sabouraud medium (10 mL), and a 0.1—1.0% solution of the studied substance (0.1—1.0 mL) were placed in the tubes. Nutrient media in which distilled water (1 mL) was introduced instead of the studied solutions served as references. Seedings were incubated in the thioethylene glycol medium for 48 h at 35 °C and in the Sabouraud medium for 72 h at 20—25 °C. The estimates were made with account of the presence and the character of culture growth on the nutrient media.

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