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NANOBIOLOGY

Hybrid Systems of Delivery of Long-Acting Drugs Based on Gentamicin Sulfate, Silver, and Copper Nanoparticles, and Gelatin Biopolymer Matrices

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Abstract—Using the method of cryochemical synthesis, systems of prolonged release of gentamicin sulfate modified with silver (2–30 nm) and copper (1–9 nm) nanoparticles from cryostructured biopolymer matrices based on gelatin with a pore size of 10–50 μm are obtained. The composition and structure of the systems are confirmed by the data of IR, UV, and NMR spectroscopy; TEM; SEM; and thermoanalytical methods of analysis, and the rate of release of the drug is determined by conductometry. Hybrid composites based on metals and gentamicin sulfate showed greater activity in suppressing the growth of *E. coli 52* and *S. aureus 144* than their components separately.

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INTRODUCTION

Gelatin is a biocompatible, biodegradable, and multifunctional biopolymer. It is widely used in food, pharmaceutical, and cosmetic industries due to its unique mechanical and technological properties. In medicine and pharmacy, gelatin is used in the production of capsules; as a matrix for implants; device coatings; and as a stabilizer in measles, mumps, rubella, Japanese encephalitis, rabies, diphtheria, and tetanus vaccines [1]. The special properties of gelatin make it an attractive carrier in the systems of directional delivery and prolonged release of drugs. However, gelatin is water-soluble and, therefore, in currently developed prolonged controlled-release dosage forms (which do not require frequent ingestion and provide the maintenance of a constant concentration of drugs in the blood or target organs), biopolymers obtained by the chemical cross-linking of gelatin in the form of gelatin microspheres [2–5] and massive gelatin hydrogels [6–9] are used.

Antibacterial drugs are included as a drug in such systems [8]. Also, metal nanoparticles (Ag, Cu) can be included in these systems, which are active against antibiotic-resistant strains [10] and for which, together with antibiotics, a synergistic increase in antibacterial activity is observed [10–13].

The use of low-temperature methods of synthesis and modification makes it possible to control the structure and porosity of the resulting biopolymer matrices [14], reduce the size and change the structure of the particles of drugs [15, 16], and include metal particles in the particles of drugs [11–13].

The aim of this work was the cryochemical synthesis of hybrid systems of prolonged release on the basis of gentamicin sulfate, metal nanoparticles (silver, copper), and gelatin biopolymer matrices, as well as the determination of the antibacterial activity of the forms that were obtained against bacterial strains of *Escherichia coli* (*E. coli 52*) and *Staphylococcus aureus* (*S. aureus 144*).

EXPERIMENTAL

The substance of gentamicin sulfate, corresponding to the pharmacopoeial article FS 42-2628-00, colloidal silver of KND-S-K brand (TU 9154-024-74107096-2008), copper II chloride, and hydrazine

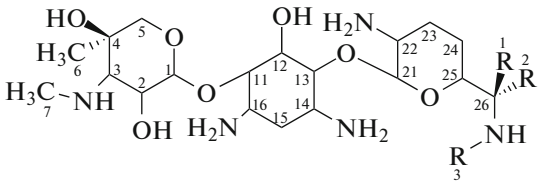

Fig. 1. Structural formula of the main components of gentamicin sulfate.

hydrate of analytical grade, were used without additional purification. Copper nanoparticles were obtained by reducing an aqueous solution of copper chloride by hydrazine hydrate [17]. Low-temperature synthesis of gelatin biopolymer matrices was performed according to [14].

Gentamicin sulfate fine powder and gentamicin sulfate hybrid nanocomposites with silver (Ag/gentamicin sulfate) or copper (Cu/gentamicin sulfate) nanoparticles were prepared by spraying an aqueous solution containing gentamicin sulfate and metal nanoparticles (1 wt % of gentamicin sulfate, 0.005 wt % of Ag, or 0.02 wt % of Cu) through a pneumatic nozzle into liquid nitrogen; then the frozen solutions were subjected to cryosublimation drying for 24 h [15, 16]. Gentamicin sulfate, as well as Ag/gentamicin sulfate and Cu/gentamicin sulfate nanocomposites, were included in the disks of gelatin cryostructured matrices as follows: spongy disks were immersed for 30 min in aqueous solutions of gentamicin sulfate and metal nanoparticles (1 wt % of gentamicin sulfate, 0.02 wt % of Ag, or 0.02 wt % of Cu), then the disks were removed from the solution, frozen in liquid nitrogen, and subjected to cryosublimation drying for 24 h. The samples based on gelatin (Ag/gentamicin sulfate/gelatin and Cu/gentamicin sulfate/gelatin) contained 11.5 wt % of gentamicin sulfate and 0.46 wt % of metal.

X-ray phase analysis of the samples was performed on a Rigaku D/MAX-2500 diffractometer (Rigaku, Japan) using CuKα radiation (λ = 1.54056 Å). The IR spectra of the samples were obtained in the range of 4000–400 cm^{-1} on a Bruker Tensor II spectrometer (Germany) with an ATR platinum attachment. The IR spectra of powdered samples were recorded by diffuse reflection. The UV spectra of the samples were recorded on a Jasco V-770 spectrophotometer (Jasco, Japan) in the range of 300–500 nm. Nuclear magnetic resonance (^{1}H NMR) spectra were recorded in a solution in deuterated water (D_2O) using a high-resolution VXR-400 NMR spectrometer (Varian, United States). Thermoanalytical studies were performed on a TG 209 F1 Perseus thermogravimetric analyzer (NETZSCH, Germany) and a DSC 204 F1 Phoenix differential scanning calorimeter (NETZSCH, Germany).

The kinetics of release of gentamicin sulfate from cryostructured matrices was monitored conductometrically using a Mettler Toledo conductometer (Switzerland) with an LE703 electrode.

The microstructure of the samples was studied using transmission electron microscopy (TEM) on an LEO 912 AB Omega electron microscope (ZEISS, Germany) at ×80–×500000 magnifications and scanning electron microscopy (SEM) on a Phenom scanning electron microscope (FEI Company, Germany) at ×20–×4000 magnifications.

Determination of the antibacterial activity of the samples was carried out by the disk-diffusion method [18] using disks of filter paper and gelatin cryostructured matrices (4 mm in diameter and 2 mm in height). Bacterial cells obtained from the collection of bacterial cultures of the Department of Microbiology, Faculty of Biology, Moscow State University: *E. coli 52*, *S. aureus 144* (Catalog of the collection of microorganisms of the Department of Microbiology, Biological Faculty, Moscow State University) were used as test cultures. The experiments were carried out in Petri dishes containing 20 mL of agar nutrient medium dried during the day (thickness of the medium layer 4 mm). Measurement of growth inhibition zones (GIZ) of the test cultures was carried out

Table 1. Functional groups to the structural formula of the main components of gentamicin sulfate (Fig. 1)

Main components of gentamicin sulfate	R_1	R_2	R_3
Gentamicin C1	$-CH_3$	–H	$-CH_3$
Gentamicin C1a	–H	–H	–H
Gentamicin C2	$-CH_3$	–H	–H
Gentamicin C2a	–H	$-CH_3$	–H
Gentamicin C2b	–H	–H	$-CH_3$

Table 2. ^{1}H NMR spectra (D_2O) of hybrid composites of gentamicin sulfate with silver nanoparticles

Carbon atom number (Fig. 2) associated with the hydrogen atom(s)	δ
1	5.08; *d*
2	3.78; *dd*
3	2.57; *d*
5a	4.04; *d*
5b	3.31; *d*
6	1.20; s
7	2.50; s
11/13	3.25; *t*/3.30; *t*
12	3.57; *t*
14/16	2.77-2.96; *m*
15eq	1.96; *dt*
15ax	1.17–129; *m*
21	5.14; *d*
22	2.81; *dd*
23eq/23ax	1.55–1.83; *m*
24eq/24ax	1.40–168; *m*
25	3.73–3.90; *m*
26	2.67; *dd*
R_1 ($-CH_3$)	1.05; *d*
R_2 ($-CH_3$)	1.04; *d*
R_3 ($-CH_3$)	2.32; *s*

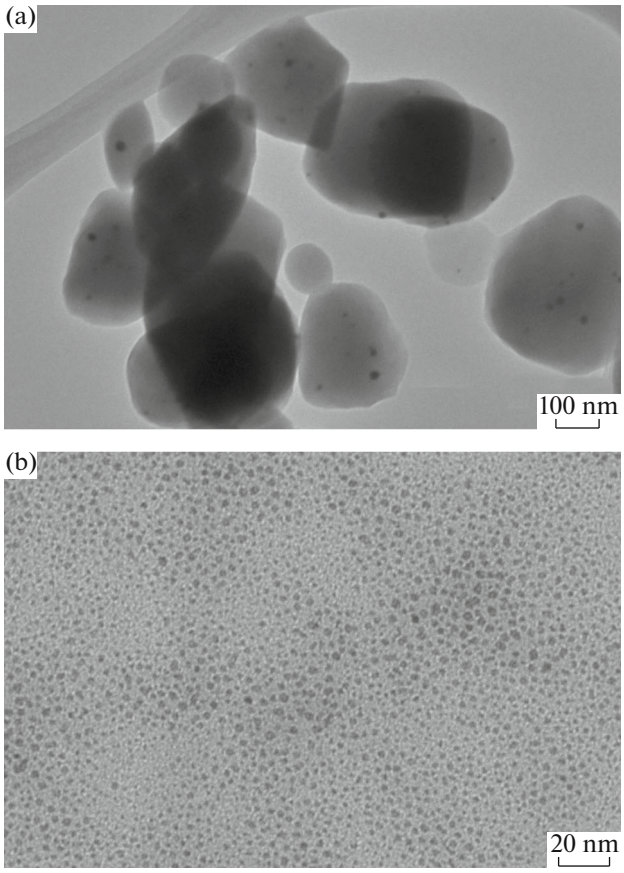

Fig. 2. TEM micrographs of (a) hybrid composites of gentamicin sulfate with silver nanoparticles and (b) Cu/gentamicin sulfate/gelatin system.

after 24 h of incubation. Statistically reliable results were obtained by a ninefold repetition of the GIZ measurements for each series of samples.

RESULTS AND DISCUSSION

To find the composition and structure of the samples, they were characterized by physicochemical analysis. The IR spectra of hybrid composites of gentamicin sulfate with silver and copper nanoparticles corresponded to the IR spectrum of gentamicin sulfate, except for the band corresponding to the oscillations of HSO_4^- and C–O, which when passing from gentamicin sulfate to its hybrid composites with silver and copper nanoparticles shifted from 1040 to 1056 cm^{-1}. After the composites were incorporated into gelatin-based matrices, the IR spectrum of the resulting systems was a superposition of the IR spectra of gelatin and gentamicin sulfate. The bands Amide A (NH) 3280 cm^{-1}, Amide B (CH) 2929 cm^{-1} Amide I (CO, CN) 1634 cm^{-1}, Amide II (CH, NH) 1530 cm^{-1}, Amide III (CN, NH) 1235 cm^{-1}, characteristic of gelatin [19] were present in it. The IR spectrum of the

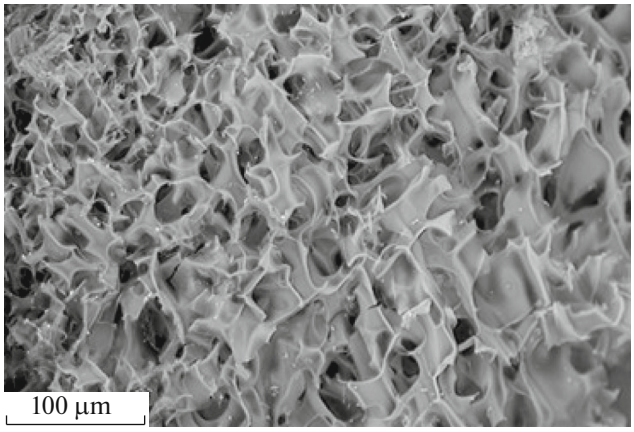

Fig. 3. SEM micrograph of Cu/gentamicin sulfate/gelatin system.

sample also shows bands of NH_2^+, NH_3^+ groups at 1520, 1625, and 3280 cm^{-1}, HSO_4^- and C–O at 1059 cm^{-1} and SO_2 at 607 cm^{-1}, characteristic of gentamicin sulfate [20].

The ^1H NMR spectra of hybrid composites of gentamicin sulfate with silver and copper nanoparticles, as well as compounds released from the biopolymer matrix (Fig. 1, Tables 1, 2), corresponded to literature data for gentamicin sulfate [21].

In the case of Ag/gentamicin sulfate composites, at high silver contents in the sample (4%) in the 405 nm region, weak absorption is present, caused by the surface plasmon resonance of silver nanoparticles. The low intensity of this absorption, as well as the absence of a band corresponding to the plasmon absorption of copper nanoparticles in the UV spectrum of an aqueous solution of the Cu/gentamicin sulfate composites, are associated with the low content of metal nanoparticles in the samples.

Gentamicin sulfate, which is part of the resulting hybrid composites, as well as gentamicin sulfate released from the surface of gelatinous matrix, interacts with salicylic aldehyde when heated to form a characteristically colored salicylidene derivative that absorbs at 403 nm.

Thermoanalytical studies were carried out for the systems. In the case of gentamicin sulfate, thermogravimetric analysis shows the loss of chemically and physically bound water at a temperature of about 85°C, which takes place with an endothermic effect of 317 J/g and is about 8 wt % of the sample. At 250 and 298°C, further decomposition of the antibiotic, with endothermic effects of 367 and 270 J/g and a weight loss of 22 and 34%, occurs. Gelatin cryostructured biopolymer loses water at 84°C with an endothermic effect of 191 J/g and a 3% reduction in the mass of the sample. Thermal decomposition of the biopolymer proceeds at 221 and 310°C with endothermic effects of

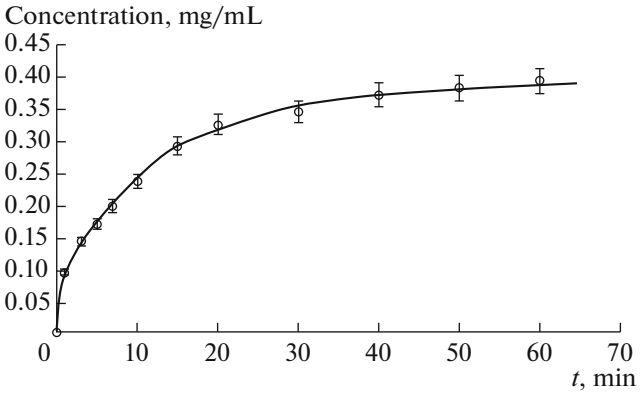

Fig. 4. Kinetic curve for gentamicin release from gelatin biopolymer matrix.

114 and 286 J/g and a total weight loss of 57%. In the case of Ag/gentamicin sulfate/gelatin and Cu/gentamicin sulfate/gelatin systems obtained by the method of cryochemical synthesis, no loss of water and the corresponding endothermic effects are observed. When the sample is decomposed, three endothermic effects are observed: at 221°C 85 J/g (relates to gelatin), at 267°C 145 J/g (probably relates to gentamicin sulfate bound to the carrier), and at 311°C 191 J/g (which is a superposition of the endothermic effects of gentamicin sulfate and gelatin).

According to transmission electron microscopy (TEM) data, the Ag/gentamicin sulfate and Cu/gentamicin sulfate nanocomposites consist of organic particles (50–350 nm in size), inside of which nanosized particles, the electron diffractograms of which correspond to silver or copper, are included. On TEM micrographs of Ag/gentamicin sulfate/gelatin and Cu/gentamicin sulfate/gelatin systems, silver and copper nanoparticles are also visible (Fig. 2). The size of metal nanoparticles in the obtained systems is 2–30 nm (average size 11 nm) for silver and 1–9 nm for copper (average size 5 nm).

SEM micrographs (Fig. 3) indicate that gelatin cryostructured biopolymer matrices are wide-pore matrices with a pore size of 10–50 μm. The inclusion of gentamicin sulfate and metal nanoparticles does not significantly affect their size, which indicates that the particle size of gentamicin sulfate applied to the matrix is much smaller than the pore diameter of the matrix. A spot elemental analysis of the sample surface shows that, in addition to the elements forming the matrix, the samples contain sulfur (which is part of gentamicin sulfate), as well as silver or copper.

A typical kinetic curve for the release of gentamicin from the biopolymer matrix is shown in Fig. 4. The release of gentamicin from the gelatin matrix takes 65 min. Changing the structure of the matrix by varying the conditions of its synthesis may allow us to further reduce the rate of the release of gentamicin sulfate and get a longer effect of controlled release of the drug component.

For the systems obtained, antibacterial activity against microbial cells of *E. coli 52* and *S. aureus 144* was determined. It turned out that modifying gentamicin sulfate with metal nanoparticles that are active against pathogenic microorganisms results in an increase in its antibacterial activity (Tables 3, 4). The inclusion of hybrid composites of gentamicin sulfate with metal nanoparticles into gelatinous matrices does not violate the observed pattern, and their activity exceeds the activity of metal nanoparticles or gentamicin sulfate included in similar matrices. Thus, obtained for hybrid systems of gentamicin sulfate with silver and copper nanoparticles, a synergistic increase in antibacterial activity is observed with respect to *E. coli 52* and *S. aureus 144* when compared with the effect of individual components.

Table 3. *E. coli 52* and *S. aureus 144* growth-inhibition zones around filter paper disks impregnated with solutions* of gentamicin sulfate, colloidal silver, copper, and gentamicin sulfate hybrid composites with silver and copper nanoparticles

Bacterial strain	GIZ, Ag, mm	GIZ, Cu, mm	GIZ, gentamicin sulfate, mm	GIZ, Ag/ gentamicin sulfate, mm	GIZ, Cu/ gentamicin sulfate, mm
E. coli 52	0	0	33.8 ± 0.8	36.5 ± 0.6	35.0 ± 0.8
S. aureus 144	0	0	18.0 ± 0.6	21.1 ± 0.6	20.2 ± 0.6

*Gentamicin sulfate 0.3 wt %, Ag 0.0015 wt %, and Cu 0.006 wt %.

Table 4. *E. coli 52* and *S. aureus 144* growth-inhibition zones around gentamicin sulfate, Cu and Ag nanoparticles, and gentamicin sulfate hybrid nanocomposites with metal nanoparticles (Ag and Cu) included in gelatin biopolymer matrices

Bacterial strain	GIZ, Ag, mm	GIZ, Cu, mm	GIZ, gentamicin sulfate, mm	GIZ, Ag/ gentamicin sulfate, mm	GIZ, Cu/ gentamicin sulfate, mm
E. coli 52	4 ± 1.2	0	32 ± 0.6	36 ± 1.2	34 ± 1.2
S. aureus 144	0	0	21 ± 0.6	24 ± 1.2	23 ± 1.2

CONCLUSIONS

A low-temperature synthesis of gentamicin sulfate hybrid composites with metal nanoparticles active against antibiotic-resistant microorganisms was carried out. Cryochemically synthesized nanocomposites are nanoparticles of gentamicin sulfate, 50–350 nm in size, containing silver (2–30 nm) and copper (1–9 nm) nanoparticles. Previously, such gentamicin-based nanocomposites have not been studied. The obtained hybrid systems turned out to be more active to the processes of suppressing the growth of *E. coli 52*, *S. aureus 144* than individual components. This result is consistent with previously published data by the authors of the article [10–13] on the synthesis and antibacterial activity of composites of another drug of dioxidine with silver and copper nanoparticles. In the case of dioxidine nanocomposites with silver and copper, a synergistic increase in antibacterial activity was also observed when compared with the action of individual components.

The inclusion of drug composites in gelatin biopolymer matrices not only did not affect their composition and the observed effect of the synergistic increase in the antibacterial activity of the antibiotic and metal nanoparticles, but it also provided a gradual release of the active drug components from the wide porous polymer matrix, i.e., the prolonged action of the synthesized hybrid nanosystems.

Thus, the systems are promising precursors for dosage forms of the prolonged release of drug components.

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