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Creation and analysis of hybrid nanocomposites based on dioxidine and nanoparticles of bioactive metals embedded in cryostructurated gelatin matrices

A V Nuzhdina^{1,2}, T I Shabatina^{1,2}, O I Vernaya² and V P Shabatin²

¹ Bauman Moscow State Technical University, Faculty "Fundamental Sciences", Moscow, Russia

² Lomonosov Moscow State University, Faculty of Chemistry, Moscow, Russia

Email: anastasia.nuzhdina@emtc.ru

Abstract. This paper is devoted to the creation of hybrid nanocomposites based on the antibacterial drug dioxidine and bioactive metals - silver and copper. The obtained materials were analyzed using the methods of NMR, UV and IR spectroscopy, low-temperature adsorption of argon, SEM and TEM. It is shown that they are silver nanoparticles with a size of 2-30 nm or copper nanoparticles with a size of 10-40 nm, incorporated into dioxidine particles with a size of 50-350 nm. The obtained drug nanocomposites were embedded into wide-porous biopolymer cryostructurated gelatin-based matrices. The possibility of the release of drug components from a biopolymer carrier and manifestation of its antibacterial activity has been shown. At the same time, hybrid nanocomposites based on metals and antibacterial drugs showed increased activity to suppress the growth of microbial cells of Escherichia coli 52, Staphylococcus aureus 144, Mycobacterium cyaneum 98, then their components separately.

1. Introduction

It is known that the efficacy of medicinal substances is largely influenced by their solubility. When reducing the particle size to 20-200 nm not only increases the solubility of the substance, but also improves the permeability of biological barriers, which leads to an overall increase in bioavailability [1]. The effectiveness of pharmacopoeial drugs can be improved, including through the creation of new hybrid forms containing known medicinal substances together with nanosized bioactive metal nanoparticles [2-4]. In this case, mixing of two or more components on a nanoscale scale can contribute to the change in existing and the acquisition of new properties by the resulting hybrid systems [5].

In addition, the current task is to create drugs with controlled release of medicinal substances, including prolonged dosage forms, in which the release of the drug substance takes longer than for the usual dosage form [6-9]. An important advantage of the dosage forms of prolonged action is to reduce the peak concentration and the absence of temporary fluctuations in the concentration of the active substance in the blood and tissues, concomitant therapy with conventional medicines. One of the methods for obtaining such dosage forms is the binding of the pharmacologically active component to the biopolymer matrix, which provides a gradual release of the drug substance from it and a long-term maintenance of its required concentration in the body or locally in a specific target organ. As biocompatible carriers for the so-called "depot forms" of medicinal substances, wide porous gelatinous matrices formed by cryostructuring techniques are of interest [10–13].

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The purpose of this paper was the synthesis of hybrid nanocomposites based on the antibacterial drug dioxidine and silver and copper metal nanoparticles, the inclusion of the obtained nanocomposites in gelatinous cryostructurated matrices to ensure their prolonged release, as well as the determination of the antibacterial activity of the resulting hybrid drug nanoforms with respect to *E. coli bacterial cells 52*, *S. aureus 144 and M. cyaneum 98*.

2. Experimental part

2.1. Sampling

Dioxidine substance corresponding to Pharmacopoeia Article 42-2308-97, КНД-С-К colloidal silver (TU 9154-024-74107096–2008), copper chloride II, hydrazine hydrate of qualification "analytical grade" were used without further purification. Copper nanoparticles were obtained by reducing an aqueous solution of copper chloride to hydrazine hydrate [14]. Low-temperature synthesis of gelatin cryostructurated matrices was performed according to previously published methods [15, 16].

Highly dispersed dioxidine powder and hybrid dioxidine nanocomposites with silver nanoparticles (Ag-dioxidine) or copper (Cu-dioxidine) were prepared by spraying an aqueous solution containing dioxidine and metal nanoparticles (1 wt% Dioxidine, 0.005 wt% Ag or 0.02 wt% Cu) through a pneumatic nozzle into liquid nitrogen, then the frozen solutions were subjected to cryosublimation drying for 24 hours [17–20].

Dioxidine, as well as Ag/dioxidine, Cu/dioxidine nanocomposites were included in the disks of cryostructurated matrices as follows: sponge gelatin disks were immersed for 30 minutes in aqueous solutions of dioxidine and metal nanoparticles (1 wt% dioxidine, 0.02 wt% Ag or 0.02 wt% Cu), then the discs were removed from the solution, frozen with liquid nitrogen and subjected to cryosublimation drying for 24 hours. The obtained samples of Ag-dioxidine-gelatin and Cu-dioxidine-gelatin contained 11.5 wt% dioxidine, 0.46 wt% metal.

2.2. Determination of physico-chemical properties

The ¹H NMR spectra were recorded in a saturated solution in deuterated water (D_2O) on a VXR 400 high resolution NMR spectrometer (Varian, USA). The IR spectra of the samples were obtained in the range of 4000–400 cm⁻¹ on a Bruker Tensor II spectrometer (Germany) with the prefix ATR platinum. The IR spectra of powdered samples were recorded by the method of diffuse reflection. UV spectra of 0.01 wt% aqueous solutions of samples were taken on a Jasco V-770 spectrophotometer (Jasco, Japan) in the range of 200–700 nm. The release of dioxidine from cryostructurated matrices was monitored spectrophotometrically at 360 nm.

The microstructure of the samples was studied by transmission electron microscopy (TEM) on a LEO 912 AB Omega electron microscope (Zeiss, Germany) at magnifications of 1000–20000 times and scanning electron microscopy (SEM) on a Phenom scanning electron microscope (FEI Company, Germany) at magnifications of 20 to 4000 times.

The determination of the specific surface area (S_{sp}) of the samples was performed by the method of low-temperature adsorption — thermal desorption of argon on a laboratory setup based on a Chrom 5 chromatograph. Pre-adsorbed gases were removed from the surface of the samples in a vacuum unit. The average particle size of dioxidine was calculated by the formula: $a = 6 / (\rho S_{ud})$, where ρ is the dioxidine density.

2.3. Determination of antimicrobial activity

The antibacterial activity of hybrid nanocomposites Ag-dioxidine and Cu-dioxidine in comparison with dioxidine and solutions of colloidal silver and copper was determined by a disk-diffusion method [21] using Krasnaya Lenta filter paper disks (5 mm diameter) and gelatin cryostructurated matrices disks

(4 mm dia and 2 mm high). As test cultures the bacteria were used obtained from a cultures collection from the Department of Microbiology, Biological Faculty, Moscow State University, named after M. V. Lomonosov (*E. coli 52, S. aureus 144* and *M. cyaneum 98*). The experiments were performed in Petri dishes containing 20 ml of agar nutrient medium, dried for 24 hours (thickness of the medium layer 4 mm). Measurements of the zone of inhibition of growth of test cultures were performed after 16 hours of incubation. Statistically reliable results were obtained by repeating measurements of the zone of inhibition of growth three times for each series of samples.

3. Results and discussion

The determination of the chemical composition of the obtained samples was carried out by various methods of physico-chemical analysis. Comparison of the 1H NMR spectra of the starting dioxidine and its hybrid nanocomposites showed that, as part of the hybrid nanocomposites Ag-dioxidine and

Cu-dioxidine, the studied drug compound remained unchanged in chemical form (see below). Initial dioxidine.

Spectrum ¹H NMR (D2O, δ, ppm, 600 Hz): 4.93-5.21 (m, 4 H, CH₂); 7.85-8.05 (m, 2 H, Ar); 8.38-

8.52 (m, 2 H,

Ar).

Hybrid dioxidine nanocomposites.

Spectrum ¹H NMR (D2O, δ, ppm, 600 Hz): 4.92-5.25 (m, 4 H, CH₂); 7.84-8.03 (m, 2 H, Ar); 8.35-

8.51 (m, 2 H,

Ar).

The IR spectra of the source dioxidine and the resulting hybrid composites Ag-dioxidine and Cudioxidine were identical (Figure 1). The UV spectra of dioxidine aqueous solutions and Ag-dioxidine and Cu-dioxidine compositions also completely coincided (λ max = 241, 259, 360 and 375 nm) and corresponded to chemically unchanged dioxidine [22, 23]. In addition, absorption spectra corresponding to dioxidine were also present in the UV spectra of aqueous extracts of samples of the Ag-dioxidine-gelatin matrix and Cu-dioxidine-gelatin matrix. Thus, on the basis of NMR, IR and UV spectroscopy data, we confirmed the presence of chemically unchanged dioxidine in the composition of hybrid nanosystems Ag-dioxidine, Cu-dioxidine, Ag-dioxidine-gelatin matrix and Cu-dioxidinegelatin matrix.

The kinetic curve of dioxidine release from the gelatin matrix is shown in Figure 2. The release of dioxidine from the biopolymer matrix occurs within 60 minutes, that is, relatively quickly. To achieve a more prolonged effect of controlled isolation of the drug component from the carrier matrix, it is necessary to change the structure of the matrix by varying the conditions of its synthesis.



Figure 1. IR spectra of pharmacopoeial dioxidine (a) and hybrid compositions of Ag-dioxidine (b) and Cu-dioxidine (c).



Figure 2. Kinetic curve of dioxidine release from the gelatin matrix

The TEM data indicate that Ag-dioxidine and Cu-dioxidine hybrid nanocomposites consist of organic particles 50–350 nm in size, inside of which silver particles of 2–30 nm in size or copper 10–40 nm in size are enclosed. Examples of micrographs of the Ag-dioxidine and Cu-dioxidine systems are shown in Figure 3.



Figure 3. TEM micrographs of hybrid composites Ag-dioxidine (a) and Cu-dioxidine (b).

The specific surface values and the average particle size calculated for these values for Agdioxidine nanocomposites were 31 $m^{2} \times g^{-1}$ and 130 nm, and for Cu-dioxidine nanocomposites - 24 $m^{2} \times g^{-1}$ and 166 nm, respectively. Thus, the average particle size obtained by the method of lowtemperature adsorption of argon is consistent with the data obtained by the TEM method.

SEM photomicrographs (Figure 4) indicate that gelatin cryostructurated matrices are wide porous matrices with a pore size of tens of microns. The inclusion of dioxidine and metal nanoparticles does not significantly affect their size.



Figure 4. SEM micrographs of hybrid composites Ag-dioxidine (a) and Cu-dioxidine (b) included in gelatin cryostructurated matrices.

The antibacterial activity of various hybrid dioxidine-based nanosystems has been determined for bacterial strains *E. coli 52*, *S. aureus 144* and *M. cyaneum 98*. The data obtained are summarized in Tables 1 and 2. The antibacterial activity of hybrid nanocomposites Ag-dioxidine and Cu-dioxidine was higher than that of individual

nanoparticles of metals and dioxidine. The same trend was observed when testing nanocomposites included in gelatin cryostructured matrices. Thus, a synergistic increase in the antibacterial activity of the resulting hybrid systems with respect to a number of bacterial cells was demonstrated in comparison with the effect of individual medicinal components.

Bacterial strain	Growth	Growth	Growth	Growth Inhibition	Growth Inhibition
	Inhibitio	Inhibiti	Inhibition Zone,	Zone,	Zone,
	n Zone,	on	dioxidine, mm	Ag/dioxidine, mm	Cu/dioxidine, mm
	Ag, mm	Zone,			
		Cu, mm			
E. coli 52	0	0	26 ± 1.2	36.7 ± 0.6	32.1 ± 1.2
S. aureus 144	0	0	30.3 ± 0.6	37.4 ± 0.6	33.0 ± 1.2
M. cyaneum 98	0	0	26.1 ± 0.8	36.3 ± 0.6	31.5 ± 0.8
S. aureus 144 M. cyaneum 98	0 0 0	0 0 0	20 ± 1.2 30.3 ± 0.6 26.1 ± 0.8	36.7 ± 0.6 37.4 ± 0.6 36.3 ± 0.6	$ \begin{array}{r} 32.1 \pm 1.2 \\ 33.0 \pm 1.2 \\ 31.5 \pm 0.8 \\ \end{array} $

Table 1. Growth Inhibition Zone for *E. coli 52*, *S. aureus 144* and *M. cyaneum 98* around filter paper discs soaked with sample solutions.

^aDioxidine - 0.3 wt%, Ag - 0.0015 wt%, Cu - 0.006 wt%

Table 2.	The growth i	nhibition zor	ne of E. co	oli 52 around	dioxidine,	Cu and Ag na	anoparticles,	and
dioxidine	hybrid nano	composites v	vith metal	nanoparticles	s included	in gelatinous	cryostructur	ated
matrices.								

Cu, Ag, mm mm	Growth Inhibitio n Zone	ion Growth Inhibition ne, Zone Cu/Dioxidine, mm	Growth Growth Inhibition Inhibitio Zone Dioxidine, n Zone mm	Growth Inhibition Zone Ag/Dioxidine, mm
$0 40 \pm 12 350 \pm 06 373 \pm 12$	Cu, mm 0	373+12	Ag, mm 4.0 ± 1.2 35.0 ± 0.6	38.1 ± 1.2

4. Conclusion

In the research we obtained hybrid nanocomposites consisting of particles of dioxidine 50–350 nm in size, inside of which silver nanoparticles with a size of 2–30 nm or copper 10–40 nm in size are enclosed. These nanocomposites were incorporated into gelatinous cryostructurated matrices, which allowed us to achieve a gradual release of the drug within 60 minutes. Tests of antimicrobial activity against strains of E. *coli* 52, S. *aureus* 144, M. *cyaneum* 98 showed that the compositions have a higher antibacterial activity against cells compared to the original dioxidine and metal nanoparticles.

The resulting bioactive nanomaterials exhibit significantly greater antibacterial activity against pathogenic strains (S. *aureus 144*, *M*. cyaneum 98), compared with E. *coli* cells that are part of the natural bacterial composition of the human body. This eliminates the possible increased toxicity of the resulting hybrid nanocomposites and biopolymer nanomaterials based on them and opens up prospects for the creation of new dosage forms for targeted delivery and controlled release of drug components.

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