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Ion Release and Bactericidal Activity of Ag /Tryptophan and Ag/Cu/Tryptophan Complexes in the Structure of Cotton Tissue

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Abstract

The work quantitatively studied the leaching into water of Ag and Cu ions, from Ag and Ag/Cu nanoparticles (NPs) fixed on the surface of bactericidal tissues –cotton fabrics (cf) in the form of complexes with tryptophan (Trp) molecules. The effect of the modification of Ag/Cu NPs in cotton tissues by Trp on their bactericidal activity is studied. Trp adsorption isotherms demonstrate an increased sorption capacity of Ag/Cu-cf compared to Ag - cf. A significant part of NPs (about 70-80 %) is desorbed from the surface of the tissue upon contact with water in the form of complexes with Trp. The release of ions from Ag-cf and Ag/Cu -cf in the absence of Trp is about 30-55%. A strong relationship between the residual amounts of NP-Trp complexes with tissue can be explained by the presence of binding sites of the modifier with the active groups of the tissue surface — glycosidic residues, hydroxyl groups, and others in the heterogeneous structure of cotton. The bactericidal activity of tissues containing tightly bound complexes (Ag/Cu/Trp-cf and Ag/Trp-cf), with a significantly lower content of metal NPs (1-5 mg NP per 1 g of tissue) is comparable to the activity of composites without amino acid (Ag/Cu-cf and Ag-cf), (6-30 mg NPs per 1 g of tissue) in relation to a number of bacteria: *E. coli, K. Pneumoniae, P. Aeruginoss, S. Aureus, E. Faecalis, C. albicans*. However, after 7 days of contact with the bacterial medium, secondary bacterial growth is detected that is not observed during use Ag/Cu-cf and Ag-cf. The work continues in the direction of determining the optimal ratio of NP-Trp on the surface of tissue samples in terms of their biocidal action and the dynamics of release of modifiers (NPs, Trp and their complexes) from the surface of tissues in aqueous solution.

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Introduction

This article continues a series of publications devoted to the production and study of bactericidal tissues modified with nanoparticles of silver (Ag NPs), copper (Cu NPs) and their bimetallic compositions (BMNPs) (Ag/Cu NPs). Nanosized metals and their oxides in tissues have a pronounced bactericidal action against a wide class of microorganisms *(E. coli, K. pneumonia, P. aeruginos, S. aureus, E. faecalis)*, as well as antimycotic activity. BMPs also have high antitumor efficacy [1-6]. The dynamics of the release of silver and copper ions into water from bactericidal cotton fabrics (cf) modified by Ag NPs and BMNPs and physico-chemical properties of materials are studied by us [5]. It was found that the largest number of Ag and Cu ions is released during the first 24 hours of contact of the tissue with the aqueous medium, after 48 hours of contact the amount of released ions drops sharply, and practically stopped after 72 hours. Besides, bactericidal activity of tissues after leaching of metal ions from the surface of nanoparticles remained high, which was explained by the reduction of evolved ions by glycoside residues of the tissue surface [6]. The obtained results explain the high bactericidal activity of modified cotton textiles precisely in the first day of their use for disinfecting wound surfaces from a number of bacteria due to the active diffusion of silver and copper ions that are formed on the surface of NPs when in contact with the humid environment. The NPs are fixed on textiles due to electrostatic interaction with textile components including functional surface groups as well as porous structure of tissue [7,8]. Antimicrobial properties of the AgNPs and binary Ag/Cu NPs are studied in [9-13]. To prevent toxic side effects of metal NPs on the body, simultaneously biomolecules or drugs are used for complex therapy of infectious-inflammatory diseases, and the most commonly used is the amino acid Trp, Silver nanoparticles prepared with tryptophan were found to effectively prevent the formation and kill bacteria in established biofilm structures and may be useful for the prevention and treatment of biofilm-related infections [14-26 and references therein].

The adsorption modification of cotton fabrics contained Ag and Ag/Cu nanoparticles by amino acid tryptophan and their properties are shown also in [27]. It has been shown that Ag/Trp coordination compounds at therapeutic doses are non-toxic, exerting an antimicrobial effect. However, along with the large number of articles on the interaction of biomolecules with metal NPs to reduce the toxic effect, it is interesting and important to compare the biocidal activity of NP-Trp-fabric composites with that of samples without Trp. In this work, we determined the amount of silver and copper ions released into water from the surface of Ag/Trp-cf and Ag/Cu/Trp-cf materials and studied the effect of Trp in complexes on the surface of textiles on their bactericidal activity.

Experimental

A simple and effective method for the production of cotton fabric modified with Ag NPs and BMNPs by means of a soft heat treatment without the use of chemical reducing agents and stabilizers, with an enhanced antibacterial effect compared with monometallic Ag and Cu particles is presented in refs [1,5,6]. Briefly, the technique for preparing of bactericidal textile is as follows: Cotton fabric (cf)-madapollam, a density of 94 g/m^2, was impregnated with salts of silver and copper nitrates. 10 g of tissue was kept in 100 ml of salt solution with c=10^{-7} – 10^{-1} M for 30 min for measuring adsorption isotherms. Ironing of the squeezed fabric at 200°C is accompanied by the appearance of yellow (in the case of Ag-cf) or yellow-brown (in the case of Ag/Cu-cf) color. Samples of tissue contained the ions of silver, copper and both Ag^+ and Cu^{2+} ions were prepared for comparison and dried during 24 h at room temperature on a paper substrates. We also found a quantitative dependence of the formation of copper nanoparticles on the tissue on the nature of the anion - nitrate, acetate or copper sulfate. This issue is not discussed in this article and will be the subject of further consideration.

The Diffusion-Reflectance Spectra (DRS) of the fabrics with NPs were registered by means of spectrophotometer Perkin Elmer Lambda Bio UV-vis with the integrating sphere of Labsphere RSA – PR- 20 in the range of waves 200-1000 nm.

For SEM analysis it was used a HITACHI SU5000 Schottky Field-Emission Scanning Electron Microscope with both SEM (surface image) and EDS (Cu and Ag confirmation) capabilities.

To determine the amount of released ions from the modified tissues, atomic absorption spectroscopy method has been used. Ag-cf and Ag/Cu/-cf (0.12 g) were kept in 10 ml of water for 24 hours following which the amount of ions desorbed from tissues was analyzed. These procedures were repeated every 24 hours for three days, similarly to described in [5]**.** The total amount of metal on the fabric was determined by the same method. To determine the total content of metals in the tissues, wet digestion of a tissue sample (0.024 g (2 × 2 cm) was carried out with a solution of 5 ml of HNO_3 + 5 ml of H_2O_2. After decomposition, the amount of Cu in the solution was determined with electrothermal atomic absorption spectroscopy to determine the amount of substance in tissues containing monometallic NPs, and with flame atomic absorption spectroscopy for tissues containing bimetallic NPs. Experimental conditions of the analysis are listed in Table 1. All measurements was carried out with use of Shimadzu AA-6800 atomic absorption spectrometer.

Table 1: Experimental condition of atomic-absorption analysis

Element	Cu	Ag
Technique	Electrothermal AAS, platform tube Pyrolysis: 900 °C Atomization: 2200 °C	Flame AAS C_2H_2 2 L/min Air: 15 L/min
Wavelength,nm	324.7	328.1
Spectral slit, nm	0.5	0.2
Lamp mode	Deuterium background correction	No background correction

The effect of obtained fabrics on bacteria has been studied using classic microbiological method similar to presented in [1]. Petri dishes were filled with the respective test cultures agar for bacteria. Then cotton fabrics were cut into equal-sized round pieces app. 12mm x 12mm and placed on the cooled agar the test-cultures of bacteria (10^6 CFU), carefully triturated with a spatula Drygalski on the surface of the agar. After drying up of inoculation the tissue of investigated samples were applied onto the agar surface (1 cm x 1 cm) and Petri dishes were cultured in the conditions of thermostat at 37 ^{o}C for 24 hours. A zone of inhibiting the growth of bacteria was measured by a ruler after 24 hrs, 48 hrs and 7 days. The relative error in measuring of the inhibition zone of bacteria growth is 5%.

Results and Discussion

The aldehyde function of cellulose promotes the process of silver and copper ions reduction and the corresponding NPs formation on the tissue' surface under treatment at 200 - 220^0 C. The distribution of NPs along the surface of the tissue is not completely uniform, which can be explained by the structure of the tissue that is heterogeneous at the micro level. Figure. 1 shows electron microscopy images of Ag-cf and Ag/Cu-cf cotton fabrics.

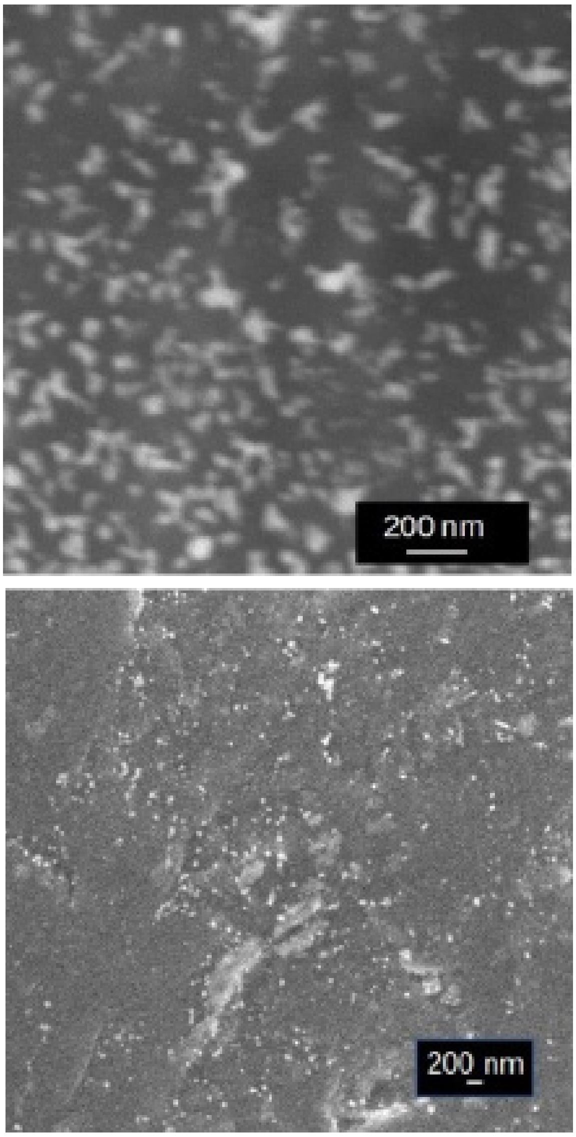


Figure 1: SEM of Ag-cf and Ag/Cu –cf

The release of silver and copper ions in water from Ag NPs and Cu NPs is discussed in [28-30]. In accordance with [29] the amount of released silver from textile varies between 1% and 45% of total silver amount. We have previously shown the release of silver and copper ions in water from the textile modified with corresponding NPs [5]. It was found out that during the 24-hours soaking cycles in water, approximately 56% of the Ag ions are overall released from the Ag/cotton fabric , as well as 14% Ag^+ + 17% Cu^{2+} from the Ag/Cu/cotton fabric [5].

Release of silver and copper ions from Ag-cf and Ag/Cu-cf in water depending on soaking time is shown in Figure 2.

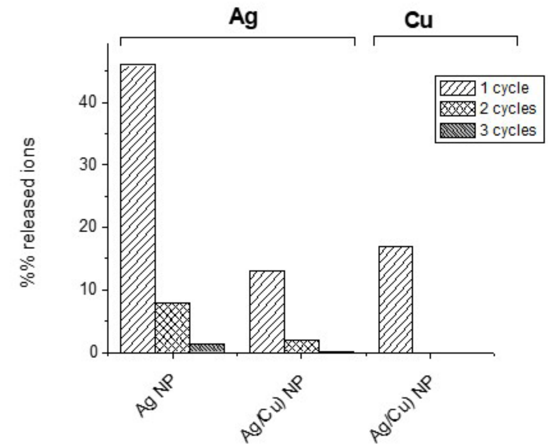


Figure 2: The amount of silver and copper ions for three consecutive soaking cycles in water (24, 48 and 72 hours) of Ag-cf and Ag/Cu-cf for two consecutive soaking

Besides, bactericidal activity of tissues after leaching of metal ions from the surface of nanoparticles remained high, what can be explained in particular due to the reduction of part of the released ions in solution by free glycoside groups on the tissue surface.

Tissues modified with Ag/Cu-cf are more active in their bactericidal and antimycotic effects than Ag-cf [1, 6].On the surface of nanoparticles immobilized on tissue, large amount of ions are formed upon contact with water or biological fluid, which determines high bactericidal activity of the tissue. Tryptophan adsorbed on the surface of nanoparticle-modified tissues forms strong complexes with metal ions during adsorption. The formation of Ag/Cu/Trp and Ag/Trp complexes on the surface of tissue accompanied by quenching of Trp fluorescence [28]. The affinity of Trp for tissue' structure is very low and it increases in the presence of silver and copper. Wherein adsorption isotherms exhibit significantly greater adsorption capacity of Ag/Cu-cf to Trp molecules compared with Ag-cf (Figure 3). The corresponding logarithm stability constants (Lg K1 CuTrp =8.71, $CuTrp_2$ =16.61, Ag Trp = 4,1) are given in the book [31]. Excess of Trp can interfere with the contact of the NP with the bacterial wall.

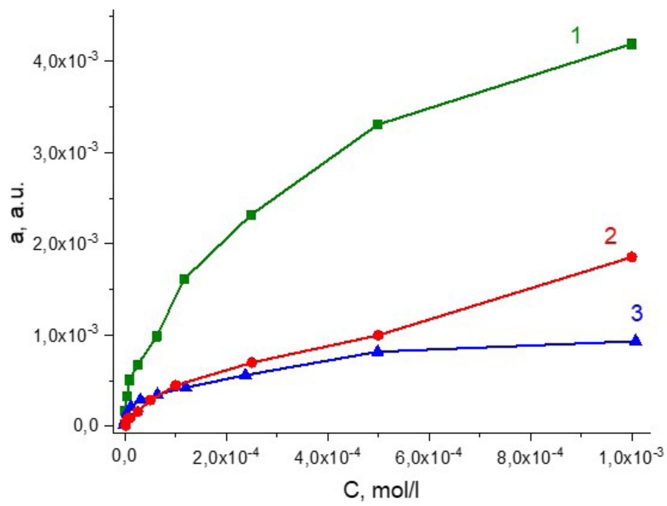


Figure 3: Adsorption isotherms of Trp on Ag/Cu-cf (1), Cu-cf (2), Ag-cf (3).

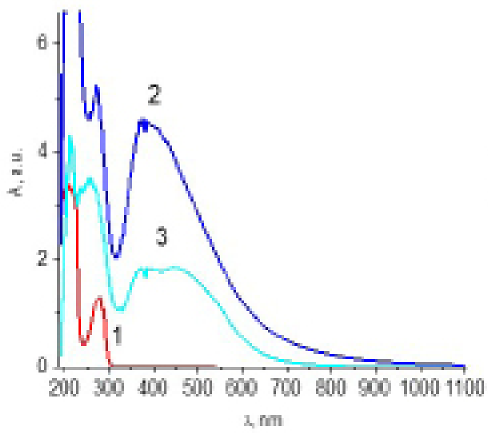
The diffuse reflectance spectra of tissues modified by complexes of metal nanoparticles with Trp indicate the consumption of metal ions for interaction with Trp (Figure 4). The intensity of the SPR band of silver NPs decreases, the band broadens due to the formation of complexes (Figure 4a). The Ag/Cu/Trp-cf spectrum also clearly shows a decrease in the intensity of the SPR band of silver NPs (max 400 nm) as an ion source and copper ions (max app. 800 nm,) for the formation of complexes with Trp (Figure 4b, inset). In water or biological fluid, the complexes of silver and copper with Trp pass into solution.

The resistance of the dry samples of fabric with immobilized metal species is 2700 – 3000 kOhm which is rather high. This fact confirms that metal particles are distributed separately one from another on the surface of the fabric samples. The resistance decreased up to 320-100 kOhm after soaking of the fabric with the double distilled water. It can be a result of the releasing of metal ions from the surface of the particles and their further migration.

The amounts of silver and copper NPs on the surface of the modified Ag/Trp-cf and Ag/Cu/Trp-cf tissues before and after washing are shown in Table 2.

These data indicate a high degree of desorption of metal complexes with Trp from the surface of the initial samples into water, i.e., the loss of metals from the surface of the modified tissue. The bactericidal activity of tissue samples containing weakly adsorbed complexes was not determined since they are not a clear characteristic of the finished material that can be recommended for use. We studied the activity of tissues with strong bound complexes with the surface shown in columns 2 and 3.

(a)



(b)

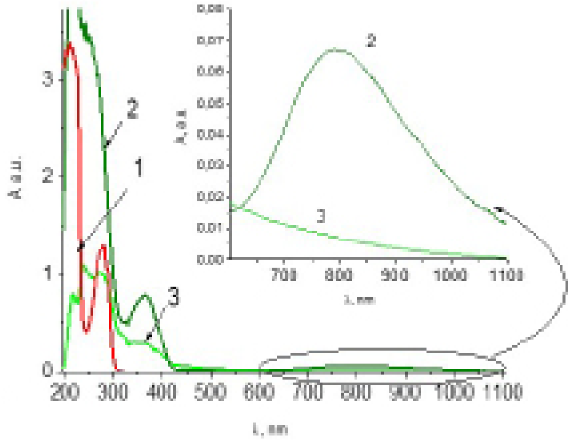


Figure 4: Absorption spectrum of Trp in water solution (1), DRS of Ag-cf before (2) and after (3) adsorption of Trp (a). DRS of Ag/Cu-cf before (2) and after (3) adsorption of Trp.

Table 2: The amounts of silver and copper NPs on the surface of the modified tissues after Trp adsorption (1st column) and after desorption (2nd and 3rd columns) of occluded complexes by two cycles of tissue washing in water, mg/g.

Initial amount of Ag in the tissue Ag/Trp-cf **5.7**	The amount of Ag after desorption Ag/Trp complexes **1.3**	The amount of Ag after repeated washing of Ag/Trp-cf **1.2**
Initial amount of Ag in the tissue Ag/Cu/Trp -cf **4.7**	The amount of Ag after desorption Ag/Cu/Trp complexes **0.79**	The amount of Ag after repeated washing of Ag/Cu/Trp-cf **0.75**
Initial amount of Cu in the tissue Ag/Cu/Trp – **1.18**	The amount of Cu after desorption Ag/Cu/Trp complexes **0.16**	The amount of Cu after repeated washing of Ag/Cu/Trp-cf **0.15**

Table 3: Microbial growth inhibition zone, mm

Modifier Bacterial culture	**Initial sample of tissue**		**After Trp adsorption**		**After Trp desorption**	
	Ag Np	Ag/Cu Np	Ag Np	Ag/Cu Np	Ag Np	Ag/Cu Np
E. coli	16	12	11	11	11	11
K. pneumoniae	11	11	11	11	11	11
P. aeruginoss	13	13	12	11	11	11
S. aureus	13	13	12	11	11	11
E. faecalis	12	13	14	11	11	11
C. albicans	0	13	0	0	0	11

In relation to *Penicillium spp*., A. niger, A. flavus the studied samples do not have a biocidal effect, with the exception of Ag-cf. The work continues in the direction of determining the optimal ratio of NP-Trp on the surface of tissue samples in terms of their biocidal action and the dynamics of release of modifiers (NPs, Trp and their complexes) from the surface of tissues in aqueous solution.

Conclusion

The work studied the leaching into water of Ag and Cu ions from Ag and Ag / Cu nanoparticles (NPs) fixed on the surface of bactericidal tissues –cotton fabrics (cf) in the form of complexes with tryptophan (Trp). A significant part of NPs (about 70-80 %) is desorbed from the surface of the tissue upon contact with water in the form of complexes with Trp. The release of ions from Ag-cf and Ag/Cu-cf in the absence of Trp is about 30-55 %. A strong relationship between the residual amounts of NP-Trp complexes with tissue can be explained by the presence of binding sites of the modifier with the active groups of the tissue surface — glycosidic residues, hydroxyl groups in the heterogeneous structure of cotton. The bactericidal activity of tissues containing tightly bound complexes (Ag/Cu/Trp-cf and Ag/Trp-cf), with a significantly lower content of metal NPs (1-5 mg NP per 1 g of tissue) is comparable to the activity of composites without amino acid (Ag/Cu-cf and Ag-cf), (6-30 mg NPs per 1 g of tissue) in relation to a number of bacteria: E. coli, K. Pneumoniae, P. Aeruginoss, S. Aureus, E. Faecalis, C. albicans. However, after 7 days of contact with the bacterial medium, secondary bacterial growth is detected that is not observed during use Ag/Cu-cf and Ag-cf. The work continues in the direction of determining the optimal ratio of NP-Trp on the surface of tissue samples in terms of their biocidal action and the dynamics of release of modifiers (NPs, Trp and their complexes) from the surface of tissues in aqueous solution. Metallized tissues modified with tryptophan show bactericidal activity at a significantly lower content of silver and copper than samples without Trp. The mechanism of action of such composite materials requires further research.

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