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Enhanced Bactericidal Activity of Cotton Fabrics Modified by Binary Ag/Cu Nanoparticles

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Abstract: In the present work we demonstrate formation of bimetallic Ag/Cu NPs (BMNP) on the surface of cotton tissue. Simple and cheap method of tissue preparation includes the impregnation with the salts solution followed by ironing at app. 200°C. Enhanced bactericidal action of obtained tissue samples against series of microorganisms occurred due to the synergetic effect. BMNP are distributed on the tissue surface enough homogeneously, but Ag:Cu ratio within bimetallic NPs slightly differs from each other on various parts of tissue due to irregularity of textile structure on which NPs are fixed. The increased antimicrobial action of Ag/Cu/ tissue compounds is probably a result of the release of bioactive Ag^+ and Cu^{2+} ions from the surface of BMNP and their availability in combination interact with bacterial membranes. Tissues modified by Ag/Cu bimetallic NPs have great potential in the preparation of drugs against bacteria.

Keywords: Ag/Cu–Modified Tissue, Nanoparticles, Bacteria, Enhanced Bactericidal Activity

1. Introduction

The use of nanosized silver and copper particles (NPs) as bactericides is the subject of growing interest. Nanosized silver is the most frequently used nanoparticle due to its antimicrobial property including application in antibacterial fabrics, cosmetics, water filters and for biomedical application. Copper in solid state and ionic form posses antimycotic and bactericide activity being much cheaper than silver. Now the actual problems in the development of new antibacterial materials based on metal NPs are the following: simplifying and making cheaper of nanosized metal particles synthesis; stabilization of easy-oxidized metal NPs; reducing of their toxic effect. For today many industrial soft goods are made with content of silver NPs (napkins, socks, linen etc). The ability of fabric to hold firmly of metal NPs on the surface is very important. When two different metal NPs are within the proximity, especially being fixed on the tissue surface, their electronic, optical, biological properties can be drastically enhanced.

Silver, as an antimicrobial agent, is capable to destroy the wide spectrum of antibiotic-resistance bacteria [1-3]. The mechanisms of Ag NPs' effect on a bacterial cell are discussed in the literature as the following: anchoring to cell wall, then the accumulation of NPs, the destruction of cell membrane by free radicals, the interaction of silver with respiratory enzymes, release of reactive (singlet) oxygen, destruction of cell, interaction with the sulphur and phosphorus atoms of DNA [4 and references therein]. Copper in solid state and ionic form posses antimycotic and bactericide activity [1, 4, 5]. Unique properties have also Cu NPs, which are less toxic, than ions of copper, and initial substances for a Cu NPs production is on a few orders cheaper, than those that are used for the synthesis of Ag NPs. Synergetic action of the combination of the two different metals are noted in a number of publication [6-8].

Recently we reported effective and economical method of the bactericidal tissues production by impregnation of cotton fabric with solutions of corresponding salts followed by soft heat treatment [9] without the use of chemical reducing agents, stabilizing agents and subsequent cleaning procedures.

In this paper we characterize obtained Ag/Cu/tissue samples by SEM, EDS and by the absorption spectroscopy methods in the UV-visible region to confirm BMNPs formation. The bactericidal action of bimetallic Ag/Cu-modified fabrics against a series of the microorganisms (Klebsiella pneumoniae, *Enterobacter aerogenes*, Pseudomonas aeruginosa, Staphylococcus aureus, Enterococcus faecalis, *Proteus mirabilis* and *Escherichia coli*) are demonstrated also. The electrical conductivity of the NPs within fabrics was studied in the dry state and in a wet environment.

2. Methods

2.1. Preparation of Modified Fabrica

To prepare the material modified with bimetallic nanoparticles Ag / Cu we used cotton fabric madapollam with density 94 g/m^2 impregnated with aqueous solutions of silver nitrate and copper sulfate in a molar ratio Ag: Cu = 1: 1 similarly to [9]. Briefly note that impregnation time was 30 min. with the subsequent squeezing of tissues and their ironing at 205 ÷ 220°C.

2.2. Optical Spectra of Fabrics with NPs

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.

2.2.1. Scanning Electron-Microscopy (SEM) and Energy-Dispersive X-Ray Spectroscopy (EDS) Measurements

For SEM - EDS 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. For EDS analysis on each sample several point scans have been performed in Variable Pressure – low vacuum (30 Pa) with Ultra Variable pressure Detector (UVD).

2.2.2. The Electrical Resistance Measurements

The electrical resistance of the modified tissues, portable professional multifunction digital multimeter, HYELEC MS8229 (China) similar to noted in [10] has been used. The electrode surface area was 1 mm^2; the distance between electrodes during measurements was 1 cm; the surface area of the fabric was 4.5 cm^2.

2.3. Bactericide Effect of Modified Fabrics Investigation

To determine the effect of fabric materials with Ag and Ag/Cu NPs on bacteria we used classic microbiological method. Petri dishes were filled with the respective test cultures agar for bacteria. Then cotton fabrics were cut into equal-sized round pieces 10mmx10mm 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°C for 24 hours. The number of repeated measurements for each culture of bacteria is 3. A zone of inhibiting the growth of bacteria was measured by a ruler. This is the distance (millimeters) from the edge of the bactericidal napkin, located on the surface of a nutrient with bacteria to the edge of the inhibition zone. If the specimen does not exhibit bactericidal properties, then the inhibition zone is zero, if it manifests itself from the edge of the sample, there is a "rim" in which there are no bacteria. The linear value is a zone of growth retardation of bacteria. The relative error in measuring of the inhibition zone of bacteria growth is 5%. The number of repeated measurements for each culture of bacteria is 3. The significance of the differences for the two groups of data obtained (the zone of bacterial growth inhibition for mono- and BMNP) was estimated using the Student's test. The significance level α in the study of the Ag and Ag / Cu nanoparticle activity on the tissue was 0.02; 0.01; 0.005; 0.2; 0.02 and 0.001 for *Klebsiella pneumoniae, Enterobacter aerogenes, Pseudomonas aeruginosa, Staphylococcus aureus, Proteus mirabilis and Escherichia coli,* respectively.

3. Results

As it was mentioned early [9] the reduction of the Ag^+ and Cu^{2+} to NPs, occurred under heat treatment of impregnated cotton at app. 200°C with participation of glycosidic part of cotton as simultaneously the reductant of ions to metal NPs and stabilizer of appearing NPs. The formation of Ag_2O and Cu_2O was registered in the DRS and EDS spectra also. Presumably silver ions are recovered directly on the surface of the textile and visible light promotes charge transfer process in a complex which silver ions form with OH groups of glucose residues in the structure of cotton.

Metal NPs are located in the structural micropores and on the surface of the fabric. Ag/Cu/tissues had yellow-brownish to red-brownish color the tint of that appeared because of formation of Ag_2O and copper protoxide Cu_2O on the surface of BMNP core [10]. In optical absorption spectra of Ag / Cu NPs on the tissue surface are the bands with maxima 430nm - the SPR band of Ag NPs and CuO (800 nm) (Figure 1). Wide absorption band with max 480nm belongs to the absorption spectrum of Ag/Cu BMNPs and is located close to the SPR band of Ag NPs. Ionizing potential of Ag NPs with the sizes of 1-2 nm is lower on 1.5 eV as compared to bulk silver. It means that the Ag+ ions are considerably easier generated from developed surface of NPs. Due to marked difference of oxidizing potentials of Cu (0.337 V) and Ag (0.799 V), copper facilitates the reduction of silver ions to NPs [11].

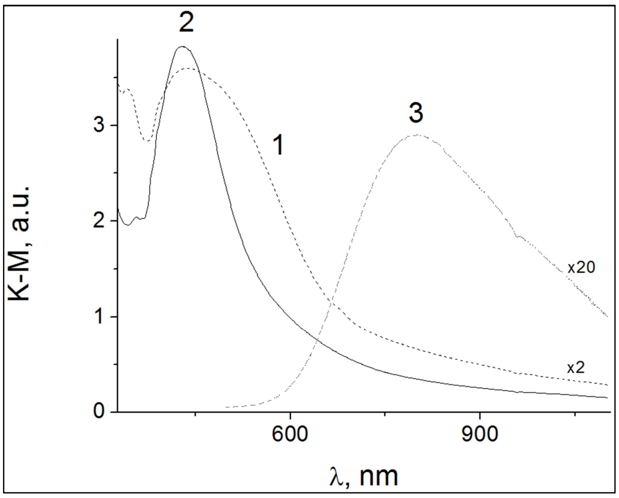
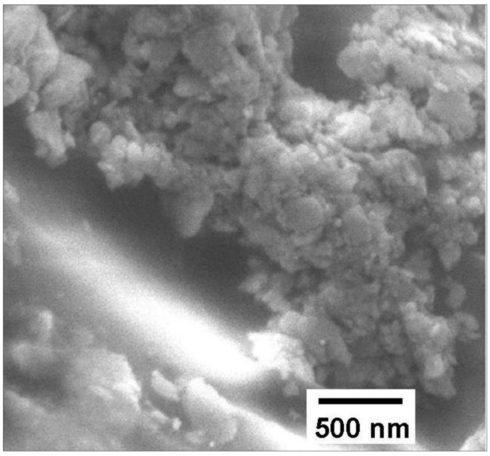


Figure 1. DRS of the tissue samples modified with Ag/Cu (1), Ag (2), Cu (3) NPs. The Y-axis is shown in the coordinates of the Kubelka-Munk function.

SEM images of tissues modified with Cu (a), Ag (b), BMNPs Ag/Cu (c) and tissue (d) are shown on the Figure 2. Images of tissues with Ag and Cu monoparticles are showen for comparison with BMNPs.



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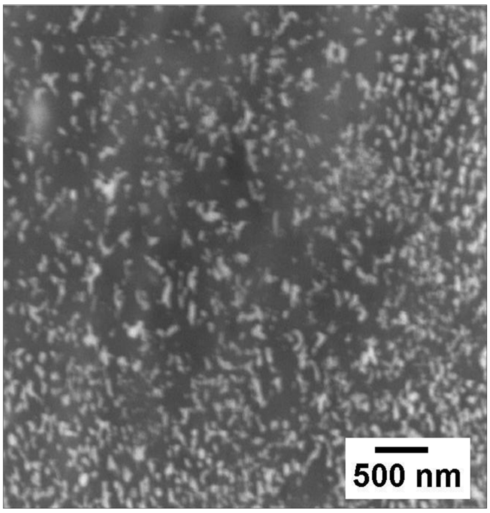

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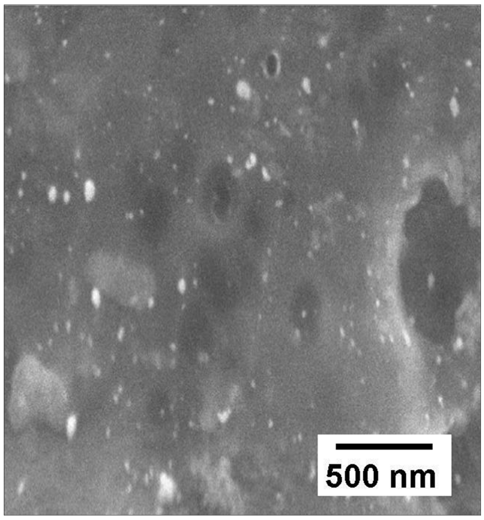

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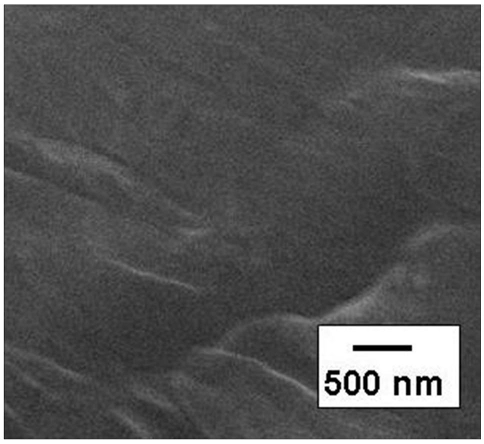

d

Figure 2. SEM images of tissues modified with Cu (a), Ag (b), Ag/Cu NPs (c) and clean tissues (d).

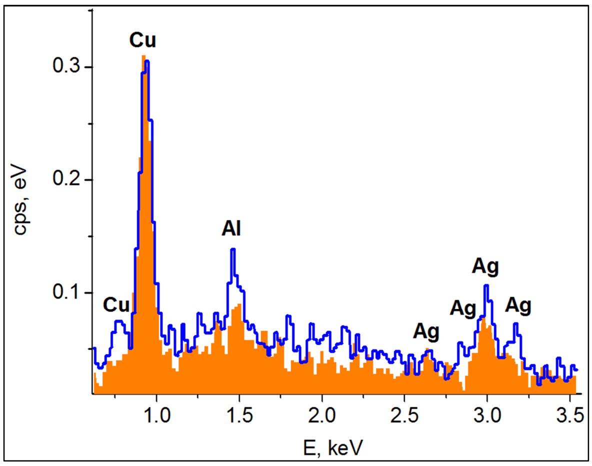

Figure 3. EDS point scan analysis of Ag/Cu NPs performed on two different places of tissue.

There are the aggregates of copper particles (Figure 2 a), Ag NPs (b) and BMNPs (c). Ag and Cu NPs are distributed on the tissue surface enough homogeneously. The electrical resistance of dry BMNPs -modified fabrics is more than 4 MOhm, the same for non-modified dry tissue. It should confirm that metal particles firmly hold out on fabric as individual clusters separated from each other. After wetting of the fabric with double-distilled water or with solution of KNO_3 the electrical resistance of the modified samples reduced sharply. Metal ions are generated on the wetted tissue' surface from BMNPs which leads to increase of the conductivity, namely electric resistance in case of wet Ag/Cu tissue is 320 ± 5 кOhm, after wetting with 0.1M solution of KNO_3 it is only 55 ± 5 кOhm as a result of significant contribution of ionic conductivity of KNO_3 to the overall electrical conductivity.

The number of Ag and Cu NPs on the surface of modified tissue calculated by us early by the method of flaming atomic-absorbing spectroscopy are app. 2.4 10^{17} atom/cm^2 for each metal that corresponds to the initial concentrations of metal salts used for the impregnation [10]. However application of EDS method showed that Ag:Cu ratios some differed on the different areas of the same sample of tissue (Figure 3).

The weight%% of the both metal within one particle registered on the different spots of tissue are shown in the Table 1.

Table 1. *Cu:Ag ratios, wt.%%, within one BMNP on 6 different spots of tissue, the values are in the ranges from 2 to 5.*

Spectrum Label	Ratio, wt.%%						Statistics			
	spot1	spot2	spot3	spot4	spot5	spot6	Min	Max	Average	Standard Deviation
Cu	5.70	4.02	2.79	2.48	2.36	6.61	2.36	6.61	3.40	3.233
Ag	1.54	1.31	1.42	0.88	1.58	1.85	0.88	1.85	1.48	0.106

It should be noted that the Ag: Cu ratio could inverse in case we used other initial Cu salt [10]. In case of copper nitrate, Ag: Cu ratio on tissue surface is app. 2:1 (does not showed in this article), because when copper sulphate was used, part of silver forms a slightly soluble in water sediment.

The results of the action of fabrics impregnated with Ag NPs and Ag/Cu composites are shown in Figure 4. The Ag/Cu modified fabrics have higher antimicrobial activity comparing with samples containing only silver.

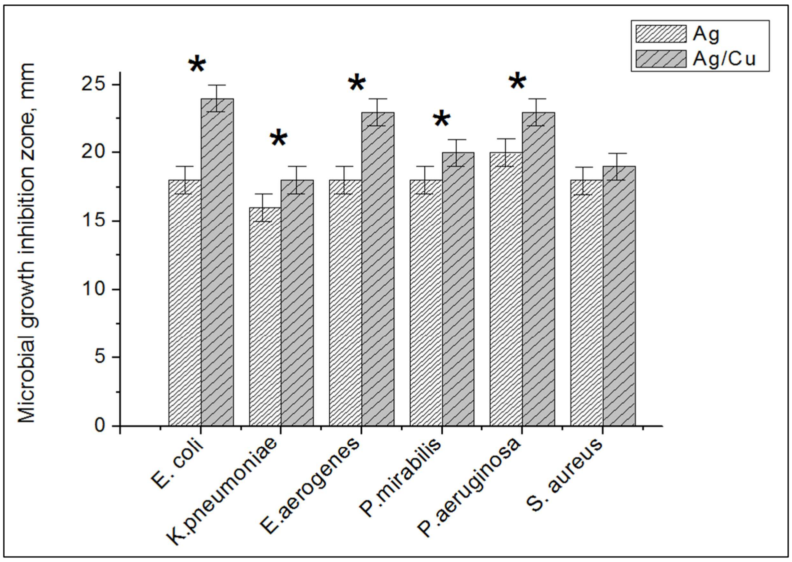

Figure 4. *Antimicrobial activity of tissue samples modified with Ag and Ag/Cu Nps. Statistically significant pairs of values of the growth inhibition zone are indicated in the figure with the symbol "*."*

4. Discussion

Metal NPs can be embedded in fibers of the tissue or coated on their surface. It is known, that silver, copper and their compounds are ionized in the presence of water and biological fluids. Both NPs and released ions are toxic for bacteria. The real contribution of NPs and the corresponding ions in the bacterial destruction is unclarified till now. Bactericidal action of cotton tissue madapollam impregnated with ions Ag+ and Cu^{2+} (without reduction to NPs) is high and comparable with the activity of the NPs/tissue samples (non-published results yet). The bactericidal activity of tissues with NPs does not change after washing with detergent, and allows them to be used several times, which was shown by us earlier [10]. We suppose that on the surface of NPs a double electric layer containing Ag^+ and Cu^{2+} in outer layer is formed. Released ions of silver and copper bind to different functional groups of bacteria and block them. Thus, the enhanced antimicrobial action of Ag/Cu compounds is probably a result of simultaneous release of Ag^+ and Cu^{2+} ions from the surface of BMNPs and their availability in combination interact with bacterial membranes. The authors [3] noted that silver ions and Ag_2O breakage the enzymes disulfide (S-S) bonds, Cu ions and Cu_2O denaturize the protein structures on viral surfaces, which confirms our assumption. Positively charged silver and copper ions form electrostatic bonds with the negatively charged surface of microorganism cells and interact with proteins.. Preliminary experiments reveal that the amount of silver and copper ions released from the surface of the Ag/Cu-modified tissue in a water solution during 24 hours is 13% and 17% respectively (non-published results yet). A similar trend- enhanced action - was observed also in the study of antitumor properties of bimetallic nanoparticles Ag/Au [12] with more pronounced action compared to the monometallic ones.

5. Conclusion

Enhanced bactericidal action of easy-produced cotton tissues modified with bimetallic Ag/Cu NPs in comparison with monometallic ones against series of bacteria occurred due to the synergetic effect. This action is probably a result of the simultaneous release of Ag^{+} and Cu^{2+} ions from the surface of BMNPs and their availability in combination interact with bacterial cell membranes. Tissues modified with Ag/Cu bimetallic NPs have great potential in the preparation of drugs against bacteria.

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