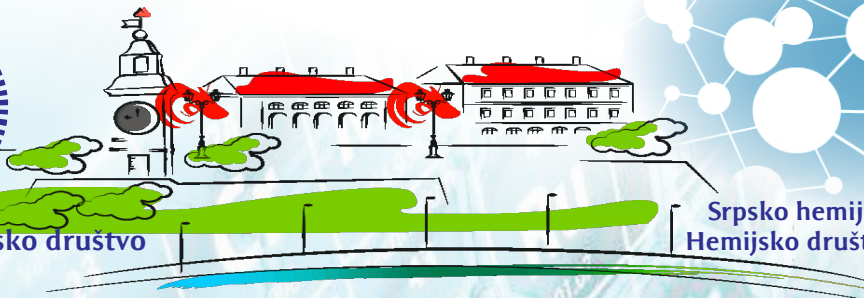




Srpsko hemijsko društvo



Srpsko hemijsko društvo  
Hemijsko društvo Vojvodine

# 55. savetovanje Srpskog hemijskog društva

# KNJIGA RADOVA

55<sup>th</sup> Meeting of  
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## Antibacterial activity of Cu-based nanoparticles synthesized on the cotton fabrics previously modified with succinic and citric acids

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### Introduction

Cu-based nanoparticles (NPs) are increasingly gaining scientific attention due to the abundance of this metal, its relative inexpensiveness compared to Ag and excellent antimicrobial activity against various bacteria strains. Gram-negative bacteria *E. coli* and Gram-positive bacteria *S. aureus* are especially sensitive to the action of Cu-based NPs which can be utilized for treatment of burns, surgical wounds and diabetic foot ulcer infections<sup>1</sup>. Recently, the potential application of copper in different forms (metallic, ionic, copper oxides, copper complexes) for fabrication of medical textiles with antimicrobial activity became the focus of several research groups<sup>2-4</sup>. The exploitation of Cu, Cu<sub>2</sub>O and CuO NPs or their mixtures for imparting antibacterial activity to cotton fabrics is of particular interest. The dominant method for synthesis of Cu-based NPs relies on the introduction of carboxyl groups to cotton fibers by coating them with adequate polymer or chemical modification of cellulose, adsorption of Cu<sup>2+</sup>-ions and their reduction.

Our approach for fabrication of Cu-based NPs on cotton fabrics was oriented towards modification of fabrics with polycarboxylic acids. It was assumed that ester bonds between the hydroxyl groups of cotton and the carboxyl groups of acids are formed while the rest of the free carboxyl groups can be utilized for adsorption of Cu<sup>2+</sup>-ions from aqueous solution of CuSO<sub>4</sub>. To get better insight into the influence of carboxyl group number in the acid structure and further, on the cotton fabric, the study was carried out using a dicarboxylic succinic acid and tricarboxylic citric acid.

### Experimental

Desized and bleached cotton (Co) woven fabric (117.5 g/m<sup>2</sup>, 52 picks/cm, 27 ends/cm, thickness of 0.26 mm) was used as a substrate. Co fabrics were cleaned in the bath containing 0.1% nonionic washing agent Felosan RG-N (Bezema) at liquor-to-fabric ratio of 50:1. After 15 min of washing at 50 °C, the fabrics were rinsed first with warm water (50 °C) and then thoroughly with cold water. The samples were dried at room temperature.

Modification of Co fabrics with succinic and citric acids was carried out by immersion of 0.50 g of the sample in 20 mL of the acid aqueous solution (10 w/v) in the presence of 2.06 g of the catalyst sodium hypophosphite (SHP) for one hour. After drying at 80 °C for 10 min the samples were cured at 170 °C for 3 min. The samples were then rinsed in distilled water and dried at room temperature. Co fabrics modified with succinic and citric acids are marked as Co+SUC and Co+CA, respectively.

Subsequently, 0.50 g of Co+SUC and Co+CA fabrics were soaked in 25 mL of 10 mM solution of  $\text{CuSO}_4$  for 2 h. In order to eliminate the excessive  $\text{Cu}^{2+}$ -ions, the samples were rinsed three times with deionized water. 0.050 g of sodium borohydride ( $\text{NaBH}_4$ ) was dissolved in 25 mL of 0.1 mM NaOH solution and the samples were immediately dipped into the solution where the reduction process took place in the following 30 min at room temperature. The samples were thoroughly rinsed with deionized water and left to dry at room temperature. These samples are marked as Co+SUC+Cu and Co+CA+Cu.

Determination of carboxyl content in oxidized Co fabric was based on the calcium acetate method described by Kumar and Yang and modified by Praskalo *et al.*<sup>5,6</sup>.

Fourier transform infrared (FTIR) spectra of the control Co fabric, Co fabrics modified with polycarboxylic acids and Co fabrics modified with polycarboxylic acids after rinsing in 0.1 M NaOH solution were recorded in the ATR mode using a Nicolet 6700 FTIR Spectrometer (Thermo Scientific) at  $2\text{ cm}^{-1}$  resolution, in the wavenumber range  $500\text{--}4000\text{ cm}^{-1}$ .

The morphology of the control and Co fibers impregnated with Cu-based NPs was assessed by field emission scanning electron microscopy (FESEM, Tescan Mira3 FEG). The samples were coated with a thin layer of Au prior to analysis.

The amounts of adsorbed  $\text{Cu}^{2+}$ -ions on the Co+SUC and Co+CA fabrics from  $\text{CuSO}_4$  solution were calculated on the basis of the concentration of residual  $\text{Cu}^{2+}$ -ions in the solution which was measured using a Spectra AA 55 B (Varian) atomic absorption spectrometer (AAS). AAS was also used for the measurement of the total Cu content in the Co fabrics after reduction process. Dry impregnated Co fabrics were dissolved in the 1:1  $\text{HNO}_3$  solution.

X-ray photoelectron spectroscopy (XPS) measurements were performed in order to evaluate the chemistry of the control Co, Co+SUC+Cu and Co+CA+Cu fabrics. The XPS analysis was carried out using a K-Alpha spectrometer (Thermo Scientific, UK) utilizing a monochromated Al  $K\alpha$  ( $h\nu = 1486.6\text{ eV}$ ) X-ray source.

The antibacterial activity of Co fabrics was tested against Gram-negative bacteria *E. coli* ATCC 25922 and Gram-positive bacteria *S. aureus* ATCC 25923 using a standard test method for the determining the antimicrobial activity of immobilized antimicrobial agents under dynamic contact conditions ASTM E 2149-01 (2001). The percentage of bacterial reduction ( $R$ , %) was calculated by the following equation:

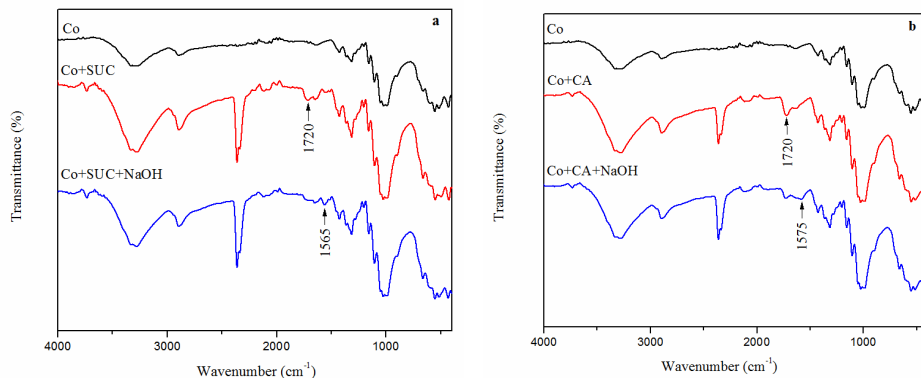
$$R = \frac{C_0 - C}{C_0} \cdot 100$$

where  $C_0$  (CFU – colony forming units) is the number of bacteria colonies on the control fabric and  $C$  (CFU) is the number of bacteria colonies on the fabric with NPs.

## Results and discussion

Chemical changes induced by modification of Co fabrics with polycarboxylic acids were assessed by FTIR spectroscopy. FTIR spectra of the Co+SUC and the Co+CA fabrics are shown in Fig. 1a and 1b, respectively. The bands characteristic for cellulose are clearly identified in all spectra. The appearance of the band with a peak centered at  $1720\text{ cm}^{-1}$  in the spectra of the Co+SUC and Co+CA fabrics proved the formation of ester bonds between hydroxyl groups of cellulose and carboxyl groups of polycarboxylic acids<sup>7,8</sup>. An immersion of the samples in 0.1 M solution of NaOH caused the deprotonation of free carboxyl groups i.e. a formation of carboxylates. The presence of carboxylates was confirmed by appearance of the band at  $1565/1575\text{ cm}^{-1}$ . The existence of both bands at  $1720\text{ cm}^{-1}$  and  $1565/1575\text{ cm}^{-1}$

in these spectra showed that applied polycarboxylic acids were bound to cellulose by ester bonds, but a certain number of carboxyl groups of the SUC and CA remained free.



**Figure 1.** FTIR spectra of the control samples and the samples modified with succinic acid (a) and citric acid (b)

The content of free carboxyl groups in the Co+SUC and Co+CA fabrics was determined titrimetrically. The results in Table 1 show that the content of free carboxyl groups was higher in the Co+CA than in the Co+SUC fabric. This indicates that the higher the content of carboxyl groups in precursor acid the higher content of free carboxyl groups in the sample. Free carboxyl groups introduced to Co fabrics are considered as potential sites for binding of Cu<sup>2+</sup>-ions and they were exploited for their adsorption. The Cu<sup>2+</sup>-ions uptakes of Co fabrics modified with polycarboxylic acids after 2 hours long adsorption in aqueous solution of CuSO<sub>4</sub> are also summarized in Table 1. As expected, the larger the number of free carboxyl groups, the larger the uptake of Cu<sup>2+</sup>-ions. The total Cu content in Co fabrics impregnated with Cu-based NPs after reduction of adsorbed Cu<sup>2+</sup>-ions was determined by AAS (Table 1). A trend of increase in total Cu content with an increase of free carboxyl groups in Co fabrics was maintained.

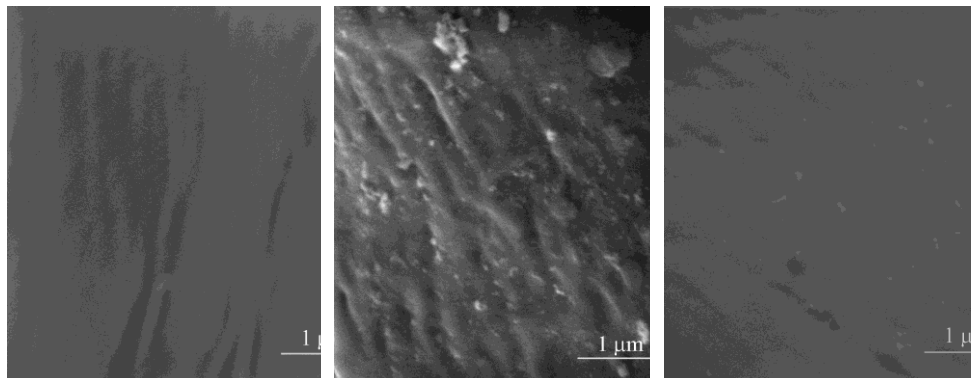
**Table 1** Carboxyl groups content, Cu<sup>2+</sup>-ions uptake and total content of Cu after Cu<sup>2+</sup>-ions reduction in Co fabrics modified with polycarboxylic acids

Sample	COOH content, μmol/g	Cu <sup>2+</sup> -ions uptake, μmol/g	Total content of Cu after reduction, μmol/g
Co+SUC	304±59	81±13	58±3.7
Co+CA	436±62	102±4.5	91±1.8

The presence of Cu-based NPs was confirmed by FESEM analysis. The FESEM images of the control, Co+SUC+Cu and Co+SUC+Cu samples are shown in Fig. 2a, 2b and 2c, respectively. As can be seen, Cu-based NPs are unevenly distributed over the surface of the fibers.

In order to get insight into the oxidation state of the copper in fabricated NPs a single points on the surface of the Co+SUC+Cu and Co+CA+Cu samples were analyzed by XPS. A high resolution scans of the Co+SUC+Cu and Co+CA+Cu samples were accomplished in the Cu2p region and they are shown in Fig. 3a and 3b, respectively. Fig. 3 shows two main peaks corresponding to Cu2p<sub>3/2</sub> and Cu2p<sub>1/2</sub> followed by shakeup satellites in the Cu2p spectra of all investigated samples.

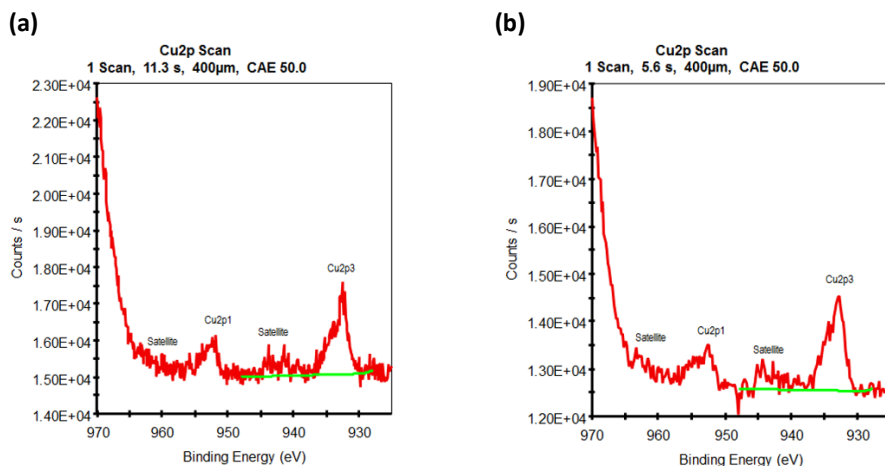




**Figure 2.** The FESEM images of the control (a), Co+SUC+Cu (b) and Co+CA+Cu (c) fibers

Asymmetric  $\text{Cu}2p_{3/2}$  peak can be deconvoluted into two components: the peak related to metallic Cu or  $\text{Cu}_2\text{O}$  and the peak assigned to CuO. There is no doubt that copper appeared in the form of CuO in studied NPs since the shakeup satellites are evident in both  $\text{Cu}2p$  spectra. The shakeup satellites are characteristic of materials possessing a  $d^9$  configuration like in a  $\text{Cu}^{2+9}$ . On the basis of XPS results it was not possible to distinguish metallic Cu from the  $\text{Cu}_2\text{O}$  form due to their spectral overlap<sup>9</sup>. The difference in binding energies of the  $\text{Cu}2p_{3/2}$  signal for these two forms is only 0.1 eV (932.6 and 932.7 eV for Cu and  $\text{Cu}_2\text{O}$ , respectively). Therefore, it is suggested that synthesized Cu-based NPs consist of the mixture of Cu/ $\text{Cu}_2\text{O}$  and CuO.

The antibacterial activity of the Co fabrics was tested against Gram-negative bacterium *E. coli* and Gram-positive *S. aureus*. The Co+SUC and Co+CA samples alone did not show any antibacterial activity. The results summarized in Table 2 show that the presence of the Cu-based NPs in the Co+SUC+Cu and Co+CA+Cu fabrics ensured 99.9 % bacterial reduction of both bacteria colonies. It is generally accepted that the larger the amount of loaded Cu-based NPs on the textile substrates, the stronger the antibacterial activity. However, we cannot discuss the results from this point of view as obtained Cu contents were sufficiently high for imparting desired level of antibacterial activity to both studied Co fabrics.



**Figure 3.** XPS high resolution spectra of the Co+SUC+Cu (a) and Co+CA+Cu (b) fabrics in the  $\text{Cu}2p$  region

**Table 2** Antibacterial activity of the Co fabrics impregnated with Cu-based NPs

Sample	Number of bacterial colonies (CFU)	R, %	Number of bacterial colonies (CFU)	R, %
	<i>E. coli</i>		<i>S. aureus</i>	
Control Co	3.5×10 <sup>5</sup>		2.6×10 <sup>4</sup>	
Co+SUC+Cu	<10	99.9	<10	99.9
Co+CA+Cu	<10	99.9	<10	99.9

### Conclusions

Modification of cotton fabrics with succinic and citric acids ensured carboxyl groups required for adsorption of Cu<sup>2+</sup>-ions. Dicarboxylic citric provided higher content of free carboxyl groups on the fabric which led to a larger uptake of Cu<sup>2+</sup>-ions and generation of larger amounts of Cu-based nanoparticles which were detected by FESEM analysis. XPS analysis suggested that nanoparticles existed in the form of mixture of CuO and Cu/Cu<sub>2</sub>O. The presence of Cu-based nanoparticles on both cotton fabrics modified with polycarboxylic acids provided 99.9 % reduction of Gram-negative bacteria *E. coli* and Gram-positive bacteria *S. aureus*, indicating that fabricated textile nanocomposite exhibit excellent antibacterial activity.

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### Antibakterijska aktivnost nanočestica na bazi Cu sintetisanih na pamučnim tkaninama prethodno modifikovanim ćilibarnom i limunskom kiselinom

U ovom radu je diskutovana mogućnost *in situ* sinteze nanočestica na bazi Cu na pamučnim tkaninama prethodno modifikovanim ćilibarnom i limunskom kiselinom u cilju dobijanja tekstilnih nanokompozita sa antibakterijskom aktivnošću. Utvrđeno je da veći broj karboksilnih grupa u prekursoru obezbeđuje veći broj slobodnih karboksilnih grupa na tkanini, a time veću sorpciju Cu<sup>2+</sup>-jona iz rastvora CuSO<sub>4</sub> i veći sadržaj nanočestica nakon redukcije. Prisustvo nanočestica na pamučnoj tkanini potvrđeno je SEM analizom, a XPS analizom je ustanovljeno da se sintetisane nanočestice sastoje iz metalnog Cu/Cu<sub>2</sub>O i CuO. Ispitivani nanokompoziti ostvarili su maksimum redukcije bakterija Gram-negativne *E. coli* i Gram-pozitivne *S. aureus*.

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