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Serbian Young Chemists' Club



**54. SAVETOVANJE
SRPSKOG HEMIJSKOG DRUŠTVA**

**5. KONFERENCIJA
MLADIH HEMIČARA SRBIJA**

**KRATKI IZVODI
i
KNJIGA RADOVA**

**54th MEETING OF
THE SERBIAN CHEMICAL SOCIETY**

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KRATKI IZVODI I KNJIGA RADOVA

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***In situ* synthesis of Cu/Cu₂O nanoparticles on the TEMPO oxidized cotton fabric**

Darka Marković, Matea Korica, Mirjana Kostić*, Željko Radovanović, Zoran Šaponjić**, Miodrag Mitrić**, Maja Radetić*

Innovation Center of the Faculty of Technology and Metallurgy, University of Belgrade, Karnegijeva 4, Belgrade, Serbia

**Technology and Metallurgy, University of Belgrade, Karnegijeva 4, Belgrade, Serbia*

***Institut of Nuclear Sciences "Vinča", University of Belgrade, P.O. Box 522, Belgrade, Serbia*

Introduction

Recent breakthrough in nanotechnologies had large impact on research in the field of medical textiles. So far many efforts have been made to fabricate antimicrobial textile materials with incorporated metal (Ag, Cu) and metal oxide (TiO₂, ZnO, CuO, Cu₂O) nanoparticles (NPs) and to learn more about the mechanism of their action¹⁻³. The possible application of Cu, CuO and Cu₂O NPs for antibacterial protection of cotton fabrics became the focus of many research groups since the copper in different forms (ionic, copper oxides, copper complexes) is very active against various bacterial strains and precursor salts of copper are much cheaper than those of silver.

Unlike in the case of Ag NPs where the dip-coating of cotton fabrics, *in situ* synthesis of Cu, CuO and Cu₂O NPs seems to be the method of choice in published studies until now^{4,5}. Namely, the dominant approach relies on the introduction of carboxyl groups to cotton fibers by coating them with appropriate polymer or chemical modification of cellulose, adsorption of Cu²⁺-ions and their reduction. In our study we applied the TEMPO/NaClO/NaClO₂ system under neutral conditions for selective oxidation of cellulose in order to enhance its ability to bind Cu²⁺-ions. We focused our study on much shorter oxidation times since the aim was to preserve the structure of cellulose fibers i.e. their mechanical strength. Therefore, the main goal of our study was to discuss the influence of the functional groups generated by TEMPO-mediated oxidation of cotton fabric at different reaction times on the amount, activity and stability of *in situ* synthesized Cu/Cu₂O NPs using sodium borohydride as a reducing agent at room temperature.

Experimental

Desized and bleached cotton (Co) woven fabric (117.5 g/m², 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.

The TEMPO-mediated oxidation was carried under neutral condition (pH 7) according to the method described previously^{5,6}. Co fabric (10 g) was soaked in 0.05 M sodium phosphate buffer solution at pH 7 (50 mL phosphate buffer solution/g Co fabric), containing TEMPO (20 mg TEMPO/g Co fabric). Sodium chlorite (50 mg NaClO₂ /g Co fabric) and sodium hypochlorite solution (5 mmol NaClO/g Co fabric) were added to the flask and stirred at 200 rpm and 60°C for fixed periods of time: 15 and 45 min. After stirring for a designed time, the oxidation was quenched by adding ethanol (ca. 5 mL). The oxidized Co fabric was thoroughly washed with water by filtration, subsequently with ethanol, and dried at room temperature. Depending on the duration of oxidation, TEMPO oxidized Co fabrics are marked as Co15 and Co45 in the following text.

To obtain oxidized Co fabric containing only carboxyl groups, the aldehyde groups were selectively oxidized to carboxyl groups with NaClO₂ at pH 4–5. Oxidation was carried out by stirring the mixture comprising of distilled water (50 mL), 5 M CH₃COOH (10 mL), NaClO₂ (0.905 g) and Co fabric (1 g) at room temperature for 48 h, followed by washing thoroughly with water by filtration. 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.^{7,8}.

A procedure for the *in situ* synthesis of Cu-based NPs was adopted from Errokh et al.⁵, though several changes have been made including the introduction of sodium borohydride as a reducing agent instead of hydrazine and hydroxylamine. Briefly, 0.50 g of TEMPO oxidized Co fabric was soaked in 25 mL of 10 mM solution of CuSO₄ for 2 h. In order to eliminate the excessive Cu²⁺-ions, the samples were rinsed for three times (1 min) in deionized water. 0.050 g of sodium borohydride (NaBH₄) 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 Co15+Cu/Cu₂O and Co45+Cu/Cu₂O.

The amounts of adsorbed Cu²⁺-ions on the TEMPO oxidized Co fabrics from CuSO₄ solution were calculated on the basis of the concentration of residual Cu²⁺-ions in the solution which was measured using a Spectra AA 55 B (Varian) atomic absorption spectrometer (AAS). AAS was also utilized for determination of total Cu content in the Co fabrics after reduction process. Dry impregnated Co fabrics were dissolved in the 1:1 HNO₃ solution. Additionally, AAS was used for the evaluation of Cu²⁺-ions release from the sample in physiological saline solution.

The morphology of Co fibers impregnated with Cu/Cu₂O NPs was assessed by field emission scanning electron microscopy (FESEM, Mira3 Tescan). The samples were coated with a thin layer of Au prior to analysis.

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:

$$R = \frac{C_0 - C}{C_0} \cdot 100 \quad (1)$$

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.

Cu²⁺-ions release was tested by immersing the sample of impregnated fabric in physiological saline solution (9 g/L NaCl) at 37 °C in static conditions. The concentration of released Cu²⁺-ions was measured after 1, 3, 6 and 24 hours by Spectra AA 55 B (Varian) atomic absorption spectrometer.

Results and discussion

The duration of TEMPO-mediated oxidation can be used as an efficient parameter for tailoring the carboxyl group contents in oxidized Co fabrics. Table 1 shows the relation between oxidation time and carboxylic group content in the oxidized Co fabrics. The longer the oxidation time the larger the amounts of carboxylic groups. Generated carboxylic groups were the potential sites for adsorption of Cu²⁺-ions from aqueous CuSO₄ solution. The values of Cu²⁺-ions uptake after 2 hours long adsorption are also given in Table 1. Evidently, an increase in carboxylic group content brought about an increase in Cu²⁺-ions uptake.

A change in the Cu²⁺-ions uptake can be also visually evaluated by change of the color of fabrics from white to different shades of blue. The photographs of samples after adsorption of Cu²⁺-ions are presented in Fig. 1. Obviously, the larger the Cu²⁺-ions uptake the bluer the samples.

Table 1 COOH groups content in TEMPO oxidized Co fabrics under different conditions, Cu²⁺-ions content after adsorption and total Cu content after reduction of Cu²⁺-ions

Sample	COOH content, $\mu\text{mol/g}$	Cu ²⁺ -ions uptake, $\mu\text{mol/g}$	Total content of Cu after reduction, $\mu\text{mol/g}$
Co15	631.9 \pm 23.9	142 \pm 1.13	134 \pm 8.76
Co45	792.9 \pm 18.5	148 \pm 10.1	146 \pm 9.61

Cu²⁺-ions adsorbed on the TEMPO oxidized Co fabrics were in the next step reduced with sodium borohydride in alkaline conditions at room temperature. Both samples immediately changed the color from pale blue to dark brown as can be seen in Fig.1. It is supposed that the brown color appeared due to reduction of Cu²⁺-ions and formation of Cu NPs. This color change was confined only to the fabrics. In other words, it could not be visually detected in the solution containing reducing agent. These results are practically confirmed by the values of Cu content that are summarized in Table 1. It is obvious particularly in the case of Co45 sample that the whole amount of Cu²⁺-ions was converted into Cu/Cu₂O NPs.

However, obtained brown color gradually changed during the drying of samples at room temperature. The samples became green (Fig. 1). Again, the color yield depended on the Cu content in the samples. The larger the Cu content the greener the sample. The instability of metallic Cu NPs in the air, particularly when borohydride is used as a reducing agent is well known feature. As our samples were dried and stored in the air, it is very likely that oxidation process took place in these phases. Cady et. al. observed the same behavior⁴. They suggested that the reduction of Cu²⁺-ions initially resulted in the formation of Cu NPs and then the drying in the air caused a partial oxidation to Cu₂O. These authors also assumed that the core/shell form of NPs (Cu core/Cu₂O shell) was obtained, but we do not have strong proof for such assumption.

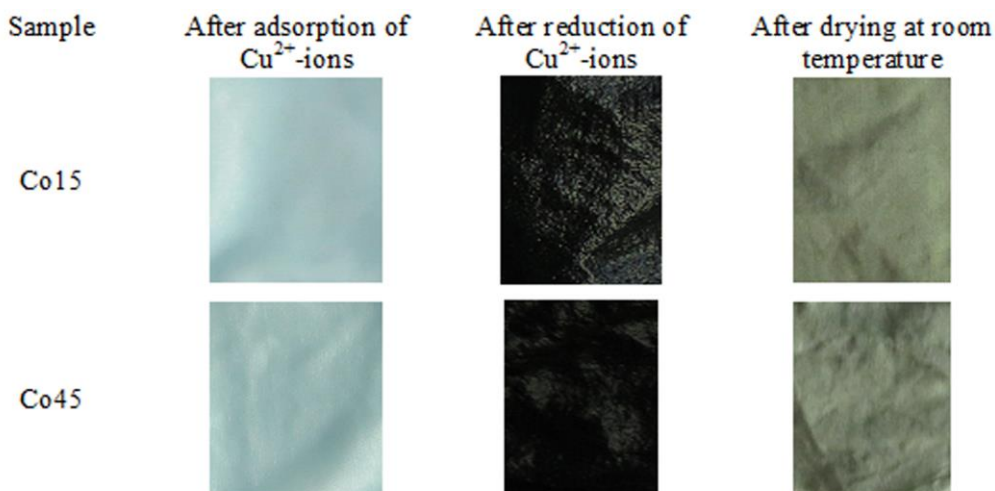


Figure 1. The photographs of the fabrics with adsorbed Cu²⁺-ions, the fabrics immediately after Cu²⁺-ions reduction and the fabrics after drying in air at room temperature

The morphology of fibers surface was evaluated by SEM analysis. SEM images of the control fiber and the TEMPO oxidized fibers impregnated with Cu/Cu₂O NPs are shown in Fig. 2. Presented images confirmed the presence of unevenly distributed Cu/Cu₂O NPs with an average dimension around 50 nm. It is also evident that NPs agglomerated on the fiber surface.

Antibacterial activity of Cu/Cu₂O NPs immobilized on the TEMPO oxidized Co fabrics was tested against Gram-negative bacteria *E. Coli* and Gram-positive bacteria *S. aureus*. The results from Table 3 indicate that both investigated samples provided maximum bacterial reduction (99.9 %) for both

bacteria strains. It is generally accepted that the larger the amount of Cu-based NPs, the stronger the antibacterial activity⁵. In our study such tendency was not observed likely due to sufficiently large amounts of NPs that could impart desired antibacterial activity to Co fabrics.

Taking into account the key role of Cu^{2+} -ions in bacteria cell inactivation, it was necessary to study their release from fabricated samples. In order to simulate the body fluids physiological saline solution was applied as a release medium. Since both investigated samples provided equivalent antibacterial activity, the sample Co15+Cu/Cu₂O was chosen for release experiments due to shorter TEMPO oxidation time. Cumulative release of Cu^{2+} -ions in the physiological saline solution is presented in Fig. 3. Keeping in mind that the potential application of such textile nanocomposite would be for the production of wound dressings, a controlled leaching of Cu^{2+} -ions during short time interval (less than 24 h) would be preferable for infection prevention. It is evident from Fig. 3 that fabricated sample meets this important demand.

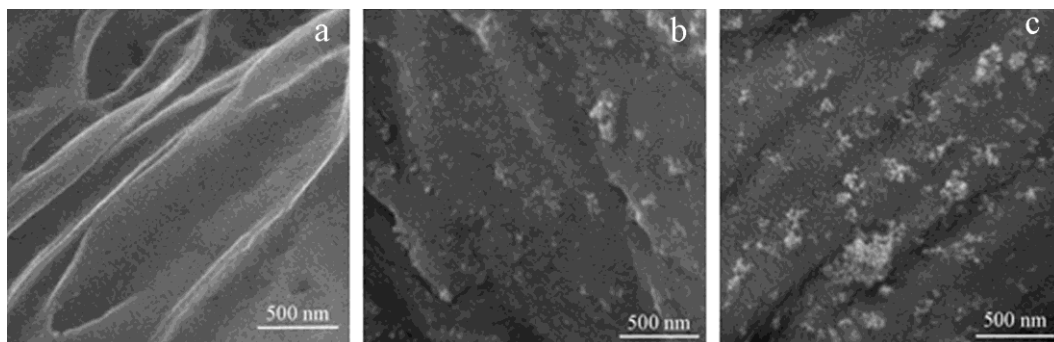


Figure 2. SEM images of the (a) untreated Co fiber, (b) Co15+Cu/Cu₂O fiber and (c) Co45+Cu/Cu₂O fiber

Table 2 Antimicrobial activity of Co fabrics impregnated with Cu/Cu₂O NPs

Sample	Number of bacterial colonies (CFU)		R, %	
	<i>E. coli</i>	<i>S. aureus</i>		
Control Co	2×10^5	3×10^4		
Co15+Cu/Cu ₂ O	<10	<10	99.9	99.9
Co45+Cu/Cu ₂ O	<10	<10	99.9	99.9

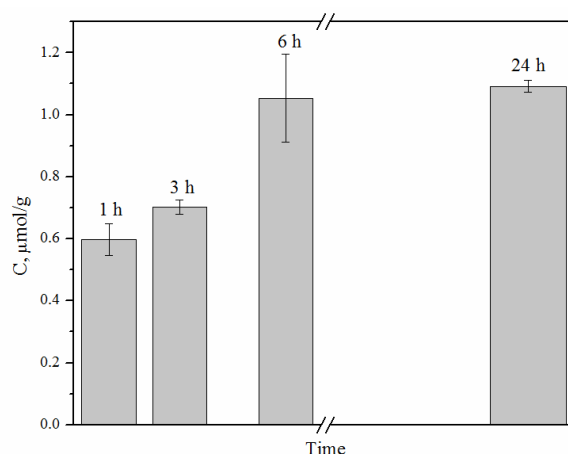


Figure 3. Release of Cu^{2+} -ions from the Co15+Cu/Cu₂O fabric into physiological solution

Conclusions

Cu/Cu₂O nanoparticles were successfully *in situ* synthesized on the surface of TEMPO-oxidized cotton fabrics. TEMPO-mediated oxidation of cellulose enhanced the adsorption of Cu²⁺-ions due to formation of carboxyl groups. The larger the amounts of carboxyl groups the larger the Cu²⁺-ions adsorption. Reduction of Cu²⁺-ions with sodium borohydride resulted in the formation of Cu/Cu₂O nanoparticles. SEM analysis revealed uneven distribution of agglomerates of nanoparticles with an average dimension of approximately 50 nm.

Obtained textile nanocomposites provided maximum bacterial reduction of 99.9 % against Gram-negative *E. coli* and Gram-positive *S. aureus*. Controlled release of Cu²⁺-ions from the samples into physiological solution was achieved, making them viable candidates for medical applications.

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In situ sinteza Cu/Cu₂O nanočestica na TEMPO oksidovanoj pamučnoj tkanini

*In situ sinteza nanočestica Cu/Cu₂O na pamučnoj tkanini diskutovana u ovom radu bazirana je na adsorpciji Cu²⁺-jona na karboksilnim grupama formiranim TEMPO oksidacijom celuloze i njihovoj redukciji natrijum-borhidridom. Hemijske promene nastale TEMPO oksidacijom su praćene titrimetrijski određivanjem sadržaja karboksilnih grupa u pamuku. Prisustvo nanočestica Cu/Cu₂O na pamučnoj tkanini potvrđeno je SEM i AAS analizama. Promene obojenja tkanina su određene refleksionom spektrofotometrijom. Antibakterijska aktivnost generisanih nanočestica Cu/Cu₂O je testirana prema Gram-negativnoj bakteriji *E. coli* i Gram-pozitivnoj bakteriji *S. aureus*.*

*Produžavanje vremena TEMPO oksidacije za rezultat je imalo povećanje sadržaja karboksilnih grupa na pamučnoj tkanini. Stoga su veće količine Cu²⁺-jona mogle da se adsorbuju na supstratu. Ovo je dalje za posledicu imalo stvaranje većih količina nanočestica Cu/Cu₂O. Svi ispitivani tekstilni nankompoziti su obezbedili maksimum redukcije bakterija *E. coli* i *S. aureus*. Takođe je utvrđeno da pamučne tkanine sa inkorporiranim nanočesticama Cu/Cu₂O pružaju kontrolisano otpuštanje Cu²⁺-jona u fiziološkom rastvoru što je imperativ za prevenciju infekcija.*

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