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Physicochemical and antibacterial characterization of ionocity Ag/Cu powder nanoparticles

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ABSTRACT

Metal ion in bimetallic nanoparticles has shown vast potential in a variety of applications. In this paper we show the results of physical and chemical investigations of powder Ag/Cu nanoparticles obtained by chemical synthesis. Transmission electron microscopy (TEM) experiment indicated the presence of bimetallic nanoparticles in the agglomerated form. The average size of silver and copper nanoparticles is 17.1(4) nm (Ag) and 28.9(2) nm (Cu) basing on the X-ray diffraction (XRD) data. X-ray photoelectron (XPS) and Raman spectroscopies revealed the existence of metallic silver and copper as well as Cu_2O and CuO being a part of the nanoparticles. Moreover, UV–Vis spectroscopy showed surface alloy of Ag and Cu while Time of Flight Secondary Ion Mass Spectroscopy (ToF-SIMS) and Energy Dispersive X-ray Spectroscopy (EDX) showed heterogeneously distributed Ag structures placed on spherical Cu nanoparticles. The tests of antibacterial activity show promising killing/inhibiting growth behaviour for Gram positive and Gram negative bacteria.

1. Introduction

Metallic nanoparticles have been intensively studied systems since 1980s. Their role in daily life rapidly rises year by year [1]. Colloidal, powder or thin film metallic nanoparticles such as silver [2,3], copper [1,4], platinum [5], zinc [6] or gold [7] are well-known for their optical, catalytic, electronic, magnetic and biological properties [8–21] with differs properties or similar with the bulk form [22]. Silver nanoparticles are commonly investigated due to their antibacterial properties and possibility of their potential usage in variety applications. Monometallic Ag nanoparticles have recently been applied to cleaning products in order to intensify their antibacterial properties as well as to eliminate used nowadays toxic chemicals from the environment. Moreover, antibacterial properties of nanoparticles are also used in the connection with cotton (silver nanoparticle hybrids) as an antibacterial underwear [23]. Latterly, Hajipour et al. [14] showed that impact of metallic and bimetallic nanoparticles on the environment and living organism depends on their structure, properties, transport into cell and interaction with microorganisms.

The influence of nanostructures on microorganism cell growth and activity is very complicated. Nanoparticles can interact with the cell walls and cytoplasmic membranes leading to changes in their structure and properties (liquidity, permeability). Effects like uncontrolled flow and transport of ions/metabolites from the cell into the environment have been recently observed [14,15].

Additionally nanoparticles can penetrate through cell binding with various organelles and cause increased negative effect on the functioning as a whole cell [17]. According to literature [24] it has been shown that influence of Ag nanoparticles on *Escherichia coli* cells causes the deformation of their cell membrane and osmotic stress. Moreover, Precibal at al. [25] and Jung et al. [19] showed that low concentration of Ag^+ nanoparticles had influence on electrochemical gradient through the cell membrane. It causes depolarization of cell membrane which leads to cell death. Also C. Santo et al. [10] reported that dry copper kills bacteria at the contact with copper surfaces.

Furthermore, metallic nanoparticles, due to their properties for example optical, particularly color switching can be used as UV indicators or fashionable accessories in textiles [9]. Recently, it has been noticed that, obtained by relatively low-cost, copper nanoparticles may replace commonly used silver or gold nanoparticles in production of antibacterial and conductive materials. Additionally, copper nanoparticles can effectively absorb aqueous arsenic species [26] and have potential application in the cooling systems [27]. To use the attributes of both copper and silver nanoparticles, we are considering bimetallic system contain both copper and silver nanoparticles. Improved properties such bimetallic systems, comparing to single metallic ones have been recently reported. For example bimetallic nanoparticles Ag-Cu can be

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applied as precursors of conductive ink [28]. Recently, Czaplinska et al. [29] and Shin et al. [30] have pointed to their good catalytic properties. It is worthwhile to note that the bimetallic nanoparticles can also be used in electronic devices especially in medicine area. Jin et al. [31] latterly showed, that CuO-AgO_2 system can be applied as sensors of non-enzymatic glucose.

Metallic and bimetallic nanoparticles can be obtained by several methods such as thermal reduction [32], vacuum vapor deposition [33], microwave irradiation methods [34], laser ablation [35], laser-assisted galvanic replacement [36], chemical reduction [1,4,22,37] and polyol method [29,38]. Depending on a fabrication method such a system may exhibit different physical and chemical properties. The aim of this paper is to report the process of chemical synthesis of powder Ag/Cu nanoparticles (NPs) and characterize their properties with the use of X-ray diffraction (XRD), X-ray photoelectron spectroscopy (XPS), Raman Spectroscopy (RS), UV–Vis spectroscopy as well as Scanning (SEM) and Transmission (TEM) Electron Microscopy. The biological influence of bimetallic nanoparticles on *Escherichia coli* and *Bacillus subtilis* is also discussed.

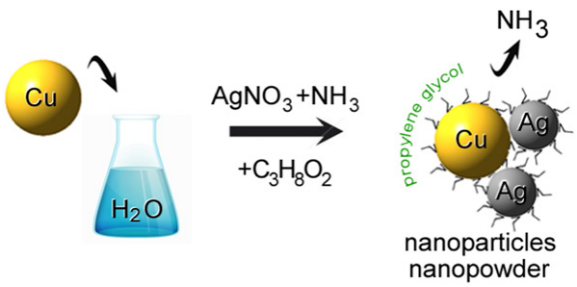
1.1. Experimental procedure

1.1.1. Synthesis of the silver - copper powder nanoparticles

The bimetallic silver - copper powder nanoparticles were obtained by chemical synthesis. In the first step the copper nanoparticle synthesis was made by polyol method as described in our earlier work [38]. All chemical reagents used in the experiment were of analytical grade and were obtained without purification. According to Tsuji et al. [39] the $Cu(OAc)_2$ was used as a base substance. The amount of 0.99 g copper nanoparticles obtained in the first step of synthesis was suspended in 100 ml deionized water in the ultrasonic bath. Next the whole mixture was stirred by the magnetic stirrer and 100 g propylene glycol with concentrated ammonia was added to the solution drop-wise. Next, the separately prepared mixture of 0018 g $AgNO_3$ suspended in 5 ml deionized water with 1 ml propylene glycol was added drop-wise to the mixture of copper nanoparticles at the room temperature. The final mixture was stirred to complete the reaction for 5 min and separated on the high-speed centrifuge with 18,000 RPM by 10 min. Subsequently, the nanoparticles produced in such process were washed in 50 ml deionized water and 150 ml ethyl alcohol in the ultrasonic bath for 10 min. In the last step, the Ag/Cu nanoparticles were filtered on the micro cellulose filter in the Buchner funnel. The process is illustrated in Scheme 1.

1.1.2. Characterization of nanoparticles

The X-ray photoelectron spectroscopy (XPS) measurements were taken with the use of PHI 5700 Physical Electronics spectrometer. Monochromatized Al Kα radiation was used. The survey spectra for bimetallic nanoparticles showed principal core levels of C, O, Ag and Cu. The spectra were corrected for the background signal using the iterated Shirley algorithm, while the bands were fitted by a composition of Gaussian and Lorentzian lines.



Scheme 1. Synthesis process of Ag/Cu powder nanoparticles.

The powder patterns were recorded with the use of Empyrean Panalytical X-ray diffractometer in the Bragg-Brentano geometry using Cu Kα radiation (λ = 1.54 Å). The step-scan covered the angular range 20–110° with the step of 0.02°. Scanning electron microscopy (SEM) images were obtained by a FE-SEM 7600F Jeol instrument equipped with an energy dispersive X-ray spectroscope (EDX). Transmission Electron Microscopy (TEM) micrographs were collected with the use of JEOL 3010 microscope working at 300 kV equipped with 2 k × 2 k Orius TM 833 SC200D Gatan CCD camera. The data collected by Time of Flight - Secondary Ion Mass Spectrometry (ToF-SIMS) were obtained by ION-TOF - TOF.SIMS.5 spectrometer equipped with a bismuth liquid metal ion gun. High lateral resolution maps of ions distribution were obtained by applying the Fast Imaging Mode. Focused high-energy primary ion beam (30 keV Bi^+ ions at an ion current of about 0.1 pA) was aimed at the sample surface causing the emission of secondary ions.

The Raman measurements were made by a WITec confocal CRM alpha 300 Raman spectrometer equipped with a laser operating at 532 nm. A dry Olympus MPLAN 100×/0.9NA objective was used to collect Raman spectrum with the integration time of 20 s and resolution of 3 cm^{-1}. The Raman scattering line produced by a silicon plate (520.7 cm^{-1}) was used to signal calibration while the measured data were analyzed using Voigt peak fittings procedure by GRAMS software package.

The UV–Vis absorbance spectra for copper oxide and Ag/Cu nanoparticles were collected at room temperature using a CRAIC Technologies microspectrophotometer in the 300–700 nm range, a standard halogen lamp, a dry Olympus MPLAN 15×/0.28NA objective and an absorbance mode. The recorded optical absorbance spectra were transformed using Kubelka-Munk function from the reflectance data.

The influence of silver-copper nanoparticles on the growth of bacteria was examined on two laboratory strains Gram-negative *Escherichia coli* (*E. coli*) and Gram-positive *Bacillus subtilis* (*B. subtilis*), according to the procedure showed by Valodkar et al. [40]. The bacteria strains were taken from −80 °C stock (laboratory collection), streaked onto Luria-Bertani Broth (LB) and shaken (130 rpm) overnight at 37 °C. Then 100 μl of refreshed bacterial culture was used to inoculate LB medium and also incubated at the same conditions as described above until the mid-logarithmic phase had been attained. A range of dilutions of nanoparticles (0.001, 0.005, 0.025, 0.05, 0.1 and 0.2 $mg \cdot ml^{-1}$) in sterile liquid standard NB medium (nutrient broth, Biocorp, Spain) were used for determination of the minimum inhibitory concentration of Ag/Cu ($MIC_{Ag\text{-}Cu}$) in the samples. The sterile NB medium was supplemented with Ag/Cu nanoparticles in proper concentration and sonicated in 20 kHz for 2 h. In the next step bacterial suspension was added to the medium supplemented with nanoparticles into the final optical density OD = 0.1. The bacterial cultures were grown by shaking samples in the darkness at 130 rpm in 37 °C for *B. subtilis* and *E. coli*. To determine antibacterial impact of Ag/Cu nanoparticles, the microorganisms were enumerated by the plate-count method for viable cells. For this purpose 0.1 ml of bacterial liquid culture was washed twice in 0.85% NaCl solution to remove of nanoparticles. In the next step washed culture was introduced on solid TSA medium (Trypticase soy agar, BioMérieux, France) after 15, 60 and 120 min of incubation. The numbers of *B. subtilis* and *E. coli* colonies were enumerated after 24 h of incubation at 37 °C. At the same time the control culture without nanoparticles in NB liquid medium for *B. subtilis* and *E. coli* culture was made. The presented results are expressed as $CFU \cdot ml^{-1}$ (CFU, colony forming units) and graphically as log $CFU \cdot ml^{-1}$. All analyses were conducted in three replicates.

2. Results and discussion

2.1. Physicochemical analysis

X-ray photoelectron spectroscopy (XPS) was used to determine chemical environment and oxidation states in Ag/Cu nanoparticles