PHYSICAL CHEMISTRY 2014

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on Fundamental and Applied Aspects of
Physical Chemistry

The Conference is dedicated to the
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with

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ANTIBACTERIAL ACTIVITY OF COPPER NANOPARTICLES

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ABSTRACT

Copper nanoparticles (CuNPs), with an average particle size of about 5 nm, was prepared by the simple chemical reduction procedure. TEM and UV–Vis spectroscopy contributed to the analysis of size and optical properties of CuNPs, and their antibacterial activity was evaluated toward human pathogenic bacteria *Escherichia coli* and *Staphylococcus aureus*, in a concentration dependent manner. The changes in the cell membrane morphology of tested strains were investigated by atomic force microscope (AFM), after 2 h of their contact with CuNPs. It was found that CuNPs cause different types of cell membrane disruptions, as well as that *S. aureus* bacteria were slightly resistant to the cell membrane damage than *E. coli*.

INTRODUDTION

As a result of growing microbial resistances to multiple antimicrobial agents and development of resistant strains, there is increasing demand for novel antibacterial materials, such as metal NPs. Their main advantage is high surface area to volume ratio compared to bulk material, which provide a large active surface in the contact with microorganisms. Because of high cost of metals like silver and gold, material chemist have focused their attention on exploring possibilities of using CuNPs as ultimate antimicrobial agent [1,2]. Copper toxicity originates not only from the generation of oxidative stress but also fromits tendency to alternate between its cuprous, Cu(I), and cupric, Cu(II), oxidation states, differentiating CuNPs from other metal NPs. The both ions-mediated bactericidal mechanisms involve the

inhibition of cellular energy-transducing capabilities and accumulated damage at multiple cellular sites. In this study, the rapid inactivation of the two prokaryote cells by small and bare CuNPs is presented.

EXPERIMENTAL

Synthesis of CuNPs. The Cu hydrosol was prepared under inert atmosphere and vigorous stirring, by the chemical reduction of the weakly acidic (pH = 3 – 4) copper (II) chloride hydrate (0.5 mM) solution, using $NaBH_4$ (13.2mM) as a reducing agent and ascorbic acid (1.25mM) as a protective agent.

Antimicrobial assays. The antimicrobial activity of the CuNPs was quantitatively assessed in a physiological saline solution (8.5 g NaCl in 1 L of distilled water). *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923) bacteria were used like a test species. Microbial inoculum was prepared in tryptone soy broth (TSB, Torlak, Serbia), supplemented with 0.6% Yeast Extract (Torlak, Serbia), and left overnight at 37 °C. 3 mL of test solutions with different concentrations of the Cu hydrosol were inoculated with 1% inoculum (30 μL) as well as control sample without CuNPs. The Cu hydrosol was used in the form in which it had been prepared. All tubes were first incubated at 37 °C for 2 h, and after that, 100 μL aliquots was removed and subjected to decimal dilution with sterile physiological saline. From all dilutions 100 μL aliquots were placed in sterile Petri dishes, covered with trypton soy agar (Torlak, Serbia) and incubated 24 h at 37 °C. After incubation period the colony forming units (CFU) of each plate was determined. The percentage of microbial growth reduction (R, %) was calculated using the equation $R=[(C_0-C)/C_0)]\cdot 100$, where C_0 is the number of CFU from control sample and C is the number of CFU from the treated samples.

Characterization. Particles characterizations were performed with Termoscientific Evolution 600 spectrophotometer and transmission electron microscopy (TEM) JEOL–1200EX. Morphological changes of bacterial cell were recorded by AFM (Quesant-Scope Universal Scanning, USA).

RESULTS AND DISCUSSION

Initially, the formation of CuNPs was visually indicated by the color change of the reaction mixture from light blue to reddish-brown. The observed color originates from the strong absorption of the CuNPs when the frequency of the electromagnetic field becomes resonant with the coherent electron motion. As a results, the surface plasmon resonance (SPR) peak at 562 nm is appeared in the absorption spectrum of the CuNPs (Fig. 1). During the NPs grow, metal-catalyzed hydrolysis of borohydride ions and association

of negative charges (BH_4^- and BO_3^{3-}) with the CuNPs, took place [3]. These ions can temporarily stabilize the CuNPs by adsorption onto their surface, providing Coulomb repulsions between them and preventing their aggregation, which was also corroborated by TEM measurement (Fig. 1, inset). The dark spots on the TEM image present almost spherical CuNPs, with an average diameter of 5.3 ± 0.1 nm.

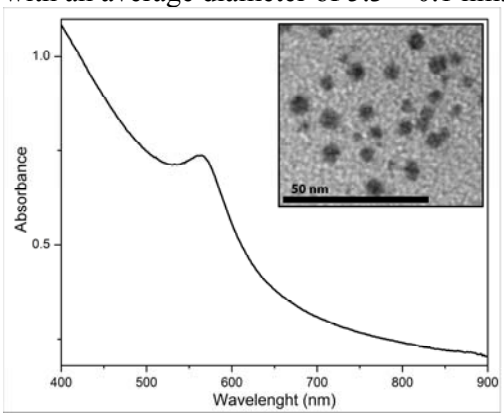

Figure 1. UV – Vis spectrum of CuNPs; Inset: TEM image of CuNPs

Results of the biological activity trials on the tested strains showed that microbial sensitivity toward different CuNPs concentracion (8, 16 and 32 ppm), vary depending on the microbial species. After 2 h of their contact, CuNPs were able to reduce 99.9 % of *E. coli* and 98.0 % *S. aureus* strains, at high CuNPs concentrations of 32 ppm. It was found that microbial reduction reached maximum against *E. coli* at all CuNPs concentration, but only with concentration of 32 ppm there was no microbial growth (<10). Besides, Cu NPs were shown good antibacterial activity against *S. aureus*, but only one or two logarithm units reduction of initial number of colonies in the presence of 8 and 16 ppm or 32 ppm of the CuNPs concentration, respectively. The results indicate that all tested concentration of CuNPs are more effective against *E. coli* than *S. aureus*.

Antibacterial activity of CuNPs was visually confirmed by AFM measurements, before and after 2h of their contact with CuNPs (Fig. 2). It can clearly be seen that surface of untreated rod–shaped *E. coli* and *S. aureus* spheres is smooth and compact, (Fig. 2a, 2b insets). Electrostatic interaction between Cu ions and membrane phospholipids causes disruption of the *E. coli* cell wall in the form of grooves and pores, and splitting strains' apical ends (Fig. 2a). The shape of treated cocci cell appeared to be damaged with greatly roughened surface texture as a consequence of reaction amine and carboxyl groups in peptidoglycan layer with Cu ions, (Fig. 2b). Comparing the data of these two strains, the effect of the CuNPs on the cell morphology was much less intense in the case of *S. aureus* than for *E. coli*, probably because of the thicker peptidoglycan layer and intrinsic difference in the cell wall structure. To conclude, the changes in cells morphology, as well as the possible damage caused by there cycling redox

reactions between Cu^{2+} and Cu^{+} at the surface of the cells and their electrostatic interactions with peptidoglycans and lipids will affect the bacteria in processes such as the respiratory chain, and cell division, finally causing the death of the cell.

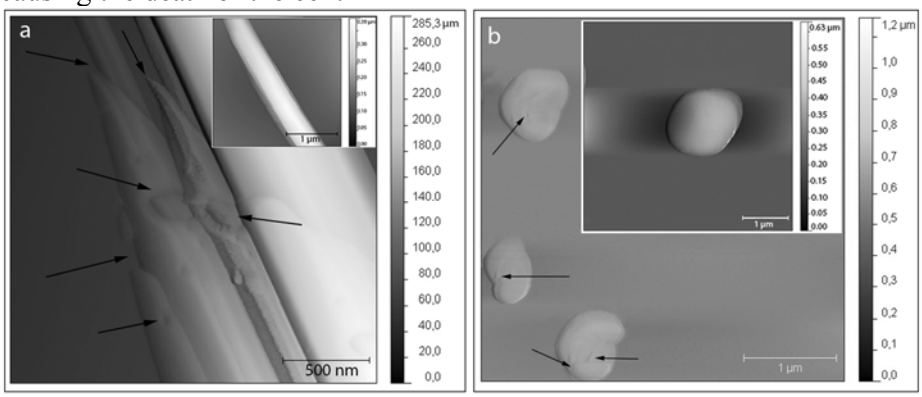

Figure 2. AFM images of (a) treated and untreated *E. coli* (inset); (b) treated and untreated *S. aureus* (inset).

CONCLUSIONS

The above set of results have shown evidence that prepared CuNPs with large and free surface to liberate ions, have a high and rapid antibacterial activity, visible after 2 h of their contact. These NPs have the advantage to closely interact with cell membrane, cousing its demages and disturb its permability and respiration function. Fast control of pathogenic microbes by these NPs, offering an inexpensive disinfectant for the rapid treatment of wastewater generated from hospitals.

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REFERENCE

[1] M. Raffi, S. Mehrwan, T.M. Bhatti, J.I. Akhter, A. Hameed, W. Yawar, M.M. Hasan, Annals of Microbiology, 2010, 60, 75-80.
[2] U. Bogdanović, V. Lazić, V. Vodnik, M. Budimir, Z. Marković, S. Dimitrijević, Materials Letters, 2014, 128, 75–78.
[3] V.V. Vodnik, J.M. Nedeljković, Journal of Serbian Chemical Society, 2000, 65, 195-200.