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ANTIMICROBIAL AND PHOTOCATALYTIC PROPERTIES OF DOPED AND UNDOPED ZINC OXIDE NANOPARTICLES

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Abstract: In this paper, zinc oxide nanoparticles doped with copper (ZnO/Cu NP) were synthesized by using black and green tea, vitamin C and trisodium citrate as a reduction agent. Antimicrobial and photocatalytic properties were tested. The antimicrobial activity of the doped synthesized ZnO NP against the clinical isolates of *Acinetobacter baumannii* and methicillin resistant *Staphylococcus aureus* (MRSA) was performed by the agar well diffusion method. ZnO NP with all four reduction agents showed good antimicrobial efficiency against both microorganisms, with similar inhibition zone. Photocatalytic activity was more pronounced in case of undoped, pure ZnO nanoparticles, while the best results for doped ZnO samples were obtained for ZnO/Cu NPs using black tea.

Keywords: zinc oxide; nanoparticles; antimicrobial activity; photocatalysis.

1. INTRODUCTION

Zinc oxide is well known multifunctional material; it is used in optoelectronics [1], biotechnology [2], microbiology field [3] etc. ZnO has a unique ability to form different nanoforms with different morphology: nanorods nanobelts, nanorings, nanosticks, nanospheres and nanoflowers. These ZnO nanoforms have better chemical and photochemical stability, greater surface area, optical permeability and biocompatibility. Due to its non-toxicity and antimicrobial properties ZnO is very often used as a cosmetic or pharmaceutical ingredient [4]. There are some reports in which antimicrobial activity is connected with generation of reactive oxygen species on the surface of inorganic oxides [5]. Also, inorganic oxides contain mineral elements which are essential to humans and exhibit activity when taken in small amounts [6]. Even though ZnO is a multifunctional material, its usage is somehow restricted due to inability to interact with solar system effectively and due to high recombination rate of electron and hole [7]. It is important to emphasize that nanomaterials have the ability to upgrade their properties due to incorporation of some other elements which significantly contributes to their application area [8,9]. This presents hypothesis of the presented research work. ZnO material was chosen because this material proved to be the most popular host material for metal incorporation [10]. There are great numbers of synthesis methods for ZnO reported in scientific literature. Some of them are sol-gel [11], solvent-free method [12], hydrothermal method [13], electrodeposition [14], pyrolysis [15], etc. The choice of synthesis method is very connected to nanomaterial properties such as uniformity, particle size and ultimately with its application. If application process requires good electrical and magnetic properties, the first evaluation step of a nanomaterial is toward photocatalytic abilities. On the other hand, if application goes in medical direction antimicrobial efficiency is very important. It was reported that ZnO nanoshapes keep their photocatalytic activity but lose their active sites upon aggregation due to absence of capping agents [7]. Chang et al reported that with higher concentration of Zn toxicity prevail over health benefit for Zn acting as essential mineral [16]. In order to overcome these disadvantages of pure ZnO, there are some reports in literature dealing with possibility to change absorption characteristic of ZnO *via* incorporation of some other elements in ZnO nanostructure. Silver, gold and copper are mostly used for better performances of ZnO properties.

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This research has a focus on photocatalytic and antimicrobial properties of undoped ZnO and copper doped ZnO (ZnO/Cu) nanoparticles, where copper was produced by green chemistry method using green tea, black tea, vitamin C and trisodium citrate. The aim of this paper is to study structural and optical properties of pure ZnO and ZnO/Cu samples in order to enhance photocatalytic and antimicrobial properties of ZnO due to modification of ZnO structure.

2. EXPERIMENTAL

Zinc Nitrate Hexahydrate ($Zn(NO_3)_2\times 6H_2O$, ≥99% Sygma Aldrich), Glycerol (Merck), Copper (II) Sulfate Hexahydrate ($CuSO_4\times 6H_2O$, ≥99% Sygma Aldrich), Trisodium citrate (Merck), Vitamin C (Alkaloid, Skopje), Green and Black Tea (Market products) were used for synthesis of ZnO NP and ZnO/Cu NPs.

Zinc nitrate hexahydrate ($Zn(NO_3)_2 \cdot 6H_2O$) was mixed with 165 µL of glycerol ($C_3H_8O_3$) for 30 minutes and then sample was calcinated for 2 hours at 300°C. The initial temperature was set to 50°C and then the temperature was slowly raised up to 300°C (approximately 3 °C/min) in order to avoid overheating and eventual mass losses. Cu NPs were synthesized from $CuSO_4x5H_2O$ with four different reduction agents: ascorbic acid ($C_6H_8O_6$), trisodium citrate ($Na_3C_6H_5O_7$), green tea and black tea. In order to synthesis ZnO/Cu composite 1 g of ZnO powder was dispersed in 10 mL of distilled water and then doped with 10 mL of Cu NP by stirring for 4h on magnetic stirrer and 1h on ultrasonic bath. All samples then were vaporated and calcinated for 2h on 300°C.

Antimicrobial activity of ZnO and ZnO/Cu NP against clinical isolates of methicillin resistant *Staphylococcus aureus* (MRSA) and *Acinetobacter baumannii* was performed by the agar well diffusion assay. Bacterial isolates were suspended in sterile 0.9 % NaCl solution to achieve concentration of $1.5x10^8$ cfu/mL and inoculated with sterile swabs onto Muller Hinton agar. Wells of 5 mm diameter were made on Mueller Hinton agar using sterile gel puncture and inoculated with 50 µL of ZnO and ZnO/Cu NPs. After overnight incubation in 37°C, inhibition zone around NPs was measured using measuring scale. All experiments were performed in triplicate.

Photocatalytic study was performed by using sunlight as a light source. In order to determinate photocatalytic power of ZnO/Cu NPs we used 0,1 g of ZnO/Cu nanopowder and with 100 mL of Methylene Blue (10 ppm) and we followed degradation of Methylene Blue in time. For absorption experiment Perkin Elmer Lambda 25 spectrophotometer was used.

FTIR spectrophotometer Bruker „Tensor 27“ was used for structure assessment of ZnO and ZnO/Cu samples in range 4000 to 400 cm^{-1}.

3. RESULTS AND DISCUSSION

In order to get insight in synthesized ZnO and ZnO/Cu structures, FTIR spectroscopy was performed. Figure 1. presents FTIR spectra of ZnO/Cu samples obtained using green tea (down black spectrum), black tea (middle red spectrum) and trisodium citrate (upper blue spectrum) with designated wavenumbers. IC spectrum of pure ZnO was published previously [12]. As earlier reported existence of peaks in the range below 800 cm^{-1} is observed for inorganic compounds [10,17,18]. Well-defined peaks in the wavelength number range from 420 to 480 cm^{-1} correspond to Zn-O bond [10, 17-19]. Depending on the synthesis procedure, as well as, used precursors, peaks with lower intensity at 520, 570 and 670 cm^{-1} are also characteristic for Zn-O bond [18, 19]. Peaks with frequency from 450 to 480 cm^{-1} are associated with stretching vibration of Zn-O bond in tetrahedral coordination, while peak at 670 cm^{-1} is associated with stretching vibrations of Zn-O, also, but in octahedral arrangements. [17]. In ZnO/Cu samples obtained with black tea and trisodium citrate one can see a peak at 1325 cm^{-1} which, according to literature data, correspond to asymmetric stretching vibration of CO- group because of Lewis acidity, while peaks present in ZnO/Cu (green tea) sample at 1550-1560 cm^{-1} are assigned to symmetric stretching vibrations of CO group due to Bronsted acidity. All three samples also contain peaks at 2367 cm^{-1} attributed to CO_2 molecules from air or alcohol and peaks at 3450-3745 cm^{-1} resulting from water existence onto the nanoparticle surface [19].

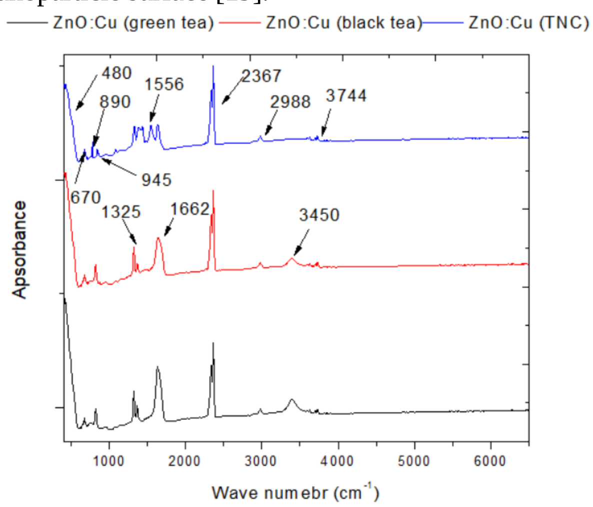


Figure 1. FTIR spectra of ZnO/Cu samples synthesized by using green tea (down black spectrum), black tea (middle red spectrum) and trisodium citrate (upper blue spectrum) as a reducing agent

Synthesized nanoparticles of ZnO and ZnO doped with copper were tested as potential catalysts. ZnO belongs to a group of wide bang gap materials with E_g = 3.3 eV which means that only UV part of sunlight spectrum can be used for generation of charge carriers. In a mission to improve photocatalytic properties of pure ZnO, ZnO structure was modified with copper nanoparticles. Using Tauc's plot and Tauc's equation $\alpha = A(h\nu-E_g)^n/h\nu$ we calculated band gaps for pure ZnO and ZnO doped with copper obtained by different reducing agents by green chemistry method. In Tauc's equation, α presents absorption coefficient, E_g is absorption band gap, A is constant and n depends on the transition nature i.e. allowed direct, allowed indirect, forbidden direct and forbidden indirect transitions [12]. Obtained band gaps (Table 1) confirmed that doping of ZnO with copper using different reduction agents surely affect absorption characteristics of pure ZnO nanoparticle. As seen from results, ZnO/Cu (vitamin C) has a lower band gap value comparing to pure ZnO, while three other samples with green tea, black tea and trisodium citrate showed higher values of band gap. Lower value of band gap indicates that more sunlight energy can be used to create electron holes [7]. Based on this we can conclude that ZnO/Cu sample with vitamin C gave the best results for improvement of photocatalytic properties of ZnO. Furthermore, band gap value is directly connected to nanoparticle size i.e. higher value of band gap – smaller nanoparticle which can be valuable for adsorption and kinetic processes. ZnO/Cu with black tea proved to be adequate formulation for synthesis of nanoparticles with smaller diameter.

Table 1. Values of λ_{max} and E_g from Tauc's plot for pure ZnO and ZnO/Cu samples

Sample	Λ_{max}, nm	E_g, eV
Pure ZnO	366	3,06
Vitamin C	578,77	1,94
Black tea	312	4,23
Trisodium citrate	313	4,20
Green tea	328	4,04

Photocatalytic study

Photocatalytic abilities of ZnO and ZnO/Cu samples were evaluated through degradation of methylene blue (MB) which is a phenothiazine cationic dye. It is worth saying that degradation of dyes by reduction process is favorable from the thermodynamical point of view, but from kinetics point of view it is not [20]. Our synthesized samples of ZnO and ZnO/Cu therefore can contribute to degradation of MB working as photocatalysts. In order to get insight in potential application of ZnO and ZnO/Cu as catalysts, degradation of MB was monitored from kinetic point of view, using UV/VIS spectrophotometer at specific wavelength at λ_{max}= 664 nm.

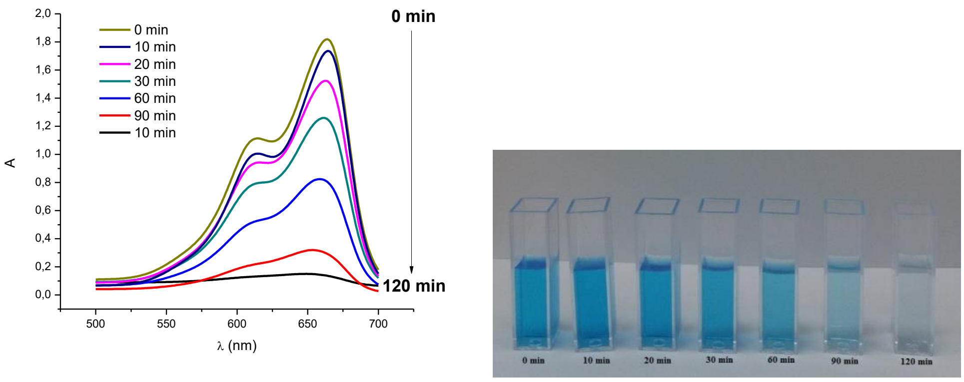


Figure 2. Absorption spectra for MB (c = 10 mg/L) vs. time for pure ZnO

Figure 2. presents photochemical reaction of MB for undoped ZnO as a function of time at following time intervals: 10 min, 20 min, 30 min, 60 min, 90 min and 120 min and the reduction of MB concentration was calculated using the following equation:

$$R\ (\%) = \frac{(1-c_t)}{c_0} * 100\% \quad (1)$$

Where c_0 and c_t present initial concentration of MB and MB concentration in some time *t*. The analogue calculations were conducted also for ZnO/Cu NP samples obtained using different reduction agents: green tea, black tea, vitamin C and trisodium citrate. Summarized photo effect is presented in Figure 3. Obtained results showed that the best photocatalytic behavior was shown by pure ZnO NP (93% of MB was degraded) comparing to ZnO/Cu NP samples. One of the reasons for better catalytic activity of pure ZnO NP can be contributed to thermal instability of doped metal since doped ions serve as blocking agents of surface sites which are responsible for photocatalytic activity [7]. In case of ZnO/Cu NP the best results were obtained by using black tea, where 89% of MB was decomposed.

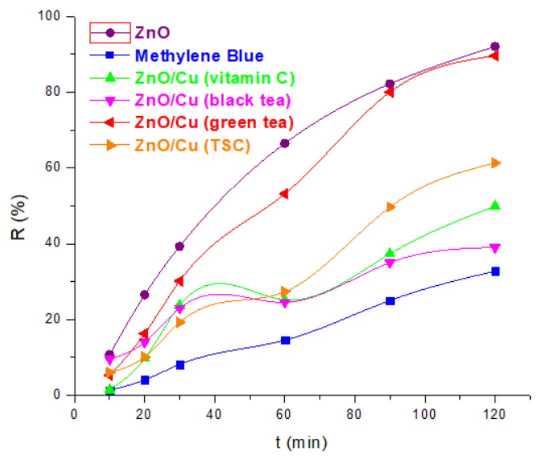


Figure 3. Extend of decomposition (reduction) of the MB dye solution with respect to time intervals over pure ZnO nanoparticles and ZnO/Cu nanocomposite under natural sunlight irradiation of MB; R (%) = f(t)

Photocatalytic reactions in which ZnO was used as catalyst are mainly described by Langmuir-Hinselwood model, where k_r – constant rate (mg L^{-1} min^{-1}), C_A (mg L^{-1}) concentration of substance whose photocatalysis is being performed and θ_A presents surface area coverage of photocatalyst with substance which undergo the photocatalytic process.

$$v_A = \frac{dC_A}{dt} = k_r\theta_A \quad (2)$$

and according to literature [26-27], with the following mechanism presented ono Equations 3-7:

$$hv + ZnO \rightarrow ZnO\ (h^+ + e^-) \quad (3)$$

$$ZnO\ (h^+) + H_2O_{\ (ads)} \rightarrow ZnO + OH^{\cdot}_{(ads)} + H^+ \quad (4)$$

$$ZnO\ (h^+) + H_2O^-_{(ads)} \rightarrow ZnO + OH^{\cdot}_{(ads)} \quad (5)$$

$$ZnO\ (e^-) + O_{2(ads)} \rightarrow ZnO + {}^{\cdot}O_2^-{}_{(ads)} \quad (6)$$

Formed radicals initiate a series of reactions in which bonds between organic molecules and aromatic rings are breaking, as well as oxidation of intermediate species which all lead to final degradation products:

$$OH^{\cdot}_{(ads)} / {}^{\cdot}O_2^-{}_{(ads)} + \text{intermediate} \rightarrow \text{products} \quad (7)$$

Figure 4. presents scheme for photocatalysis using ZnO as photocatalyst. In order to follow kinetics of ZnO and ZnO/Cu a graph ln(c/c_o) vs. t was constructed (Figure 5). It is visible from Figure 5. that ZnO and ZnO/Cu contribute degradation of MB. Just by looking at the slopes it is more than obvious that our synthesized catalyst (ZnO and ZnO/Cu) affect MB degradation. The steepest slope is noticeable for pure ZnO and for ZnO/Cu samples the best results were obtained using black tea.

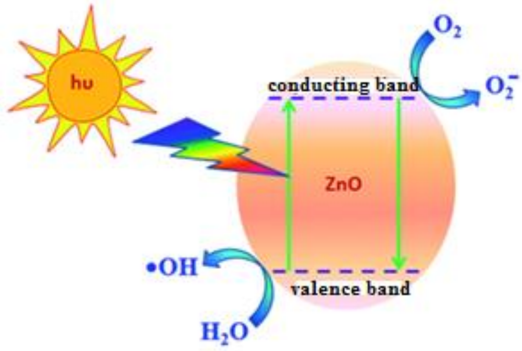


Figure 4. Photocatalytical scheme for ZnO [4]

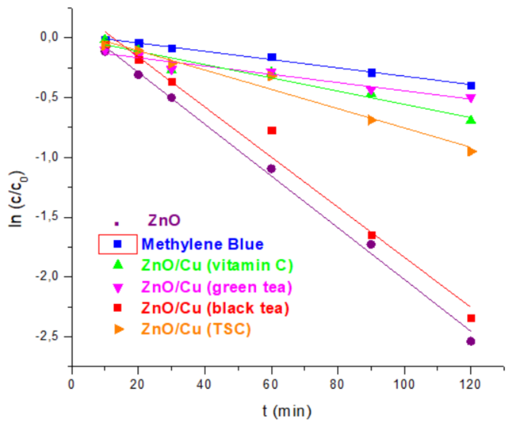


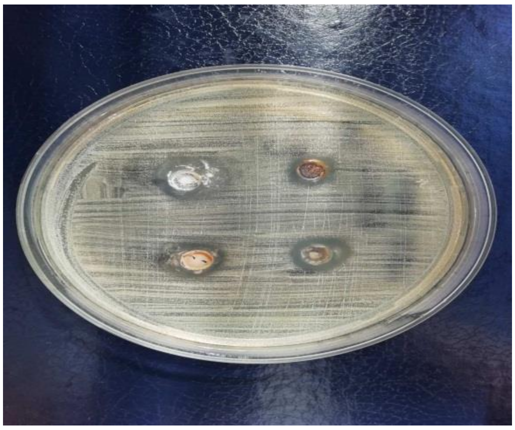
Figure 5. Comparison of the apparent rate constants of MB dye solution in the presence of pure ZnO and ZnO/Cu nanocomposite photocatalysts under natural sunlight irradiations.

This result for black tea is actually not surprising taking into account that we concluded above that according to E_g value, ZnO/Cu samples with black tea gave the smallest particles and the highest percent of MB degradation. Particle size plays an important role in adsorption processes, by decreasing the particle size the adsorption of reactants is enhanced on the catalyst surface [21].

In general, we can conclude that pure ZnO samples have high level of photocatalytic activity, while samples of ZnO/Cu reduced with different agents showed variation in degradation kinetic, but the most suitable results were provided by ZnO/Cu with black tea. Obtained differences in photocatalytic properties are probably due to different amount of copper incorporated in ZnO structure. All of this go in favor of postulated hypothesis that synthesis procedure directly affects physicochemical characteristics of inorganic nanoparticles, which must be taken into account for nanoparticles application.

Microbiological study

Antimicrobial activity of ZnO nanoparticles is extensively investigated, but the exact toxicity mechanism is not completely illuminated and still controversial. The antimicrobial activity of the metal oxide NPs, such as ZnO, is generally dependent on several factors like their size, surface area, shape, internalization of particles, and chemical functionalization [22]. Distinctive mechanisms of interaction between bacteria and NPs explain their antimicrobial activity: direct contact of ZnO-NPs with cell walls, resulting in destruction of bacterial cell integrity, and liberation of antimicrobial ions with formation of toxic reactive oxygen radicals [23]. Antimicrobial testing displayed good activity of ZnO NPs doped with all four reduction agents against both bacteria. Inhibition zones for both tested clinical isolates were similar, respectively for MRSA strain were about 10 mm (Figure 6), and for *A. baumannii* isolate were about 8 mm (Figure 7). Our results are in conclusion with other investigators, who have found better activity of nanoparticles against Gram positive bacteria (e.g. *S. aureus*), then against Gram negative bacteria (e.g. *Acinetobacter baumannii*) [24, 25]. Proposed explanation for our results could be related to differences in the cell wall structure, cell physiology, metabolism or degree of contact between bacteria and NPs .



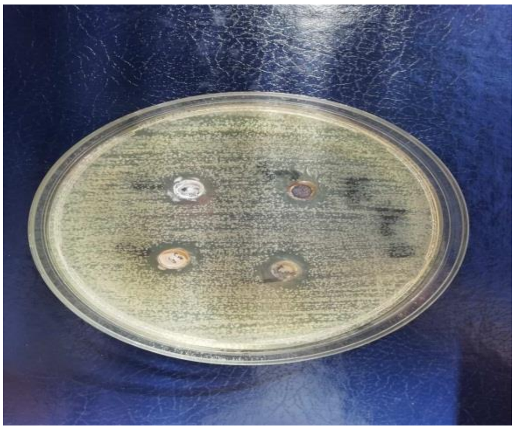


Figure 6. (left) and Figure 7. (right) Antimicrobial activity of ZnO/Cu NPs against MRSA strain and Antimicrobial activity of ZnO/Cu NPs against A. Baumannii

4. CONCLUSIONS

ZnO is suitable host for enhancement of photocatalytic and microbial properties of materials. ZnO and ZnO/Cu NPs samples synthesized by solvent-free method and green chemistry method were characterized by IC spectroscopy, UV/VIS spectrophotometry and by applying Tauc's plot energies of band gap were calculated. Enhanced photocatalytic activity and antimicrobial properties were shown by pure ZnO NPs and ZnO/Cu NPs with black tea. Antimicrobial testing of all ZnO/Cu NPs samples showed good activity both against MRSA and *A. baumannii* isolates. These results are very encouraging and show potential for their future medical application.

5. ACKNOWLEDGEMENTS

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АНТИМИКРОБНЕ И ФОТОКАТАЛИТИЧКЕ ОСОБИНЕ ДОПИРАНИХ И НЕДОПИРАНИХ НАНОЧЕСТИЦА ЦИНКА

Сажетак: У раду су проучаване наночестице цинк-оксида допиране са бакром (ZnO/Cu NP) уз помоћ сљедећих редукционих средстава: црни, зелени чај и три-натријум цитрат. Испитиване су антимикробне и фотокаталитичке особине. Антимикробна активност допираних наночестица ZnO NP на сојеве *Acinetobacter baumannii* и метицилин резистенти *Staphylococcus aureus* (MRSA) испитана је дифузионим методом у агару. ZnO NP са сва четири редукциона средства показале су подједнако добру антимикробну активност на обје испитиване бактерије. Фотокаталитичка активност је више изражена код недопираних честица цинк оксида, док је код допираних честица најбоље резултате дала синтеза са црним чајем.
Кључне ријечи: цинк-оксид, наночестице, антимикробна активност, фотокатализа.

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