



Antimicrobial properties of ZnO nanoparticles incorporated in polyurethane varnish

Dušan Zvekić^{1,*}, Vladimir V. Srdić¹, Maja A. Karaman², Milan N. Matavulj²

¹Department for Materials Science, Faculty of Technology, University of Novi Sad, Bul. Cara Lazara 1, 21000 Novi Sad, Serbia

²Department of Biology and Ecology, Faculty of Natural Sciences, University of Novi Sad, Trg Dostiteja Obradovica 3, 21000 Novi Sad, Serbia

Received 15 February 2011; received in revised form 29 March 2011; accepted 31 March 2011

Abstract

The antimicrobial effect of the ZnO nanoparticles dispersed in polyurethane varnishes was investigated. Antimicrobial activity was evaluated against three bacteria (*Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*) and one strain fungi (*Sacharomyces cerevisiae*) by the “pour-plate” test. It was shown that the ZnO nanoparticles efficiently inhibit growth of the colonies of *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Saccharomyces cerevisiae*, but their activity against *Escherichia coli* was found to be questionable suggesting more detailed research.

Keywords: ZnO nanoparticles, polyurethane, nanocomposite coating, antibacterial activity

I. Introduction

Composites are very important materials for human population. The history of the use of composite materials dates back to ancient history when Egyptians used various techniques for strengthening their building materials. Recently, need for designing new materials with improved properties have forced fast development of nanostructured materials, especially nanocomposites. Thus, researches have been focused on investigation of materials at the atomic, molecular and macromolecular level, with the aim to understand and manipulate the features that are substantially different from the processing of materials on micro-scale. Polymer-based nanocomposites, with inorganic nanoparticles dispersed in polymer matrix, are interesting because of their improved properties, simple processing steps and relatively low costs. A particular challenge in creating the polymer-based nanocomposites is considerable improvement of mechanical properties. However, newly developed nanocomposites with bactericidal properties occupy considerable attention in recent years, not only because of their impact on hu-

man health and safety but also because of the possibility of extended lifetime of materials used in everyday life. Possible applications of these materials are very broad: i) different types of sterile materials are important in hospital, where often wounds are contaminated with microorganisms, in particular fungi and bacteria [1], ii) purification of water, i.e. the removal or inactivation of pathogenic microorganisms, is necessary for the treatment of wastewater [2], etc.

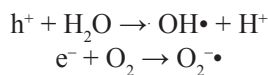
During the past few decades, several investigations have been carried out concerning the use of polymer films, synthetic and natural zeolites and particles with different metal ions (Ag, Cu, Zn, Hg, Ti, Ni, Co) as materials with bactericidal properties [2-4]. Among inorganic antibacterial agents, silver nanoparticles have been employed most extensively. Silver nanoparticles liberate silver ions in liquids that show a broad spectrum of the antimicrobial activities [5-7]. The mechanism of the antimicrobial effect of silver is still not fully understood. It is believed that DNA loses its replication ability and cellular proteins become inactivated upon treatment with silver ions. In addition, it has also been shown that silver ions bind to the functional groups of proteins, resulting in protein denaturation [8,9]. On the other hand, it is believed that ZnO (semi-

* Corresponding author: tel: +381 65 857 4555
e-mail: dusanz76@yahoo.com

conductor with a wide band gap of 3.37 eV and tetragonal wurzite structure) nanoparticles have bactericidal properties primarily due to its photocatalytic activity. Similarly to titania [10], ZnO can absorb light (UV or visible) which induces a separation of charge, generating a hole (h^+) in the valence band and an electron (e^-) in the conduction band:



At the surface of the excited ZnO particle, the valence band holes abstract electrons from water and/or hydroxyl ions, generating hydroxyl radicals ($\text{OH}\cdot$). In addition, electrons can reduce O_2 to produce the superoxide anion $\text{O}_2^{\cdot-}$ [10]:



The obtained $\text{OH}\cdot$ and $\text{O}_2^{\cdot-}$ can induce lipid peroxidation in membranes, DNA damage due to strand breakage or oxidized nucleotides and oxidation of amino acids and protein catalytic centers [11]:



Another possibility is destruction of organic material in a direct reaction with positively charged ZnO particles. It was observed that ZnO shows bactericidal properties also in case of complete absence of light.

In this work, the role of zinc oxide nanoparticles in possible antibacterial activity was investigated in toxicity tests with the three bacteria (*Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*) and one strain fungi (*Sacharomyces cerevisiae*).

II. Experimental

2.1 Sample preparation

Polyurethane varnish with 50% gloss and small amount of silica particles (Milesi, Italy) was used for preparation of polyurethane based nanocomposite coatings. Three different types of polyurethane varnishes, with 0, 0.4 and 0.7 wt.% of ZnO nanoparticles

(Degussa, Germany), were prepared by careful dispersion of ZnO nanoparticles in polyurethane with intensive mixing under magnetic stirring for 10 min. The prepared polyurethane varnishes were deposited as coatings on standard wood floor samples, having size of 10×10 cm.

2.2 Characterization

The precursor powder and prepared samples were characterized by BET, XRD, SEM and TEM. Specific surface area of ZnO nanopowder was determined by low-temperature nitrogen adsorption, on Autosorb 1C Quantachrome, USA, using the BET method. X-ray diffraction of ZnO nanopowder was performed on PANalytical X-ray diffractometer, X'Pert PRO, The Netherlands, using Cu K α radiation at 40 kV and 40 mA in 2θ region 20-120° and with step size of 0.03°. The size and morphology of ZnO nanoparticles were determined using transmission electron microscopy on FEI Tecnai F20, Japan at working voltage of 200 kV. The microstructure of the deposited nanocomposite coatings was analyzed by scanning electron microscopy JEOL SEM 6450 LV, Japan.

2.3 Antimicrobial test

The antimicrobial activity of the nanocomposite coatings was conducted against three bacteria: *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 8739) and *Pseudomonas aeruginosa* (ATCC 9027) and one strain eukaryotic fungi (*Sacharomyces cerevisiae*) by the "pour-plate" test (spillover of sample in Petri dish with inoculated test strain). The Mueller-Hinton agar (TORLAK, Beograd) was used as a nutrient medium for initiating the growth of bacterial and fungi colonies. In accordance with the procedure CLSI [12] bacteria were incubated at 35°C overnight. The first counting of grown colonies was performed after 24 h and checked after 36 h. The malt agar (TORLAK, Belgrade) was used as a nutrient medium for inducing the growth of colonies of fungi and incubated at 22 °C for additional 4 days.

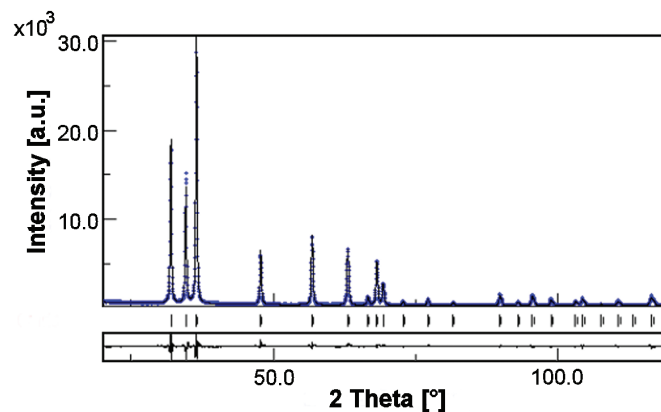


Figure 1 XRD pattern of ZnO powder refined by MAUD program

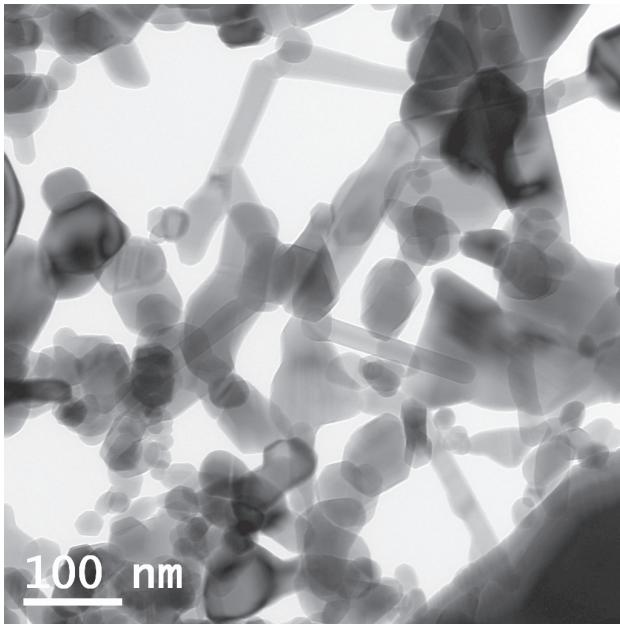


Figure 2 TEM micrograph of ZnO powder

III. Results and discussion

ZnO powder with purity of 98.5 % has specific surface of 23.3 m²/g. High crystallinity of ZnO powder was confirmed by X-ray diffraction. The structural analysis

of X-ray diffraction data was performed by the Rietveld analysis, using MAUD program. The background was defined by a fifth-parameter polynomial and refined simultaneously with the zero-point and scale. Both, the instrumental and sample intrinsic profiles were described by a convolution of Lorentzian and Gaussian components, and the TCH-pseudo Voigt profile function was used. Parameters characterizing the instrumental resolution function were obtained from a LaB₆ standard powder sample. Refinements were undertaken in space group *P63mc* for hexagonal ZnO with all atoms in general positions. Refined XRD pattern ($Sig = 2.0258224$, $R_w(\%) = 7.542874$, $R_b(\%) = 5.9602365$, $R_{exp}(\%) = 3.7233639$) is presented in Fig. 1 and confirmed that ZnO particles have pure hexagonal wurzite structure with unit cell parameters $a = 3.2520 \text{ \AA}$ i $c = 5.2104 \text{ \AA}$ and crystallite size of 63.2 nm. Transmission electron microscopy image of the zinc oxide powder is illustrated in Fig. 2 and shows that ZnO powder is nonagglomerated and has slightly elongated particles with broad size distribution and the average size of ~60 nm.

The microstructures of the obtained nanocomposites (polyurethane varnishes deposited on standard wood floor samples) were examined by SEM analyses and are illustrated in Fig. 3. The nanocomposite coatings consist

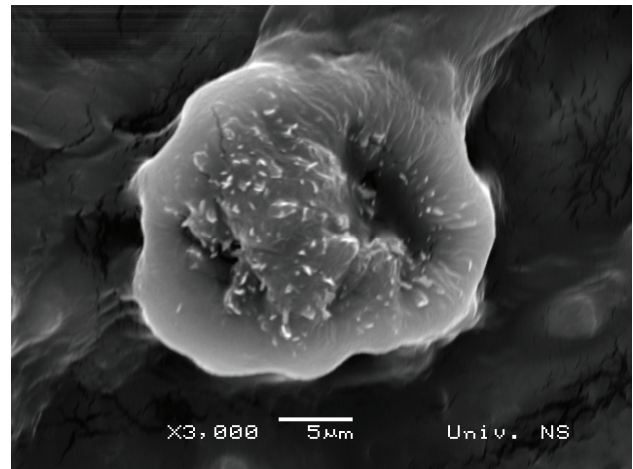
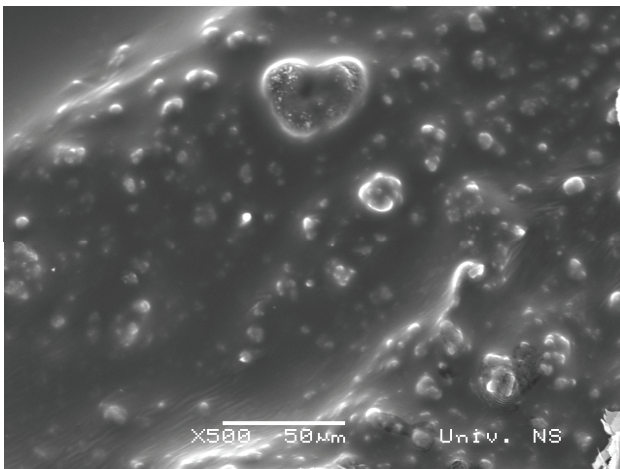


Figure 3 SEM micrographs of nanocomposite coating (polyurethane varnishes with 0.7 wt.% of ZnO nanoparticles deposited on standard wood floor samples)

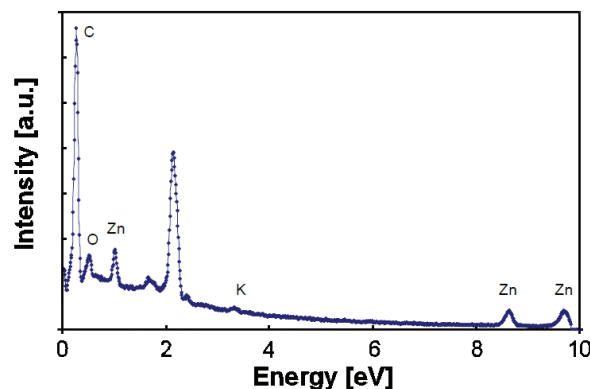


Figure 4 EDS spectra of nanocomposite coatings (polyurethane varnishes with 0.7 wt.% of ZnO nanoparticles deposited on standard wood floor samples)

of polymer matrix in which micrometer silica particles were uniformly dispersed (Fig. 3a). Presence of ZnO nanoparticles was confirmed by EDX analysis (Fig. 4) and by SEM when higher magnification was used (Fig. 3b). However, it was observed that ZnO nanoparticles are not uniformly dispersed in polyurethane matrix.

The antimicrobial effect of the ZnO nanoparticles dispersed in polyurethane varnishes against three bacteria and one strain fungi was conducted by the “pour-plate” method. A parallel test on the polyurethane varnishes without the ZnO nanoparticles was also carried out for comparisons. The counted number of bacterial colonies and photographs of investigated samples after the tests are given in Table 1 and Fig. 5, respectively. The results clearly indicate that the pure polyurethane varnishes deposited on standard wood floor samples do not show antimicrobial properties. On the other hand, the colonies of *Pseudomonas aeruginosa* and *Saccharomyces cerevisiae* are completely not recorded at the surface of polyurethane varnishes with the ZnO nanoparticles. In addition, 0.4 and 0.7 wt.% of the ZnO nanoparticles inhibit growth of *Staphylococcus aureus* by more than 85% and 95% respectively (Table 1). However, activity of the ZnO nanoparticles against *Escherichia coli* was discutable since after 36 hours of incubation, colonies of test strain started to grow from the very edge of the wood samples treated with ZnO nanoparticles, suggesting certain adaptation on the used agents (Fig. 5d).

IV. Conclusions

Polyurethane based nanocomposites with 0.4 and 0.7 wt.% of a commercial ZnO nanoparticles were prepared by deposition of polyurethane varnishes on standard wood floor samples. The obtained nanocomposite coatings consist of polymer matrix in which micrometer silica and nanosized zinc oxide particles are dispersed. It was shown that the pure polyurethane varnishes do not show antibacterial properties, but the antimicrobial effect of the ZnO nanoparticles was confirmed. Thus, growth of the colonies of *Pseudomonas aeruginosa* and *Saccharomyces cerevisiae* was completely inhibited in presence of the ZnO nanoparticles. In addition, 0.4 and 0.7 wt.% of the commercial ZnO nanoparticles inhibit growth of *Staphylococcus aureus* by more than 85% and 95% respectively (Table 1), and only activity of the ZnO nanoparticles against *Escherichia coli* was found to be questionable suggesting more detailed research.

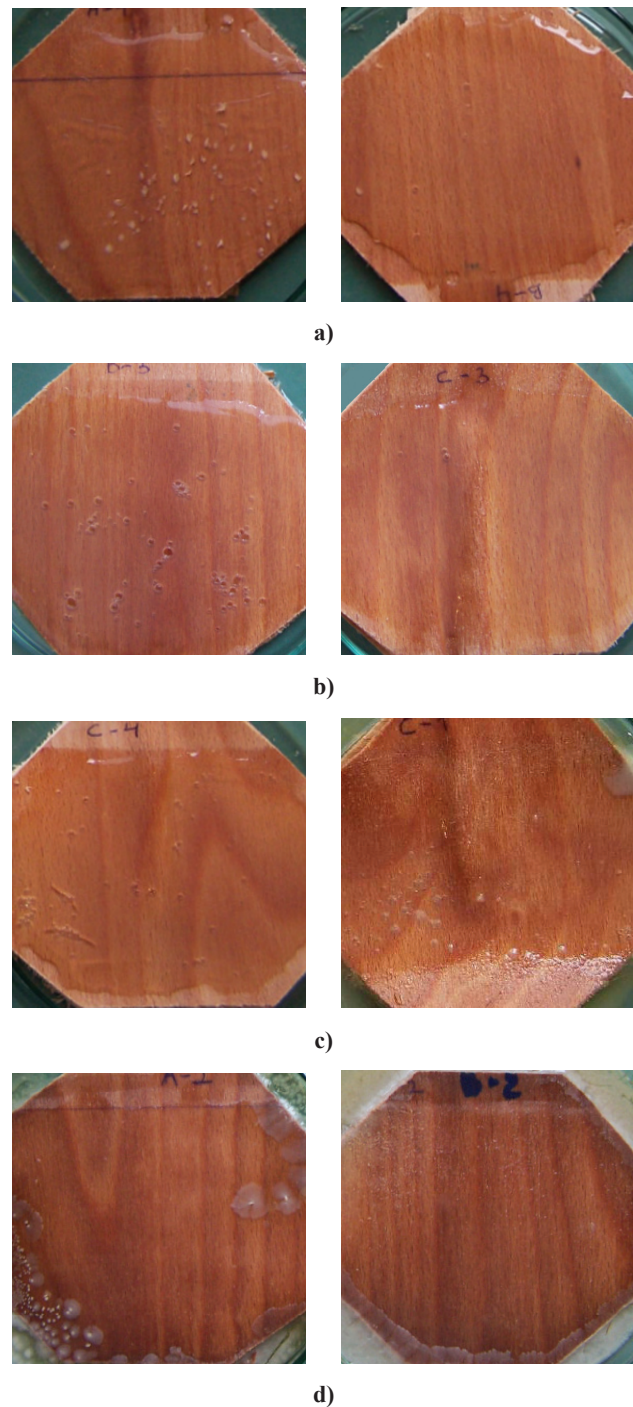


Figure 5. Photographs of the antimicrobial results for polyurethane varnishes without (left) and with ZnO nanoparticles (right) for: a) *Saccharomyces cerevisiae*, b) *Pseudomonas aeruginosa*, c) *Staphylococcus aureus* and d) *Escherichia coli*

Table 1 Counted number of colonies on different polyurethane varnishes

	Pure polyurethane varnish	Polyurethane varnish with 0.4 wt% ZnO	Polyurethane varnish with 0.7 wt% ZnO
<i>Saccharomyces cerevisiae</i>	>100	0	0
<i>Pseudomonas aeruginosa</i>	12	0	0
<i>Staphylococcus aureus</i>	45	8	2
<i>Escherichia coli</i>	16	22	16

References

1. J.S. Kim, E. Kuk, K.N. Yu, J-H. Kim, S.J. Park, H.J. Lee, S.H. Kim, Y.K. Park, Y.H. Park, C-Y. Hwang, Y-K. Kim, Y-S. Lee, D.H. Jeong, M-H. Cho, “Antimicrobial effects of silver nanoparticles”, *Nanomedicine: Nanotechnol. Biol. Medic.*, **3** (2007) 95–101.
2. P. Jain, T. Pradeep, “Potential of silver nanoparticle-coated polyurethane foam as an antibacterial water filter”, *Biotechnol. Bioeng.*, **90** [1] (2005) 59–63.
3. A.E.H. Shearer, J.S. Paik, D.G. Hoover, S.L. Haynie, M.J. Kelley, “Potential of an antibacterial ultraviolet-irradiated nylon film”, *Biotechnol. Bioeng.*, **67** (2000) 141–146.
4. P.K. Stoimenov, R.L. Klinger, G.L. Marchin, K.J. Klabunde, “Metal oxide nanoparticles as bactericidal agents”, *Langmuir*, **18** (2002) 6679–6686.
5. M. Kawashita, S. Tsuneyama, F. Miyaji, T. Kokubo, H. Kozuka, K. Yamamoto, “Antibacterial silver-containing silica glass prepared by sol–gel method”, *Biomater.*, **21** (2000) 393–398.
6. S. Pal, Y.K. Tak, J.M. Song, “Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the gram-negative bacterium *Escherichia coli*”, *App. Environ. Microbiol.*, **73** [6] (2007) 1712–1720.
7. W-C. Lin, C-N. Chen, T-T. Tseng, M-H. Wei, J.H. Hsieh, W.J. Tseng, “Micellar layer-by-layer synthesis of TiO₂/Ag hybrid particles for bactericidal and photocatalytic activities”, *J. Eur. Ceram. Soc.*, **30** (2010) 2849–2857.
8. Q.L. Feng, J. Wu, G.Q. Chen, F.Z. Cui, T.N. Kim, J.O. Kim, “A mechanistic study of the antibacterial effect of silver ions on *Escherichia coli* and *Staphylococcus aureus*”, *J. Biomed. Mater. Res.*, **52** (2000) 662–668.
9. M. Vukčević, A. Kalijadis, S. Dimitrijević-Branković, Z. Laušević, M. Laušević, “Surface characteristics and antibacterial activity of a silver-doped carbon monolith”, *Sci. Technol. Adv. Mater.*, **9** (2008) 015006.
10. L. Brunet, D.Y. Lyon, E.M. Hotze, P.J.J. Alvarez, M. Wiesner, “Comparative photoactivity and antibacterial properties of C60 fullerenes and titanium dioxide nanoparticles”, *Environ. Sci. Technol.*, **43** (2009) 4355–4360.
11. E. Cabiscol, J. Tamarit, J. Ros, “Oxidative stress in bacteria and protein damage by reactive oxygen species”, *Int. Microbiol.*, **3** (2000) 3–8.
12. Clinical Laboratory Standards Institute, *Performance standards for antimicrobial susceptibility testing*, Wayne, PA: CLSI, 2005

