OPTOELECTRONICS AND ADVANCED MATERIALS – RAPID COMMUNICATIONS Vol. 3, No. 8, August 2009, p. 795 - 799

New composite powders with high antifungal properties

S. GAVRILIU*, M. LUNGU, F. GRIGORE, N. BURUNTIA, C. GROZA
National Institute for Research and Development in Electrical Engineering, INCDIE ICPE-CA, 313 Splaiul Unirii, 030138 Bucharest - 3, Romania

The paper presents a study concerning the preparation and characterization of a new type of composite powders as silver nanoparticles deposited on ZnO microcrystalline particles. This system is destined to antifungal applications. The preparation procedure is based on the reduction of the Ag^+ from an $AgNO_3$ solution containing the fine dispersed ZnO particles. The method allows the achievement of spherical silver nanoparticles having an average diameter of 16.5…88.5 nm. The silver nanoparticles weight content varied between 0.1…25 wt. %. AAS, UV-Vis spectroscopy, dynamic light scattering technique, TEM and SEM analyses were carried out for chemical and morphological characterisations. The antifungal assay by the antibiograme method has demonstrated that these composite powders are suitable for health care and consumer goods items.

(Received May 18, 2009; accepted July 20, 2009)

Keywords: Composite powders, Silver nanoparticles, Antifungal, TEM, SEM

1. Introduction

The noble metal colloids and the composites powders containing silver nanoparticles (SNPs) received a special attention due to their high application potential in bioscience, healthcare and consumer goods.

The silver colloids are situated in top due to some especial characteristics such as antimicrobial activity, high electrical and thermal conductivity, catalytic activity or unique optical properties that depend on the size and the shape of the particles. The SNPs solutions kill over 650 types of germs and do not lead to mutant germs growing [1]. These are known as non-toxic, skin friendly and do not causes skin irritation [2]. SNPs were included in mouth rinse formula (20 mg/l) because of their better tolerance for topical applications. The best way to obtain a high antibacterial effect is the achievement of fine SNPs, which in turn, develop a high surface area. As a result, maximal silver ions are released [3, 4]. Usually, the colloidal silver solutions (CSSs) contain about 70 % Ag^0 from the total silver content (the rest of 30 % consist of Ag^+ ions), which is gradually released in its ionized form. The Ag^+ ions have a bactericidal activity at concentrations over 1 ppm, while the colloidal Ag^0 has a bactericidal activity at concentrations of 1-20 ppm [5, 6].

Conventionally, SNPs have been prepared by various methods such as physical, chemical, electrochemical or photochemical reduction of metals in different media [7…10]. But, the most of these methods have limitations in the controlling of particle size, colloidal stabilities, production on industrial scale and dispersion ability, especially. Some surfactants have been used to avoid the particle agglomeration and to achieve a stabilization of colloids [11]. The manufacture of some composite colloids consisting of nanometric silver-coated supports is a valuable way for carrying out a controllable metal nanoparticles synthesis for shape, dimensions, dispersion ability and reproducibility [12].

ZnO is a functional oxide with an important advantage for biomedical applications, consisting in its bio-safety and biocompatibility. The applications of SNPs/ZnO composite powders as active ingredients for some new advanced materials like antifungal gels, creams and dyes with multifunctional characteristics such as the protective, esthetic, antifouling and antimicrobial ones, require particles having high dispersion ability, compatibility with the other components of the products and a suitable silver ions release, too.

The present paper shows some research results regarding to the obtaining of new SNPs/ZnO composite powders by chemical deposition of SNPs on ZnO fine particles dispersed in aqueous suspensions.

2. Experimental

In the experiments, the following starting materials were used: silver nitrate aqueous solutions of concentrations of 0.017…0.085 wt. % ($AgNO_3$, p.a., crystallized, Fluka), ZnO powder (ZnO p.a., Chemapol), tri-sodium citrate solutions of concentrations of 0.5…2 wt. % ($C_6H_5Na_3O_7 \cdot 2H_2O$, Merck), sodium borohydride solutions of concentrations of 0.075 wt. % ($NaBH_4$, Merck), poly [1-vinyl-2-pyrrolidone] $(C_6H_9NO)_x$ solutions of concentrations of 0.5…0.7 wt. % (PVP with M = 10,000 of Sigma – Aldrich) and deionized water.

ZnO powder having a mean diameter of 200 nm was used as support particles for SNPs chemical deposition. This was dispersed by sonication in an $AgNO_3$ aqueous solution. In this suspension, the ZnO content was ranged between 8…15 wt. %.

The SNPs content of the experimental samples was ranged between 0.1…25 wt. %.

Further, for exemplification we will present the research results obtained for the SNPs/ZnO samples with 0.1 wt. % SNPs (sample I) and 25 wt. % SNPs (sample II), respectively.

In order to manage the deposition of the SNPs on the ZnO particles, we used the reduction method of Ag^+ ions from the $AgNO_3$-ZnO aqueous suspension by tri-sodium citrate and $NaBH_4$ in proper experimental conditions relating to concentration, temperature, pH, food rate and stirring. The tri-sodium citrate solution was poured in drops into the $AgNO_3$-ZnO aqueous suspension heated at 80 °C under vigorous stirring and after that, the poly vinyl-pyrrolidone (PVP) solution was added. These conditions were kept up to a milky yellow color appeared. Then in this suspension that was rapidly cooled at 4 °C, a $NaBH_4$ solution in a molar ratio of 1:1…1:3 against $AgNO_3$ content was dropped. The sequence of the reagent addition is an important factor in the preparation of some SNPs/ZnO composite powders with high dispersion degree and desired functional characteristic. In the first step, it is necessary the silver nuclei formation and, immediately, their protection against uncontrolled growing and flocculation. The appearance of the yellow color reveals SNPs formation. Because the SNPs have a high tendency to agglomeration, which leads to flocculation even during the synthesis process, we used PVP, which is an excellent polymer surface agent for steric stabilization. Also, tri-sodium citrate acts as an ionic capping agent for electrostatic stabilization.

The obtained slime was centrifuged at 9000 rpm for 30 minutes. Finally it was washed with deionized water. The composite powders were dried in vacuum at 50 °C for 3 hours.

For the characterization of the SNPs and SNPs/ZnO, some tests such as atomic absorption spectroscopy (AAS) using a SOLAAR S4 - Thermo Electron Corporation apparatus, dynamic light scattering technique (DLS) using a 90 Plus - Brookhaven apparatus, UV-Vis spectroscopy (UV-Vis) using a V 570 - Able & Jasco apparatus, transmission electronic microscopy (TEM) using a CM 100 – Philips microscope and scanning electronic microscopy (SEM) using a Quanta 600 – FEI microscope were carried out.

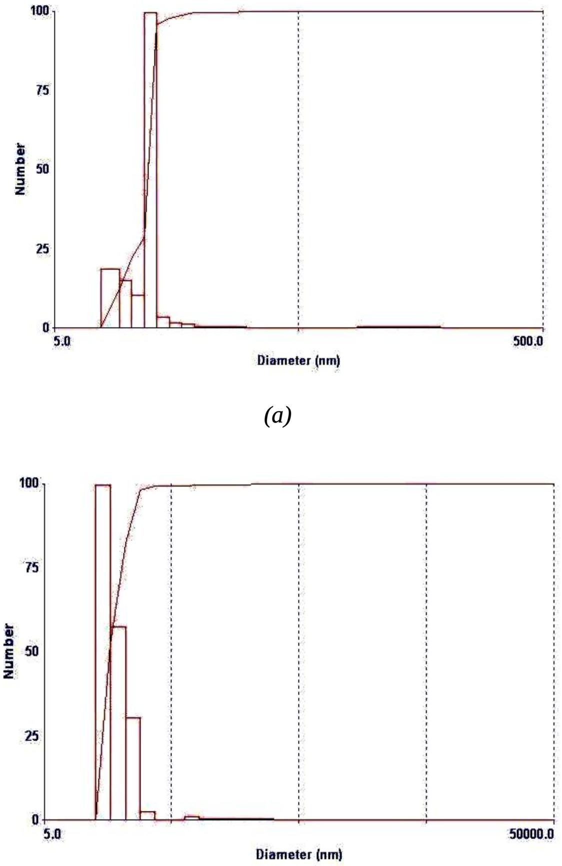
For antifungal assays of the ZnO and SNPs/ZnO powders, the specific microbiological tests were carried out according to the normative in force [13]. The antibiogram method was used upon the following germs: Aspergillus species, Paecilomyces varioti, Penicillium species, Chaetomium globosum, Myrothecium verrucaria, Stachybotrys atra, Scopulariopsis brevicaulis and Trichoderma viride. The moulds growth was monitored daily during two weeks.

3. Results and discussion

The above presented method led to a SNPs/ZnO deposition yield ranging between 93…95 %. The remained SNPs content in the supernatant at the end of the synthesis, measured by AAS and UV-Vis techniques, was ranged between 5 - 60 ppm, depending on the SNPs content from the precipitated SNPs/ZnO samples.

Fig. 1 presents the grain size distributions obtained by DLS technique of the extracted SNPs from sample I and sample II.

The both grain size distributions curves present enclosed ranges of the nanoparticle dimensions. It is well known that the $AgNO_3$ solutions with low concentrations used as Ag precursors lead to fine and stable silver colloids. So, an average diameter of 16.5 nm was measured for the extracted SNPs from the sample I, which was obtained starting from a diluted $AgNO_3$ solution (concentration of $AgNO_3$ of 0.017 wt. %). The extracted SNPs from the sample II, which was obtained starting from a higher concentrated solution (concentration of $AgNO_3$ of 0.085 wt. %) have an average diameter of 88.5 nm.

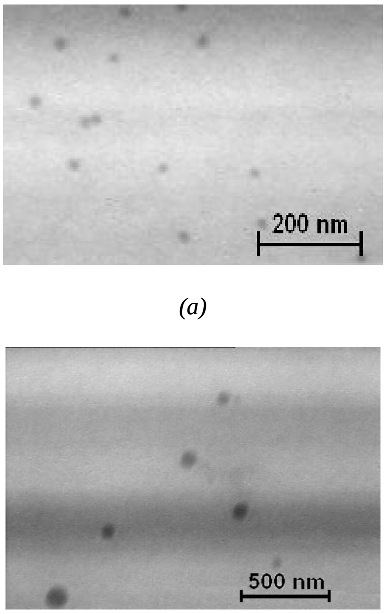


(a)

(b)

Fig. 1. Mean diameter of the colloidal solutions of the extracted SNPs from sample I (a) and (b)sample II.

The TEM images of the extracted SNPs from sample I and sample II are presented in Fig. 2. These images show a spherical geometry of the SNPs, which were deposited on the ZnO powder for the both types of samples. The results of measurements on 200 particles, shown average diameters of 14 nm and 80 nm for sample I and sample II, respectively.



(a)

(b)

Fig. 2. TEM image of the extracted SNPs from sample I (a) and (b) sample II.

Fig. 3 presents the UV-Vis absorbance spectra of the SNPs colloidal solutions extracted from sample I and sample II, respectively.

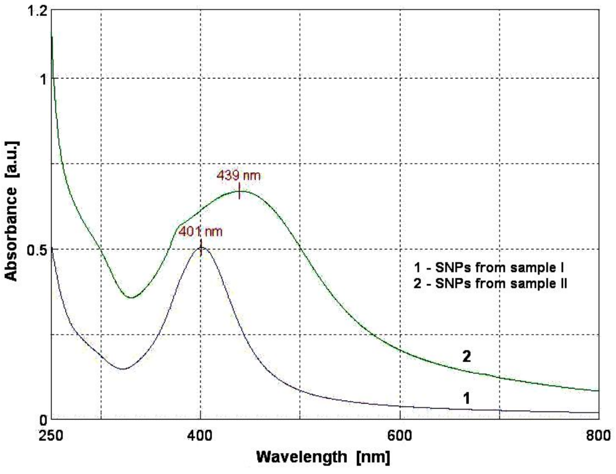


Fig. 3. UV-Vis spectra of the colloidal solutions of extracted SNPs from sample I and sample II.

The maximal absorbance obtained from UV-Vis spectra at the wavelength of 401 nm for sample I and at the wavelength of 439 nm for sample II, are in accordance with the results obtained by the DLS and TEM analyses. As we can observe, the maximal absorbance is shifted to red region with increasing of the particle diameter.

Fig. 4 presents the reflectance spectra of the ZnO, sample I and sample II, in form of powders, which were obtained using the integrative sphere of the spectrophotometer.

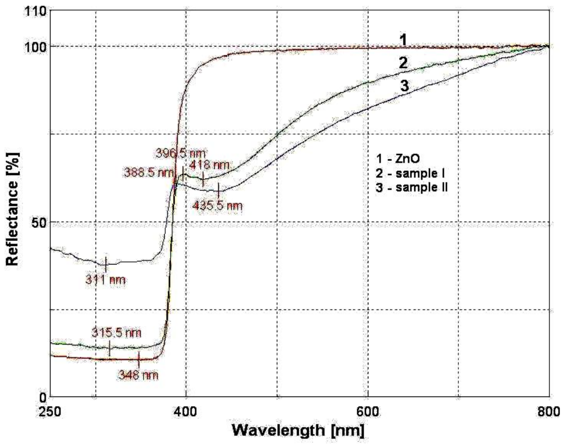


Fig. 4. Reflectance spectra of the ZnO, sample I and sample II powders.

From the recorded reflectance spectra, the SNPs/ZnO powders present higher values of the maximal reflectance comparatively with the pure ZnO powder. The maximal reflectance recorded at the wavelength of 418 nm shows for the sample I some low dimensions of the SNPs deposited on the ZnO, while the red shift of the maximal reflectance recorded at the wavelength of 435.5 nm, confirm the higher dimensions of the SNPs deposited on the ZnO in the sample II.

The SEM image of the sample I is presented in Fig. 5.

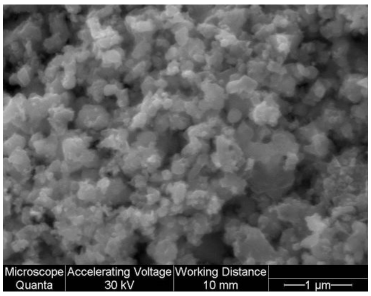


Fig. 5. SEM image of the sample I.

As it was expected, the ZnO particles are evident visible in form of micro crystallites having polyhedral geometry.

The crystal structure of ZnO is a wurtzite structure consisting of a number of planes composed of tetrahedral coordinated O^{2-} and Zn^{2+} ions, which are stacked alternately along the c-axis. The four most common face terminations of ZnO are the polar Zn terminated (0001) and O terminated $(000\bar{1})$ faces (c-axis oriented), and the non-polar (1120) (a-axis) and $(10\bar{1}0)$ faces, which both contain an equal number of Zn and O atoms [14]. The polar faces are known to posses different chemical and physical properties [15]. So, the Zn^{2+} positively charged faces may be considered as suitable deposition places for the SNPs, which have a nucleophile feature due to the covering layer of citrate ions used as electrostatic stabilizer.

The tests of the antifungal activity presented different behaviours as a result of the difference between SNPs sizes of the both samples. The sample I presents the best antifungal action as can be seen in Figs. 6-9.

Fig. 6. The aspect of the SNPs solution sample containing 5 ppm of SNPs with a mean diameter of 16.5 nm, after 14 days from inoculation.

Fig. 7. The aspect of the ZnO powder, after 14 days from inoculation.

Fig.8. The aspect of the sample I, after 14 days from inoculation.

Fig. 9. The aspect of the sample II, after 14 days from inoculation

If a clear zone of inhibition remains around the samples after 14 incubation days, it is considered that these have a good antifungal activity.

The SNPs solution samples containing 5 ppm of SNPs with a mean diameter of 16.5 nm, after 14 days inhibit all the species without Trichoderma viride (Fig. 6). The ZnO sample, after 14 days, inhibits only the growth of Aspergilus niger (Fig. 7).

The inhibition zone of the sample I, after 14 days remains clearly on a wide of 2…10 mm around it, which means that all species were inhibited (Fig. 8). The antifungal activity of this sample is better than the one of the SNPs solution sample.

The fine dispersed SNPs deposited on ZnO particles have an antifungal activity, which is not obtainable either for SNPs or for ZnO particles independently. Therefore a synergistic effect was proved.

The inhibition zone of the sample II remains clearly after 14 days, having a wide of 3 mm around the sample. An opaque zone appeared along of the circumference as a result of Trichoderma and Myrotecium germs growth (Fig. 9).

4. Conclusions

The paper presents a new type of composite powders consisting of SNPs deposited on ZnO particles.

The developed method for obtaining of these composite powders is very efficiently, ecologically, environmental friendly and can be up-scaled. This consists of ZnO particles dispersion by sonication in an $AgNO_3$ aqueous solution, followed by Ag^+ ions reduction with a tri-sodium citrate solution at 80 °C, under vigorous stirring, in order to obtain the SNPs nuclei. A final reduction was realized at 4 °C using a $NaBH_4$ solution. The obtained nanoparticles were stabilized electrostatic and steric with tri-sodium citrate and PVP, respectively.

The performed tests proved the high effectiveness of the fine SNPs from the antifungal point of view. Also, a synergistic effect was proved on the fine dispersed SNPs deposited on ZnO particles, which is not obtainable independently, either for SNPs or for ZnO particles.

The obtained composite powders are biocompatible and suitable antifungal semi-finished products for health care and consumer goods items.

References

[1] K. Y. Hwang, US Patent Appl. Publ., No. 0278534 A1, (2006).

[2] H. J Lee. S. Y. Yeo, S. H. Jeong, J. of Material Science **38**, 2199 (2003).

[3] J. L. Elechiguerra, J. L. Burt, J. R. Morones, A. Camacho-Bragado, X. Gao, H. H. Lara, J. of Nanobiotechnology **3**, 1 (2005).

[4] R. J. Holladay, H. Christensen, W. Moeller, US Patent, No. 7,135,195 B2, (2006).

[5] N. Vermerie, C. Malbrount, M. Azar, P. Arnaud, Pharmacy World & Science, **19**(4), 197 (1997).

[6] S. Y. Yang, US Patent Appl. Publ., No. 0247690 A1, (2004).

[7] G. V. Krylova, A. M. Eremenko, N. P Smirnova, Theoretical and Experimental Chemistry **41**(2), 105 (2005).

[8] I. Lee, S. Han, K. Kim, J. of Raman Spectroscopy **32**, 947 (2001).

[9] G. Rodriguez-Gattorno, D. Diaz, L. Rendon, J. Phys. Chem. B **106**, 2482 (2002).

[10] Y. Bingsheng, M. Houyi, W. Shuyun, J. Phys. Chem. B **107**, 8898 (2003).

[11] Y. S. Shon, Dekker Encyclopedia of Nanoscience and Nanotechnology, Marcel Dekker, Inc., 1, 2004.

[12] T. Yadav, A. Vecoven, US Patent Appl. Publ., No. 0008861 A1, (2005).

[13] ***, IEC 60068-2-10 Ed. 6.0 b:2005, Environmental testing - Part 2-10: Tests - Test J and guidance: Mould growth.

[14] Wang Z. l.: Materials Today **6**, 26 (2004).

[15] V. A. Coleman, C., Jagadish, Properties and Applications, edited by C. Jagadish and S. J. Pearton, 1, 2006.

---

[*] Corresponding author: stefgav@icpe-ca.ro