

SILVER AND ZINC OXIDE BASED NANO POWDERS AND THEIR POLYMER BASED NANOCOMPOSITES FOR ANTIBACTERIAL APPLICATION

Ezgi ABATAY¹, Tuğçe ÖZMEN¹, Alper ARSLANOĞLU² and Metin TANOĞLU^{3*}

¹Department of Biotechnology and Bioengineering, Faculty of Engineering, Izmir Institute of Technology, Gulbahce Campus, 35430 Urla, Izmir, Turkey

²Department of Molecular Biology and Genetics, Faculty of Science, Izmir Institute of Technology, Gulbahce Campus, 35430 Urla, Izmir, Turkey

³Department of Mechanical Engineering, Faculty of Engineering, Izmir Institute of Technology, Gulbahce Campus, 35430 Urla, Izmir, Turkey

* e-mail address of the corresponding author: metintanoglu@iyte.edu.tr (M. Tanoglu)

Keywords: Nano powder, Nanocomposites, Antibacterial properties.

Abstract

*The microorganisms cause some serious infections. It is a requirement and a necessity to create sterile fields such as hospital, food processing and public places. Composite stones are one of the main building materials that have been used in buildings due to their high resistant to abrasives, chemicals and mechanical impacts. The silver (Ag), zinc oxide (ZnO), and also nano Ag loaded ZnO (ZnO/Ag) nanopowders have demonstrated capability for the preparation of the polymer based antibacterial nanocomposite materials. In this study, Ag/polyester, ZnO/polyester, Ag/ZnO/polyester and their nanocomposites were prepared and tested with various weight fractions. The microstructure and surface morphology of these nanocomposites were investigated by means of scanning electron microscopy (SEM/EDX). The thermal properties were analyzed by differential scanning calorimetry (DSC) and thermal gravimetric analysis (TGA). Finally, The antibacterial properties of nanocomposites were analyzed against gram positive (*Bacillus subtilis*) and gram negative bacteria (*Escherichia coli*).*

1.Introduction

Recent studies focused on various antibacterial inorganic substances. Among them; Ag, ZnO and TiO₂ are traditionally well-known antimicrobial materials. Forming antibacterial activity with these powders has some potential since human beings are often infected by microorganisms such as bacterium, mold, yeast, virus and so on, in the living environment. It is a requirement and a necessity to create sterile fields in areas such as hospital, food processing and public places.

The objective of this study is to investigate the preparation routes and characterization techniques of nano-sized Ag or ZnO filled polyester based nanocomposites and ZnO/Polyester/Quartz and Ag(ZnO)/Polyester/Quartz composite stones. In addition, Ag coated ZnO particles were prepared using AgNO₃ solutions. Similarly, Ag/ZnO polyester nanocomposites were prepared and characterized. The nanopowders used within the study were characterized by SEM, XRD, FTIR, TG/DTA. The antibacterial properties of the prepared nanocomposites were analyzed against gram positive and gram negative bacteria.

2. Experimental procedure

2.1. Materials

In order to prepare nanocomposites, Ag, ZnO and ZnO/Ag powders were used as the filler, polyester was used as the matrix material.

2.2. Production of Nanocomposites

The polyester based nanocomposites were prepared by using mechanical mixing method. In this technique; polyester, cobalt octoat (CoNaP, 0.2 wt% with respect to resin) and methyl ethyl ketone peroxide (MEKP, 2 wt% with respect to resin) were mixed together with quartz which have different size until a homogenous dispersions were obtained. Then, samples were prepared by the addition of antibacterial nanopowders (3 wt% with respect to resin). The powders were separately added to resin system and stirred continuously for half an hour. The blends obtained were casted into silicon molds and the samples were cured at 80⁰C. After completion of the curing, samples were demolded and polyester nanocomposites were obtained. Finally, these are subjected to surface preparation and polishing process.

2.3. Production of Ag/ZnO Nanopowder

Ag/ZnO powders were prepared through a reduction process. In this process, ZnO and ionized water was mixed for half an hour. After adding 1wt% AgNO₃, this mixture stirred continuously at 4.5 pH for 4 hours at 70 ⁰C. Nitrate ions were filtered and washed with distilled water for purification. The remaining Ag/ZnO in filter paper was dried at 400 ⁰C for 3 hours in the oven and Ag/ZnO powders were obtained. These powders were added to the polyester system based on the procedure described above.

2.4. Characterization

Ag, ZnO and Ag/ZnO polyester nanocomposites were tested with bacterial test using E.coli and antibacterial properties of nanocomposite were determined. Overnight grown culture of E.coli was diluted, and plated on LB agar. Samples were kept on the plates and incubated at 37 ⁰C for 16 h. The plates were taken out, and the inhibition area was observed.

The particle morphology of silver nanoparticles (Ag), zinc oxide (ZnO) and silver/zinc oxide (Ag/ZnO) was characterized by means of Scanning Electron Microscopy (SEM) using FEI Quanta 250 FEG equipment to observe the shape and size of the powder. The Backscattered Electrons Detector (BSED) was used to detect the silver element coated onto the zinc oxide

surfaces. The crystallographic structures of the materials were analyzed by X-ray Diffraction (XRD) method on an Philips X'Pert Pro model. FTIR analysis was used to determine the structure of the functional groups of organic compounds by Shimadzu IRPrestige-21 FTIR-8400S. Dynamic light scattering (DLS) was used to determine the particulate sizes.

Also, The particle morphology of silver (Ag), zinc oxide (ZnO) and silver/zinc oxide (Ag/ZnO) nanocomposite was characterized by means of Scanning Electron Microscopy (SEM) using FEI Quanta 250 FEG equipment to observe the shape of the nanocomposites. The Backscattered Electrons Detector (BSED) was used to detect the nanopowders on the nanocomposite.

3. Results and Discussions

3.1. Microstructural properties of powders

The SEM micrographs of silver nanoparticles are shown in Figure 1. Based on SEM image, the average size of Ag NPs were measured about 100 nm.

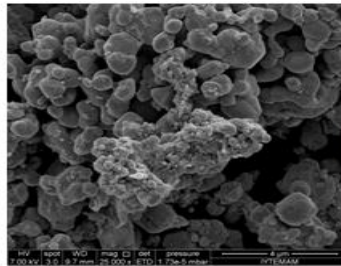


Figure 1: SEM images of Ag NPs (25000 x).

The SEM micrographs of zinc oxide particles are shown in Figure 2. Similarly, the average size of zinc oxide nanoparticles are measured about 160 nm.

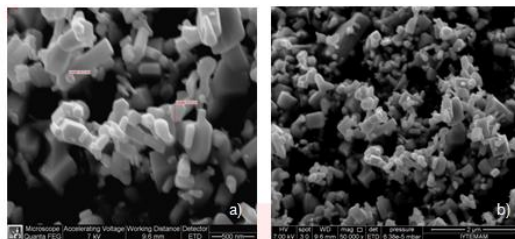


Figure 2: SEM image of zinc oxide (ZnO) particles, a) 100 000 x, b) 50 000 x.

The SEM micrographs of zinc oxide/silver (Ag/ZnO) powders are given in Figure 3. SEM images were used to evaluate the surface morphology of the silver deposited on the surface.

The Backscattered Electrons Detector (BSED) was used to reveal the presence of silver on the ZnO surfaces. Figures 3c-d show the BSED images. In BSED images, Ag is visible with brighter appearance as compared to ZnO.

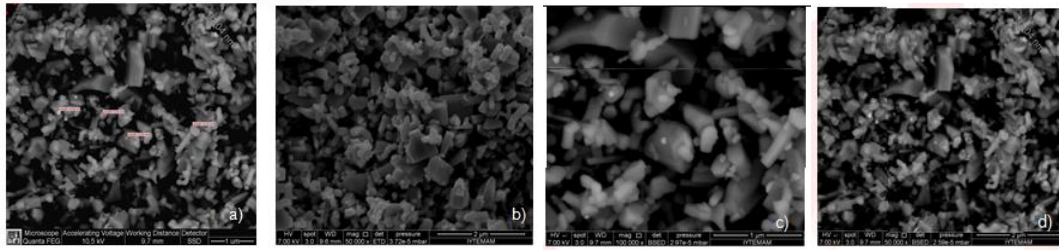


Figure 3: SEM images of zinc oxide/silver (ZnO/Ag) particles, a) 100 000 x, b) 50 000 x. BSED images of ZnO/Ag particles, c) 100 000 x, d) 50 000 x.

Ag/ZnO powder was also characterized by SEM-EDX (Energy-dispersive X-ray spectroscopy) as shown in Figure 4. Table 1 list the elemental analysis results.

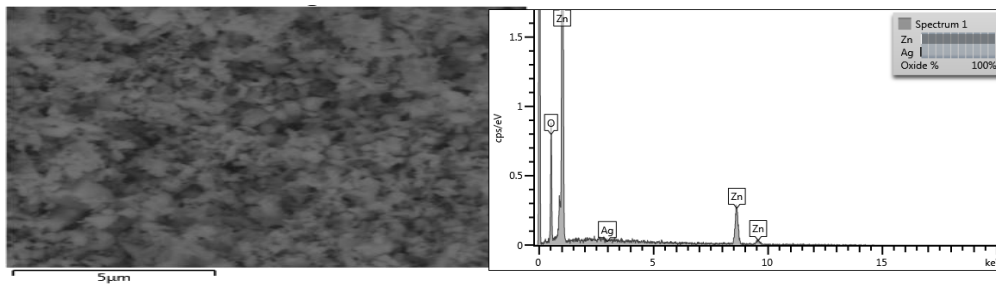


Figure 4: SEM image of Ag/ZnO powder and EDS spectrum.

Element	Wt%	Atomic %
O	19.64	49.98
Zn	80.19	49.95
Ag	0.17	0.07
Total:	100.00	100.00

Table 1: SEM-EDX analysis of Ag/ZnO powders prepared

3.2. Characterization of composite materials

The BSED image of zinc oxide powder loaded nanocomposite stone are shown in Figure 5.

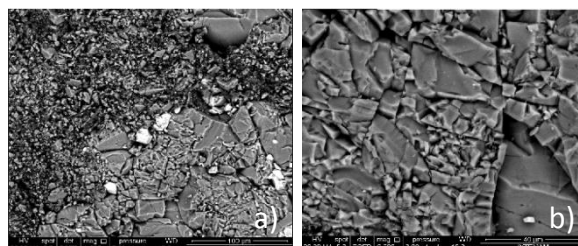


Figure 5: BSED images of a) ZnO/Polyester/Quartz NCs stone (1000 x), b) ZnO/Polyester/Quartz NCs stone (2500 x)

The BSED image of Ag(ZnO) powder loaded nanocomposite stone are shown in Figure 6.

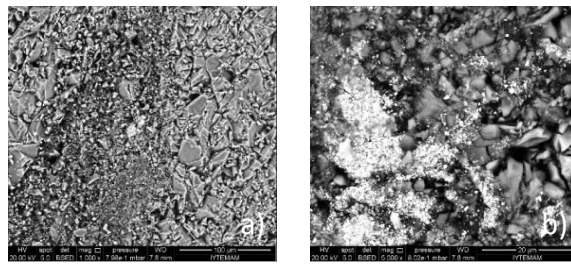


Figure 6: BSED images of a) Ag(ZnO)/Polyester/Quartz NCs stone(1000x), b) Ag(ZnO)/Polyester/Quartz NCs stone (5000x).

The BSED image of neat nanocomposite stone are shown in Figure 7. The powders are bright on the stone according to SEM images.

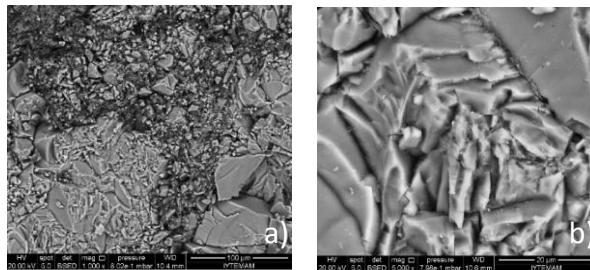


Figure 7: BSED images of a) Neat Polyester/Quartz NCs stone (1000x), b) Neat Polyester/Quartz NCs stone (5000 x).

The SEM image and EDS spectrum of Ag/ZnO nanocomposite stone including polyester and quartz are shown in Figure 8 and Table 2.

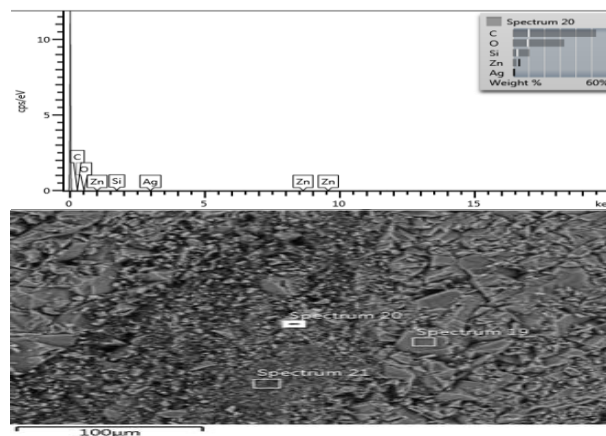


Figure 8: SEM image and EDS spectrum of Ag(ZnO)/Polyester/Quartz NCs stone.

Table 2 shows the elemental analysis of carbone, oxygene, silicone were observed from the quartz, zinc and silver. The silver amount of the system is very low that seen from Table 2.

Element	Wt%	Atomic %
C	53.06	64.02
O	32.84	29.75
Si	10.79	5.57
Zn	2.53	0.56
Ag	0.78	0.11
Total:	100.00	100.00

Table 2: EDX analysis of Ag(ZnO)/Polyester/Quartz NCs stone

The XRD patterns of Ag, ZnO and Ag/ZnO particles are shown in Figures 9. The Ag nanoparticles show four sharp peaks at $2\theta = 38.11^\circ$, 42.28° , 64.44° , 77.37° . The crystal structure of ZnO particles' peaks at $2\theta = 31.67^\circ$, 34.31° , 36.14° , 47.40° , 56.52° , 62.73° , 66.28° , 67.91° , 69.03° , and 72.48° indicating that the samples were polycrystalline wurtzite structure. In addition, the XRD pattern of Ag/ZnO particles are shown in Figure 9.

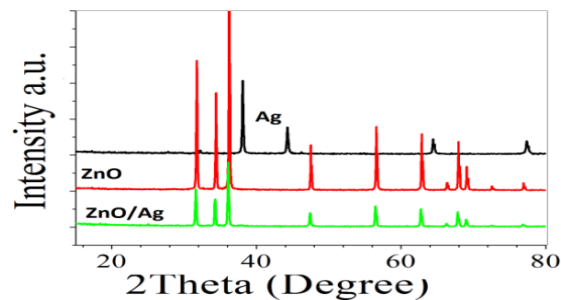


Figure 9: XRD pattern of Ag, ZnO, Ag/ZnO nanoparticles.

Infrared spectra of used nanoparticles are shown in Figure 10. Each peak of the sample spectra was examined. The peak of 2346, corresponds to C=O stretching peaks [Paulo *et al.*, 2011]. The aromatic CH stretch appears at 3100-3000 cm^{-1} . There are aromatic CC stretch bands (for the carbon-carbon bonds in the aromatic ring) at about 1500 cm^{-1} [Paulo *et al.*, 2011]. Band characteristic of nitrate ions is at 1349 cm^{-1} but this band is absent in our system, so this may indicate that NO_3^- was removed from the during the filtration system.

Therefore, this spectrum confirmed the absence of the NO_3^- on the silver nanoparticles.

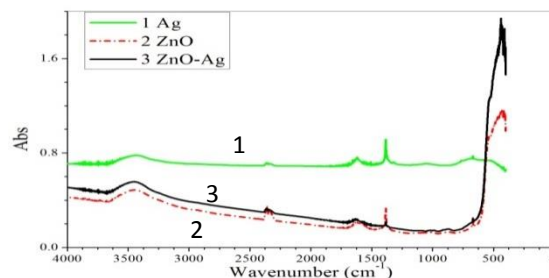


Figure 10: FTIR spectra of Ag-doped ZnO powder, Ag powder, ZnO powder.

Dynamic light scattering (DLS) was used to determine the particulate sizes. Nano silver powder has narrow diameter distribution and average particle diameter was around 73,13nm in Figure 11.

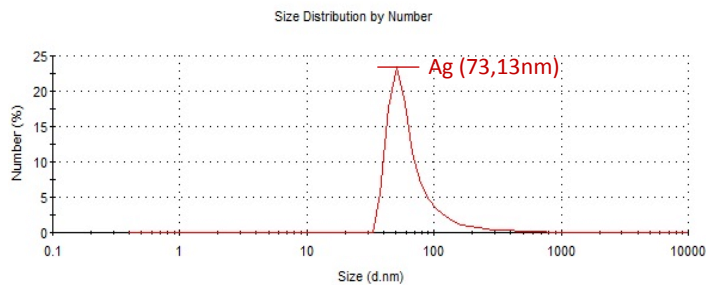


Figure 11: Dynamic light scattering analysis of silver powder.

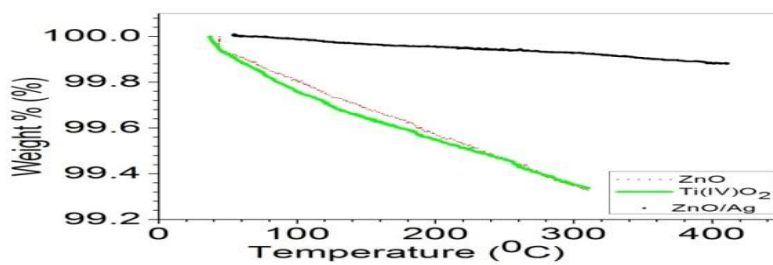


Figure 12: TGA thermogram for ZnO, TiO₂ and ZnO/Ag.

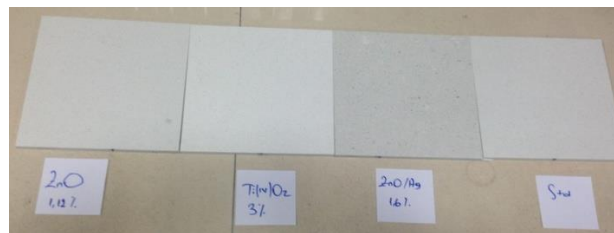


Figure 13 : Final product of ZnO/Polyester/Quartz and Ag(ZnO)/Polyester/Quartz and Neat Polyester/Quartz NCs stone.

The polyester based nanocomposites were prepared using mechanical mixing method. The characterization of nano powders were successfully analyzed and antibacterial nanocomposites were prepared by the addition of nanopowders.

4. Antibacterial Properties

The antibacterial activity of the polyester based Ag, ZnO, Ag/ZnO nanocomposites against E.coli was tested based on colony-count method. The results show that the nanocomposites have good efficacy against these bacteria. Fig. 14 shows the antibacterial activity of the Ag, ZnO, Ag/ZnO polyester nanocomposites via colony numbers.

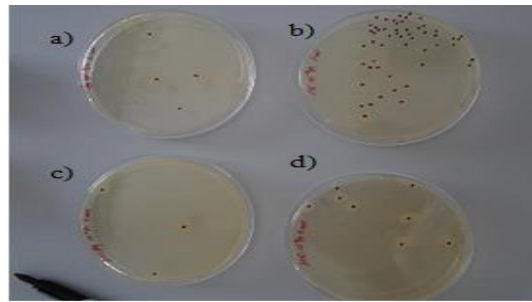


Figure 14: Colony numbers test against E.coli bacteria for Ag, ZnO, Ag/ZnO polyester nanocomposites of (a) Ag/Polyester, (b) neat Polyester, (c) Ag/ZnO Polyester, and (d) ZnO Polyester.

The prepared Ag, ZnO and Ag/ZnO polyester sites both have a strong antibacterial activity.

Test Specimen	Contact Time		
	“ 0 “	“ 1 hour “	Percent(%) Reduction
1. Polyester	$4,12 \times 10^5$ cfu / ml	$13,06 \times 10^5$ cfu / ml	9,56
2. Polyester	$4,12 \times 10^5$ cfu / ml	$13,13 \times 10^5$ cfu / ml	9,07
3. %1 Ag Polyester	$4,12 \times 10^5$ cfu / ml	$11,00 \times 10^5$ cfu / ml	23,82
4. %3 Ag Polyester	$4,12 \times 10^5$ cfu / ml	$3,79 \times 10^5$ cfu / ml	73,75
5. %5 Ag Polyester	$4,12 \times 10^5$ cfu / ml	$2,87 \times 10^5$ cfu / ml	80,12
6. %1 ZnO Polyester	$4,12 \times 10^5$ cfu / ml	$12,57 \times 10^5$ cfu / ml	12,95
7. %3 ZnO Polyester	$4,12 \times 10^5$ cfu / ml	$11,24 \times 10^5$ cfu / ml	22,1
8. %5 ZnO Polyester	$4,12 \times 10^5$ cfu / ml	$10,62 \times 10^5$ cfu / ml	26,45
9. %1 TiO ₂ Polyester	$4,12 \times 10^5$ cfu / ml	$12,57 \times 10^5$ cfu / ml	12,95
10. %1 Ag/ZnO Polyester	$4,12 \times 10^5$ cfu / ml	$13,40 \times 10^5$ cfu / ml	7,20
11. %3 Ag/ZnO Polyester	$4,12 \times 10^5$ cfu / ml	$11,24 \times 10^5$ cfu / ml	22,16
12. %5 Ag/ZnO Polyester	$4,12 \times 10^5$ cfu / ml	$6,26 \times 10^5$ cfu / ml	56,79
13. Control	$4,12 \times 10^5$ cfu / ml	$14,44 \times 10^5$ cfu / ml	—

Table 3: Antibacterial test result of test specimen

And finally, this table 3 shows the antibacterial test result. These are our test specimen. Initial bacteria is contact time is zero. After one our contact time, we observed the reduction obacteria. This reduction is calculate according to control group which is contact time 1h.

We observed the highest antibacterial reduction is five weight percent polyester silver composite materials. It is about eighty percent reduction.

The other one is 3 weigth percent polyester silver composite materials. And Ag/ZnO polyester composite has fifty persent reduction. According to the litrature, We say that this metarial has a antibacterial properties because reduction ratio should be higher than fifty percent.

5. Conclusion

Ag, ZnO, Ag/ZnO nanoparticles incorporated polyester nanocomposites have been synthesized for antibacterial applications using a simple in situ reduction method using AgNO₃ as a reducing agent under ambient conditions. Ag/ZnO particles were obtained as an alternative filler. The Ag/ZnO nanocomposites have been also evaluated as an antimicrobial agent and showed enhanced contact antimicrobial efficacy against Gram negative bacteria (E. coli). Based on antibacterial activity, Ag/ZnO nanocomposites exhibited a stronger antibacterial activity as compared to ZnO. These reveals that the Ag/ZnO nanocomposites have a potential to be considered as effective and long-lasting bactericidal surface coating material in future antibacterial and biomedical applications.

Acknowledgements: This study is supported by AKG Yalıtım ve İnşaat Malzemeleri Sanayi ve Ticaret A.Ş. and Ministry of Science and Technology of Industry, Republic of Turkey (SAN-TEZ, Project No. 1209-STZ.2012-1).

References

- [1] P. Allas. *Silver polymeric nanocomposites as advanced antimicrobial agents: Classification, synthetic paths, applications, and perspectives*. Advances in Colloid and Interface Science, 166:119-135, 2011.
- [2] K. B. Dermenci, B. Ebin, S.Gürmen. *Production of Spherical Ag/ZnO Nanocomposite Particles for Photocatalytic Applications*. World Academy of Science, Engineering and Technology, 67:682-684, 2012.
- [3] A. Zapata, Laura Tamayo, Maritza Pérez, et al. *Nanocomposites based on polyethylene and nanosilver particles produced by metallocenic “in situ” polymerization: synthesis, characterization, and antimicrobial behavior*. Macromolecular Nanotechnology, 47:1541–1549, 2011.
- [4] Y. Xu. *Study on the preparation and properties of silver-doped borosilicate antibacterial glass*. Journal of Non-Crystalline Solids, 354:1341–1345, 2008.