Original Article

Preparation of ZnO-Polystyerne Composite Films and Investigation of Antibacterial Properties of ZnO-Polystyerne Composite Films

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ABSTRACT

Background & Objectives: **Nanotechnology is one of great important part of technology. Nanoparticles can be used in different applications for industrial, medical, military and personal use. The objectives of this study were preparation of Polystyrene/ZnO nanocomposite films via a simple method and investigation of antibacterial activity of them.**

Materials and Methods: **Polystyrene/ZnO nanoparticle (PS/nano-ZnO) composite films were prepared via simple method with 0, 0.1, 1 and 2.5% wt concentration of ZnO and characterized by scanning electron microscopy (SEM). The antibacterial properties of the product were investigated against *Listeria monocytogenes, Escherichia coli, Staphylococcus aureus* and *Bacillus cereus*.**

Results: **The survival ratio of *L. monocytogenes, E. coli, S. aureus* and *B. cereus* decreased with increase of ZnO content on PS/nano-ZnO composite films and the best antibacterial activity was obtained with 2.5% wt ZnO-PS composite films for all bacteria. Results show the larger sensitivity of the *S. aureus* compared to other bacteria.**

Conclusion: **The treated fabric with ZnO NP indicates significant improve for antibacterial properties for polystyrene fabric.**

Keywords: **Anti-Bacterial Agents, Nanoparticle, Polystyrenes**

Introduction

Bactericidal materials are used in various industries such as medicine, textile and food (1, 2).

The disadvantages of using conventional organic substances for the elimination of microorganisms are toxicity and susceptibility to high temperature and pressure (1–3). For these reasons, researches have tried conserning the development of new biocidal agents such as: metal oxides (1, 3, 4). These new biocidal agents are effective at killing of microorganisms (5). They are non- toxic and

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withstand in harsh conditions (3-6). Metal or metal ions are also essential to the human beings (7-9).

Recently, nanotechnology is one of great important part of technology. Nanoparticulate formulations can be used in different applications for industrial, medical, military. Recently, the use of metal oxides nanoparticle to control and prevent the spread of microorganisms are an expanding field of research (10). Also, metal and metal oxide nanoparticle containing formulations may be utilized for external uses as surface coatings on various substrates (11-14).

Recent advances in the field of textiles lead to the development of antibacterial film by incorporating metal nanoparticles into polymeric nanofibres. The antibacterial textiles have been used in practical applications. Many textile materials also are used in hospitals and community which can be led to spread of infections in both community and hospital environments. In general, antimicrobial material can be used as an active ingredient in textile on account of its antibacterial properties. However, antibacterial textile is effective in controlling the growth of various microorganisms (15).

Nanoparticles are much more reactive than larger particles because of their small size and large surface area. They have many advantages including high surface to volume ratio, good chemical stability, biocompatibility and easy fabrication which make them appropriate for preparing hygienic surfaces (16). Fabrics coated with silver nanoparticles are quite common (16, 17). To our knowledge, little is known about the preparation of fabric modified by ZnO nanoparticle (18). ZnO nanoparticle is currently being investigated as an antibacterial agent against bacteria (19). The ZnO nanoparticles are non-toxic for human body and animal cell at very low concentration (20, 21). ZnO is one of the five zinc compounds that are currently listed as a substance generally recognized as safe (GRAS) by the Food and Drug Administration of the United States of America (21CFR182.8991). Zinc salt has been used for the treatment of zinc deficiency (22).

This paper focuses on the preparation and characterization of Polystyrene/ZnO (PS/ZnO) nanocomposite films. In addition, investigation of antibacterial activity of Polystyrene/ZnO (PS/ZnO) nanocomposite films was conducted.

Materials and Methods

Bacterial strains, media and materials

The following bacterial strains were used in this study: *Listeria monocytogenes* PTCC1163, *Escherichia coli* PTCC1394, *Staphylococcus aureus* PTCC1431 and *Bacillus cereus* PTCC1015 were obtained from the culture collection of the I.R. Dept. (Tehran, Iran). Moreover, Stock cultures were stored in the -80˚C freezer. The strains were propagated on Tryptic Soy Agar (TSA; Merck, Darmstadt, Germany) at 37˚C and maintained at 0– 2˚C before use. Zinc oxide nanoparticles were purchased from TECONAN, Spain (particle diameters: 20–25 nm; purity: 99.98%).

Polystyrene/ZnO (PS/ZnO) nanocomposite films

Polystyrene textile was first dissolved in toluene. The ZnO nanoparticles were dissolved into methanol and then directly added into the PS toluene solution. Three concentrations of 0.1, 1 and 2.5 wt% of ZnO in PS were prepared. The solution mixtures were then placed into glass plate and the solvents were evaporated in a vacuum oven at room temperature. The PS/ZnO nanocomposite films were obtained after evaporation and then heated at 80˚C to remove any solvents trapped in the polymer. For comparison purposes, a neat PS film without ZnO was also prepared according to the above mentioned method (23).

Scanned electronmicroscopy (SEM)

ZnO-PS films were cut with surgical scissors into 3 × 5 mm pieces and mounted directly on specimen stubs with 2-sided adhesive tabs of carbon (Electron Microscopy Sciences, Hatfield, Pa., U.S.A.). Mounted film strips were sputter coated with a thin layer of gold using a Scan coat Six Sputter Coater (BOC Edwards, Wilm-ington, Mass., U.S.A.). Digital images of topographical features of the filmstrips were collected using a LEO 1450 VP scanning electron microscope (LEO 1450 VP, England, Australia, and South African) operated in the high vacuum/secondary electron imaging mode at an accelerating voltage of 10 kV.

Liquid culture test

ZnO film was placed in a TSB medium and inoculated with overnight cultures of *L. monocytogenes*, *E. coli*, *S. aureus* and *B. cereus*, respectively. The glass tubes contained 5mL of liquid. The final cell density in a medium was approximately 1×10^7 cells per milliliter in each bacterial inhibition test. The tubes were shaken at 50 rpm at 25 ± 1˚C. The inoculated media were sampled (1.0 mL) at 24 h. Specimens were serially diluted, and then pour plated onto TSA. Plates were incubated at 37˚C for 24 h. Zinc oxide free inoculated PS/ZnO nanocomposite films served as a control (23).

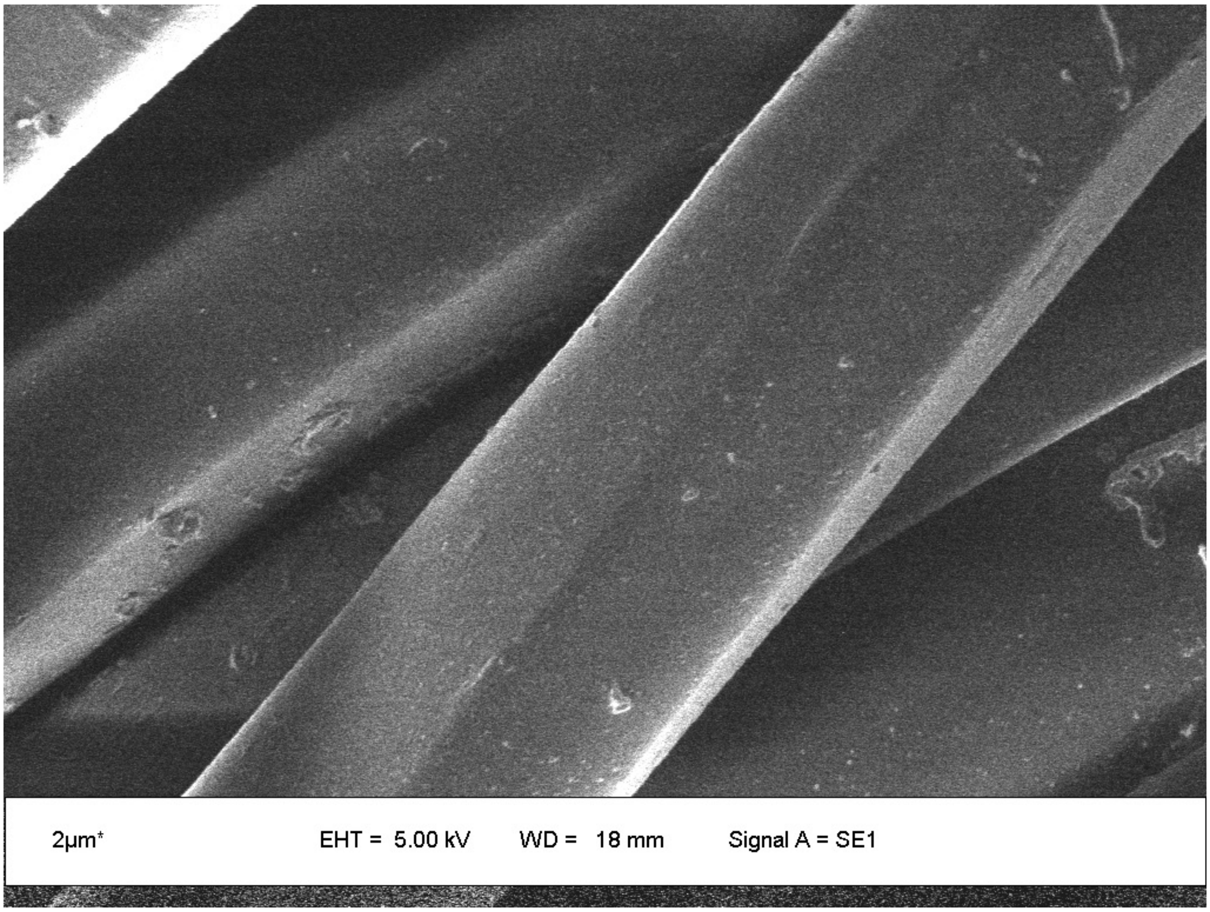
Agar diffusion test

The agar diffusion test was especially used for film samples. The inoculum of *L. monocytogenes*, *E. coli*, *S. aureus* and *B. cereus* was spread on the surface of TSA, respectively. Each film sample was placed on surface-inoculated TSA agars and incubated at 37˚C for 24 h. Inhibition zone around film specimens was used to indicate antibacterial activity of each film sample (24).

Results

SEM image of nanocomposite films

SEM was used to characterize the surface of PS treated with ZnO. SEM images of treated PS and untreated sample are shown in Fig. 1. SEM images (Fig. 1) of the coated PS textile show the presence of compound coating on the fibres.



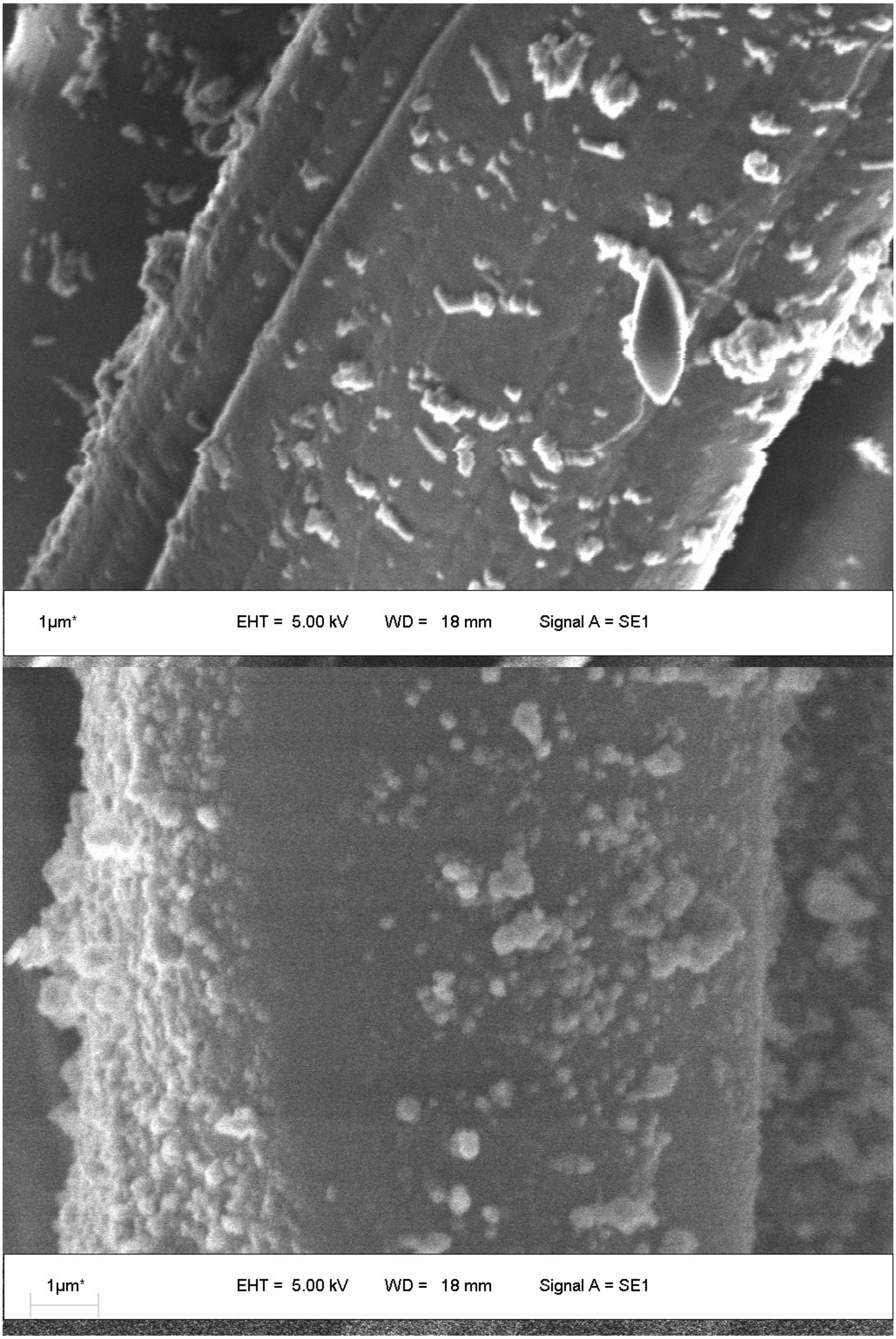


Fig.1: Scanning electron microscope photomicrographs of; a) Uncoated; b) Coated Polystyrene textile by 0.1% ZnO; c) Coated Polystyrene by 1% ZnO.

Antibacterial studies

Antibacterial activity of the PS-ZnO composite films with different ZnO contents was tested using *L. monocytogenes*, *E. coli*, *S. aureus* and *B. cereus* in comparison with the pure PS film, as displayed in Table 1. Table 1 clearly says that all samples with 0.1% wt ZnO have not inhibition zone. Samples with 1% wt ZnO have not inhibition zone but caused growth reduction of bacteria. Table 1 also clearly says all samples with 2.5% wt ZnO have inhibition zone on all of bacteria.

Results of liquid culture tests showed that the treatment with ZnO-PS film had no effect on suppressing the growth of all of bacteria in the liquid culture test.

Table 1- Inhibition zone diameter of 1 cm of ZnO-PS film

Concentration of composite%	*Listeria monocytogenes mm*	*Staphylococcus aureus mm*	*Bacillus cereus mm*	*Escherichia coli mm*
Control	-*	-	-	-
0.1	-	-	-	-
1	Gr**	Gr	Gr	Gr
2.5	11	14	11	11

*No Inhibition Zon. ** Growth Reduction

Discussion

Antibacterial properties of ZnO-PS film treated with ZnO are measured according to the inhibition zone method against *L. monocytogenes*, *E. coli*, *S. aureus* and *B. cereus*. Result have shown that growth reduction and low inhibition zone against all of bacteria for ZnO PS films with 1% and 2.5% wt ZnO, respectively. The survival ratio of *L. monocytogenes*, *E. coli*, *S. aureus* and *B. cereus* decreased with increase of ZnO content and the best antibacterial activity was obtained with 2.5% wt ZnO for all of bacteria. Results showed that the larger sensitivity of the *S. aureus* compared to other bacteria. The treatment with ZnO-PS film had no effect on suppressing the growth of bacteria in the liquid culture test. These data implied that ZnO was not released from the film into the growth broth. This suggests that the ZnO molecules are tightly bound within the film, which prevents ZnO release and expression of the antimicrobial action in broth medium.

Bacterial infection control is a serious problem in public health worldwide and food industry, so that the development of antimicrobial drugs and coated surface has attracted increasing attention in recent years. Recently use inorganic material for overcoming antibiotic resistant bacteria has been explored as a promising in alternative to the current antimicrobial drugs approaches (25, 26). Previous studies have indicated that nanocrystalline metals, (25, 27, 28) and metal oxides nanoparticle can be used as antibacterial formulations (27). The inorganic material can be used in various forms, such as powder (25, 26, 28), coated on cellulose fibers (29), or as a part of organic/inorganic nanocomposite coating (23). Nano-ZnO/high-density polyethylene (HDPE) composite films were prepared by Li *et al.* The properties of HDPE, including ultraviolet absorption, mechanical and antibacterial activity of the films, and plasticizing behavior of the HDPE, were investigated. The results demonstrated that the incorporation of modified nano-ZnO to the HDPE matrix significantly improved the performance of the nanocomposites (30).The antibacterial activities of the CuO–fabric composite were tested against *E. coli* and *S. aureus* cultures by Perelshtein *et al.* This study has indicated that CuO–fabric composites are efficient at killing these bacteria (31).

Xue-Yong *et al.* in 2009 have prepared flower-like ZnO nanowhiskers (f-ZnO) composed of uniform nanorods. Waterborne polyurethane/

flower-like ZnO nanowhiskers (WPU/f-ZnO) composite with different f-ZnO content (0–4.0 wt%) was synthesized by an in situ copolymerization process. Antibacterial activity of WPU/f-ZnO composite films against *E. coli* and *S. aureus* was tested. The results showed that the antibacterial activity influenced by the content of f- ZnO (29). ZnO/carboxymethyl chitosan bionano-composite was prepared at different temperatures. The bionano-composite was used as a finishing agent for cotton fabric to impart UV protection and antibacterial properties to cotton fabric. The results demonstrated that the incorporation of ZnO/carboxymethyl chitosan bionanocomposite to the cotton fabric significantly improved the antibacterial activity of the finished cotton fabric which influenced by the content of composite. Also the UV protection properties was influenced by the increasing the temperature of curing (15). Polyurethane-based coatings (PU) by ZnO nanoparticles were prepared. PU films with loading of ZnO nanoparticle compared to neat PU, the yielded composite films shows many excellent features. Mechanical properties, tensile strengths, abrasion resistance and antibacterial properties of ZnO-composite films, however, are better than those of neat PU. (32). A series of polypropylene carbonate (PPC)/ZnO nanocomposite films with different ZnO contents were prepared via a solution blending method by Seo *et al.* The PPC/ZnO nanocomposite films exhibited antibacterial activity (33).

However, antimicrobial coatings have the potential for protection of the surface, since survival of microorganisms on surfaces can lead to the spread of infections in both community and hospital environments. The factors that facilitate the spread of coating applications are better safety and stability, while nanoscale powders may pose a hazard upon inhalation. Toxicity of nanoparticles is generally larger than larger particles of the same material. Therefore, recent advances in the field of nanotechnology have led to the development of new antimicrobial nano structured coatings (25).

Conclusion

In the present study, a simple method for the preparation of ZnO nano composite film / PS has been developed for the expression of functional properties. The treated fabric shows antibacterial activity for fabric polystyrene. Thus the fabric may be able to protect the body against solar radiation, bacterial action, and for other technological applications.

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