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Original Research Article

## Investigation of Morphology and Antibacterial Properties of Nylon 6, 6/PANI/ZnO Nanocomposite

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### ABSTRACT

In this paper, the Nylon 6,6/Polyaniline/Zinc oxide nanocomposite with different weight loadings of each component were prepared. Antibacterial properties of the prepared nanocomposites were investigated against gram-positive Staphylococcus and gram-negative Escherichia coli (E. coli) bacteria using Resazurin indicator and optical density measurements. The results showed that the addition of equal quantities of Polyainine and ZnO nanoparticles with 5% wt. of each component to Nylon 6,6 produce the best antibacterial effect. The antibacterial effect of nanocomposite is higher on gram-negative bacteria in compared to gram-positive one. Investigation of the morphologies of optimum nanocomposite by FESEM showed its proper morphology, sufficient porosity, and high surface area for contact with bacteria. Elucidation of functional groups in optimum specimen by FT-IR revealed the existence of them in nanocomposite representing the successful formation of nanocomposite.

**Keywords:** *Polyaniline, ZnO, Nylon 6,6, Antibacterial*

## Introduction

As a challenge in food industry, microbial contamination reduces the shelf-life of foods and increases the risk of foodborne illness. Traditional methods in preserving foods include thermal processing, drying, freezing, refrigeration, addition of chemicals, modified atmosphere-packaging, and irradiation have some disadvantages such as deforming of food products, uneconomic, and limited preservation time [1]. A relatively novel approach to food preservation is antimicrobial food packaging [2]. Antimicrobial packages have been studied in the last thirty years and active packages with incorporated antioxidants [3] and UV-light absorbers [4] are available. Examples of antimicrobial materials evaluated for food packages include imazalil-impregnated low-density polyethylene to control mold growth on cheese [4] and bell peppers [5], and benomyl coupled to Surlyn to inhibit molds [6]. Cohen et al. [7] demonstrated that UV-irradiated nylon exhibits antimicrobial activity, suggesting that it might provide a route to an antimicrobial packaging material. Another important property in food-packing is its antistatic properties. The previous reports represent that the incorporation of a conducting polymer in the insulating package matrix may resolve this issue and perform antistatic properties in final package [8]. Among the intrinsically conducting polymers, polyaniline (PANI) is one of the most studied conducting polymers due to its easy synthesis, good environmental stability and reversible acid–base chemistry in aqueous solutions [9]. Recently, zinc oxide (ZnO) nanoparticles have received great attention because of their unique catalytic, electrical, electronic, optical, and antibacterial properties as well as their low cost and possible applications in diverse areas [10]. ZnO-NPs provide antimicrobial activity for food packaging. Once they are introduced in a polymeric matrix, it permits interaction of food with the packaging possessing functional part in the conservation [11]. In this work, we employed Nylon 6,6 as supporting matrix for preparation of nanocomposite with a simple rout. Dissolving of Nylon 6,6 was carried out in acid formic, followed by dispersion of PANI and ZnO nanoparticles via ultrasonic irradiation. Finally, the antibacterial properties of various formulations of nanocomposites were investigated against gram-positive and gram-negative bacterium. Furthermore, some characterization techniques were employed to elucidate the structure of optimum nanocomposite.

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## **Experimental**

### **Materials**

Nylon 6,6, ZnO nanoparticles and formic acid were all purchased from Sigma. Resazurin (camlab chemicals CB4WE) was used as indicator.

### **Instruments**

Fourier Transform Infrared Spectroscopy (FTIR) of the samples was recorded on Bruker Optics TENSOR 27 spectrometer using KBr pellets. Surface morphology imaging was carried out on field-emission scanning electron microscopy (FE-SEM MIRA3 FEG-SEM, Tescan, Czech). To measure antimicrobial activity of nanocomposites Hitachi U-2900 UV-Vis spectrophotometer was used.

### **Antimicrobial**

Test Firstly, 100 ml of Nutrient Broth one cabbage of resazurin (camlab chemicals CB4WE) were added to each cultivate well. Then, for each well 0.5 McFarland of each bacteria in separate experiments was added. Finally, with the addition of equal amounts of each nanocomposite to cultivate wells, their antimicrobial activity was elucidated by incubating at 37 for 24 h.

### **Preparation of nanocomposite**

For preparation of nanocomposites, firstly, the required amounts of Nylon 6,6 was dissolved in 60ml formic acid. Then, the desired amounts of PANI and ZnO powder was added to it and agitated by ultrasonic irradiation. Then, the mixture was left to dry at room temperature. The Nylon 6,6/PANI/ZnO nanocomposites with different weight percentages of PANI and ZnO was prepared. The formulations are represented in Table 1.

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**Table 1.** The composition of prepared nanocomposites

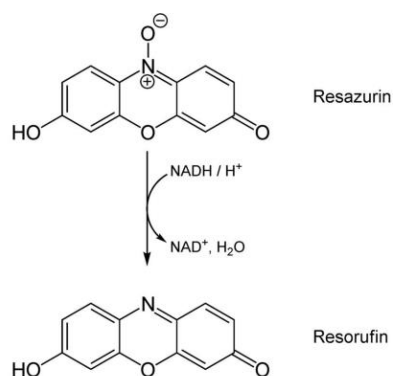
ZnO (g)	PANI (g)	Nylon 6,6 (g)	composition (% wt.)	Sample
0.006	0.03	0.56	1% ZnO 5% PANI 94% Nylon 6,6	<b>1</b>
0.006	0.066	0.534	1% ZnO 10 % PANI 89 % Nylon 6,6	<b>2</b>
0.006	0.126	0.474	1% ZnO 20 % PANI 79% Nylon 6,6	<b>3</b>
0.018	0.048	0.552	3% ZnO 5 % PANI 92% Nylon 6,6	<b>4</b>
0.018	0.078	0.552	3% ZnO 10 % PANI 87% Nylon 6,6	<b>5</b>
0.018	0.138	0.462	3% ZnO 20 % PANI 77% Nylon 6,6	<b>6</b>
0.03	0.03	0.54	5% ZnO 5 % PANI 90% Nylon 6,6	<b>7</b>
0.03	0.06	0.51	5% ZnO 10 % PANI 94% Nylon 6,6	<b>8</b>
0.03	0.15	0.45	5% ZnO 20 % PANI 75% Nylon 6,6	<b>9</b>

## Results and discussion

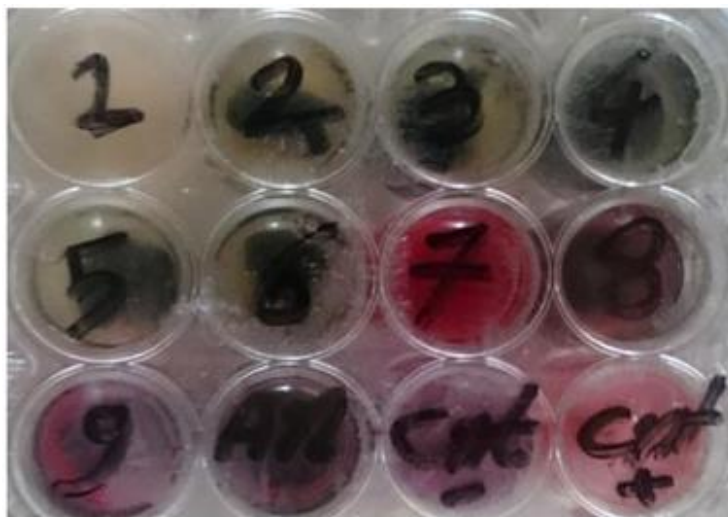
### Antibacterial tests

Staphylococcus aureus as gram-positive and Escherichia coli (E. coli) was employed as gram-negative bacteria for elucidation of antibacterial effects of nanocomposites. The growth of bacterium cause the production of enzymes which reduces the resazurin to resorufin and the purple color of the medium will disappear as seen in scheme 1. Fig.1 shows the photos of each culture medium for all nanocomposites and for Control samples including Ampicillin (Am), positive control (Cnt<sup>+</sup>), and negative control (Cnt<sup>-</sup>) for Staphylococcus aureus bacterium. The Am sample caused the death of bacterium in which due to absence of NADH and enzymatic effects, the primary purple color of medium has

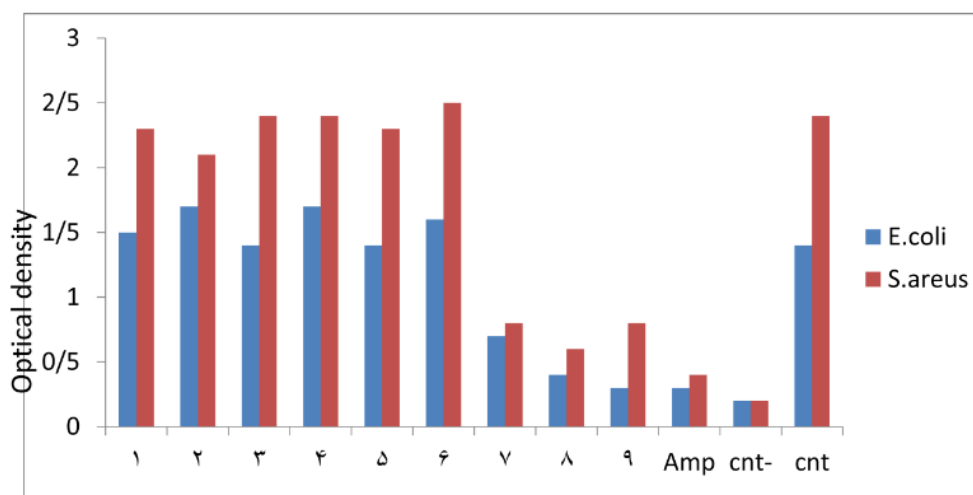
not changed. A similar situation was also observed for Cnt – sample which it has not bacterium. The color change was clearly seen for Cnt + sample which contain *Staphylococcus aureus* bacterium. Moreover, the color change was also observed for samples 1 to 6. But for samples 7-9 and especially for sample 7 the initial purple color have not changed. These results show that nanocomposites of 7-9 have antibacterial effects. Furthermore, the sample 7 with equal amounts (5 %wt.) of ZnO and PANI has the best antibacterial effect among all of the nanocomposites. For precise investigation of antibacterial effects of nanocomposites, UV-Vis spectroscopy was employed and the absorption at 600 nm for each sample as a sign of bacterial content was measured. Fig. 2 represents the results of UV-Vis measurements. As seen, the samples of 1-6 along with Cnt + have high absorption intensity which verifies the results of resazurin assay. Moreover, the samples 7-9 show antibacterial effects with low absorption intensities like as Am and Cnt – samples. The lack of antibacterial activity for nanocomposites 1-6 may be arises from the low weight loadings of ZnO (1-3 %wt.). The other observation is that the antibacterial effect is higher in the case of gram-negative *E Coli* bacterium compared to gram-positive *Staphylococcus aureus* bacterium which arises from the thinner cell wall of the former compared to later. Also the results of OD are in agreement with resazurin assay but the former results show the least bacterial mass for sample 8. By considering the cost-effectiveness of additives, the sample 7 with the composition of Nylon 6,6 90 % wt./PANI 5% wt./ZnO 5% wt. was selected as optimum nanocomposite and in the following its structural and morphological peroperties were investigated by FE-SEM technique.



**Scheme 1.** The reduction of purple Resazurin to colorless Resorufin in the presence of NADH which produced from bacterium



**Fig.1** Photos representing the color of culture mediums including nanocomposite samples from 1 to 9 and control samples including Ampicillin (Am), negative control (Cnt -), and positive control (Cnt +)



**Fig 2.** Optical density results for all specimens and control samples for both of bacteria

### Images of Scanning Electron Microscopy

The FESEM images of Nylon 6,6 90 % wt./PANI 5% wt./ZnO 5% wt. at different magnifications are seen in Fig. 3. At 10 kx magnification, nanofibers of PANI and flakes of nylon are seen with interpenetrated structure. At higher magnifications of 50 and 100 kx, nanofibers of PANI are seen which entered into Nylon matrix which both of them have fluffy structures. Due to fluffy structure of the matrix, the ZnO nano particles are not be recognizable. The morphology analysis shows that the nanocomposites have proper morphology, sufficient porosity, and high surface area for contact with bacteria.

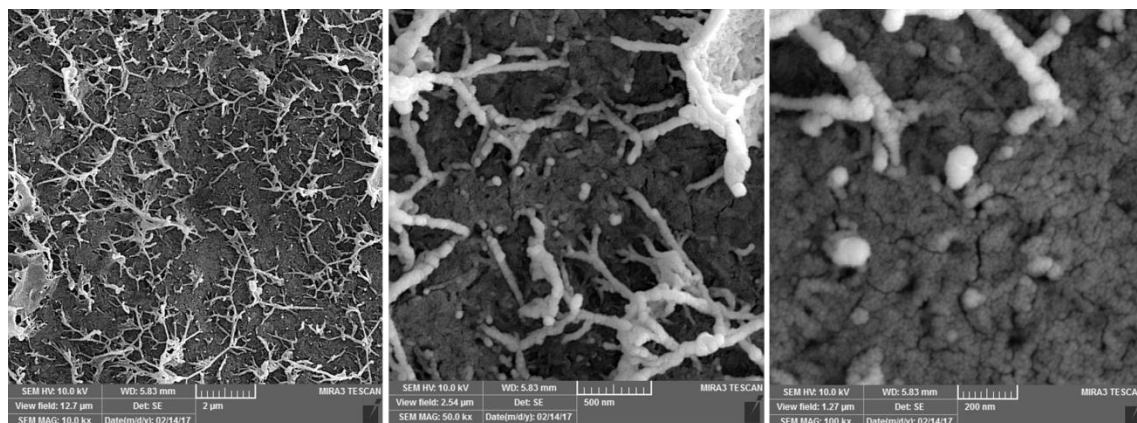


Fig. 3. FESEM images of Nylon 6,6 90 % wt./PANI 5% wt./ZnO 5% wt. at different magnifications

### FTIR Spectroscopy

The FT-IR spectra for Nylon 6,6 and optimum nanocomposite are seen in Fig. 4. For Nylon 6,6 the peaks appeared at  $1192$  and  $1273$   $\text{cm}^{-1}$  may be assigned to  $-\text{NH}-\text{CO}-$  skeletal vibrations,  $1368$   $\text{cm}^{-1}$  to  $\text{CH}_2$  wagging and  $1545$   $\text{cm}^{-1}$  to  $\text{N}-\text{H}$  bending,  $1640$   $\text{cm}^{-1}$  to amide stretching,  $2860$   $\text{cm}^{-1}$  to  $\text{CH}_2$  symmetric stretching and  $3299$   $\text{cm}^{-1}$  to  $\text{NH}$  stretching vibrations.

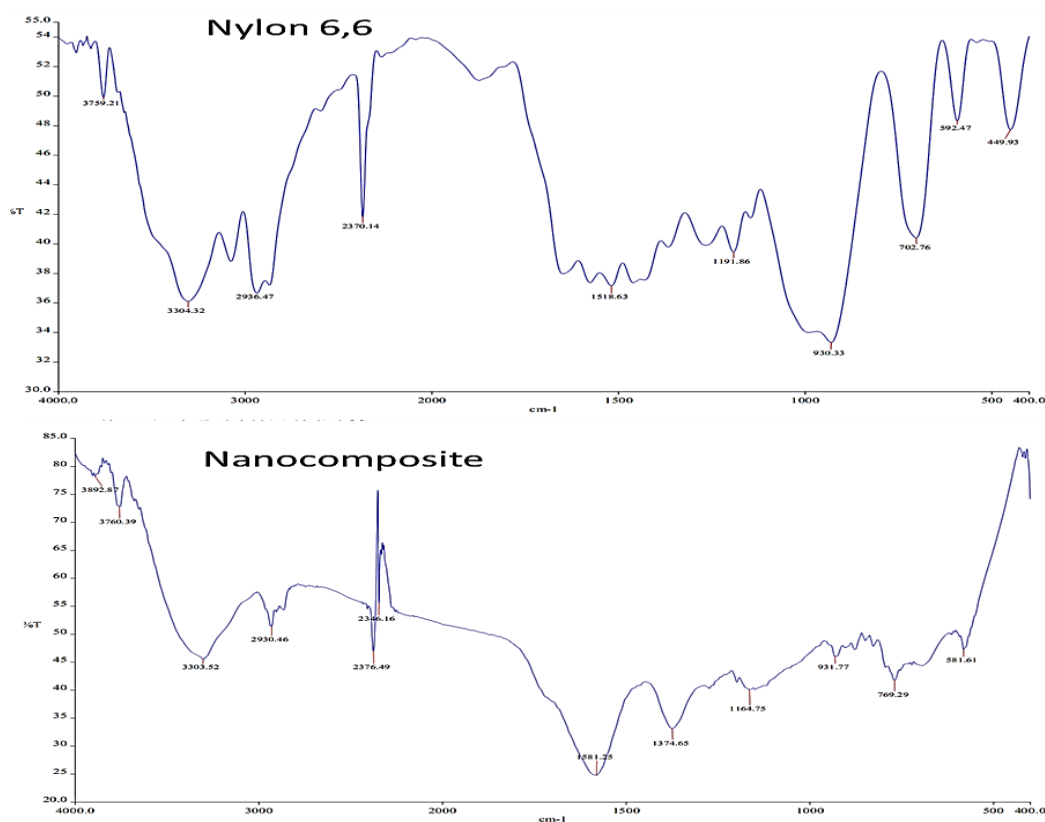


Fig. 4 The FT-IR spectra for Nylon 6,6 and optimum nanocomposite

For the nanocomposite, the broad peak ranging between 440 and 550  $\text{cm}^{-1}$  may be assigned to ZnO. The presence of PANI in the nanocomposite was verified by the band corresponding to the out-of-plane bending vibration of C–H bond of p-disubstituted benzene rings appears at 826  $\text{cm}^{-1}$ , the bands corresponding to vibration mode of N=Q=N ring and stretching mode of C–N bond appear at 1147  $\text{cm}^{-1}$  and 1305  $\text{cm}^{-1}$ , respectively, and by bands at 1586 and 1495  $\text{cm}^{-1}$  assigned to C=C stretching vibration of quinoid (N=Q=N) and benzenoid (N–B–N) rings, respectively, where Q=quinoid and B=benzenoid [11]. As a result, the FT-IR spectrum of nanocomposite reveals the peaks corresponds to ZnO, PANI; thus, the successful preparation of nanocomposite was verified.

## Conclusions

The results showed that the addition of equal quantities of Polyamine and ZnO nanoparticles with 5% wt. of each component to Nylon 6,6 produce the best antibacterial effect and the Nylon 6,6 90 % wt./PANI 5% wt./ZnO 5% wt. formulation has the best performance among them. The antibacterial effect of nanocomposite is higher on gram-negative bacteria in compared to gram-positive one due to the thinner cellular wall of former. Investigation of the morphologies of optimum nanocomposite by FESEM showed its proper morphology, sufficient porosity, and high surface area for contact with bacteria. Elucidation of functional groups in optimum specimen by FT-IR revealed the existence of them in nanocomposite representing the successful formation of nanocomposite.

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### **How to Cite This Article**

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