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ANTIMICROBIAL ACTIVITY OF Ag:ZnO/CHITOSAN COMPOSITES

Mariana (BUȘILĂ) IBĂNESCU[1*], Dana TUTUNARU[2], Viorica MUȘAT[1]
[1]“Dunărea de Jos” University of Galaţi, Centre of Nanostructures and Functional Materials-CNMF,
111 Domnească Street, 800008, Galaţi, Romania
[2]“Dunărea de Jos” University of Galaţi, Faculty of Medicine and Pharmacy,
111 Domnească Street, 800008, Galaţi, Romania
[*]Corresponding author
e-mail: mariana.ibanescu@ugal.ro

ABSTRACT

This paper deals with Ag, ZnO and Ag-doped ZnO nanoparticles embedded in chitosan (CS) matrix, prepared by hydrolysis of zinc acetate in isopropanol in the presence of lithium hydroxide (LiOH) in low temperature conditions.

The nanoparticles embedded in the chitosan matrix were characterised by X-ray diffraction (XRD) and scanning electron microscopy (SEM).

The antimicrobial activity of Ag/CS, ZnO/CS and Ag:ZnO/CS composites with respect to simple CS polymer was investigated by using the paper disc method on Mueller-Hinton agar without blood against the Gram-negative bacteria, Escherichia coli (E. coli) and the Gram-positive bacteria, Staphylococcus aureus (S. Aureus) on Mueller-Hinton agar with blood. The nanocomposites materials showed good antimicrobial activity, that recommends them for applications in medical and food packaging fields.

KEYWORDS: chitosan, ZnO nanoparticles, antimicrobial activity

1. Introduction

Polymer nanocomposites containing semiconductive zinc oxide nanoparticles have attracted a great interest due to their unique chemical and physical properties. The need of elaboration of non-toxic and cost-effective antimicrobial finishing of fabrics grows with progressive production increase of medical, healthcare and protective materials [1]. Embedding of semiconductor nanoparticles in polymeric matrices provide a way for a better exploitation of their optical properties, catalytic and antimocrobiene characteristic. On the other hand, the host polymer may influence the growth and spatial arrangement of the nanoparticles in situ during the synthesis, making them suitable for the preparation of nano patterns with different morphologies [2].

Furthermore, by selecting the polymer with certain favorable properties such as biocompatibility [3], conductivity [4] or photoluminescence [5], it is possible to obtain the nanocomposite materials for various technological purposes. In the last decades the use of biopolymers in research and industry has significantly increased due to their low cost, renewability of sources, and nontoxic, environment friendly (e.g. ‘green’) processing. This has also been reflected in the metal–polymer nanocomposite research area where polysaccharide biopolymers, such as starch [6-7], and chitosan [8-12], proved to be good environments for controlled growth of semiconductive oxide nanoparticles.

Chitosan, a polysaccharide biopolymer obtained by deacetylation of chitin, due to the presence of both amino and hydroxyl groups in its monomers, exhibits excellent chelating and film-forming properties [13].

Another, widely unnoticed, property of chitosan is its photoluminescence in the ultraviolet region of electromagnetic radiation [14]. Recently, has been was shown that biopolymers containing ultraviolet emitting chromophores can be used to induce photochemical reactions [15-16]. The combination of inorganic agents Ag, ZnO, SiO_2, TiO_2 with organic polymer-chitosan has attracted intensive research interest because of its important biological applications especially in bactericidal effect. It has a significant potential for preventing infections, for healing wounds [17] and it has anti-inflammatory properties.

Hence, these composites have been incorporated into textile fabrics, polymers, dental material, medical device and burn dressing to eliminate microorganisms.

This paper reports studies on Ag:ZnO/chitosan composites with high antibacterial activities prepared by successfula chemical procedure. The resulting materials were investigated using electron microscopy (SEM). Also, the antimicrobial activity of the as prepared materials was measured.

2. Experimental details

2.1. Materials and microorganisms

Chitosan (low molecular weight), silver nitrate ($AgNO_3$), zinc acetate dihydrate ($Zn(O_2CCH_3)\cdot 2(H_2O)_2$) and lithium hydroxide (LiOH) were purchased from Sigma–Aldrich and used as received.

Antimicrobial susceptibility was performed on isolated bacteria from urine culture Gram-negative bacteria, *Escherichia coli (E. coli)* and the Gram-positive bacteria, *Staphylococcus aureus (S. Aureus)* on Mueller-Hinton agar.

2.2. Synthesis

ZnO is prepared by hydrolysis of zinc acetate in 2-propanol using lithium hydroxide.

Different amounts of $AgNO_3$ (0.1; 5 and 15 at%) and ZnO nanoparticles (0.035 M) were dissolved in 100 mL of 1% of aqueous solution to obtain silver and zinc cations. And 1.0 g of chitosan was added to the above solution. Then the mixture was sonicated for 20 minutes after magnetic stirring and then acidity was adjusted by adding 0.1 M NaOH solution (pH 4.8) to obtain clear sol.

Figure 1 presents the composite sols of ZnO/CS, Ag/CS and Ag:ZnO/CS.

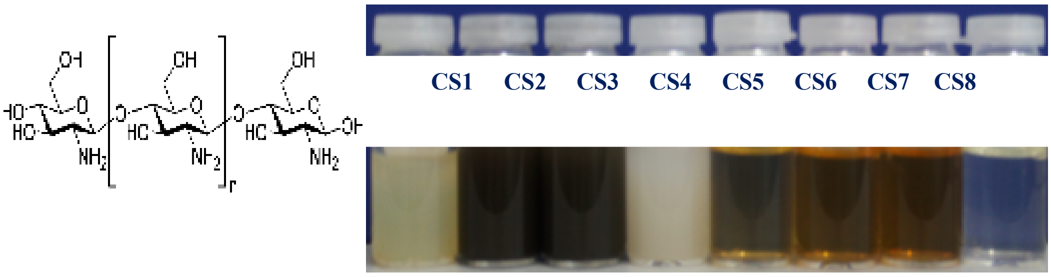

***Fig. 1**. Aspect of composite sols of ZnO/CS, Ag/CS and Ag:ZnO/CS*

2.3. Characterization of Ag:ZnO/chitosan composites

The crystal structures of the product were identified by X-ray diffraction patterns DRON-3 diffractometer system (Burevestnik, USSR) with CoKα radiation, $\lambda = 1.789$ Å.

The morphology and the composition of the product were examined by scanning electron microscopy (SEM-Quanta 200), X-ray Energy Dispersive Spectrometer (EDS-FEI).

2.4. Antimicrobial testing

The antimicrobial activity of Ag/CS, ZnO/CS and Ag:ZnO/CS composites with respect to simple CS polymer was investigated by using the paper disc method on Mueller-Hinton agar without blood against the Gram-negative bacteria, *Escherichia coli (E. coli)* and the Gram-positive bacteria, *Staphylococcus aureus (S. Aureus)* on Mueller-Hinton agar with blood. For this has been used sterilized paper disc of 6 mm in diameter impregnated with 10 μL (5 mg/1 mL) solution of the composite. In each sterilized culture dish, 0.2 mL fresh broth cultured for 24 h was added followed by 20 mL melted nutrient agar medium at approximately 50 °C. The dishes were then cooled down to room temperature before being ready for use.

The samples were then gently pressed against the medium plate to have good contact with the inoculated agar, then turned flat. The samples stayed in a constant temperature incubator at 37 ± 1 °C for 24 h, before the inhibition zones were measured.

The method for antimicrobial testing was standardized by correlation of zone diameters with minimal inhibitory concentration determined in broth.

3. Results and discussions

3.1. XRD analysis

Fig. 2 shows the X-ray diffraction patterns of pure CS film (Fig. 2a) and Ag:ZnO/CS composites films (Fig. 2b). The structure of CS was affected by incorporation of Ag and ZnO nanoparticles which disrupted the regular order of polymer chains [18]. Compared to Fig. 1a, the diffraction pattern of the Ag:ZnO/CS composite films exhibited three additional peaks at 35.8°, 36.8° and 38.4° (Fig. 1b), which were assigned to the (1 0 0), (0 0 2), (1 0 1), planes of specific hexagonal zinc oxide nanoparticles. Besides, the peak at 39.5^o indicated the presence of Ag as shown in Fig. 2b. These data revealed that it is the successful formation of Ag:ZnO/CS composites.

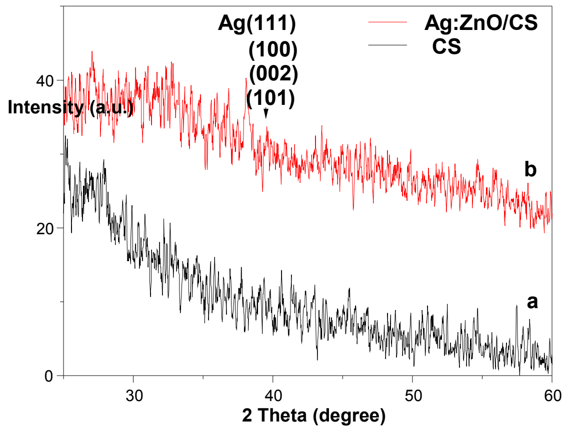

Fig. 2. *XRD patterns for a) CS pure film; b) Ag:ZnO/CS composite film*

3.2. Surface morphologies

Fig. 3 presents SEM images of pure CS film and Ag:ZnO/CS composite.

The pure chitosan film displayed a smooth surface (Fig. 2d). With doping Ag and ZnO, the surface of blend films became uneven and studded dense granule (Fig. 3a-c). The composite Ag:ZnO/CS is observed more even distribution compared to ZnO/CS where ZnO grain is higher due to agglomeration. Silver plays an important role in the distribution of nanoparticles in the chitosan matrix, as it can be observed in Fig. 3c.

The size of Ag and ZnO nanoparticles was in the range of 20–70 nm. Moreover, the particles had uniform distribution within chitosan polymer. Because the Ag content in the Ag:ZnO/CS composites was lower beyond the detection limit of EDX and Ag signals and the signals of noise coexisted, it only indicated the presence of Ag qualitatively. Fig. 4 illustrates the EDX spectrum of Ag:ZnO/CS, ZnO/CS, Ag/CS and CS nanocomposites. As shown in Fig. 4, C, Zn, O and Ag elements were identified. This result agreed well with XRD analysis.

3.3. Antimicrobial activities

The antimicrobial mechanism of chitosan was attributed to interaction with the strongly electronegative microbial surface [19].

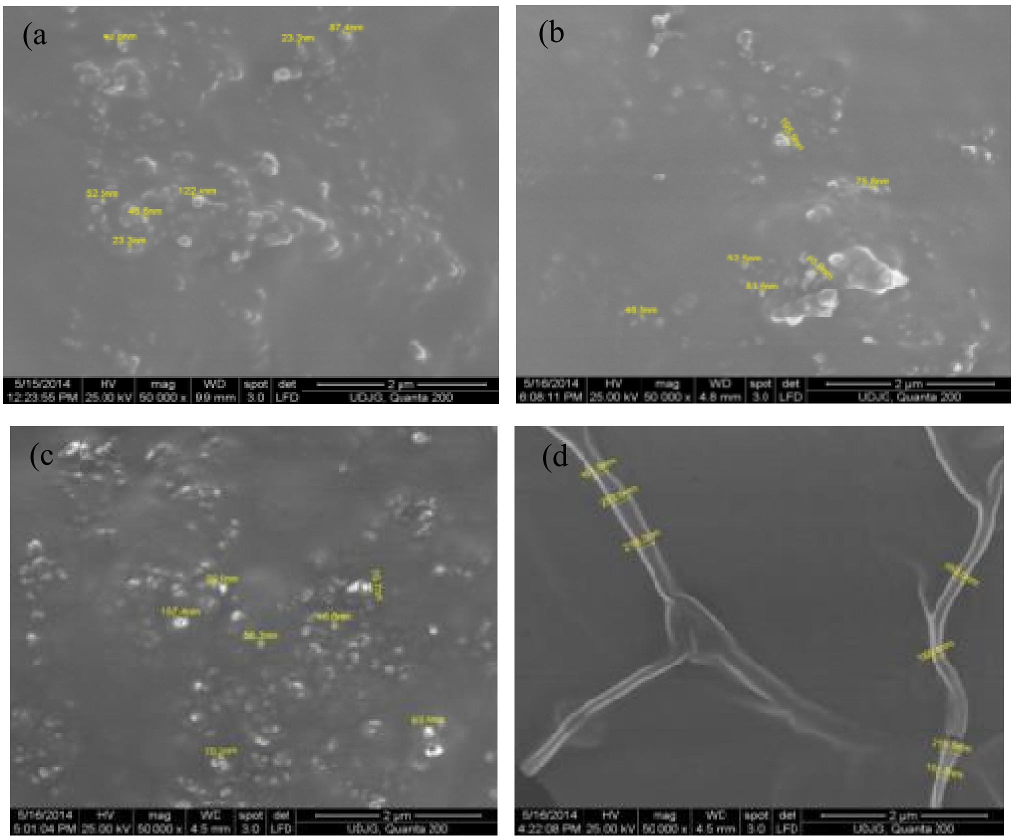

Fig. 3. *SEM images for a) Ag:ZnO/CS; b) ZnO/CS; c) Ag/CS and pure CS*

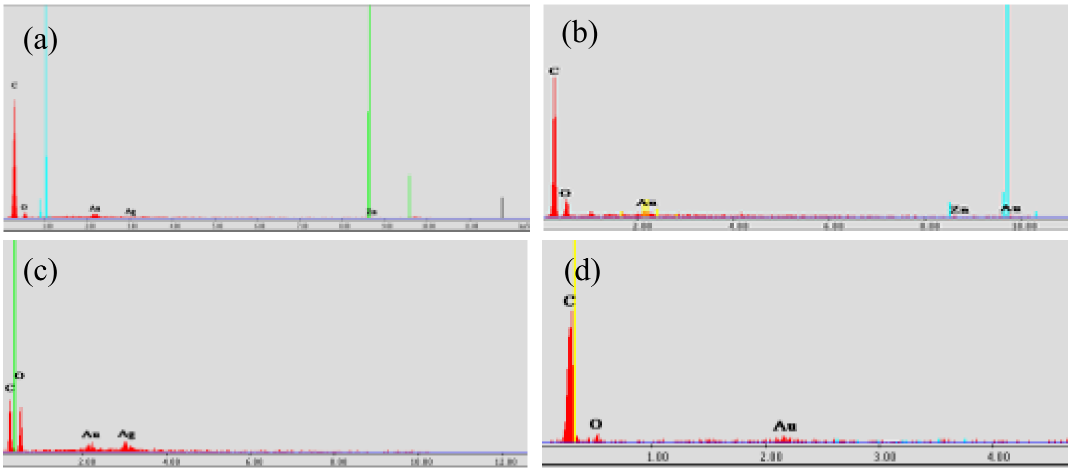

Fig. 4. *EDX spectrum for a) Ag:ZnO/CS; b) ZnO/CS; c) Ag/CS and pure CS*

Ag and ZnO nanoparticles embended in chitosan matrix depicted an enhanced antibacterial property. The experimental proofs suggest that the mechanisms of dominant bacterial ZnO is based on the production of reactive oxygen species (ROS - in particular in the presence of ultraviolet light), which chemically interact with the bacterial cell. [20-25]. Under light irradiation ZnO NPs (Eg = 3.37 eV) generated electron–hole pairs. The hole (h^+) reacted with OH^- on the surface of NPs, generating hydroxyl radicals ($OH^•$), superoxide anion (O^{2-}) and perhydroxyl radicals ($HO_2^•$). These highly active free radicals damaged the cells of microorganism as a result of decomposition and complete destruction [26-27].

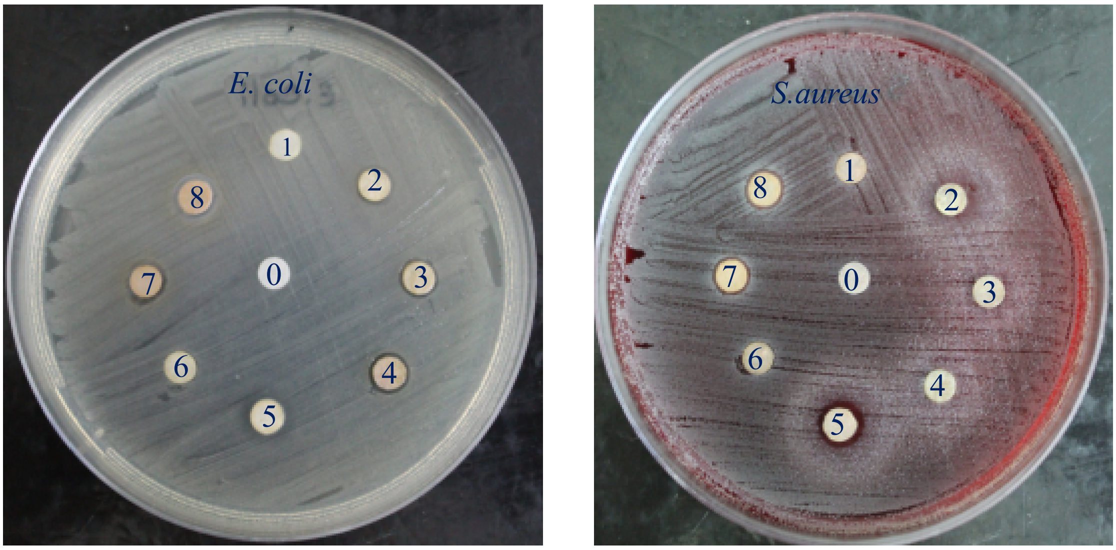

Fig. 5. *Zone of inhibition for 1) pure Chitosan (CS8); 2) Ag:ZnO/CS (CS1); 3) Ag:ZnO/CS (CS2); 4) Ag:ZnO/CS (CS3); 5) ZnO/CS (CS4); 6) Ag/CS (CS5); 7) Ag/CS (CS6); 8) Ag /CS (CS7)*

On the other hand, silver ions disrupt the DNA replication and cell division. Both antimicrobial agents appear to compromise the integrity of bacterial membrane because of chemical interactions.

In Fig. 5 is presented the antimicrobial activity of Ag:ZnO/CS, ZnO/CS, Ag/CS and CS nanocomposites. All the samples showed antimicrobial activity with bacterial viability.

The test of antimicrobial activities showed that Ag-doped ZnO/chitosan composite had higher antimicrobial activities than Ag/CS or ZnO/CS, indicating that the Ag-doped ZnO nanoparticles enhanced the antimicrobial activities of chitosan based composite.

The CS4 sample (Ag:ZnO/CS blend films with 15 wt.% Ag and ZnO) showed excellent antimicrobial activities. Therefore, the presence of Ag and ZnO significantly enhanced antimicrobial ability of chitosan.

This points out that Ag-doped ZnO nanoparticles composite has potential application in medical and food packaging fields.

4. Conclusions

Ag:ZnO nanoparticles/chitosan composite were prepared via the sol-gel method. The dispersed ZnO and Ag-doped ZnO nanoparticles with spherical morphology had uniform distribution within chitosan polymer.

The final formulation was applied on filter paper discs for measuring the antimicrobial activities against *E.coli* and *S. Aureus*, using the disc difusion method.

All the samples showed antimicrobial activity with bacterial viability. The test of antimicrobial activities showed that Ag-doped ZnO/chitosan composite had higher antimicrobial activities than Ag/CS or ZnO/CS, indicating that the Ag-doped ZnO nanoparticles enhanced the antimicrobial activities of chitosan based composite. This points out that Ag-doped ZnO nanoparticles composite has potential application in medical and food packaging fields.

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