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Synthesis of Cs-ZnO-Ag nanocomposite from crustacean shells and its antibacterial activity

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ABSTRACT

Colloidal ZnO-Ag nanowire was stabilized into a degradable biopolymer of chitosan which is derived from *Scylla serrata* shells via a new cost effective green method. Chitosan contains numerous hydroxyl and amino groups which reduces the metal oxide and metal. Physicochemical properties of the nanocomposites were determined by UV-visible spectroscopy analysis, XRD pattern, and SEM. The result reveals that ZnO nanoparticles and Ag nanowire had been uniformly distributed around chitosan polymer. Ag nanowire formation over biopolymer-ZnO was explained. The antibacterial activity of chitosan, zinc oxide and silver nanoparticles are well known. Here combined effect of CS-ZnO-Ag wire nanocomposite was studied. The antibacterial activity of CS-ZnO and CS-ZnO-Ag against various pathogens was evaluated. Silver-loaded CS-ZnO nanocomposites shows higher impact over various bacteria tested than CS-ZnO nanocomposites. Results also show that the inhibition effect of composite against positive pathogens was higher than negative pathogens. HOM inactivation model was proposed to express microbial inactivation.

Keywords: Crustacean cells, CS-ZnO-Ag nanocomposite, Antibacterial activity.

INTRODUCTION

Chitin is a natural carbohydrate polymer found extensively in crustacean shells such as crab, prawn, shrimp etc. Chitosan are used in dietary supplements, textiles, water treatment, food preservation, agriculture, ophthalmology, cosmetics, pulp & paper and medicative application (Shahidi et al., 1999, Vartiainen et al., 2004). There has been an outsized increase in chitosan analysis throughout the past decade. This is due to its non-toxicity, biocompability, biodegradability and other distinctive properties like antimicrobial activity, chelation and adsorption properties and film forming ability. Due to its polycationic nature, chitosan can be used as heavy metal trapper and act as chelating agent and flocculating agent.

Chitosan, zinc and silver have a good antimicrobial activity. In an effort to tackle the growing menace of the continued appearance of antibiotic resistance in pathogenic and opportunistic microorganisms, several new drugs were introduced in recent years. However, none of them have improved activity against multidrug-resistant microorganism (Conlon et al., 2004).

Silver nanoparticles and their composites have been used to cut back the microbial infection in burn wound and prevention of microbial colonization on varied surface devices such as catheters, prosthesis (Parikh et al., 2005; Gosheger et al., 2004). However, as per our knowledge there are no reports of the antibacterial activity of CS-ZnO-Ag nanocomposite. Hence ours is the first parallel group to report an antibacterial activity of synthesized CS-ZnO-Ag nanocomposite against various pathogens.

MATERIALS AND METHODS

Materials: Crab shell wastes were collected from a local food processing unit. All chemicals used in this study were of analytical grade. Chemicals such as sodium hydroxide, hydrochloric acid, zinc oxide, acetic acid and silver nitrate were purchased from Sisco research laboratories. Organisms used in this study were purchased from Microbial Type Culture Cultivation (MTCC).

Synthesis of Chitosan from crab shells: Crab shells were cleaned and washed with water until all protein substances were removed. They are then dried at 60 °C and grinded using mortar and crusher. Powdered shell was treated with 1 % NaOH at 60 °C for 8 hr to remove chitin. Higher solid to alkali solution ratio was preferred. The powder was filtered and demineralized using 5 % hydrochloric acid at 45 °C. It was then washed and decolorized in direct sunlight for about 10 hr. Chitin obtained was deacetylated by treating it with 50 % NaOH at 85 °C for 2 hr. Finally chitosan was synthesized from crustacean shells.

Synthesis of Chitosan-ZnO nanocomposites: 1 gm of ZnO powder was dissolved in 200 mL of 1 % acetic acid. To this solution 1 gm of Chitosan was added and the mixture was sonicated for 30 min. Solution was autoclaved at 15 kg/cm^2 for 1 hr. After autoclaving, 1 % NaOH was added to mixture until pH reaches 10. Reaction solution was heated in a water bath at 50 °C for 30 min. Obtained Chitosan-ZnO nanocomposites were washed several times with distilled water and fully dried at 60 °C (Li et al., 2010).

Synthesis of Chitosan-ZnO-Ag nanocomposites: 1 gm of synthesized Chitosan-ZnO nanocomposites were dissolved in 100 mL deionized water. 10 mL of freshly prepared 1 mM $AgNO_3$ solution was added followed by 1 % acetic acid was added until solution reaches 4 pH. The mixture was magnetically stirred for 3 hr in absence of light and maintained at 65 °C. Excess nitrate ion was removed by washing several times with deionized water, dried at 70 °C and pure Chitosan-ZnO-Ag nanocomposites were obtained.

Characterization studies: UV-vis spectra were measured by UV-3150 (Japan) spectrophotometer over the range 200 to 1000 nm with a 0.5 nm resolution. X-ray diffraction patterns were recorded using RigakuUltima III, RINT-2000/PC. Scanning Electron Microscopic (SEM) images were obtained using a field emission microscope at acceleration voltage of 10 kV.

RESULT AND DISCUSSION

CS-ZnO-Ag composite results were shown in figure.1. The samples UV result exhibit two surface plasmon resonance peaks; absorption peak at 338 nm can be attributed to the plasmon response of long Ag NWs. The shoulder peak at 380 nm was an optical characteristic similar to bulk silver, which is usually been observed for Ag nanowires (You et al., 2009). Typical XRD patterns of CS-ZnO and CS-ZnO-Ag nanocomposites indicates high crystallinity of nanocomposites with lattice planes of (1 0 0), (0 0 2), (1 0 1), (1 0

3), (1 1 0), (1 0 3) and (1 1 2) obtained at 32.12°, 34.75°, 36.34°, 47.76°, 56.83°, 63.14° and 68.13° respectively are corresponding to ZnO hexagonal structure (JCPDS No. 36-1451) (Anandhavelu S. and S. Thambidurai 2011). The patterns exhibit three characteristic peaks for CS at 17.27°, 19.42° and 23.28°.33 Three more peaks are obtained for CS-ZnO-Ag nanocomposite. These peaks obtained at 39.15° (1 1 1), 43.45° (2 0 0) and 77.09° (3 1 1). (JCPDS No. 4-783) are associated with FCC silver nanoparticles.

From SEM results the flake shaped structures are Chitosan particles over which ZnO particles can be clearly seen at the tip of every Chitosan structure. After the addition of silver ions to the nanocomposite, it forms silver nanowires around the composite. The antibacterial effects of synthesized nanocomposites were investigated against Bacillus subtilis, Salmonella yphimurium, Staphylococcus aureus, Escherichia coli, Enterococcus faecalis, Enterococcus faecium and Pseudomonas aeruginosa using the agar diffusion method (Table.1).

Table.1.Antimicrobial activity of CS-ZnO and CS-ZnO-Ag composite

Organism	Gram positive/negative	Zone of Inhibition (in mm) for		Increase % of inhibition zone
		CS-ZnO	CS-ZnO-Ag	
Bacillus subtilis	Positive	25	29	13.79
Staphylococcus aureus	Positive	24	27	11.11
Enterococcus faecalis	Positive	20	23	13.04
Enterococcus faecium	Positive	18	22	18.18
Pseudomonas aeruginosa	Negative	18	20	10
Salmonella typhimurium	Negative	15	18	16.67
Escherichia coli	Negative	18	21	14.29

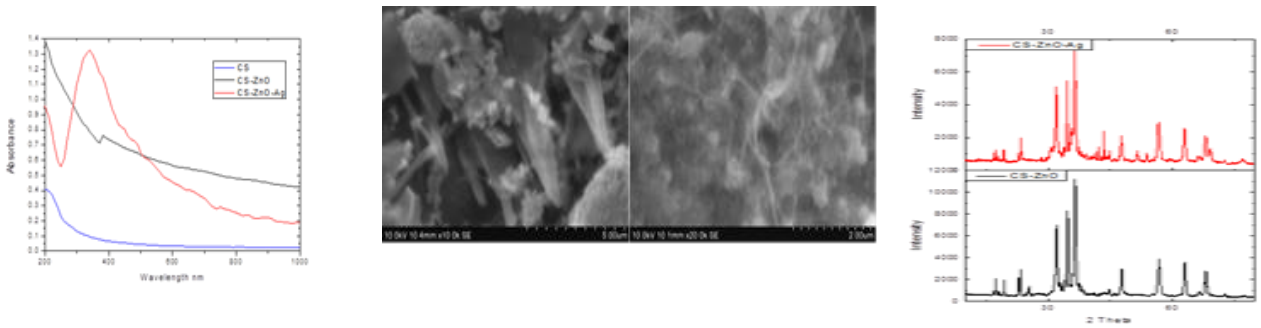

Figure.1. UV, SEM and XRD results of CS, CS-ZnO and CS-ZnO-Ag

CONCLUSION

In summary, CS-ZnO-Ag nanocomposites were synthesized from crab shell using Sono-chemical method. The nanocomposites were characterized by UV-vis, FTIR, XRD, TGA and SEM analyses which confirmed the formation of CS-ZnO-Ag nanocomposites. Additionally, the antibacterial activity of the nanocomposites was measured by agar diffusion method. Results of this study demonstrated that the synthesized nanocomposites have antibacterial activity against Bacillus subtilis, Salmonella typhimurium, Staphylococcus aureus, Escherichia coli, Enterococcus faecalis, Enterococcus faecium and Pseudomonas aeruginosa.

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