International Journal of Biological Macromolecules 77 (2015) 266–272

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

Applications of chitosan powder with *in situ* synthesized nano ZnO particles as an antimicrobial agent

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ARTICLE INFO

Article history:
Received 24 December 2014
Received in revised form 24 March 2015
Accepted 25 March 2015
Available online 1 April 2015

Keywords:
Chitosan nanocomposites
Antimicrobial activity
Immobilization of nano ZnO particles

ABSTRACT

ZnO nanoparticles are immobilized on the chitosan matrix by an *in situ* sol–gel conversion of precursor molecules in a single step. Three different composites are prepared by varying the concentration of sodium hydroxide with same quantity of chitosan and zinc acetate dihydrate. The composites were characterized by FTIR, UV–visible spectra, and XRD. The observed decrease in the band width corresponding to —OH and —NH_2 group in the composites is ascribed to the reduction of hydrogen bond due to the presence of ZnO nanoparticles. The direct evidence of the immobilization of nano ZnO particles in the matrix was identified by SEM. The average particle size values obtained for the nanoparticles, using Debye–Scherrer equation from XRD, is in the range 10–18 nm. Optical studies proved that all the three composites studied have the same band gap energy (3.28 eV) in agreement with the reported values. We observed that the composites possess excellent antimicrobial activity against Gram negative bacteria *Escherichia coli* (*E. coli*) and Gram positive bacteria *Staphylococcus aureus* (*S. aureus*) than chitosan. Based on the above studies, the biocompatible, eco-friendly and low-cost composite powder could be applied in various fields as an antimicrobial agent.

1. Introduction

Chitosan is the second-most plentiful natural polymer and has excessively studied in large number of applications [1] and the properties of chitosan can be tuned by changing its structure as well as adding nanoparticles to its polymer matrix [2]. In recent years much attention has been devoted to the hybrid materials of nanosized metal oxides and chitosan owing to their unique properties such as photo catalysts, antimicrobial agents, *etc.* This hybridization could enhance the properties of each single component counterpart of composites. [3,4]. Since chitosan molecules contain a large number of reactive hydroxyl (—OH) and amino (—NH_2) groups, they can act as a natural capping agent during the synthesis of nanoparticles [5]. Recent studies on chitosan nanocomposites—chitosan-CdS, chitosan-niobium (V) oxide, and chitosan cuprous oxide (*p*-type semiconductor)—reports their photocatalytic activities against organic dye molecules [6–10]. It has been reported that that chitosan–nano ZnO composites act as sensors for DNA [11], cholesterol molecules [2], dye adsorption [12], and semiconductor quantum dots [13]. Several authors have reported the antimicrobial properties of chitosan nano-silver and silver oxide composites [14–16]. Among the various inorganic metal compounds, ZnO is preferably used for the synthesis of chitosan nanocomposite due to its unusual properties such as wide band gap energy (3.37 eV) [17], high surface area, high catalytic efficiency, nontoxicity, chemical stability [2] antibacterial character, UV protection ability [18,19], *etc.*

Literatures survey reveal that, only a few reports are available on the hybrid of chitosan and nano ZnO composites and in all these pre-synthesized nano ZnO particles were used to develop the composites [2,12,20–22]. In the present work, a simple method for the fabrication of chitosan/nano ZnO composite powder has been described. The nano ZnO particles were formed by *in situ* sol–gel conversion of precursor Zinc acetate dihydrate in the presence of NaOH. The highlights of this method include the easiness of fabrication process, lower temperature requirement, *in situ* formation of nanoparticles, biodegradable, and biocompatible polymer matrix to stabilize the nanoparticles. The utility of the composite powder was analyzed for its antibacterial efficacy against Gram negative bacteria *Escherichia coli* (*E. coli*) and Gram positive bacteria *Staphylococcus aureus* (*S. aureus*).

It is a serious concern that several bacteria have developed antibiotic resistance against synthetic antibiotics [12], therefore, the design of efficient antibacterial agents are the need of hour.

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http://dx.doi.org/10.1016/j.ijbiomac.2015.03.058
0141-8130/

In recent years, there have been extensive investigations on the utilization of inorganic nanoparticles as antimicrobial agents. In view of this, the present work focused on the development of novel antibiotic composites containing chitosan and nano ZnO particles. The fabrication was aimed at the co-utilization of antibacterial properties of chitosan as well as nano zinc oxide particles [23]. It is already known that chitosan has inherent antimicrobial activity against Ggram negative and Gram positive bacteria, but the exact mechanism of its antimicrobial activity is not clearly known. The ability of chitosan to inhibit or kill microorganism can be tuned by varying its molecular weight, degree of deacetylation, temperature, pH, and by immobilizing metal oxides in its matrix [24]. Owing to higher film forming property, chitosan and their various composites have been investigated as an antimicrobial packing material for preservation of food articles, vegetables, and fruits [25]. The inherent antimicrobial activity of chitosan is believed to be due to the presence of the positively charged amino groups which interact with negatively charged cell membranes of pathogens, leading to the leakage of proteinaceous and other intracellular constituents and finally to the death of microorganism [26]. The sensitivity of any antibacterial film forming solution was determined by measuring the diameter of the inhibition zone and they are classified as: (a) not sensitive for diameters less than 8 mm; (b) sensitive for diameters of 9–14 mm; (c) very sensitive for diameters of 15–19 mm, and (d) extremely sensitive for diameters larger than 20 mm [27].

The present work involves the fabrication of a novel, eco-friendly antimicrobial composite containing chitosan and ZnO nanoparticles by *in situ* sol–gel conversion. The objective of this work was to formulate a simple and cost-effective bionanocomposite for the possible uses as an antimicrobial wound dressing materials, cotton fabrics, and packing materials for food stuffs.

2. Experimental

2.1. Materials

Chitosan with 85% degree of deacetylation was purchased from Sigma-Aldrich Co. Ltd. (USA). Acetic acid, sodium hydroxide, and zinc acetate dihydrate were purchased from Merck (Germany). Mineral salt broth and nutrient agar are obtained from Himedia Chemicals (India). *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) were prepared at the microbiology laboratory, Department of Life Science, University of Calicut. Clinical sample of *E. coli* and *S. aureus* were obtained from Santhi hospital, Omessery, Calicut, India. Deionized water was used to prepare all the solutions. All the chemicals were of analytical grade reagents and used without further purification.

2.2. Preparation

Chitosan solution was prepared by dissolving 0.5 g of chitosan in 2% acetic acid solution in room temperature. The solution was then filtered to remove undissolved particles and added 30 mL of 15% zinc acetate dihydrate solution and shaken well. To this solution, 30 mL of 45% sodium hydroxide solution was added slowly at a temperature of 70° C and the entire mixture was stirred at 70° C for 4 h. The white precipitate formed was allowed to settle by keeping the mixture for 24 h. The supernatant solution was discarded, the precipitate was rinsed with distilled water for several times to remove excess of NaOH present (if any), filtered and dried by keeping in an air oven maintained at a temperature of 50° C for 12 h. The white dried precipitate was powdered well in a mortar by using a pestle and this sample was designated as CN45. The above process was repeated with decreased concentration of sodium hydroxide, 30 and 15%, the corresponding composites formed were designated as CN30 and CN15, respectively.

2.3. Characterization

The FTIR spectra of the samples were recorded in KBr pellets using Fourier Transform IR Spectrometer (Model: JASCO FTIR 4108). The absorption spectra of powder was recorded by using a UV–visible spectrophotometer (Model: JASCO V-550) in the wavelength range of 200–800 nm. X-ray diffraction (XRD) patterns of composites were recorded with a X-ray diffractometer (Model: Rigaku Minifex 600) using Cu Kα radiation (kα = 0.15406 nm) at a scanning rate of 1 min^{-1}. The thermal stability and degradation behavior of composites were assessed using a thermogravimetric analyzer (TG) (Model: TGA/DTA851e) under flowing air (at a flow rate of 60 μL min^{-1}) in the temperature range, 40–740° C at a heating rate of 10° C min^{-1}. The SEM images of chitosan and composites were recorded using a Field Emission Scanning Electron Microscope (Model: HITACHI SU6600 FESEM).

2.4. Antibacterial activities

The antimicrobial activity of chitosan and chitosan-based nanocomposite powders were evaluated by agar well diffusion method. Gram negative bacteria *E. coli* (MTCC 1687) and Gram positive bacteria *S. aureus* (MTCC 737) were used as test organisms. Sterile NA plates were prepared and 0.1 mL of the inoculums of test organism was spread uniformly over it. Wells were prepared by using a sterile borer of diameter 6 mm and the samples of pure chitosan (C), CN15, CN30, and CN45 were added in each well separately. The plates were incubated at 35–37 °C for 24 h, a period of time sufficient for the growth. The zone of inhibition of microbial growth around the well was measured in mm. The antimicrobial efficacy of samples against clinical bacteria (*E. coli* and *S. aureus*) was evaluated by disc diffusion method. The antimicrobial disc was prepared as described earlier [28]. Whatman No.1 filter paper was cut into disc shape using a circular knife and autoclaved. This paper discs were soaked separately into 5 mL of 1% acetic acid solution containing 0.05 g of C, CN45, CN30, and CN15 and dried at room temperature to adsorb the antimicrobes on the paper surface. Antibacterial properties of filter paper disc treated with bionanocomposites are measured by the inhibition zone method. The presence of any clear zone that formed around the disc on the plate medium was recorded as an indication of inhibition against the microbial species.

3. Results and discussion

3.1. FTIR spectroscopy

Fig. 1 shows the FTIR spectra of pure chitosan and chitosan–nano ZnO composites powder. The characteristic broad band at 3413 cm^{-1} observed in Fig. 1a is attributed to the stretching vibrations of —NH_2 and —OH groups of chitosan. The bands at 2930 and 2847 cm^{-1} are due to the asymmetric stretching vibration of —CH_3 and —CH_2 groups and the peaks observed at 1647 and 1468 cm^{-1} are designated respectively to the stretching vibrations of C═O and scissoring vibrations of —NH_2 groups of chitosan. The band observed at 1019 cm^{-1} is due to the stretching vibration of —C—O—C— of glycosidic linkage of the polymer [29].

The FTIR spectrum of ZnO composites (Fig. 1b, c, and d) are similar to that of pure chitosan but in contrast to Fig. 1a, the band corresponding to stretching vibrations of —NH_2, and —OH are reduced significantly in composites, possibly due to the reduction of inter and intramolecular hydrogen bonds formed in the presence of nanoparticles. During the formation of composites, the