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Original Research Paper

Immobilization of ZnO on Chitosan-Neem seed composite for enhanced thermal and antibacterial activity

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Natural and inorganic materials of Chitosan-Zinc oxide-Neem seed (CS-ZnO-NS) hybrid composite were synthesized by chemical precipitation method. The obtained CS-ZnO-NS hybrid composites were characterized for functional group confirmation by Fourier transform infrared spectroscopy and UV–Visible spectroscopy. The ZnO particles connected to biopolymers exhibited small grains and rod, bullet like structure confirmed by scanning electron microscopy and transmission electron microscopy analysis. The size of the prepared CS-ZnO-NS hybrid composite was found to be 20–80 nm. The crystalline behaviors were determined by X-ray diffraction analysis. The surface area of the prepared hybrid composite was determined using BET analysis. The elemental composition was analyzed by energy dispersive X-ray spectroscopy and thermal behaviors by thermo gravimetric analysis. The obtained result shows that zinc oxide was well able to incorporate into chitosan-neem seed composite which enhances the thermal stability. Further, the antibacterial activity evaluated by agar well diffusion method against Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli* bacteria exhibits higher inhibition effect because of ZnO particles presence in the chitosan-neem seed. Hence the CS-ZnO-NS is a promising material for biomedical applications.

1. Introduction

Microbial pollution and its contamination are harmful to living organisms and are serious threat to public health [1,2]. To meet the challenges, alternate antimicrobial agents from inorganic, organic and natural antibacterial agents have been rising [3,4] and hence development of antibacterial agents has been gaining more attention in recent years. Based on this concept, chitosan (CS) is a natural cationic biopolymer with β-(1–4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit), which is obtained from deacetylation of chitin, widely available from seafood-processing wastes. It was chosen because it has unique properties such as biocompatibility, nontoxic, low cost, biodegradability, excellent film forming ability and renewability due to the presence of reactive amino and hydroxyl functional groups in the structural moiety [5–8]. Antimicrobial effects of chitosan and chitooligosaccharides were studied by Fernandes et al. [9]. Blending of natural and synthetic polymers gives new desirable materials with better property and reduced cost.

Currently, antimicrobials extracted from plants could be useful in food, dairy and pharmaceutical industries to prevent contamination by limiting the microbial growth [10]. Among these, the biopolymer is *Azadirachta indica*, commonly known as neem, belonging to the Meliaceae family. It is an evergreen tree growing in tropical and subtropical countries like India. It contains most valuable phytochemicals called limonoids such as azadirachtin, nimbin, nimbinin and salanain are found in neem every part (leaves, seeds, bark and roots). These compounds are responsible for strong biological activities against antibacterial, antiviral and antifungal activities against various microorganisms [11–14]. It was reported that, Shakeel Ahmed et al. prepared silver nanoparticles using *Azadirachta indica* aqueous leaf extract and showed antibacterial activities against gram positive and gram negative bacteria [15]. Elumalai and Velmurugan revealed the better antibacterial activities of ZnO nanoparticles from leaf extract of Azadirachta indica (L) antibacterial activity against *E. coli* and *S. aureus* [16]. Recently, hybrid composite based materials have been developed, including polymers, metal nanoparticles and oxide agents, due to outstanding properties of individual components and excellent synergistic effects simultaneously [17,18].

Inorganic materials, such as metal and metal oxides, have attracted more attention for antimicrobial properties due to their

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ability to withstand harsh process conditions and are generally regarded as safe materials to human beings and animals for the past decade [19,20]. The metal oxide nanoparticles like CuO, TiO_2 and ZnO have significant antibacterial activities [21]. The reason for choosing zinc oxide is a safe material, which is compared to organic materials, inorganic materials possess greater durability, better selectivity and heat resistance, can produce increased levels of reactive oxygen species (ROS) [22,23]. Zinc oxide is an n-type semiconductor with wide band gap (3.37 eV) and large exciton binding energy [24]. It has several unique properties such as, nontoxic, biocompatible, antibacterial, antifungal UV-blocker in sun screens wound healing and many biomedical applications [25–27]. Mostly, ZnO nanoparticles are strongly inhibited the growth of microorganisms due to disintegration of the cell membrane, thereby increases the membrane permeability [28]. There are some studies investigating the antibacterial activity of ZnO nanoparticles against pathogens. Bhadra et al. demonstrated that chitosan-capped ZnO nanoparticles exhibited higher antibacterial activity against *E. coli* compared with uncapped ZnO nanoparticles [29] and Jayasuriya et al. found that the ZnO NPs can improve the mechanical properties of pure CS films; only a low percentage of ZnO NPs can be applied for biomedical applications [30].

In our previous work we had prepared chitosan incorporated neem seed extract (*Azadirachta indica*) for medical textiles applications [31]. In the present work it is aimed to increase these properties by incorporation ZnO nanoparticles into chitosan and neem seed by chemical precipitation method. The synthesized hybrid composites functional groups were characterized by FTIR analysis. The crystalline size and surface morphological structures were analyzed by XRD, HR-SEM and TEM analysis. Thermal degradation of hybrid composite was evidenced by TGA and the surface area was determined by BET analysis. The antibacterial activity of hybrid composite was evaluated against gram positive (*S. aureus*) and gram negative (*E. coli*) bacteria. The purpose of the present work was to incorporate ZnO nanoparticles onto chitosan-neem seed composite by chemical precipitation method for enhancement of antibacterial activity and thermal stability.

2. Materials and methods

2.1. Materials

Zinc nitrate hexahydrate, sodium hydroxide, acetic acid were purchased from Fisher chemic Ltd, Chennai, India. All chemicals were analytical grade and without further purification. Chitosan was supplied by M/S south India Sea Foods, Kochi, India. Millipore water was used throughout this investigation. Dry neem (*Azadirachta indica)* seeds were collected from local market at Karaikudi, Tamilnadu, India.

2.2. Preparation of neem seed extracts (NS)

The collected seeds were washed thoroughly several times with Millipore water. Then, neem seed kernels were obtained by removing shell and then ground using a mortar. 3 gm of paste is taken. 30 mL of water is added to it. It is stirred for 40 min in a magnetic stirrer. Then, it is filtered using Whatmann filter paper No. 1. For a second time 20 mL of water is added, stirred continued for further 30 min and then filtered. The obtained filtrate was used for the following experiments.

2.3. Synthesis of CS-ZnO-NS hybrid composite

The CS-ZnO-NS hybrid composite was synthesized using chemical precipitation method. 0.5 g of chitosan dissolved in 25 mL (5%) of acetic acid, the solution was stirred for 30 min and then 25 mL of zinc nitrate hexa hydrate (0.5 M) solution was added to the above solution and continuously stirred for 3 h at 100 °C. After that, 25 mL of prepared neem seed extract mixed with the above mixture and stirring is continued for further 2 h at 80 °C. With this solution, 50 mL of sodium hydroxide (1 M) solution was added drop by drop till the precipitate was formed. The formed white precipitate was allowed to settle for 24 h. Then, the supernatant solution was discarded and the precipitate was washed more times with Millipore water, filtered using suction pump and dried at 100 °C for 5 h, which is designed as CS-ZnO-NS hybrid composite. It was clearly demonstrated in (Scheme 1). In similar manner, the NS-ZnO was prepared without addition of CS which is designed as NS-ZnO and was also prepared without the addition of NS, which is designed as CS-ZnO.

2.4. Characterizations techniques

X-ray diffraction study was carried out using X-ray diffractometer (model XPERT-PRO), (Rigaku diffractometer with Cu Kα radiation), (K = 1.5406A^0). The functional groups of the samples were analyzed using Fourier Transform Infrared Spectrophotometer (FT/IR-4600 type A, Detector-TGS using KBr pellets, in the range of 400–4000 cm^{-1}). The absorption spectra were studied using UV–Vis spectrophotometer (JASCO UV–Vis NIR (V-670)) in the wavelength range of 200–800 nm. The surface morphology and elemental analysis of the hybrid composite were analyzed using a HR-SEM (FEI Quanta FEG 200) at an accelerating voltage of 10.0 kV and TEM images of the synthesized samples were depicted using Field Emission Gun–Transmission Electron Microscope 300 kV (FEG-TEM 300 kV). Thermal stability of the hybrid composites was analyzed by thermo gravimetric analyzer (Model: STA 409 PC/PG, NETZCH). Nitrogen adsorption–desorption isotherms were performed with an Quantachrome analyzer at 77.35 K, and the surface area (BET) was determined by Brunauer–Emmett–Teller (BET) method.

2.5. Antibacterial activity of synthesized hybrid composite against human pathogens

The antibacterial activities of ZnO, NS-ZnO, CS-ZnO and CS-ZnO-NS hybrid composites were determined by agar well diffusion method with two strains of bacteria such as gram positive bacteria *Staphylococcus aureus* (*S. aureus*) and gram negative bacteria *Escherichia coli* (*E. coli*). The commonly used antibiotics (Amikacin) were taken as a positive control. The pathogenic bacteria were cultured individually on Muller-Hinton broth at 37 °C for 24 h. The 100 L of microbial culture was uniformly distributed on the plate. The synthesized material was placed on the plate. The plates were incubated for 24 h at 37 °C. After the incubation period, the zone of inhibition was measured and recorded.

3. Results and discussion

3.1. FTIR spectroscopy

Neem seed and chitosan functional groups interacting with ZnO particles are studied using Fourier transform infrared spectroscopy. The FTIR spectra of ZnO, NS-ZnO, CS-ZnO and CS-ZnO-NS hybrid composites are shown in Fig. 1. The peaks situated at 3419, 1382, 491 and 418 cm^{-1} are corresponding peaks of ZnO (Fig. 1a). A strong absorption peak at 3419 cm^{-1} is attributed to the stretching vibration of –OH group due to the physically adsorbed water molecule [32,33]. The absorption peak at 1382 cm^{-1} is assigned to (CO_3^{2-}) antisymmetric stretching

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