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Preparation, characterization and antimicrobial activities of chitosan/Ag/ZnO blend films

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ABSTRACT

Novel chitosan/Ag/ZnO (CS/Ag/ZnO) blend films were prepared via a new method of sol-cast transformation. The blend films were characterized by UV–vis absorption spectroscopy (UV–vis), X-ray diffraction (XRD), scanning electron microscopy (SEM), transmission electron microscopy (TEM) and Energy Dispersive X-Ray Fluorescence Spectrometry (EDX). The results revealed that ZnO and Ag nanoparticles (NPs) with spherical and granular morphology had uniform distribution within chitosan polymer. The product had excellent antimicrobial activities against *B. subtilis*, *E. coli*, *S. aureus*, *Penicillium*, *Aspergillus*, *Rhizopus* and *yeast*. And CS/Ag/ZnO blend films had higher antimicrobial activities than CS/Ag and CS/ZnO blend films. Moreover, the blend films almost maintained the initial color of chitosan, which have potential application as antibacterial materials.

1. Introduction

Chitosan is one such polymer with antimicrobial activity, which is the second most plentiful natural biopolymer and has the advantages of biodegradability, biocompatibility and excellent film-forming ability [1–4]. However, as a monocomponent antimicrobial agent, chitosan has been far from meeting requirements for some special conditions. So, hybrid materials that were based on CS with other antibacterial materials have attracted much attention during the last few years. For instance, the combination of inorganic agents Ag, Zn, SiO_2, TiO_2 has been reported [5–10]. Among them, chitosan–Ag nanoparticle composite had significantly high antibacterial activity with the presence of a small content (2.15%, w/w) of Ag NPs in the composite [5]. But the composite suffered from a change in color as a result of high content of Ag, which seriously limited their applications. On the other hand, the antibacterial activities of the composite were not ideal when the content of Ag was low. If there is other white metal oxide that can partly replace Ag and be simultaneously incorporated into the chitosan matrix, it would not only enhance the antibacterial property, but also still maintain the color of the chitosan.

Zinc oxide has attracted wide interest because of its good photocatalytic activity, high stability, antibacterial property and non-toxicity [11–13]. Nevertheless, it is well known that CS is only soluble in aqueous solutions of organic or mineral acids. Zinc oxide reacts easily with acidic substance, and it is difficult to synthesis CS/ZnO composite.

In this study, novel chitosan/Ag/ZnO (CS/Ag/ZnO) blend films with high antibacterial activities were successfully prepared for the first time via a mild one-step method of sol-cast transformation. In this method, Ag NPs were generated by using chitosan as the reducing agent under hot alkaline condition and at the same time ZnO NPs successfully formed in the composite. Especially, the product with low concentration of Ag maintained the initial color, which may have potential application as antimicrobial materials.

2. Experimental and materials

2.1. Materials

Chitosan, with degree of deacetylation of 85.46% and molecular weight of 2.6×10^5, was purchased from Jinan Haidebei Marine Bioengineering Co., Ltd. (China). Zinc oxide, silver nitrate, sodium hydroxide, sodium chloride, sucrose, glucose and acetic acid (>99%) were analytical grade and purchased from Guangdong Xilong Chemical Co., Ltd. Beef extract, peptone and agar powder were bacteriological grade. Potatoes were purchased from the nearby market. *Bacillus subtilis* (*B. subtilis*), *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), *Penicillium*, *Aspergillus*, *Rhizopus* and *yeast* were prepared by Microorganism Lab of Xiangtan University. Deionized water was used to prepare for all the solutions in this paper.

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Table 1
The amounts of added ZnO and $AgNO_3$ and mass ratios of Ag and ZnO to CS (the chitosan amount was 1.0 g).

Sample	Added ZnO amount (g)	Added $AgNO_3$ amount (mg)	Mass ratio of ZnO to CS (wt.%)	Mass ratio of Ag to CS (wt.%)
CS-1	0.01	1.6	1	0.1
CS-2	0.03	1.6	3	0.1
CS-3	0.05	1.6	5	0.1
CS-4	0.1	1.6	10	0.1
CS-5	0.01	7.9	1	0.5
CS-6	0.03	7.9	3	0.5
CS-7	0.05	7.9	5	0.5
CS-8	0.1	7.9	10	0.5
CS-9	0	1.6	0	0.1
CS-10	0.1	0	10	0

2.2. Preparation

Appropriate amounts of $AgNO_3$ and ZnO powders were dissolved in 100 mL of 1% (v/v) acetic acid to obtain silver and zinc cations. And 1.0 g of chitosan was added to the above solution. Then the mixture was sonicated for 30 min after magnetic stirring and then adjusted acidity by 0.1 M NaOH solution (pH 4.8) to obtain the clear sol. The resulting sol was cast onto glass plates and the transparent films were obtained after solvent evaporating slowly in air. Lastly the transparent films with glass were immersed in 0.1 M NaOH solution for 12 h at 333 K. After being removed from the glass plates, CS/Ag/ZnO blend films were obtained through sufficiently washing with deionized water and drying in an infrared oven. The amounts of added ZnO and $AgNO_3$ and mass ratios of Ag and ZnO to CS were shown in Table 1.

2.3. Characterization

The structure of the blend films was measured using UV–vis spectrophotometer (UV-2450, Shimadzu, Japan) and X-ray diffractometer (D 8 Advance, BRUKER, Germany) of Cu Kα (λ = 0.15406 nm) radiation in the 2θ ranging from 5° to 80°. The pure CS film and CS/Ag/ZnO blend films with approximately 20-μm thickness were prepared for the XRD test. The morphologies of the films were studied using HITHACH-4300 scanning electron microscope (Japan Electronic Company, Japan) equipped with EDX elemental composition analyzer. The dried samples were freeze-fractured in the liquid nitrogen and then coated with gold before observing. TEM was carried out using FEI TECNAI G2 20 TEM operating at 200 kV.

2.4. Antimicrobial activities

The agar plate method was taken to evaluate the antimicrobial activities of chitosan/Ag blend films and chitosan/Ag/ZnO blend films, compared with the positive control and pure chitosan film [14]. The antimicrobial activities of the films were tested against seven strains of microorganism, i.e., *B. subtilis*, *E. coli*, *S. aureus*, *Penicillium*, *Aspergillus*, *Rhizopus* and *yeast*. The culture of each microorganism was diluted by sterile distilled water to 10^7 to 10^8 CFU/mL. A loop of each suspension was inoculated on nutrient plates with the sample films spread (size: 2 cm × 2 cm). Then the plates with bacteria and fungus were incubated under light at 310 K for 72 h and at 301 K for 48 h, respectively. A corresponding plate without any films was used as a positive control. Especially, antimicrobial test of CS-4, CS-9 and CS-10 in the dark was taken to provide useful data for synergistic effects of Ag and ZnO NPs. Finally, the antimicrobial activities of the samples were evaluated by the diameter of the test microorganism. All the experiments were repeated five times. The results were averaged.

3. Result and discussion

3.1. XRD analysis

Fig. 1 depicts the X-ray diffraction patterns of pure CS film and CS/Ag/ZnO blend films. Two crystal forms existed in pure CS film (Fig. 1a): form I and form II depicted the major crystalline peaks at 10.2° and 19.9°, respectively [15]. However, these two main peaks (Fig. 1b) of CS at 10.3° and 20.3° were affected by doping Ag and ZnO. This indicated that the incorporation of Ag and ZnO particles disrupted the regular order of polymer chains [9]. Compared with Fig. 1a, the diffraction pattern of the CS/Ag/ZnO blend films exhibited six additional peaks at 31.9°, 34.6°, 36.4°, 56.8°, 62.9° and 68.2° (Fig. 1b), which were assigned to the (1 0 0), (0 0 2), (1 0 1), (1 1 0), (1 0 3) and (1 1 2) planes of hexagonal zinc oxide (JCPDS No. 36-1451). Besides, the peak at 38.3° indicated the presence of Ag as shown in Fig. 1b. These data revealed the formation of Ag and ZnO in blend films by the method of sol-cast transformation.

3.2. UV–vis analysis

Fig. 2 presents UV–vis absorbance spectra of pure CS film and CS/Ag/ZnO blend films. As shown in Fig. 2a, the absorption peak of pure CS membrane was not obvious in that there were not conjugated double bonds in the CS molecules. Compared with pure CS film, CS/Ag/ZnO blend films showed two absorption bands (Fig. 2b). One absorption band at 343 nm in Fig. 2b was attributed to the presence of ZnO. Compared with that of the macrocrystalline ZnO

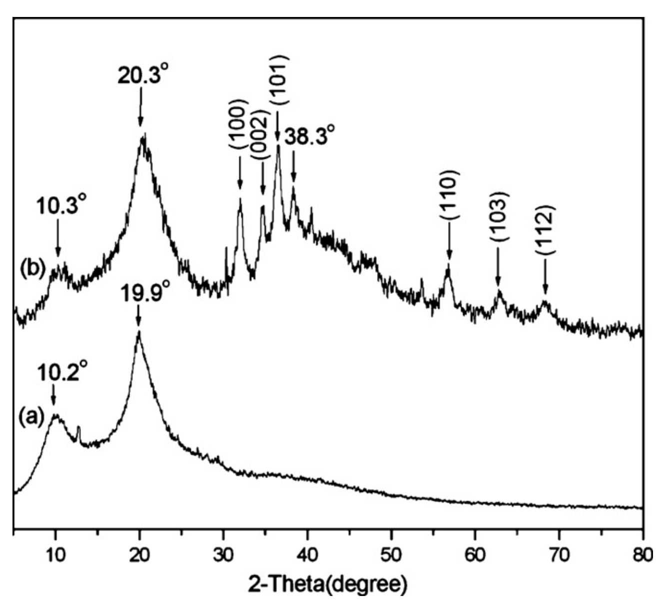

Fig. 1. X-ray diffraction patterns of the samples: (a) pure CS film and (b) CS/Ag/ZnO blend films with 0.5 wt.% Ag and 10 wt.% ZnO.