STRUCTURE, MECHANICAL PROPERTIES AND ANTIMICROBIAL ACTIVITY OF NANO-ZnO/CELLULOSE COMPOSITE FILMS

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This study was aimed at developing biopolymer-based antimicrobial films for reducing environmental pollution caused by accumulation of synthetic packaging. Nano-ZnO was modified and incorporated as antimicrobial material into natural cellulose and the solvent casting method was used to prepare active nanocomposite films. Methods, such as FT-IR, AFM and XRD, were used to characterize the modified nano-ZnO and the nanocomposite films. Mechanical and antimicrobial properties of the composite films were also tested. Results showed that the modified ZnO nanoparticles possessed good dispersion in the films. When the concentration of nano-ZnO was 2.5 wt%, the composite film had the best mechanical properties, as well as significant antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus*. It could be concluded that the novel prepared nanocomposite films can be used as a promising material for antimicrobial packaging applications.

Keywords: modified nano-ZnO, nano-ZnO/cellulose composite film, antimicrobial activity

INTRODUCTION

One of the biggest problems causing increasingly serious environmental pollution is the wide use of oil-based plastic products. In recent years, an increasing amount of research work has been conducted to explore natural biopolymers for the development of environmentally friendly packaging materials as alternatives to non-biodegradable petroleum-based synthetic plastic materials.[1] Polysaccharides, proteins and lipid materials from plant and animal origin are usually utilized for this purpose.

Cellulose as the most abundant biopolymer in nature is produced in amounts of about 100 billion metric tons per year.[2] Natural cellulose fibers have many excellent advantages, such as biodegradability, renewability, environmental friendliness, perfect processability, low cost *etc*.[3-4] Moreover, the cellulose products are non-toxic, keeping the character of good biocompatibility and stability. As a result, they are being extensively investigated for composite reinforcement, soft-tissue replacement, artificial bones, dental prostheses, packaging, building materials *etc*.[5-6] Nevertheless, biodegradable natural packaging materials usually have poor properties, especially with regard to mechanical behavior.[7] Meanwhile, the antibacterial properties of pure polymers are often insufficient for food packing applications.[8-9] These drawbacks limit the

further application of cellulose. In an attempt to overcome these limitations, a few years ago research proved that polymer/inorganic nanocomposites present dramatic enhancements in stiffness and strength compared with traditional integrated materials.[10]

Nano-ZnO, with sizes in the range of 1-100 nm, is one of the most interesting metal oxide based semiconductors, a relatively bio-safe and biocompatible material, which is fairly suitable for film/textile applications.[11] In order to remedy the drawbacks of cellulose, ZnO nanoparticles were incorporated into the cellulose film, due to the safety (GRAS) and antimicrobial activity of this compound against food borne pathogens.[12-13] The advantages of ZnO nanoparticles have been found limited, however, because of their aggregation and incompatibility with organic polymers. Consequently, they require some modification to turn their surface from hydrophilic to organophilic.[14] To the best of our knowledge, there are limited studies on the development of cellulose/nano-ZnO composite films with modified nano-ZnO. Therefore, the main objective of the present study was to develop active bio-based nanocomposite films with high mechanical behavior and antibacterial properties by mixing natural cellulose with modified nano-ZnO. The films were characterized by FT-IR, AFM and XRD methods. Furthermore, the mechanical properties of the composite films and their antimicrobial activities were also tested.

EXPERIMENTAL

Materials

The reagents and chemicals used in this study were all of analytical grade. Bleached hardwood pulp (DP = 1064) was provided by Tianjin Zhongchao Paper Industry Co. Lithium chloride (LiCl) and N,N-dimethylacetamide (DMAc) were obtained from Tianjin JiangTian Chemical Reagent Company. Acetone was supplied by Tianjin Tian Yi Chemical Reagent Company. ZnO nanoparticle, beef extract, peptone and sodium chloride were obtained from Sinopharm Chemical Reagent Co., Ltd. Agar was received from BASF. Silane coupling agent (KH550) was gained from Nanjing Shuguang Chemical Group Co., Ltd. *Escherichia coli* (*E. coli* ACTT 25923) and *Staphylococcus aureus* (*S. aureus* ACTT 25922) were provided by China General Microbiological Culture Collection Center. Distilled water was obtained in our laboratory.

Modification of ZnO nanoparticles

Figure 1 shows the procedure of ZnO nanoparticle modification and the preparation of the nanocomposite film. First, ZnO nanoparticles were dried at 120 °C for 24 h to remove moisture. Second, ZnO nanoparticles (1 g) and KH550 (10 wt% ZnO) were mixed into absolute ethanol (100 mL) and were ultrasonicated for 30 min. Third, the dispersed solution was filtrated and washed with absolute ethanol to remove the unreacted KH550 three times. Lastly, the filter mass was vacuum dried at 80 °C for 12 h and the modified nano-ZnO powder was obtained.

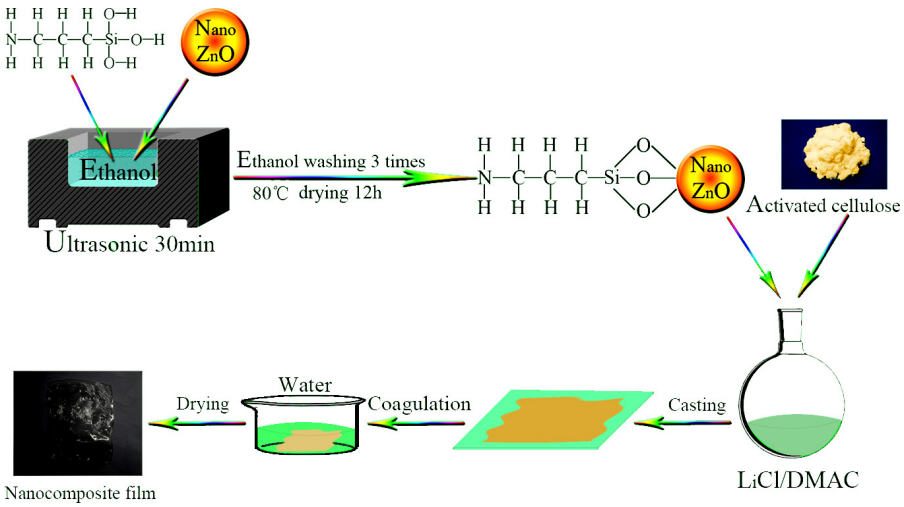

Figure 1: Procedure of ZnO nanoparticle modification and preparation of nanocomposite film

Preparation of cellulose/nano-ZnO composite films

Bleached hardwood pulps were vacuum dried at 80 °C for 12 h. LiCl was vacuum dried at 80 °C for 2 h. The LiCl (11 wt% DMAc) was dissolved in the DMAc and then cellulose (7 wt% DMAc) and unmodified or modified nano-ZnO were added into the solvent, the mixture was heated at 100 °C for 2-3 h with a thermometer and magnetic stirrer under nitrogen atmosphere, then a homogeneous and transparent cellulose gelatin was obtained after standing for 12 h. The solution was cast on a glass pane after being vibrated for 0.5 h by using ultrasonic oscillations, quickly followed by immersion in deionized water coagulation baths. After that, an asymmetric film was obtained. The residue of LiCl/DMAc was washed away with deionized water, and then the films were plasticized in 30 wt% glycerol solution and dried at room temperature. The mean thickness of the films was 60.0 μm, measured by a high accuracy film thickness tester DRK (204) (Drick Instruments Co. Ltd., China) and three measurements were carried out for each sample.[15-16]

Characterization

The Fourier transform infrared (FT-IR) spectra were recorded in the wavenumber range of 400-4000 cm^{-1} (Bruker VECTOR 22, Germany) and the sample was ground with dried potassium bromide (KBr) powder, compressed into a disc and then subjected to analysis.

The surface morphology of the cellulose/nano-ZnO composite films was observed by AFM (JEOL JSPM-5200, Japan). Images were obtained in random areas of 2×2 μm^2, by scanning in tapping mode.

The X-ray diffraction (XRD) pattern of the film samples was analyzed by an X-ray diffractometer (Rigaku RINT2000 vertical goniometer, Japan). The spectra were recorded using Cu-Ka radiation and a nickel monochromator filtering wave at 40 kV and 100mA with scanning at 2 theta from 5 to 50°.

The tensile strength of the nano-ZnO cellulose films was measured by using a Universal Testing Machine (INSTRON 3369) according to the Chinese standard method (GB/T 1040-2006). The thickness of the film was gauged by using a thickness meter. Three samples from each film were trimmed into the standard size (150 × 15 mm) before testing. Afterward, all samples were tested with an effective length of 50 mm at a speed of 50 mm/min. In the end, the average value was calculated.

The antimicrobial activity of the nano-ZnO cellulose films was determined by using the colony count method.[17] The composite films with varied concentrations of nano-ZnO (0.5, 1, 1.5, 2, 2.5, 3%) were tested against the bacterial strains of *Escherichia coli* (*E. coli* ACTT 25923) and *Staphylococcus aureus* (*S. aureus* ACTT 25922). The liquid nutrient medium was composed of beef extract (5.0 g), peptone (10 g), sodium chloride (5.0 g), distilled water (1000 mL) and pH 7.3. The broth culture media were sterilized in an autoclave at 121 °C for 20 min and three square slices (3 cm×3 cm) of each nano-ZnO cellulose film were placed in the center of the broth culture medium. After that, the broth culture medium was inoculated with *S. aureus* and *E. coli* for which the bacterial suspension concentrations were of about 1×10^5 CFU/mL, respectively. All the plates were incubated in a bacteriological incubator at 37 °C for 24 h. The number of visible colonies was counted after the incubation. All samples were tested in triplicate. The antimicrobial activity of the nano-ZnO cellulose films was determined by the percentage of bacterial reduction (R%), which was calculated by the following equation:

$$R\% = \frac{Num_{control} - Num_{sample}}{Num_{control}} \times 100\% \quad (1)$$

where R is the bacterial reduction rate of the nanocomposite film, $Num_{control}$ and Num_{sample} are the numbers of bacteria in the control and in the treated sample, respectively.

Statistical analysis was performed using the SPSS package program version 18 (SPSS Inc., Chicago, Illinois, USA). All data from triplicate measurements for each treatment were analyzed by variance (one-way ANOVA model) and the means were compared with the least significant differences (LSD) test at a significance level of $P<0.05$. The values were reported as means with their standard deviations for all results. Thus, each of the points in the figures was attributed to the average of the data taken from three samples.

RESULTS AND DISCUSSION

FT-IR Spectroscopy

The introduction of nanoparticles into a polymer can change the intermolecular interaction of the matrix.[18] However, nanomaterials easily agglomerate during the preparation of polymer/nanoparticle composites because of the small particle size and wide surface area. This agglomeration phenomenon limits the excellent physical and chemical properties of nanoparticles, such as strength, toughness and antimicrobial properties. Therefore, it is extremely important to

modify the surface of nanoparticles and improve their dispersed state in the matrix when preparing polymer/nanoparticle composites. In this study, before mixing with the polymer, the surface of nanoparticles was modified with a silane coupling agent.

Figure 2 shows the FT-IR spectra of original nano-ZnO (a) and modified nano-ZnO (b). The absorption peak at 420 cm^{-1}, 423 cm^{-1} is characteristic of nano-ZnO. It can be observed that there are many hydroxyl groups on the surface of nano-ZnO: the peaks ranging between 3100~3450 cm^{-1} and at 1628 cm^{-1} correspond to the stretching vibrations of hydroxyl groups of the nanoparticles. The absorption at 1408 cm^{-1}, which appears in the spectrum of KH550-ZnO represents the asymmetric and symmetric stretching vibrations of C-H in CH_2. In comparison with the spectrum of the as-received nano-ZnO, in the spectrum of KH550-ZnO, the bending of N-H at 1551 cm^{-1} of KH550 is observed. In addition, the bands at 1170 cm^{-1}, 992 cm^{-1} and 831 cm^{-1} in the spectrum of KH550-ZnO indicate the existence of Si-O-Si, C-O-C and Si-O-C, respectively. As expected, these data prove that KH550 was chemically connected to the surface of nano-ZnO during the modification processes.

Atomic-Force Microscopy (AFM)

The morphological characteristics of the modified ZnO nanoparticles present in the cellulose film are clearly shown in Figure 3A. It may be noticed that the cellulose film has a granular surface, and most particles have diameters between 50 and 80 nm.

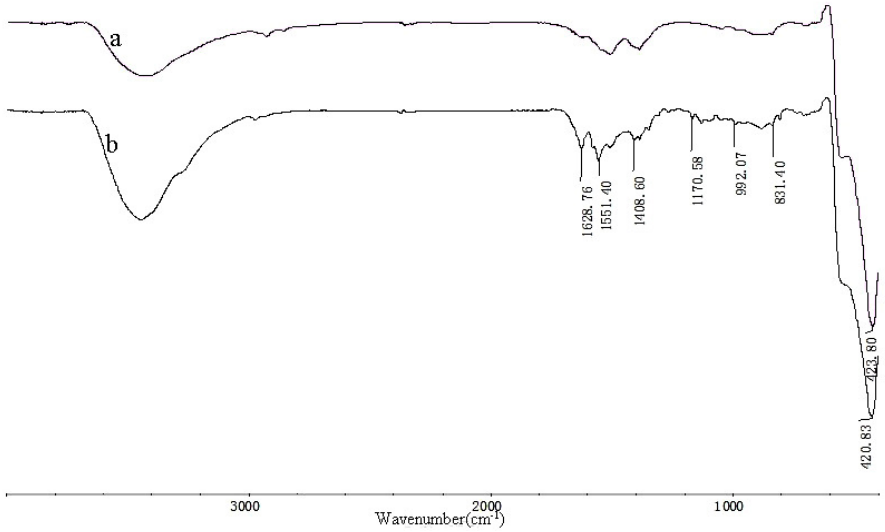

Figure 2: FT-IR spectra of original nano-ZnO (a) and modified nano-ZnO (b) with KH550

Table 1
Crystal parameters of nano-ZnO/cellulose composite films and pure cellulose film

Type	Diffraction angle (°)	Grain size (Å)	Crystallinity (%)
Cellulose film	12.4	51	12.3
	20.8	40	
	26.9	73	
Nano-ZnO/cellulose films	7.5	288	15.0
	20.7	91	
	26.7	39	

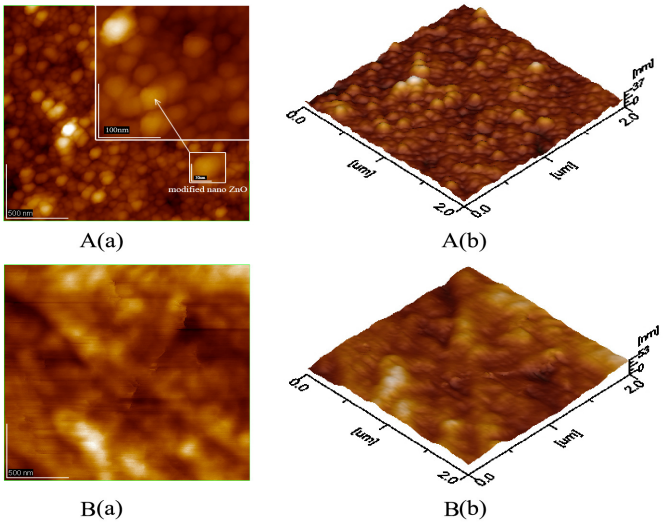

Figure 3: AFM flat (a) and three-dimensional images (b) of the modified nano-ZnO cellulose film (A) and unmodified nano-ZnO cellulose film (B)

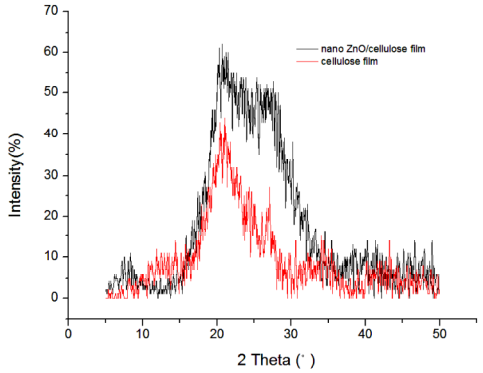

Figure 4: XRD patterns of the nano-ZnO/cellulose composite film and of the pure cellulose film

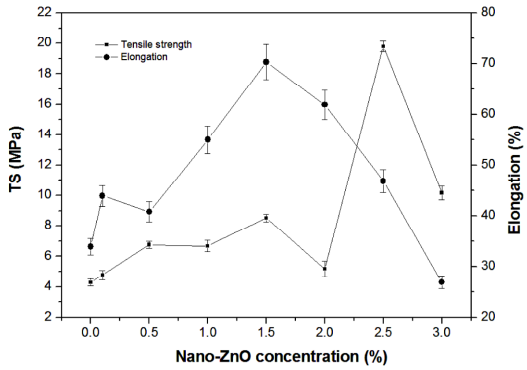

Figure 5: Effects of modified nano-ZnO on mechanical properties of the cellulose film

However, the particles are obviously larger in Figure 3B, with diameters between 100 and 200 nm. This implies that the modified ZnO nanoparticles are distributed well on the surface of the cellulose film; the silane coupling agent KH550 being responsible for this phenomenon. Furthermore, the unmodified ZnO nanoparticles present some agglomerations on the surface of the cellulose films.

X-Ray Diffraction (XRD)

The XRD technique is used to reveal the crystallographic ultrastructure of the material studied.[11] Pure cellulose films and nano-ZnO/cellulose composite films were analyzed using the XRD technique (Fig. 4, Table 1). The results showed the main diffraction crystalline peaks of cellulose film (observed at 2 theta = 12.4º, 20.8º and 26.9º), which represent cellulose II crystalline structure. Cellulose has a high proportion of amorphous structure and a natural partial crystallinity. After adding the nano-ZnO, intense diffraction peaks were visible in the nanocomposite films at 2 theta = 7.5º, 20.7º and 26.7º.

Each phase has its corresponding XRD spectrum, similarly to the fingerprints of people. If cellulose has no interaction with nanomaterials or the interaction is weak in the composite film, it will have its own crystalline region in the

spectrum and would superpose in the blending components briefly. In the diffraction diagram of the nanocomposite film, the peaks of 31.72º, 34.38º, 36.20º and 47.54º, which belong to the nano-zinc oxide, were not found (JPCDS card number: 36-1451). The peak of 7.5º would belong to a dissimilar characteristic peak of cellulose, but the 12.4º diffraction, which belongs to the cellulose disappeared. The peak intensities of the nanocomposite film were enhanced, the grain sizes of the crystal area were increased and the crystallinity of the films was increased from 12.3% to 15%. This indicated that the interaction happened between nano-ZnO and cellulose. Although the crystalline structure of the composite film had no obvious changes, the addition of nano-ZnO interfered with its original space structure.

Tensile strength (TS)

The tensile strength is the most important parameter for packaging films. The TS test of the nano-ZnO cellulose films was carried out and the values are presented in Figure 5. The TS values slowly increased in the initial range of nano-ZnO concentration (0-1.5%). There was an obvious decline from 8.5 to 5.2 MPa at 2% concentration, however, a strong increase in the TS value was observed at 2.5% nano-ZnO concentration (up to 19.8 MPa). These results indicate that the 2.5% nano-ZnO cellulose film possessed the most effective tensile strength for a final product. These observations could be explained by the fact that while the fiber network of cellulose is intrinsically inhomogeneous, the added modified ZnO nanoparticles act as bonding agents and improve fiber-fiber interaction, which increases the tensile strength of cellulose.[19]

Antimicrobial activity of nanocomposite films

ZnO nanoparticles were effective in killing Gram-positive and Gram-negative bacteria and also in inhibiting the growth of fungi.[20-22] The antimicrobial activity of the nanocomposite films is illustrated in Table 2. The number of *E. coli* colonies declined as the concentration of ZnO nanoparticles in the cellulose film increased. It was found that the 2.5% particle loaded cellulose film had the highest reduction rate of *E. coli*, while there was a lower reduction rate at 2% concentration, which could be attributed to the agglomeration of ZnO nanoparticles. Similarly, the nano-ZnO has the most effective activity against *S. aureus* at 2.5%. As for the PE film, the reduction of *E. coli* and *S. aureus* was of -20% and -31%, respectively, which means that the PE film possesses lower antimicrobial property compared with the composite film. Overall, the nanocomposite film exhibited excellent antimicrobial activity against *E. coli* and *S. aureus*. Thus, the developed cellulose/nano-ZnO composite films proved a potential material for inhibiting microbial activity.

Table 2
Antimicrobial activity of nano-ZnO cellulose films

C (%)	*E. coli*		*S. aureus*	
	Number $\times 10^6$	R (%)	Number $\times 10^6$	R (%)
0	11.3 ± 0.35	0	6.5 ± 0.30	0
0.5	7.4 ± 0.28	35 ± 3	4.1 ± 0.23	37 ± 2
1	3.4 ± 0.10	70 ± 1	2.7 ± 0.16	58 ± 1
1.5	4.1 ± 0.37	64 ± 3	2.6 ± 0.21	60 ± 2
2	2.8 ± 0.24	75 ± 2	1.5 ± 0.08	77 ± 1
2.5	1.3 ± 0.12	89 ± 2	1.0 ± 0.26	85 ± 3
3	2.1 ± 0.06	81 ± 1	3.0 ± 0.21	54 ± 2
PE	13.6 ± 0.30	-20 ± 3	8.5 ± 0.38	-31 ± 3

C (%): concentration of ZnO nanoparticles; R (%): reduction in viability

CONCLUSION

This study demonstrated the preparation and the properties of a biodegradable cellulose nanocomposite film. The modification of nano-ZnO with KH550 increased the compatibility of nano-ZnO with the cellulose film, while the presence of the modified nano-ZnO improved the tensile strength and the antimicrobial activity of the nanocomposite film.

The dispersion of modified ZnO nanoparticles in the mixed system was favorable, the addition of nano-ZnO interfered with its original space structure, and the nanocomposite films showed a strong antimicrobial activity against *E. coli* and *S. aureus*, and the highest tensile strength of 19.8 MPa, which is 4.6 times that of the normal cellulose film. When the concentration of nano-ZnO was 2.5 wt%, the composite film had the best tensile strength and antimicrobial properties. Therefore, the nano-ZnO/cellulose composite film proves to be a promising material and its development could be a new approach for improving the properties of cellulose films, inhibiting the growth of bacteria. However, the antibacterial mechanism needs further research.

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