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Structure and properties of chitin whisker reinforced chitosan membranes

Bomou Ma, Aiwen Qin, Xiang Li, Xinzhen Zhao, Chunju He*

State Key Lab for Modification of Chemical Fibers and Polymer Materials, College of Material Science & Engineering Donghua University, Shanghai 201620, PR China

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ABSTRACT

In this paper, rod-like chitin whisker was used as a filler to reinforce the chitosan membrane, and a series of composite membranes were prepared by casting–evaporation method. Mechanical testing shows that tensile strength of the resulting composite membrane with 3 wt% chitin whisker content reaches up to 110.3 MPa, which is about 2.8 times than that of neat chitosan membrane (38.5 MPa), and moisture regain of all composite membranes presents a decreasing tendency with increasing content of chitin whisker. Furthermore, SEM was used to investigate the morphology difference between neat chitosan membrane and composite membranes, to understand the reinforce mechanism of chitin whisker. Wide angle x-ray diffraction and Fourier transform infrared spectroscopy were used to visualize the structure change before and after the compositing processes. Besides, the bacterostatic test shows that this composite membrane presents effective inhibitory effect on *Staphylococcus aureus*, *Escherchia coli* and *Corinebaterium michiganence* respectively, which indicates it a promising material for packaging and wound dressing.

1. Introduction

Chitosan, an abundant natural biological macromolecule in the world, has attracted high consideration due to its nontoxicity, biocompatibility and biodegradability [1]. Chitosan membrane, an important form of chitosan, presents potential application in tissue engineering, food preservation, wastewater purification, environmental protection, fuel cell and separate technology [2–11]. Nonetheless, the poor mechanical property of chitosan membrane limits its further development. In recent years, much work has been carried out to improve its mechanical property through crosslinking, blending and the addition of reinforcements [12]. And the addition of reinforcement has received great attention since it is simple and effective. Sun et al. used carbon nanotube (CNT) to reinforce chitosan membrane with 99.2 MPa tensile strength [13]. Furthermore, it was reported that the parallel aligned graphene oxide and unzipped multiwalled carbon nanotube oxides presented excellent strength enhancement, and tensile strength of the prepared chitosan membrane reached up to 133.1 and 142.7 MPa respectively [14,15]. Although the inorganic addition can reinforce the chitosan membrane significantly, they are not suitable candidate as biomaterial since they are non-degradable. Therefore, it is very important to develop fully biodegradable chitosan composite membrane with high strength. Chitin whisker, a hydrolysis product of chitin with rod-like shape, is biodegradable and biocompatible, and presents remarkable reinforcement in many bio-based polymer matrixes. Watthanaphanit et al. and Wongpanit et al. confirmed the enhancement of chitin whisker for alginate fibers and silk fibroin sponges respectively [16,17]; and Ji et al. reported that chitin whisker was a good reinforcing material for the polycaprolactone matrix [18].

Chitin and chitosan, both of them are natural biodegradable materials with similar structure, which guarantees the excellent compatibility and contributes to form the strong electrostatic. Moreover, since chitin and chitosan are renewable natural macromolecules, it is of great significance to develop chitin whisker/chitosan composite materials with the shortage of petroleum. In this paper, a series of these composite membranes were prepared through conventional casting-evaporation method. From the results of mechanical testing, it is shown that tensile strength of the composite chitosan membrane with 3 wt% chitin whisker content reaches up to 110 MPa, which is about 2.8 times than that of neat chitosan membrane. SEM images visualize the status of chitin whisker in chitosan matrix, which helps us to understand the reinforcing mechanism. Besides, the bacteriostatic results show that the composite membranes present effective inhibitory effect on *Staphylococcus aureus*, *Escherchia coli* and *Corinebaterium michiganence* respectively, indicating it a promising material for packaging and wound dressing.

* Corresponding author. Tel.: +86 21 6779 2842; fax: +86 21 6779 2855.
E-mail address: chunjuhe@dhu.edu.cn (C. He).

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2. Experimental

2.1. Materials and reagents

Chitin powder was made from snow crab with viscosity average molecular (Mv) mass about 1.53×10^5, which is calculated by the equation $[\eta] = 2.4 \times 10^{-1} \times Mv^{0.69}$, where dimethyl acetamide containing 5% lithium chloride (w/w) was chosen as a solvent; chitosan flake (degree of deacetylation 90%, source: snow crab), its Mv is about 9.76×10^5, which is calculated by the equation $[\eta] = 7.8 \times 10^{-2} \times Mv^{0.76}$, where 0.2 M acetic/0.3 M sodium acetate was chosen as a solvent [19]. Both of them were purchased from Introduction of Jinhu Crust Product Co. Ltd (Qingdao, China). Hydrochloric acid (HCl, 36–38 wt%), acetic acid and other reagents were purchased from Sinopharm Chemical Reagent Co. Ltd., and used as received.

2.2. Preparation of chitin whisker

Chitin whisker was prepared by hydrolysis method to remove the amorphous parts of chitin powder [20,21]. Briefly, 3 mol/L HCl aqueous and chitin powder, at the weight ratio of 30:1, was added to a round-bottomed flask fitted with magnetic stirring and condensation at 90 °C for 3 h. After acid hydrolysis, the suspension was filtrated under suction to obtain the residue. The hydrolytic process was repeated three times. For the third time, filtration is very difficult due to the very small size of residue, therefore, centrifugation was used to wash the residue until neutral. Finally, chitin whisker slurry was collected and lyophilized to obtain the dried chitin whisker, the yield is about 40%.

2.3. Preparation of chitin whisker/chitosan composite membranes

In order to make the chitin whisker well dispersed in chitosan solution, firstly, a certain amount of chitin whisker was dispersed in deionized water under ultrasonic treatment for 10 min to obtain a milky, homogeneous suspension. And then, a desired amount of chitosan flake, chitin whisker suspension, deionized water and acetic acid were added to round-bottomed flask under magnetic stirring for 12 h until a homogeneous mixed solution was prepared. The mass ratio of chitin whisker to chitosan were controlled at 1:99, 3:97, 5:95, 10:90, 15:85 and 20:80, respectively. To prepare the chitin whisker/chitosan composite membrane, the mixed solution was casted onto a glass plate and evaporated at 60 °C for 3 h, then immersed in 2 wt% sodium hydroxide aqueous solution to be regenerated. The obtained membranes were washed and dried to afford the chitin whisker/chitosan composite membranes.

2.4. Characterization

2.4.1. Scanning electron microscopy (SEM)

Morphology of the chitin whisker was observed under S-4800 field emission scanning electron microscope (Hitachi Ltd., Japan). For the chitin whisker/chitosan composite membranes, a crack was made intentionally and sputter-coated with gold. JSM-5600LV scanning electron microscope (Jeol Ltd., Japan) was used to observe the tearing surface.

2.4.2. Transmission electron microscopy (TEM)

The chitin whisker was dispersed in deionized water by ultrasonic treatment. The obtained suspension was dropped on a carbon-coated copper grid, gradually evaporated under infrared lamp, and observed under 2100F transmission electron microscopy (Jeol Ltd., Japan).

2.4.3. Wide angle x-ray diffraction (WAXD) and Fourier transform infrared spectroscopy (FT-IR)

WAXD of all membranes was obtained from Rigaku D/Max-2550 (Rigaku Corp., Japan) with Cu radiation operated at 40 kV and 200 mA, from 5° to 60° in steps of 0.02°. FT-IR was recorded on Nicolet 8700 (Thermo Electron Corp, USA) in the range of 4000–400 cm^{-1} with attenuated total reflection (ATR) accessory.

2.4.4. Mechanical testing

Tensile strength of all the membranes was performed on a WDW3020 materials testing system (Changchun Kexin Instrument Co. Ltd., China) at room temperature with a crosshead speed of 10 mm/min. The samples were cut into strips of 80 × 10 mm, and five strips were measured for each sample.

2.4.5. Moisture regain studies

A piece of membrane was dried at 105 °C for 1 h, and weighed it on Mettler XS105, then, its weight was recorded at certain time intervals until constant weight under equilibrium condition (25 °C and 65% relative humidity). The collected data were used for further calculations and analysis.

2.4.6. Bacteriostatic test

Disk diffusion method was used to investigate the bacteriostatic activity of the composite membrane (with 3 wt% chitin whisker) against *S. aureus*, *E. coli* and *C. michiganence* qualitative. Membrane disks (diameter = 70 mm), after sterilization, were placed on the inoculated culture medium (pH = 6.0) and cultured at 37 °C for 24 h to observe the growth of bacteria.

3. Results and discussion

3.1. Characterization of chitin whisker

Morphology of the prepared chitin whisker was shown in Fig. 1. Nanoscale microcrystals could be clearly seen from Fig. 1a, but its width and length was difficult to evaluate due to the crossing and overlapping. The transmission electron microscopy in Fig. 1b clearly shows that the average length and width of chitin whisker was about 300 and 20 nm respectively, with an average aspect ratio of 15. This is in accordance with the data of crab shell chitin whiskers reported before [22]. Here, it must be pointed out that the dimensions of chitin whisker prepared from different sources will have a little difference. Besides, it was found that the chitin suspension was composed of chitin individual microcrystals and aggregated microcrystals. The shape of individual microcrystals was a single slender rod with sharp points, which had relative narrow distribution in size. The shape of aggregated microcrystals was a bunch of single slender rod, which had a broad distribution in size. In addition, the chitin whisker suspension exhibited colloidal behavior due to the presence of positive charge on chitin whiskers, which guarantees the stability of the suspension [16,17,23].

3.2. Mechanical properties

A series of chitin whisker/chitosan composite membranes were prepared through the conventional casting-evaporation method. Their typical stress–strain profiles and related bar charts in Fig. 2 clearly show that tensile strength of the chitin whisker/chitosan composite membranes sharply increases as compared with that of neat chitosan membrane (38.5 MPa) with increasing whisker content, and reaches a maximum value (110.3 MPa) at 3 wt% whisker content, the result is in accordance with the published reference [23]. However, the tensile strength decreases gradually with further increasing whisker content. A similar tendency is observed for the elongation at break. Elongation of the chitin whisker/chitosan