Structure and properties of chitin whisker reinforced chitosan membranes

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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 bacteriostatic test shows that this composite membrane presents effective inhibitory effect on *Staphylococcus aureus*, *Escherchia coli* and *Corinebacterium michiganense* respectively, which indicates it a promising material for packaging and wound dressing.

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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 bio-compatible, and presents remarkable reinforcement in many bio-based polymer matrixes. Watthanaphant 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 *Corinebacterium michiganense* respectively, indicating it a promising material for packaging and wound dressing.
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 [n] = 2.4 × 10^{-1} × Mv^{0.76}, 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 [n] = 7.8 × 10^{-2} × 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 filled 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 flakes, 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 microscopy (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 Nicoet 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. michiganense 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
composite membrane reaches a maximum value (11.8%) at 3 wt% whisker content, which is higher than that of neat chitosan membrane (7.9%). After that, the elongation presents a decreasing tendency, and reaches a minimum value (1.9%) at 20 wt% whisker content.

According to Ref. [16], mechanical properties of nanocomposites depend not only on the amount, size, shape, and the alignment of the fillers, but also on the ability of matrix to transfer the stress. Chitin whisker is a rod-like short fiber and possesses high crystallinity degree, which guarantees its high modulus and strength. Moreover, both chitin whisker and chitosan are natural polysaccharide with similar structures, this contributes to form the strong electrostatic interactions and hydrogen bonds between them, as shown in Fig. 3. Therefore, the interfacial adhesion strength and compatibility between chitin whisker and chitosan is very excellent. All the factors make the added chitin whisker act as a strengthened point in membrane matrix. When the load is applied to the membrane, they are transferred to the chitin whisker quickly. Because the chitin whisker is very strong, this enables the chitin whisker/chitosan composite membranes present excellent tensile strength at low whisker content. However, the decrease of the tensile strength at higher chitin whisker content, i.e. greater

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**Fig. 1.** SEM (a) and TEM (b) images of the prepared chitin whisker.

**Fig. 2.** Typical stress strain behavior of neat chitosan membrane and composite membranes with different chitin whisker content (a), and their corresponding bar charts of stress at break (b), elongation at break (c), initial modulus (d).
than 3 wt%, may be ascribed to the aggregation of the whisker. The explanation for the tensile strength can also be used for the elongation, the electrostatic interactions and hydrogen bonds between chitosan and chitin whisker make the loaded stress to be easily transferred to the whisker. At the same time, they produce the drag force which delays the fracture of samples. However, as the content of chitin whisker is further increased, the flaws caused by stress concentration dominate the properties of composite chitosan membrane, which leads to its fracture at low strain. As shown in Fig. 2d, the introduction of chitin whisker increases the initial modulus of chitosan matrix gradually at the expense of elongation. This can be attributed to the increasing crystallinity of composite chitosan membranes with increasing the chitin whisker content.

3.3. Morphology of the tearing surface

Scanning electron microscopy was a visualized method to study the composite materials. However, the status of chitin whisker in chitosan matrix is illegible from the surface and cross section of chitin whisker/chitosan composite membrane, which may be attributed to the good adhesion and compatibility between chitin whisker and chitosan [24]. For this, a crack was made intentionally on the composite membrane to detect the status of chitin whisker, the results are shown in Fig. 4, which presents the tearing surface’s morphology of composite membranes loaded chitin whisker at 0%, 3%, 10% and 20% respectively. It can be seen that the tearing surface of the neat chitosan membrane is relatively smooth (Fig. 4a), and that of the composite membrane with 3% chitin whisker is relatively rough (Fig. 4b) without visible large agglomeration or cluster. This structure guarantees the high-strength of chitin whisker/chitosan composite membrane at low chitin whisker content. Some aggregations are observed in Fig. 4d with further increasing chitin whisker contents to 20%, which leads to the tensile strength decreases of composite membrane.

3.4. Structure characterization

Fig. 5 shows the FT-IR spectra and WAXD patterns of neat chitosan membrane, chitin whisker/chitosan composite membranes and chitin whisker respectively. From Fig. 5a, the broad band at about 3360 cm\(^{-1}\) is attribute to the characteristic absorption of O–H and N–H stretching vibrations in chitosan membrane, and the band at 2850 and 2920 cm\(^{-1}\) is characteristic of methyl (CH\(_3\)) antisymmetric stretching vibration and symmetric stretching vibration respectively [25]. The strong vibrations at 1640 and 1600 cm\(^{-1}\) is assigned to the stretching of amides I and II respectively. The peaks at 1420 and 1050 cm\(^{-1}\) correspond to the flexural vibration of CH\(_2\) and stretching vibration of C–O, respectively [26]. For chitin whisker, the characteristic absorption peaks of O–H and N–H are observed at 3448, 3267, and 3106 cm\(^{-1}\), respectively [27]. The other peaks are also observed at 1660 and 1622 cm\(^{-1}\) (amide I bands; single H-bonded and doubly H-bond, respectively), 1558 cm\(^{-1}\) (amide II bands), 1154 and 1064 cm\(^{-1}\) (secondary alcohol and primary alcohol, respectively) [16,17]. By comparing all the FT-IR spectra, it is clearly seen that spectra of composite membranes is very similar to that of chitosan membrane, and the characteristic absorption peaks of chitin whisker is invisible due to the coverage of strong absorption of chitosan matrix.

From Fig. 5b, WAXD pattern of chitosan membrane shows two weak diffraction peaks at 2θ angles of 10.1° and 20.5° respectively, which suggests that they present very low qualitative apparent degree of crystallinity. For chitin whisker, there presents two major diffraction peaks at 2θ angles of 9.5° and 19.5° as well as three other weak peaks at about 21.0°, 23.6° and 26.5° respectively. It is clearly seen that intensity of the chitin whisker is far higher than that of chitosan membrane, which indicates that the chitin whisker possess relatively high apparent degree of crystallinity. For all the chitin whisker/chitosan composite membranes, their WAXD patterns are more similar to that of chitosan membrane, regardless of the content of chitin whisker. This suggests that the presence of chitin whisker have little effect on the crystallinity degree of the chitosan matrix. However, the 2θ angle of composite membranes is more close to that of chitin whisker with increasing whisker content, which indicates that the presence of chitin whisker affects the crystalline structure of the chitosan matrix.

3.5. Moisture regain studies

Moisture regain is an indicator of materials’ water uptaking property. It is well known that chitosan is easy to swell in water, which leads to its membrane relatively low mechanical properties at wet conditions [28]. One effective way to improve this properties is to reduce its swelling through hydrophobic modification [25]. Fig. 6 shows the moisture regain behavior of neat chitosan membrane, chitin whisker/chitosan composite membranes and chitin whisker respectively, which present a rapid initial moisture uptake followed by a leveling off. It is clearly shown that moisture regain of the neat chitosan membrane (9.6 wt%) is far higher than that of chitin whisker (2.0 wt%), and moisture regain of the composite membranes are between that two values, which indicates that the addition of chitin whisker can improve the water resistance
properties of chitosan matrix. According to reference [27], the equilibrium water uptake by any polymeric system involves sorption, diffusion, and permeation process, and sorption is the most prominent one that drives the water uptake in chitosan/chitin whisker system. The addition of chitin whisker into chitosan matrix makes the composite membranes possess numerous nanocrystals on their surface, which has lower affinity and reactivity to water. At the same time, the NH2 groups are the most reactive species in the composite membranes, which facilitates its interaction with the water molecules. However, the addition of chitin whisker results in a reduction in the number of amino groups on the membrane surface. On the other hand, the highly crystallinity of chitin whisker hinders the movement of water molecules between the polymer chain. All the factors contribute to the decrease of moisture regain of the composite membranes, as compared with that of neat chitosan membrane. It can be inferred that the water sensitivity and low mechanical properties at wet condition of chitosan membrane can be improved through the introduction of the chitin whisker.

3.6. Bacteriostatic results

Inhibitory effect of the composite membrane (with 3 wt% chitin whisker) against S. aureus, E. coli and C. michiganence was investigated qualitative, and the neat chitosan membrane as control. As shown in Fig. 7, the neat chitosan membranes do not show any inhibitory effect on three kinds of bacteria. However, the chitin whisker/chitosan composite membranes present effective inhibitory effect. Since the inhibitory effect of chitosan is significantly associated with its molecular weight, the chitosan with low molecular weight presents strong bacteriostatic and
bactericidal activity [29]. The chitosan molecular weight here used is very high, which guarantees the high mechanical properties of composite membranes at the sacrifice of the bacteriostatic property. For the composite membrane, the chitin here used holds low molecular weight and about 20% degree of deacetylation, which contributes the chitin has positive charge under weak acidic environment. Furthermore, the positive charge will flocculate the negative charge in cytoplasm, and achieves to the bacteriostatic and bactericidal activity [30]. These results confirm that the chitin whisker reinforced chitosan membrane will be a promising material for packaging and wound dressing.

4. Conclusion

Chitin whisker is a good reinforcing material for chitosan membrane due to their excellent compatibility, affinity and similar structure. Tensile strength of the composite membrane with 3 wt% chitin whisker reaches 110.3 MPa, which is about 2.8 times than that of neat chitosan membrane (38.5 MPa). With further increasing chitin whisker content, the tensile strength decreases gradually due to the aggregation of whisker. So does moisture regain. It can be inferred that chitin whisker improves the water resistance of chitosan membrane and further improves its mechanical properties at wet condition. Besides, this kind of composite membrane presents effective inhibitory effect on S. aureus, E. coli and C. michiganence respectively. In summary, the chitin whisker reinforced chitosan membrane holds not only excellent mechanical properties, but also inhibitory effect on typical bacteria, this indicates it a promising material for packaging and wound dressing.

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