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Preparation and characterization of novel curdlan/chitosan blending membranes for antibacterial applications

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ABSTRACT

Antibacterial membranes were prepared from a mixture of hydrolyzed curdlan and chitosan. The elongation-at-break and water vapor transmission rate of curdlan/chitosan blended membranes were substantially improved when the temperature was high (90 °C) due to curdlan gel formation and the interaction between the hydroxyl groups of curdlan and the amino groups of chitosan. This was confirmed by Fourier transform infrared spectroscopy. The membranes were further investigated for their miscibility between the two components using differential scanning calorimetric measurements, dynamic mechanical analysis, optical microscopy, and scanning electron microscopy. High reaction temperatures (90 °C) and low curdlan contents caused better interactions between components, and allowed membranes to be prepared with higher storage moduli and lower water absorption rates. The 4 bacteria strains were chosen as target bacteria using the optical density method to prove the antibacterial activity of the membranes obtained. The resulting curdlan/chitosan membranes exhibited outstanding antibacterial activity.

1. Introduction

In recent years, degradable membranes have attracted widespread interest due to the increasing problem of environmental pollution caused by the use of synthetic polymers based on petroleum sources. Renewable natural polymers show wider application prospects in the food, packaging, and medical industries compared to their synthetic counterparts because such renewable polymers exist abundantly in nature and are available at low cost (Liu, Qin, He, & Song, 2009).

Chitosan is a linear biopolymer that has become of enormous interest because of its many advantages, including biocompatibility, biodegradability, hydrophilicity, non-toxicity, non-antigenicity, and antimicrobial activity, as well as bioadherence and cell affinity (Wan, Lu, Dalai, & Zhang, 2009). In the biomedical field, chitosan and its derivatives have a great number of applications, ranging from wound dressings, contact lenses, sorbents and enzyme supports to drug delivery systems and tissue engineering (Klinkaewnarong, Swatsitang, & Maensiri, 2009). The healing of dermal wounds using macromolecular agents, such as natural polymers, is preferred to skin substitutes because such polymers offer many advantages, such as biocompatibility, non-irritant and non-toxic properties, and ease and safety of dermal application (Sezer et al., 2007). Chitosan has been used as a wound dressing in burn healing for the activation and proliferation of inflammatory cells in granulation tissue, and has wound cleaning and re-epithelization properties (Gouda & Keshk, 2010). However, the mechanically inferior features of chitosan in the wet state have limited its utility. Although chitosan membranes are highly impermeable to oxygen, they have relatively poor water vapor barrier characteristics due to their excellent hydrophilicity, making their use as artificial skins difficult (Willför, Sundberg, Tenkanen, & Holmbom, 2008). Srinivasa reported that adding plasticizers to chitosan reduced its barrier properties despite the positive effects on its mechanical capabilities (Srinivasa, Ramesh, & Tharanathan, 2007). Much effort has been exerted to improve the water barrier capability of chitosan by weakening its hydrophilic property, amongst which the blending chitosan with other hydrophobic materials has attracted the greatest interest.

Curdlan, a microbial polysaccharide, which occurs naturally as a linear (triple helix) polysaccharide composed of 1,3-β-linked D-glucose units produced by a strain of *Alcaligenes faecalis* (McIntosh, Stone, & Stanisich, 2005). Since its discovery by Harada in 1966, it has been used in food and non-food related research due to its unique physicochemical properties. Curdlan possesses the ability to form ‘high-set’ thermo-irreversible gels and ‘low-set’ thermo-reversible gels at two distinctive temperatures, which is a unique type of behavior compared to other heat-set gels

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(Nishinari & Zhang, 2004). In addition, (1,3)-β-D-glucan belongs to a group of compounds known as biological response modifiers that enhance or restore normal immune defences (McIntosh et al., 2005). Therefore, most non-food related studies are concentrated on the immunomodulation and anti-tumour activity of this bacterial exo-polysaccharide (Gao et al., 1997). As reported previously (Gao et al., 2010), curdlan has potentially inhibitory effects against AIDS virus infections and blood anti-coagulant activity, as well as low toxicity in vitro and in vivo. More importantly, curdlan is insoluble in water, and has the potential to improve a material's water barrier capability. However, it is soluble in alkaline aqueous solutions ($pH > 12$) because of its ability to undergo conformational changes. This polysaccharide has been actively investigated as a drug delivery carrier due to its gelation property, which varies according to temperature and pH, and its anti-HIV and anti-tumour activities (Kim, Na, & Choi, 2005).

In the present work, chitosan was blended with curdlan to form a heterogeneous membrane. The mechanical properties, miscibility of components, and water vapor transmission rate of the curdlan/chitosan blend membranes are studied as a function of the interaction between chitosan and curdlan. In addition, the membranes were tested to determine their antibacterial properties, which will determine some applications in the medical field.

2. Experimental

2.1. Materials

Chitosan from shrimp shells was purchased from Jinhu. Co. Ltd., China. Its deacetylation degree was 92%, and its molecular weight (Mw) was 400 kDa. Curdlan, with a polymerization degree of 450–500, was purchased from Shandong Zhongke Biological Technology Co., Ltd. Glycerin (purchased from Sinopharm Chemical Reagent Co. Ltd., Shanghai, China) was diluted into a 25% solution and used as a plasticizer. Other chemicals used were purchased from Sinopharm Group Chemical Reagent Co. Ltd. (China). The strains of *Escherichia coli*, *Staphyloccocus aureus*, *Klebsiella pneumoniae* and *Psudomonas aeruginosa* were isolated from clinical samples. The *E. coli* strain was resistant against ampicillin and cefotetan tested. The *S. aureus* strain was resistant against ampicillin, clindamycin, gentamicin, rifampicin and tetracycline tested. The *K. pneumoniae* strain was resistant against ampicillin, cefotetan, gentamicin and levofloxacin tested. The *P. aeruginosa* strain was resistant against ampicillin, cefotetan, cotrimoxazole and nitrofurantoin tested.

2.2. Preparation of curdlan/chitosan membrane

Chitosan powder and curdlan powder were first dissolved in 1% acetate solution and 1% sodium hydroxide under continuous stirring to produce two solutions each with concentrations of 20 mg/mL. Two types of solutions were mixed together at different weight ratios, and the final volumes of each were adjusted to 90 mL by the addition of 1.5 mL glycerin. The mixtures were continuously stirred at 60 and 90 °C for several hours, poured into Teflon-coated dish (12 cm × 12 cm), and then evaporated in an oven at 60 °C overnight. The uniform membranes obtained had an average thickness of around 0.5 mm, and were collected for mechanical property determinations and characterization measurements. Pure chitosan and curdlan membranes were also produced using the same technique and used as controls.

2.3. Characterization

2.3.1. Measurement of stress–strain properties

The stress–strain properties, such as tensile strength (TS) and elongation-at-break (EB), of the curdlan/chitosan films were measured using an Instron universal testing machine (Model 4204) with a 100 N load cell and an extension speed of 50 mm/min. Each tested film had an average length of 80 mm, width of 10 mm, and thickness of 0.5 mm, and the applied gauge length was 20 mm. Prior to testing, all samples were conditioned under standard atmospheric conditions (i.e., 50 ± 2% relative humidity (RH) and 21 ± 1 °C) for 24 h.

2.3.2. Water absorption

The water absorption of the curdlan/chitosan membranes was tested as follows: membranes were equilibrated at room temperature in a desiccator overnight and then immersed completely in deionized water for a second night. After sufficient swelling, the membranes were taken out from the water and the excess water on their surfaces was wiped off with filter paper until a constant weight was obtained. The water absorption rate (denoted as WA%) of curdlan/chitosan membranes was calculated as $WA = (W_w - W_d)/W_d \times 100\%$, where W_w and W_d are the weights of the wet and dry membranes, respectively.

2.3.3. Water vapor transmission rate

The water vapor transmission rate ($g\,m^{-2}\,h^{-1}$) (denoted as WR) was measured via Fujun's method (Liu et al., 2009). Membrane specimens were placed over glass cups (Ø 3.5 cm) containing 15 mL of distilled water (about 1/2 cup). The cups were placed in an environmental chamber, where the value of RH was confirmed to be 50% with the help of a hygrometer, at 25 °C for 10 h, so that the water vapor transmission process could be induced by the difference in RH between the exterior (50%) and interior (100%) of the cup. The weights of the cups were recorded every hour to the calculate water vapor transmission rate and an average value was obtained.

2.3.4. Fourier-transform infrared characterization

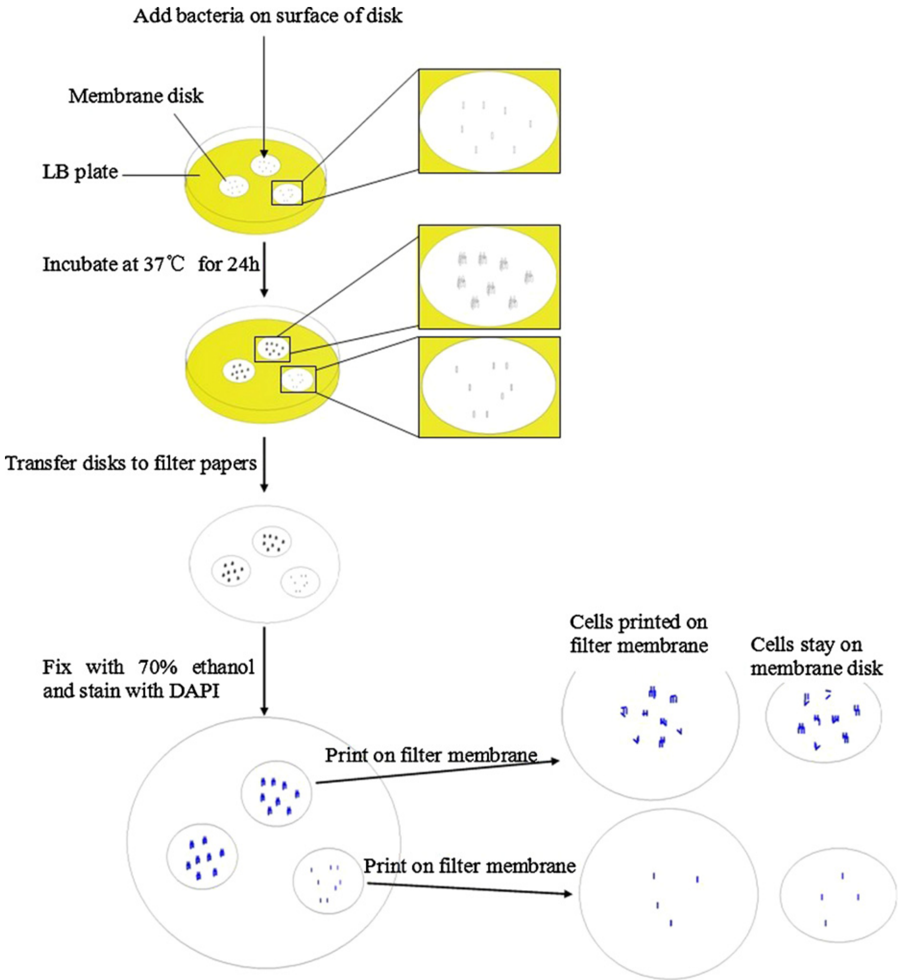
Fourier-transform infrared (FT-IR) spectra of membranes with different curdlan/chitosan ratios and different reaction temperatures (60 and 90 °C) were obtained using an IR spectrometer (FT-IR, PerkinElmer 1600X, USA) in attenuate total reflection (ATR) mode. To examine the interactions between curdlan and chitosan, 64 consecutive scans at 1 cm^{-1} resolution were averaged.

2.3.5. Thermal transition characteristics

The thermal transition characteristics of the curdlan/chitosan samples were analyzed by differential scanning calorimetry (DSC) using a PerkinElmer (DSC-6) instrument and Pyris 3 software. Approximate 3–7 mg of each sample was weighed into a standard crimped aluminium DSC pan and DSC scanning was performed under a nitrogen atmosphere with a gas flow rate of 20 mL/min. The applied temperature range was from −30 to 300 °C. The heating and cooling rates were both 10 °C/min. Isothermal heating and cooling were kept constant for 5 min. In the first heating cycle, each sample was heated from 20 to 130 °C, and in the second heating cycle, heating was performed from −30 to 300 °C. The second heating scans of the samples are reported in the present study. Prior to the analysis of thermal transitions by DSC, all films were dried using a desiccator under identical condition of 25 ± 1 °C for a week.

2.3.6. Dynamic mechanical analysis

The storage modulus (E') of the blend membranes was recorded on a dynamic mechanical analyzer (DMA 242C, NETZSCH-Gerätebau GmbH) from −100 to 300 °C at a frequency of 1 Hz and a



Scheme 1. Membrane antimicrobial activity test. The areas indicated by black squares are magnified as insets.

heating rate of 3 °C/min under an atmosphere of 150 mL/min nitrogen.

2.3.7. Morphology observations

The morphologies of the curdlan/chitosan membranes were observed under an optical microscope (Olympus BX51) under phase contrast mode (ph1 mode and condenser at lowest position).

The cross-sectional morphologies of the blend films were examined using a scanning electron microscope (Hitachi, S-4800, FE-SEM). Cross-sectional samples were prepared by fracturing films in liquid nitrogen. Prior to observation, samples were arranged on metal grids, using double-sided adhesive tape, and coated with gold under vacuum.

2.3.8. Antimicrobial activity of membranes

The curdlan/chitosan membranes and “mixed cellulose esters membrane filter” (a kind of hydrophilic filter membrane that has no antimicrobial activities.) were cut into Ø 1 cm disks and placed on solid LB medium-covered plates. Then, 50 μL of *E. coli*, *S. aureus*, *K. pneumoniae* and *P. aeruginosa* cultures were added to the surfaces of the membranes, respectively. After the liquid on the surface of the membrane disks had been absorbed, the plates were incubated upside-down at 37 °C for 24 h.

After incubation, the membrane disks were transferred onto filter papers, fixed with 70% ethanol solution, and stained with 10 μg/mL 4,6-diamidino-2-phenylindole dihydrochloride. The stained cells were printed on new “mixed cellulose esters membrane filters” (see Scheme 1). Cell proliferation was evaluated by observing the assembly of blue fluorescent bacterial cells using an Olympus BX51 optical microscope under UV mode.

3. Results and discussion

3.1. Mechanical properties

Chitosan consists of a relatively rigid six-membered ring structure backbone and an intra-chain hydrogen bonding network, along with ionic side groups; thus, it often exhibits high mechanical performance (Ghosh, Azam Ali, & Walls, 2010). Polysaccharides blends with different polymer ratios can be useful for improving mechanical properties. In this study, chitosan was blended with curdlan to form blend membranes. The TS and EB of the different films were measured and were shown in Table 1. Table 1 shows that when the reaction temperature was 60 °C, the TS and EB of the curdlan/chitosan blend membranes all significantly decreased compared to the pure chitosan membrane. The low TS and EB values could be attributed to the lack of formation of intermolecular hydrogen bonding between the NH_3^+ of the chitosan backbone and the OH^- of the curdlan. Although curdlan is soluble in dilute sodium hydroxide aqueous solutions, the existence of chitosan probably restrained the formation of hydrogen bonds between curdlan molecules. When the reaction temperature was 90 °C, curdlan gels formed adequately due to hydrogen bond interactions and glycerin was used as a bridging or cross-linking agent to modify and improve the mechanical properties of chitosan and its conjugates

Table 1
Effect of the mass ratio of curdlan and chitosan on maximal tensile strength (TS), elongation at the break point (EB), water absorption rate (WA), and water vapor transmission rate (WR) of curdlan/chitosan membranes (25 °C, 50% RH).

Amounts of curdlan/chitosan (w/w)	60 °C				90 °C			
	TS (MPa)	EB (%)	WA (wt.%)	WR ($g\,m^{-2}\,h^{-1}$)	TS (MPa)	EB (%)	WA (wt.%)	WR ($g\,m^{-2}\,h^{-1}$)
Pure chitosan	3.66 ± 0.12	14.87 ± 0.70	59.20 ± 2.66	264.42 ± 9.25	3.00 ± 0.11	14.60 ± 0.60	34.51 ± 0.96	304.4 ± 14.00
1/2	1.92 ± 0.10	4.64 ± 0.21	29.80 ± 1.31	328.53 ± 13.14	4.92 ± 0.24	24.69 ± 1.01	19.20 ± 1.27	360.5 ± 14.24
1/1	1.16 ± 0.05	3.02 ± 0.12	49.50 ± 2.03	327.30 ± 13.75	2.28 ± 0.08	8.81 ± 0.38	30.40 ± 1.53	304.7 ± 12.48
2/1	1.39 ± 0.06	2.76 ± 0.11	35.10 ± 1.61	296.49 ± 10.97	1.83 ± 0.54	8.21 ± 0.34	30.80 ± 1.52	336.5 ± 13.46

with curdlan. Gelation occurred when curdlan in low concentrations of NaOH was heated above 60 °C. The gelation mechanism would be correlated to the transition of the molecular association from an associated state to a dissociated state in the dilute regime (Tada, Matsumoto, & Masuda, 1999). The molecular association of curdlan is characterized as a multi-helical conformation at high pH. However, at low pH, the molecular association is characterized as a heterogeneously aggregated coil, which consists of a hydrophilic surface and a hydrophobic core (Kim et al., 2005). However, no data about mechanical properties of pure curdlan membranes were obtained, because pure curdlan membranes prepared in this study were brittle and easily break. Water absorption and water vapor transmission rates were not tested in this work for the same reason.

3.2. *Water absorption and water vapor transmission rate*

Water sensitivity is another important property for the practical applications of curdlan/chitosan membranes. To analyze the hydrophobic/hydrophilic characters of the obtained membranes, their WA was tested, and the results are shown in Table 1. The WA of blend membranes decreased significantly compared to the pure chitosan membrane. With increasing swelling time, WA increased until it reached equilibrium. Comparing the WA of membranes with different curdlan contents, it can be observed that the hydrophobicity of the blend membranes is enhanced to a maximum value when the mass ratio of curdlan and chitosan is 1/2. This results from the hydrophobic chains of curdlan and the improved formation of intermolecular hydrogen bonding between the NH_3^+ of the chitosan backbone and the OH^- of curdlan. When the reaction temperature was 60 °C, the WA of the curdlan/chitosan blend membrane (mass value: 1/2) was 29.80%, and when the reaction temperature was 90 °C, the WA was 19.20%.

The WR is assumed to be independent of the water vapor pressure gradient applied across the membranes. However, hydrophilic materials such as chitosan deviate from this ideal behavior due to the interactions of the permeating water molecules with the polar groups in the film structure. Chitosan films have relatively poor water vapor barrier properties, which result from their hydrophilicity (Rao, Kanatt, Chawla, & Sharma, 2010). Because curdlan has different gel properties under different reaction temperatures, it changes the interchain distance in the polymer network, promoting water clustering by competing with water at the active sites of the polymer matrix and forming micro cavities in the polymer network structure. The water is absorbed into the film matrix, leading to a less dense structure where chain ends are more mobile and increasing the transmission rate (Diab, Biliaderis, Gerasopoulos, & Sfakiotakis, 2001). Thus, the WR value of the curdlan/chitosan blend membrane (mass value: 1/2) is much larger than that of the pure chitosan films. When the reaction temperature was 60 °C, the WR of the curdlan/chitosan blend membrane (mass value: 1/2) was 328.53 $g\,m^{-2}\,h^{-1}$, and when the reaction temperature was 90 °C, the WR was 360.5 $g\,m^{-2}\,h^{-1}$.

3.3. *Morphology of curdlan/chitosan blend membranes*

To explore the relationship between the morphology and different reaction temperatures of membranes, samples with curdlan-to-chitosan mass ratio of 1:2 prepared at 60 and 90 °C were tested by optical microscopy. Digital images from the procedure are displayed in Fig. 1. Under transmission mode, the phase separation of curdlan/chitosan blend membranes prepared at 60 °C can be easily observed (Fig. 1A). In contrast, at 90 °C, a symmetrical blend membrane was formed (see Fig. 1B). Curdlan is a thermo-gelable bacterial polysaccharide that forms two types of gel depending on its heating temperature. The resulting gel is called a low-set gel when the temperature is 60 °C, and the cross-linking within a low-set gel is accomplished by hydrogen bonds between molecules. The resulting gel is called a high-set gel when the solution is heated to a temperature >80 °C, and cross-linking is accomplished by hydrophobic interactions (Funami, Yada, & Nakao, 1998). In addition, the chain structures of curdlan and chitosan are similar,

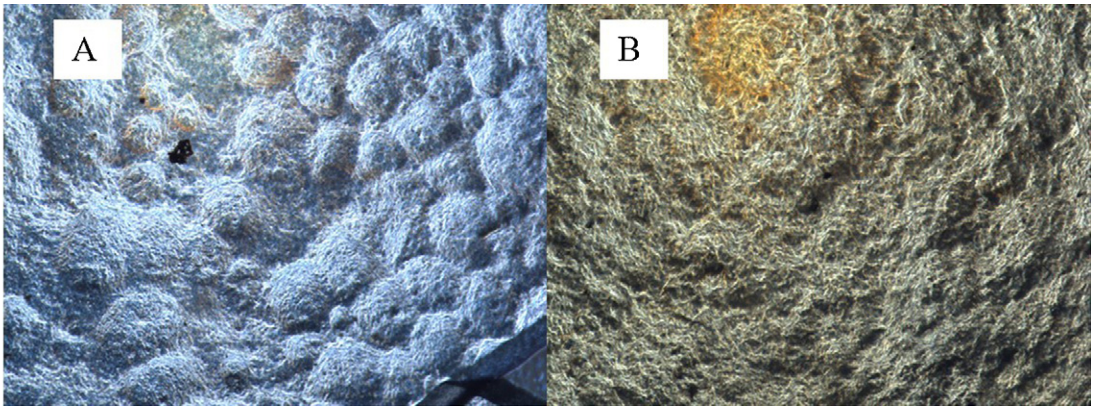


Fig. 1. Optical microscope pictures of curdlan/chitosan blend membranes ($W_{curdlan}/W_{chitosan} = 1/2$) at different reaction temperatures: (A) 60 °C and (B) 90 °C (magnification: 40×).

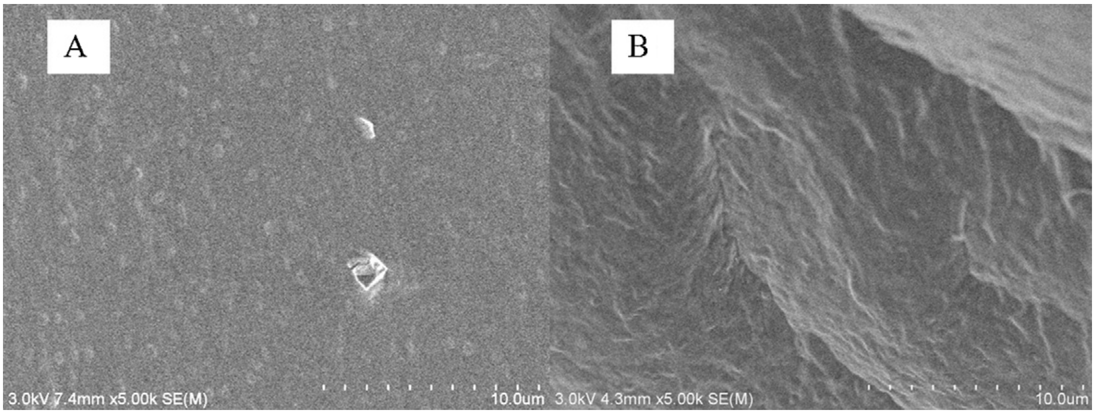


Fig. 2. SEM micrographs taken of the fracture surface of the curdlan/chitosan blend membranes ($W_{\mathrm{curdlan}}/W_{\mathrm{chitosan}} = 1/2$) at different reaction temperatures: (A) 60 °C and (B) 90 °C.

except for the groups at the C-2 positions, so the two can theoretically form a uniform mixture. When the reaction temperature was 90 °C, a symmetrical blend membrane between curdlan and chitosan was formed (Fig. 1B), increasing the mechanical properties of curdlan/chitosan blend films. However, when the reaction temperature was 60 °C, no sufficient intermolecular hydrogen bonding between the NH_3^+ of the chitosan backbone and the OH^- of the curdlan was formed.

Fig. 2 shows typical SEM images of the fracture surface of the curdlan/chitosan blend membranes ($W_{\mathrm{curdlan}}/W_{\mathrm{chitosan}} = 1/2$) after brittle fracturing in liquid nitrogen. Fig. 2A shows that at 60 °C, the curdlan/chitosan blend films exhibited a phase-separated morphology containing nanoparticles with diameters of about 300–400 nm dispersed in the continuous chitosan matrix. In Fig. 2B, the curdlan was distributed homogeneously through the chitosan component at 90 °C, and curdlan-aggregated domains could not be found. These results are in agreement with the results obtained from our optical microscope analysis (see Fig. 1).

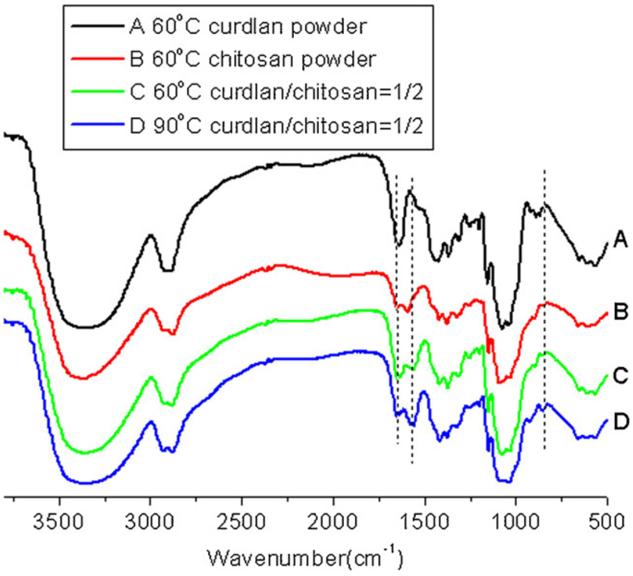


Fig. 3. FT-IR curves of (A) curdlan powder, (B) chitosan powder, and (C) curdlan/chitosan membranes with curdlan-to-chitosan ratios of 1:2, prepared at 60 and (D) 90 °C.

3.4. FTIR spectra

Fig. 3 shows the FT-IR spectra of pure curdlan, pure chitosan, and the curdlan/chitosan blend films ($W_{\mathrm{curdlan}}/W_{\mathrm{chitosan}} = 1/2$) at different reaction temperatures (60 and 90 °C). The main chain elements of curdlan and chitosan are similar to each other, except for an amino group, which exists only in chitosan at the C-2 position, and an –OH group, which exists in curdlan at the same position. The peak at 1644 cm^{-1} in the spectrum of pure curdlan (Fig. 3A) is attributed to the existence of water, which does not seem to be completely eliminated from the sample. A similar phenomenon is found for some other polysaccharides, such as konjac glucomannan (Jin, Zhang, & Nishinari, 2006). The peaks at 1650 and l605 cm^{-1} in the spectrum of pure chitosan (Fig. 3B) are assigned to C═O stretching (amide I) and NH bending (amide I), respectively. The FT-IR spectra of curdlan/chitosan blend films ($W_{\mathrm{curdlan}}/W_{\mathrm{chitosan}} = 1/2$) at different reaction temperatures, 60 and 90 °C, are shown in Fig. 3C and D, respectively. Both curdlan/chitosan blend films ($W_{\mathrm{curdlan}}/W_{\mathrm{chitosan}} = 1/2$) prepared at 60 and 90 °C exhibit an absorption peak at 1570 cm^{-1}, which is ascribed to NH bending (amide II). Compared to pure chitosan (Fig. 3B), curdlan/chitosan blend films (Fig. 3C and D) show the disappearance of the NH_2-associated band at 1605 cm^{-1} attributed to N–H bending in the primary amine (Wan, Sun, & Li, 2008). These findings confirm the chemical interactions between curdlan and chitosan. The absorption band of the blend membrane prepared at

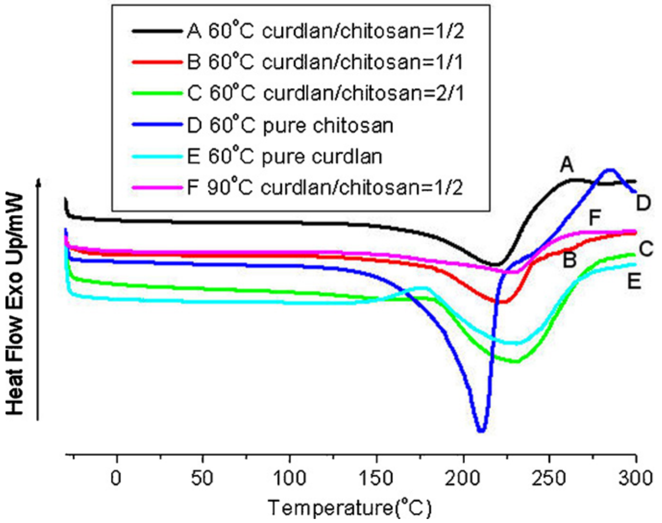


Fig. 4. DSC spectra of chitosan, curdlan, and curdlan/chitosan blend membranes.

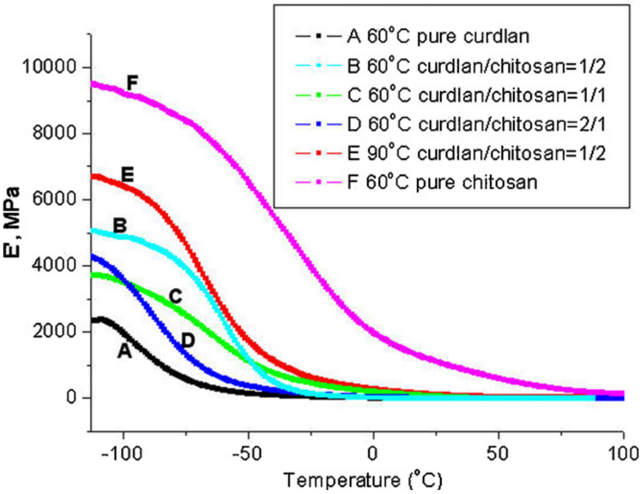


Fig. 5. DMA curves of the storage modulus as a function of temperature for curdlan, chitosan, and curdlan/chitosan blend membranes prepared at 60 and 90 °C.

90 °C is more obvious than that of the blend membrane prepared at 60 °C, indicating that the interactions between curdlan and chitosan in the blend films are easier to form when the reaction temperature is 90 °C.

3.5. DSC measurements

Fig. 4 presents several DSC thermograms for curdlan, chitosan, and the curdlan/chitosan blend membranes prepared at 60 and 90 °C with different curdlan-to-chitosan ratios. In the DSC curve of the chitosan membrane (second heating run; Fig. 4D), a new and wide endothermic peak centered around 210 °C formed, which is probably related to the breakdown of ionic cross-linking interactions between the hydroxyl groups of chitosan and the hydroxyl groups of glycerin (Wan, Sun, & Li, 2009). An exothermic peak near 285 °C is possibly linked to the decomposition of chitosan, which starts at about 240 °C (Wan, Lu, et al., 2009). No glass-transition temperature (T_g) was observed in chitosan. Chitosan is a semi-crystalline polymer with strong inter- and intra-molecular hydrogen bonds. It also has a rigid amorphous phase because of its heterocyclic units. As a result, when chitosan is heated within a certain temperature range below its decomposition temperature, the variations in heat capacity related to the change in specific volume near T_g are too small to be detected by DSC. The DSC curve of curdlan (Fig. 4E) shows a broad and shallow exothermic peak at 145–185 °C, and a very large endothermic peak at 185–275 °C. The exothermic peak may be caused by the formation of spindle-shaped microfibrils with a pseudo-crystalline form, while the endothermic one may be ascribed to the melting of curdlan gel (Zhang, Huang, Nishinari, Watase, & Konno, 2000). Fig. 4A–C present several DSC thermograms for curdlan/chitosan blend membranes with different mass ratios of curdlan and chitosan. The DSC thermogram of curdlan/chitosan prepared at 60 °C in Fig. 4A–C shows nearly similar thermal behaviors. Nevertheless, a clear difference is noticed: the higher the weight ratio of curdlan, the higher the endothermic temperature peak at about 220 °C moves. This suggests that some interactions occurred between the hydroxyl groups of curdlan and the amino groups of chitosan. In addition, when the weight ratio of curdlan:chitosan was 1:2 and the reaction temperature was increased from 60 to 90 °C, the endothermic peak of the curdlan/chitosan blend membrane moved from about 220 °C to 230 °C. While the hydroxyl groups of curdlan and the amino groups of chitosan could form interactions, it is also possible that, when the reaction temperature is 90 °C, hydrophobic interactions occur between the methyl groups at C-6 of D-glucosyl residues on different molecules of curdlan. This may result in more intense intermolecular hydrophobic interactions when the temperature is

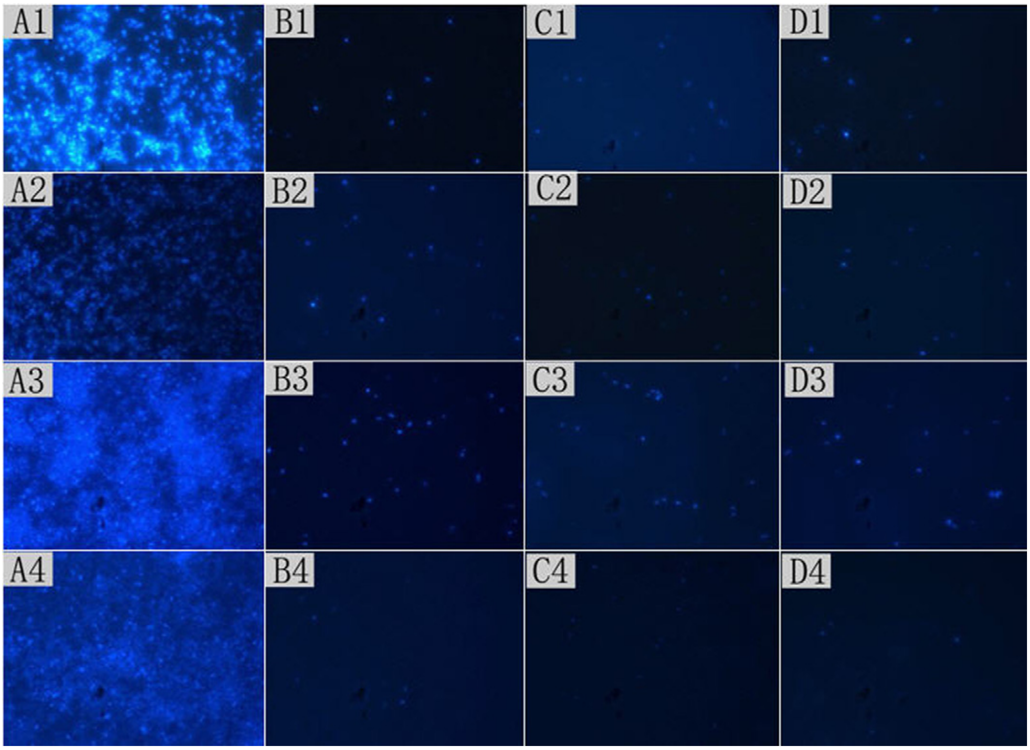


Fig. 6. Antimicrobial activity test: (A) mixed cellulose esters membrane filters, (B) pure chitosan membrane, (C) curdlan/chitosan = 1/2 blend membrane prepared at 60 °C, (D) curdlan/chitosan = 1/2 blend membrane prepared at 90 °C. The numbers 1, 2, 3 and 4 following A, B, C and D represented *Escherichia coli*, *Staphyloccocus aureus*, *Klebsiella pneumoniae* and *Psudomonas aeruginosa*, respectively.

increased, forming an irreversible gel (Tako & Hanashiro, 1997). Thus, compared to the curdlan/chitosan blend films prepared at 60 °C (Fig. 4A), the endothermic peak related to the breakdown of intra- or inter-actions between curdlan and chitosan, which was featured by the curdlan/chitosan blend membranes prepared at 90 °C (Fig. 4F), moved to a higher temperature. These results are in agreement with the results obtained from our SEM analysis (see Fig. 2).

3.6. DMA analysis

The synergistic effects of curdlan and chitosan prepared at different reaction temperatures on the properties of curdlan/chitosan blend membranes can be further demonstrated by the measurement of their dynamic mechanical properties. The E' of the curdlan, chitosan, and curdlan/chitosan blend membranes, with different ratios of curdlan to chitosan prepared at different reaction temperatures, as a function of temperature is shown in Fig. 5. Compared with pure curdlan, an increase in E' was found in curdlan/chitosan blend membranes (Fig. 5). Compared to pure chitosan, however (Fig. 5F) an obvious decrease in E' is seen for curdlan/chitosan blend films over the entire temperature range investigated. In addition, the E' of the curdlan/chitosan blend films prepared at 60 °C reaction shifted to considerably higher values when the chitosan content in the membranes was increased, suggesting that the interactions between curdlan and chitosan are strong enough to allow very efficient load transfers to chitosan and providing high mechanical strengths. The E' of curdlan/chitosan blend membranes prepared at 90 °C (Fig. 5E) is much higher than that of the blend membranes prepared at 60 °C (Fig. 5B) for the entire temperature ranges investigated. It is possible that the curdlan/chitosan blend membranes prepared under different reaction temperatures showed different E' due to the varying degrees of cross-linking reactions amongst curdlans. When the reaction temperature was 90 °C, hydrophobic interactions occurred between the methyl groups at C-6 of the D-glucosyl residues on different curdlan molecules. This may have resulted in more intense intermolecular hydrophobic interactions and the production of irreversible gels. Thus, increases in the cross-linking degree of the blend membrane with increasing reaction temperature are again supported.

3.7. Antimicrobial activity analysis of curdlan/chitosan blend films

The inhibitory activity of curdlan/chitosan blend membranes was measured based on a comparison of cell assemblies between growth conditions of bacteria on mixed cellulose ester membrane filters, pure chitosan membrane and curdlan/chitosan blend membranes. Mixed cellulose ester membrane filters, which were used as a negative control, showed no antimicrobial activity. *E. coli*, *S. aureus*, *K. pneumoniae* and *P. aeruginosa* grew on such membranes at normal speed and colonies appeared after incubation at 37 °C for 24 h (Fig. 6-A1, A2, A3 and A4). Chitosan has antimicrobial activities, so the growth of the 4 gram positive or negative bacteria on the pure chitosan membrane was largely suppressed and no cell colonies were observed after incubation at 37 °C for 24 h (Fig. 6-B1, B2, B3 and B4). As shown in Fig. 6-C1, C2, C3, C4, D1, D2, D3 and D4, no cell colonies were observed in the curdlan/chitosan blend membranes (curdlan/chitosan = 1/2). At the same time, different preparation temperatures (60 and 90 °C) did not appear to result in significant discrepancies in antimicrobial activity.

4. Conclusion

Curdlan/chitosan blending membranes (with a component ratio of 1/2) prepared at 90 °C exhibit outstanding mechanical and permeable properties. SEM, optical microscopy, DSC, and DMA analyses indicate excellent compatibility between curdlan and chitosan in blend membranes prepared at appropriate conditions. The blending membranes obtained show antimicrobial activities identical to pure chitosan membranes. In addition, the preparation temperature of the blend membranes significantly influenced the strength and elongation of the blend membrane largely. Diverse antimicrobial membranes could be prepared by adjusting the preparation temperature and component ratio. Compared to common antibacterial membranes, curdlan/chitosan blend membranes may have broader application potential.

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