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Structure and Properties of Films Fabricated from Chitin Solution by Coagulating with Heating

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ABSTRACT: In the present work, chitin films were fabricated from chitin solution dissolved in 8 wt % NaOH/4 wt % urea aqueous systems at low temperature by coagulating with heating without using any coagulant. The formation of the chitin films was confirmed to be an entirely physical process, namely chitin inclusion complex associated with NaOH, urea and water in the solvent was broken by heating, leading to the aggregation of the chitin chains through the hydrogen bonding. A schematic model was proposed to describe the regeneration mechanism of the chitin, indicating the physical crosslinking and entanglement of the chitin chains to form the films. Glycerol was used as plasticizer, and with an increase of the glycerol content, the elongation at break (ε_b) of chitin films increased rapidly from 7.8 to 83.1%, whereas the tensile strength (σ_b) decreased from 89.3 to 51.7 MPa. This work provided a "green" pathway for the researches and developments of chitin materials. © 2013 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 39538.

KEYWORDS: physical crosslinking; chitin film; glycerol; plasticizer; coagulate with heating

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INTRODUCTION

Chitin, poly(β-(1→4)-*N*-acetyl-D-glucosamine), is the most abundant natural polymer after cellulose, which mainly occurs as ordered crystalline microfibrils in exoskeletons of crabs and shrimps.[1,2] Chitin has several useful properties, such as nontoxicity, being odorless, high crystallinity, biocompatibility and biodegradability.[3–5] Despite its huge annual production and easy accessibility, chitin still remains as an unutilized biomass resource primary because of its intractable bulk structure, and thus, only limited attention has been paid to chitin, principally from its biological properties.[6–8] The microfibrils of chitin consist of nanofibers about 2–5 nm diameter and about 300 nm in length embedded in a protein matrix, thus various methods have been developed for preparing chitin nanofibers (or whiskers),[9–11] including acid hydrolysis,[12] TEMPO-mediated oxidation,[13] ultrasonic technique,[14] and electrospining method.[15] Owing to the semicrystalline structure of chitin with extensive hydrogen bonding, the cohesive energy density is very high and so it is insoluble in all usual solvent.[16] So far, only a few solvent systems for chitin have been found, such as, LiCl/*N*,*N*-dimethylacetamide,[17] NaOH/urea aqueous solution,[18–20] $CaCl_2$/MeOH,[21] and ionic liquids.[22,23] Although chitin has been blended with other polymer to prepare materials, for example, hybrid scaffolds composed of β-chitin and collagen,[24] composite gels and films based on chitin and cellulose,[25] and complex beads composed of alginate and carboxyl chitin,[26] the pure chitin materials prepared directly from the chitin solution have been reported scarcely except for our laboratory, because of the difficult dissolution and regeneration of chitin.

In our laboratory, chitin has been successfully dissolved in 8 wt % NaOH/4 wt % urea aqueous through freezing/thawing method to prepare chitin hydrogel,[19] aerogel,[18] and film.[20] However, we found that the regeneration condition affected the properties of the chitin materials much. Moreover, the regeneration mechanism of the chitin solution has not been identified clearly. In the present work, we attempted to use a new way to regenerate the chitin film without using any solvent but just by the heating method, and the chitin film was plasticized. Usually, plasticizers can attract the water molecules, reduce the intermolecular interactions between the biopolymer chains, and increase the flexibility of films.[27] Glycerol is a relatively small polar molecule and common plasticizer for starch, protein, and other biopolymers.[28–30] The plasticized films can be prepared through solution casting method.[31] The structure of chitin films were characterized by Fourier transform infrared (FTIR) spectroscope, solid-state ^{13}CNMR, X-ray diffraction (XRD), and scanning electron microscopy (SEM), and the thermo and mechanical properties were studied by thermo-gravimetric analysis (TGA), dynamic mechanical analysis (DMA), and tensile testing.

Table I. The Composition and Properties of the Chitin Films

Code	Composition (chitin : glycerol, w/w)	T_{max1} (°C)[a]	T_{max2} (°C)	T_g (°C)[b]	σ_b(MPa)	ε_b(%)
CF0	100 : 0	-	312.3-341.6	-	88.6	7.6
CF1	87.4 : 12.6	208.9	336.6	52.7	55.4	34.6
CF2	79.2 : 20.8	207.8	349.5	45.3	51.3	82.9

[a] Temperature at the maximum rate of mass loss was measured by TG. [b] Glass transition relaxation temperature was determined by DMA.

EXPERIMENTAL

Materials

Chitin was purchased from Jinke Chitin (Zhejiang, China). The degree of acetylation (DA) was determined by elemental analysis to be 0.98, and the weight-average molecular weight (M_w) was determined to be 3.2×10^5 in 5% (w/v) LiCl/DMAc by dynamic light scattering (DLS, ALV/GGS-8F, ALV, Germany). Glycerol, NaOH, and urea were reagent grade without any further purification.

Preparation of Chitin Film

To prepare chitin solution, chitin powders were dispersed into 8 wt % NaOH/4 wt % urea/88 wt % water mixture with stirring for 5 min, and then was stored under refrigeration (−30°C) for 4 h. The frozen solid was thawed and stirred extensively at room temperature. The freezing/thawing cycles were repeated three times to obtained a transparent chitin solution with chitin concentrations of 2 wt %. Subsequently, chitin solution was transferred into a dish, which was placed into a 50°C oven for 1 h to form a gel. Finally, chitin films were obtained by immersing gels in distilled water to remove residue for 3 days, and dried in the air. The chitin films prepared without glycerol were coded as CF0. For plasticization, wet chitin films were immersed in 2 and 4 wt % glycerol aqueous solution for 1 day and dried in air. The glycerol content in the films were determined to be 12.6 and 20.8%, and coded as CF1 and CF2, respectively (Table I).

Characterization

Chitin films were cut into particle-like size after freezing-dried and then vacuum-dried for 24 h at 50°C before measurements. The dried films were analyzed in KBr discs by FTIR (Perkin Elmer Spectrum one, Wellesley, MA). Cross-polarization/magnetic angle spinning (CP/MAS) solid-state ^{13}C NMR spectra were recorded on an Infinity Plus 400 Spectrometer (^{13}C frequency = 100.12 MHz) with a CP/MAS unit at ambient temperature. The spinning rate and the contact time were 5.0 kHz and 5.0 ms, respectively. Pulse width was 2.10 μs, spectral width 50.000 kHz, acquisition time 20.48 ms, and the spectrum accumulated 2000 times. The wide-angle X-ray diffraction (XRD) pattern of the dried sheets was recorder on a XRD instrument (XRD-6000, Shimadzu, Japan) with Cu K_α radiation (λ = 0.154 nm). XRD data were collected from $2\theta = 4$ to 40° at a scanning rate 2° min^{-1}. Scanning electron microscope (SEM) was taken with a Hitachi X-650 microscope (Japan). The chitin films in both wet and dry state were frozen in liquid nitrogen and snapped immediately, and then freeze-dried. The fracture surface of chitin films were sputtered with gold, and than observed and photographed.

Measurements of Thermal and Mechanical Properties

Thermo-gravimetric analysis (TGA) of the dry samples (5 mg) was carried out on a Pyris TGA linked to a Pyris diamond TA Lab System (Perkin-Elmer) at a heating rate of 10°C min^{-1} from 100 to 500°C under nitrogen atmosphere. Dynamic mechanical thermal analysis was performed on a dynamic mechanical analyzer (DMA, TA instrument Q800 series) in tensile mode at a frequency of 1 Hz. Samples were 5×1 cm^2 (length × width), and the test temperature ranged from −100 to 100°C with a heating ratio of 4°C min^{-1}. The tensile strength (σ_b) and elongation at break (ε_b) of the films (7×1 cm^2) were measured on a universal testing machine (CMT6350, Shenzhen SANS Test Machine, Shenzhen, China) with a tensile rate of 5 mm min^{-1} according to ISO527-3: 1995(E). An average value from three replicates of each sample was taken.

RESULTS AND DISSCUSION

Structure of Chitin Films

Figure 1(a) shows the FTIR spectra of original chitin film and chitin films plasticized by glycerol. In the spectra of CF0, two absorption peaks were observed at 3433 and 3266 cm^{-1}, which can be assigned to the stretching of —OH groups and —NH groups in chitin chains, respectively. The split absorbance peaks at 1660 and 1621 cm^{-1} were attributed to the amide region, and the absorbance band at 1561 cm^{-1} was corresponded to the amide II region.[32] These results indicated that the spectrum of the CF0 was in good agreement with that of pure α-chitin.[31,33] In comparison with native chitin, the spectra of the chitin films plasticized by glycerol (CF1 and CF2) exhibited similar absorbance peaks, and the peak at 3433 cm^{-1} became sharper, attributing to the increase of —OH groups,[34] as a result of the introduction of glycerol, which lead to the intermolecular hydrogen bonding between chitin and glycerol. Furthermore, the absorption bands at 2935 and 2880 cm^{-1} which were assigned to the asymmetric and symmetric stretching vibration of —CH_2— groups in the films of CF1 and CF2 were enhanced indicating the existence of glycerol.

The solid-state ^{13}C NMR spectra for native chitin and chitin films are presented in Figure 1(b). Signals of eight groups can be distinguished in the spectra of native chitin: sharp peaks at 174.5 and 23.8 ppm were attributed to carbonyl and methyl carbons, respectively; the peaks at 104.8, 83.9, 76.3, 74.5, 61.5, and 56.1 ppm were ascribable to the resonances of C1, C4, C5, C3, C6, and C2 on the *N*-acetyl-D-glucosamine unit of chitin,

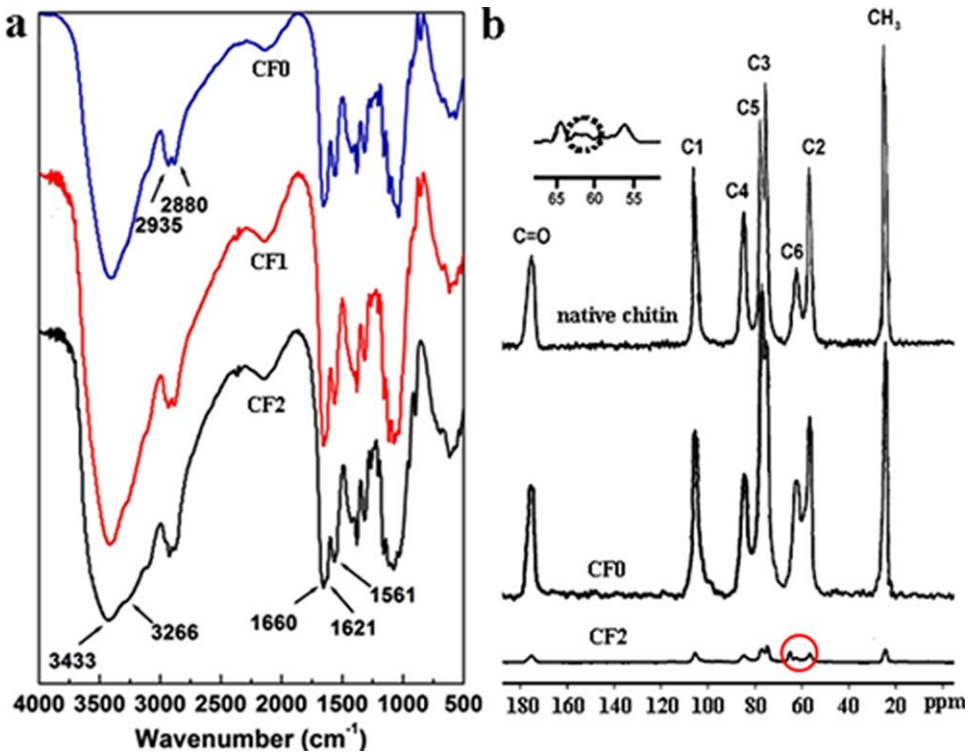

Figure 1. (a) FTIR spectrum of chitin films: CF0, CF1, and CF2. (b) Solid state ^{13}C NMR spectra for native chitin, chitin film, and CF2. Insert: amplified spectra of CF2. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

respectively.[35] In the spectra of CF0 and CF2, the chemical shifts of eight carbons in the spectra of CF0 were similar to that of the native chitin, indicating the dissolution and regeneration process hardly changed the structure of chitin. The signals assigned to C3 and C5 of the native chitin and regenerated chitin (CF0) existed here as two peaks, indicating the same α−chitin structure.[33,36] Interestingly, the intensities of the eight carbons of chitin obviously decreased due to the addition of glycerol in the chitin film. The broad bands between 65 and 55 ppm (in the red circle), including 64.5, 62.4, 61.1, 59.3, and 56.0 ppm, were observed in the spectra of CF2 [see the insert of Figure 1(b), in the black circle]. These peaks could be assigned to the carbons of glycerol and chitin (C6, C2). These results demonstrated that glycerol existed as a plasticizer in the chitin films, and the broad bands were referred to the strong interaction between glycerol and chitin molecules.

X-ray powder diffraction was performed to monitor the changes in crystallinity of chitin. Figure 2 shows the X-ray diffraction patterns of native chitin and the chitin films. All of the chitin samples showed six diffraction peaks at $2\theta = 9.3°$, 12.6°, 19.2°, 20.4°, 23.3°, and 26.3°, which corresponded to (020), (021), (110), (120), (130), and (013) planes, respectively, were typical crystal patterns of α-chitin.[12,37] These results further supported the conclusion from the FT-IR and ^{13}C-NMR. The intensities of the chitin films were evidently lost compared with those of the native α-chitin [Figure 2(b–d)]. In view of these results, the dissolution and regeneration were physical process, and the heating induced the destruction and rearrangement of the hydrogen bonding of chitin, leading to the formation of regenerated chitin films. The crystallinity of the chitin film was lower than that of native chitin. Moreover, the crystallinity of the chitin films further decreased after plasticized by glycerol. These results suggested that hydrogen bonding between the chitin molecules in the CF1 and CF2 was further broken through the strong interaction between chitin and glycerol.

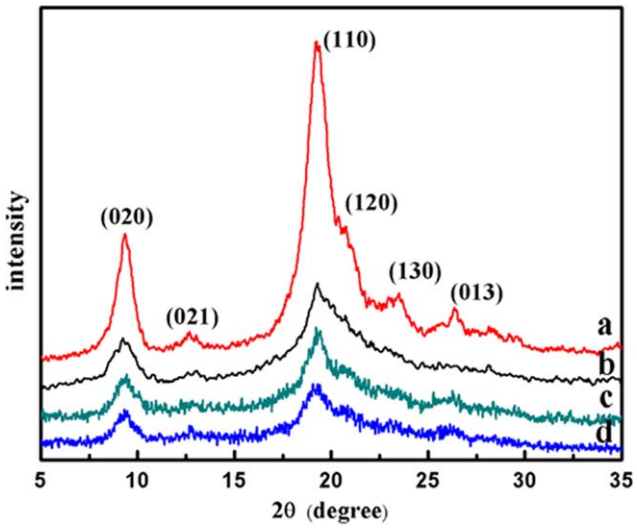

Figure 2. XRD patterns of chitin films: (a) native chitin, (b) CF0, (c) CF1, and (d) CF2. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

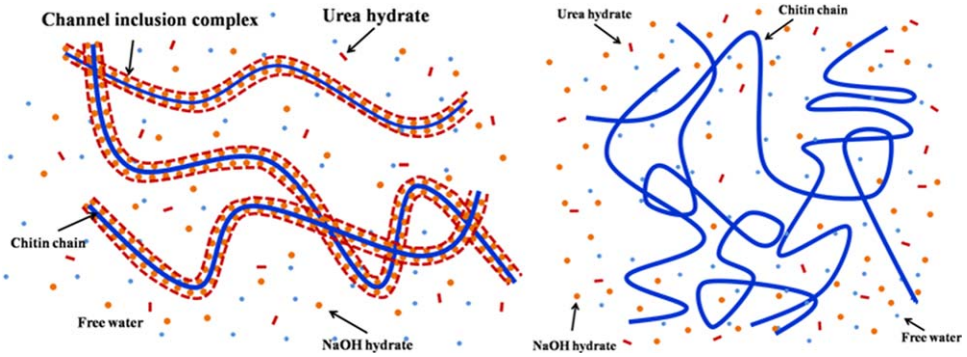

Figure 3. A schematic model to describe the transition from the chitin solution (left) to gel (right). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Effects of Heating and Plasticization on the Chitin Regeneration

It has been reported that the chitin had the similar dissolution mechanism to cellulose due to their similar structure. Namely, the urea hydrates can possibly be self-assembled at the surface of the NaOH hydrogen-bonded cellulose to form a channel inclusion complex (IC), leading to the good dissolution.[19,20,38,39] In the present study, the inclusion complex associated with chitin, NaOH, urea and water led to the chitin dissolution. However, the chitin inclusion complex could be destroyed by heating, leading to the chitin gelation through the formation of new hydrogen bonding networks between the chitin chains.[39,40] A schematic model was proposed to describe the regeneration mechanism of the chitin solution, as shown in Figure 3.The inclusion complex associated with chitin, NaOH, urea and water (Figure 3, left) was broken by heating, so a gel transition of the chitin complex solution occurred through the physical cross-linking and entanglement of the chitin chains (Figure 3, right), supported in Figures 1 and 2. This could be explained that the chitin inclusion complex was broken at elevated temperature, leading to the exposing of the chitin —OH groups, which aggregated easily. The aggregation between the chitin molecules formed the gel sheets, and then became films by drying. In our findings, it was a "green" way to regenerate the chitin film by heating at 50°C, because there was not any coagulant such as organic or inorganic agents. Therefore, the fabrication of the chitin regenerated by heating was a "green "process.

It is noted that the chitin films prepared by different methods have different morphologies.[20] The morphological observation of chitin films in both wet (freeze-dried) and dried (air-dried) states were carried out by scanning electron microscopy (SEM). Figure 4 shows SEM images of the cross sections of the chitin films by freeze drying. All samples displayed porous structure which was formed after the sublimation of solid ice during the freeze-drying process. However, the chitin films with different glycerol content (CF1 and CF2) exhibited relatively looser structure than that of CF0 without glycerol. SEM images of the cross sections and surface of the chitin films by air drying are shown in Figure 5. These films exhibited relatively homogeneous interior surface (cross section). The surface of chitin films with low

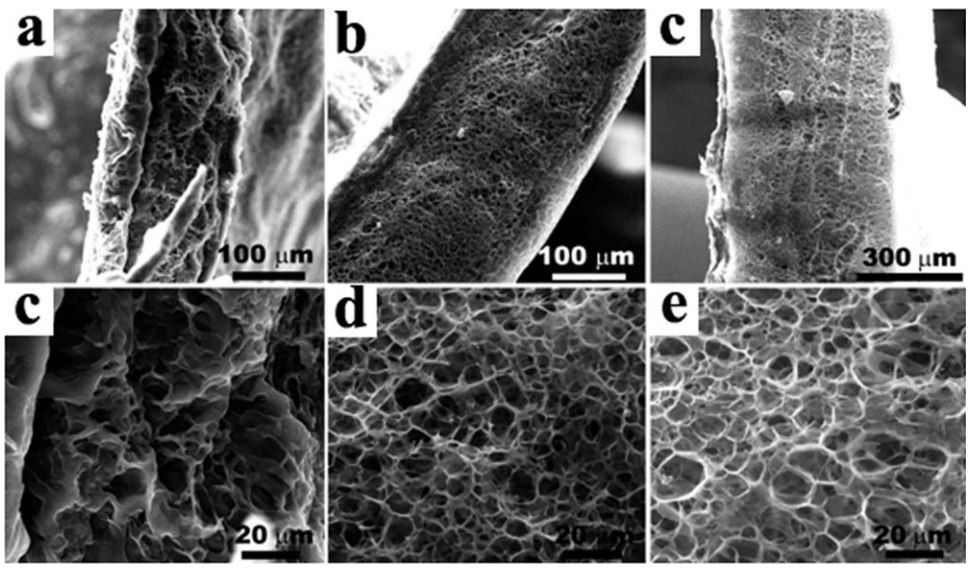

Figure 4. SEM images of the cross sections of chitin films by freeze drying: CF0 (a, d), CF1 (b, e), and CF2 (c, f).

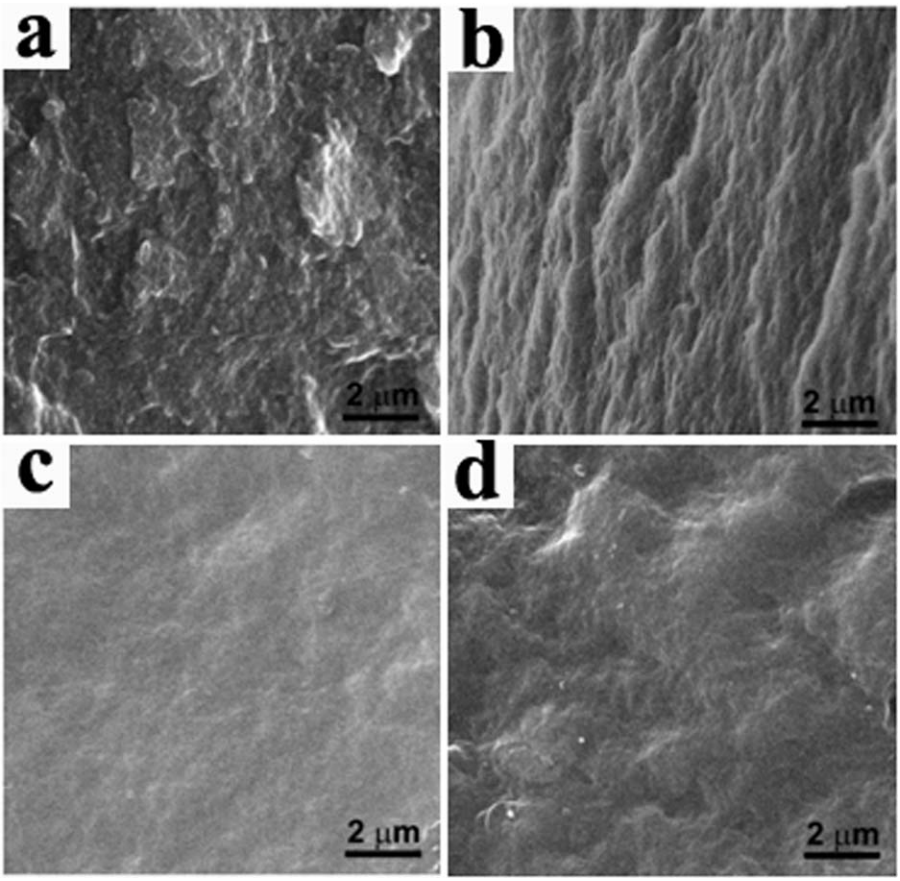

Figure 5. SEM images of the cross sections (top) and surface (bottom) of chitin films dried in the air: CF0 (a, c) and CF2 (b, d).

glycerol content (CF2) became relatively rough compared to that of CF0, but its surface became homogeneous with an increase of glycerol content. In the view of above results, the chitin films prepared by air drying had homogenous morphology in both cross section and surface on the whole, indicating a good regeneration and plasticization. These results suggested that the crystalline structure of chitin was further destructed by glycerol and the space among chains was increased, which was supported by the results of SEM and XRD. Clearly, the compatibility of chitin and glycerol was good, leading to appropriate plasticizing effect.

Thermal and Mechanical Properties of Chitin Films

Figure 6 shows the TG and DTG traces of the chitin films under nitrogen atmosphere. The thermal degradation behavior of chitin films could be divided into two steps: the evaporation of residual moisture and plasticizer occurred at 100–250°C (T_{max1}) and the degradation of chitin appeared at 250–400°C (T_{max2}).

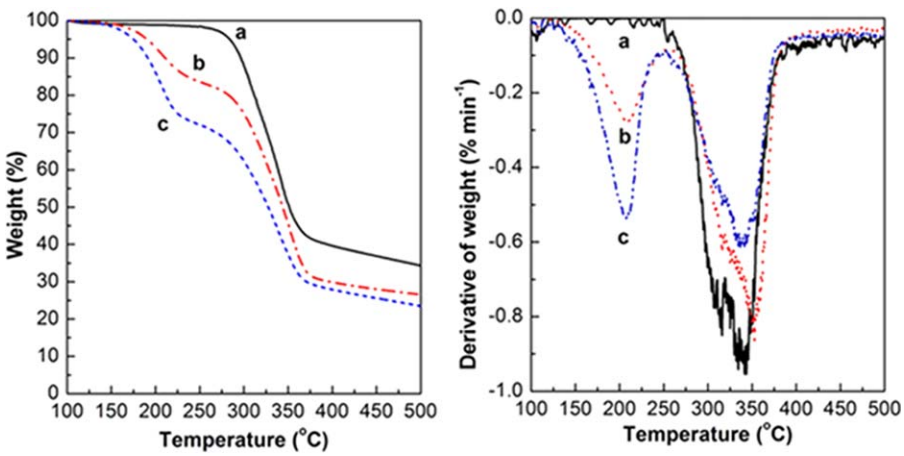

Figure 6. TG and DTG curves of chitin films: (a) CF0, (b) CF1, and (c) CF2. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

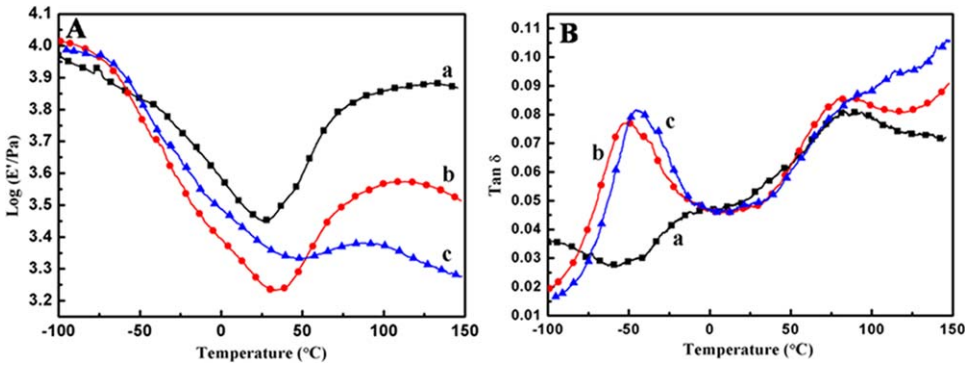

Figure 7. Temperature dependence of the storage modulus (E') (A) and the loss peak (tan δ) (B) for chitin films: (a) CF0, (b) CF1, and (c) CF2. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

From the DTG curve of the pure chitin films (CF0), no peak was observed around T_{max1}. However, the sharp peaks around T_{max1} in the curves of the glycerol plasticized chitin films and the area of the peak increased with the increase of the glycerol content in the chitin films. Therefore, the peaks at T_{max1} could be assigned to the release of glycerol from the samples. The pure chitin films showed two T_{max2} values at 314 and 340°C, which merged into peaks with the incorporation of chitin and glycerol in glycerol plasticized chitin films.

The thermal and mechanical properties of the chitin films were characterized by using DMA. The results for the temperature dependence of the storage modulus (E') and the loss factor (tan δ) of the chitin films are presented in Figure 6. Interestingly, the storage modulus of chitin films firstly decreased as the temperature increased, and then increased with an increase of temperature. The chitin films had the lowest storage modulus at around 27, 34, and 52°C for CF0, CF1, and CF2, respectively. The storage modulus values of chitin films at −100°C increased from 0.93 to 1.03 GPa with an increase of glycerol content, proving the enhancement of modulus in plasticized films with improved compatibility. The glass transition temperature of viscoelastic materials is considered to depend on the chain stiffness of the polymers and the effectiveness of intermolecular forces.[41]

To analyze the the molecular motion in the chitin films, the glass transition peaks in tangent δ (tan δ) are shown in Figure 7(b). There was no peak for pure chitin films (CF0) in the temperature range from −100 to 100°C, whereas obvious peaks can be observed at −52.7 and −45.3°C for CF1 and CF2, respectively. These relaxation peaks could be attributed to the glass transition relaxation (T_g), corresponding to the cooperative motion of the chitin chains and glycerol molecules. The results further confirmed that the strong hydrogen bonding between chitin and glycerol existed in the chitin films, leading to the increase of the chitin chain mobility.

The chitin films plasticized with glycerol (CF2) exhibited flexible and translucent appearance, as shown in Figure 8(a,b). The engine oil on the chitin film decreased the light scattering and as a result, the chitin film polished oil exhibited good light transparency as shown in Figure 8(b). The stress–strain (σ–ε) curves for the chitin films with different glycerol content are shown in Figure 8(c). In the case of the pure chitin film (CF0), the tensile strength (σ_b) was 89.3 MPa and elongation at break (ε_b) was 7.8%, indicating that CF0 was relatively rigid and brittle. The chitin films plasticized with glycerol had lower σ_b value but higher ε_b value, compared with the pure chitin film, indicating that the improvement of the flexibility. The results

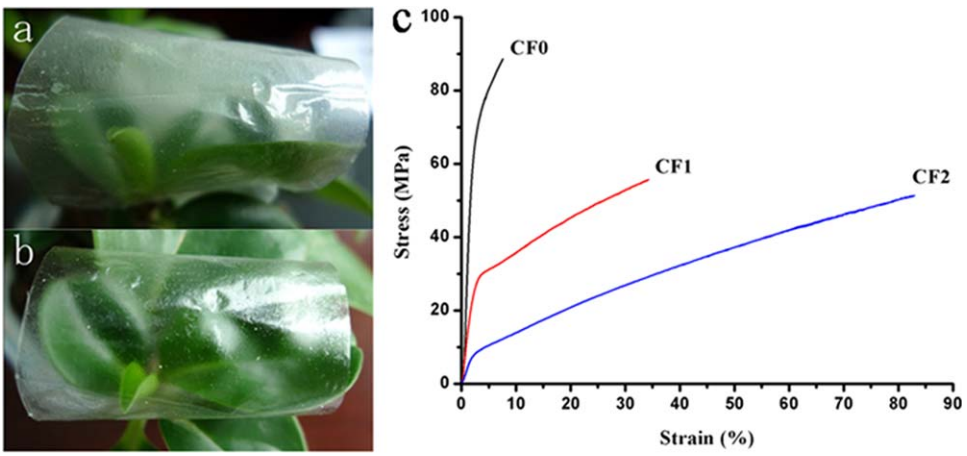

Figure 8. Photograph of original (a) pure chitin film, (b) pure chitin film polished with engine oil. (c) Stress–strain curves of chitin films: CF0, CF1, CF2. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

revealed that the mechanical behaviors of the chitin films strongly depended on the introduction of glycerol. The ε_b values increased from 7.8 to 83.1%, whereas the σ_b values of the chitin films decreased from 89.3 to 51.7 MPa with the increasing glycerol content from 0 to 20.8%. The results indicated the flexibility of chitin films obviously improved when glycerol was incorporated into chitin matrices as plasticizer.

CONCLUSION

Chitin films were successfully prepared through dissolving chitin solution in 8 wt % NaOH/4 wt % urea aqueous system at low temperature, and then by the physical crosslinking and entanglement of the chitin chains via heating way. The chitin inclusion complex associated with NaOH, urea and water in the solution was broken by heating, leading to the aggregation of chitin chains to form the gel sheets, resulting in the regeneration to give pure chitin film. Glycerol was used as a plasticizer to be added into the pure chitin film to improve its flexibility. The homogeneous structure, relatively high mechanical properties and good miscibility of the plasticized chitin films were as a result of the strong hydrogen bonding interaction between chitin and glycerol. The regeneration of chitin solution by the heating method without any organic or inorganic coagulants was a "green" pathway.

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