Biodegradable Polylactide/Chitosan Blend Membranes

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Received January 26, 2006

Biodegradable blend membranes based on polylactide and chitosan with various compositions were prepared via a two-step processing pathway. In the first step, solutions of each component were properly mixed and cast into a gelatinous membrane, and in the second step, the obtained membrane was immersed into a mixed solution for the solvent extraction followed by a drying procedure to finally generate a well-blended membrane. An acetic acid−acetone solvent system was selected for poly(DL-lactide)/chitosan membranes, and another solvent system for poly(L-lactide)/chitosan membranes consisted of acetic acid and dimethyl sulfoxide. Some processing parameters, such as the concentration of component solutions and the composition ratio of mixed solvents and extraction solvents, were optimized by primarily considering whether the directly visible phase separation occurred during the processing procedures. Morphologies of these blend membranes were viewed using SEM. It was found that the processing parameters exerted quite notable impacts on the morphology of the membranes. The hydrophilicity of membranes was examined by measuring their water contact angle and swelling index. These blend membranes were also investigated for their miscibility using IR spectra, X-ray diffractograms, TG, DSC, and dynamic mechanical analysis methods. Although the presence of phase separation at a microscopic level was detected for these membranes, pronounced interactions between components were confirmed. The obtained results shown that some membranes prepared under optimized processing conditions had a partially miscible structure.

Introduction

Lactic acid-based polymers, termed polylactides, have been extensively investigated for many years for various biomedical purposes due to their biocompatibility, biodegradability, and absorbability. Their main applications include surgical devices, artificial organs, drug delivery systems with various routes of administration, carriers of immobilized enzymes and cells, biosensors, ocular inserts, materials for orthopedics and biodegradable packaging,[1−3] and, in particular, temporary implants for fixations and supports in tissue regeneration,[4−6] which are commonly known as tissue engineering scaffolds.

Polylactides are commonly divided into two categories: poly(L-lactide) (PLLA) and poly(DL-lactide) (PDLLA), which are produced from L-lactide and DL-lactide, respectively. PLLA is a chiral polyester and usually found to be semicrystalline.[7] Its crystallinity, glass transition temperature (T_g), and melting point can alter within a certain range depending on the polymerization routes and the molecular weight of the resultant products. PDLLA is a sort of amorphous polyester with a T_g varied from around 50 to 60 °C and a wide range of melting temperatures, generally depending on its molecular weight. Since PLLA and PDLLA both show a relatively poor mechanical characteristic, mainly involving their brittleness at room temperature,[8,9] they have been selectively blended with other biodegradable aliphatic polyesters or copolyesters to obtain desirable mechanical properties.[10−12] In addition to their mechanical concerns, several other disadvantages have also been realized when polylactides are employed for porous tissue engineering scaffolds:[13−16] (1) hydrophobicity, hindering the cells from penetrating into the pores of scaffolds; (2) absence of cell recognition sites on the surface of the scaffolds, leading to poor cell affinity and adhesion; and (3) acidic degradation products (lactic acid and acidic oligomers), causing a decline of local and systemic pH and resulting in some side effects such as a inflammatory response.

Chitosan is a naturally occurred biopolymer composed of β-1,4-linked 2-acetamino-2-deoxy-D-glucopyranose and 2-amino-2-deoxy-D-glucopyranose. It is generally obtained by alkaline deacetylation of chitin, which is the main component of the exoskeleton of crustaceans and the second most abundant biopolymer in nature after cellulose.[17] Chitosan has received a great deal of attention for decades in medical and pharmaceutical applications because of its nontoxic, nonantigenic, biocompatible, and biodegradable properties, which allow its use in various medical areas such as topic ocular application,[18] implantation,[19] or injection.[20]

Chitosan has amino groups and hydroxyl groups on its backbone, which on one hand makes chitosan itself hydrophilic and on the other hand brings chitosan a polycationic property. Several studies have indicated that chitosan is also bioadhesive[21,22] and has excellent nerve cell affinity.[23] With the above-mentioned problems described in the case of polylactide, it is easy to conclude that chitosan can effectively provide its merits to offset the demerits of polylactides if these two kinds of biopolymers are successfully blended into a new one. Apparently, the expected polylactide/chitosan blends would possess

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10.1021/bm0600825 CCC: $33.50
Published on Web 03/25/2006

hydrophilic and bioadhesive properties, and an important new advantage should be that the chitosan component can effectively buffer the acidic degradation products of polylactides because of the basic characteristic of chitosan. However, it is found that blending chitosan with polylactide at a highly miscible level is very difficult to achieve for two main reasons: (1) Chitosan has a high T_g[24] and will probably start to decompose before melting; thus, it is impossible to blend chitosan with other polymers by the melting processing technique though PDLLA and PLLA are both thermoplastic. (2) So far, to our knowledge, there are no shared common solvents reported for chitosan, PDLLA, or PLLA. Chitosan can only be dissolved in very few kinds of dilute aqueous acidic solutions because of its specific structure, but PDLLA and PLLA can normally be dissolved in some organic solvents. Nevertheless, several efforts have still been made to modify polylactides using chitosan. Li et al. directly pressed PDLLA together with dry chitosan powder to produce porous PDLLA/chitosan blend membranes with a melting molding method at 160 °C, while using salt as a porogen.[25] Obviously, the desirable miscibility between two components for their blend membranes cannot be expected, although there were no corresponding data provided. Suyatma et al. reported blend films made from chitosan and PLLA using 1% aqueous acetic acid solution as solvent for the former and chloroform for the latter, respectively.[26] Those films were prepared via a solution-casting method and directly dried in air, and consequently, there was no miscibility between the two components. In those cases, since chloroform has poor solubility in water and will also evaporate much more rapidly than water from the casting membranes during the dry procedure, inevitably the membranes obtained will contain separated phases. Very recently, Chen et al. investigated PLLA/chitosan composite particles. These particles were obtained by using acetic acid and dimethyl sulfoxide (DMSO) as solvents and directly precipitating in acetone.[27] The thermal properties of the particles were studied, but the T_g and other thermal characteristics regarding the chitosan component were not detected. In addition, some details for the preparation of samples were not released in their report.

On the basis of the facts mentioned above, it is obviously necessary to obtain a better understanding for these kinds of polylactide/chitosan composites or blends. We have prepared a series of PDLLA/chitosan and PLLA/chitosan blend membranes using a two-step method. In the first step, an appropriate solvent system is selected in order to ensure that the two components are effectively blended together and the resulting mixtures can be easily shaped into membranes without the occurrence of visible separate phases. In the second step, the obtained membranes are introduced into other solvent systems to extract the original solvents inside the membranes, to potentially avoid further possible phase separation during the dry procedures of the membranes. By optimizing the processing parameters, some well-blended membranes were obtained. Although some studies on polylactide/chitosan blends have been done, to the best of our knowledge, this two-step method is a new one for preparing polylactide/chitosan blend membranes, especially considering the second step. Some results regarding the structure and morphology as well as the related properties of these blend membranes are presented here.

Experimental Section

Materials. Chitosan was received in the form of flake from Fluka. The first derivative UV spectroscopy of chitosan was recorded on a spectrophotometer (VARIAN Cary 300 Bio UV−visible) and used for calculating the degree of deacetylation (DDA) of chitosan.[28] A calibration curve from *N*-acetyl-D-glucosamine was generated using the method of Tan et al.[29] The water content of chitosan samples was analyzed via thermogravimetric measurements, and it was considered the largest contributing factor to the error in the DDA calculation. The viscosity-average molecular weight of chitosan was determined using 0.25 M CH_3COOH/0.25 M CH_3COONa as a solvent system according to our previously reported method.[30] The DDA value and viscosity-average molecular weight of chitosan were 86.3 (± 2.7) % and 1.71 (± 0.19) × 10^6, respectively.

PDLLA and PLLA were supplied by Birmingham Polymers Inc. (U.S.A.). Their viscosity-average molecular weight was measured in a Ubbelohde-type viscometer in chloroform at 30 °C and calculated using the following different relations

$$\text{For PDLLA:}^{31} \quad [\eta] = 2.21 \times 10^{-4} M_v^{0.77} \text{ (dL/g)} \quad (1)$$

$$\text{For PLLA:}^{32} \quad [\eta] = 5.45 \times 10^{-4} M_v^{0.73} \text{ (dL/g)} \quad (2)$$

M_v values for PDLLA and PLLA were obtained as 1.13 (± 0.17) × 10^6 g/mol and 1.52 (± 0.24) × 10^5 g/mol, respectively.

Other chemicals (acetic acid, acetone, dimethyl sulfoxide (DMSO), ethanol, sodium acetate, *N*-acetyl-D-glucosamine, sodium hydroxide, and chloroform) were all obtained from Aldrich and used as reagent grades.

Preparation of Blend Membranes. Chitosan solution was prepared by dissolving chitosan in 1.0% (v/v) acetic acid aqueous solution with a 1.0 wt % chitosan concentration. To this solution, a certain amount of acetone or DMSO was introduced to produce different mixtures. In a typical experiment for preparing PDLLA/chitosan membrane, 0.5 g of chitosan was first dissolved into 50 mL of 1.0% (v/v) acetic acid aqueous solution, and then, a certain amount of acetone was slowly added with vigorous stirring for 2 h, followed by adding 2% (w/v) PDLLA solution in acetone drop by drop with vigorous stirring for additional 2 h. The composition ratio between chitosan and PDLLA was adjusted by changing the amount of PDLLA solution. Subsequently, this newly obtained gelatinous mixture was degassed using a centrifuge (Sorvall Rc 5C Plus) and cast into a membrane on a Petri dish. The membrane with dish was then immersed into NaOH−CH_3CH_2OH aqueous solution (NaOH was dissolved in 80% ethanol aqueous solution with an ~0.5 wt % NaOH concentration) for 24 h. Finally, this solidlike membrane was exhaustively washed with distilled water until neutrality was achieved and dried slowly in air at room temperature for about 5 days.

In the case of PLLA/chitosan membrane, a similar method was employed, but a few changes were made. DMSO was first introduced into 1.0 wt % chitosan solution in 1.0% (v/v) acetic acid to produce a concentrated solution, and then, 2% (w/v) PLLA solution in DMSO was added dropwise at a very slowly rate with vigorous stirring. The composition ratio of the mixture was controlled via varying the amount of PLLA solution. Afterward, the steps were kept the same as those performed in the first case until a dry membrane was received.

Two sets of samples were prepared. One set was named PDLLA/ch-25, PDLLA/ch-50, and PDLLA/ch-75, and another set was coded PLLA/ch-25, PLLA/ch-50, and PLLA/ch-75; the number in both sets following ch denotes the weight fraction of chitosan in the membrane.

Membrane Characterization. Small membrane samples were coated with gold−palladium, and the cross-sectional areas of the samples were viewed for their morphologies using scanning electron microscope (SEM, Philips, XL-30).

IR spectra were collected on a Nicolet 510P FTIR spectrometer in transmission mode at 2 cm^{-1} intervals and 64× scanning. A piece of membrane sample was cut into very small pieces, and these pieces were ground further into powder as small as possible. Powderlike samples were mixed with KBr, and the resulting mixture was pressed into disks (0.5 mm in thickness).

Thermogravimetric (TG) analysis was performed on TGA 2050 (TA Instruments). Each membrane sample (ca. 10 mg) was run from 25 to 400 °C at a scanning rate of 10 °C/min under a nitrogen atmosphere.

Differential scanning calorimetry (DSC) measurements were carried out on DSC 2010 (TA Instruments). DSC curves were obtained by heating samples from room temperature to the required high temperature at a heating rate of 10 °C/min under a nitrogen atmosphere.

DMA (dynamic mechanical analysis) measurements were conducted to observe T_g of the blend membranes. The storage modulus E', loss modulus E'', and mechanical damping tangent (tan δ) of the blend membranes were recorded on a dynamic mechanical analyzer (DMA 2980, TA Instruments) from 30 to 250 °C at a frequency of 1 Hz and a heating rate of 2 °C/min in an atmosphere of 150 mL/min nitrogen.

A SCINTAG X1 X-ray diffractometer was used to inspect the diffractograms of the blend membranes at 23 °C. The X-ray source was Ni-filtered Cu Kα radiation (45 kV and 40 mA). The dry membranes were mounted on aluminum frames and scanned at a speed of 2°/min from 5° to 40° (2θ). To measure the relative crystallinity percentage (X_c) of the membranes, the amorphous areas and the areas of the crystalline peaks were measured, and X_c was calculated from the diffractograms of the membranes with the following relationship:[33]

$$X_c = [A_c/(A_c + A_a)] \times 100\% \quad (3)$$

where A_c and A_a are the areas of the crystalline and amorphous regions, respectively. Four specimens were measured for each sample.

Contact angle measurements were performed at room temperature by the sessile drop method using a goniometer equipped with a high-speed camera (CAM 200, KSV Instruments). A 5-μL water droplet was initially placed on the surface of a membrane sample, and the advancing or receding contact angle of water was then measured by increasing or decreasing the drop size of water, respectively, until the three-phase boundary line moved. The test liquid less than 2 μL was added into the original droplet or withdrawn from it each time using a microsyringe with a flat-tipped needle at a normal rate of 0.5 μL/s. The values reported were an average of at least four measurements on different surface regions of the membranes.

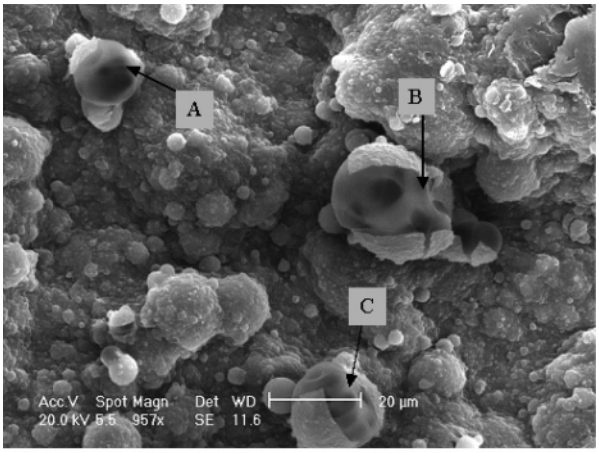
Dry membranes were immersed in an excess amount of deionized water at ambient temperature for 2 h. The mass of the wet membrane was obtained after the gentle removal of surface water with blotting paper. The swelling index (SI) was calculated on the basis of the following formula:

$$\mathrm{SI} = [(W_s - W_d)/W_d] \times 100\% \quad (4)$$

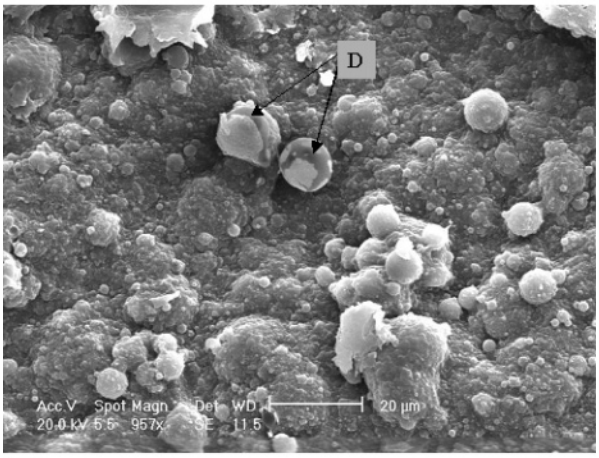
where W_d and W_s are the masses of dry and swollen samples measured at different times, respectively. For each reported value, at least five replicate measurements were averaged.

Results and Discussion

Effect of the Concentration of Component Solutions on the Morphology of Membranes. It is known that chitosan can only be dissolved in a few kinds of dilute acidic aqueous solutions, but PDLLA or PLLA is usually soluble in some organic solvents such as chloroform, hexafluoro-2-propanol, dichloromethane, and the like. In the present study, since the solution-casting method is employed, selecting proper solvent systems should be taken into account first. We have found that directly blending the PDLLA or PLLA solutions with chitosan solutions by randomly selecting respective solvents to prepare a well-blended membrane is generally unsuccessful, because in these cases, PDLLA or PLLA could rapidly precipitate out from the mixtures during the blending procedure or relatively slowly separate out from the resulting membrane during the followed drying procedure. To effectively blend two components



(a)



(b)

Figure 1. SEM micrographs of blend membranes prepared with very thin component solutions (extraction solvent, 80% ethanol containing 0.5 wt % NaOH). (a) PDLLA/ch-50; (b) PLLA/ch-50.

of each pair (PDLLA/chitosan or PLLA/chitosan), properly mixed solvents have been employed by considering the solubility of organic solvents in water, and thus, acetone and DMSO were chosen for PDLLA and PLLA, respectively. It is also worth pointing out that the sequence for processing these membranes is also limited as a one-way procedure, that is, introducing PDLLA or PLLA solutions into the chitosan solutions but not contrary, because the chitosan solution is quite viscous even though its concentration is as low as 1 wt %, and PDLLA or PLLA solution having a concentration higher than 5% (w/v) can still be considered a relatively thin solution.

In principle, it is generally accepted that a well-mixed solution could be achieved if component solutions of greatly reduced concentration are employed. However, in the present cases, it is observed that if two component solutions are too thin the resulting membranes made from the mixed solutions will also have a microscopically phase separated structure, although they usually still show a miscible appearance. This observation is true for both PDLLA/chitosan and PLLA/chitosan membranes. SEM images for the two kinds of membranes obtained by using very thin component solutions are presented in Figure 1. In Figure 1a, the mixed solvent consisted of 1% aqueous acetic acid solution and acetone with a volume composition ratio of 1:0.5 and the concentration of chitosan solution in the mixed solvent was 0.25 wt %; the concentration of PDLLA solution in acetone was 0.5% (w/v). Correspondingly, in Figure 1b, the mixed solvent was composed of 1% aqueous acetic acid solution

and DMSO with a volume composition ratio of 1:0.5, and the concentration of chitosan solution in the mixed solvent and the concentration of PLLA solution in DMSO were 0.25 wt % and 0.5% (w/v), respectively. It can be seen in Figure 1 that two types of blend membranes possess a clear two-phase structure, i.e., a great number of dispersed small balls are embedded into a continuous matrix. Further examinations conducted by comparing these micrographs with the SEM images of the controls (pure chitosan, PDLLA, and PLLA membranes obtained using corresponding mixed solvents and the same processing methods; images have not been shown), indicate that the continuous matrixes should be ascribed to the chitosan, and those dispersive balls are composed of PDLLA (Figure 1a) or PLLA (Figure 1b). An interesting observation is that some small balls with a relatively large diameter are hollow inside. Using strong electron beams (corresponding to a very high magnification) under SEM to melt these balls, their inside hollow structures can be clearly viewed (see those balls indicated by A, B, C in Figure 1a and the balls denoted D in Figure 1b). Since 0.25 wt % chitosan solution was still markedly viscous due to the addition of acetone or DMSO, once PDLLA or PLLA solution was introduced via blending the component solutions, the introduced PDLLA or PLLA probably initially stayed inside the many small liquid balls formed and dispersed by stirring forces; and thus, generated gelatinous mixtures could maintain a structure like an oil-in-water emulsion system because of the very low viscosity of PDLLA or PLLA solution. After that, the diffusion of PDLLA or PLLA molecules from the inside of these small liquid balls into the chitosan phase could become very slow or even finally stopped, because the PDLLA or PLLA solution was too thin, and viscous forces between balls and chitosan solution were not strong enough to break these balls into smaller ones; and meanwhile, the surface or interface tensions of the balls also hindered PDLLA or PLLA molecules from freely diffusing into the chitosan phase. During the period of solvent extraction at the later stage of preparation, once the solvents (acetone or DMSO) inside the balls were almost entirely extracted out, many smaller balls could shrink into solid ones, but those relatively large balls might act in a quite different behavior. PDLLA or PLLA molecules inside a bigger ball could move toward to the surface of the ball while this ball was shrinking, and as a result, the gathered PDLLA or PLLA molecules could form a shell due to the diffusion forces of solvents, leaving a hollow ball inside the blend membranes after the membranes was completely dried. To obtain well-blended membranes, the concentrations of component solutions, therefore, have been optimized at 1.0 wt % for chitosan and 2.0% (w/v) for PDLLA or PLLA for all of the following samples.

Effect of Mixed Solvent on the Morphology of Membranes. The composition ratio of mixed solvents is another factor which can significantly influence the morphology of blend membranes. In many cases, if an inappropriate composition ratio is applied, directly visible phase-separation phenomena will be immediately observed. By keeping the concentration and volume of the chitosan solution constant and varying the amount of acetone or DMSO, the effect of the composition ratio of mixed solvents on the morphology of blend membranes were inspected, and results are given in Table 1. The indication of phase separation, which is referred to by "+", means that white solid precipitate inside the solidlike membranes (well-blended gelatinous mixtures containing PDLLA or PLLA solutions are colorless) was observed during the preparation procedure before the membranes were dried. Otherwise, using a "−" sign indicates that there was not any directly visible phase separation

Table 1. Composition Ratio of Mixed Solvents and Its Effect on the Morphology of Membranes[a]

	composition ratio (I) (v/v)[b]					
sample	1:0.4	1:0.6	1:0.8	1:1	1:1.2	1:1.4
PDLLA/ch-25	+	+	−	−	+	+
PDLLA/ch-50	+	+	−	−	−	+
PDLLA/ch-75	+	+	−	−	−	+
	composition ratio (II)(v/v)[c]					
sample	1:0.4	1:0.6	1:0.8	1:1	1:1.2	1:1.4
PLLA/ch-25	+	+	−	−	+	+
PLLA/ch-50	+	+	−	−	+	+
PLLA/ch-75	+	−	−	−	+	+

[a] Extraction solution consisted of 80% ethanol aqueous solution containing 0.5 wt % NaOH; extraction time was 3 days. [b] Composition ratio (in volume) of aqueous acetic acid solution to acetone. [c] Composition ratio (in volume) of aqueous acetic acid solution to DMSO.

observed for the relevant membranes. It is clear in Table 1 that, if the amount of acetone or DMSO used for the mixed solvents is too small or large beyond a limit, the phase separation could take place at the early stage during which the membranes are being prepared. Although both acetone and DMSO are soluble in water, chitosan solutions have limited tolerance for them, because they are not good solvents for chitosan. Adding acetone or DMSO into a chitosan solution will, on one hand, dilute the content of acetic acid and, on the other hand, force the available water to compete with chitosan, which will finally create a more concentrated and gelatinous mixture. After introducing PDLLA or PLLA solution into such an obtained mixture and if the total accumulated amount of acetone or DMSO is too large for the tolerances of the chitosan solutions, PDLLA or PLLA molecules will stay inside the acetone phase (for the former) or the DMSO phase (for the latter), and phase separation inside the resulting membranes will become inevitable. The results listed in Table 1 suggest that the composition ratios of the mixed solvents should be selected between 1:0.8 and 1:1.2 for PDLLA/chitosan mixtures and between 1:0.8 and 1:1.1 for PLLA/chitosan mixtures. The value of 1:1 seems to be an appropriate one for both.

Effect of Extraction Solution on the Morphology of Membranes. Apart from the effects mentioned above, the dry procedures also critically impose a strong impact on the morphology of membranes. Directly drying the gelatinous membranes obtained using the above optimized processing conditions in air will still result in the appearance of phase separation inside the membranes because of far different evaporation speeds of each component of the mixed solvents. A solvent-extracting step has been demonstrated to be greatly essential for the preparation of the membranes in the present cases. Since the chitosan solutions contained only 1.0% (v/v) acetic acid, the concentration of NaOH for the extraction solutions was selected as 0.5 wt %, which was strong enough to neutralize the obtained gelatinous membranes. In addition, it was found that the time spent on extracting the gelatinous membranes also obviously affected the morphology of the resulting membranes. All gelatinous membranes, therefore, were immersed into the extraction solutions for at least 48 h to almost completely extract the organic solvents inside, followed by exhaustive washes with distilled water. By doing this, the effect of extraction time could be virtually ignorable. Under the above-mentioned processing conditions, the effect of the ethanol concentration on the morphology of the blend membranes was mainly investigated, and the results are listed in Table 2. It is noted that directly visible phase separation could occur for both

Table 2. Effect of Ethanol Concentration on the Morphology of Membranes[a]

sample	95%	90%	80%	70%	60%
PDLLA/ch-25	+	−	−	−	+
PDLLA/ch-50	+	−	−	+	+
PDLLA/ch-75	+	−	−	+	+
PLLA/ch-25	+	−	−	−	+
PLLA/ch-50	+	−	−	−	+
PLLA/ch-75	+	−	−	−	+

[a] Composition ratio of mixed solvents for both types of membranes was 1:1. Extraction solution contained 0.5 wt % NaOH; extraction time was 3 days. "+" refers to the appearance of directly visible phase separation. "−" denotes that there is not any directly visible phase separation.

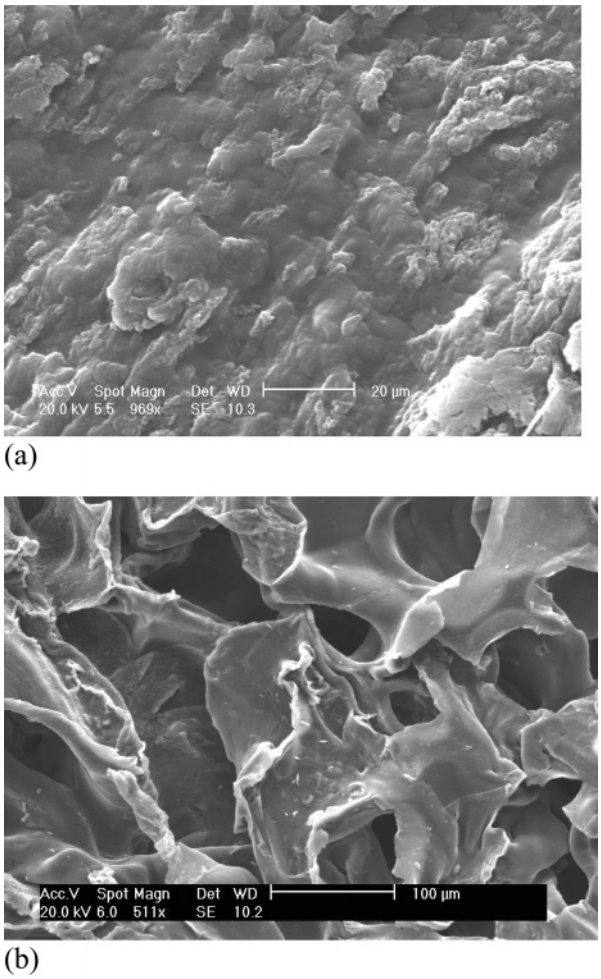


Figure 2. SEM micrographs of PDLLA/ch-50 membrane in the cross-sectional areas (composition ratio (v/v) of aqueous acetic acid solution to acetone, 1:1; extraction solvent, 80% ethanol containing 0.5 wt % NaOH). (a) Before etching; (b) after etching.

kinds of blend membranes if the concentration of ethanol is higher than 90%, and moreover, the phase separation could appear again, provided that the concentration of ethanol is lower than 80% for PDLLA/chitosan membranes or lower than 70% for PLLA/chitosan membranes. Apparently, extraction procedures in the present cases have involved so many intricate diffusion mechanisms and tangled diffusion dynamics of different solvents that it is too difficult to clarify the details for these diffusion processes at a microscopic level due to the tremendous complication of the present multiphase system. However, the overall results in Table 2 reveal that a proper concentration of ethanol used for the extraction solution should be controlled within 80−90% for both types of membranes.

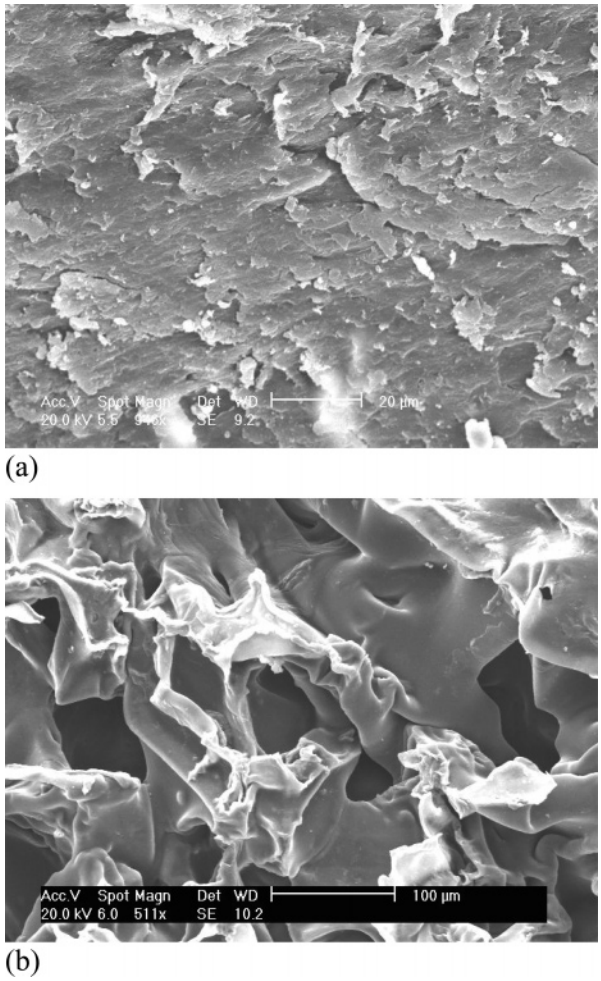


Figure 3. SEM micrographs of PLLA/ch-50 membrane in the cross-sectional areas (composition ratio (v/v) of aqueous acetic acid solution to DMSO, 1:1; extraction solvent, 80% ethanol containing 0.5 wt % NaOH). (a) Before etching; (b) after etching.

Internal Morphology of the Blend Membrane Obtained under the Optimized Processing Conditions. Several blend membranes were first prepared under the optimized processing conditions, and their PDLLA or PLLA component was then removed using an etching method for the observation of their internal structure and morphology. The etching processing involved two steps. In the first step, small membrane samples were etched inside chloroform for 2 days at ambient temperature with stirring, and in the second step, these membrane samples were further extracted in a Soxhlet extractor by use of chloroform for 24 h, followed by drying to constant weight in a vacuum. The cross-sectional areas of the membranes were viewed using SEM, and two representative SEM micrographs are represented in Figures 2 and 3. Peesan et al. employed a similar method to investigate the phase morphology of hexanoyl chitosan/ploylactide blend film;[34] clear phase separation was evidenced by the presence of numerous unconnected voids inside their blend films. In our cases, as shown in Figures 2 and 3, there is no phase separation observed for both types of membranes before the etching, and many irregular and interlocked pores with pore sizes of less than 100 μm appear for both kinds of membranes after PDLLA or PLLA is removed, revealing that the PDLLA or PLLA component has been intertwined tightly with the chitosan component; and so, the obtained membranes display a well-blended internal microstructure. Hence, all of the following blend membranes will be

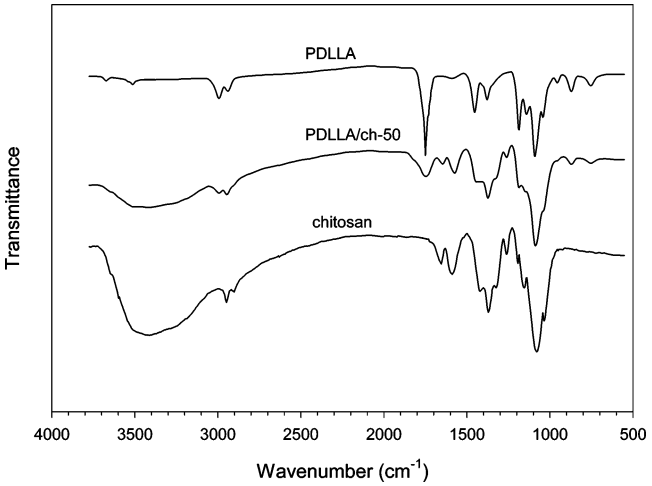


Figure 4. IR spectra of PDLLA, chitosan, and PDLLA/ch-50 membranes.

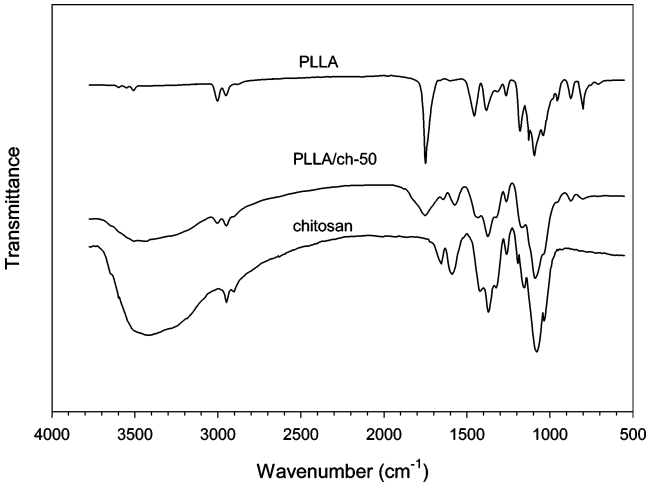


Figure 5. IR spectra of PLLA, chitosan, and PLLA/ch-50 membranes.

prepared under optimized conditions for further investigations unless otherwise stated.

IR Analysis. IR spectroscopy is a common technique for investigating the intermolecular and intramolecular interactions in polymers. Figures 4 and 5 present IR spectra of PDLLA, PLLA, chitosan, and two representative blend membranes: PDLLA/ch-50 and PLLA/ch-50. Several characteristic bands of PDLLA are located at 750 and 865 cm^{-1} (CH bend); 1042, 1090, 1138, and 1186 cm^{-1} (═C—O stretch); 1375 cm^{-1} (CH_2 wag); 1452 cm^{-1} (CH_3 bend); 1751 cm^{-1} (C═O stretch, ester group); 2941 cm^{-1} (CH stretch); 2993 (CH_3 stretch); 3510 and 3664 cm^{-1} (OH stretch, end group). When compared to PDLLA, PLLA shows a quite similar IR spectrum but exhibits a few differences in the following characteristic bands at 798 cm^{-1} (CH bend, crystalline-sensitive band), 1267 cm^{-1} (═C—O stretch), and 3504−3631 cm^{-1} (OH stretch, end group). The main bands in the IR spectrum of chitosan can be seen as follows: a broad and strong overlapped band at around 3400 cm^{-1} (OH and NH stretch); two weak bands at 2913 and 2859 cm^{-1} (CH stretch); two middle strong bands at 1655 and 1589 cm^{-1} (amide I and amide II); 1380, 1326, and 1255 cm^{-1} (deformation of C—CH_3 and amide III); 1153, 1071, and 1029 cm^{-1} (saccharide structure).[35] To more clearly observe the shifts of the bands, several corresponding IR spectra with higher resolution are provided in Figures 6 and 7.

It can be observed that in Figures 4 and 6 several noticeable changes occur in the spectrum of PDLLA/ch-50 in

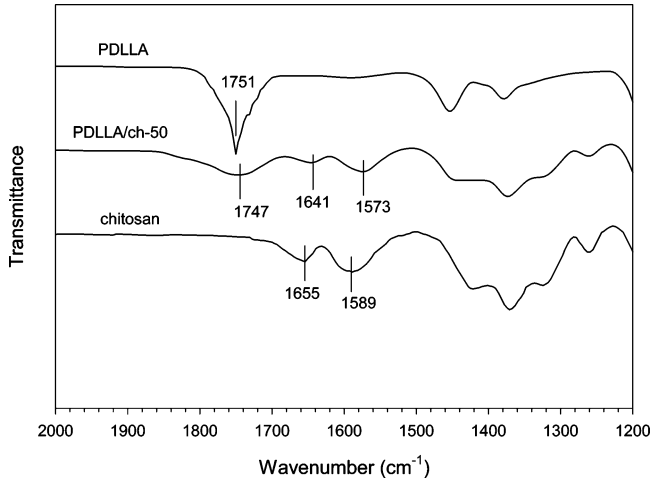


Figure 6. IR spectra of PDLLA, chitosan, and PDLLA/ch-50 membranes with higher resolution.

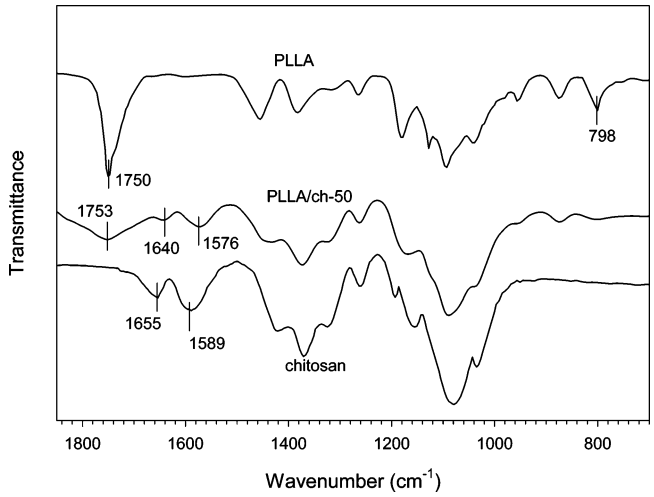


Figure 7. IR spectra of PLLA, chitosan, and PLLA/ch-50 membranes with higher resolution.

comparison with the spectrum of each component. The two original bands of the chitosan component at 1655 and 1589 cm^{-1} for amide I and amide II are shifted to 1641 and 1573 cm^{-1}; a clearly measurable decrease in wavenumber of about 14 cm^{-1} for both peaks is recorded. An original strong band of the PDLLA component at 1751 cm^{-1} for the ester group becomes significantly weaker and is markedly wider. The intensity of the stretching bands overlapped and centered near 3400 cm^{-1} for the hydroxyl and amino groups pronouncedly decreased. Two bands at 750 and 865 cm^{-1} for the CH bend are weakened in intensity by more than half. A band at 1186 cm^{-1} for the carboxyl stretch almost disappears. All these registered events indicate that there are obvious interactions among the amino, carboxyl, and hydroxyl groups of the two components inside the blend membrane. These interactions should be attributed to the hydrogen bonds possibly formed between amino (in chitosan) and carboxyl groups (in PDLLA) or hydroxyl (mainly in chitosan) and carboxyl groups, because there is no covalent interaction between PDLLA and chitosan chains.

Similar results are found in the case of the PLLA/ch-50 membrane in Figures 5 and 7. In particular, in the spectrum of PLLA/ch-50, an original crystalline-sensitive band at 798 cm^{-1} in the spectrum of the PLLA component almost disappears, further revealing that the crystal structures of the PLLA component have been evidently damaged and implying that a

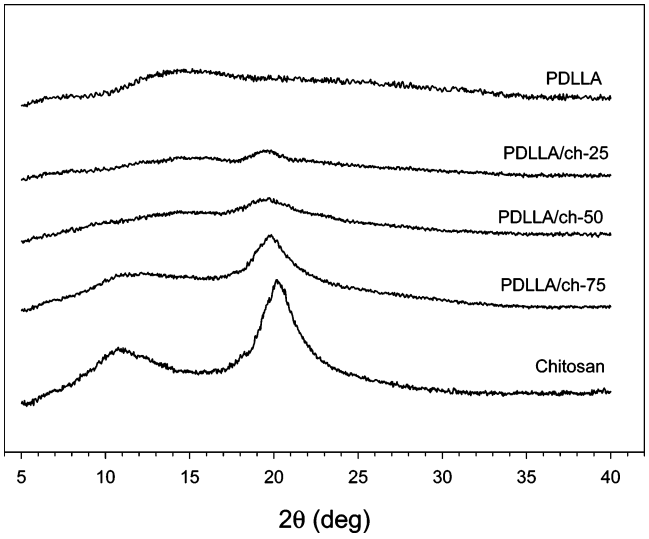


Figure 8. X-ray diffraction patterns of PDLLA, chitosan, and PDLLA/chitosan membranes.

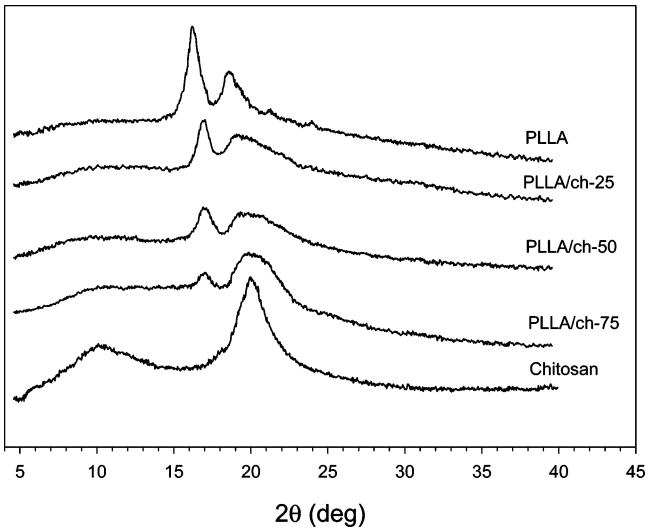


Figure 9. X-ray diffraction patterns of PLLA, chitosan, and PLLA/chitosan membranes.

great number of chitosan molecules have probably been entangled with PLLA at a molecular level.

Crystalline Property of Blend Membranes. Since both chitosan and PLLA are semicrystalline polymers, their crystalline property will certainly affect the structure and property of blend membranes. Figures 8 and 9 illustrate the diffraction patterns of the membranes, whereas the corresponding readable diffraction angle (2θ) and calculated crystallinity are listed in Table 3. The X-ray pattern of the pure chitosan membrane shows two characteristic peaks located at 2θ: about 10.2° and 20.2°. It is known that chitosan always contains bound water (~5%) even it has been extremely dried. The incorporation of bound water molecules into the crystal lattice, commonly termed hydrated crystals, generally gives rise to a more dominated polymorph which can be normally detected by a broad crystalline peak in the corresponding X-ray pattern, and therefore, the crystalline peak centered at around 10.2° is attributed to the hydrated crystalline structure of chitosan.[36] Another peak registered near 20.2° is reported to be the indication of the relatively regular crystal lattice (110, 040) of chitosan.[37,38] The diffractogram of the pure PLLA membrane consists of two main diffractive peaks at angle 2θ of around 16.2° and 18.5°. The peak recorded at $2\theta = 16.2°$ is recognized as the (200, 110) reflection of the α-form crystals,[39,40] and the other peak appeared at 18.5° corresponds to the (010) reflection.[41] The amorphous property of PDLLA is evidenced by its flat X-ray pattern. For the blend membranes of PDLLA/ch-25 and PDLLA/ch-50, one original characteristic peak of chitosan at about 10.2° totally disappears (see Figure 8), and another original crystalline peak at around 20.2° has been slightly shifted to a smaller diffraction angle at ca. 19.5° with a very low crystallinity of about 6%. In the case of the PDLLA/ch-75 membrane, although two crystalline peaks still remain, its crystallinity has been decreased by nearly half compared to that of pure chitosan membrane. These results reveal that, after the chitosan is effectively blended with PDLLA, the original crystalline structure of chitosan could have been partially destroyed or seriously modified, and as a consequence, the blend membranes obtain a partially miscible structure with more amorphous domains due to the interactions between chitosan and PDLLA.

Table 3. Diffraction Angle and Crystallinity of Membranes

sample	peak I (°)	peak II (°)	X_c (%)
PDLLA	*a*	*a*	*a*
PDLLA/ch-25	*a*	19.5 ± 0.2	5.1 ± 1.6
PDLLA/ch-50	*a*	19.4 ± 0.2	6.2 ± 1.4
PDLLA/ch-75	11.4 ± 0.2	19.6 ± 0.1	11.7 ± 1.9
chitosan	10.2 ± 0.1	20.4 ± 0.2	22.4 ± 1.3
PLLA	16.2 ± 0.1	18.5 ± 0.2	24.2 ± 1.3
PLLA/ch-25	16.9 ± 0.1	19.3 ± 0.1	14.4 ± 2.1
PLLA/ch-50	17.1 ± 0.2	19.4 ± 0.2	11.6 ± 1.9
PLLA/ch-75	17.0 ± 0.2	19.7 ± 0.2	12.9 ± 2.3

[a] No readable crystalline peak.

Some distinct changes in diffractograms of PLLA/chitosan membranes are also observed. As shown in Figure 9, the original peak of chitosan component at 10.2° almost completely vanishes in all X-ray patterns of the blend membranes. Although two crystalline peaks are recorded for each membrane, a narrow peak presumably matched with PLLA has been shifted to around 17°, and another broad peak registered at about 19.5° differs from the original peaks of the PLLA or chitosan component in both the shape and the position. Furthermore, the crystallinity of all membranes conspicuously decreases by about 10% compared to that of their each component. Apparently, if no intermolecular interactions exist between the two components, each component will form its own crystalline domains, and thus, the diffraction patterns of the blend membranes should be the simple superposition of those of each component. The obtained results signify that two components, chitosan and PLLA, have interacted with each other in a certain manner so that the original crystalline structures of each component have been disturbed or partially damaged to a different extent, leading to various crystalline structures of the blend membranes. Considering the interlocked morphology, notably decreased crystallinity, and IR spectra of blend membranes prepared under the optimized conditions, it can be deduced that these blend membranes have a partially miscible structure.

Thermal Behaviors. Figures 10 and 11 exhibit TG curves of PDLLA, PLLA, chitosan, PDLLA/ch-50, and PLLA/ch-50 membranes. All data for two sets of samples, regarding their T_{onset} (the onset temperature of thermal degradation, defined as a temperature corresponding to a weight loss of 5%), T_{max} (temperature at maximum degradation rate, determined by derivative TG curves), and WL_{max} (weight loss at maximum degradation rate), are summarized in Table 4.

It is generally accepted that, if two components of a blend have a significant difference in their T_{onset} (the same is true of

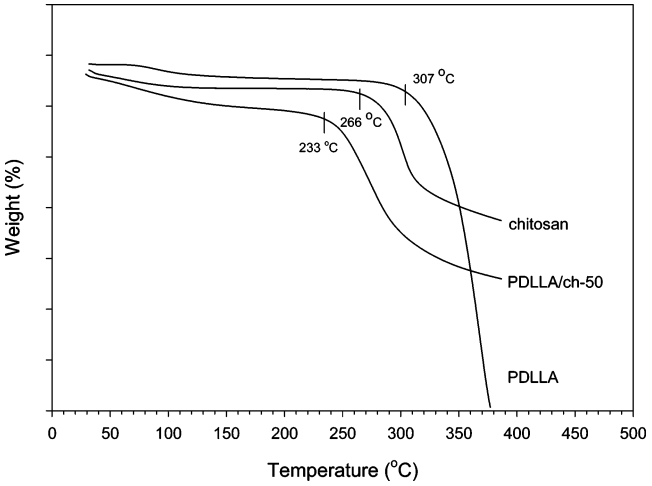


Figure 10. TG thermograms of chitosan, PDLLA, and PDLLA/ch-50 membranes.

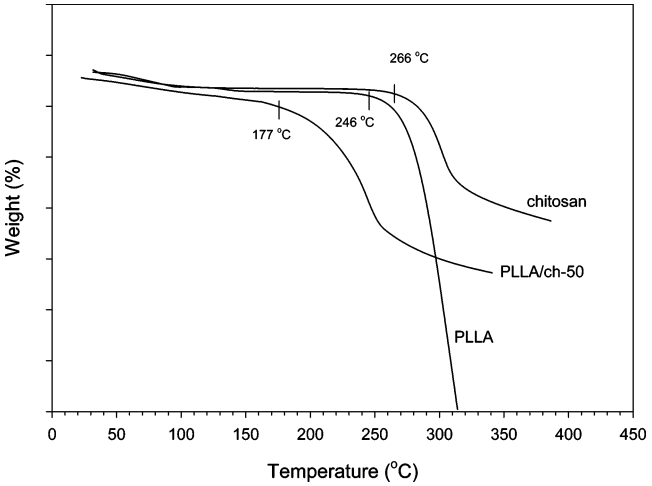


Figure 11. TG thermograms of chitosan, PLLA, and PLLA/ch-50 membranes.

Table 4. Data Collected from TG and DTG Thermograms[a]

sample	T_{onset} (°C)	T_{max} (°C)	WL_{max} (%)
PDLLA	307	335	23
PDLLA/ch-25	259	291	25
PDLLA/ch-50	233	272	29
PDLLA/ch-75	252	286	22
chitosan	266	299	19
PLLA	246	280	22
PLLA/ch-25	189	251	27
PLLA/ch-50	177	229	28
PLLA/ch-75	201	263	26

[a] The values in the table are the average values from four specimens for each sample.

the T_{max} case) and no interaction exists between these two components, the TG thermogram of the blend would show its thermal degradation in two stages matching each T_{onset} of its components, i.e., there would be two T_{onset}'s for the blend. Figures 10 and 11 show that PDLLA/ch-50 and PLLA/ch-50 have only one T_{onset}. In fact, each blend membrane in the two sets of samples has only one T_{onset}, though their TG curves have not all been represented. These results suggest that a considerable amount of interactions may exist between components in each blend membrane, which most probably comes from hydrogen bonds.

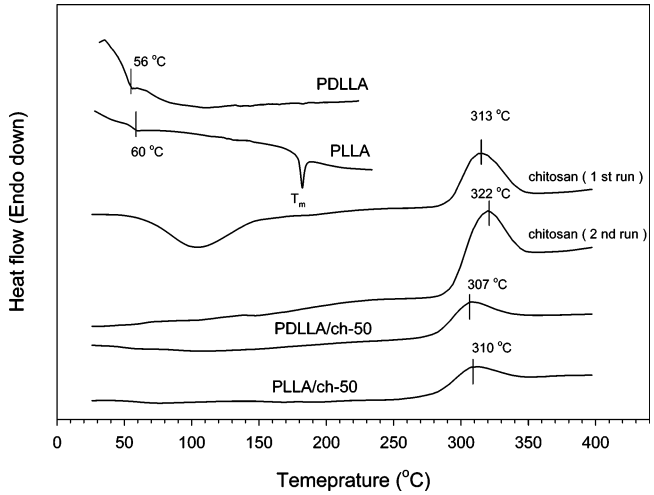


Figure 12. DSC traces of PDLLA, PLLA, chitosan, PDLLA/ch-50, and PLLA/ch-50 membranes.

A general behavior observed from Figures 10 and 11 as well as in Table 4 is that the T_{onset} of each membrane is remarkably lower than that of each of its components. In the case of the PDLLA/ch set, PDLLA is an amorphous component, but chitosan is a crystalline one; the X-ray patterns of PDLLA/ch membranes in Figure 8 indicate that the interactions between the two components have partially destroyed the original crystalline structure of chitosan, which inevitably decreases the strength and thermal stability of the chitosan component, and as a result, a markedly lower T_{onset} is observed for these blend membranes. In the case of the PLLA/ch set, since both PLLA and chitosan are crystalline components, the molecular chains of the two components could be deeply entangled with each other during the solution-processing procedures so that they are not able to efficiently recrystallize again after they are shaped into a membrane and experience a drying process, as evidenced in Figure 9, which definitely will reduce the original strength and thermal stability of each component and, consequently, lead to a much lower T_{onset} for the blend membranes.

One important parameter frequently used to assess whether two polymers are miscible in the amorphous phase is the glass transition temperature T_g. In principle, if two polymers are completely miscible with each other and blended into a new one, only one T_g observed between the T_g values of each component should appear in the DSC thermogram of the resulting blend; if they are partially miscible, the resulting polymer blend would have two T_g's related to each component, but the T_g value corresponding to one component could be affected by the other one, usually depending on the composition ratio. Figure 12 shows several DSC thermograms of PDLLA, PLLA, chitosan, PDLLA/ch-50, and PLLA/ch-50 membranes. The T_g's of PDLLA and PLLA can be clearly read through the baseline steps as ~56 and ~60 °C, respectively. Another peak at around 180 °C recorded in the DSC curve for PLLA can be assigned to its melting point. In the DSC curve of chitosan (first heating run), a wide endothermic peak centered around 106 °C with a large temperature interval is probably attributed to absorbed moisture, and another exothermic peak centered at near 313 °C is possibly linked to the decomposition procedure of chitosan, which starts at around 280 °C and is also basically in good agreement with the TG analysis as shown in Figure 10. However, chitosan does not show any features in its DSC curve to which its T_g can be related.

Numerous studies on the thermal properties of chitosan have suggested that using a quench technique could effectively

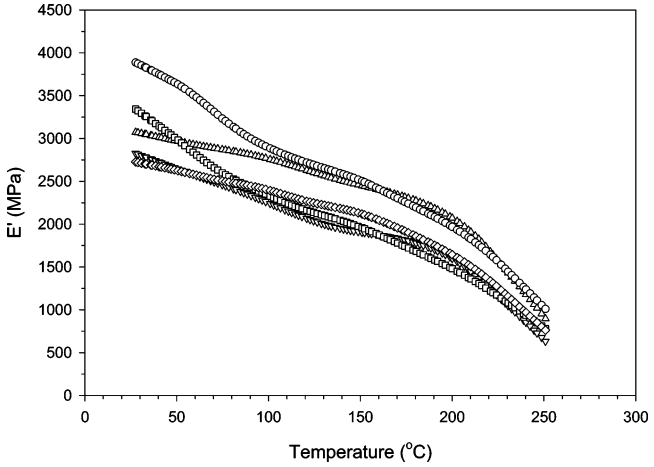


Figure 13. Temperature dependence of storage modulus (E'). ○−PDLLA, □−PLLA, △−chitosan, ◊−PDLLA/ch-50, ▽−PLLA/ch-50.

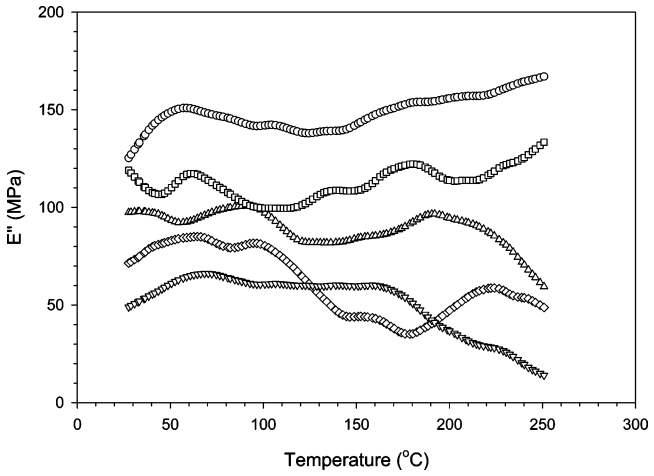


Figure 14. Temperature dependence of loss modulus (E''). ○−PDLLA, □−PLLA, △−chitosan, ◊−PDLLA/ch-50, ▽−PLLA/ch-50.

measure the T_g of chitosan via DSC scanning.[42,43] Chitosan samples, therefore, were heated from 23 °C at a rate of 10 °C/min up to 190 °C and cooled to room temperature at the same rate under nitrogen atmosphere in the first heating run, and then, the samples were reheated again until 400 °C. A thus-obtained DSC curve for chitosan in the second heating run is also presented in Figure 12. Unfortunately, in our case, there is no obviously readable T_g for chitosan in this plot, and the only change detected is that the original endothermic peak entirely disappears. This observation could be ascribed to the structure and properties of chitosan. It is known that chitosan is a semicrystalline polymer due to its strong intramolecular hydrogen bonds on the backbone, and it also has a rigid amorphous phase because of its heterocyclic units; as a result, when it is heated within a certain range of temperature below its decomposition temperature, the variations in heat capacity corresponding to the change in specific volume near T_g is probably too small to be detected by the DSC technique. In fact, by observing two other DSC thermograms for PDLLA/ch-50 and PLLD/ch-50 shown in Figure 12, difficulties can be encountered again in finding out new T_g's for these two blend membranes. A more sensitive method, the DMA technique, is therefore employed to further investigate the miscibility of the membranes.

Dynamic Mechanical Properties. Dynamic mechanical analysis is a sensitive technique to investigate relaxation processes in relation to the molecular motions associated with the internal changes that occur in a polymer.[44] Figure 13 displays

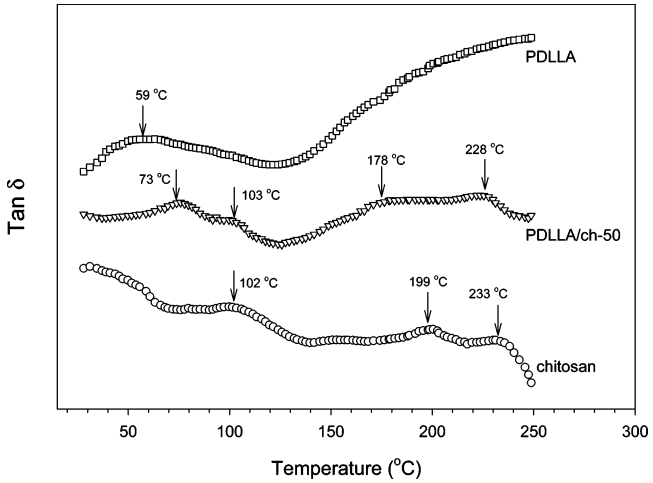


Figure 15. Tan δ curves for PDLLA, chitosan, and PDLLA/ch-50 membranes.

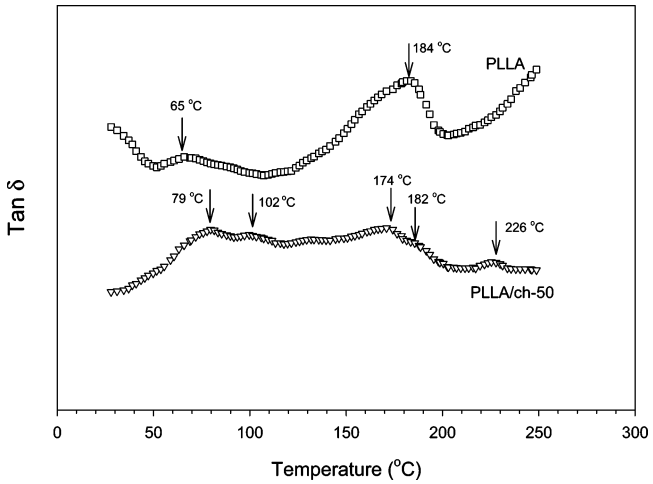


Figure 16. Tan δ curves for PLLA and PLLA/ch-50 membranes.

the storage modulus (E') as a function of temperature for PDLLA, PLLA, chitosan, PDLLA/ch-50, and PLLA/ch-50 membranes, and Figure 14 illustrates the temperature dependence of the loss modulus (E'') for these membranes. Their corresponding thermograms for damping tangent (tan δ) are presented in Figures 15 and 16. Although the storage modulus of each membrane does not register any notably readable peaks in the each plot, it shows significant changes over the temperature domain, indicating a progressive loss at the elastic property of the membranes. Some peaks are registered in the plots of E'' or tan δ, as shown in Figures 14−16. In principle, both E'' and tan δ can be used to measure the characteristic temperatures matched with various relaxation processes, even though a very small quantitative difference may be frequently found between the temperature values determined by E'' or tan δ.[45−47] Since the peaks recorded in the plots of tan δ seem to be more clearly and easily located, in the present case, tan δ will be primarily used. In Figure 15, the plot of PDLLA shows one relaxation peak at around 59 °C, which should correspond to an increase in the free volume of the sample with the temperature and is possibly ascribed to its T_g according to the DSC curve displayed in Figure 12. Three peaks registered at around 102, 199, and 232 °C are observed for chitosan. Several reports have suggested that the T_g of chitosan is higher than 170 °C;[42,48,49] hence, the peak at about 199 °C should be ascribed to the α-relaxation of chitosan, corresponding to its T_g. Another tan δ maximum at 102 °C (β-relaxation) may be caused by partial acetamide groups

attached to the C-2 position in the chitosan backbone.[50] Boyal et al. suggested that a possible kind of transition, named the liquid−liquid transition, in a polymer which is being exposed to a thermal environment at a temperature higher than its T_g, corresponds to the motion of very long chain segments or even the whole chain from imaginary liquid to the real liquid.[51] As stated previously, chitosan has a T_{onset} at around 260 °C based on the observations from its TG thermogram; the peak appearing near 232 °C in its tan δ curve cannot be assigned to any other kind of transition; it thus may correspond to a sort of possible liquid−liquid transition mentioned above. In Figure 16, two relaxation peaks for PLLA at about 65 and 184 °C are observed. The first one at the lower temperature should be considered its T_g, and the second one likely indicates the melting of crystal phrase. This observation is basically in agreement with its DSC analysis.

In the case of the PDLLA/ch-50 membrane, its DMA plot in Figure 15 shows four detectable relaxation peaks recorded at about 73, 103, 178, and 228 °C, respectively. In comparison with the original DMA spectra of its components, it is reasonable to believe that the peaks at 73 °C should correspond to the T_g of the PDLLA component, which is around 14 °C higher than its original T_g, and a peak at 178 °C most probably matches the T_g of the chitosan component, which has shifted toward a lower temperature with a difference of ca. 21 °C compared to its original T_g of 199 °C. Two T_g's for this blend membrane are detected, and the difference between them is large (∼105 °C), suggesting the presence of significant phase separation in the membrane at a microscopic level although this membrane did not exhibit any visible phase separation during the preparation procedure. However, these two T_g's reveal the fact that the spectrum of the PDLLA/ch-50 membrane is not a simple superposition of those of the pure components.

In general, it is known that interactions between polymer chains, such as ionic interaction, hydrogen bond, and cross-linking, can cumulatively contribute to shift the T_g of polymer. It has been reported that, in the case of two-component polymer blends, provided that the two components are not completely miscible but one component is capable of interacting with the other one, the T_g's of the components would shift to the inside of their respective original T_g's.[52,53] The fact that the two T_g's of PDLLA and chitosan shift to the inside suggests that there exist marked interactions between PDLLA and chitosan which probably result from hydrogen bonds formed between chitosan and PDLLA, implying that PDLLA and chitosan are partially miscible.

A similar behavior was also observed in the case of the PLLA/ch-50 blend membrane. The original T_g of the PLLA component at 65 °C has shifted to a higher temperature at about 79 °C, and the original T_g of the chitosan component at 199 °C has been relocated at 178 °C, indicating that these two components are also partially miscible.

All blend membranes were measured using the DMA technique. The data focused on the T_g of each component in the blend membranes are collected and listed in Table 5. It is seen that for each blend membrane two T_g's are recorded, and the difference between the two T_g's is large and alters very slightly. Hence, it can be approximately concluded that the composition ratio of the membrane does not substantially affect the miscibility of membranes.

Hydrophilicity of Blend Membranes. To examine the hydrophilicity of blend membranes, their water contact angles and swelling indexes were measured, and the results are provided in Table 6. It can be seen that both PDLLA and PLLA have an advancing contact angle of >90° and a receding contact angle near 90°, indicating their hydrophobicity. By incorporating chitosan, the contact angle of the blend membrane distinctly decreases with increasing content of chitosan for both types of blend membranes, and meanwhile, the corresponding SI of the blend membranes has concomitantly increased at a more pronounced span. These results suggest that, by employing an appropriate processing method and controlling the processing conditions, the hydrophilicity of the PDLLA/chitosan or PLLA/chitosan membrane has been significantly improved. Since the data listed in Table 6 exhibit basically uniform alterations, the results may imply that the two components in each blend membrane have coordinately coexisted together, because otherwise, those data shown in Table 6 for contact angle and SI would quite irregularly vary.

Table 5. Glass Transition Temperature of Component in Blend Membranes[a]

sample	T_g of chitosan (°C)	T_g of PDLLA (°C)	ΔT_1 [b] (°C)
PDLLA		59.6	
PDLLA/ch-25	171.3	69.9	101.4
PDLLA/ch-50	177.4	72.8	104.6
PDLLA/ch-75	184.4	67.2	117.2
chitosan	199.8		
sample	T_g of chitosan (°C)	T_g of PLLA (°C)	ΔT_2 [c] (°C)
PLLA		65.7	
PLLA/ch-25	178.7	75.1	103.6
PLLA/ch-50	173.5	78.2	95.3
PLLA/ch-75	180.5	73.3	107.2

[a] The values in the table are the average values from four specimens for each sample. [b] $\Delta T_1 = T_g$ (chitosan) $- T_g$ (PDLLA). [c] $\Delta T_2 = T_g$ (chitosan) $- T_g$ (PLLA).

Table 6. Water Contact Angle and Swelling Index of Blend Membranes

sample	θ_a (°)[a]	θ_r (°)[b]	SI (%)
PDLLA	96.5 ± 1.9	87.3 ± 2.2	
PDLLA/ch-25	89.3 ± 1.4	78.8 ± 1.7	14 ± 3
PDLLA/ch-50	84.5 ± 2.5	73.4 ± 2.3	31 ± 3
PDLLA/ch-75	78.2 ± 1.6	66.6 ± 2.1	52 ± 2
chitosan	71.1 ± 1.5	60.9 ± 1.8	73 ± 2
PLLA	97.7 ± 1.4	89.8 ± 1.7	
PLLA/ch-25	90.1 ± 2.1	81.8 ± 2.2	11 ± 2
PLLA/ch-50	85.2 ± 1.8	74.5 ± 1.9	28 ± 4
PLLA/ch-75	78.9 ± 1.6	67.7 ± 2.4	49 ± 3

[a] θ_a: advancing contact angle. [b] θ_r: receding contact angle.

Conclusions

Biodegradable blend membranes composed of polylactide and chitosan with various compositions have been successfully prepared. The presented method involving a solution-casting and solvent-extracting processing technique has been confirmed to be successful. It has been found that the morphology of the obtained blend membranes can be significantly influenced by the processing conditions. The blend membranes will possess a phase-separated morphology at a microscopic level if component solutions that are too thin are employed even if other processing conditions are feasible. Inappropriate mixed solvents and extraction solvents usually bring blend membranes with directly visible phase-separated morphologies. The membranes prepared under the optimized processing conditions can exhibit an interlocked internal structure and morphology. Although phase separation at a microscopic level is still observed, the

cumulative results obtained from IR spectra, TGA and DMA thermograms, as well as X-ray diffractograms all suggest that there exist pronounced interactions that probably result from hydrogen binds between different components, and the blend membranes prepared under the optimized processing conditions have a partially miscible structure. In addition, it is also found that the composition ratio of the blend membrane does not substantially affect the miscibility of the membranes.

Acknowledgment. The authors gratefully acknowledge Professor Ze Zhang at Départment de Chirurgie, Université Laval, Canada, for providing some PDLLA and PLLA materials as gifts.

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BM0600825