Improvement in Ductility of Chitosan through Blending and Copolymerization with PEG: FTIR Investigation of Molecular Interactions

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Chitosan is an important biomaterial used widely in medical applications. One of the key concerns about its use is the fragile nature of chitosan films. By comparing the component molecular interactions using FTIR, this study attempts to understand how the ductility of chitosan can be improved by blending and copolymerizing with poly(ethylene glycol) (PEG). An improvement in ductility was obtained for all compositions of blend as manifested by a decrease in modulus and an increase in strain at break. For comparable PEG composition (~30%), the properties of the solution-cast blend were better than those of the grafted copolymer. Therefore, blending may be a more efficient way to improve ductility of chitosan. FTIR characterization of the materials revealed subtle decreases in molecular interactions upon annealing the partially miscible blend. These may not be apparent in DSC or X-ray diffraction, yet they play a key role in the mechanical behavior. It appears that in the case of the graft copolymer the improvement in the properties comes from suppression of the crystallinity of each component and not from component interactions. On the other hand, in the blend, the improvement appears to come predominantly from the “well-dispersed”, “kinetically trapped” phase morphology and from the intermolecular interactions. Therefore, annealing the blend leads to decreased intermolecular interactions, phase coarsening, and deterioration in properties.

1. Introduction

Chitosan is a biodegradable, biocompatible, naturally occurring polymer. It is one of the major components used in vascular surgery, tissue culture, and tissue regeneration as a hemostatic agent. Chitosan scaffolds have been investigated for use in tissue engineering. Improving the fragile nature of films and membrane permeability are the key challenges that need to be addressed for improving chitosan as a biomaterial.

Chitosan has a high modulus of elasticity along with low strain to break owing to the high Tg and crystallinity. Morphology and ductility can be improved by either blending or copolymerizing with other polymers. Synthetic hydrophilic biocompatible polymers may be the ideal candidates for such modification of chitosan. Significant work on the morphology and the miscibility issues on the resulting materials has been carried out. Several blends of chitosan with other synthetic polymers have been evaluated, but poly(ethylene glycol) (PEG) is of particular interest. This is due to the hydrophilic, biocompatible, and biodegradable nature of PEG. A number of PEG-based technologies are now finding applications in commercial therapeutic, research, and diagnostic products.

Chitosan/poly(ethylene oxide) (PEO) blends were found to be miscible below 50% (w/w) of chitosan. Recently, it was found that a PEO/chitosan blends may be amorphous up to 20% PEO content. At higher weight fractions, PEO crystallizes by forming spherulites in the blend. Evmenko and co-workers suggested the significant improvement in mechanical properties of chitosan upon addition of PEG. Roberts’ group investigated the effect of acetyl content on the miscibility of chitosan and PEO blends and found that interfacial interaction was stronger at higher acetyl content. FTIR characterization of these blends and pure chitosan with different degrees of deacetylation has been addressed previously.

In this paper, we address the component interactions responsible for the change in properties of chitosan when blended and copolymerized with PEG. We characterize and compare the properties of chitosan/PEG blends at different compositions and compare it with a copolymer. This is achieved by using a combination of FTIR spectroscopy, X-ray diffraction, and optical microscopy. The effects of annealing are also explored with a view to understanding the effect of temperature on molecular interactions.

2. Materials and Methods

2.1. Materials. Chitosan (degree of deacetylation (DD) = 93%, molecular weight = 600 000 g/mol) and poly(ethylene glycol)-Ald were purchased from Fluka. Pure PEG (molecular weight = 10 000 g/mol) was purchased from Aldrich.

2.2. Sample Preparation. Blends. Appropriate amounts of chitosan and PEG were dissolved in a 1% solution of glacial acetic acid in water to obtain a 4% solution of blend.
The solution was stirred for $\frac{1}{2}$ h to obtain a clear solution and centrifuged at a speed of 5000 rpm for 15 min at 25 °C to remove the air bubbles present. Approximately 10 mL of this solution was added in a Petri dish, and ammonia was passed through it to obtain a uniform gel. This gel was allowed to dry for 24 h and washed with ethanol and water to remove excess acetic acid present. The resulting film had an uniform thickness ranging from 25 to 40 μm. The compositions of blends analyzed were 70/30, 60/40, and 50/50 of chitosan and PEG, respectively.

Graft Copolymer Films. Chitosan was dissolved in 1% acetic acid solution and derivatized with PEG-Ald.15 The films of this solution were prepared by the similar technique employed in preparation of blend films described above. A sample with a PEG graft level of 31 wt %, as measured by Howard Matthew’s research group at Wayne State University.16

2.3. Sample Analysis. Bionix MTS system was used to perform the mechanical testing. The strain rate used was 1 mm/min. The average and standard deviation of measurements from five samples are reported. The films used for these measurements were of size 3 cm × 1 cm and had thickness of approximately 100 μm. All of the measurements were performed at room temperature on dry films.

Film X-ray diffraction patterns were obtained by using a Nicolet R3 diffractometer equipped with a $\theta-\theta$ goniometer. The operating conditions during the experiment were 40 kV and 50 mA with Cu Kα radiation. For pure PEG analysis, powder was used instead of film.

A Bio-Rad FTS 6000 FTIR spectrometer (Digilab Division, Bio-Rad Laboratories, Cambridge, MA) was used to probe the molecular interaction between the two components. To enable FTIR spectrum measurement at various temperatures, the temperature-controlled environmental chamber fitted with IR transparent potassium bromide windows was used. For all of the measurements, the spectra were obtained in the rapid scan mode with open aperture setting. Fifty scans were co-added with the resolution of 4 cm$^{-1}$. Films of blends were cut to a size of 3 cm × 2 cm and mounted on Minimat stretcher (manufactured by Rheometric scientific). Preparation of PEG films was more challenging because of high crystallinity of PEG. Therefore, a solution of PEG in water was prepared and a drop of solution was added on a 3M Teflon (PTFE) card, and this solution was allowed to dry and subsequently analyzed. For this sample, the PTFE spectrum was used as background. For the annealing experiment, the film was mounted on the minimat stretcher, and a room-temperature scan was acquired. Subsequently, the sample was heated (or cooled) to a specified temperature (30, 40, 45, 50, 60, 70, and 80 °C), and 30 min were allowed for the sample to attain thermal equilibrium.

Nikon inverted optical microscope DIAPHOT 300 was used to characterize the morphology of the blend and copolymer. The images were acquired at 40 × magnification.

3. Results and Discussions

3.1. Mechanical Testing. The mechanical properties of blends and the copolymer are characterized and compared with the pure chitosan. Pure chitosan film was brittle with a modulus of elasticity ($E$) of 166 MPa and strain at break ($\varepsilon_b$) of 8%. However, in the 60/40 and 70/30 blend films, $E$ increased and $\varepsilon_b$ decreased with increasing chitosan composition, as expected (Table 1). At comparable compositions (70/30) to the copolymer, the blend had a lower $E$ and higher $\varepsilon_b$, suggesting that blending may be an effective and simpler way to improve the ductility of chitosan compared to copolymerization.

To understand the importance of component interactions and the phase morphology, we performed annealing experiments. The semicrystalline PEG has a glass transition temperature ($T_g$) significantly below room temperature and a melting point at 63 °C (estimated from the DSC curve midpoint). On the other hand, chitosan has an estimated $T_g$ at 203 °C.17 Therefore, one may expect further phase separation to take place in the blend at temperatures higher than the melting point of PEG. When the blend films were annealed at 80 °C for 30 min, an increase in elastic modulus of blend films was observed. For 70/30 and 60/40 blends, $E$ increased to values comparable to that of pure chitosan, whereas the effect was moderate for the “bicontinuous” 50/50 blend. However, for chitosan-g-PEG copolymer, no changes were observed in $E$ and $\varepsilon_b$ upon annealing. The Flory–Huggin’s interaction parameter ($\chi$) for chitosan–PEG is not known. Therefore, it is not clear whether the copolymer is already microphase-separated at 80 °C. If the microphase separation was occurring solely because of the annealing at 80 °C, we would expect significant change in properties, which is not seen. Therefore, there appears to be no morphological change in the copolymer upon annealing at these temperatures. On the other hand, the blends appear to be undergoing further microphase separation upon annealing. Therefore, the $E$ and $\varepsilon_b$ values reflect the properties of the major component (chitosan) upon annealing. This also suggests to sustain the improved ductility in blends, preparation and annealing conditions should be carefully controlled.

In going from the 50/50 blend to the 60/40 blend, there is an increase in the modulus (as expected) and an increase $\varepsilon_b$ (not as expected). The reason for the increased strain at break is not completely clear. We believe that this may be related to the morphology change going from the 50/50 to the 60/40 blend. The 50/50 blend would be expected to have a bicontinuous phase structure, characterized by a high interfacial area. Therefore, in this blend, the sample may fail even in the “more brittle” PEG phase. This sample does not show a large difference in the $\varepsilon_b$ even upon annealing (it changes from 18% to 22%). In the blends with chitosan as a major

| Table 1. Modulus of Elasticity ($E$) and Strain at Break ($\varepsilon_b$) Data for the Blend and Copolymer Samples |
|---|---|---|---|
| sample | $E$ (MPa) | $\varepsilon_b$ (%) | $\varepsilon_b$ (%) |
| before annealing | after annealing | before annealing | after annealing |
| pure chitosan | 165.8 ± 6 | 165.8 ± 8 | 8 ± 3 | 8 ± 3 |
| 50/50 blend | 71.7 ± 8 | 99.5 ± 11 | 18 ± 4 | 22 ± 4 |
| 60/40 blend | 91.5 ± 12 | 146.8 ± 8 | 31 ± 3 | 10 ± 6 |
| 70/30 blend | 107.7 ± 9 | 148.8 ± 9 | 20 ± 4 | 8 ± 5 |
| 69/31 copolymer | 121.2 ± 10 | 128.3 ± 12 | 15 ± 3 | 15 ± 4 |
phase, failure would be expected to occur in the chitosan phase. In the unannealed 60/40 blend, the PEG minor phase will have a small drop size, which can act as crack stoppers, increasing \( \varepsilon_b \). If the small drop size is the reason, annealing would increase the drop size and decrease \( \varepsilon_b \). In fact, we see that \( \varepsilon_b \) changes from 31% to 10% upon annealing. The strong effect of annealing was observed in the 70/30 blend also.

3.2. X-ray Diffraction. Figure 1a shows the diffraction patterns of pure chitosan and pure PEG. Pure chitosan shows a strong reflection at 19.7° and a relatively weak reflection centering at 10°, which are associated with the crystalline regions and have been used to estimate crystallinity.18 Pure PEG shows strong reflections at 18.74° and 22.86° and weak reflections at 26.77°, 30.5°, 35.9°, and 40° suggesting the crystalline nature.19 We monitor the qualitative changes in these peaks to observe the crystalline characteristics of each component in the blend and copolymer. In the 70/30 blend film, the 10° reflection for chitosan is absent and the strong 19.7° reflection is diminished. There is a broad peak in the 18°–21° region, which may have contributions from both chitosan and PEG. This indicates that there is a decrease in chitosan crystallization upon blending. For PEG, the presence of reflections at 26.77°, 30.5°, 35.9°, and 40° provides evidence of the crystallinity of PEG in the blend. Estimation of crystallinity by comparing the intensity count values suggests 18% reduction in degree of crystallinity of PEG in the blend compared to pure PEG. This is probably due to the significant effect of the stiff chitosan chain on mobility in the overall mixture, inhibiting the crystal growth of PEG.9 On the other hand, the copolymer does not show any well-defined PEG crystalline peaks or chitosan crystalline peak (10°). There is a broad peak at 17°–25°. This suggests that there may be a significant reduction in the crystallinity in the copolymer compared to the blend at the “same” composition. Upon annealing, there was no significant difference in the X-ray diffraction pattern of the blend. This further suggests that crystallinity may not be an important factor for deterioration in ductility after annealing. The intermolecular interaction in miscible regions of the blend may be the driving force. These findings will be correlated with the FTIR findings in the next section.

Figure 1. X-ray diffraction pattern (a) of chitosan (A) and PEG (B) (b) of 70/30 blend (A) and 69/31 graft copolymer (B).
3.3. FTIR Characterization. The blend and copolymer films were characterized by FTIR to investigate the molecular changes responsible for improvement in ductility before annealing and deterioration after annealing. The normalized IR spectra are reported (reference peak = 2880 cm\(^{-1}\)). The normalization addresses any potential variation due to a change in the film thickness, thereby allowing for better quantitative analysis.

Pure chitosan shows a distinct amide I band and amide II band at 1650 and 1580 cm\(^{-1}\), respectively.\(^5,20\) The area between 1000 and 1150 cm\(^{-1}\) was saturated, maybe because of the presence of three distinct vibrational modes of C–O–C, C–OH, and C–C ring vibrations. N–H stretching and O–H stretching vibrations can be characterized by the broad peak in the region of 3200–3500 cm\(^{-1}\) (Figure 2a).\(^5,20\) Pure PEG shows distinct peaks at 1280, 947, and 843 cm\(^{-1}\) (Figure 2b).

(i) Effect of Annealing on Pure Chitosan and Pure PEG. When pure chitosan was heated and annealed, the absorbance and the areas of the peaks in the 1500–1700 cm\(^{-1}\) region decreased with increase in the temperature, but these changes were reversible, in agreement with the previous results.\(^3\) Similar results were shown by pure PEG in the region between 800 and 1500 cm\(^{-1}\). The absorbance of peaks at 1280, 947, and 843 cm\(^{-1}\) decreased significantly in the heating run. But the peak intensities recovered during the cooling run with no net changes in the absorbance as shown in Figure 3. These reductions in absorbances may be due to the melting of crystals of PEG during the heating run. This melting appears to occur even though we are at 50 °C, which is well below the estimated midpoint for melting of PEG at 63 °C. But while cooling, the recrystallization produced the original peaks again. Therefore, the peaks at 1280, 947, and 843 cm\(^{-1}\) may be associated with the crystalline regions of PEG.

(ii) Blend and Copolymer at Room Temperature. FTIR spectrum of the 70/30 blend (Figure 2c) exhibited most of the characteristics of chitosan and PEG but with some important differences. The disappearance of peaks at 1650 cm\(^{-1}\) from chitosan and 1760 cm\(^{-1}\) from PEG (carbonyl stretching vibration), coupled with the increase in the absorbance of the 1580 cm\(^{-1}\) peak compared to that in the pure chitosan spectrum, suggests an attractive intermolecular interaction between chitosan and PEG, as suggested in the literature.\(^24\) The 843, 1280, and 947 cm\(^{-1}\) peaks can be clearly seen, which are the contributions from the crystalline region in PEG. These findings are supported by X-ray diffraction. The IR scans of blends at other compositions were similar in nature. The appearance of interaction peaks in the blend suggests partial miscibility for all of the blend compositions tested, in agreement with literature.\(^9\) Therefore, the improvement in ductility could be because of the partial miscibility of blends at room temperature.

On the other hand, peaks at 1650 and 1580 cm\(^{-1}\) were observed in chitosan–g–PEG copolymer (Figure 2d). The absorbances of peaks at 843, 1280, and 947 cm\(^{-1}\) were decreased compared with the blend spectrum. Therefore, for chitosan–g–PEG, it appears that intermolecular interaction is absent even though chitosan and PEG are part of the same molecule. Also, the decrease in absorbance of peaks at 843, 1280, and 947 cm\(^{-1}\) suggests decrease in crystallinity of PEG, which agrees well with X-ray diffraction findings.

In summary, the FTIR and X-ray diffraction studies show that (a) the blend is partially miscible and is characterized by strong intermolecular interactions and (b) the copolymer shows stronger crystallinity suppression and lack of intermolecular interactions.

(iii) Effect of Annealing on Blend and Copolymer. Annealing experiments were performed with the procedure described in Materials and Methods. The IR spectra of the blend film at room temperature, at 50 °C, and at room temperature after cooling are shown in Figure 4. The results are reported only for the 50/50 blend because similar quantitative results were obtained with all blend compositions tested.

(a) Chitosan Peaks. The absorbance of the 1580 cm\(^{-1}\) peak changed irreversibly, decreasing during the heating run and
practically remaining constant during the cooling run. Also a peak shift from 1580 to 1591 cm\(^{-1}\) was observed. The peak at 1650 cm\(^{-1}\) became more pronounced and shifted to 1658 cm\(^{-1}\) with the subsequent heating and cooling. To get quantitative analysis, the absorbance ratio of the 1580 cm\(^{-1}\) peak to the 1650 cm\(^{-1}\) peak as a function of temperature was compared, and it was found that the ratio kept decreasing during both heating and cooling (Figure 5a). This ratio may be a measure of the importance of decrease in intermolecular interactions causing an increase in phase separation. In addition, in the broad region between 3190 and 3500 cm\(^{-1}\) corresponding to the N–H stretching region, change in slope was observed as shown in Figure 5b. N–H stretching mode has been used as a measure of intermolecular interaction and the subsequent miscibility.\(^{21,22}\) The change of slope in this region is a further measure of decrease in intermolecular interactions, which leads to further phase separation upon annealing.

(b) PEG Peaks. The peaks associated with PEG in the blend showed two distinct types of responses (Figure 6). Analysis of peaks at 843 and 1280 cm\(^{-1}\) revealed that the absorbance decreased during the heating but increased with the subsequent cooling with the major slope change occurring at 50 °C. These correspond to crystalline regions of PEG as discussed earlier. The significant slope change observed during the heating run between 40 and 50 °C suggests “melting” of PEG crystals, and recrystallization can be observed with increase in slope during cooling run in a reversible manner. This suggests that the annealing has no effect on the crystalline regions of the PEG in blend. The absorbance of peaks at 1344 and 1411 cm\(^{-1}\) decreased with the heating run and remained constant in the cooling run. Consistent with the observation for interaction peak at the 1580 cm\(^{-1}\) chitosan peak, these peaks may also be sensitive to the chitosan/PEG interactions and suggest further phase separation upon annealing.

Figure 4. Effect of annealing on blend. Decrease in absorbance of the 1580 cm\(^{-1}\) peak and appearance of the 1650 cm\(^{-1}\) peak can be seen.

Figure 5. Change in intermolecular interactions in blend upon annealing: (a) ratio of 1580/1650 peaks; (b) N–H stretching region.
The changes mentioned above were also confirmed by the difference spectroscopy technique (results not shown), which enables us to monitor the small changes in spectrum. The room-temperature spectrum of the blend after cooling was subtracted from the room-temperature spectrum before heating. Spectrum indicated that the changes due to the peaks at 1580, 1650, and 1411 cm\(^{-1}\) were appreciable.

On the other hand, for chitosan-\(g\)-PEG, no significant changes in the spectrum were observed after annealing (Figure 7). Also the ratio of absorbance at 1580 to that at 1650 cm\(^{-1}\) remained almost constant during annealing as shown in Figure 8. In contrast to the blend, the absence of significant changes in the copolymer IR spectrum during annealing indicates that there was no phase coarsening in the copolymer, an agreement with X-ray diffraction data.

### 3.4. Optical Microscopy

Pure chitosan is semicrystalline, while pure PEG is highly crystalline with well-defined crystal structures. Homogeneous phase morphology was observed in blend films (Figure 9a) before annealing, but coarsening of this morphology was seen after annealing (Figure 9b).

Homogeneous morphology of blend films suggests (i) partial miscibility of the blend before annealing and (ii) a kinetically trapped well-dispersed blend system. Phase separation occurs after annealing, which is responsible for deterioration of mechanical properties of blend films. We believe that this is just phase coarsening and not the change in overall crystallinity as suggested by X-ray diffraction and FTIR. No significant change was observed in the morphology of chitosan-\(g\)-PEG after annealing (Figure 9c,d). This suggests the absence of macroscopic phase separation in the copolymer. The results are in agreement with FTIR findings.

Therefore, in the one component copolymer, grafted PEG appears to diminish the crystallinity of both of the components. This is shown by a lack of the crystalline IR peaks and X-ray diffraction peaks associated with PEG and chitosan. Consequently, the property improvement may be predominantly due to reduced crystallinity. This is also consistent with the lack of any phase coarsening observed upon annealing at 80 °C. On the other hand, in the blend, PEG appears to be partially miscible. Therefore, in chitosan-rich and PEG-rich regions, crystallization appears to occur. This is evidenced by the presence of the PEG crystallinity peak in the IR spectrum, the PEG X-ray diffraction pattern...
of the blend, and optical microscopy. The improvement in properties in the blend appears to come from the well-dispersed phase morphology in which large-scale phase separation is prevented by the “rapid” solvent evaporation and the low temperature. This view is supported by the fact that upon annealing at 80 °C, the phase coarsening occurs, causing an increase in $E$ and decrease in $\epsilon_b$. Therefore, by manipulating the formulation conditions, compositions, and annealing, one may use blending as an effective way to improve the ductility of chitosan. FTIR spectroscopy appears to provide the change in the molecular interactions in the blend in a very sensitive way.

4. Conclusions

The improvement in ductility of chitosan was obtained by modification of chitosan by preparing blend and grafted copolymers with PEG. Three different blend compositions and one copolymer composition were analyzed for mechanical properties before and after annealing. Molecular interactions and phase morphology were characterized using FTIR spectroscopy, X-ray diffraction, and optical microscopy, respectively. Our results suggest the following: (i) Blending chitosan with poly(ethylene glycol) improves the ductility of chitosan with the 50/50 blend showing the lowest $E$ and 60/40 blend showing highest $\epsilon_b$ among the compositions of blend tested. The decrease in $E$ was 56%, while increase in $\epsilon_b$ was 125% for the 50/50 blend. (ii) On the other hand for chitosan-g-PEG (31% PEG), the decrease in $E$ was 28%, while the increase in $\epsilon_b$ was 88%. (iii) FTIR spectra during heating and subsequent cooling suggest that blend is partially miscible and that the miscibility may be driven by the intermolecular interactions. For the graft copolymer, no change was observed upon annealing because it is a one-component system. Surprisingly, we do not observe intermolecular interaction peaks in the copolymer. (iv) For the same PEG composition, the ductility of the blend was “better” than that for the graft copolymer. This suggests that solution blending causes “kinetically limited” small droplets of crystalline PEG to form in a chitosan matrix. Such “microphase” structure appears to be more effective in improving the properties of chitosan than those of one-component graft copolymer, at least for the compositions used in this study. However, the property improvements due to blending may be lost if the blend is annealed at higher temperatures because of further phase separation.

The method used in this study to characterize component interaction in blends can be extended to different systems of polymers with chitosan such as collagen and dextran.

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References and Notes
