Relationship between Composition and Properties for Stable Chitosan Films Containing Lipid Microdomains

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Received 30 March 2005; accepted 9 September 2005
DOI 10.1002/app.23178
Published online in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: Biocompatible, biodegradable films composed of a hybrid blend of chitosan and egg phosphatidylcholine (ePC) were characterized in terms of composition, morphology, and performance-related properties. The miscibility between chitosan and ePC for blends of 1 : 0.2 to 1 : 2.5 chitosan : ePC (wt/wt) was examined by differential scanning calorimetry and X-ray diffraction analysis. The partial miscibility exhibited between chitosan and ePC provided an understanding of the microdomain morphology that was visualized by laser scanning confocal fluorescence microscopy of the films. The stability of the films in physiologically relevant media was assessed by percent weight loss over time. The mechanical properties of the chitosan–ePC films were determined by dynamic mechanical analysis and tensile tests. Interestingly, the dry film composed of a high lipid formulation (1 : 2.5 (wt/wt) chitosan: ePC) had the lowest tensile strength, contained lipid microdomains (10–30 μm in size), and provided the highest degree of stability. Following immersion in phosphate buffer solution, the Young's modulus of the film was found to decrease by more than two orders of magnitude and could be further manipulated by decreasing the lipid content within the film. In this way, relationships between the composition and the physical as well as mechanical properties of the chitosan–ePC blends were established. Furthermore, this study demonstrates the potential usefulness of partially miscible chitosan-based blends for biomedical purposes. J Appl Polym Sci 103: 3453–3460, 2007

Key words: phospholipids; miscibility; films; blends; biopolymers

INTRODUCTION

In recent years, various groups have examined hybrid blends such as mixtures of organic and inorganic materials as well as combinations of polymer and lipid.[1,2] Blending is often used as a means to combine the favorable characteristics of distinct families of materials and to create a composite with improved properties. The miscibility of two materials, determined by enthalpic and entropic factors, influences the phase morphology within the blend (i.e., presence of domains). In turn, the phase morphology, as well as the inherent characteristics of each individual component, determines the performance-related properties of the blend. For use of blends in biomedical applications, the performance-related properties that are of most interest include: rheological and mechanical properties as well as biodegradability, biocompatibility, bioadhesion, swelling behavior, and stability.[3]

To date, chitosan-based materials have been assessed for a wide variety of medical applications including drug delivery, tissue engineering, bone repair, wound healing, blood dialysis, and ocular retention.[4–8] Chitosan is a polysaccharide that is naturally present in crustacean shells, some microorganisms, and fungi, but can also be obtained from the deacetylation of chitin. Chitosan contains mainly β-(1→4) linked 2-amino-2-deoxy-D-glucopyranose units and is a positively charged crystalline polymer that becomes increasingly soluble in low pH media (i.e., 1% acetic acid solution, pH = 5).[9] Chitosan also has excellent film forming properties that may be manipulated by the type and pH of solvent used as well as the temperature employed during processing.[10] However, films formed from chitosan alone are limited in terms of application due to their brittleness under dry conditions and instability in aqueous environments.[11]

To prepare stable chitosan films with improved mechanical properties, chemical crosslinking agents and synthetic grafting methods have been explored extensively.[12–14] By comparison, physical blending is a straightforward and effective method to prepare chitosan films. The miscibility of materials in polymer blends is dependent on specific interactions between the components such as: the degree of hydrogen bonding, ionic and dipole, π-electron and charge-transfer complexes. From the literature, there is evidence of inter- and intramolecular hydrogen bonding and/or ionic interactions between chitosan and the following homopolymers: silk fibroin,[15] poly(acrylic acid),[16] poly(3-hydroxybutyric acid),[17] poly(ethylene glycol),[18] poly(vinyl pyrrolidone),[19] nylon-4,[20] pectin,[21] poly(caprolactone),[22] poly(vinyl alcohol),[23] and

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Contract grant sponsor: NSERC.

collagen.[24] Chitosan has also been reported to be immiscible with poly(lactic acid),[25] nylon-6,[20] and cellulose.[26] In general, most polymer blends are immiscible two-phase systems that have properties that render them suitable for specific applications.[27] For example, chitosan–nylon blends have been explored for use in cell culture and tissue engineering as they have increased cell adhesion and result in more cytokine production than pure nylon membranes.[28]

Recently, our group evaluated films formed from blends of chitosan and egg phosphatidylcholine (ePC) for localized delivery of hydrophobic drugs.[29,30] This unique blend of polymer and lipid materials has been shown to produce films with low swelling and a high degree of stability in aqueous media. In addition, the chitosan–ePC films are biocompatible, biodegradable, and capable of providing delayed release of highly hydrophobic drugs over periods of several months.[29,30] The objective of the present study was to gain an understanding of the relationship between the composition (i.e., ratio of chitosan to ePC) and several of the performance-related properties of chitosan–ePC films. Differential scanning calorimetry (DSC) was used to examine the thermal behavior of the blends. X-ray diffraction analysis was used to determine the miscibility between the polymer and lipid components. The morphology of chitosan–ePC films was examined by laser scanning fluorescence confocal microscopy. The Young's modulus and storage modulus of the blends were measured from tensile tests and dynamic mechanical analysis (DMA), respectively. The stability of the films was assessed over a 6-week period in biologically relevant media. Overall, these experiments allow for many of the composition–property relationships of the chitosan–ePC system to be established. Also, the organization of the polymer and lipid materials within the blends is realized. Interestingly, this research further demonstrates the potential usefulness of phase-separated chitosan-based blends.

EXPERIMENTAL

Materials

Chitosan (MW ~400,000 and ~85% deacetylation) was purchased from Fluka BioChemika (Buchs, Switzerland). ePC was obtained from Northern Lipids (British Columbia, Canada). The fluorescent probe, 1, 2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine-*N*-(7-nitro-2-1,3-benzoxadiazol-4-yl) (NBD-DPPE), was purchased from Avanti Polar Lipids (Alabaster, AL). Ethanol was obtained from Commercial Alcohols (Ontario, Canada). Crude ePC, Canada origin fetal bovine serum (FBS), and all other chemicals were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO).

Preparation of chitosan–ePC films

A 2% (wt/wt) chitosan solution was prepared in distilled water containing 1% (v/v) acetic acid. ePC (pure or crude) was dissolved in ethanol (50–800 mg/mL) and blended with chitosan. The ratio of chitosan to ePC in the formulations ranged from 1 : 0.2 to 1 : 3.3 (wt/wt). A total volume of 13 mL of the chitosan–ePC solutions were poured into teflon-coated dishes and dried in a dessicator for 5 days at room temperature.

Thermal analysis of chitosan–ePC films

Thermal analysis of chitosan, ePC, and chitosan–ePC blends was performed using a DSC Q100 system (TA Instruments, New Castle, DE). For DSC analysis, the sample weights of the chitosan and chitosan–ePC films were 5–7 mg. Pure ePC was dissolved in ethanol and dried for 5 days prior to DSC analysis. The samples were cooled to –40°C using a refrigerated cooling system and then heated to 70°C at a temperature ramp speed of 5°C/min under nitrogen purge. The second heating cycle was used for the analysis of the DSC thermograms. TA universal analysis software was used for all thermal observations.

X-ray diffraction analysis of chitosan–ePC films

X-ray diffraction patterns of chitosan powder, chitosan film, chitosan–ePC blends (1 : 0.2 and 1 : 2.5 (wt/wt)), and pure ePC were obtained using a Siemens D5000 $\theta/2\theta$ diffractometer with Cu Kα source operating at 50 kV, 35 mA. The secondary beam was monochromatized by a Kevex solid detector. Chitosan and chitosan–ePC solutions were cast onto aluminum disks and dried in a dessicator at room temperature. Patterns were obtained using a step width of 0.02° 2θ between 3 and 38° 2θ at ambient temperatures. Pure ePC was also examined in the short θ range by a SAXS Nanostar Bruker AXS diffractometer. A parallel Goebel-mirror beam from a Cu Kα source was used and the data were collected on transmission mode with a 2D detector.

Microscopic evaluation of chitosan–ePC films

The phase morphology of the chitosan–ePC films containing low and high amounts of lipid (i.e., 1 : 0.2 and 1 : 2.5 (wt/wt) chitosan: ePC) were imaged on a Zeiss LSM 510 laser scanning fluorescence confocal microscope (Zeiss, Germany). Briefly, pure ePC and 1 mol % of the fluorescent phospholipid NBD-DPPE ($\lambda_{ex} = 460$ nm, $\lambda_{em} = 534$ nm) were dissolved in ethanol and mixed. The lipid solution was blended with chitosan prior to casting onto a glass slide. Cover slips were placed on the solution to prevent optical reflectance

and the formulation was dried in a dark room overnight. The fluorescent-labeled films were excited at a wavelength of 488 nm by an argon ion laser. The pinhole aperture was set at 98 μm, resulting in a 0.8-μm optical section. The optical sections were acquired by moving the focal plane from the surface of the film in the z direction at 1-μm intervals between optical slices. The stack size was 146 × 146 μm^2 (512 × 512 pixels) in the xy plane and 31 μm in the z direction. The images were processed using the Zeiss LSM image browser software package.

DMA of chitosan–ePC films

The storage moduli of chitosan and chitosan–ePC films were measured with a DMA Q800 V3.13 (TA Instruments, New Castle, DE). Thin dry films containing increasing amounts of pure ePC were analyzed in the tensile mode at a constant frequency (1 Hz). The films were between 0.1 and 0.4 mm in thickness, 5.4 mm in width, and 25 mm in length. The strain amplitude imposed (5–15 μm) was small enough to maintain the films' mechanical response in the linear elastic regime. The films were cooled to −40°C and then heated to 70°C at a temperature ramp speed of 2°C/min. TA universal analysis 2000 v3.8B software was used to process the measurements.

Tensile properties of chitosan–ePC films

The mechanical response of the chitosan and chitosan–ePC films (1 : 0.2 to 1 : 2.5 (wt/wt) chitosan–ePC) at high deformations was measured using a universal Instron testing apparatus. Standard tensile tests at room temperature were performed using a crosshead rate of 2.5 mm/min. Films were uniformly cut using a dye and press. The geometry of the films (0.1–0.4 mm thickness, 3 mm width, and 25 mm gauge length) and experimental conditions used were according to the specifications of the ASTM D 638 procedure. Films were also swelled in 0.01M phosphate buffer saline (PBS, pH = 7.4) solution for 24 h at 37°C and blot-dried prior to measuring the tensile strength.

Stability of chitosan–ePC films

The percent weight loss of chitosan films containing increasing amounts of crude ePC was evaluated in 0.01M PBS containing 10% FBS over a 6-week incubation period at 37°C. Films were cut 10 × 10 mm^2 in size and placed in scintillation vials containing 10 mL of the PBS/FBS solution. The solution was replaced with fresh PBS/FBS twice a week. The films were removed from the vials following 1, 2, 4, and 6 weeks, washed in ethanol and distilled water, and then dried. The percent weight loss (W_L) was calculated using the following equation: $W_L = [(W_i - W_d)/W_i] \times 100$; where W_i is the initial weight of the film and W_d is the final weight of the dry film.

RESULTS AND DISCUSSION

The compatibility of the chitosan–ePC films was found to be closely related to their composition, as blends of chitosan: ePC in ratios of 1 : 0.2 to 1 : 1.7 (wt/wt) have a homogeneous appearance, as visible to the naked eye. Yet, a film formed from 1 : 2.5 (wt/wt) chitosan: ePC has a heterogeneous appearance with apparent domains dispersed throughout the film (photograph not shown). To understand the relationships between composition and morphology for the chitosan–ePC blends, the miscibility between the materials was investigated by various methods. In a previous report, FTIR analysis confirmed the presence of ionic and hydrogen bonding interactions between chitosan and ePC.[29]

Thermal analysis of chitosan–ePC films

Thermal analysis was employed to investigate the miscibility between chitosan and ePC, as well as to determine whether domains of lipid are present within the blends. DSC thermograms corresponding to the second heating cycle for blends of chitosan and ePC are shown in Figure 1. A film prepared from chitosan alone did not show any transition over the temperature range investigated. Chitosan has been reported to exhibit two endothermic peaks at 100 and 290°C that are attributed to the evaporation of water and the onset of degradation, respectively.[31,32]

The DSC thermogram for pure ePC included a single broad endothermic transition at 12.7°C with an enthalpy of 22 J/g. This peak corresponds to the gel to liquid–crystalline phase transition (T_m) for ePC; where the hydrocarbon chains of the lipid molecules rearrange from an ordered, all-trans conformation to a disordered conformation.[33] ePC consists of a mixture of phosphatidylcholine lipids having hydrocarbon chains of different lengths and degrees of saturation. Due to the compositional heterogeneity of ePC, the T_m is unsymmetrical and broad (i.e., extends over 20°C). The values reported for the average T_m of ePC range from –17 to 110°C owing to differences in water content and purity of the sample as well as the conditions employed for DSC analysis (i.e., scan rate, sensitivity of instrument, sample size).[34,35]

As shown in Figure 1, the blending of chitosan with ePC alters the gel to liquid-crystalline transition of the lipid. In general, chitosan was found to cause the main endothermic peak for ePC to split into two components that were higher and lower in temperature in comparison with the average T_m for pure ePC. Specifically, the average transition temperatures for the two components were found to vary with a change in the

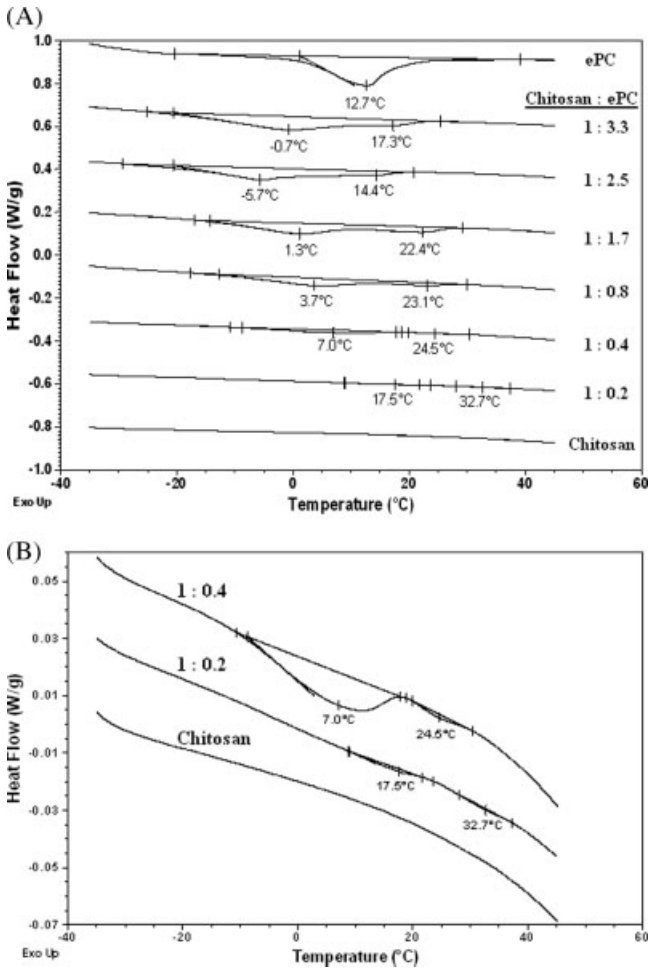

Figure 1 (A, B) DSC thermograms of pure ePC, chitosan, and chitosan–ePC films (1 : 3.3 to 1 : 0.2 (wt/wt) chitosan: ePC). The transitions for ePC within the 1 : 0.4 and 1 : 0.2 films are more evident in (B).

relative ratio of ePC to chitosan. The change in the T_m values for the lipids may imply that there is some degree of miscibility or interaction present between the lipid and chitosan components of the blends.

As observed in Table I, the transition enthalpy values for chitosan–ePC blends with high ePC content were similar to that for pure lipid, when the values are compared per unit mass of lipid. However, at low amounts of ePC (1 : 0.2 and 1 : 0.4 (wt/wt)), the enthalpy per unit mass of lipid was significantly lower than that for pure ePC (Table I). This effect may be attributed to a dispersion of the lipid molecules throughout chitosan, which decreases the potential for lipid–lipid interactions and reduces the cooperativity of the gel to liquid-crystalline transition. Similarly, Fang and coworkers showed that increasing chitosan concentrations within a pure DPPC bilayer led to a reduction in enthalpy of the melting transition due to the suppressed cohesive energy.[36] However, this behavior may also be attributed to a ''cholesterol-like'' phenomena in which the chitosan acts to

TABLE I
The Enthalpy per Unit Mass of the Lipid Component for Pure ePC and the 1 : 0.2 to 1 : 3.3 (wt/wt) Chitosan: ePC Films

Film composition (chitosan: ePC (wt/wt))	ΔH (J/g of lipid)
1 : 0 (Chitosan)	–
1 : 0.2	0.6
1 : 0.4	8.4
1 : 0.8	19.9
1 : 1.7	21.4
1 : 2.5	21.3
1 : 3.3	24.7
0 : 1 (ePC)	21.8

increase the fluidity of the lipid below the T_m and reduce the fluidity above the T_m. The addition of cholesterol to lipid membranes acts to broaden the transition of the lipid with the enthalpy of the transition reaching zero when the cholesterol content is increased above 50 mol %.[37]

X-ray diffraction analysis of chitosan–ePC films

The interaction between chitosan and ePC was further examined by X-ray diffraction analysis (Fig. 2). In agreement with previous reports, the chitosan flakes exhibited a relatively low degree of crystallinity with three broad short range orders at $2\theta = 10.5^\circ$, 20.1°, and 22.0°.[38] When a chitosan film was prepared by dissolving chitosan flakes in 1% (v/v) acetic acid solution, three frequent atomic distances of 9.9, 7.3, and

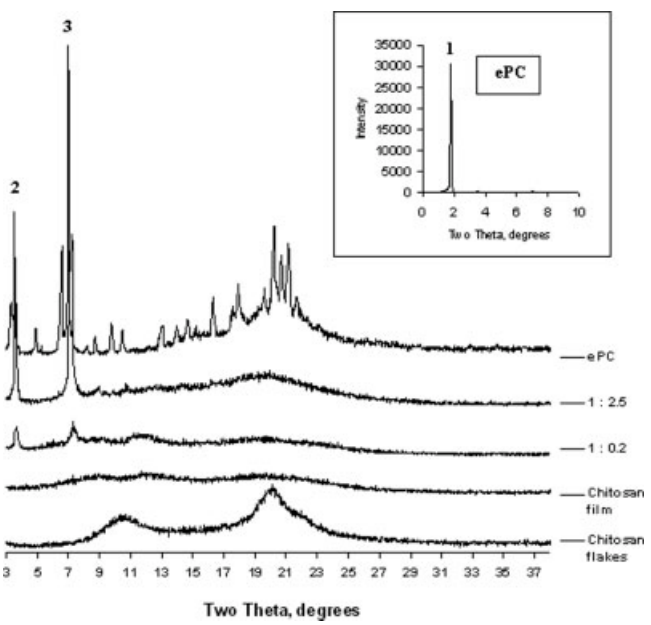

Figure 2 X-ray diffraction patterns of pure ePC, chitosan–ePC films (1 : 2.5 and 1 : 0.2 (wt/wt) chitosan: ePC), chitosan film, and chitosan flakes. Pure ePC was also analyzed in the short θ ranges as shown in the insert.

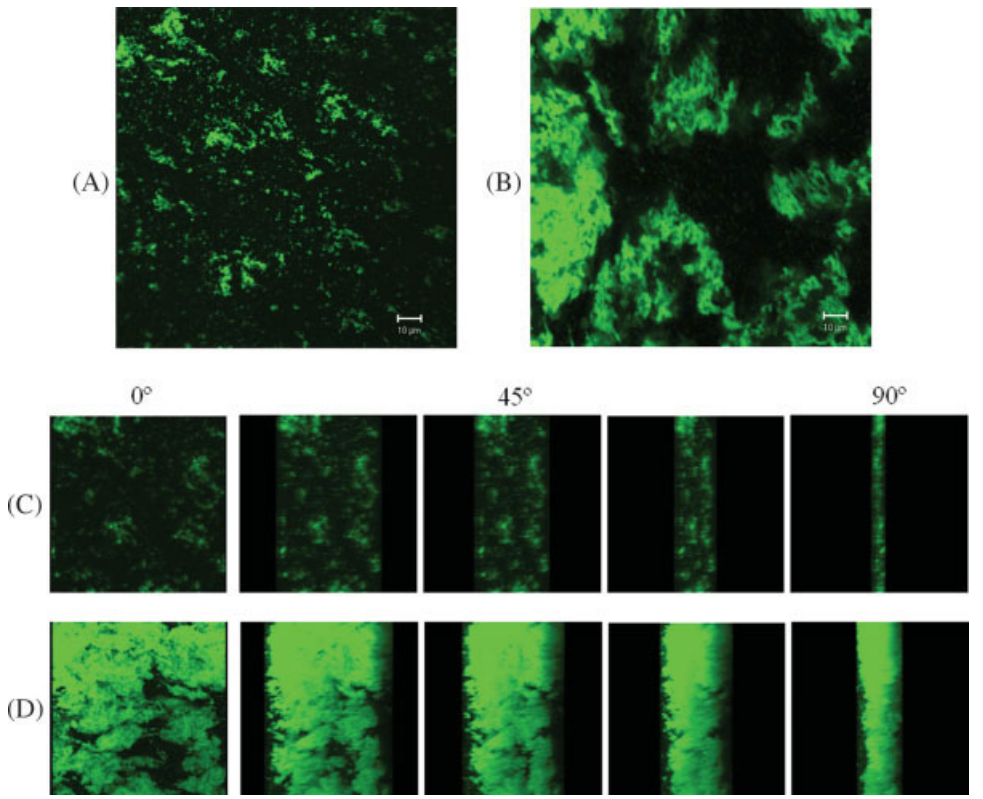

Figure 3 Fluorescence confocal microscopy of (A) low lipid film (1 : 0.2 (wt/wt) chitosan: ePC) and (B) high lipid film (1 : 2.5 (wt/wt) chitosan: ePC) (×63). The chitosan–ePC images of films containing low lipid (C) and high lipid (D) captured using a projection function and animated to observe their cross sections. The bright (fluorescent) regions represent the lipid content within the chitosan–ePC films. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

6.6 Å appeared with lower intensity at $2\theta = 9.0^\circ$, 12.1°, and 20.1°, respectively. The diffraction pattern of the chitosan film (no lipid) resembles an amorphous material, whereas ePC has a large number of strong, sharp peaks confirming that the lipid is a highly crystalline material. Among the observed reflections for ePC, there are well-defined ones (labeled 1, 2, and 3 in Fig. 2) at $2\theta = 1.8^\circ$, 3.6°, and 7.0° that are indicative of a bilayered, lamellar structure.[39,40] The additional peaks are most likely due to other three-dimensional lattices, as ePC is composed of a mixture of PC chain lengths that can have different orientations. ePC also contains amorphous regions that provide a scattering halo at 4.8–4.0 Å. In comparing the diffraction pattern for ePC and the ePC–chitosan films, it is clear that many of the crystalline peaks have disappeared once the ePC is blended with chitosan. The absence of several of the peaks in the diffraction pattern for the blends is an indication that interactions between chitosan and ePC are operative. However, the major crystalline peaks within ePC are still present in the diffraction patterns for the chitosan–ePC blends, demonstrating that there are regions enriched in lipid (i.e., all lipid is not molecularly dissolved throughout the film). From this study it is clear that domains enriched in lipid and lipid–lipid interactions are present within the chitosan–ePC films, even at low ePC content. In addition, from the X-ray analysis it appears that components of the lipid are organized into lamellar-like structures within the blend.

Microscopic evaluation of the chitosan–ePC film

The distribution of the lipid present at low and high amounts (1 : 0.2 and 1 : 2.5 (wt/wt) chitosan: ePC) within the chitosan-based films was examined by laser scanning confocal fluorescence microscopy [Figs. 3(A,B)]. Figures 3(C,D) are three-dimensional images of the low and high chitosan–ePC films being viewed as a projection (from 0° to 90°), as processed by the Zeiss LSM browser software package. The dark (black) regions represent the chitosan component of the film and the bright (fluorescent) regions correspond to the lipid. As shown, the chitosan–ePC films include a continuous chitosan matrix with lipid microdomains dispersed throughout. ePC is present in irregularly shaped domains dispersed throughout the 1 : 0.2 chitosan–ePC film. For the 1 : 2.5 film, the lipid is aggregated and assembled in circular or spiral shaped domains that are also dispersed throughout

the film, which explains its heterogeneous appearance (as discussed previously). The size of the microdomains varies from 1 to 30 μm depending on the lipid content within the blend. The presence of lipid domains within the films supports the results obtained from both thermal and X-ray diffraction analyses. Since the lipid is a low melting material, when compared with chitosan, the regions enriched in lipid may be considered ''islands of mobility''.[41]

The properties of films formed from these phase-separated blends will be determined by the morphology of the blend, the inherent characteristics of each component (i.e., chitosan and lipid), as well as the interactions stabilizing the interfacial regions. From our previous studies it was found that hydrogen bonding and ionic interactions are present between the lipid and chitosan components of the blends.[29] However, the chitosan–lipid interactions are obviously not strong enough to completely override the chitosan–chitosan and/or lipid–lipid interactions present within the films.

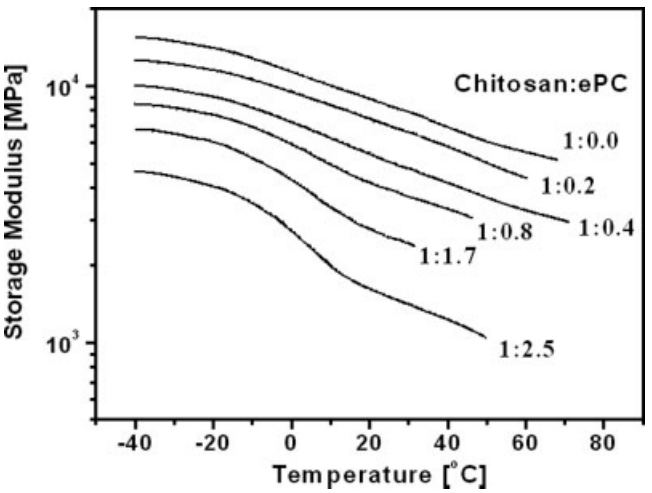

Figure 4 The storage modulus as a function of temperature of the chitosan films containing increasing amounts of ePC (1 : 0.2 to 1 : 2.5 (wt/wt) chitosan: ePC).

Mechanical properties of the chitosan–ePC film

Dynamic mechanical analysis

The mechanical properties of biomaterials are routinely used to evaluate the softness of implantable medical devices to prevent rupture or damage of surrounding tissue and ensure patient compliance.[42] Furthermore, the mechanical strength of a material at physiological temperature provides an indication of the physical deformations that it may sustain *in vivo*. The variations in the storage moduli as a function of temperature for the dry chitosan and chitosan–ePC films are plotted in Figure 4. The storage modulus is a measure of the amount of energy stored as elastic energy and is related to the stiffness of a material.[42] The storage modulus for a film formed from chitosan alone was found to decrease from 15,400 to 5100 MPa as the temperature was increased from −40 to 70°C. Specifically, at physiologically relevant temperatures (i.e., 37°C) the storage modulus for the chitosan film was ~7200 MPa, which is indicative of a strong plastic material.[27]

The storage modulus of the pure ePC could not be measured at room temperature using this method, as the lipid alone does not form a film. Therefore, the addition of ePC to chitosan was used to decrease the modulus of chitosan to produce a softer film. From our results, the storage modulus decreased as the amount of ePC was increased over the entire temperature range examined (Fig. 4). Specifically, at 37°C the 1 : 0.2 and 1 : 2.5 chitosan–ePC films had a storage modulus of ~6000 and 1300 MPa, respectively. The mechanical response of the chitosan–ePC films as a function of temperature supports the results from the DSC analysis.

Pronounced decreases in the storage modulus were observed for the films containing the higher lipid content (1 : 1.7 and 1 : 2.5 (wt/wt)) in the temperature range that corresponds to the T_m of ePC (Fig. 1). In this way, the ePC incorporates ''islands of mobility'' within the chitosan matrix and to some extent disrupts chitosan's hydrogen-bonding network.[41]

Tensile tests

The Young's moduli of the dry chitosan and chitosan–ePC films are in agreement with the trend observed for the storage moduli (Table II). Specifically, the moduli of the chitosan–ePC films decreased as the amount of ePC was increased within the blend. For the 1 : 0.2 and 1 : 2.5 (wt/wt) chitosan: ePC film, the Young's moduli were 2400 and 350 MPa, respectively. The values obtained for the Young's moduli of the chitosan–ePC films are comparable to other chitosan-based blends. For example, Cheng and coworkers found that the Young's moduli of chitosan–gelatin films could be reduced from 1240 to 430 MPa by increasing the gelatin content.[43] For chitosan–keratin blends, chitosan was added to increase the strength and stiffness of the keratin films.[44]

Films used for drug delivery or other medical applications will often be subjected to different volumes of fluid and physical deformation. For this reason, the Young's moduli of wet chitosan–ePC films were measured (Table II). It should be noted that a film composed of chitosan alone dissolved within 24 h when placed in a buffer solution. For the wet chitosan–ePC films, the average Young's modulus (i.e., average modulus = 0.92 ± 0.33 MPa) was 3 orders of magnitude lower than that for the dry films (i.e., average modulus = 1865 ± 1256 MPa). This decrease in

TABLE II
A Summary of the Young's Modulus for Both Dry and Wet Chitosan–ePC Films

Film composition (chitosan:ePC) (wt/wt)	Young's modulus for dry films (MPa)	Young's modulus for wet films (MPa)
1 : 0 (Chitosan)	3849.0 ± 0.7	–
1 : 0.2	2399.1 ± 0.6	0.66 ± 0.04
1 : 0.4	2358.7 ± 0.4	0.58 ± 0.02
1 : 0.8	1177.0 ± 0.2	0.91 ± 0.11
1 : 1.7	1057.6 ± 0.3	1.01 ± 0.06
1 : 2.5	349.3 ± 0.1	1.42 ± 0.2

Films prepared from chitosan alone swelled rapidly when immersed in 0.01*M* PBS and dissolved within 24 h, preventing mechanical analysis. Each value represents the mean value ± standard error of the mean ($n = 3$).

the modulus is mainly attributed to the plasticizing effect of water.[45] The moduli of the wet films were also found to increase as the amount of ePC increased within the blend. This trend is related to the stability of the films as discussed later. The values obtained for the Young's modulus of the wet chitosan–ePC films are comparable to the reported values for poly(lactide-*co*-glycolide) films, which have been commonly explored for biomedical applications.[46,47]

Stability of chitosan–ePC films

The percent weight loss (W_L) of the chitosan–ePC films were examined following incubation in a PBS/FBS solution over a 6-week period at 37°C. Films composed of chitosan alone dissolved within 24 h in the PBS/FBS solution. Chitosan is only soluble in aqueous media with pH < 6; therefore, the complete dissolution of the chitosan film is likely due to the acetic acid that remains within the film following preparation.[29] By contrast, the stability of the chitosan–ePC films increased as the amount of ePC increased within the films and the films remained intact for the entire incubation period. Specifically, after 1 week in the PBS/FBS solution, chitosan: ePC films composed of 1 : 0.2, 1 : 0.8, and 1 : 2.5 (wt/wt) were reduced in weight by 50% ± 4%, 33% ± 1%, and 12% ± 5%, respectively. Following 2-week incubation, the weight of the films was further decreased (65% ± 3%, 45% ± 5%, and 26% ± 7%, respectively); while, after 4 weeks of observation, the low lipid formulations (1 : 0.2 and 1 : 0.8 (wt/wt)) were too fragile to measure the degree of weight loss. At 6 weeks, the film composed of 1 : 2.5 (wt/wt) chitosan : ePC retained its structural integrity and a 33% ± 1% weight loss was recorded. For weeks one and two, a linear relationship was observed between the amount of lipid present and the percent weight remaining. The stability of the film with high lipid content is likely attributed to the overall increase in hydrophobicity of the film that results from the presence of the lipid, as well as the specific interactions present between chitosan and ePC. As discussed in our previous report, and confirmed by FTIR analysis, the presence of interactions between chitosan and ePC lead to the displacement of acetic acid from the film during preparation.[29] As evidenced in these studies, the displacement of acetic acid from the films during preparation prevents the dissolution of the film upon incubation in aqueous media. Following preparation, the chitosan–ePC interactions act to enhance the structural integrity of the films. Therefore, it seems that the lipid component of the film is largely responsible for the film's stability. However, not only is ePC alone unable to form a film, but phase separation is more prevalent when the amount of ePC is increased within the blend (Fig. 3), which proves the materials are not completely miscible. Overall, this demonstrates that phase-separated blends of partially miscible materials may be considered for preparation of stable films.

CONCLUSIONS

From our results, it is clear that chitosan and ePC form phase-separated blends and are compatible, partially miscible biomaterials. Furthermore, these studies reveal that specific components of the ePC lipid interact with chitosan. Fluorescence scanning confocal microscopy of the chitosan–ePC films demonstrated that the lipid is present as microdomains, dispersed throughout the chitosan matrix. The composition and morphology of the film were also related to the mechanical properties of the blend. Specifically, as the amount of lipid increased within the blend, the size of the microdomains increased and the stiffness of the dry chitosan–ePC films decreased. It was also found that the stability as well as the Young's modulus for the wet films increased as the amount of ePC increased within the film. Therefore, as the extent of phase separation increased, the mechanical properties and the stability of the films improved. Overall, this study further demonstrates that partially miscible chitosan-based materials may be exploited for specific applications. Future studies will employ the relationships established in this work, to tailor the biological performance of the chitosan–ePC system for use in drug delivery.

The authors thank Arpana Bhatnagar, Dr. Zhihui Yin, and Dr. Sergei Petrov for their assistance.

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