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Chitosan–g-PLGA copolymer as a thermosensitive membrane

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ABSTRACT

A thermosensitive copolymer was synthesized by graft copolymerization of poly(lactide-co-glycolide) (PLGA) copolymers onto the surface of chitosan membranes. Acryloyl chloride was used as a coupling reagent for the covalent attachment of PLGA to the chitosan membranes. FTIR spectroscopy and DSC analysis were used to characterize the resulting graft copolymer. Thermosensitive swelling behaviors of the copolymer were investigated as well. The membranes exhibited reversible swelling–shrinking behavior; higher swelling ratios were obtained observed at higher temperatures. Drug permeation studies were carried out using vancomycin hydrochloride and betamethasone sodium phosphate as the model drugs. The permeability coefficient of vancomycin was found to be a discontinuous function of temperature; the permeability increased steeply above the upper critical solution temperature (UCST) of the membranes. Considering the high biocompatibility of chitosan and PLGA, these thermosensitive chitosan–g-PLGA membranes might be used to develop an intelligent drug delivery system.

1. Introduction

Chitosan, a polysaccharide derived from naturally abundant chitin (Abdou, Nagy, & Elsabee, 2008), is currently receiving an enormous deal of interest for medical and pharmaceutical applications due to its low toxicity, good biocompatibility and biodegradability (Alves & Mano, 2008; Jian, Sharma, & Vyas, 2006; Muzzarelli, 2009; Ravi-Kumar, 2000; Ta, Dass, & Dunstan, 2008). In the past few years, a great deal of attention has been paid to chitosan-based temperature-sensitive copolymers, which undergo reversible volume or sol–gel phase transitions in response to temperature. Thermosensitive solutions and hydrogels based on combinations of chitosan and glycerophosphate disodium salt has been developed (Chenite, Buschmann, Wang, Chaput, & Kandani, 2001; Ganji, Abdekhodaie, & Ramazani, 2007; Ruel-Gariepy, Leclair, Hildgen, Gupta, & Leroux, 2002). The formation of three-dimensional physical hydrogels of chitosan via the enzymatic hydrolysis of urea has been investigated by Chenite, Gori, Shive, Desrosiers, and Buschmann (2006). Thermally sensitive hydrogels of chitosan have been developed by graft copolymerization of chitosan and *N*-isopropylacrylamide (Gholap & Badiger, 2004; Lee, Ha, Cho, Kim, & Lee, 2004; Qing & Yue, 2006), graft copolymerization of chitosan and poly(ethylene glycol) (PEG) (Bhattarai, Ramay, Gunn, Matsen, & Zhang, 2005) and block copolymerization of chitosan and PEG (Ganji & Abdekhodaie, 2008).

In the present work, a thermosensitive membrane consisting of chitosan and poly(lactide-co-glycolide) (PLGA) is presented PLGA and other poly(α-hydroxy acid)s, such as poly(lactide) (PLA) or poly(glycolide) (PGA), have been used as carriers for controlled delivery of a wide range of bioactive agents, such as hormones, steroids, antibiotics and anti-cancer agents (Mohamed & Van Der Walle, 2008; Wang, Wu, Li, & Feng, 2000). Many methods have been used to combine chitosan with poly(α-hydroxy acid)s (Mi, Lin, Wu, Shyu, & Tsai, 2002; Perugini, Genta, Conti, Modena, & Pavanetto, 2003; Ravi-Kumar, Bakowsky, & Lehr, 2004; Vila, Sànchez, Tobío, Calvo, & Alonso, 2002; Wang et al., 2003). Surface modification of poly(L-lactic acid) (PLLA) by entrapment of chitosan and its derivatives was reported by Liu, Jiao, Zhang, and Zhou (2007). This surface modification significantly promoted the compatibility of PLLA films with osteoblasts. Recently, chitosan–PLGA composite fibrous scaffolds were developed by homogeneous blending of chitosan and PLGA at a ratio of 50:50 (w/w) (Moshfeghian, Tillman, & Madihally, 2006). Fabrication of fibrous matrices using this technique resulted in a characteristic soft and strong mechanical property that could not be obtained by either PLGA or chitosan fibers alone.

Although there are some experimental investigations concerning chitosan/PLGA combinations, to the best of our knowledge, few studies have been reported on the grafting of PLGA and chitosan. In this study, the chitosan–g-PLGA membranes were prepared by grafting PLGA onto the surface of chitosan films.

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Fourier-transform infrared spectroscopy (FTIR) and differential scanning calorimetry (DSC) techniques were used to characterize the synthesized copolymers. Dynamic and equilibrium swelling of the chitosan–g-PLGA membranes and the chitosan films were compared. Drug permeation studies with the chitosan–g-PLGA copolymer were carried out using vancomycin hydrochloride and betamethasone sodium phosphate as the model drugs to evaluate thermosensitivity of the membranes.

2. Materials and methods

2.1. Materials

Medium molecular weight chitosan ($M_w = 2.5 \times 10^5$, DDA = 82.5%) and vancomycin hydrochloride and betamethasone sodium phosphate were purchased from Sigma–Aldrich Chemical Co. (USA). PLGA (RG 504, M_w = 48,000 Da) having a lactide/glycolide ratio of 50/50 was purchased from Boehringer Ingelheim (Germany). Acryloyl chloride, triethylamine, dried 1,2-dichloromethane, glacial acetic acid, dimethylformamide (DMF) and acetone were purchased from Merck (Germany) and used as received. Ethyl ether was purchased from Panreac (E.U.) and dehydrated carefully.

2.2. Preparation of thermosensitive membranes

The process of membrane preparation consisted of three steps: (1) synthesis of acrylate-terminated PLGA (PLGA-A), (2) preparation of chitosan films and (3) synthesis of chitosan–g-PLGA membranes.

2.2.1. Step 1: Preparation of acrylate-terminated PLGA

In a 250 mL, two-necked, round-bottomed flask with a glass stirrer, 5 g of PLGA was dissolved in 100 mL of dehydrated dichloromethane and cooled to 4 °C for 30 min. Next, 5 mL of triethylamine was added drop-wise to the cold PLGA solution while it was stirred, and the final solution was mixed for another 15 min. An excess amount of acryloyl chloride was added to the reaction vessel, and the solution was stirred at room temperature under light vacuum for 48 h. The resultant mixture was filtered to remove solid by-product. Placing the filtrate in an excess amount of anhydrous ethyl ether extracted the PLGA-A. Purification of the product was achieved by extensive extraction with ethyl ether. The precipitant was dried under vacuum for 72 h to remove all solvents.

2.2.2. Step 2: Preparation of chitosan films

A typical solvent cast-evaporation method was used for the preparation of chitosan films. Clear solutions of chitosan were obtained by dissolving chitosan (2% w/v) in an aqueous solution of acetic acid (0.1 M). The polymer solution was cast onto the petri-dishes, and the solvent was slowly evaporated at room temperature and dried under high vacuum. Disc-shaped films were fabricated using a punch with a diameter of 1 cm.

2.2.3. Step 3: Synthesis of chitosan–g-PLGA membranes

In order to swell the chitosan films and consequently assist the graft reaction, the dry chitosan films were placed into a DMF/glacial acetic acid mixture (50:50 ratio) containing various amounts of PLGA-A and left overnight. Over the next three hours, the reaction was carried out at 110 °C under constant stirring The resultant films were frequently washed with DMF and acetone for two days to remove the un-reacted PLGA-A. The final product was rinsed with deionized distilled water and dried under vacuum at room temperature.

2.3. Characterization of chitosan–g-PLGA membranes

Fourier-transform infrared spectra of PLGA, PLGA-A, chitosan and chitosan–g-PLGA copolymer were recorded on a Mattson 1000 FTIR spectrometer to confirm the success of the graft copolymerization. Spectra of PLGA, PLGA-A and chitosan were obtained using the potassium bromide disk method, while the copolymers were acquired directly.

The glass transition temperatures (T_g) of the membranes were determined by a Perkin-Elmer DSC-7 differential scanning calorimeter. The instrument was calibrated with an indium standard and distilled water. The membrane samples were accurately weighed into aluminum pans and sealed. The DCS runs were conducted over a temperature range of 0–250 °C at 10 °C/min under a dry nitrogen flow (40 mL/min). The T_g values were measured as the temperature at which a change in heat capacity occurred.

2.4. Membrane swelling

Dried polymer disks were weighed and immersed in a phosphate buffer solution (PBS, pH = 7.4) in a shaker bath held at 30 °C. The samples were taken out at regular intervals, blotted to remove excess liquid and immediately weighed. The swelling index of the membranes was quantified using the following equation (Perugini et al., 2003):

$$\text{Swelling Index, } S = (W_2 - W_1)/W_1 \quad (1)$$

where W_1 and W_2 are the weight of the film before and after immersion in the swelling medium, respectively.

To check the thermosensitive properties of chitosan–g-PLGA copolymers, the temperature-dependent swelling behaviors of the membranes were determined over the temperature range of 15–40 °C in 5 °C steps. The dry membranes were placed in a phosphate buffer solution (PBS, pH = 7.4) in a shaker bath at a fixed temperature and allowed to reach equilibrium. The equilibrium swelling ratio was calculated based on the following equation:

$$\text{Equilibrium Swelling Ratio} = (W_{eq} - W_1)/W_1 \quad (2)$$

where W_{eq} is the weight of swollen membranes at equilibrium.

2.5. Drug permeability

Two-compartment glass diffusion cells were used to determine the permeability of the membrane as a function of temperature. The donor cell was filled with 25 mL of drug solution, and 25 mL of distilled water was placed into the receptor cell. The thickness of the polymer membrane was 0.8 mm, and the available diffusion area was 0.89 cm^2. The temperature of the diffusion cell was maintained by circulating water in its glass jackets. An internal bar magnet stirred at 600 rpm throughout all experiments. The temperature was varied from 24 to 36 °C in 3 °C steps. At appropriate intervals, aliquots (5 mL) were withdrawn from the receptor cell and replaced with fresh distilled water. The concentration of permeated drug was assayed at 285 nm using a Schimadzu UV-160 spectrophotometer.

3. Results and discussion

3.1. Preparation of thermosensitive membranes

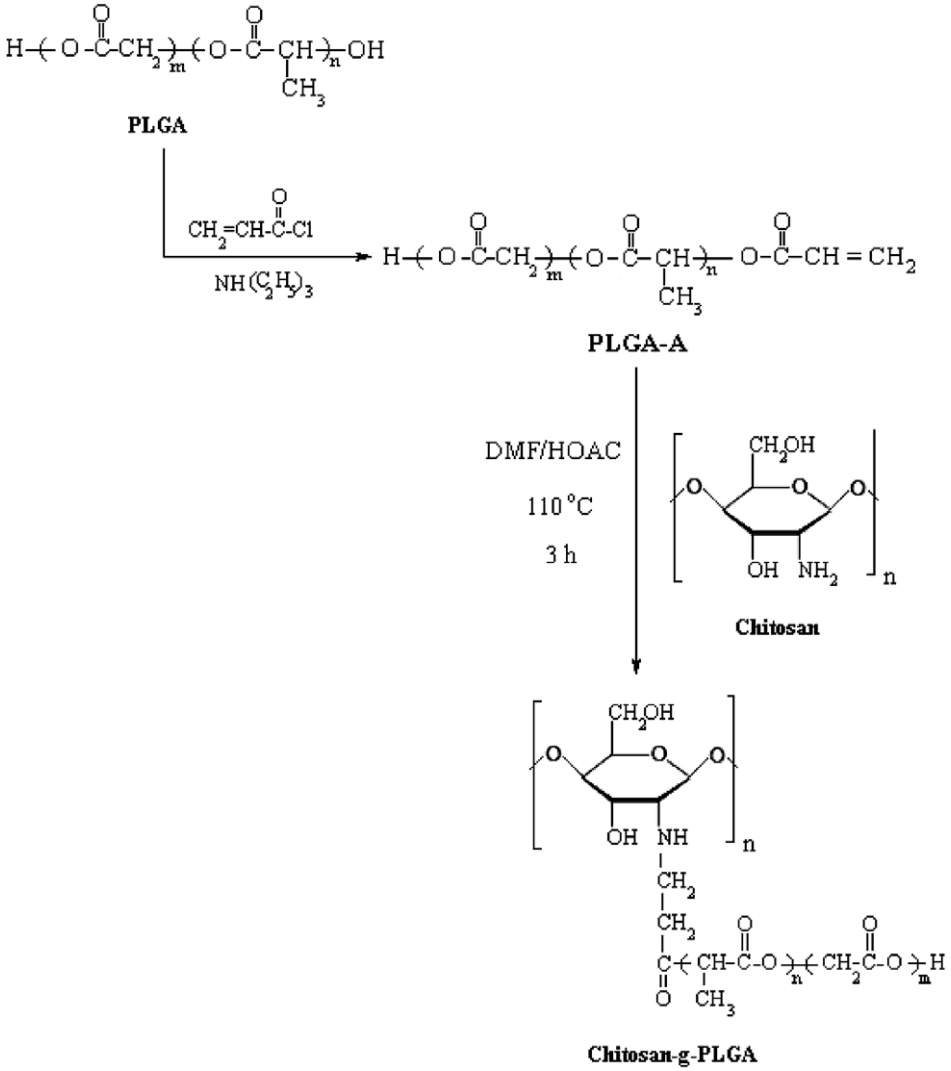
The PLGA macromere was copolymerized with chitosan macromolecules in order to impart hydrophobicity and thermosensitivity to the chitosan membranes. The PLGA hydroxyl end-groups reacted with acryloyl chloride to form activated PLGA (PLGA-A). The activated PLGA was grafted on the previously swollen chitosan

membranes (Scheme 1). It has been reported that a mixture of DMF and acetic acid in the ratio of 50:50 is a good swelling agent for chitosan powder (Silva, Menezes, & Garcia, 2003). Due to the presence of amino groups, chitosan swells in acidic media. This swelling is in response to the electrostatic repulsion between the NH_3^+ of chitosan chains, which leads to enhanced swelling of the polymer network. Therefore, a DMF/acetic acid mixture in the ratio of 50:50 was chosen as the reaction media. Various amounts of PLGA-A were used to obtain different compositions of the chitosan–g-PLGA copolymers (Table 1).

The grafting reaction of PLGA-A onto the surface of chitosan membranes is occurred mainly through Michael addition reaction. The Michael addition involves the addition of a nucleophile, also called a 'Michael donor' to an activated electrophilic olefin, the 'Michael acceptor', resulting in a 'Michael adduct' (Mather, Viswanathan, Miller, & Long, 2006). Scheme 1 shows the grafting of PLGA-A onto chitosan would occur by reaction between the NH_2 groups in chitosan (Michael donor) with the unsaturated C=C double bond in the activated PLGA (Michael acceptor). Similar results were published for Michael addition of polyurethane onto the surface of chitosan films (Silva et al., 2003). Utilizing the Michael addition in heterogeneous conditions, Silva et al. showed that the NH_2 groups are more reactive than OH groups in the chitosan repeating units. In a same work, Tsubokawa et al. modified the chitosan surface amine groups through the Michael reaction with methyl acrylate followed by alternate reactions with ethylene diamine and further methyl acrylate (Tsubokawa & Takayama, 2000). Modification of chitin with small molecules was also achieved through Michael addition to pendant amine groups. Reaction with acrylonitrile yielded cyanoethylated chitin (Mather et al., 2006; Tokura, Nishi, Nishimura, & Ikeuchi, 1983) and reaction with ethyl acrylate afforded ester-containing chitin (Aoi et al., 2000; Mather et al., 2006).

3.2. FTIR study of chitosan–g-PLGA membranes

A comparative FTIR spectrum of PLGA, PLGA-A, chitosan and chitosan–g-PLGA copolymers is shown in Fig. 1. The spectrum of PLGA shows absorbance peaks at 745 (CH-bend), 1095 and 1180 (C—O stretch), 1395 (CH-bend), 1750 (C=O ester) and 2900 (CH_2-bend) cm^{-1} (Fig. 1a). A broad peak at 3550 cm^{-1} illustrates the vibration of OH terminal groups in PLGA. The reaction between acryloyl chloride and hydroxyl groups of PLGA leads to a decrease of the OH-stretching vibration peak around 3450 cm^{-1} (Fig. 1b). Furthermore, two new peaks appearing at 1430 and 1630 cm^{-1} could be assigned to the presence of carbon–carbon (C=C) double bonds. The FTIR spectrum of chitosan is shown in Fig. 1c. A sharp band at 3450 cm^{-1} is due to NH_2 and OH-stretching vibration in



Scheme 1. Schematic representation of the graft copolymerization of chitosan and PLGA.

Table 1
Compositions and characteristics of samples prepared with different molar ratios of reactive agents.

	Samples No.			
	CP1	CP2	CP3	CP4
Chitosan concentration (% w/v)	2	2	2	2
PLGA-A concentration in reaction medium (% w/v)	10	15	20	25
PLGA-A graft wt%[a]	5.3	8.9	14.5	18.4
Ratio of carbonyl groups per chitosan repeating unit[b]	0.09	0.13	0.24	0.31
Equilibrium swelling ratio	17.4	15.9	10.5	8.1
T_g (°C)	203	158	126	98

[a] The graft wt% was calculated by the following relation: $(W_t - W_c)/W_t \times 100$, where W_t and W_c are the weight of chitosan–g-PLGA and chitosan membranes, respectively.
[b] Ratio of carbonyl groups per chitosan repeating unit in the product determined by ^{1}H NMR spectra.

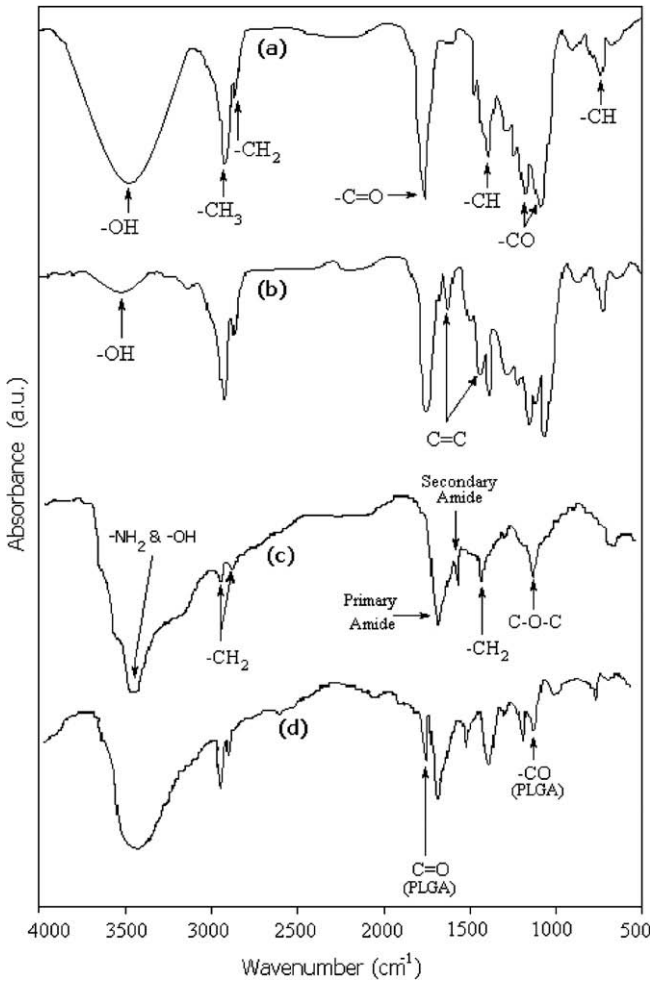


Fig. 1. FTIR spectra of (a) PLGA, (b) PLGA-A, (c) chitosan and (d) the chitosan–g-PLGA copolymer.

the chitosan matrix. Further, in the C—H stretch region of the FTIR spectrum, the higher intensity peak at 2940 cm^{-1} and the lower intensity peak at 2890 cm^{-1} are assigned to the asymmetric and symmetric modes of CH_2, respectively. In addition, the characteristic band due to CH_2 scissoring, which usually occurs at 1400 cm^{-1}, was also present in the spectrum. The peaks at 1660, 1550 and 1120 cm^{-1} are assigned to strong N—H bending vibration of the primary and secondary amides and C—O stretching vibration of the ether linkage in the chitosan backbone, respectively. Fig. 1d illustrates the FTIR spectrum of the chitosan–g-PLGA copolymer. The presence of a new peak at 1740 cm^{-1} could point to the existence of ester carbonyl groups in the chitosan chain, which come from PLGA-A. The absence of the two peaks at 1420 and 1630 cm^{-1} that were assigned to unsaturated carbon–carbon double bounds, together with a displacement of the secondary amide deformation band from 1550 to 1480 cm^{-1}, suggests that the grafting reaction occurred mainly by the reaction between the NH_2 groups in chitosan and the CH_2═CH— groups in PLGA-A.

3.3. DSC of chitosan–g-PLGA membranes

Differential scanning calorimetry was performed to confirm the chemical reaction between chitosan and PLGA. The DSC scans for pure PLGA and chitosan were carried out in the temperature range of 0–250 °C (Fig. 2a). The glass transition temperature (T_g) for PLGA was detected at 55 °C; however, there was no evidence of any endothermal melting peak. The DSC thermogram of chitosan showed a broad endothermic peak between 60 and 100 °C, which could be attributed to the moisture content in the polysaccharide backbone. To eliminate the effect of moisture, two cycles of heating and cooling runs were adopted; the results of the second heating run are also shown in Fig. 2a. The method is reported to eliminate the effect of moisture on T_g (Dong, Ruan, Wang, Zhao, & Bi, 2004), and the same results were obtained in this study. Although chitosan has crystalline regions, the glass transition temperature is not found because the rigid-rod polymer backbone has strong inter- and intra-molecular hydrogen bonding. It has been reported that chitosan exhibits an exothermic degradation peak around 300 °C (Dong et al., 2004), which is not observed in the present temperature range. The glass transition temperatures for copolymers CP1, CP2, CP3 and CP4 were detected around 203, 158, 126 and 98 °C, respectively (Fig. 2b). Apparently, the glass transition temperatures of copolymers were much higher than that of PLGA and increased with increasing chitosan content. These results confirm the successful grafting of PLGA with chitosan membranes.

3.4. Swelling of the membranes

The effect of the modification of chitosan membranes with PLGA is evident in the dynamic swelling study; the water absorption capacity of copolymers is much lower than that of chitosan (Fig. 3). Swelling measurements were not completed for chitosan membranes due to their physical instability in aqueous medium. Bonding the hydrophobic PLGA copolymer onto the chitosan films improves the water resistance properties and reduces their water uptake capability.

In order to check the thermosensitive properties of membranes, studying the swelling behavior or wettability assessment of membranes at different temperature is a common method (Wu, Li, Han, & Liu, 2006; Yang, Yang, Lin, Wu, & Chen, 2008; Zhang et al., 2009). The equilibrium swelling ratios of different chitosan–g-PLGA membranes at various temperatures are illustrated in Fig. 4. Apparently, the membranes show thermo-positive swelling behavior, where higher swelling ratios were obtained at higher temperatures. It is also clear that the swelling of the membranes decreased with

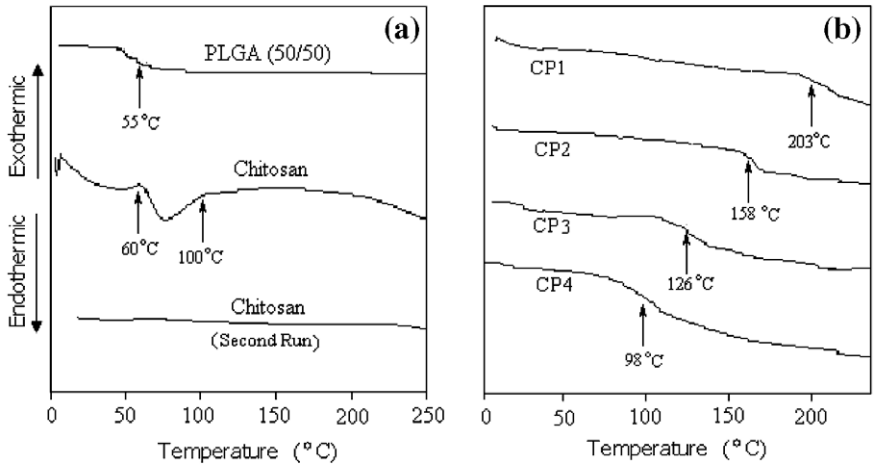


Fig. 2. DSC thermograms of (a) PLGA and chitosan samples and (b) chitosan–g-PLGA copolymers obtained from the second run at a heating rate of 10 °C/min.

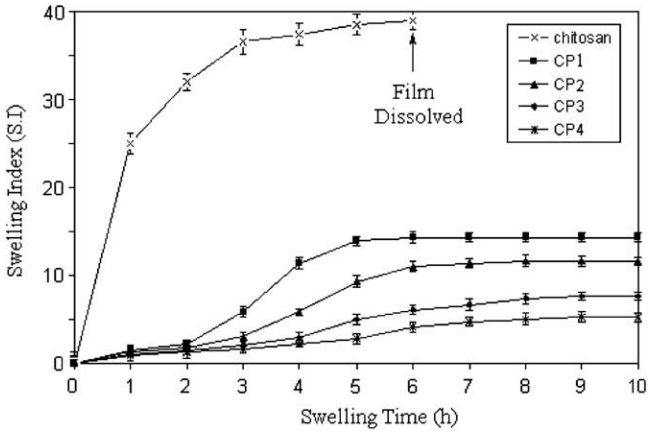


Fig. 3. Dynamic swelling behavior of membranes versus time in a buffer solution (pH = 7.4, T = 30 °C).

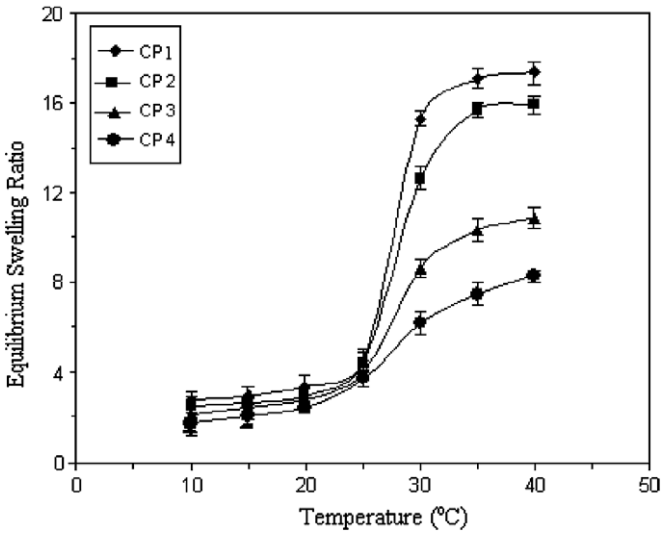


Fig. 4. Effect of temperature on the equilibrium swelling ratio of chitosan–g-PLGA membranes.

increasing degree of PLGA grafting (Table 1). The swelling of chitosan–g-PLGA membranes varied only slightly until 25 °C, then it increases rapidly with increasing temperatures from 25 to 35 °C (Fig. 4).

Since chitosan is more hydrophilic than PLGA, the chitosan–g-PLGA copolymers exhibit different degrees of hydration depending on the chitosan/PLGA ratio in the copolymer. The presented results indicate that the ratio of hydrophilic and hydrophobic co-monomers in chitosan–g-PLGA membranes plays an important role in their swelling behavior (Table 1 and Fig. 4). The minimum amount of grafted PLGA that results in the desired swelling characteristics is approximately 5%. On the other hand, excess PLGA grafting (>18.5%) suppresses the equilibrium swelling ratios. The copolymers CP1 and CP2 showed a sharp change in the swelling ratios as the temperature rose from 25 to 35 °C, while those of CP3 and CP4 varied less (Fig. 4).

The swelling behavior of chitosan–g-PLGA membranes may due to chitosan crosslinking. This phenomenon suggested the possibility of the formation of a growing chain on the activated-PLGA macromere. Therefore, we are now conducting a new series of experiments aiming to further study the governing reaction mechanism.

Fig. 5 represents temperature-dependent swelling behavior of CP1 in response to consecutive temperature change between 25 and 35 °C. The presented results indicate that the swelling behavior exhibited by the CP1 and CP2 was reversible. Obviously, the swollen membrane reverted back to the shrink state while lowering the temperature from 35 to 25 °C. Again, while increasing the temperature from 25 to 35 °C, the membrane attained the swelling state.

The thermo-reversibility of swelling in polymeric membranes may be related to the competition between the different molecular forces involved in hydrogel swelling. Based on the Flory–Rehner theory, the thermodynamic force of mixing and the retractive forces of the polymer chains are the two opposing forces compromising the swelling behavior. The electrostatic repulsion between the NH_3^+ as well as the hydrophobic interactions, originate from polarity variation due to temperature changes in chitosan chains (Karlström, Carlsson, & Lindman, 1990), are the most important forces governing the water uptake in chitosan-based membranes. Since the hydrophobic forces are known to be temperature dependent (Chenite et al., 2000), changing the medium temperature could results to the probable competition between the electrostatic repulsion and hydrophobic forces of chitosan chains.

3.5. Permeation of drugs through chitosan–g-PLGA membranes

To confirm the thermosensitivity of chitosan–g-PLGA membranes, the drug release profiles for two drug agent, vancomycin

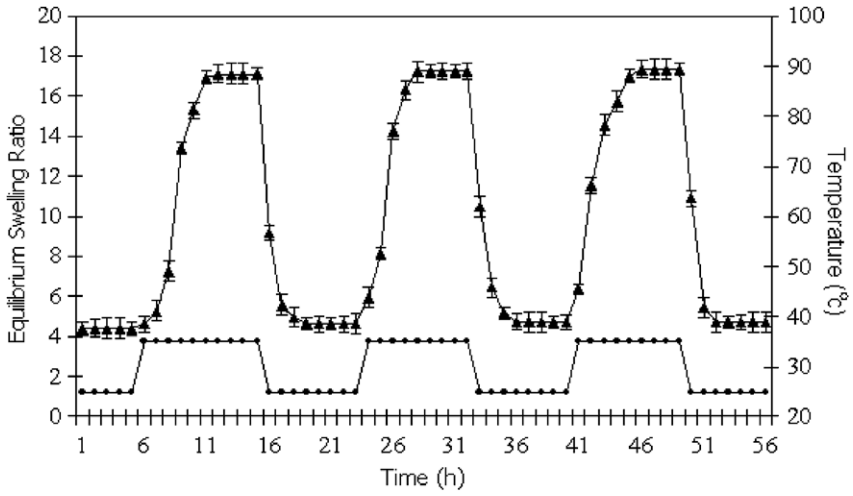


Fig. 5. Reversible changes in swelling ratios (▲) of CP1 membrane in response to consecutive temperature (●) change between 25 and 35 °C.

hydrochloride and betamethasone sodium phosphate were investigated.

Fig. 6a shows the permeated amount of vancomycin hydrochloride through CP1 membrane at different temperatures versus time. The presented results indicate that the permeated amount of vancomycin through the CP1 membrane was a function of temperature. Almost no permeation of vancomycin was observed below 24 °C. The permeation started at 25 °C and increased rapidly with increasing temperature. The amount of vancomycin that permeated at 36 °C was nearly 13 times higher than that at 27 °C. The same results were obtained for betamethasone sodium phosphate (Fig 6b), indicating the thermosensitive release from CP1 membrane. However, the overall release rate of betamethasone is higher than vancomycin, which could be assigned to its higher water solubility.

The values of vancomycin permeability were determined from the slope of the permeation curves shown in Fig. 6. The effect of temperature on the permeability of vancomycin is shown in Fig. 7. Permeability of vancomycin changed slightly until 27 °C and then it markedly increased with an increase in temperature to 30 °C. This interesting result could be assigned to the steep swelling behavior of the chitosan–g-PLGA membranes.

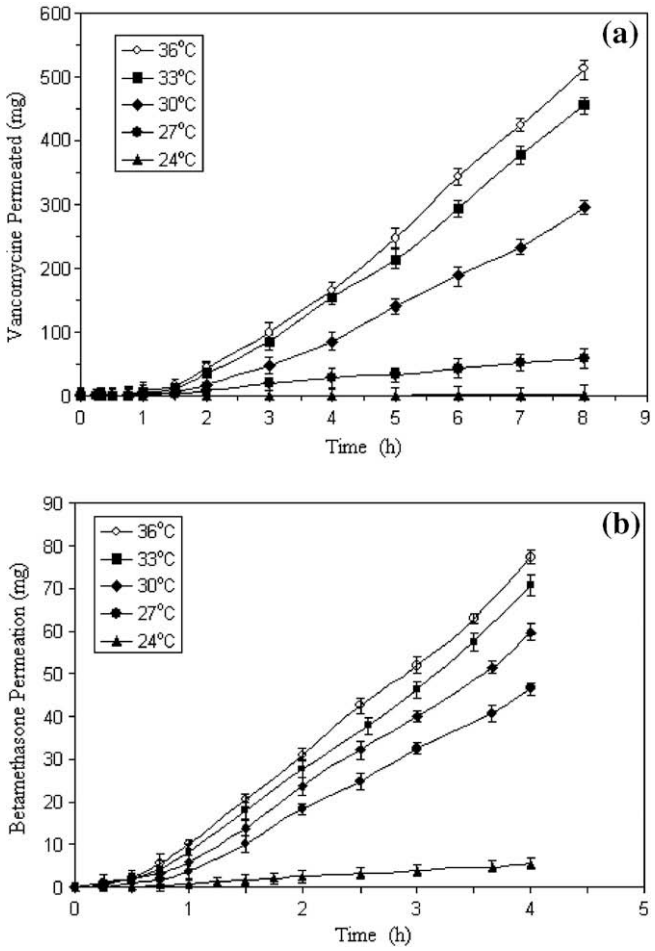


Fig. 6. Effect of temperature on (a) vancomycin and (b) betamethasone permeation through CP1 membrane.

4. Conclusion

A chitosan–g-PLGA copolymer was synthesized and characterized as a thermosensitive membrane by graft copolymerization of previously activated poly(lactide-co-glycolide) (PLGA) copolymers onto the surface of chitosan membranes. Chitosan was graft copolymerized with PLGA copolymer, which had been previously activated. These membranes underwent a sharp temperature-dependent swelling–shrinking behavior around body temperature. Drug permeation studies indicated that the chitosan–g-PLGA

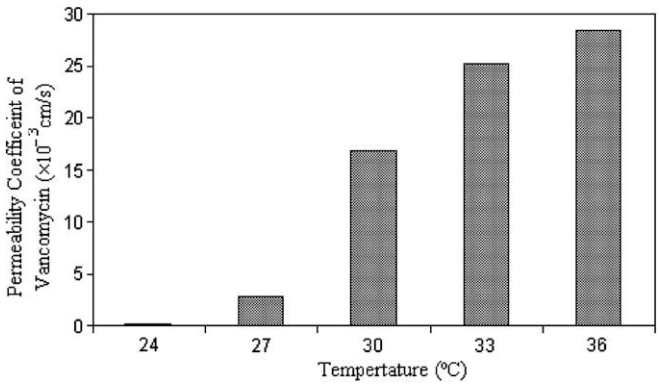


Fig. 7. Effect of temperature on steady-state permeability coefficient (*P*) of vancomycin hydrochloride through CP1 membrane.

membranes could be used as an efficient on–off drug delivery system for biomedical applications.

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