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Biodegradable MPEG-g-Chitosan and methoxy poly(ethylene glycol)-b-poly (ε-caprolactone) composite films: Part 1. Preparation and characterization ☆

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ABSTRACT

In this paper, composite films of MPEG-g-Chitosan (MPEG-g-CS) and methoxy poly(ethylene glycol)-b-poly(ε-caprolactone) (MPEG-b-PCL) were prepared by casting/solvent evaporation method. MPEG-b-PCL was synthesized by ring-opening polymerization of ε-caprolactone initiated by MPEG (M_n = 2000). And MPEG-g-CS was synthesized by alkylation of chitosan followed by Schiff base formation. Characterization of composite films was performed by fourier transform infrared spectroscopy (FTIR), differential scanning calorimeter (DSC), thermogravimetric analysis (TGA) and scanning electron microscopy (SEM). Chemical composition of composite film had strongly influenced the thermal properties, morphology, water absorption and *in vitro* degradation behavior of films. With the increase of MPEG-g-CS content in composite films, the water absorption and degradation rate increased accordingly. The composite films based on MPEG-g-CS and MPEG-b-PCL prepared in this work might have great potential in the application of wound healing and tissue engineering.

1. Introduction

Chitosan, the only cationic polysaccharide in nature, is composed of N-acetylglucosamine (GlcNAc) and glucosamine (GlcN) residues. As the product of deacetylation of chitin, chitosan could only be dissolved in some acidic solution when pH value was below 6 (Jayakumar, Prabaharan, Reis, & Mano, 2005; Mourya & Inamdar, 2008). However, it is currently being studied intensively for its applications in pharmaceutical science, cosmetics, biomedical fields, agriculture, and food industries as well as in sewage treatment, paper industry, etc., because of its favorable properties such as low-toxicity, good biocompatibility, biodegradability, mucoadhesive, etc. (Cathell et al., 2008; Hejazi & Amiji, 2003; Ravi Kumar, 2000; Sashiwa & Aiba, 2004). Recently, chitosan and its derivatives have been fabricated into films for various medical applications such as tissue engineering, wound healing and so on (Li, Chen, Yin, Yao, & Yao, 2007; Ravi Kumar, 2000). However, some shortcomings made chitosan film unwelcome in medical application due to its high sensitivity to water as well as only dissolution in acidic solution during the preparation process. In order to exploit the unique properties of this versatile cationic polysaccharide, attempts are being made to modify chitosan, realizing its full potential (Jayakumar et al., 2005; Ravi Kumar, 2000; Sashiwa & Aiba, 2004). Among these chitosan derivatives, PEGlation chitosan has gained considerable attention because PEG is a polymer that bas been approved by Food and Drug Administration (FDA) for excipient. According to the previous report of Muslim et al. (2001), we also successfully synthesized the PEGlation of chitosan to improve the water-solubility of chitosan and optimize its properties.

Polymer blending technology is an effective way to obtain new polymeric materials with optimized properties without the complicated process of synthesizing the totally new ones. Other advantages of this technology include versatility, simplicity, and inexpensiveness (Li et al., 2007; Rodrigues et al., 2008; Thanpitcha, Sirivat, Jamieson, & Rujiravanit, 2006). Blending method has been successfully used to mix some hydrophobic biodegradable polyesters such as poly(ε-caprolactone) (PCL), and polylactide (PLA) with chitosan film to improve its water-resistant properties (Jiao, Liu, & Zhou, 2007; Wu, 2005). MPEG-b-PCL is a biodegradable and biocompatible copolymer, has been widely used in the pharmaceutical applications (Zhou, Deng, & Yang, 2003). Here, we report on casting/solvent evaporation method to prepare a novel composite film based on MPEG-b-PCL and MPEG-g-CS. A series of composite films with various weight ratios of MPEG-g-CS and MPEG-b-PCL were prepared. Thermal properties and morphological characterization as well as the water absorption and *in vitro* degradation behavior of films were investigated and discussed in this paper.

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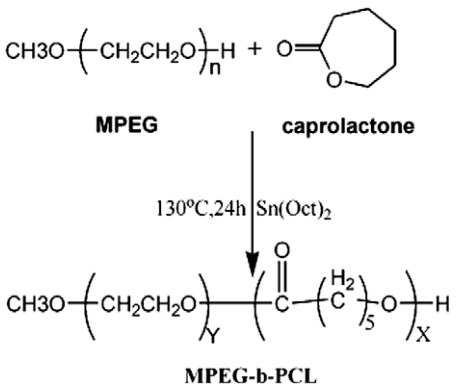
The further application of these composite films in wound healing or tissue engineering will be reported later.

2. Materials and methods

2.1. Materials

ε-Caprolactone (CL), poly(ethylene glycol) methyl ether (MPEG, M_n = 2000), stannous octoate ($Sn(Oct)_2$), and chitosan (with 92% degree of deacetylation (DD)) with ~200 kDa were obtained from Sigma–Aldrich. All the materials used in this article were analytic reagent (AR) grade, and used as received.



Scheme 1. Synthesis scheme of MPEG-b-PCL.

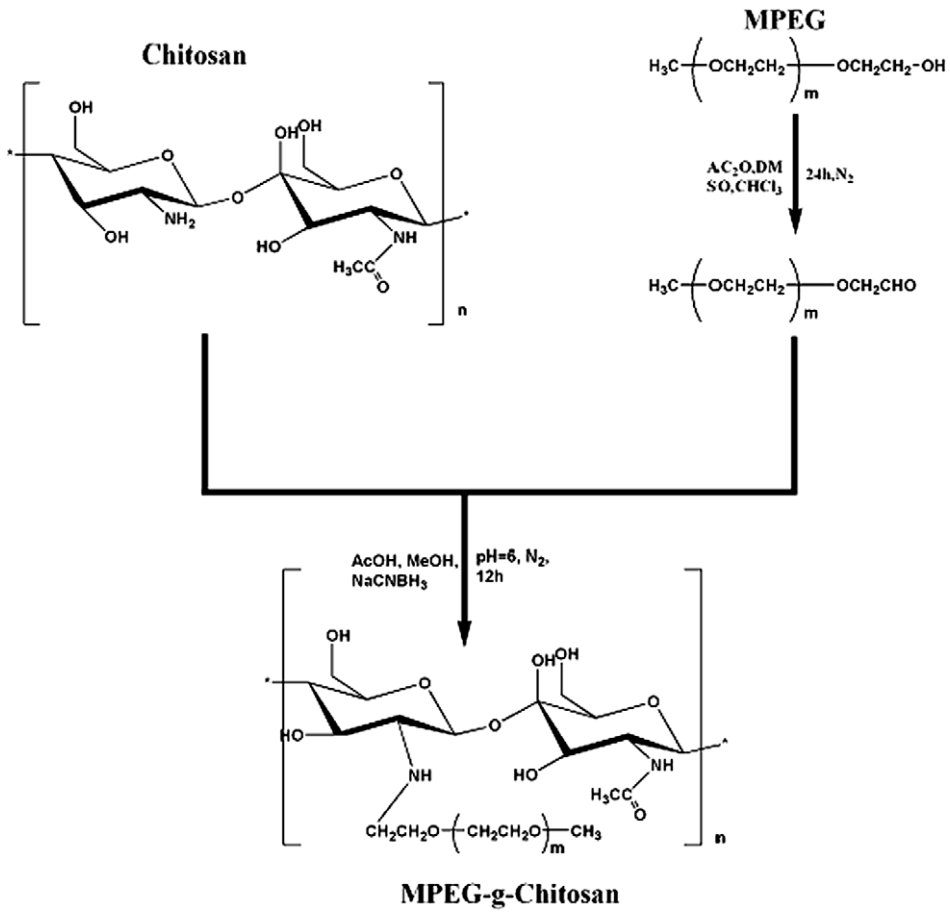
2.2. Synthesis of MPEG-b-PCL

MPEG-b-PCL was synthesized by ring-opening polymerization of ε-caprolactone (0.1 mol) at 130 °C for 24 h initiated by MPEG (0.01 mol) using stannous octoate ($Sn(Oct)_2$) as catalyst, which is similar to the method reported previous (Gong et al., 2007). The process of synthesis was presented in Scheme 1. The obtained MPEG-b-PCL was precipitated from petroleum ether and dried in vacuum at 25 °C. The purified polymers were kept in desiccators before the further use.

2.3. Synthesis of MPEG-g-CS polymer

According to the report of Muslim et al. (2001), MPEG-g-CS was prepared by the same method with little modification as shown in Scheme 2. Briefly, MPEG–aldehyde was first prepared by oxidation of MPEG with DMSO/acetic anhydride. After MPEG was completely dissolved into anhydrous DMSO/chloroform solution (90/10, v/v), acetic anhydride was added gradually into the reaction system under the protection of nitrogen atmosphere. The molar ratio of acetic anhydride to MPEG was 12. The reaction system was kept at ambient temperature for 24 h under the nitrogen atmosphere and finally precipitated with excess pre-cold diethyl ether. The mixture was filtered and the obtained crude product of MPEG–aldehyde was re-precipitated twice from chloroform solution with pre-cold diethyl ether. After the drying process, the white PEG–aldehyde powder was obtained and kept in desiccators for further use.

MPEG-g-CS was synthesized by alkylation of chitosan followed by Schiff base formation. MPEG–aldehyde and chitosan with the



Scheme 2. Synthesis scheme of MPEG-g-Chitosan.

molar ratio of 0.6/1 were added into a mixture of acetic acid and methanol (2/1, v/v). And the pH value of reaction system was adjusted to 6 with aqueous 1 M NaOH solution. Subsequently, aqueous $NaCNBH_3$ solution was added dropwise into the reaction system maintaining the pH value at 6. The reaction system was performed for 12 h at ambient temperature. Finally, the resulting solution was dialyzed against distilled water and 0.05 M NaOH solution alternatively for 3 days, and the solution inside of bag filter was subsequently freeze-dried. The final product of MPEG-g-CS was obtained by removal of unreacted MPEG with excess acetone.

2.4. Characterization of MPEG-b-PCL and MPEG-g-CS

The chemical structure of obtained MPEG-b-PCL and MPEG-g-CS were confirmed by ^{1}H NMR (Varian, USA) at 400 MHz using $CDCl_3$ and D_2O as the solvent, respectively. The macromolecular weight of MPEG-b-PCL polymer was determined by ^{1}H NMR. According to the previous report of Chan, Kurisawa, Chung, and Yang (2007), degree of substitution (DS) of MPEG grafted onto chitosan chain could be calculated from the relative peak area of methylene group of PEG to acetyl group of the monosaccharide residue.

2.5. Preparation of MPEG-b-PCL and MPEG-g-CS composite films

The casting/solvent evaporation technology was employed to prepare the composite films of MPEG-b-PCL and MPEG-g-CS. Initially, aqueous MPEG-g-CS solution at the concentration of 10 mg/ml and aqueous MPEG-b-PCL solution at the concentration of 20 mg/ml were prepared separately. Subsequently, MPEG-g-CS and MPEG-b-PCL solution were mixed together at weight ratio (w/w) of 0/100, 30/70, 50/50, 70/30, 100/0, and dried in glass Petri dish at 50 °C for 2 days. Finally, the composite films were obtained and peeled from Petri dish for further use. The obtained composite films (MPEG-g-Chitosan/MPEG-b-PCL, w/w) were named 30/70 composite film, 50/50 composite film and 70/30 composite film, respectively.

2.6. Characterization of composite film

2.6.1. Fourier transform infrared spectroscopy (FTIR)

FTIR (KBr) spectra were performed at room temperature using NICOLET 200SXV Infrared Spectrophotometer (USA). The characteristic absorption bands of the composite films and polymers were detected at wavenumbers ranging from 500 to 4500 cm^{-1}.

2.6.2. Morphological analysis

The morphological characterization including surface and cross section of composite film was performed by scanning electron microscopy (JSM-5900LV, JEOL, Japan). Composite film samples were placed at cabinet drier for 24 h before observation. The cross section of film was obtained by cutting film after dealing with liquid nitrogen.

2.6.3. Thermal properties

The thermal properties of composite films were characterized by a differential scanning calorimeter (DSC, NETSCZ 200, Germany). The purified and dried samples were used for DSC test. Sample was first heated from 20 °C to 200 °C under nitrogen atmosphere at a heating rate of 10 °C/min, and reheated to 200 °C at the same rate after quenched to 20 °C, at last sample was cooled to 20 °C again at the cooling rate of 10 °C/min. Thermogravimetric analysis (TGA) was performed by a thermogravimetric analyzer (TA 2910, DuPont, USA) under a steady flow of nitrogen atmosphere at a heating rate of 10 °C/min in the range of 20–600 °C.

2.7. Water absorption study

Films swelling properties were evaluated by determining the percentage of hydration. The water absorption of composite film formulation was detected by weighing the film pieces after placing in pH 7.4 phosphate-buffered solution. Each film was divided into portions of 1 cm^2 (1 cm × 1 cm) and cut, weighed and placed in buffer solution for predetermined periods of time (5, 10, 20, 40, 60 and 90 min) as described by Rodrigues et al. (2008). At the predetermined time, the films were taken from the medium and weighed after removal of the surplus surface water using filter paper. The percentages of water absorption were calculated by the following equation:

$$\text{Water absorption } (\%) = \frac{W_{90} - W_0}{W_0} \times 100 \quad (1)$$

where W_{90} is the weight of wet film at time 90 min and W_0 is the original film weight at zero time, respectively. Here the water absorption of composite film after immersed in PBS solution for 90 min was defined as equilibrium-swelling ratio. This experiment was performed in triplicate.

2.8. In vitro degradation test

In vitro degradation behavior of composite films (1 cm × 1 cm) was performed in 5 ml phosphate-buffered solution (PBS, pH 7.4) at 37 °C containing 1.5 μg/ml of lysozyme. The concentration of lysozyme was chosen to correspond to the concentration in human serum (Porstmann et al., 1989). Briefly, films with calculated dry weight were incubated in the lysozyme solution with gentle agitation for the period of study. Lysozyme solution was refreshed daily to ensure continuous enzyme activity (Masuda, Ueno, & Kitabatake, 2001). Samples were removed from the medium at predetermined time (7, 14 and 28 days), and rinsed with distilled water, finally dried under vacuum and weighed. The degree of *in vitro* degradation was calculated by the weight loss:

$$\text{Weight loss } (\%) = \frac{W_0 - W_t}{W_0} \times 100$$

where W_0 is the dry weight before degradation test and W_t is the dry weight at predetermined time t. To separate between enzymatic degradation and dissolution, control samples were stored for 28 days under the same conditions as described above, but without the addition of lysozyme.

3. Results and discussion

3.1. Characterization of MPEG-b-PCL

The chemical structure of MPEG-b-PCL was confirmed by ^{1}H NMR spectrum as presenting in Fig. 1. The characteristic peaks of product were marked in this figure. Peaks at 1.40, 1.65, 2.32, and 4.06 ppm are assigned to methylene protons of $-(CH_2)_3-$, $-OCCH_2-$, and $-CH_2OOC-$ in PCL units, respectively. The sharp peak at 3.65 ppm is attributed to methylene protons of $-CH_2CH_2O-$ in PEG units in block copolymer. The weaken peaks at 4.23 and 3.82 ppm are attributed to methylene protons of $-O-CH_2-CH_2-$ in PEG end unit that linked with PCL blocks, respectively.

Macromolecular weight (M_n) of MPEG-b-PCL polymers and PCL/PEG ratio in these copolymers were calculated from ^{1}H NMR spectra following with the following equations:

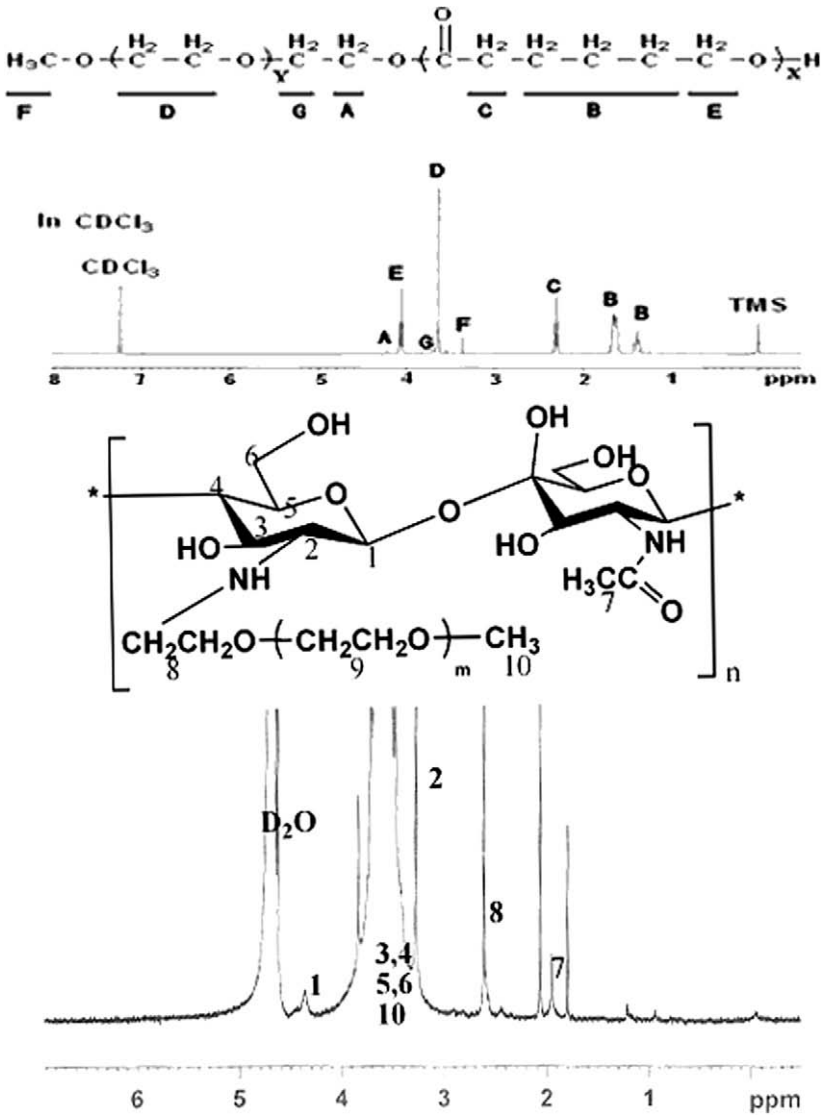


Fig. 1. ^{1}H nuclear magnetic resonance (^{1}H NMR) of MPEG-b-PCL and MPEG-g-CS.

$$\frac{2(2(x-1))}{I_E} = \frac{4}{I_A} \quad (2)$$

$$\frac{4(y-2)+4}{I_D} = \frac{4}{I_A} \quad (3)$$

$$M_{n(\text{MPEG-b-PCL})} = M_{n(\text{MPEG})} + M_{n(\text{PCL})} = 44x + 144y \quad (4)$$

where I_E, I_A, and I_D were integral intensities of methylene protons of –CH2OOC– in PCL units at 4.06 ppm, methylene protons of –O–CH2– in PEG end unit at 4.23 ppm, and methylene hydrogen of homosequences of PEG oxyethylene units at 3.65 ppm, respectively; x and $y$were, respectively, the corresponding block number of MPEG and PCL in MPEG-b-PCL polymers.

According to the result of DSC examination (Fig. 2), MPEG-b-PCL had a melting temperature (ΔH_m) at 47.1 °C and 80.6 J/g, respectively. As expected, the crystalline polymer of MPEG-b-PCL was a MPEG block because the T_m and ΔH_m of MPEG (M_n = 2000) were 57 °C and 176.5 J/g, whereas the T_m and ΔH_m of PCL (100% crystallinity) were 60 °C and 139.5 J/g, respectively (Lopez-Rodriguez, Lopez-Arraiza, Meaurio, & Sarasua, 2006; Niamsa, Puntumchai, Sutthikhum, Srisuwan, & Baimark, 2008).

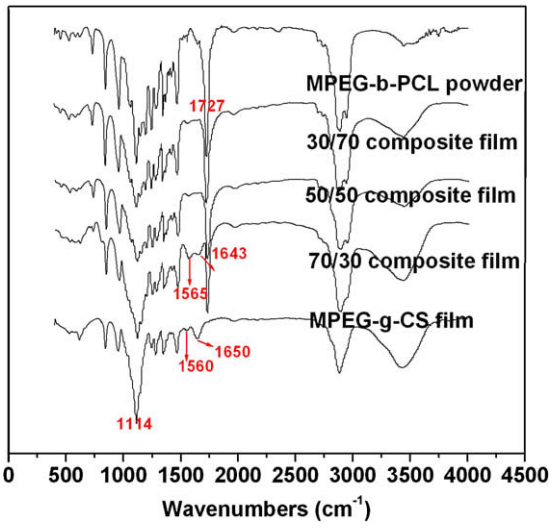


Fig. 2. Fourier transform infrared spectroscopy (FTIR) spectrum of MPEG-b-PCL, MPEG-g-CS, and composite films.

3.2. Characterization of MPEG-g-CS

For PEGylation of chitosan (MPEG-g-CS), MPEG (M_n = 2000) was adopted to react with amine groups of chitosan to provide a physiologically stable amide linkage (Scheme 2). Methoxy poly(ethylene glycol) (MPEG) was selected for PEGylation of chitosan instead of PEG in order to avoid cross-linking of the copolymers (Gorochovceva, Naderi, Dedinaite, & Makuška, 2005). Successful synthesis of MPEG-g-CS was confirmed by ^{1}H NMR spectra as presented in Fig. 1. The linkage between chitosan and MPEG was confirmed by the appearance of a new peak of –NH–CH_2CH_2O– at 2.45–2.60 ppm (Signal 8) in ^{1}H NMR spectra compared with that of pure chitosan. The deacetylation degree (DD) of chitosan was evaluated with Signal 7 at 2.0–2.1 ppm from the acetyl group (–COCH3) and Signal 2 at 2.95–3.10 ppm from the monosaccharide residue (CH–NH–), being about 92%. DS of MPEG onto chitosan chain was determined from the relative peak area of methylene group of MPEG (Signal 8) to acetyl group (Signal 7) of the monosaccharide residue as the report of Chan et al. (2007). This result suggested that MPEG-g-CS with DS of 1.8 was successfully synthesized in this work.

3.3. Composite films characterization

3.3.1. Fourier transform infrared spectroscopy (FTIR)

According to the characteristic spectra of the polymers and composite films in Fig. 2, an attempt was made to determine the eventual presence of interactions (possible interactions) between the polymers. The characteristic absorption bands of MPEG-b-PCL were around 1727 cm^{-1} which confirmed the presence of –COO– in polymer. The characteristic absorption bands of MPEG-g-CS have been described previously (Du & Hsieh, 2007) and they corresponded to: –NH_2 (1650 and 1560 cm^{-1}), C–O–C (1114 cm^{-1}) and the band at 3480 cm^{-1} belonged to the stretching vibrations of hydroxyl groups. As shown in Fig. 2, we could find that the intensity of amino stretching bands decreased as the MPEG-b-PCL ratio increased in composite films. The lower wave number shift as well as the intensity of amino stretching bands of MPEG-g-CS in composite films could be explained by the presence of molecular interaction between MPEG-g-CS and MPEG-b-PCL (Niamsa et al., 2008).

3.3.2. Thermal properties

Differential scanning calorimeter (DSC) and thermogravimetric analysis (TGA) were selected to characterize the thermal properties of composite films. As presented in Fig. 3, MPEG-g-CS had a melting temperature (ΔH_m) at 57 °C and 18.1 J/g which also confirmed the linkage of MPEG onto chitosan chain. Meanwhile, the melting temperature (ΔH_m) of MPEG-b-PCL powder was 47 °C (80.6 J/g). Composite films were thermal stable and the degradation did not occur ranging from 20 °C to 200 °C. As shown in Fig. 3 and Table 1, we could find that ΔH_m of composite films increased (from 18.1 J/g to 80.6 J/g), whereas T_m of composite films decreased (from 57 °C to 47 °C) as the MPEG-g-CS ratio increased in composite films. This might be attributed to crystallization disturbance of MPEG-g-CS in the composite state. Sarasam and Madihally (2005) have reported that the presence of chitosan in MPEG-b-PCL composite film could interfere the crystallization of MPEG-b-PCL film resulting in the alteration of thermal properties of composite film. They also suggested that chitosan and MPEG-b-PCL were miscible and could be blended in amorphous phase.

Thermal decomposition behaviors of MPEG-g-CS films, composite films and MPEG-b-PCL powder were performed by TG analysis. According to the thermogram (TG) presented in Fig. 4, the temperature of degradation (T_d) of MPEG-b-PCL powder was 396 °C. Whereas, degradation of MPEG-g-CS has two stages with the first stage loss of 60% weight at 336 °C and then completely degraded at 399 °C which might attribute to the graft of MPEG onto chitosan chain. The thermal decomposition behaviors of 70/30 and 50/50 composite films also displayed two-stage degradation which had higher T_d than that of MPEG-g-CS film but lower T_d than that of MPEG-b-PCL powder. However, the thermal decomposition behavior of 30/70 composite film was similar with that of pure MPEG-b-PCL powder with T_d at 390 °C, but lower than that of pure MPEG-b-PCL. These results also suggested the presence of molecular interaction between MPEG-g-CS and MPEG-b-PCL as identified by FTIR.

Table 1
The samples prepared in this work.

Samples	Weight ratio of MPEG-g-CS/MPEG-b-PCL	DSC	
		T_m (°C)	ΔH_m (J/g)
100/0	100/0	57	18.1
70/30	70/30	54	34.7
50/50	50/50	53	61.2
30/70	30/70	51	65.0
0/100	0/100	47	80.6

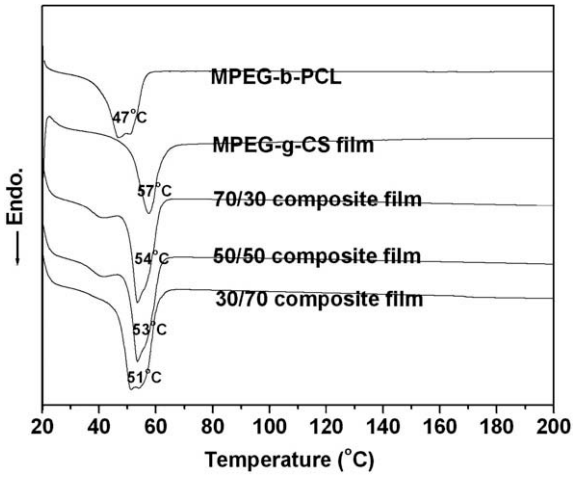


Fig. 3. Differential scanning calorimeter (DSC) spectrum of MPEG-b-PCL, MPEG-g-CS, and composite films.

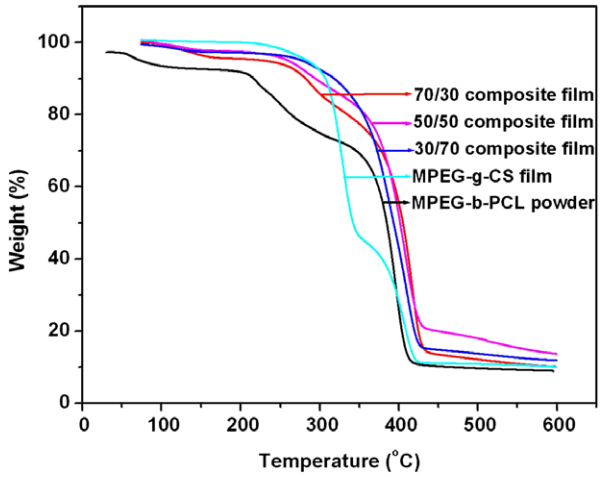


Fig. 4. Thermogravimetric analysis (TGA) of MPEG-g-CS film, MPEG-b-PCL powder and composite films.

3.4. Morphological characterization

According to the report of SEM images, we could find that the average thicknesses of the composite films are approximately 20 μm except 70/30 composite film (about 70 μm). Three typical scanning electron microphotographs of surfaces and cross sections of composite films were, respectively, presented in Fig. 5. The surfaces and cross sections of MPEG-g-CS were smooth (data not shown), whereas the composite films had rough surface. And the roughness of surface increased as the MPEG-b-PCL ratios increased. The blending ratio also greatly influenced cross sections of films. The cross section of 70/30 composite film and 30/70 composite film, respectively, presented in Fig. 5(A-2) and (C-2) were stuffed at 2000× magnification absence of hollows which could be observed more clearly in magnification. But the appearance of these two cross sections have some distinctions, cross section of 30/70 composite films was more rough than that of 70/30 composite films, which might attribute to the high ratio of MPEG-b-PCL present in composite film resulting in the separation of MPEG-b-PCL from composite films. Niamsa et al. (2008) also observed that the separation of MPEG-b-PCL from composite films as MPEG-b-PCL ratio increased in chitosan/MPEG-b-PCL blend films. However, composite film of 50/50 was hollow cross section as presented in Fig. 5(B-2) which could be more clearly observed in magnification. The reason for this phenomenon why the hollow cross section was obtained in 50/50 composite film is still unclear which calls for further study.

3.5. Water adsorption of composite films

The ability for a film to preserve water is one of the most important aspects in skin tissue engineering especially for wound healing (Tanigawa, Miyoshi, & Sakurai, 2008). The water adsorption behavior of these composite films is shown in Fig. 6. All of the prepared MPEG-g-CS/MPEG-b-PCL composite films showed the great ability of water adsorption. As the MPEG-g-CS ratio increased in composite film, the greater capacity of water adsorption could be gained. Composite film of 70/30 had the largest water absorption of 535% and composite film of 30/70 had the smallest water absorption of 316% among these composite films. These results indicated that the blending of water-insoluble MPEG-b-PCL could decrease the water adsorption of composite films as well as alter

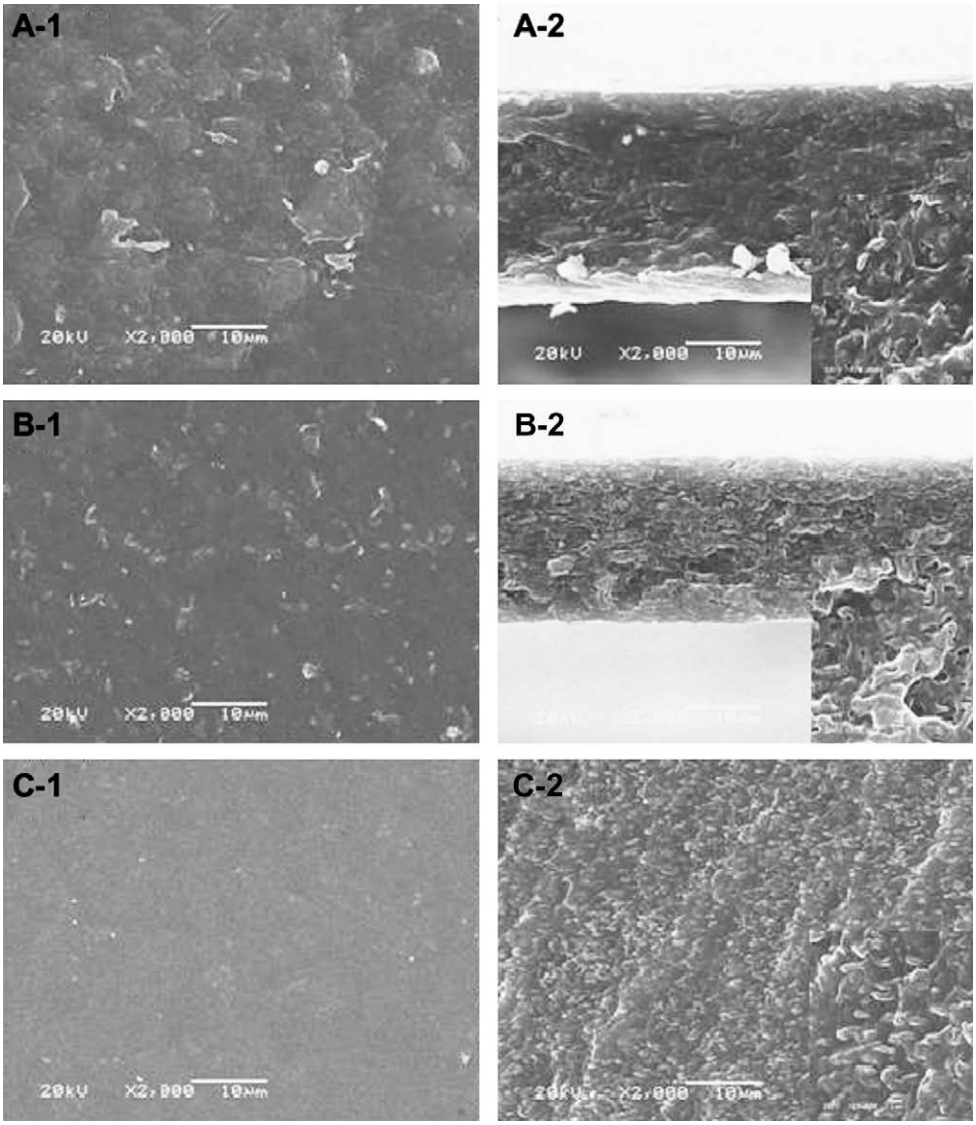


Fig. 5. Scanning electron microscopy (SEM) micrographs of surfaces of (A-1) 30/70 composite film, (B-1) 50/50 composite film, (C-1) 70/30 composite film and cross sections of (A-2) 30/70 composite film, (B-2) 50/50 composite film, (C-2) 70/30 composite film.

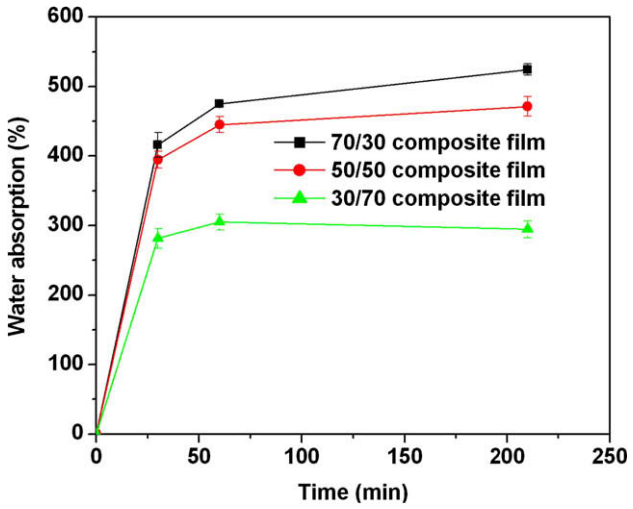


Fig. 6. The water absorption of composite films in PBS solution at 37 °C.

the transparency of composite films as immersed in PBS solution (the higher ratio of MPEG-b-PCL in composite films, the more whiteness of observation of film).

3.6. In vitro degradation test

It is well known that, in human serum, chitosan is mainly depolymerized enzymatically by lysozyme, and not by other enzymes or other depolymerization mechanisms (Nordtveit, Vårum, & Smidsrød, 1996; Varum, Myhr, Hjerde, & Smidsrød, 1997). The enzyme biodegrades the polysaccharide by hydrolyzing the glycosidic bonds present in the chemical structure. The *in vitro* degradation behavior of chitosan and its composite/blend films have already been performed by a number of studies (Freier, Koh, Kazazian, & Shoichet, 2005; Hirano, Tsuchida, & Nagao, 1989; Lee, Ha, & Park, 1995). However, most of the studies were carried out with accelerated conditions using low pH for optimum lysozyme activity as well as high enzyme concentrations. To our knowledge, long-term *in vitro* degradation studies of MPEG-g-CS/MPEG-b-PCL composite film at physiological pH with enzyme concentration correspond to the concentration in human serum has not yet been published. As presented in Fig. 7, we could find that

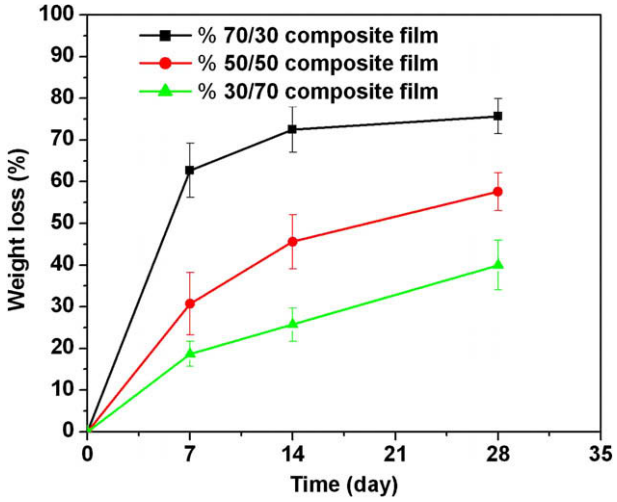


Fig. 7. *In vitro* degradation behavior of composite film in PBS solution with lysozyme at 37 °C.

the degradation rate of composite film increased as the MPEG-g-CS content increased in composite film. 70/30 composite film had the greatest degradation among the three composite films with 70% weight loss after 28 days, which is corresponding to the ratio of MPEG-g-CS in composite film. This might be explained by that the lysozyme could only efficiently degrade MPEG-g-CS but not the MPEG-b-PCL (Deng, Zhou, Li, Zhao, & Yuan, 2001). It bas been reported that MPEG-b-PCL was stable in PBS solution with or without addition of lysozyme (Jiang et al., 2008). To distinguish between enzymatic degradation and simple dissolution of MPEG-g-CS in composite film, we compared with the weight loss after 28 days of samples that had been immersed in PBS without lysozyme. In spite of well solubility of MPEG-g-CS, we could find that the dissolution of MPEG-g-CS was low with only 15% weight loss after 28 days in 70/30 composite film. This result indicated that the weight loss of composite film in PBS solution with lysozyme was predominant induced by the degradation of MPEG-g-CS not by the dissolution of MPEG-g-CS. The specific degradation behavior of composite film by lysozyme might be beneficial in wound healing or tissue engineering applications because lysozyme is present in certain human body fluids.

4. Conclusion

Composite films of MPEG-g-CS and MPEG-b-PCL with different weight ratios (0/100, 30/70, 50/50, 70/30, 100/0) were successfully prepared by casting/solvent evaporation methods. MPEG-b-PCL with molecular weight of 4000 and MPEG-g-CS with DS of 1.8 was successfully synthesized. The presence of molecular interaction between MPEG-g-CS and MPEG-b-PCL was identified by FTIR and TGA. The blending ratio greatly influenced the thermal properties, morphology, water absorption and *in vitro* degradation behavior of composite films. The roughness of composite films increased as MPEG-b-CS ratio increased. Meanwhile, introduction of MPEG-b-PCL could decrease the transparency as well as the water absorption of the film. *In vitro* degradation test suggested that the composite film could efficiently degrade at PBS solution with lysozyme. The higher ratio of MPEG-g-CS in composite film, the higher degradation rate of composite film could be gained. Therefore, the prepared composite film with suitable hollow and high capacity of water adsorption could have great potential in the application of wound healing and tissue engineering.

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