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Synthesis and characterization of a new thermosensitive chitosan–PEG diblock copolymer

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ABSTRACT

A novel thermosensitive hydrogel was synthesized by block copolymerization of monomethoxy poly(ethylene glycol) macromere (PEG) onto chitosan backbone, using potassium per sulfate as a free radical initiator. This block copolymer exhibits a thermoreversible transition from an injectable solution at low temperature to a gel at body temperature. Synthesized copolymers were characterized using FT-IR, ^{1}H NMR, ^{13}C NMR, and DSC techniques. Solubility test was performed to compare water and organo-solubility of chitosan before and after copolymerization. Sol–gel transition behavior was investigated using the vial inversion method and viscosity measurements. The gelation behavior makes the chitosan–PEG block copolymers more promising and attractive materials for biomedical applications.

1. Introduction

Chitosan is a promising biopolymer (Chenite, Gori, Shive, Desrosiers, & Buschmann, 2006; Muzzarelli et al., 2007; Ratajska et al., 2003; Zhang, Li, Gong, Zhao, & Zhang, 2002) that has long been used in pharmacy (Kato, Onishi, & Machida, 2003) and medicine (Ravi-Kumar, 2000; Singh & Ray, 2000) for oral (Jian, Sharma, & Vyas, 2006), nasal (Illum, Jabbal-Gill, Hinchcliffe, Fisher, & Davis, 2001) and parenteral (Felt, Buri, & Gurny, 1998) drug administration and for peptide (Bernkop-Schnűrch, 2000) and gene (Guang-Liu & De-Yao, 2002) delivery systems. Low solubility of chitosan in both water and organic solvents resulted in many studies aimed at making water soluble derivatives of chitosan using chemical modification techniques. For example, sulfonation (Bannikova, Sukhanova, Vikhoreva, Varlamov, & Galbraikh, 2002), quaternarization (Polnok, Borchard, Verhoef, Sarisuta, & Junginger 2004), carboxymethylation (Wongpanit et al., 2005), and *N*- and *O*-hydroxyalkylation (Donges, Reichel, & Kessler, 2000; Richardson & Gorton, 2003). Furthermore, a variety of graft copolymerization of chitosan with lactic acid (Yao et al., 2003), poly acrylic acid (Shim & Nho, 2003), vinyl pyrrolidone (Yazdani-Pedram & Retuert, 1997), 3-*o*-dodecyl-D-glucose (Ngimhuang, Furukawa, Satoh, Furuike, & Sakairi, 2004), and *N*-isopropylacrylamide (Lee, Ha, Cho, Kim, & Lee, 2004) were presented and evaluated as practical biomedical materials. So far, several studies have investigated PEGylation of chitosan to improve its affinity to water and organic solvents. Poly(ethylene glycol) (PEG) is a neutral, water soluble and non toxic polymer which has been employed for pharmaceutical and biomedical applications (Harris & Zalipsky, 1997). PEG is a synthetic polymer approved by the FDA for internal consumption and injection in a variety of foods, cosmetics and drug delivery systems (Cavalla, 2001). Sugimoto et al. used reductive amination of PEG-aldehyde in aqueous organic acid as a typical method for grafting PEG onto chitosan (Sugimoto, Morimoto, Saimoto, Sashiwa, & Shigemasa, 1998). They have found that solubility of chitosan-g-PEG in water was dependent on the molecular weight of PEG, the weight ratio of PEG in hybrid and degree of substitution (DS). PEG-cross-linked chitosans and reacetylated chitosans were synthesized by Pozzo, Fagnoni, Guerrini, Benedittis, and Muzzarelli (2000). Shantha and Harding (2002) synthesized microspheres of chemically modified chitosan by graft copolymerization of PEG-diacrylate macromonomere on the chitosan backbone. Gorochovceva and Makuśka (2004) synthesized a water-soluble O-PEGylated chitosan by etherification between *N*-phthaloyl chitosan and PEG monomethyl ether iodide (MPEG-I) using Ag_2O as a catalyst. They synthesized chitosan-O-MPEG graft copolymers with different degree of substitution. However, Hu, Jiang, Xu, Wang, and Zhu (2005) found that it is difficult to remove the trace amount of Ag_2O dispersed in the final product and achieve desired solubility in water or common organic solvents unless the copolymer possesses a high value of DS. Hu and coworkers synthesized PEG-g-chitosan by N-substitution of triphenylmethyl chitosan with methoxy poly(ethylene glycol) iodide in organic medium and subsequent removal of triphenylmethyl groups. These copolymers were soluble in water over wide pH range. Also, organo-solubility

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of the copolymers in DMF and DMSO was achieved for DS value more than 24% (Hu et al., 2005). Bhattarai and coworkers synthesized chitosan-g-PEG copolymers to obtain a thermosensitive gel (Bhattarai, Matsen, & Zhang, 2005a; Bhattarai, Ramay, Gunn, Matsen, & Zhang, 2005b). Chitosan was first modified with a PEG-aldehyde to yield an imine (Schiff base) that was subsequently converted into PEG-g-chitosan through reduction with sodium cyanoborohydride ($NaCNBH_3$) (Harris et al., 1984). Despite the major advantage of thermosensitivity, the preparation of PEG-aldehyde was generally inconvenient with a low degree of conversion (Sugimoto et al., 1998). In addition, Bentley, Roberts, and Harris (1998) found that air oxidation of PEG-aldehyde could occur readily and aldol condensation might emerge during the reaction resulting in polymerization of PEG-aldehyde.

Although significant efforts have been done on synthesis of chitosan-graft-PEG copolymers, block copolymerization of chitosan and PEG has not been reported. This study aims to develop a novel injectable block copolymer of chitosan and poly(ethylene glycol) that exhibit a thermoreversible transition from an injectable sol at low temperature to a gel at body temperature. For this purpose, chitosan–PEG diblock copolymers were prepared by introducing monomethoxy poly(ethylene glycol) onto chitosan chain using potassium per sulfate (KPS) as an initiator. This new synthesize method of the block copolymer is original and more convenient than those previously reported. It may overcome the disadvantage of previously prescribe method to synthesize the graft copolymers such as remaining undesirable materials and low degree of conversion. Fourier transform infrared (FT-IR), ^1H and ^{13}C nuclear magnetic resonance (NMR) and differential scanning calorimetry (DSC) techniques were used to characterize synthesized copolymers. The solubility, viscosity, and sol–gel transition temperature were studied for the copolymer prepared with different ratios of chitosan/PEG and various polymer concentrations.

2. Experimental

2.1. Materials

Medium molecular weight chitosan was purchased from Sigma–Aldrich Chemical Co. (USA). For purification, chitosan was dissolved in 2% aqueous acetic acid solution, filtered and then precipitated by adding concentrated NaOH solution. The degree of deacetylation (DDA) of chitosan was found to be 82.5% by ^1H NMR analysis. The viscosity-average molecular weight (M_v) was determined to be 2.5×10^5 using Mark–Houwink equation (Wang, Bo, Li, & Qin, 1991). Monomethoxy poly(ethylene glycol) (MPEG, $M_w = 2000$) was purchased from Fluka Chemical Co. (USA) and thoroughly dehydrated before use to eliminate traces of moistures. Acryloyl chloride, triethylamine and dried 1,2-dichloromethane were purchased from Merck (Germany). Ethyl ether was purchased from Panreac (E.U.) and was dried carefully. Potassium per sulfate (KPS) was purchased from Sigma and used as received. Other reagents were chemical grade and used as received.

2.2. Preparation of block copolymers

The process consisted of two steps: (1) preparation of PEG macromere and (2) synthesis of diblock copolymer.

Step 1: *Preparation of PEG-macromere*. In a 250 ml two-necked round-bottomed flask equipped with a glassy stirrer, 10 g of MPEG was dissolved in 100 ml of dried dichloromethane at room temperature. Triethylamine (4.4×10^{-3} mol) was added drop-wise to the PEG solution with continued stirring and the final solution was mixed for a further 15 min. Excess amount of acryloyl chloride (9×10^{-3} mol) was added gradually to the reaction vessel, and the final solution was refluxed at 40 °C for 4 h. Obtained mixture was filtered to remove solid byproduct. Adding the filtrate in an excess amount of anhydrous ethyl ether gave the PEG macromere. The resultant product was purified by extensive extraction with dried ethyl ether. Finally, to remove all solvents the precipitant was dried under vacuum for 48 h.

Step 2: *Synthesis of diblock copolymer*. Chitosan dissolved in 0.1 M acetic acid was taken into a flask containing mixer and nitrogen inlet. Potassium per sulfate (KPS) was added to the chitosan solution and the resultant mixture was stirred for 30 min, under nitrogen atmosphere at 60 °C. PEG macromere was added gradually and the resultant mixture was stirred for 6 h. The mixture was filtered and the filtrate was then precipitated with 5% sodium hydroxide. The precipitate was obtained by centrifugation. Free PEG was well removed from the resulting product by washing with acetone. Finally, the resultant mixture was dialyzed with a dialysis membrane (M_w 12,000–14,000 cut) against distilled water. Obtained product was dried under atmosphere for 24 h and then in vacuum at 40 °C for 2 days. Block copolymers with different ratio of chitosan to the PEG macromere were prepared (Tables 1 and 2).

2.3. Characterization of copolymers

Fourier transforms infrared (FT-IR) spectra of PEG, PEG macromere, and chitosan–PEG diblock copolymers were recorded on a Mattson 1000 FT-IR spectrometer, using the potassium bromide disc method, to define the structure of the products. The chemical bonding between chitosan and PEG has been studied with ^1H NMR and ^{13}C NMR spectroscopy (Bruker 500 MHz). Thermal properties of chitosan and chitosan–PEG block copolymer were investigated by differential scanning calorimetry (DSC, Perkin-Elmer Series 7), in aluminum pans (7–8 mg) at a heating rate of 10 °C/min with nitrogen flow (40 ml/min). Solubility tests were carried out in isotonic phosphate buffered saline (PBS 0.1 M, pH 7.4), diluted acetic acid, CH_3Cl, DMF, and DMSO. 5 mg of a copolymer was added into 1 ml of solvent, and shaken for 1 day at room temperature. The solubility was determined from the residual mass after centrifugation.

2.4. Sol–gel transition of block copolymers

The sol–gel transition behavior was determined by a test tube inverting method with a temperature increment of 1 °C per step (Gupta, Tatorc, & Shoichet, 2006). Polymer solutions were prepared in 5 ml vials with inner diameter of 10 mm. The vials were immersed in a water bath at each temperature for 15 min. The sol–gel transition temperature was monitored by inverting the vials, and if there was no flow in 30 s, it was regarded as a gel.

Table 1
Effect of initiator concentration on the composition of chitosan–PEG copolymer

Sample No.	Molar ratio of PEG/CH in feed	KPS (mol/L)	Molar ratio of PEG/CH in product[a]
CP1-1	0.4	0.001	0.062
CP1-2	0.4	0.005	0.089
CP1-3	0.4	0.01	0.10
CP1-4	0.4	0.015	0.094
CP1-5	0.4	0.02	0.089

[a] Mole ratio of PEG to chitosan (CH) in product determined by ^1H NMR spectra.

Table 2
Solubility of chitosan and chitosan–PEG block copolymers

Sample	PEG/CH[a]	Solubility (%)[b]				
		PBS	HoAc	DMSO	DMF	$CHCl_3$
Chitosan	–	0	100	0	0	0
CP1	0.10	5	100	7	0	0
CP2	0.15	15	100	20	15	0
CP3	0.26	40	100	56	40	0
CP4	0.42	100	100	100	100	0
CP5	0.53	100	100	100	100	0

[a] Mole ratio of PEG to chitosan in product (PEG/CH) determined by ^1H NMR spectra.
[b] Solubility calculated based on the percentage of the initial polymer (5 mg) that is dissolved in 1 ml of solvent after 24 h. PBS, buffer solution (0.1 M, pH 7.4); HoAc, acetic acid 0.1 M.

The transition temperature was determined with ±1 °C accuracy. Phase transition behavior of chitosan–PEG diblock copolymers was also studied by measuring their solution viscosity as a function of temperature, using a Haake Viscometer (VT550) equipped with SP2P sensors. The solutions were placed in the rotor of a viscometer operated at a fixed spindle speed of 30 rps and thermostated with a water bath circulator. Measurements were made in the temperature range of 10–60 °C.

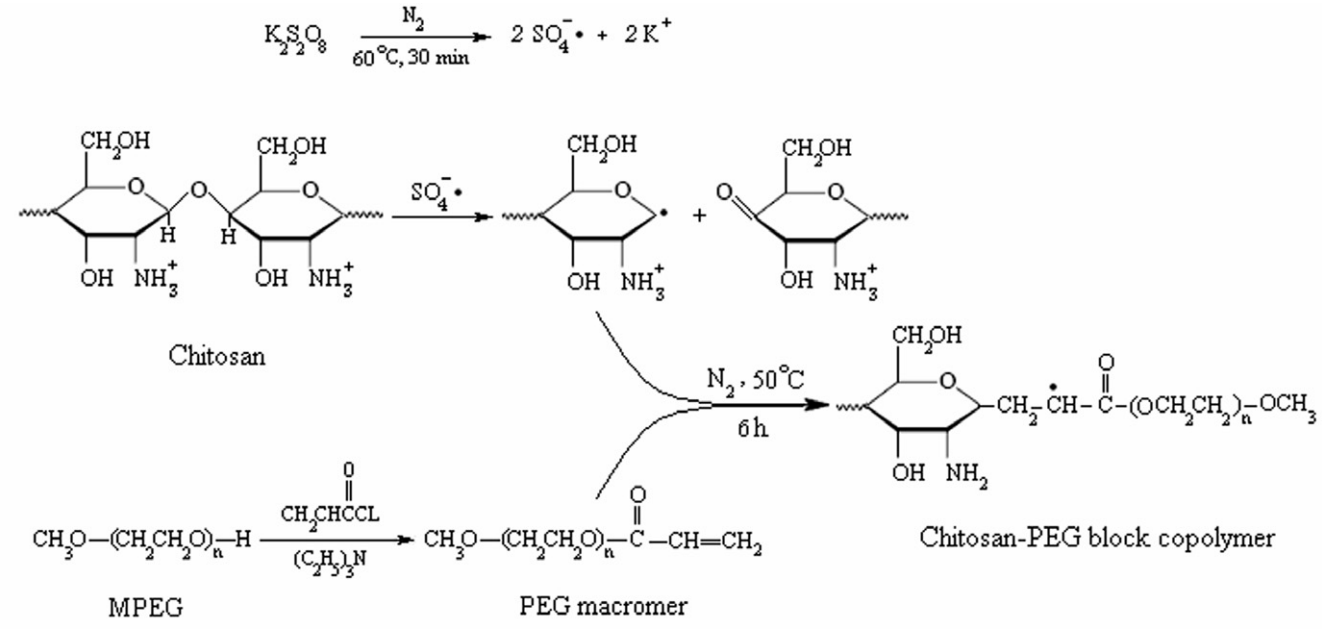
3. Results and discussion

3.1. Preparation of block copolymers

Monomethoxy poly(ethylene glycol) macromere was copolymerized with chitosan in order to impart hydrophilicity as well as thermosensitive properties to the chitosan macromolecules. KPS initiator was used in aqueous solution to effectively introduce the PEG-macromere onto chitosan backbone. It seems that mechanism of copolymerization of PEG-macromere and chitosan chain can be illustrated as it is shown in the (Scheme 1). In a pre-degradation step, the degradation of chitosan by KPS would occur through a well-known mechanism (Hsu, Don, & Chiu, 2002). Persulfate initiator ($S_2O_8^{2-}$) is decomposed under heating to generate sulfate anion radical, $SO_4^{-\bullet}$. The radical attacks the C_4 carbon of chitosan chain and transfers the radical to the C_4 carbon by subtracting the hydrogen from it. The presence of free radicals at the C_4 carbon results in the breakage of the C—O—C bond in the main chain. Therefore, as the chitosan chain was degraded two shorter chains were produced with a terminal carbonyl group at one scission end and a free radical at the other scission end. The reactive free radical end in chitosan chain could act as a powerful nucleophile; readily attacking the unsaturated carbon-carbon double bond in PEG-macromere. Therefore, the PEG macromere could grow on the chitosan chain and produce the block copolymers with a range of PEG content (Scheme 1). The effect of KPS concentration on the composition of the synthesized copolymers is illustrated in Table 1. Presented results indicate that, at the constant feed ratio of PEG/CH, as KPS molarities increases from 0.001 to 0.01 mol/L, the mole ratio of PEG/CH in the copolymers increases from 0.062 to 0.10. For the higher concentration of KPS, i.e. greater than 0.01 mol/L, the molar ratio of PEG/CH in product decreases with increasing KPS concentration (Table 1). The same results were published for emulsion polymerization of poly(methyl methacrylate) in the presence of chitosan and KPS (Hsu et al., 2002).

3.2. Characterization of copolymers

IR spectrums of MPEG, PEG macromere, chitosan, and chitosan–PEG block copolymer are shown in Fig. 1 to define the structure of synthesized copolymer. Pure MPEG has characteristic peaks at 1150, 1390, 1445, and 2880 cm^{-1}; corresponding to the presence of CH_2 and CH_3 groups (Fig. 1a). Also, a narrow peak appears at 3458 cm^{-1} illustrates the vibration of –OH terminal groups in MPEG. The IR spectrum of PEG macromere is shown in Fig. 1b. The reaction between acryloyl chloride and hydroxyl groups of MPEG leads to a large decrease in the –OH stretching vibration peak. Furthermore, a new peak appears at 1730 cm^{-1} that can be attributed to the formation of a carbonyl bond, and two new peaks appearing at 1420 and 1632 cm^{-1} seem to belong to the unsaturated carbon-carbon double bonds (C=C).



Scheme 1. Schematic representation of copolymerization of chitosan and PEG macromere.

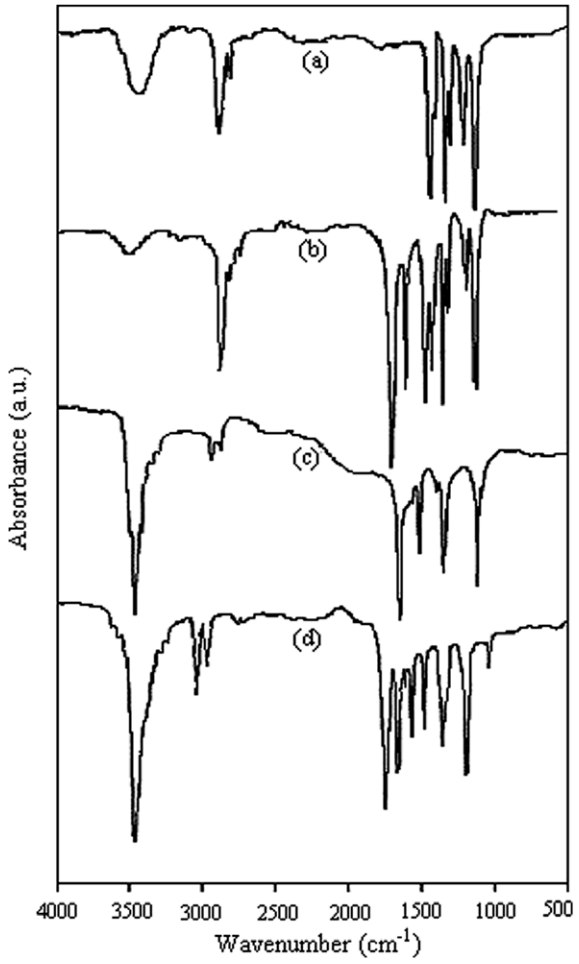


Fig. 1. FTIR spectra of (a) MPEG, (b) PEG macromere, (c) chitosan, and (d) chitosan–PEG block copolymer.

An IR spectrum of chitosan is shown in Fig. 1c. A sharp band at 3450 cm^{-1} has been attributed to $-NH_2$ and –OH group stretching vibration in chitosan matrix. Further, in the C—H stretch region of FTIR spectrum, the higher intensity peak at 2940 cm^{-1} is assigned to the asymmetric and the lower intensity peak at 2876 cm^{-1} is assigned to the symmetric modes of CH_2. In addition, the characteristic band due to CH_2 Scissoring, which usually occurs at 1400 cm^{-1} is observed in the spectrum. The peaks at 1660, 1550, and 1120 cm^{-1} could be assigned to strong N—H bending vibration of primary and secondary amide and C—O stretching vibration of ether linkage of chitosan backbone, respectively.

The IR spectrum of chitosan–PEG copolymer is illustrated in Fig. 1d. The strong peaks at 2950, 2880, 1460, 1400, and 1160 cm^{-1} could be assigned to the presence of MPEG in the chitosan backbone. Presence of a new peak at 1730 cm^{-1} could point to the existence of ester carbonyl group in chitosan chain, coming from PEG macromere. The absence of two peaks at 1420 and 1630 cm^{-1} that was assigned to unsaturated carbon–carbon double bonds, confirms the reaction scheme illustrated in Scheme 1. Also, the sharp peak at 1120 cm^{-1} that was assigned to the C—O stretching vibration of ether linkage in the chitosan backbone was changed to a weak peak at 1057 cm^{-1}. These results indicate that PEG macromere was connected to the chitosan backbone.

The molecular structure of chitosan and chitosan–PEG block copolymer was further characterized with ^1H NMR (Fig. 2). The spectra of chitosan in Fig. 2a exhibited the typical peaks, including the methyl and methane protons at 1.76 and 3.2 ppm (H_7, H_2), the ring methane protons and also the methylene protons at 3.5–3.7 ppm ($H_{3,4,5}$, H_6) and the protons on the anomeric carbon at 4.8 ppm (H_1). The spectrum of chitosan–PEG copolymer in Fig. 2c was similar to that of chitosan except two new peaks, one at 4.5 ppm ($H_{a,b}$) and another at 3.38 ppm (H_d). These new peaks were assigned to PEG macromere (Fig. 2b). Also, a non separated broad peak was appeared at 3.8–4.3 ppm from the overlap of saccharide groups of chitosan with methylene groups of PEG ($H_{3,4,5,6,1',c'}$). Furthermore, the intensity of the proton peak of the anomeric carbon (H_1, 4.8 ppm) was reduced significantly, and the proton of amino groups at 3.2 ppm was shifted to 3.3 ppm (H_2).

More confirmation for the polymerization of the block copolymer was obtained by ^{13}C NMR analysis (Fig. 3). The signals at 23.3 (C_7), 55.8 (C_2), 61.2 (C_6), 72.3 (C_3), 75.0 (C_5), 77.3 (C_4), 103.2 (C_1), and 174.5 (C_8) ppm were attributed to the polysaccharide structure (Fig. 3a). In comparison with chitosan, peak correspond to $C_{1'}$ appeared at 98.6 ppm on the ^{13}C NMR spectra of chitosan–PEG copolymer (Fig. 3b). One broad peak at 68.6 (C_d) ppm and four narrow peaks at 59.0 (C_e), 63.4 (C_a), 64.1 (C_b), and 173 (C_c) ppm were assigned to the PEG macromere.

The differential scanning calorimetry studies was also performed to confirm the chemical reaction between chitosan and PEG macromere. Fig. 4 shows the DSC scans for pure chitosan and MPEG in the temperature range of 0–350 °C. Chitosan shows a broad endothermic transition between 60 and 100 °C due to the loss of moisture content in the polysaccharide backbone. An exothermic peak at 340 °C is attributed to the degradation of the

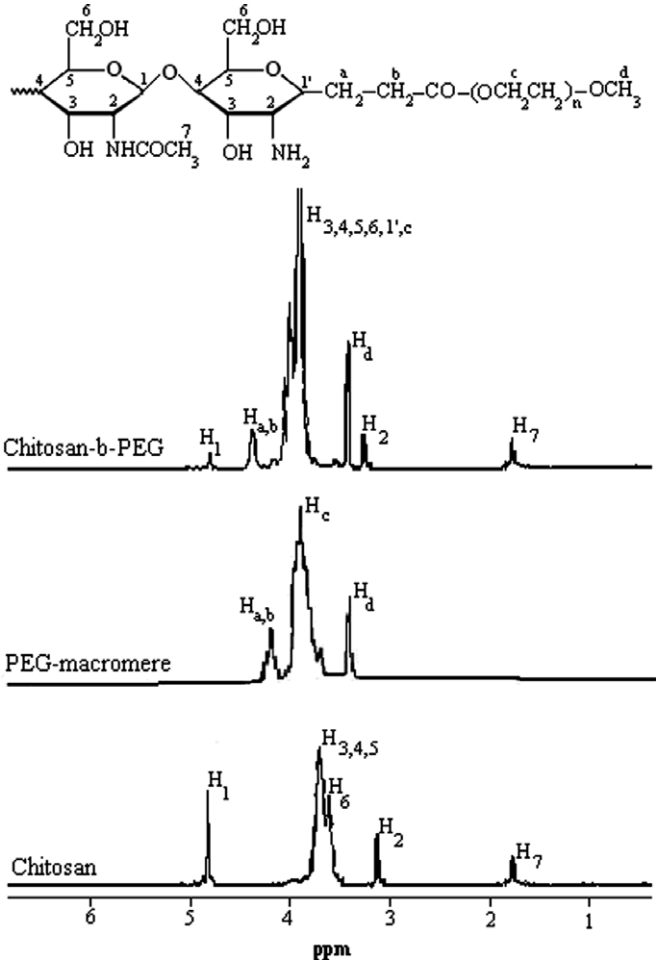


Fig. 2. ^1H NMR spectra of (a) chitosan, (b) MPEG, and (c) chitosan–PEG block copolymer in D_2O/HCl (100:1 V/V) at 80 °C.

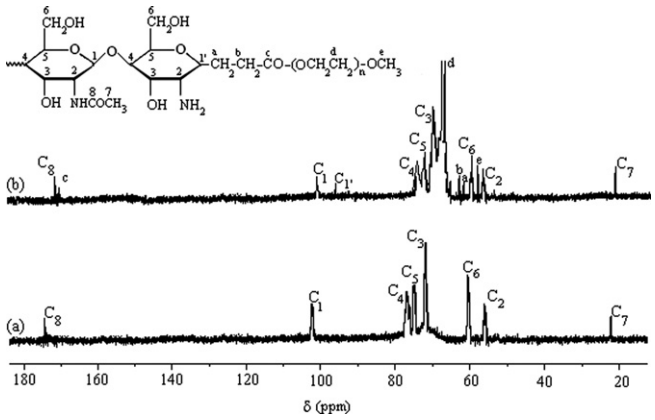


Fig. 3. ^{13}C NMR spectra of (a) chitosan and (b) chitosan–PEG block copolymer in D_2O/HCl (100:1 V/V) at 80 °C.

chitosan. Although chitosan has crystalline regions, the glass transition temperature (T_g) has no been found because of rigid-rod polymer backbone having strong inter and intra-molecular hydrogen bonding. This behavior is frequently detected in many polysaccharides such as cellulose and chitin derivatives (Dong, Ruan, Wang, Zhao, & Bi, 2004; Kim, Kim, Moon, & Lee, 1994). MPEG (M_w = 2000) is a semi-crystalline material with a melting point of 53 °C (Fig. 4). It has been reported that MPEG has a glass transition temperature at around −40 °C, which is not observed in the present temperature range. Fig. 5 shows the DSC thermograms for chitosan–PEG copolymers. To eliminate the effect of moisture, two cycles of heating and cooling runs were adopted and the results of the second heating run is shown in Fig. 5. The summary of the DSC results for block copolymers also is given in Fig. 5. Sharp endothermic peaks of CP2, CP3, CP4, and CP5 copolymers must be corresponded to the melting temperature of MPEG segment. Endothermic peak of copolymer CP1 was too weak to be detected clearly due to low content of MPEG. Apparently, the degradation temperatures of the copolymers were shifted from 325 to 344 °C, indicating the enhanced thermal stability of copolymers. This variation in thermal events supported the formation of the copolymer.

Solubility of chitosan and chitosan–PEG copolymers in different solvents is presented in Table 2. Chitosan is insoluble in water and other common solvents because of its strong intra-molecular hydrogen bonding. The copolymerization with hydrophilic PEG molecules disrupted the inherent crystalline structure of chitosan by disrupting the intra-molecular hydrogen bonding. Therefore, as the molar ratio of PEG in chitosan–PEG block copolymer increases the solubility increases (Table 2). Moreover, the enhanced organo-solubility of CP4 and CP5 would extend the application of chitosan in biomedical engineering.

3.3. Sol–gel transition

Different samples with various compositions of chitosan–PEG copolymers (CP1, CP2, CP3, CP4, and CP5 in Table 1) were tested for gelation behavior using the inverted tube test method. The solutions of CP4 and CP5, with PEG/CH ratio more than 40%, showed an obvious sol-to-gel transition around body temperature, with polymer concentrations ranging from 2 to 3 w/v%. Below the transition temperature, these solutions were viscous liquids that flowed easily and were injectable through a 22-gauge needle. As the solutions were heated to above the transition temperature, they transformed into an opaque gel. In addition, viscosity measurement method has been used to show the sol–gel transition behavior of the copolymers. Fig. 6 illustrates viscosity of CP4 copolymer versus temperature for two different polymer concentrations in the solution. A sharp increase in viscosity around 35 °C for 2 and 3% w/v indicates the beginning of the gelation process. Table 3 shows the gelation time and temperature of CP4 and CP5 solutions. The gelation time varied from 6 to 11 min. Solutions with high polymer concentrations and low PEG content gel faster than those with low polymer concentrations or high PEG content. Similar trends were observed by Bhattarai et al. (2005) for thermosensitive chitosan-g-PEG copolymers. They have shown that the chitosan-g-PEG copolymers would undergo a thermoreversible

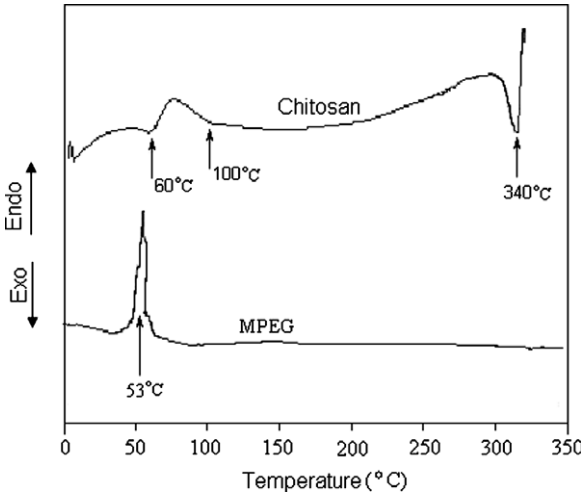


Fig. 4. DSC thermograms of MPEG and chitosan.

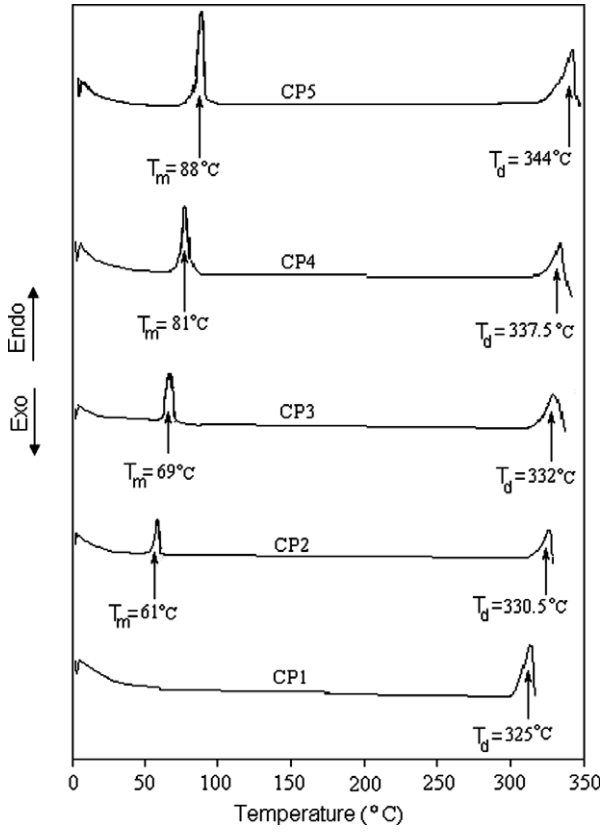


Fig. 5. DSC thermograms of chitosan–PEG block copolymers obtained from the second run at a heating rate of 10 °C/min. (T_m and T_d: melting temperature and degradation temperature of copolymers).

transition from a solution at low temperature to a transparent gel at approximately 25 °C or above. The required time for gelation of their chitosan-g-PEG copolymers varied from 10 min to 1 h, depending on polymer concentration. They have also found that the required amount of grafted PEG to have an injectable thermosensitive copolymer is approximately 36–55 wt%. Below the 36 wt% of grafted PEG, the obtained copolymers were found to be hardly soluble in water (Bhattarai et al., 2005).

So far, several mechanisms have been presented for the gelation of thermosensitive polymer solution, especially in chitosan and PEG systems (Ngimhuang et al., 2004; Richardson & Gorton, 2003; Sugimoto et al., 1998). It is well known that thermosensitive behavior in a polymer solution can generally be considered as a change in the intermolecular interactions in response to temperature. Poly(ethylene glycol) solutions are known to become less soluble and precipitate at higher temperatures in aqueous solutions due to a conformational transition to a less-polar form (Carstens et al., 2005). Polysaccharides can also be considered as ethylene glycol containing polymers, as well as some cellulose derivatives which have demonstrated reduced solubility in aqueous media upon heating (Saeki, Nobuhiro, Nakata, & Kaneko, 1967). In a chitosan/PEG copolymer, considering the polarity of chitosan chains at low temperatures (Karlstrőm, Carlsson, & Lindman, 1990), chitosan chains are covered with water molecules attached by hydrogen bonds between hydrophilic groups of PEG and water molecules. Upon heating of this solution, both chitosan and PEG polymer chains lose their attached water molecules and hence, their hydrophobic interactions between copolymer molecules increase. The physical junction zones of polymer chain segments increase and gel forms. Considering the intermolecular forces involved in the chitosan/PEG solution, it is obvious that the DS of PEG in copolymer would regulate the physical properties of the chitosan–PEG block copolymer as well as its physical properties.

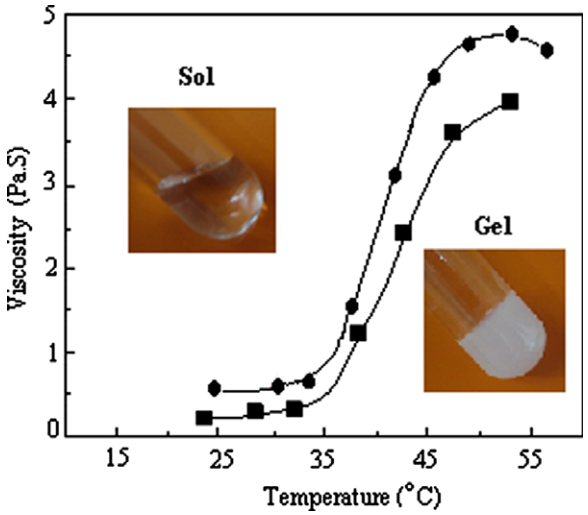


Fig. 6. Viscosity of chitosan–PEG block copolymer (sample CP4) versus temperature for two different values of polymer concentration (■): 2% w/v and (●): 3% w/v.

Table 3
Gelation temperature and gelation time of chitosan–PEG block copolymers

Sample No.	Polymer content (%w/v)	Gelation temperature (°C)	Gelation time (min)
CP4-1	2	36	9
CP4-2	3	35	6
CP5-1	2	40	11
CP5-2	3	37	8

4. Conclusion

A novel thermosensitive injectable hydrogel was developed and characterized by spectral techniques. Chitosan was block copolymerized with PEG macromere in the presence of potassium persulfate as a free radical initiator. This hydrogel undergoes a thermosensitive transition from a free flowing solution at room temperature to a gel around 36 °C. This gelation behavior makes the chitosan–PEG block copolymers more promising and attractive materials for biomedical applications such as drug delivery, cell encapsulation or tissue engineering.

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