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Synthesis and characterization of AB-crosslinked graft copolymers based on maleilated chitosan and *N*-isopropylacrylamide

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Abstract

AB-crosslinked graft copolymers based on a maleilated chitosan (MA-CS) and *N*-isopropylacrylamide (NIPAAm) were successfully synthesized through a two-step reaction. Chitosan was first modified with maleic anhydride to produce MA-CS which contained vinyl carboxylic acid groups. In the second step, NIPAAm monomer was graft-copolymerized onto MA-CS via free radical polymerization initiated by UV radiation. The chemical structures of the synthesized copolymers were confirmed by analyses of the Fourier transform infrared and nuclear magnetic resonance spectra. The grafting efficiency increased with the added amount of NIPAAm monomer and it reached a plateau value of ca. 55%. In the mean time, grafting ratio increased with the added amount of NIPAAm as well, and it had a value of 2.47 when NIPAAm monomer was added five times the amount of MA-CS. A two-stage thermal degradation behavior was observed for the copolymer samples, corresponding to the degradations of chitosan and grafted PNIPAAm chains, respectively. In addition, all copolymer samples exhibited the same glass transition temperature as pure PNIPAAm at 137 °C. All of the experimental results confirmed the AB-crosslinked structure in the formed copolymer of PNIPAAm and MA-CS. The influences of grafting ratio on the phase transition as well as swelling behavior of copolymer gels in various pH buffer solutions at different temperatures were studied.

Keywords: Chitosan; Maleic anhydride; Poly(*N*-isopropylacrylamide); Graft copolymer; Hydrogels

1. Introduction

Chitosan (CS), generally prepared via the partial deacetylation of chitin in a hot alkali solution, can be dissolved in acidic solutions and becomes a cationic polymer because of the protonation of amino groups on the C-2 position of pyranose rings. It therefore exhibits pH responsive behavior due to the large numbers of amino groups (Denuziere, Ferrier, & Domard, 1996; Lee, Park, & Ha, 1997; Park, Lee, & Ha, 1996). Because of several unique and interesting biological properties such as biocompatibility, biodegradability, and nontoxicity, chitosan has been used in a variety of biomedical fields such as drug delivery carriers, surgical thread, and wound healing materials (Muzzarelli, 1977; Shigemasa, Morimoto, Saimoto, Okamoto, & Minami, 1998). Many applications of chitosan in skin tissue engineering have been reported (Cho, Cho, Chung, Yoo, & Ko, 1999; Ma, Wang, He, & Chen, 2001), especially for its advantages in wound healing such as hemostasis, acceleration of the tissue regeneration and the fibroblast synthesis of collagen. Furthermore, chitosan has both reactive amino and hydroxyl groups that can be used to chemically alter its structure and properties under mild conditions (Don, Hsu, & Chiu, 2001; Don, King, & Chiu, 2002a, 2002b).

For more than two decades, considerable attention has been drawn to the so-called smart hydrogels which can undergo a reversible and yet discontinuous volume phase change in response to environmental stimuli such as pH (Hirokawa & Tanaka, 1984), temperature (Bae, Okane, & Kim, 1990; Hoffman, 1987; Hoffman, Afrassiabi, & Dong, 1986; Tanaka, 1979; Yan & Hoffman, 1995), ionic strength (Khare & Peppas, 1995), electric field (Beltran, Hooper, Blanch, & Prausnitz, 1990; Ilavsky, 1982; Ulbrich, Subr, Podperova, & Buresova, 1995), and so on. These smart gels have potential applications in biomedical and pharmaceutical fields (Zhang & Nicholas, 2000). Among them,

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temperature- and pH-responsive hydrogels have been most widely studied because these two factors are crucial to the human body (Nishi & Kotaka, 1989; Tanaka, Kagami, Matsuda, & Osada, 1995). One of the most well-known thermal-responsive polymers is poly(*N*-isopropylacrylamide) (PNIPAAm), which exhibits a lower critical solution temperature (LCST) at around 32 °C in an aqueous solution (Chee, Rimmer, Soutar, & Swanson, 2001; Winnik, 1990). That is, it dissolves in water below the LCST and precipitates from the aqueous solution above the LCST due to the disruption of hydrogen bonding with water and the increasing hydrophobic interactions among isopropyl groups. PNIPAAm hydrogels with crosslinked structure thus can swell in water below the LCST and shrink above it (Aoki et al., 1994; Diez-Pena, Quijada-Garrido, & Barrales-Rienda, 2002; Tanaka et al., 1995). It should be mentioned that in addition to the applications in controlled drug release, PNIPAAm-grafted surfaces have been exploited for controlling cell adhesion/detachment by changing the incubation temperature above or below its LCST, thus avoiding the usage of tripsin (Nakajima et al., 2001).

Recently, a much great attention has been drawn to the development of dual-stimuli-responsive hydrogels, especially for the delivery vehicles that respond to localized conditions of pH and temperature in the human body. They may be prepared by combining PNIPAAm with another stimulus-sensitive polymeric component, to form copolymer gels (Bokias & Hourdet, 2001; Kubota, Tasumoto, Sano, & Matsukawa, 2001; Lowman & Peppas, 1999; Peniche et al., 1999; Zhang & Peppas, 2002). The copolymer hydrogels are generally synthesized by radical polymerization of NIPAAm with acrylic acid type monomers in the presence of a crosslinking agent, most frequently *N*,*N*-methylene-bisacrylamide (MBAM). However, it would be beneficial to introduce other unique properties into the materials such as biocompatibility, biodegradability and biological functions in addition to the dual-stimuli-responsive property, especially when biomedical applications are desired (Hirasawa, Maeda, & Kitano, 1998; Liu & Fan, 2002; Nozaki, Maceda, Ito, & Kitano, 1995; Nozaki, Maceda, & Kitano, 1997). A number of biopolymers have been considered to be incorporated into PNIPAAm in this respect, and the one with the greatest potentiality is chitosan. Dual-stimuli-responsive hydrogels that combine the characteristic properties of chitosan with those of PNIPAAm have not received much attention until recently (Cho et al., 2004; Kim, Cho, Lee, & Kim, 2000; Lee & Chen, 2001; Lee, Ha, Cho, Kim, & Lee, 2004; Lee, Wen, & Chiu, 2003; Verestiuc, Ivanov, Barbu, & Tsibouklis, 2004; Wang, Fang, & Hu, 2001; Wang et al., 2000). Kim et al. (2000) synthesized graft copolymers based on chitosan and NIPAAm using ceric ammonium nitrate as the initiator. The chitosan-*g*-PNIPAAm was then crosslinked through the amino groups in CS with glutaraldehyde. They found that the equilibrium water content of the crosslinked chitosan-*g*-PNIPAAm dropped sharply when pH and temperature were greater than 7 and 30 °C, respectively. Wang et al. (2000, 2001) synthesized a semi- and a full-IPN chitosan/PNIPAAm hydrogel based on a formaldehyde-crosslinked chitosan network with a linear PNIPAAm and with a MBAM-crosslinked PNIPAAm, respectively. The authors exploited the temperature-responsive properties of the prepared IPN gels. Lee et al. (2003) synthesized a semi-IPN chitosan/PNIPAAm particle with the method of soapless dispersion polymerization using two different initiators and a common crosslinking agent, MBAM. They investigated the morphologies of the prepared particles and reported that a core-shell structure was formed when a cationic initiator was used, but not in the systems with an anionic initiator. Verestiuc et al. (2004) synthesized a semi-IPN of PNIPAAm/CS using tetraethylene glycodiacrylate as the crosslinker. The effects of the degree of crosslinking and the ratio of chitosan to PNIPAAm on the pH/temperature induced phase transition characteristics and swelling behaviors of the hydrogels were investigated. They found that as the chitosan content and crosslinking density increased, the phase transition temperature of the interpenetrated hydrogels became diffusive and shifted towards lower temperatures. Most three-dimensional networks of dual-stimuli-responsive CS/PNIPAAm hydrogels reported in the literatures were synthesized by the use of a crosslinking agent. In this study, we report a different route for the synthesis of crosslinked copolymers based on a modified chitosan and PNIPAAm without the involvement of a crosslinking agent. That is, chitosan main chains are linked at various points to the PNIPAAm chains, a novel type of structure called AB-crosslinked graft copolymer with A and B being chitosan and PNIPAAm, respectively. Several instrumental methods have been used to characterize the copolymer's structure. The influences of the grafting ratio on the phase transition behavior of the copolymer gels in solutions at different pH values and temperatures were investigated.

2. Experimental

2.1. Materials

Chitosan (TCI, Tokyo, Japan) was purified by dissolution in an acetic acid solution followed by precipitation with the addition of an alkali solution. It was then filtered and washed with an excess amount of water and subsequently with acetone. Finally, it was dried in vacuo. The degree of deacetylation (DDA, i.e. degree of substitution of amino group) of chitosan was determined by a spectrophotometric method. A certain amount of chitosan was first dissolved in 0.01 N HCl(aq) and a first-derivative spectrum was recorded using an UV spectrophotometer. From the calibration curve obtained from the first derivative value of absorbance at 203 nm of *N*-acetyl glucosamine standard, the degree of deacetylation of chitosan was found to be 85.6%. This value agreed to the result that measured previously (86%), using a colloid titration method (Don et al., 2002a, 2002b). The viscosity average molecular weight ($\bar{M}_v$) of chitosan, determined by a capillary viscometric method in a 0.2 M AcOH/0.2 M AcONa solution at 25 °C, was calculated to be 4.5×10^5 based on the Mark–Houwink equation, $[\eta]=k\bar{M}_v^{\alpha}$, where k and α were 1.38×10^{-2} and 0.85, respectively (Don et al., 2002a, 2002b). Maleic anhydride (MA) was of reagent grade and purchased from Fluka (Steinheim, Germany). *N*-isopropylacrylamide (NIPAAm) monomer was supplied by Acros (New Jersey, USA). It was purified by dissolution in toluene followed by precipitation in a large excess of hexane and drying in vacuo. The photo-initiator, 2-hydroxy-2-methylphenyl-1-propanone (HMPP), also called 'Darocure 1173', was purchased from Sigma (St Louis, USA). All the other chemical reagents were at least reagent grade and used without further purification.

2.2. Reaction of chitosan with maleic anhydride

In order to provide reactive sites on chitosan molecules that can be copolymerized with NIPAAm, a modified chitosan carrying vinyl carboxylic acid groups was designed and synthesized. Specifically, 0.750 g of chitosan was dissolved in 30.0 g of formic acid, and 3.75 g of MA was added afterwards. This solution was kept in a rotary oven for 24 h at 25(±1) °C. After the reaction was completed, the solution was dialyzed against deionized water in a dialysis bag with a cut-off molecular weight of 6000–8000 g/mol to remove residual small molecules such as formic acid and un-reacted MA. After 5 days of dialysis and during which water was replaced every 4 h, the product was poured into a large volume of acetone, and followed by filtration and drying in a vacuum oven. The degree of substitution was measured by both gravimetric and UV-spectrophotometric method. In the UV-spectrophotometric method, a calibration curve was first established using pure maleic acid as the standard with the peak absorbance at 210 nm. The molar absorptivity ($\varepsilon_{\max}$) due to the $\pi\rightarrow\pi^*$ transition (K band) at 210 nm was found to be 14,090. Thus, the amount of vinyl carboxylic acid bonded to chitosan could be found by the interpolation from the calibration curve with the measured absorbance of a specific amount of maleilated chitosan.

2.3. Copolymerization of N-isopropylacrylamide with maleilated chitosan

A specific amount of NIPAAm monomer (W_m) and HMPP photo initiator (1 wt% of monomer) were added into an aqueous solution of previously synthesized maleilated chitosan (W_{CS}). The solution was poured into a Teflon open mold and pre-dried at 50 °C for 2 h. Graft polymerization of NIPAAm onto chitosan was carried out under UV irradiation at an energy level of 750 mJ/cm^2 for 120 s. After reaction, the sample was post-dried at 50 °C. The obtained sample film was washed several times in hot water at 50 °C for the removal of un-reacted monomer. It was then dried in vacuo and weighed (W_1). Subsequently, PNIPAAm homopolymer was extracted out from the sample film with cold water at 15 °C. The final copolymer was obtained by oven-drying in vacuo at 50 °C and weighed again (W_2). The monomer conversion (X), grafting efficiency (GE), and grafting ratio (GR) were all calculated by the gravimetric method according to the following equations:

$$X=(W_1-W_{CS})/(W_m) \qquad (1)$$

$$\mathrm{GE}=(W_2-W_{CS})/(W_1-W_{CS}) \qquad (2)$$

$$\mathrm{GR}=(W_2-W_{CS})/(W_{CS}) \qquad (3)$$

For comparison, pure PNIPAAm was synthesized in the same way by UV photo-initiation. However, a crosslinking agent (1 wt% of monomer), *N*,*N*-methylene-bisacrylamide, was added to crosslink PNIPAAm.

2.4. Structure analysis

Structure analysis was carried out with a Fourier transform infrared (FTIR) spectrophotometer (model 550, Nicolet) and a solid NMR instrument (Bruker DSX400WB NMR, 100 MHz for ^{13}C nucleus and 400 MHz for ^{1}H nucleus). To obtain FTIR spectra, sample was ground into powder and mixed with KBr powder. The mixture powder was then pressed into transparency disc and scanned from 4000 to 400 cm^{-1} for 32 times with a resolution of 4 cm^{-1}. The structures of various samples were also analyzed by a solid NMR instrument, for which the samples were also ground to powders.

2.5. Thermal properties

A differential scanning calorimeter (TA 2920, TA Instruments) was used to observe the thermograms of copolymer samples. Nitrogen gas was purged into the cells. A sample (8–10 mg) was placed in an aluminum pan and scanned from 30 to 220 °C at a heating rate of 10 °C/min. Then, it was quenched and re-heated again. A mid-point method was employed to estimate the glass transition temperature (T_g). Thermal degradation behavior of various samples was investigated using a thermal gravimetric analyzer (Hi-Res TGA 2950, TA Instruments). A certain amount of sample (5–8 mg) was placed in a platinum pan and heated to 100 °C, held for 5 min to remove residual moisture, and then heated from 100 to 600 °C at a rate of 20 °C/min under nitrogen atmosphere.

2.6. Morphology observation

Low voltage (3–5 kV) scanning electron microscope (FESEM, Leo1530, Germany) was used to observe the surface and cross-sectional morphologies of copolymer samples. Samples were prepared by two different methods.

In the first method, copolymer films were oven-dried directly. In the second method, copolymer films were swollen in deionized water and then freeze-dried. To observe the cross-sectional area, cryogenic fracturing of the samples was done after freezing the samples in liquid nitrogen. All specimens were coated with a conductive layer of sputtered gold.

2.7. Phase transition temperature and swelling ratio of copolymer gels

The phase transition behavior of various copolymer samples, placed in quartz cuvettes, was studied by measuring the optical transmittance of the system at 480 nm over the temperature range, 20–50 °C. An UV–Visible spectrophotometer equipped with an online temperature controller was used to measure the optical transmittance. For comparison, the transmittance of pure chitosan and crosslinked PNIPAAm were also measured. The swelling experiments were performed in buffer solutions (Horiba) at different pH values, as well as in different temperatures. For the pH dependent swelling studies, copolymer films (approximately 0.03 g, 4×4 cm^2) were incubated in a 30.0 g of buffer solutions ranging from pH 2 to 12. For the temperature dependent swelling studies, copolymer films were swollen in a particular buffer solution at various temperatures. After 8 h of incubation, reaching equilibrium swelling, gel samples were carefully taken out from the solution, blotted with a filter paper to remove free water from the surface, and then weighed. The swelling ratio (SR, g/g) was evaluated using the equation as follows: $SR=(W_{wet})/(W_{dry})$, where W_{dry} and W_{wet} were weights of dry and wet sample, respectively.

3. Results and discussion

3.1. Synthesis and structure analysis of maleilated chitosan

In order to prepare chitosan having vinyl functional groups for further graft polymerization with NIPAAm monomer, maleic anhydride was used to modify chitosan. Pure formic acid was used as the solvent to dissolve chitosan, avoiding the possible hydrolysis of maleic anhydride which tended to occur in an aqueous solution. After completion of the substitution reaction, the chemical structure of the modified CS was characterized by NMR and FTIR spectrophotometers. Fig. 1 shows the NMR spectra of both pure and modified chitosan. Compared with the spectrum of pure chitosan, three new peaks are observed for modified CS. Obviously, the absorption peaks at 120 and 140 ppm prove the existence of C=C double bond. The absorption peak at 168 ppm was caused by the bonded carboxylic acid. Therefore, after reaction with maleic anhydride, modified chitosan contained vinyl carboxylic acid groups. Furthermore, it was found that there was almost no change in the relative peak area at 23.7 ppm from methyl absorption in *N*-acetyl group of chitosan. Thus, it was believed that deacetylation of *N*-acetyl group in chitosan did not occur ($–NH–C(=O)–CH_3\rightarrow–NH_2$) during the dissolution in formic acid and subsequently substitution reaction with maleic anhydride. In the FTIR spectrum, pure chitosan generally exhibits characteristic absorption peaks of amide groups at 1650 cm^{-1} (amide I, C=O stretching) and 1560 cm^{-1} (amide II, N–H bending). After reaction with maleic anhydride, several new peaks were found in addition to the original peaks from chitosan itself, as shown in Fig. 2. The new absorption peaks at 1705 and 1668 cm^{-1} were assigned to the free and hydrogen-bonding unsaturated carboxylic acid groups, respectively (Silverstein, Bassler, & Morrill, 1981). The hydrogen bonding of carboxylic acid was due to the existence of a large number of hydrogen-bearing functional groups in chitosan such as hydroxyl, amino and amide groups. The finding of these two new peaks agrees with the results from NMR analysis. It was originally assumed that both hydroxyl and amino groups in chitosan could have reacted with maleic anhydride, resulting in ester and amide linkage, respectively. However,

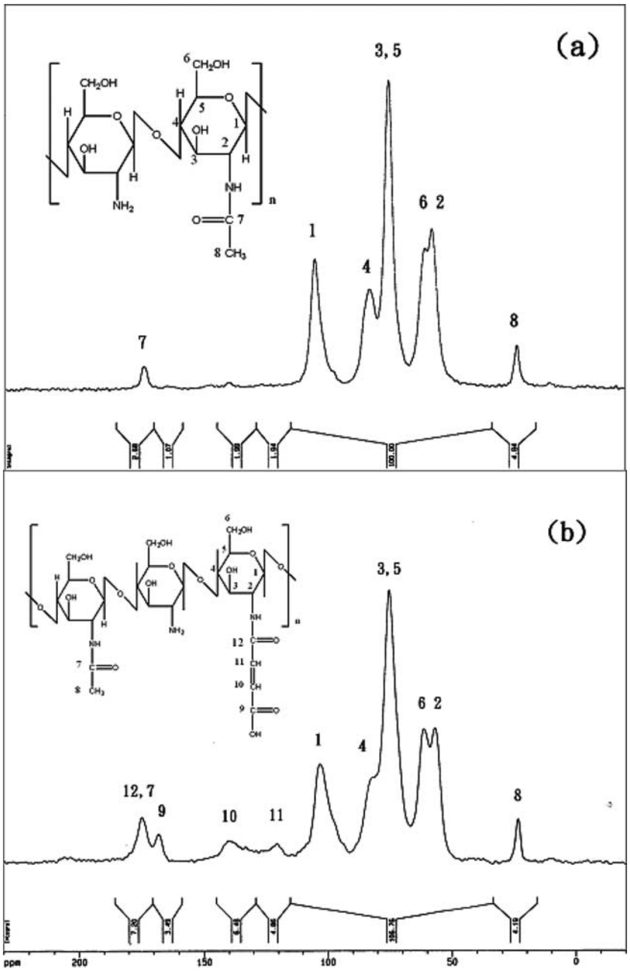


Fig. 1. ^{13}C NMR spectra of pure chitosan (a) and maleilated chitosan (b) obtained from the reaction of maleic anhydride with chitosan.

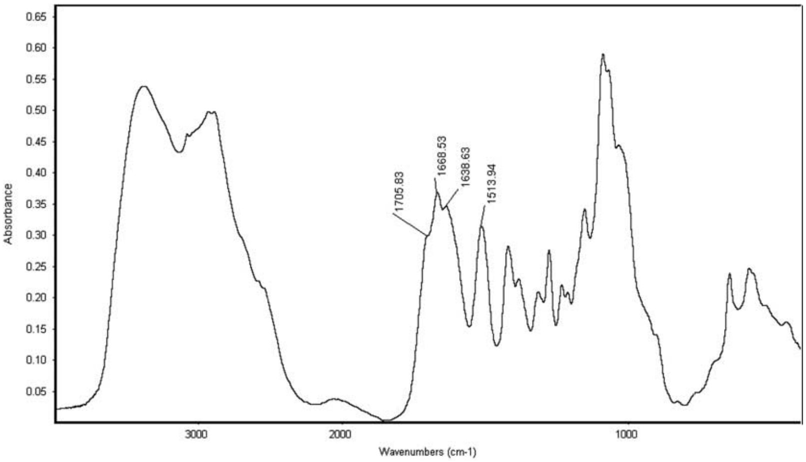


Fig. 2. FTIR spectrum of maleilated chitosan obtained from the reaction of maleic anhydride with chitosan in formic acid.

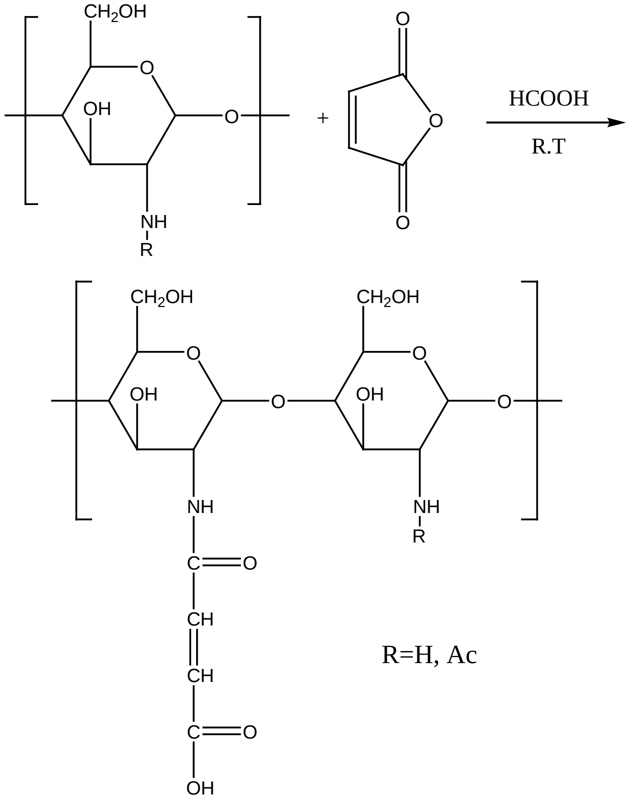
Fig. 2 did not show any absorption peaks of C═O stretching from ester linkage, which was normally found in the range between 1720 and 1740 cm^{-1}. Instead, the original amide peak (amide I) increased in intensity after reaction, and shifted to lower wavenumber due to hydrogen bonding. The results thus lead to a conclusion that the maleic anhydride preferentially reacts with amino group at C-2 position of the CS pyranose ring, as shown in Scheme 1.

From the gravimetric analysis, it was found that there was 2.04×10^{-4} mol of substituted vinyl carboxylic acid group in chitosan. This value was further converted to the degree of substitution, i.e. moles of vinyl carboxylic acid per mole of pyranose ring, with the assumption that the vinyl carboxylic acid group was only bonded at the C-2 position, suggested by the previous spectrophotometric analysis. The calculated value was 0.461. Therefore, the degree of substitution of amino group decreased from the original 0.856 to 0.395 due to reaction of amine with maleic anhydride. The degree of substitution of *N*-acetyl group remained the same as 0.144. The result was further confirmed by a UV spectrophotometric method. The degree of substitution from UV method was 0.487, which was close to the value obtained from the gravimetric method.

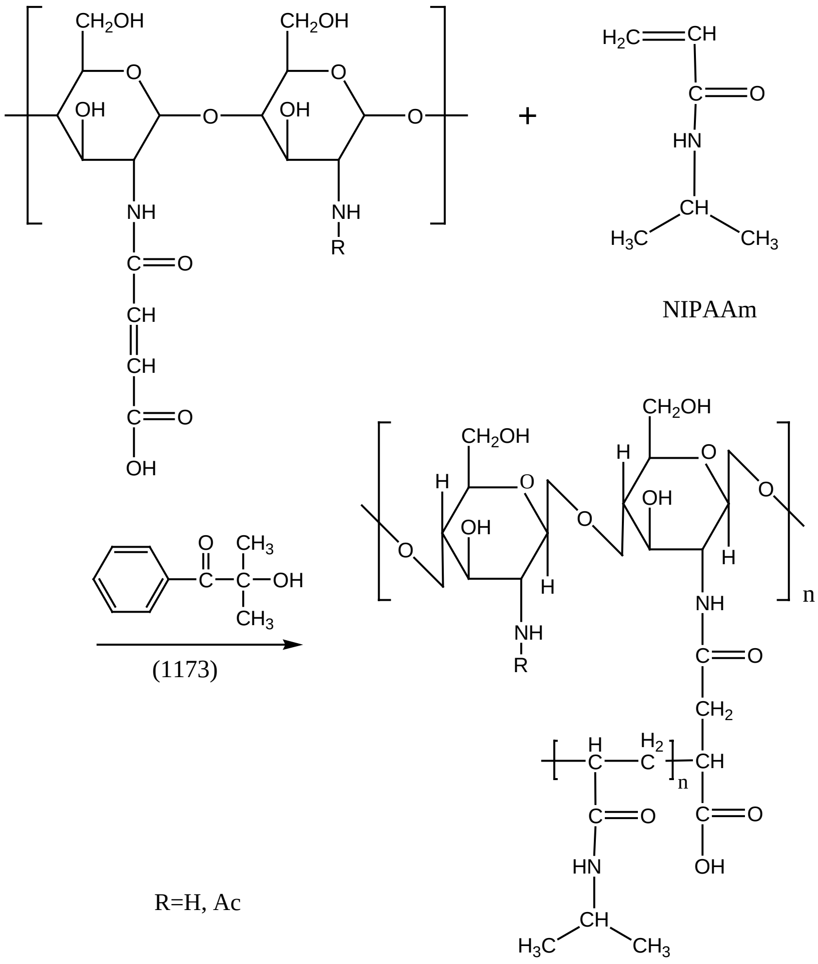
3.2. Grafting of NIPAAm onto maleilated chitosan

After the substitution reaction, the double bond in vinyl carboxylic acid became available for copolymerization with other vinyl monomers through traditional chain polymerization. The maleilated chitosan prepared previously was thus reacted with various amounts of NIPAAm monomer via UV photo initiation. After UV irradiation, a gel film was formed and dried at 50 °C. The reaction route and the chemical structure of the product are shown in Scheme 2.

From the gravimetric analysis, the monomer conversion (X), grafting efficiency (GE) and grafting ratio (GR) of various samples were calculated and listed in Table 1. A relatively high monomer conversion was observed for all samples. This high conversion was because of the long exposure time during UV irradiation at a high energy dosage on the sample. In addition, the conversion increased with the amount of photo-initiator. The increase in the concentration of photo-initiator ($[I_2]_o$) would increase the concentration of propagating chain radicals, which in turn increase the reaction rate. Accordingly, the monomer conversion increased as well. A relationship has been derived (Odian, 1991) between the monomer conversion and initiator concentration, i.e. $-\ln(1-X)=k[I_2]_0^{1/2}$, where k is



Scheme 1. The reaction of maleic anhydride and chitosan (DDA = 85.6%) in formic acid.



Scheme 2. Graft polymerization of maleilated chitosan with NIPAAm monomer.

a constant. Substitution of the values in Table 1, a good linear relationship with a correlation coefficient of 0.988 was obtained, where the proportional constant *k*, known from the slope, was 189 1/mol$^{1/2}$. In addition, the grafting efficiency and the grafting ratio also increased with the amount of monomer and photo-initiator, and the grafting efficiency seemed to reach a plateau value.

After repeated washing to remove un-reacted monomer and PNIPAAm homopolymer, graft copolymer of CS-*g*-PNIPAAm was obtained. To prove that linear PNIPAAm can be removed completely, linear PNIPAAm and chitosan were dissolved in an acetic acid solution first, and then chitosan was crosslinked by glutaraldehyde. It was confirmed that linear PNIPAAm can be washed out completely from crosslinked chitosan structure. Fig. 3 shows FTIR spectra of pure PNIPAAm and several copolymers with different grafting ratios, respectively. PNIPAAm has also the absorption peaks of amide I (1652 cm^{-1}) and amide II (1548 cm^{-1}). In addition, the characteristic isopropyl groups give the doublet absorption peaks at 1387 and 1368 cm^{-1}. In the case of the copolymer samples, the intensity of absorption peaks due to amide I and amide II clearly increase due to the copolymerization with PNIPAAm (cf. Fig. 2). In addition, the intensity of methyl peak at 2973 cm^{-1} and the doublet peaks of the isopropyl group increase with the grafting ratio of PNIPAAm. Fig. 4 shows the ^{13}C NMR spectra of various copolymer samples. The C=C absorption peaks at 120 and 140 ppm disappear completely, indicating that there is no residual double bond left in the chitosan copolymers after the reaction. Furthermore, the absorption peak at 23 ppm (–CH_3) increase in intensity with grafting ratio, due

Table 1
Conversion (*X*), grafting efficiency (GE) and grafting ratio (GR) of various systems based on maleilated chitosan and NIPAAm

Sample	NIPAAm (g)	*X*	GE	GR
P1	0.2688	0.864	0.201	0.174
P2	0.5376	0.911	0.465	0.847
P3	0.8064	0.931	0.556	1.552
P4	1.0752	0.943	0.567	2.141
P5	1.3440	0.946	0.522	2.471

For each sample, maleilated chitosan was added with the same amount as 0.2688 g, and Darocure 1173 was always added as 1 wt% of NIPAAm monomer.

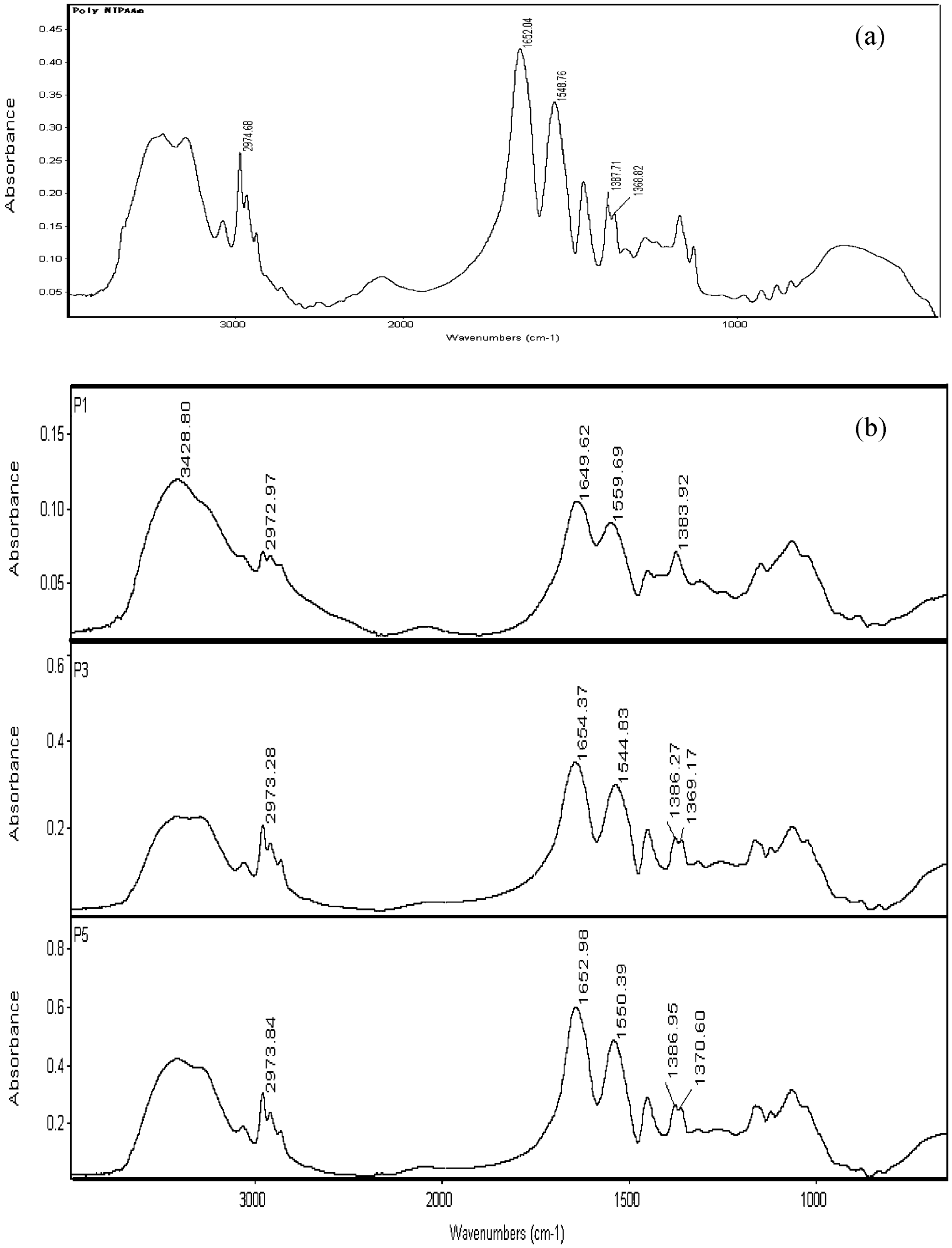


Fig. 3. FTIR spectra of pure PNIPAAm (a) and various chitosan-*g*-PNIPAAm copolymer samples (b); top: P1, middle: P3, bottom: P5.

to the isopropyl group of the grafted PNIPAAm. Another new peak appeared at 42 ppm which stems from the methine carbon in the isopropyl group. FTIR and NMR spectra thus confirmed the structure of CS-*g*-PNIPAAm copolymer. Since there were many vinyl carboxylic acids along a maleilated chitosan chain that underwent reaction with NIPAAm, an AB-crosslinked graft copolymer structure was produced. As shown in Scheme 3, chitosan chains (i.e. A) were crosslinked by the PNIPAAm chains (i.e. B) to form a network that swells but is not soluble in water.

3.3. Thermal characterization of CS-g-PNIPAAm copolymers

Fig. 5 shows the thermal degradation curves of pure chitosan and maleilated chitosan under N_2 atmosphere. The maximum-rate degradation temperature (T_{max}, first derivative peak temperature) of pure chitosan was 278 °C and it increased to 298 °C after substitution with maleic acid on the C-2 position. This increase is because of the increasing hydrogen bonding and charged complex formation between

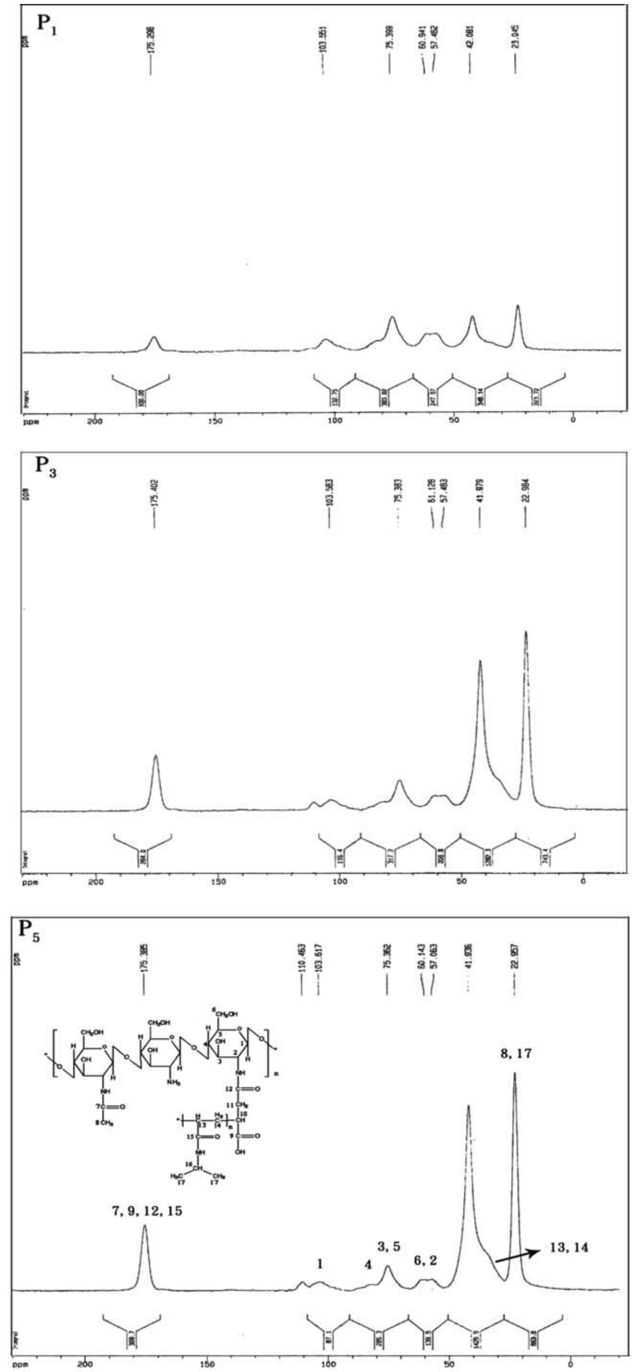


Fig. 4. ^{13}C NMR spectra of various chitosan-*g*-PNIPAAm copolymer samples; top: P1, middle: P3, bottom: P5.

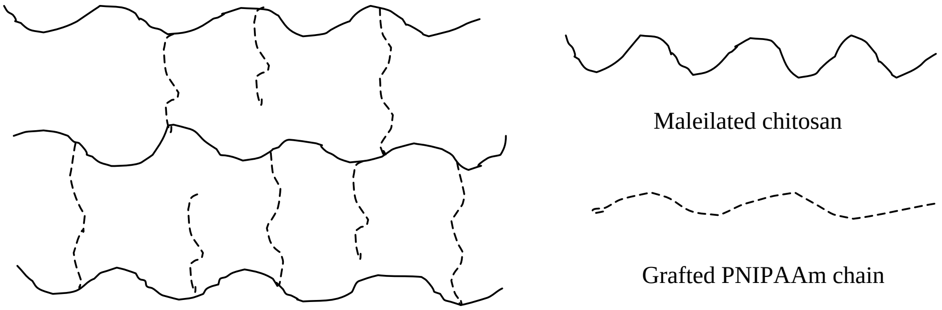
the substituted carboxylic acid group and the remaining amino group. The electrolyte complex has been observed in the system of chitosan and poly(acrylic acid) (Chavasit & Torres, 1990; Chuang, 2001), which also showed an increased degradation temperature. In the same figure, a high char yield at 600 °C was found for pure chitosan at the value of 41.5%, but decreased slightly to 35.6% for MA-CS.

Fig. 6 shows the thermal degradation curves of pure PNIPAAm and copolymer samples. Pure PNIPAAm shows one-stage degradation behavior with the $T_{\max}$ at 413 °C. However, all copolymer samples exhibit two-stage degradation behavior, where the first- and second-stage were caused by the degradation of chitosan and grafted PNIPAAm chains, respectively. The appearance of two distinct stages of degradation confirmed that the reaction products were graft copolymers. Table 2 lists the values of $T_{\max}$ and char yield ($\mathrm{CY}_{\mathrm{exp}}$) of various samples. Compared with MA-CS and PNIPAAm, both the $T_{\max}$ of copolymer samples in the first-stage and second-stage slowly increased with grafting ratio, and reached their plateau values, 309 and 423 °C, respectively. In contrast to chitosan, the char yield of pure PNIPAAm was only 1.5%. As a result, when maleilated chitosan was grafted with PNIPAAm, the char yield decreased proportionally as grafting ratio increased.

Chitosan and PNIPAAm are both hydrophilic polymers that absorb moisture. If a DSC thermal scan was carried out on pure CS and MA-CS, a broad endothermic peak centered at 104 °C was observed for both samples, which was obviously due to the absorbed moisture (Fig. 7). This endothermic peak consequently disappeared in the heating curve of the second run. However, for MA-CS, another small peak was found between 160 and 200 °C, which was absent in the thermogram for pure chitosan. This is due to the strong ionic force between the substituted carboxylic acid group and the amino group. This finding agrees with the TGA results. Because of strong inter- and intra-hydrogen bonding in CS and MA-CS, no glass transition temperature and melting peak can be observed in DSC curves. After copolymerization with NIPAAm, a DSC thermal scan was also recorded on the CS-*g*-PNIPAAm copolymer. After first run to evaporate all the moisture, the second run revealed the glass transition temperature of PNIPAAm chains (Fig. 8). All the graft copolymers had approximately the same glass transition temperature at 137 °C as the pure PNIPAAm, indicating the existence of independent PNIPAAm phase in the copolymers.

3.4. Morphology observation

After UV photo-polymerization and post-drying, a transparent copolymer film was obtained. SEM micrographs of the surface and cross-section of all copolymer samples were examined. Basically, they were all smooth and dense. To observe the morphology of swollen samples, copolymers were immersed in distilled water until equilibrium absorption was attained, and subsequently lyophilized in a freeze-dryer. After lyophilization, opaque films were obtained. Fig. 9 shows SEM micrographs of the surfaces of these films. Obviously, mass lumps of irregular shapes were seen on the surface and their size increased with the grafting ratio of PNIPAAm. This was believed due to the chemical structure of this AB-crosslinked graft copolymer, where chitosan and PNIPAAm had different swelling capabilities.



Scheme 3. AB-crosslinked graft copolymer based on maleilated chitosan and PNIPAAm.

3.5. Phase transition temperature

The phase transition behavior of various samples at different pH values (2, 4, 7, 9, and 12) was investigated by measuring the optical transmittance at 480 nm over the temperature range 20–50 °C. There was no phase transition observed for both CS and MA-CS in the investigated temperature range. However, pure PNIPAAm and PNIPAAm-grafted chitosan exhibited phase transition behavior as shown in Fig. 10 (only data at pH 2, 4 and 9 are shown here, where the optical transmittance behaviors at pH 7 and 12 were similar to those at pH 4 and 9, respectively). For pure PNIPAAm, a lower critical solution temperature (LCST) at 32 °C was clearly observed. The optical transmittance was almost 100% below 32 °C and it dropped immediately to zero once the temperature was raised to above 32 °C. The observed LCST was found to be independent of pH values. The reason for the LCST characteristic in PNIPAAm has been well exploited in literature (Aoki et al., 1994; Chee et al., 2001; Diez-Pena et al., 2002; Tanaka et al., 1995; Winnik, 1990), which the precipitation above the LCST is due to the disruption of hydrogen bonding with water and the increasing hydrophobic interaction among isopropyl groups.

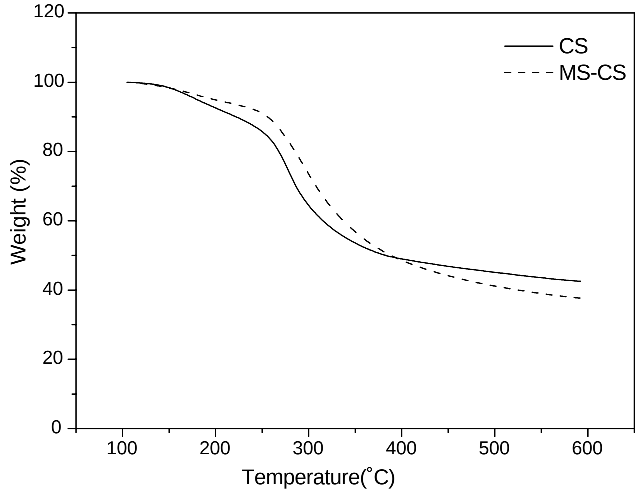


Fig. 5. Thermal degradation curves of pure chitosan and maleilated chitosan under N_2 atmosphere.

When chitosan was grafted with PNIPAAm, the optical transmittance became dependent on the grafting ratio and pH value. For those copolymer samples with higher grafting ratio (P4, P5), Fig. 10 shows that their optical transmittance maintained at a constant value at low temperatures and then started to decrease at 32 °C. The fact that the phase transition temperature was the same as the LCST of pure PNIPAAm indicates that precipitation of the grafted PNIPAAm phase was not affected by the chitosan component. This is because of its graft copolymer structure. In contrast, as reported in the literature (Ringsdorf, Simon,

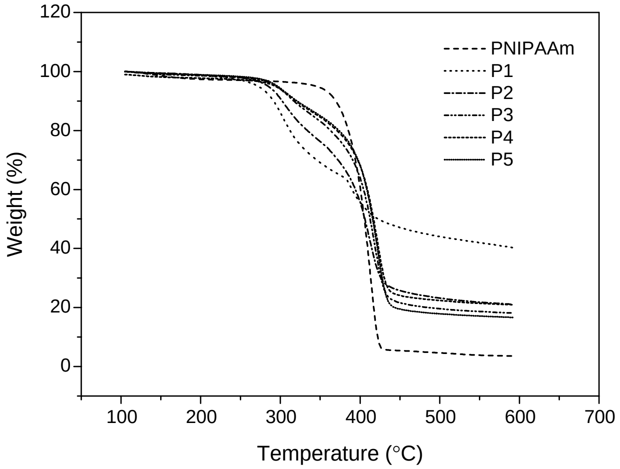


Fig. 6. Thermal degradation curves of pure PNIPAAm and various chitosan-*g*-PNIPAAm copolymer samples under N_2 atmosphere.

Table 2
First and second maximum-rate degradation temperatures ($T_{max,1}$ and $T_{max,2}$) and char yield (CY, %) of chitosan, PNIPAAm and various copolymer samples

Sample	$T_{max,1}$ (°C)	$T_{max,2}$ (°C)	CY (%)
CS	278	–	41.5
MA-CS	298	–	35.6
PNIPAAm	–	413	1.5
P1	302	388	38.0
P2	306	413	19.5
P3	308	418	18.3
P4	309	423	15.4
P5	309	422	14.0

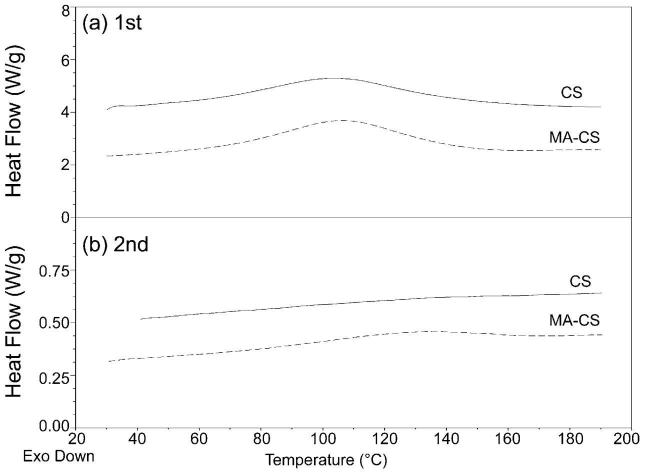


Fig. 7. DSC thermograms of pure chitosan and maleilated chitosan; (a) first heating curve, (b) second heating curve.

& Winnik, 1992; Zhou & Chu, 1998), if a 'random' copolymer was synthesized from NIPAAm with some other hydrophilic monomer such as acrylic acid, the LCST would increase with the composition of the second monomer, due to the resulted hydrophilic modification of the PNIPAAm chain. The effect of grafting ratio on the optical transmittance was clearly observed, when the temperature was raised above 32 °C. As shown in Fig. 10, the higher the grafting ratio of PNIPAAm, the sharper the phase transition was. When the grafting ratio was very low as in sample P1, the decrease in optical transmittance at transition temperature was nearly indiscernible, and it exhibited almost the same optical transmittance behavior as that of pure chitosan. As the grafting ratio increased, the phase transition became pronounced. For sample P5 with the greatest grafting ratio, the phase transition behavior became similar to pure PNIPAAm. The optical transmittance was also found to be dependent on pH value. The optical transmittance at pH 4 and 7 was lower than those at other pH values. This will be explained in detail in the following sections.

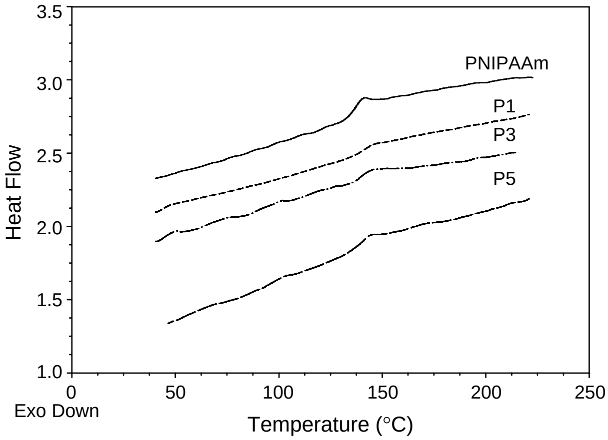


Fig. 8. DSC thermograms of pure PNIPAAm and various chitosan-*g*-PNIPAAm copolymer samples; (a) first heating curve, (b) second heating curve.

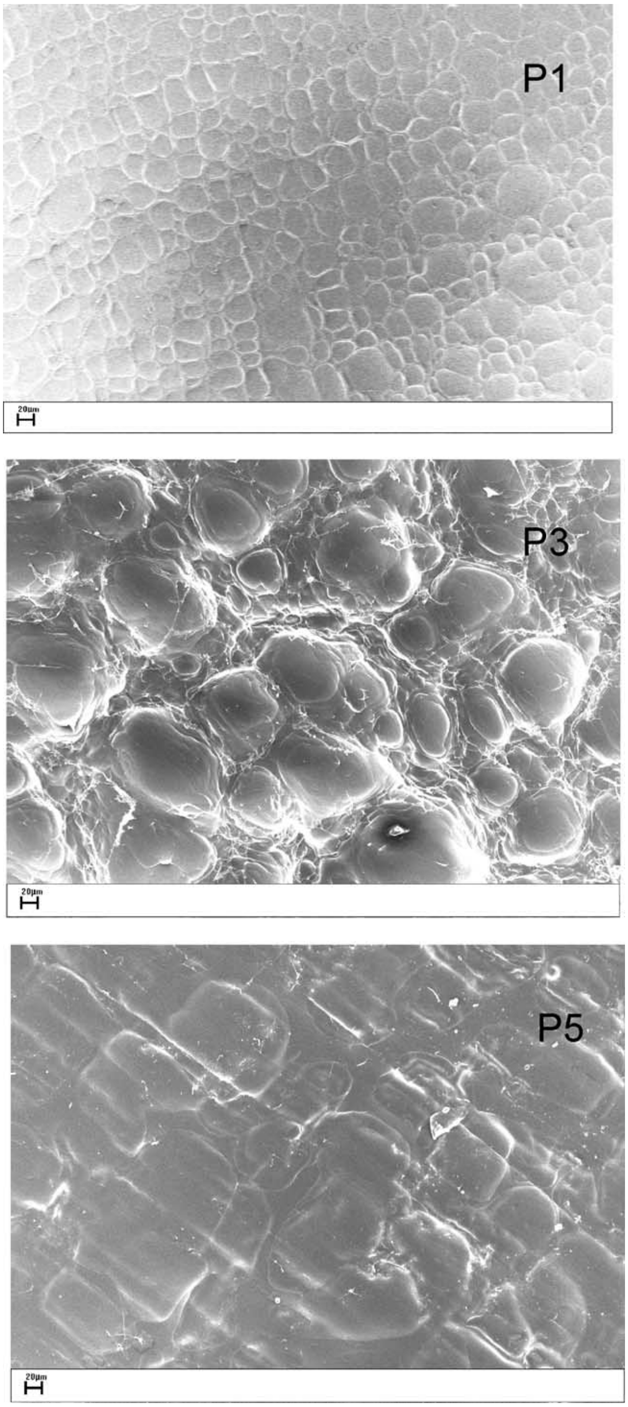


Fig. 9. SEM micrographs of the surfaces of various copolymer samples, top: P1, middle: P3, bottom: P5.

3.6. Swelling behavior

Chitosan easily dissolves in an acidic environment due to the protonation of amino groups at C-2 position. The chitosan chains extend because of the repulsive force arising

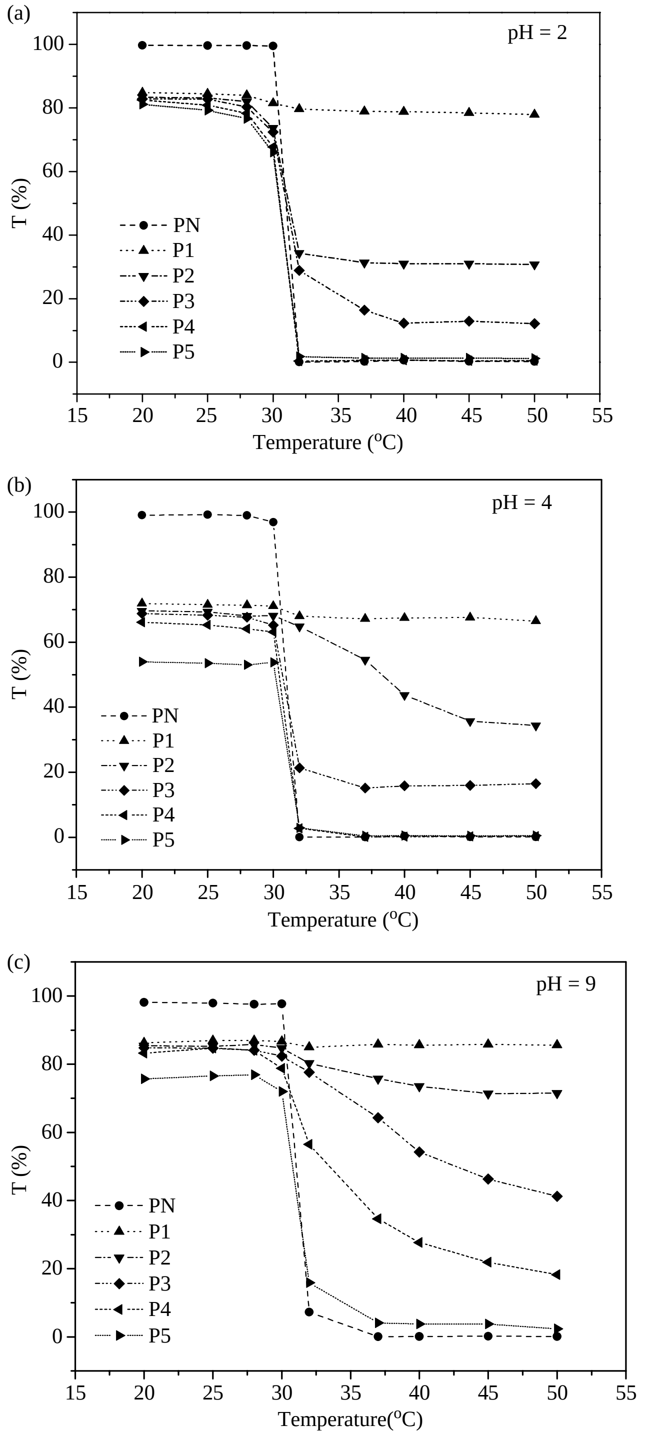


Fig. 10. Optical transmittance at 480 nm of pure PNIPAAm and various chitosan-*g*-PNIPAAm copolymer samples over the temperature range 20–50 °C, (a) pH 2, (b) pH 4, (c) pH 9.

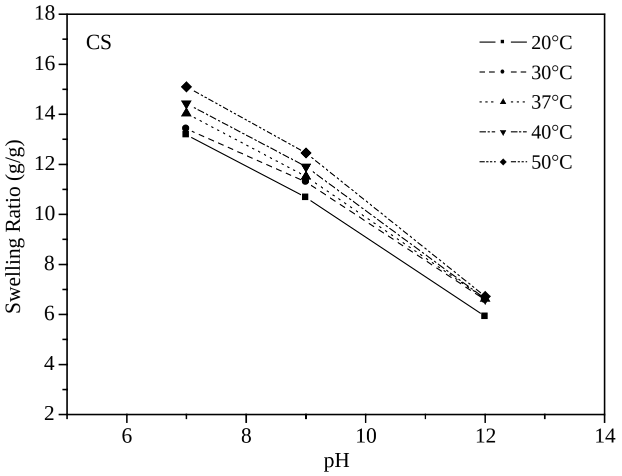


Fig. 11. Swelling ratio of pure chitosan at different temperatures as a function of pH value.

from positive charges along the chains. When the pH value is increased to greater than 6 (pK_a 6.3), chitosan no longer dissolved in aqueous solutions because of insufficient charge density along the chains. The degree of protonation of amino groups decreases with the pH value, leading to a decrease in the swelling ratio (Fig. 11). However, the situation changes when vinyl carboxylic acid group is introduced into chitosan (see Scheme 1). The prepared maleilated chitosan having almost the same number of amino group (DS = 0.40) and carboxylic acid group (DS = 0.45) became difficult to be dissolved in an acidic solution even at pH 2. The reasons are two-fold. First, substitution of carboxylic acid group reduced the original number of amino groups, which in turn reduced the positive charge density along the chitosan chain. Secondly, there was an increase in the strong inter- and intra-hydrogen bonding due to the introduction of carboxylic acid groups, as revealed in previous FTIR spectra. The swelling ratio of MA-CS, however, depended on pH value as that of pure CS. Fig. 12 shows that the swelling ratios of MA-CS had the lowest value in a solution at pH 4 or 7, and it increased when the solution became more acidic or basic. This can be explained by the extents of ionization of amino group and carboxylic acid in respond to pH values. In a strong acidic solution, almost all amino groups became protonated and positive charged ($NH_2 + H^+ \rightarrow NH_3^+$), whereas most

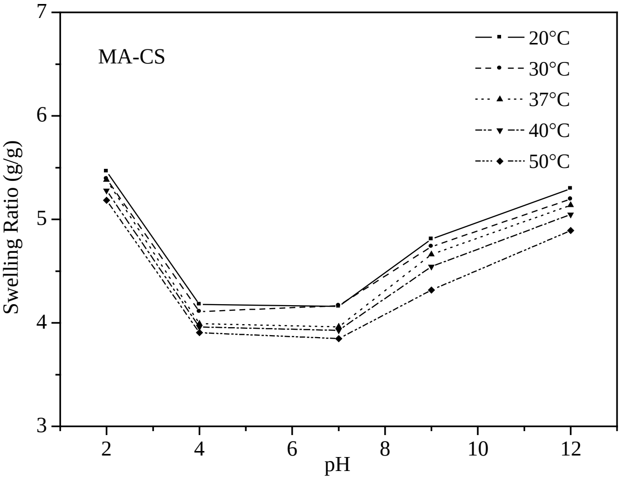


Fig. 12. Swelling ratio of the maleilated chitosan at different temperatures as a function of pH value.

carboxylic acid groups remained neutral (–COOH). On the contrary, in a basic solution, almost all amino groups were not protonated (–NH_2), but most carboxylic acids dissociated to carboxylate anions (–$COOH + OH^- \rightarrow COO^- + H_2O$) (Chavasit & Torres, 1990; Chuang, 2001). Therefore, maleilated chitosan still had considerable electric charges along the chains, namely, positive charges in a strong acidic solution and negative charges in a basic solution. It thus could swell due to the electric repulsive force at both conditions. However, at the intermediate pH values ($4 \leq pH \leq 7$), both amino and carboxylic acid groups might undergo ionization. A strong ionic force thus developed between cationic amino group and anionic carboxylate group, leading to a decrease in swelling ratio. As a result, the lowest swelling ratio was observed at pH 4 and 7. The same phenomenon has been observed for polyelectrolyte complex system of chitosan and poly(acrylic acid) (Chavasit et al., 1990; Chuang, 2001). Fig. 12 also shows that the swelling ratio of maleilated chitosan did not change substantially with temperature.

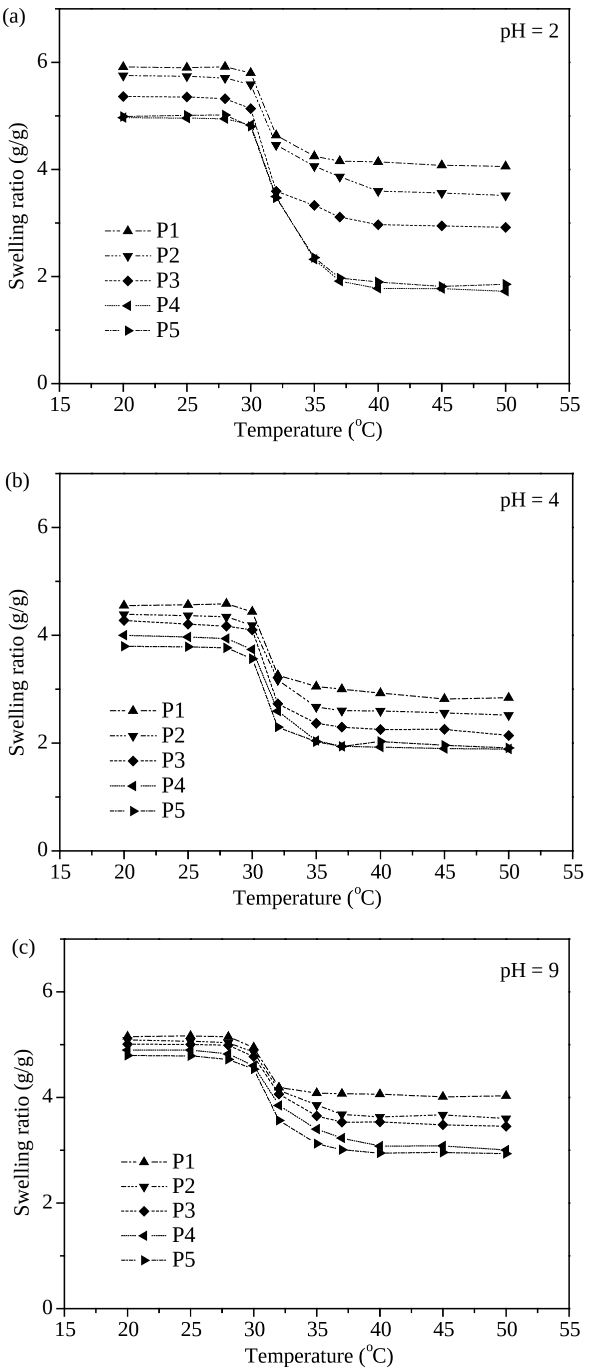


Fig. 13. Swelling ratio of various chitosan-*g*-PNIPAAm copolymer samples at different temperatures, (a) pH 2, (b) pH 4, (c) pH 9.

Pure PNIPAAm dissolves in neutral water, as long as the temperature is below its LCST. Above LCST, PNIPAAm undergoes deswelling and excludes most absorbed water. Therefore, it is a temperature responsive hydrogel. Combining PNIPAAm and chitosan in a crosslinked graft copolymer, the swelling ratio could depend on both temperature and pH value of the aqueous solution. Fig. 13 shows the swelling ratio of CS-*g*-PNIPAAm copolymers at different pH values as a function of temperature (only data at pH 2, 4 and 9 are shown here, where the swelling behaviors at pH 7 and 12 were similar to those at pH 4 and 9, respectively). All of these copolymers exhibited both pH- and temperature–responsive behavior. First, the swelling ratio decreased with an increase in the grafting ratio from P1 to P5. This was most probably due to an increase in the crosslinking density. Also, all swelling ratios started to decline at 32 °C due to the existence of grafted PNIPAAm component. The extent of decrease at phase transition also depended on the grafting ratio of PNIPAAm. An increase in the grafting ratio resulted in a larger decrease in the swelling ratio, which had the same trend as in the optical transmittance behavior. That is, the phase transition became more pronounced as the grafting ratio increased. Second, all copolymers had the same pH response as in maleilated chitosan. The swelling ratio always had the lowest values at pH 4 and 7. When the solution became more acidic or basic, the swelling ratio increased due to the decrease in ionic force between cationic amino groups and anionic carboxylic groups.

4. Conclusions

AB-crosslinked graft copolymers of chitosan and PNIPAAm were successfully synthesized through a two-step reaction. Maleic anhydride was first reacted with chitosan to provide vinyl carboxylic acid groups. In the second step, PNIPAAm was grafted onto modified CS via these vinyl groups initiated by UV irradiation. Both the grafting efficiency and grafting ratio increased with the added amount of NIPAAm monomer. The grafting efficiency reached a plateau value about 55%. Combining MA-CS and PNIPAAm in a graft copolymer, the swelling ratio depended on both pH value and temperature of the aqueous solution. The pH-dependent swelling behavior was due to the co-existence of amino groups and carboxylic acid groups in the MA-CS chain, for which the swelling ratio had the lowest values at pH 4 and 7. The temperature-dependent swelling behavior was derived from the grafted PNIPAAm component with which the swelling ratio started to decrease at 32 °C. The extent of decrease and also the sharpness of phase transition depended on the grafting ratio of PNIPAAm. The higher the grafting ratio, the more pronounced the phase transition was. Further studies on possible applications of these novel type AB-crosslinked graft copolymers in biomedical materials such as controlled drug release and scaffold are currently under investigation, and the relevant results will be reported in a forthcoming article.

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