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Preparation of thermosensitive chitosan with poly(*N*-isopropylacrylamide) side at hydroxyl group *via* *O*-maleoyl-*N*-phthaloyl-chitosan (MPCS)

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Abstract

A novel temperature-sensitive graft copolymer was prepared by grafting *N*-isopropylacrylamide (NIPAAm) onto a chitosan derivate whose amino groups were protected by phthaloyl groups. The deprotection of the phthaloyl groups yields chitosan-*g*-PNIPAAm copolymers with free amino groups. The chemical structure of the copolymers was characterized by FT-IR, ^{1}H and ^{13}C NMR spectroscopy. Our experiments reveal that the grafting degree increases with monomer concentration and reaction time but decreases with the initiator concentration and temperature. In comparison with the chitosan, chitosan-*g*-PNIPAAm shows a faster thermal decomposition. This is because PNIPAAm chains destroy the crystalline region of chitosan. Besides, the graft copolymers exhibit good solubility in aqueous solutions with a broader pH range. Like PNIPAAm, the copolymer exhibits a thermally sensitivity in aqueous solution.

Keywords: Chitosan; *N*-Isopropylacrylamide; Graft copolymer; Thermo-sensitivity

1. Introduction

Chitosan is a well-known abundant natural polymer (Roberts, 1992). Due to its biodegradability, biocompatibility and non-toxicity (Majeti & Kumar, 2000; Kurita, 2001), it has found applications in many areas such as biomedical and agriculture areas. Nevertheless, the crystallinity and poor solubility (Liu, Chen, & Fang, 2006) limit its further applications. Thus, chemical modification has been used to improve the properties of this rigid aminopolysaccharide.

On the other hand, stimuli-sensitive systems, which alter their volume and shape reversibly according to the various external physiochemical factors (Chung, Bae, Park, Lee, & Park, 2005), have received much attention in recent years. Poly(*N*-isopropylacrylamide) (PNIPAAm) is a well-known thermo-sensitive polymer with a lower critical solution temperature (LCST) around 32 °C in aqueous solution. Copolymers based on chitosan and PNIPAAm are expected to exhibit a combination of their properties.

Actually, several studies have been conducted on grafting PNIPAAm chains onto chitosan and its derivates. Because the amino group at C-2 has a higher reactivity than the hydroxyl groups at C-6 and C-3 on the chitosan backbone, most chemical modifications deal with the former. Shin, Kang, Park, and Yang (2002) synthesize a series of hydrogels composed of maleilated chitosan (MC) and NIPAAm by free radical polymerization with ammonium persulphate (APS)/*N,N,N′,N′*-tetramethyl ethylenediamine (TEMED) as the initiator. These hydrogels exhibit sensitivity in response to temperature or pH. Kim, Cho, Lee, and Kim (2000) report the graft copolymerization of NIPAAm onto chitosan using ceric ammonium nitrate as the initiator. The copolymer is soluble in formic acid but insoluble in water. A thermo-responsive comb-like chitosan copolymer has also been synthesized (Chen & Cheng, 2006) by

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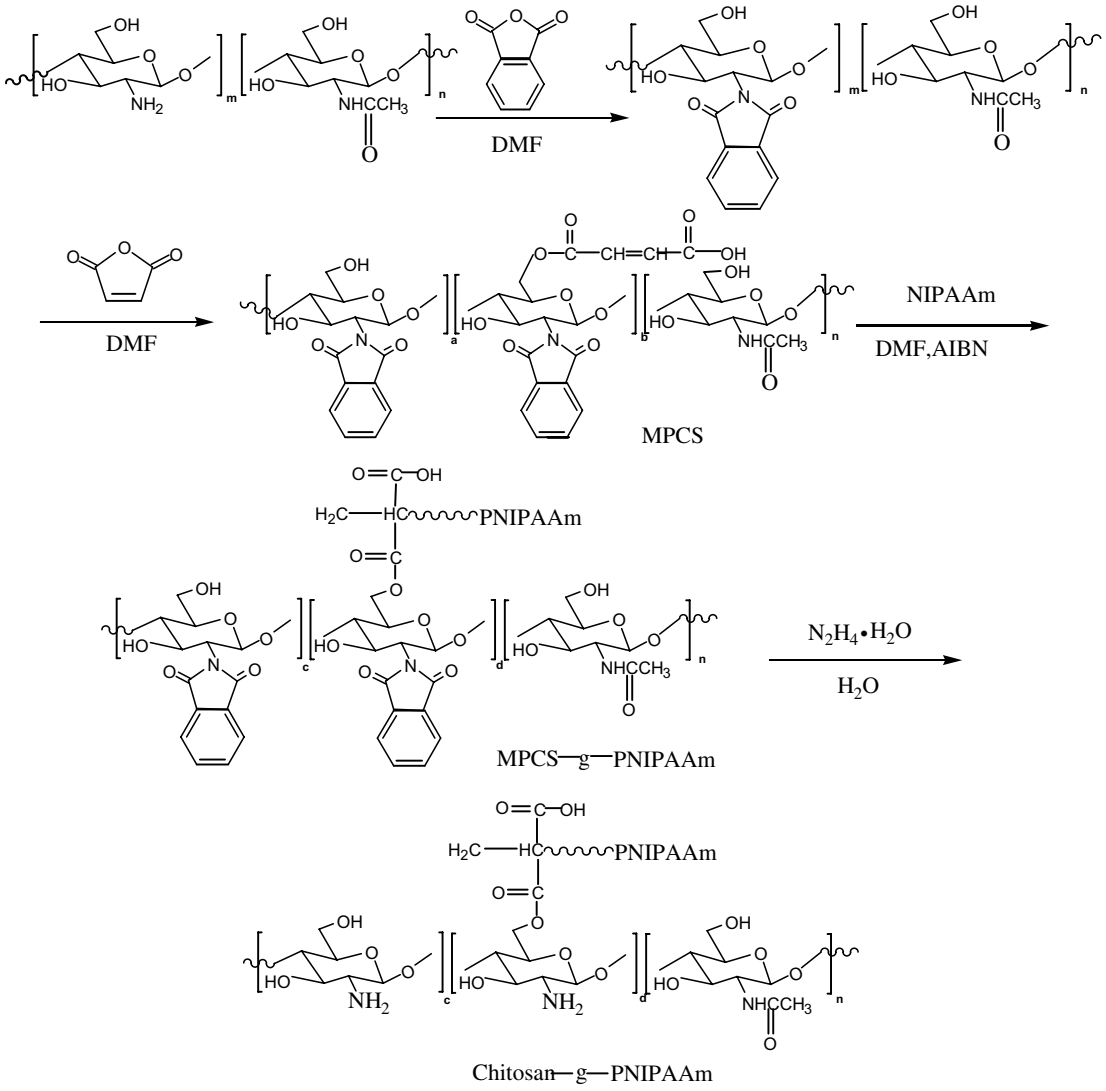
grafting PNIPAAm with a mono-carboxyl end group onto chitosan through amide bond linkages.

The amino groups in chitosan impart its various advanced functions, including biological activity and cationic polymer properties (Nishimura, Kohgo, & Kurita, 1991). To graft PNIPAAm onto chitosan and preserve the amino group of chitosan, we can prepare *O*-maleoyl-*N*-phthaloyl-chitosan (MPCS) which has double bond for the subsequent graft copolymerization of vinyl monomers. Meanwhile, the amino group can be protected by phthaloyl group. In the present work, NIPAAm is grafted onto MPCS by use of azo-bis-isobutyronitrile (AIBN) as the initiation. The deprotection of the *N*-phthanoyl groups yields chitosan-*g*-PNIPAAm copolymers with grafting sites regioselectively at the hydroxyl groups (Scheme 1). The effects of various experimental conditions on the grafting degree have been investigated. We also studied crystallinity, thermal property, solubility and thermal sensitivity of the copolymers.

2. Experimental

2.1. Materials

Chitosan (degree of deacetylation = 92%, determined from ^{1}H NMR spectrum in D_2O/CF_3COOD 95:5 v/v; viscosity average molecular weight = 7.57×10^5, determined in 0.1 M acetate acid/0.2 M NaCl aqueous solution at 25 ± 0.5 °C by means of Ubbelohde viscometer, according to the Mark-Houwink equation). *N*-Isopropylacrylamide (NIPAAm) was prepared by the reaction of acryloyl chloride with isopropylamine, and then recrystallized from the mixture of hexane and toluene (First Reagent Factory of Shanghai). Phthalic anhydride, maleic anhydride and hydrazine monohydrate were obtained from the First Reagent Factory of Shanghai (China). *N*,*N*-dimethylformamide (DMF) was distilled under reduced pressure from magnesium sulfate and stored over molecular sieves (4 Å). Azo-bis-isobutyonitrile (AIBN) was recrystallized by



Scheme 1. Grafting of *N*-isopropylacrylamide onto chitosan.

alcohol. All other commercially available chemicals were used as received without further purification.

2.2. Synthesis of chitosan derivatives

2.2.1. Phthaloylation of chitosan (PHCS)

Chitosan was heated with excess phthalic anhydride in dried DMF to yield phthaylchitosan (PHCS) (Nishimura et al., 1991; Huang & Fang, 2006). The degree of substitution (DS) of phthaloyl groups was 98% determined by elemental analysis.

2.2.2. Maleoylation of PHCS (MPCS)

A mixture of PHCS and maleic anhydride in DMF was heated in nitrogen at 120–130 °C with stirring. After reaction for 12 h, the resulting black solution was cooled to room temperature and poured into ice water. The precipitate was collected by filtration, washed completely by Soxhlet extraction with ethanol and dried to give *O*-maleoyl-*N*-phthaloyl-chitosan (MPCS) as a black powdery material. The DS of maleoyl groups was 62% determined by elemental analysis.

2.2.3. Preparation of MPCS-graft-NIPAAm copolymer

Graft polymerization of NIPAAm onto chitosan was carried out in dried DMF using AIBN as the initiator under a nitrogen atmosphere. The reaction was stirred at certain temperature for different time. The resulting solution was cooled to room temperature and poured into ice water, and then the precipitate was collected by filtration. The obtained product was purified by Soxhlet extraction with acetone for 24 h, and then dried under vacuum at room temperature. Varieties factors on the effect of the grafting degree were investigated.

The grafting degree ($G\%$) and grafting efficiency ($E\%$) were calculated as follows:

$$G\% = (W_g - W_0)/W_0 \times 100$$

$$E\% = (W_g - W_0)/W_1 \times 100$$

where W_g, W_0 and W_1 were weights of graft copolymer, MAPHCS and NIPAAm monomer, respectively.

2.2.4. Deprotection of the graft product

After phthaloyl-protected graft copolymer was dispersed in water and heated to 100 °C under nitrogen atmosphere, hydrazine monohydrate was added to deprotect the phthaloyl group. The obtained transparent solution was filtered and dialyzed against ethanol using a dialysis membrane (molecular weight cut-off 12 kDa; dialysis tubings, cellulose membrane, Sigma) to remove the impurities. The precipitate was dried to obtain the final product, chitosan-*g*-PNIPAAm.

2.3. Characterization

FT-IR spectra were recorded on a Bruker Vector-22 FT-IR spectrometer scanning from 4000 cm^{-1} to 400 cm^{-1} at room temperature. ^{13}C and ^{1}H NMR spectra were detected by a Bruker AV 300 spectrometer operating at 300.13 MHz and 100.6 MHz, respectively. For chitosan-*g*-PNIPAAm, D_2O was used as the solvent. For the other samples, DMSO-d_6 was used as the solvent. X-ray powder diffraction diagrams (XRD) were recorded with a Japan D/Max-rA X-ray diffractometer using graphite-monochromatized CuKα radiation ($\lambda = 1.54178$ Å). Elemental analyses were performed with an Elementar Vario EL-III elemental analyzer.

The thermal properties of various samples were measured by thermogravimetric analysis (TGA). Decomposition profiles of TGA were recorded with a heating rate of 10 °C/min in nitrogen between 50 °C and 500 °C. Solubility tests on different solvents were carried out at room temperature.

The phase transition behavior of chitosan-*g*-PNIPAAm in aqueous solution was studied by measuring the optical transmittance at 550 nm in the range of 25–45 °C by use of an UV–Visible spectrophotometer equipped with an online temperature controller.

3. Results and discussion

3.1. FT-IR and NMR characterization of MPCS

Based on PHCS, MPCS is synthesized by reaction of PHCS with maleic anhydride to provide vinyl functional group for graft polymerization of NIPAAm. Fig. 1 shows the FT-IR spectrum of chitosan (a) and MPCS (b). Compared with chitosan, MPCS has new peaks at 1777 cm^{-1} and 1712 cm^{-1} (carbonyl anhydride), 721 cm^{-1} (phenyl ring) and 1640 cm^{-1} (double bond of the maleoyl group) in the spectrum.

In Fig. 2(a), the ^{1}H NMR spectrum of MPCS shows the aromatic phthalimido peaks at 7.2–8.0 ppm and the peaks of chitosan backbone hydrogens at 2.8–5.0 ppm (Huang & Fang, 2006)(associated with DMSO-d_6 at 2.5 ppm and

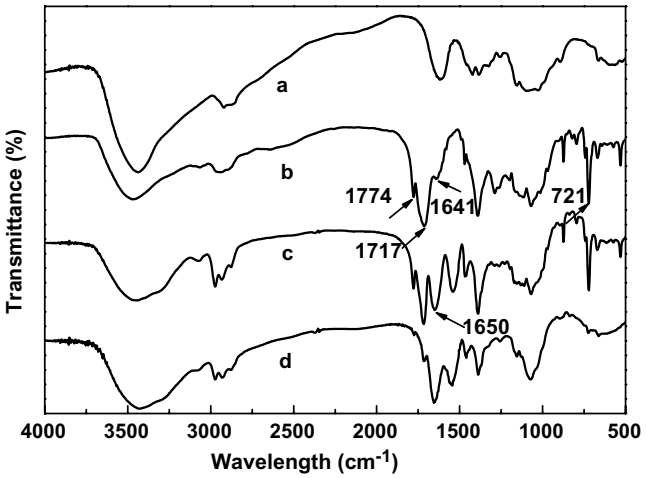


Fig. 1. IR spectra of chitosan (a), MPCS (b), MPCS-*g*-PNIPAAm (c) and chitosan-*g*-PNIPAAm (d).

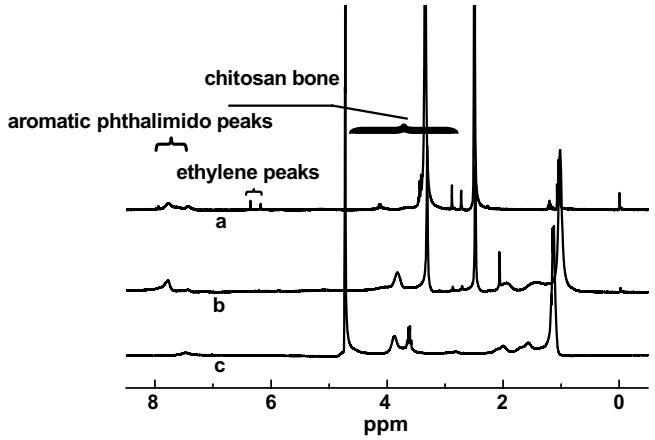


Fig. 2. ^{1}H NMR spectrum of MPCS (a) MPCS-*g*-PNIPAAm (b) in DMSO-d_6 and chitosan-*g*-PNIPAAm (c) in D_2O.

impurity H_2O at about 3.4 ppm). The weak signal at 6.0–6.4 ppm is due to the protons of CH=CH. Fig. 3 shows the ^{13}C NMR spectra. Besides the characteristic peak of chitosan (50–100 ppm) (Zhang, Ping, Zhang, & Shen, 2003), the peaks at 120–140 ppm prove the existence of carbon double bond and aromatic groups, while the peaks observed between 162 ppm and 168.2 ppm correspond to carbonyl carbon of ester groups, carboxyl groups and phthalimido groups. The FT-IR and NMR results indicate that maleic anhydride is grafted at the hydroxyl group of chitosan chain.

3.2. FT-IR and NMR characterization of graft copolymers

MPCS with vinyl group is the key intermediate for the further grafting. The structure of MPCS-*g*-PNIPAAm was examined by FT-IR spectrum (Fig. 1(c)), the peak at 1650 cm^{-1} and at 1540 cm^{-1} is attributed to carbonyl stretching vibration (amide I), and N–H bending vibration (amide II) of PNIPAAm, respectively. The evidence of stronger absorbance at 2800–3000 cm^{-1} for C–H implies significantly the graft of the PNIPAAm chain. Fig. 1(d) also shows that the characteristic peak of phthanoyl group (1777 cm^{-1}, 1712 cm^{-1}) has almost disappeared, whereas the peaks at 1650 cm^{-1} and at 1540 cm^{-1} still exist in the final grafted product. The facts indicate that the *N*-phthanoyl groups are removed after deprotection reaction, but the PNIPAAm chains are not destroyed.

The NMR spectra also confirm the graft copolymerization of MPCS and NIPAAm. In Fig. 2(b), ^{1}H NMR of MPCS-*g*-PNIPAAm exhibits the two broad peaks (–CH–CH_2–) at 1.4–1.9 ppm (Chung et al., 2005) and a peak (–NH–CH–) at 3.8 ppm which belongs to the hydrogen proton of PNIPAAm chain. What is more, the intensity of methyl group enhances significantly due to the introduction of methyl group of PNIPAAm. The ^{1}H NMR of chitosan-*g*-PNIAAm in D_2O is showed in Fig. 2(c). The characteristic peaks at $\delta = 1.4$–1.9 (–CH–CH_2–) and $\delta = 3.9$ (–NH–CH–) indicates the existence of PNIPAAm. The peak at 7.0–8.0 ppm (aromatic phthalimido) implies the incomplete deprotection.

3.3. X-ray diffraction characterization

As shown in Fig. 4, the X-ray diffraction spectrum of chitosan has low crystality and the characteristic peaks at $2\theta = 11°$ and 20° are assigned to crystal forms I and crystal forms II. Because the phthaloyl group destroys the hydrogen bond of chitosan matrix, MPCS becomes amorphous. The graft copolymerization further decreases the crystallinity of MPCS due to introduction of bulky pendant chains of grafted PNIPAAm. In comparison with chitosan, chitosan-*g*-PNIPAAm does not have the peak $2\theta = 11°$, and the intensity of the peak $2\theta = 20°$ decreases sharply. This is because the conjugation of PNIPAAm with chitosan suppresses the crystallization of both chitosan and PNIPAAm to some extent.

3.4. Thermal stability of chitosan-g-PNIPAAm

The TGA thermograms of chitosan, pure PNIPAAm and chitosan-*g*-PNIPAAm are presented in Fig. 5. In

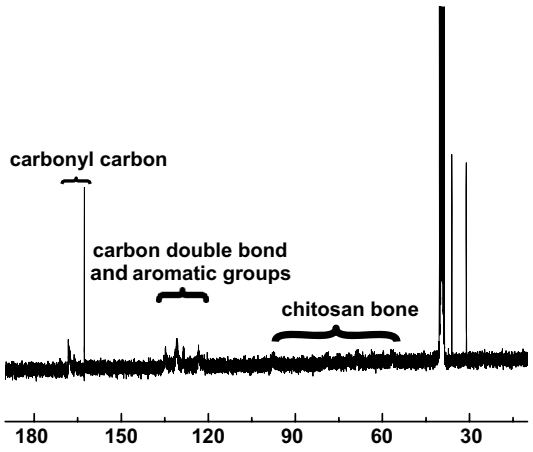


Fig. 3. ^{13}C NMR spectrum of MPCS in DMSO-d_6.

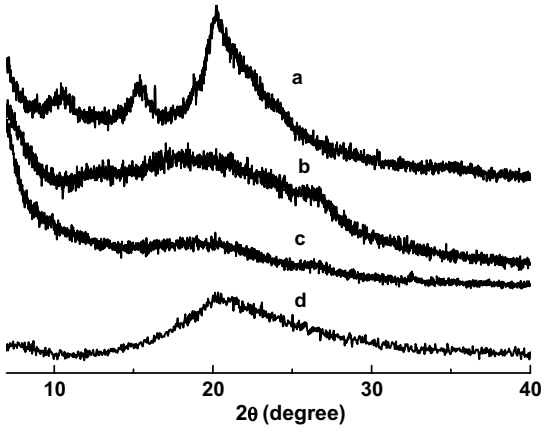


Fig. 4. X-ray diffraction of chitosan (a), MPCS (b), MPCS-*g*-PNIPAAm (c) and chitosan-*g*-PNIPAAm (d).

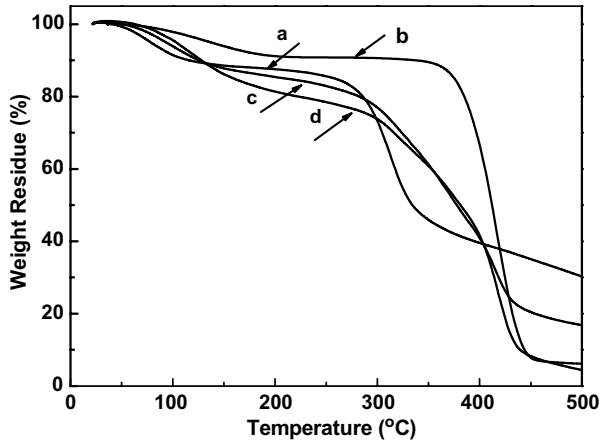


Fig. 5. TGA thermograms of chitosan (a) PNIPAAm (b), chitosan-*g*-PNIPAAm with grafting 54% (c) and 122.8% (d).

comparison with chitosan, copolymers exhibit poorer thermal stability because the introduction of the PNIPAAm destroys the crystalline region of the chitosan. Fig. 5 also shows the char yield for chitosan-*g*-PNIPAAm with high grafting ratio 122.8% is only 6.4%, lower than that of chitosan and chitosan-*g*-PNIPAAm with grafting ratio 54.2%. Namely, the degradation of copolymers increases with the increasing grafting ratio.

3.5. The effects of reaction conditions

3.5.1. Effect of NIPAAm amount in the feed

As can be seen in Fig. 6, the NIPAAm amount in the feed significantly affects the grafting of PNIPAAm onto chitosan backbone. The grafting degree and the grafting efficiency gradually increase with the increase of NIPAAm content in the feed. However, the grafting efficiency is always low, the maximal only 16.2%. This is attributed to the lower reactivity of double bond in maleoyl group compared to that of NIPAAm. As a result, it stimulates the formation of a large number of homopolymer, which inhibits the grafting reaction on chitosan chain. The grafting efficiency shown in Fig. 6 further indicates that the graft polymerization is slower than the homopolymerization of NIPAAm (Kim et al., 2000).

3.5.2. Effect of reaction temperature

The grafting degree for the grafting of NIPAAm monomer onto MPCS at different temperature is shown in Fig. 7. With temperature increasing from 60 °C to 70 °C, the grafting degree increases slightly. This might be because production of homopolymer is not so fast at 70 °C that it favors the initiation of double bond of maleoyl group. At higher temperature, the grafting degree decreases rapidly probably due to the severity of the monomer chain transfer reactions.

3.5.3. Effect of initiator content

Fig. 8 illustrates the effect of initiator concentration on the grafting of NIPAAm onto MPCS. As the initiator

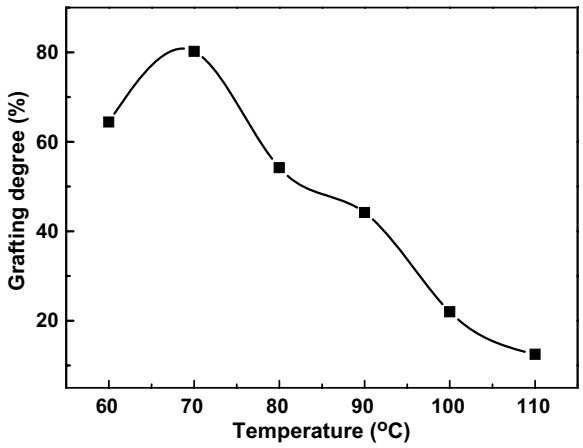


Fig. 7. Effect of temperature on the grafting of PNIPAAm onto MPCS (reaction conditions: MPCS 0.5 g, NIPAAm 2 g and AIBN 1% at 70 °C for 12 h).

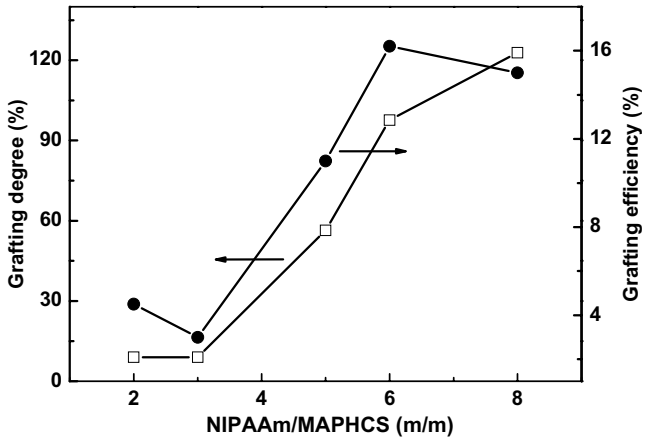


Fig. 6. Effect of monomer concentration on the grafting of PNIPAAm onto MPCS (reaction conditions: MPCS 0.5 g and AIBN 1% at 70 °C for 12 h).

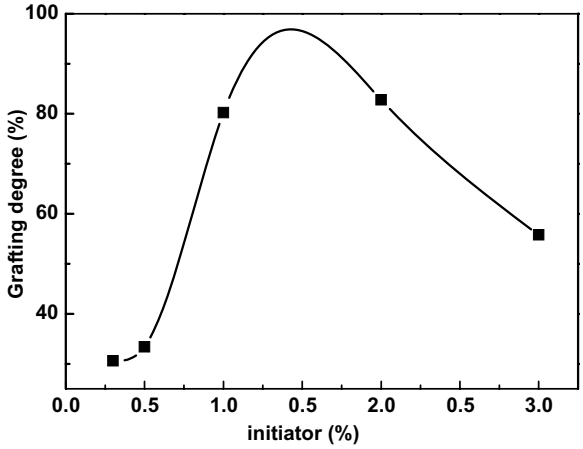


Fig. 8. Effect of initiator concentration on the grafting of PNIPAAm onto MPCS (reaction conditions: MPCS 0.5 g, NIPAAm 2 g at 70 °C for 12 h).

content increases, the grafting degree first increases rapidly and then decreases. The initial increase can be attributed to the increasing number of free radical sites on the chitosan backbone. However, higher concentration of initiator helps to the production of homopolymer due to higher activity of NIPAAm monomer, resulting in the decrease in grafting degree.

3.5.4. Effect of polymerization time

Fig. 9 shows the effect of polymerization time on the grafting of NIPAAm onto MPCS. The grafting degree initially increases slowly with time up to 240 min. Subsequently, it enhances rapidly and tends to level off. It is in agreement with the general trend in grafting reaction.

3.6. Solubility test

Chitosan, MPCS, MPCS-*g*-PNIPAAm, chitosan-*g*-PNIPAAm are used for the solubility test in several solvents. Because of strong actions of hydrogen bond, chitosan is only soluble in acidic solutions such as acetic acid. In comparison with chitosan, chitosan-*g*-PNIPAAm exhibits significantly improved solubility in the range pH 1–12. In other words, the copolymers are soluble in acidic, neutral or alkaline aqueous solutions. This is probably because the coexistence of amino groups and PNIPAAm chains significantly destroy the crystalline of chitosan.

3.7. Thermosensitive behaviors

The phase transition behavior of chitosan-*g*-PNIPAAm in water is investigated by measuring the optical transmittance at 550 nm in the range of 25–45 °C.

As shown in Fig. 10, the transparency of copolymer maintains at a constant value at low temperatures and then starts to drop until zero. Clearly, the graft copolymer exhibits a less sharp transition than PNIPAAm. This is similar to PNIPAAm grafted on a surface (Zhang, 2004; Zhang, Zhou, Li, Fang, & Zhang, 2005). This should be due to the constraint of chitosan to PNIPAAm chains.

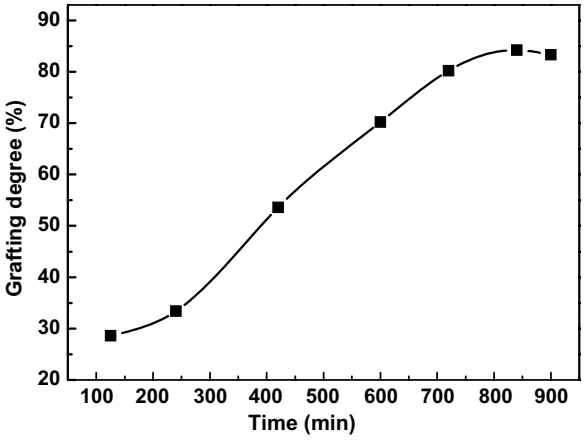


Fig. 9. Effect of time on the grafting of PNIPAAm onto MPCS (reaction conditions: MPCS 0.5 g, NIPAAm 2 g and AIBN 1% at 70 °C).

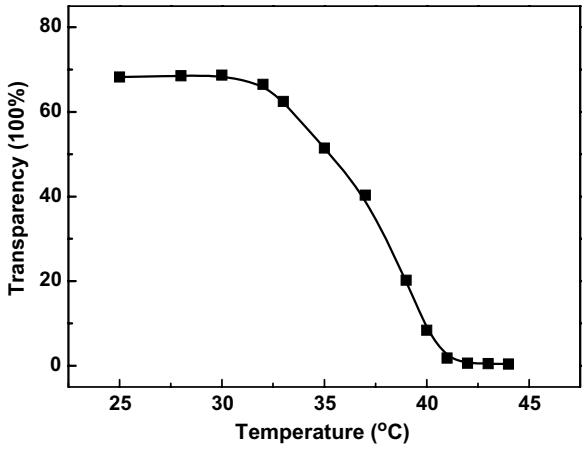


Fig. 10. Transparency of chitosan-*g*-PNIPAAm in aqueous solution at 550 nm over the temperature between 25 °C and 45 °C.

4. Conclusion

The graft copolymerization of NIPAAm onto chitosan can be conducted *via* MPCS where NIPAAm monomer is grafted regioselectively at the hydroxyl groups. The deprotection of the phthaloyl groups yields chitosan-*g*-PNIPAAm copolymers with amino groups. The grafting degree is influenced by the monomer concentration, reaction temperature, initiator concentration and reaction time. In comparison with the mother chitosan, chitosan-*g*-PNIPAAm has much better solubility in aqueous solutions in the range pH 1–12. The graft copolymer also exhibits thermally sensitivity. It is expected to be potential in biomedical applications.

Acknowledgments

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