

Preparation of dual-sensitive graft copolymer hydrogel based on N-maleoyl-chitosan and poly(N-isopropylacrylamide) by electron beam radiation

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Abstract. Organic solvent-soluble N-maleoyl-chitosan (NMCS) was synthesized by reaction of chitosan with maleic anhydride (MAH) in N,N-dimethylformamide (DMF). N-maleoyl-chitosan-graft-poly(N-isopropylacrylamide) (NMCS-g-PNIPAAm) copolymer hydrogel was prepared via free radical polymerization by electron beam (EB) irradiation. The copolymer obtained was analysed by FT-IR, XRD and thermal gravimetric analysis (TGA). It was found that the grafting yield and grafting efficiency increased with increasing radiation absorbed dose and monomer amount, and then decreased. The swelling ratio of the copolymer hydrogel was low at pH 4–5, and LCST of the hydrogel was around 32°C.

Keywords. Chitosan; N-isopropylacrylamide; thermo- and pH-sensitive; hydrogel; EB irradiation.

1. Introduction

Intelligent hydrogels have the capability to respond to small external stimulus changes. A thermosensitive hydrogel, one of the environmental stimuli-response hydrogels, collapses at elevated temperature above the lower critical solution temperature (LCST) (Chen and Hoffman 1995). Poly(N-isopropylacrylamide) (PNIPAAm) is a widely studied thermosensitive polymer because it has a LCST in the range of 30–32°C which is near that of the human body (37°C). Successfully, the copolymer of NIPAAm with other functional monomers was widely used in the fields of chemistry, materials, and biotechnology (Schild 1992; Kang *et al* 2000).

Chitosan(CS), poly[β -(1–4)-2-acetamido-2-deoxy-*D*-glucopyranose], was obtained by alkaline deacetylation of chitin (Ravi Kumar 1999). Because of its biodegradability, biocompatibility and non-toxicity, it has been used as an anticoagulant (Vongchan *et al* 2002), a wound-healing accelerator (Ueno *et al* 2001) and drug delivery carriers (Jameela and Jayakrishnan 1995). Chitosan can be dissolved in only acidic solution through the interaction between H⁺ and –NH₂, but it is insoluble under higher pH conditions (Yao *et al* 1999). It is also of limited solubility in organic solvents. In order to sort the above problems, chemical modification affords a wide range of novel chitosan derivatives, for example, carboxymethyl chitosan (Sun *et al* 2003), N-alkylated chito-

san (Li *et al* 2002), N-trimethylchitosan (Domard *et al* 1987) and N-(2-carboxybenzyl) chitosan (Lin *et al* 2007). Dong *et al* (2006) prepared water-soluble maleoyl chitosan and organic solvent-soluble maleoyl chitosans by reaction of chitosan with maleic anhydride (MAH) under different reaction conditions. As a result of introducing vinyl groups and carboxyl groups onto the –NH₂ groups of chitosan, Huang *et al* (2005) synthesized and characterized N-maleamic acid-chitosan-g-poly(butyl acrylate) polymers. Don and Chen (2005) prepared MA-CS-g-PNIPAAm copolymers based on water-soluble maleoyl chitosan and N-isopropylacrylamide (NIPAAm) by UV radiation. So far, reports on the copolymer hydrogel based on organic solvent-soluble maleoyl chitosans which was prepared by radiation was relatively sparse.

It is well known that physical, chemical and radiant methods can be applied to prepare polymer blends. Among them, radiation technique is relatively simple for the improvement or modification of polymer materials through cross-linking, grafting, or degradation (Rosiak and Ulan-ski 1999; Zhao *et al* 2003). Moreover, the product can be free from impurities such as chemical residues from initiators for no catalysts or additives are needed to initiate the reaction in radiation processing. In addition, the degree of crosslinking and grafting can be controlled by the change of radiation absorbed dose (Mesquita *et al* 2004).

In this paper, organic solvent-soluble maleoyl chitosans (NMCS) were obtained by the reaction of chitosan with MAH in N,N-dimethylformamide (DMF). And then, we prepared thermo- and pH-sensitive copolymer hydrogel by grafting NIPAAm onto NMCS by a new

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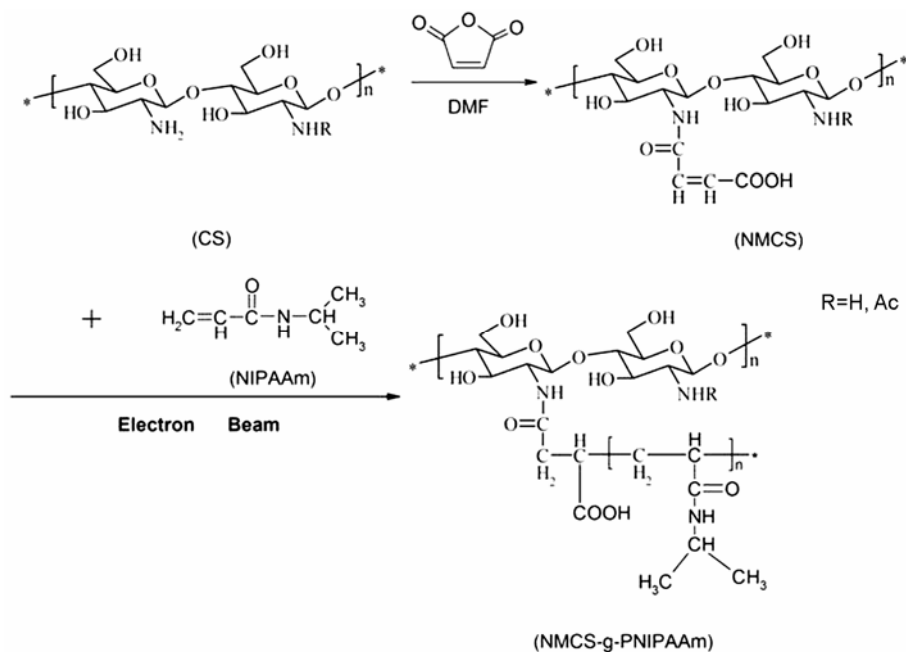


Figure 1. Synthetic scheme for NMCS and NMCS-g-PNIPAAm.

method: EB irradiation (figure 1). The synthesized copolymers were characterized by FT-IR, XRD and TGA. The effects of monomer amount and radiation absorbed dose on the grafting yield and grafting efficiency were studied in detail. Also, the swelling behaviour of the hydrogels in different temperature and pH conditions was investigated. The swelling ratio of the copolymer hydrogels was lower at pH 4–5. Like PNIPAAm, the LCST of the graft copolymer hydrogels was around 32°C.

2. Experimental

2.1 Materials

Chitosan (CS) was purchased from Sinopharm Chemical Reagent Co. Ltd (China). The average molecular weight was 4.0×10^5 g/mol and the N-deacetylation degree was above 90%. N-isopropylacrylamide (NIPAAm) (Wako Pure Chemical Industries, Japan) was recrystallized from the mixture of hexane and toluene. Maleic anhydride (MAH) was of analytical grade and obtained from the Shanghai Chemical Reagent Company. All other chemicals were analytical grade and used without any purification.

2.2 Preparation of NMCS-g-PNIPAAm copolymer hydrogels

2.2a N-maleoyl-chitosan (NMCS): Chitosan (2.0 g), and maleoyl anhydride (6.0 g) were dissolved in 100 mL of dimethylformamide (DMF). Then the mixture was

stirred at 130°C for 3 h under a nitrogen atmosphere. The reacted solution was cooled to room temperature and poured into ice water to precipitate the product. The product was collected by filtration, washed with diethyl ether for three times and then dried in vacuum at 40°C. The grayish-white powder of N-maleoyl-chitosan (NMCS) was obtained at last.

2.2b NMCS-g-PNIPAAm copolymer hydrogels: NMCS and NIPAAm with various feed ratios (weight ratio NMCS/NIPAAm, 1:1, 1:1.5, 1:2, 1:2.5, 1:3, 1:3.5, 1:4) were dissolved in 50 ml DMF. The solution mixtures of different feed ratios were poured into reaction bags, respectively and purged by bubbling N_2 gas for 30 min. The bags were then sealed and irradiated by electron beam. A Dynamitron Electron Accelerator ($E = 1.5$ MeV, $I = 10$ mA) was applied in the current of 2–4 mA and the radiation absorbed dose was selected from 40–200 kGy. The irradiated solution mixture was poured into ice water, and then the precipitate was collected by filtration. The obtained product was washed with acetone in a Soxhlet extractor for 48 h, for stripping out the unreacted monomer, homopolymer and other impurities. The copolymers were then dried in a vacuum at 40°C in a vacuum overnight. As shown in figure 2, chitosan chains were crosslinked by the PNIPAAm chains to form a three-dimensional polymer network that swells but is not soluble in water. The effects of monomer amount and radiation absorbed dose on the grafting yield and grafting efficiency were investigated.

The grafting yield ($G\%$) and grafting efficiency ($GE\%$) were defined as follows:

$$G\% = \frac{(W_g - W_o)}{W_o} \times 100\%,$$

$$GE\% = \frac{(W_g - W_o)}{W_m} \times 100\%,$$

where W_g , W_o and W_m are the weight of grafted copolymers, NMCS and NIPAAm monomer, respectively.

2.3 Characterization

2.3a FT-IR: FT-IR spectra of the copolymer products were determined by a Nicolet Avatar 370 instrument using the KBr pellet method.

2.3b X-ray diffraction: X-ray diffraction (XRD) were obtained with a D/max-2200 X-ray diffractometer using graphite-monochromatized $\text{CuK}\alpha$ radiation ($k = 1.54178 \text{ \AA}$).

2.3c Thermogravimetric analysis: The thermal properties of various samples were measured by NETZSCH STA 409 PG/PC. Decomposition profiles of TGA were recorded with a heating rate of $20^\circ\text{C}/\text{min}$ in nitrogen between 25°C and 700°C .

2.3d Swelling ratio determination: Different pH buffers with the same ionic strength ($I = 0.4 \text{ M}$, adjusted by NaCl) were used and the swelling ratios were determined gravimetrically. The dried copolymer hydrogels were immersed into buffer solutions of various pH and certain temperature for a period of time until the swelling equilibrium was reached. After blotting with filter paper to

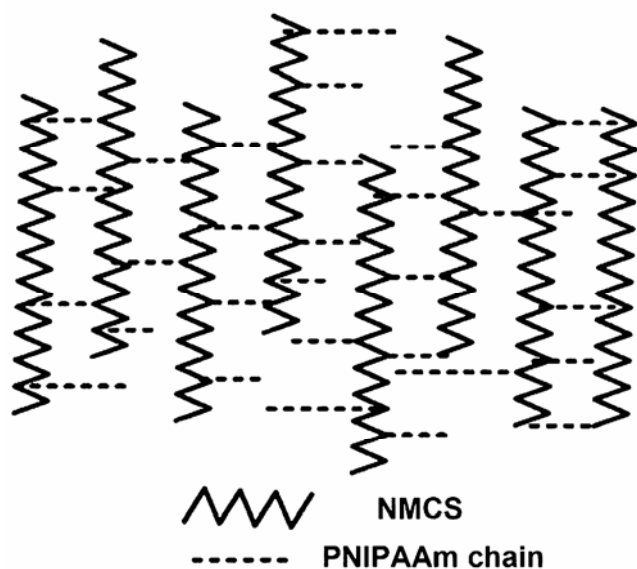


Figure 2. Grafted copolymer hydrogel based on N-maleoyl-chitosan and PNIPAAm.

remove the superabundant water on the surface, the samples were weighed immediately by an electronic balance.

The swelling ratio of the grafted copolymer hydrogels was expressed by the following formula (Nghah 2002)

$$\text{Swelling ratio (SR)} = \frac{W_s - W_d}{W_d},$$

where W_s and W_d are the weights of swelled and dried samples, respectively.

3. Results and discussion

3.1 FT-IR spectra

NMCS was synthesized by reaction of chitosan with maleic anhydride to provide vinyl functional group for graft polymerization of NIPAAm. The FT-IR spectra of chitosan, NMCS, PNIPAAm and NMCS-*g*-PNIPAAm are shown in figure 3. The FT-IR spectrum of NMCS showed a strong $\nu(\text{C}=\text{O})$ at the wavelength of 1717 cm^{-1} and $\nu(\text{C}=\text{C})$ at 1636 cm^{-1} . Meanwhile, evident $\gamma(\text{C}=\text{C})$ 825 cm^{-1} was also observed (figure 3(b)). The structure of PNIPAAm was verified from the following bands: $\nu(\text{C}=\text{O})$ 1650 cm^{-1} (amide I), $\beta(\text{N}-\text{H})$ 1540 cm^{-1} (amide II) (figure 3(c)). Contrasting with the spectrum of NMCS and PNIPAAm, $\nu(\text{C}=\text{O})$ 1650 cm^{-1} (amide I) and $\beta(\text{N}-\text{H})$ 1550 cm^{-1} (amide II) remain appearing in the spectrum of NMCS-*g*-PNIPAAm. The $\nu(\text{C}=\text{O})$ 1723 cm^{-1} demonstrates the existence of NMCS constitutional unit (figure 3(d)). In addition, the matter of absorbance at $2850\text{--}3000 \text{ cm}^{-1}$ for C-H implies obviously the graft of the PNIPAAm chain.

3.2 XRD characterization

The XRD spectrum of chitosan (figure 4(a)) has low crystallinity and the characteristic peaks at $2\theta = 11^\circ$ and 20°

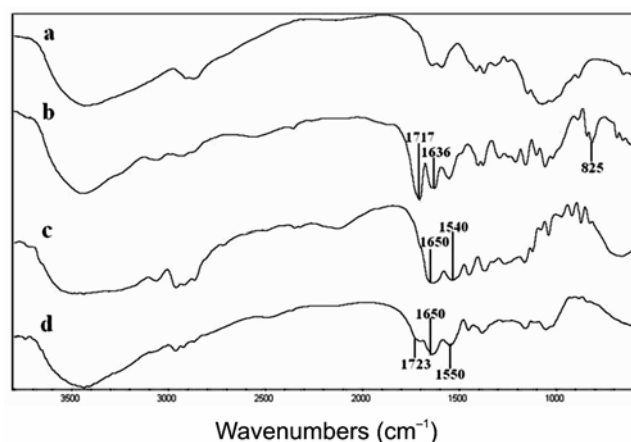


Figure 3. FT-IR spectra of (a) chitosan, (b) NMCS, (c) PNIPAAm and (d) NMCS-*g*-PNIPAAm.

are assigned to crystal forms I and II. In comparison with chitosan, NMCS does not have the peak at $2\theta = 11^\circ$ (figure 4(b)), and the intensity of the peak, $2\theta = 20^\circ$, descends sharply. Because the maleic anhydride group was introduced into chitosan, the intramolecular and intermolecular hydrogen bond of chitosan were destroyed. Since the PNIPAAm was grafted onto the vinyl group of NMCS by irradiation, the copolymer chains have relational twisting and containment. In the XRD spectrum of NMCS-g-PNIPAAm (figure 4(c)), the intensity of the peak, $2\theta = 20^\circ$, has a tendency to disappear.

3.3 TG determination of NMCS-g-PNIPAAm copolymer hydrogels

The TGA thermograms of CS, NMCS, pure PNIPAAm and NMCS-g-PNIPAAm copolymer hydrogel are presented in figure 5. The maximum rate degradation temperature (T_{\max} , first derivative peak temperature) of CS was 318°C and it increased to 365°C after substitution with maleic acid (figures 5(a) and (b)). This increase is because of the increasing hydrogen bonding and charged complex formation between the substituted carboxylic acid group and the remaining amino group. In accordance with the XRD spectrum (figure 4), the introduction of the maleoyl groups destroyed the crystalline region of the CS. Pure PNIPAAm shows one-stage degradation behaviour with T_{\max} at 410°C (figure 5(c)). However, NMCS-g-PNIPAAm copolymer hydrogels exhibit two-stage degradation behaviour, where the first and second stages were caused by the degradation of chitosan and grafted PNIPAAm chains, respectively (figure 5(d)). The appearance of two distinct stages of degradation confirmed that the reaction products were graft copolymers.

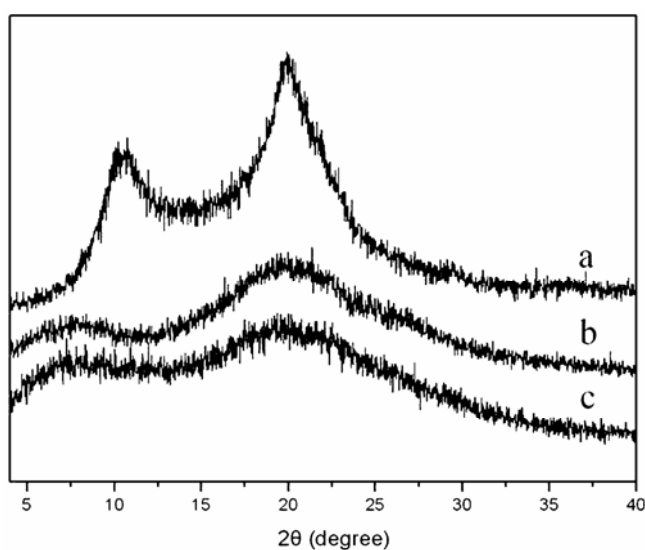


Figure 4. XRD of a. chitosan, b. NMCS and c. NMCS-g-PNIPAAm.

3.4 Effects of reaction conditions on yield and efficiency of grafting

3.4a Effect of radiation absorbed dose: In the experiment, free radicals from vinyl group was produced by EB irradiation. PNIPAAm chains were grafted onto NMCS by the assistance of free radicals. In figure 6, the yield and efficiency of grafting increased with increasing radiation absorbed dose in the range of 40–160 kGy, and then decreased. Usually, the free radicals in the reaction system increased with dose and then turned to decay. The decrease of free radicals with dose may be explained that the accumulated free radical in higher concentration makes them easier for combining each other. In this case, the free radicals began to decay around 160 kGy.

3.4b Effect of feed ratios of NIPAAm and NMCS: As is shown in figure 7, the amount of NIPAAm in the feed ratio significantly affected the grafting of PNIPAAm onto NMCS. When the ratio of NIPAAm ($W_{\text{NIPAAm}}/W_{\text{NMCS}}$) was < 2 , the yield and efficiency of grafting increased with increasing NIPAAm. The efficiency of grafting was higher at lower feed ratio of NIPAAm and then decreased with the increase of NIPAAm. From now on, it may be 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.

3.5 Effect of pH on swelling ratio of NMCS-g-PNIPAAm copolymer hydrogels

The ionizable carboxyl groups and remaining amino groups on the copolymer hydrogels lead to the potential of pH sensitivity. Figure 8 shows the effect of pH on the

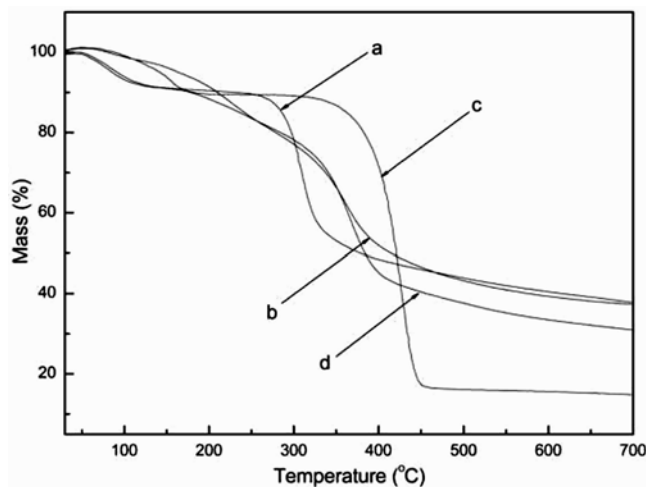


Figure 5. TGA of (a) chitosan, (b) NMCS, (c) PNIPAAm and (d) NMCS-g-PNIPAAm.

swelling ratio of the copolymer hydrogels. The NMCS-g-PNIPAAm copolymer hydrogels gave higher swelling ratio at low pH (≤ 3) and high pH (≥ 6), but lower swelling ratio in the range of pH 4–5. The NMCS-g-PNIPAAm copolymer hydrogels contain amino and carboxyl groups, which accept or release protons depending on the surrounding pH condition. In a strong acidic solution, almost all amino groups became protonated and positively charged ($\text{NH}_2 + \text{H}^+ \rightarrow \text{NH}_3^+$), whereas most carboxylic acid groups remained neutral ($-\text{COOH}$). On the contrary, in a basic solution, almost all amino groups were not protonated ($-\text{NH}_2$), but most carboxylic acids dissociated to carboxylate anions ($-\text{COOH} + \text{OH}^- \rightarrow \text{COO}^- + \text{H}_2\text{O}$) (Chavasit and Torres 1990; Sun *et al* 2007). However, at the intermediate pH values ($3 < \text{pH} < 6$), both amino and carboxylic acid groups might undergo ionization. A strong ionic force thus developed between cationic amino group and anionic carboxylate group has lead to a decrease in

swelling ratio. As a result, the lowest swelling ratio was observed at pH (~ 4) and pH (~ 5).

3.6 Thermosensitivity on swelling ratio of NMCS-g-PNIPAAm copolymer hydrogels

The thermosensitivity of NMCS-g-PNIPAAm copolymer hydrogels was evaluated by determining the effect of temperature on the swelling ratio, as shown in figure 9. There is an obvious LCST phenomena in higher pH (~ 12) and lower pH (~ 2) conditions. The swelling ratios of NMCS-g-PNIPAAm copolymer hydrogels underwent a sharp decrease around 32°C . Around pH (~ 4), the swelling ratio of the copolymer hydrogels was lower and no LCST was found, may be because of the interaction between carboxyl group and amino group, as well as carboxyl group and amide group.

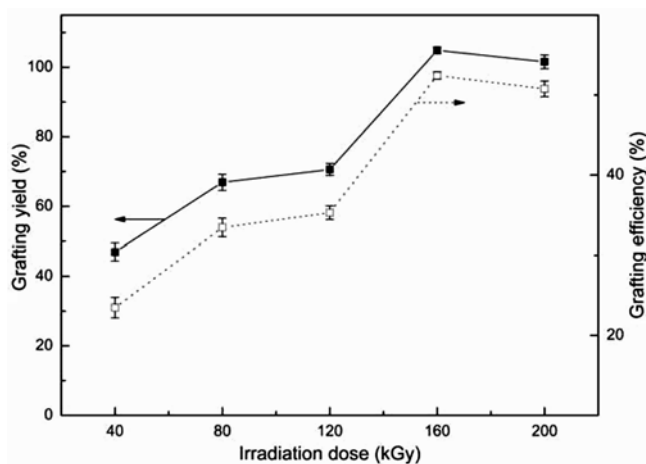


Figure 6. Effect of dose on yield and graft efficiency of grafting (feed ratio: $W_{\text{PNIPAAm}}/W_{\text{NMCS}} = 2$).

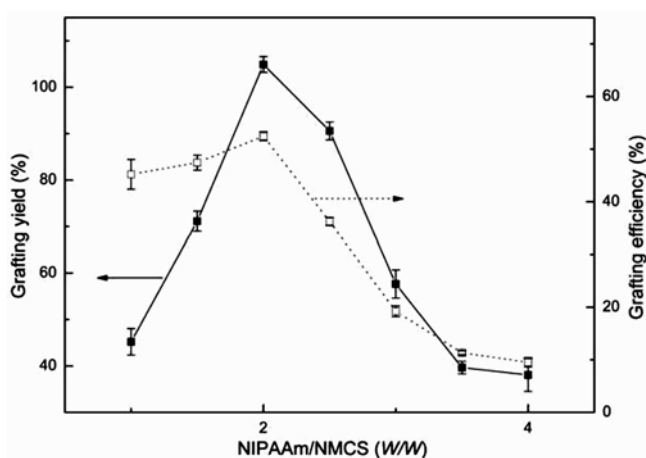


Figure 7. The grafting yield and graft efficiency with different monomer amounts (irradiation dose: 160 kGy).

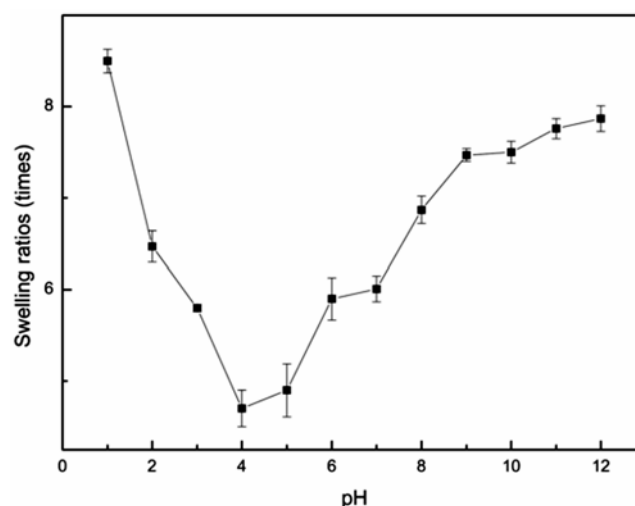


Figure 8. Effect of pH on the swelling ratio of NMCS-g-PNIPAAm copolymer hydrogel ($G = 104.8\%$).

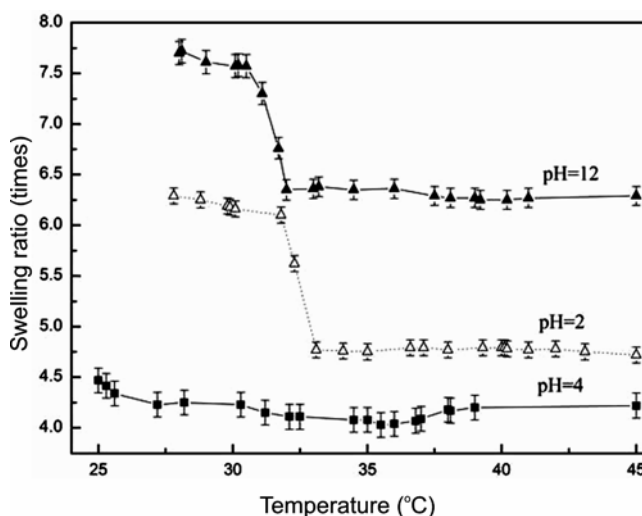


Figure 9. Effect of temperature on swelling ratio of NMCS-g-PNIPAAm copolymer hydrogel ($G = 104.8\%$).

4. Conclusions

NMCS-*g*-PNIPAAm copolymer hydrogel with thermal and pH sensitivity was synthesized by EB radiation techniques. The yield and the efficiency of grafting increased with increasing radiation absorbed dose in the range of 40–160 kGy, and then decreased. When the ratio of NIPAAm ($W_{\text{NIPAAm}}/W_{\text{NMCS}}$) was < 2 , the yield and efficiency of grafting increased with increasing NIPAAm. The copolymer hydrogel gave higher swelling ratio at lower pH (≤ 3) and higher pH (≥ 6) and lower swelling ratio in the range of pH 4–5. The thermo-sensitivity of the hydrogels appeared at higher pH and lower pH conditions and the LCST was around 32°C.

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