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Synthesis, characterization and thermal sensitivity of chitosan-based graft copolymers

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Abstract—Novel chitosan-based graft copolymers (CECTS-g-PDMA) were synthesized through homogeneous graft copolymerization of (*N*,*N*-dimethylamino)ethyl methacrylate (DMA) onto *N*-carboxyethylchitosan (CECTS) in aqueous solution by using ammonium persulfate (APS) as the initiator. The effect of polymerization variables, including initiator concentration, monomer concentration, reaction time and temperature, on grafting percentage was studied. XRD, FTIR, DSC and TGA were used to characterize the graft copolymers. Surface-tension measurements, turbidity measurements and temperature-variable ^{1}H NMR analysis were combined to investigate the thermal sensitivity of CECTS-g-PDMAs in aqueous solution.

Keywords: Chitosan; Characterization; Graft copolymerization; Grafting percentage; Thermal sensitivity

1. Introduction

Chitin, a naturally occurring polysaccharide, is one of the principal ingredients of the carapaces, crusts and shells of crustacea. It is the second most abundant organic resource next to cellulose on Earth. Deacetylation of chitin with a degree of deacetylation more than 50% gives chitosan, which is soluble in organic acids such as acetic or formic acid, and has been more widely used than chitin as films,[1–3] membranes,[4,5] fibres[6,7] and particles.[8,9]

Chemical modification of chitosan through graft copolymerization has been shown to be a promising method for the preparation of new materials, which enables one to introduce special properties into these abundant biopolymers and enlarge their fields of potential applications. In previous studies, the graft copolymerization of common monomers such as 2-hydroxyethyl methacrylate and acrylamide has been reported.[10,11] Although the homopolymer of DMA and some copolymers, including a DMA segment, have exhibited novel thermal sensitivity that is promising for potential applications such as drug delivery,[12] there are, however, few literature reports about the homogeneous graft copolymerization of DMA onto chitosan in aqueous solution. One exception is that of Singh who reported the heterogeneous graft copolymerization of DMA onto chitosan membrane by γ-radiation.[13]

The present work is focused on the graft copolymerization of DMA onto *N*-carboxyethylchitosan (CECTS) using ammonium persulfate as initiator. The modification was conducted homogeneously in aqueous solution due to the application of CECTS rather than chitosan, which renders the mixture of DMA and CECTS miscible. The purpose of this work is to investigate the effect of reaction variables on the grafting percentage of CECTS-g-PDMA, and most importantly, to exploit the thermal sensitivity of a graft copolymer, for which there are few reports in the literature, to the best of our knowledge.

2. Experimental

2.1. General methods

Chitosan, a product of Yuhuan Chitin Company (Zhejiang Province, China), was purified according to the literature procedure.[14] The degree of deacetylation

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of the purified chitosan was about 0.9 (^{1}H NMR), and the viscosity-average molecular weight was 1.7×10^5 g/mol determined using a Ubbelohde viscometer according to the literature method.[15] Monomer DMA was from Acros, which was used after the removal of impurities through a basic Al_2O_3 column. Other chemicals were of reagent grade and used without further purification except where otherwise stated.

2.2. *N*-Carboxyethyl chitosan (CECTS)

CECTS was prepared by Michael addition reaction of acrylic acid (AA) on chitosan according to the relevent literature.[16] Chitosan (0.58 g, $-NH_2$ ca. 3.2 mmol) was dissolved in 20 g of aqueous solution containing 28 mmol of AA. Magnetic stirring gave a transparent and viscous mixture solution, which was stirred at 50 °C for 12 h. The product was precipitated by the addition of excess acetone after the completion of the reaction. Dialysis of the CECTS solution against distilled water for 2 days and re-precipitation of the product in acetone, followed by drying in vacuum at 40 °C, furnished the purified powdery product. The degree of substitution (DS) of the final CECTS was about 0.27 as characterized by ^{1}H NMR analysis. (Note: DS in this case is defined as the molar ratio of the reacted hydrogen in the amino groups to the initial primary amino groups of chitosan, i.e., the theoretical maximum degree of substitution is 2).[17]

2.3. *N*-Carboxyethylchitosan-g-poly[(*N*,*N*-dimethylamino)ethyl methacrylate] (CECTS-g-PDMA)

Graft copolymer CECTS-g-PDMA was homogeneously synthesized in aqueous solution by using ammonium persulfate as initiator. The typical protocol was as follows: In a 25-mL three-necked round-bottom flask, a mixture of 0.25 g of CECTS and 10 mL of distilled water was stirred and purged with nitrogen for 30 min. The initiator (0.0228 g, 0.1 mmol) was added, followed by the addition of monomer DMA (2 g). The reaction was performed at 50 °C for 3 h under a nitrogen atmosphere. The contents of the flask were cooled to room temperature and then poured into 100 mL of acetone to precipitate the graft copolymer, which was isolated by filtration. The homopolymer of PDMA was removed by extraction with MeOH for 24 h in a Soxhlet extractor. The product that resulted was then dried under vacuum at 40 °C until a constant weight was attained. The grafting percentage (GP) and grafting efficiency (GE) were calculated as follows:

$$\mathrm{GP}\ (\%) = (W_\mathrm{g} - W_0)/W_0 \times 100$$
$$\mathrm{GE}\ (\%) = (W_\mathrm{g} - W_0)/W_\mathrm{m} \times 100$$

where W_g, W_0 and W_m denote the weights of graft copolymer, CECTS and the charged monomer, respectively.

2.4. Characterization of CECTS-g-PDMA

X-ray diffraction (XRD) patterns were obtained on a D/max-rA X-ray diffractometer using graphite monochromatized Cu Kα radiation ($\lambda = 1.54056$ nm). The scanning range was 5–70° with a scanning rate of 1°/min. Fourier transform IR (FTIR) spectra were obtained with a Perkin–Elmer FTIR Spectrum One series. All samples were prepared as potassium bromide pellets and characterized at room temperature. Differential scanning calorimetry (DSC) was performed on a DSC-Q10 instrument under a nitrogen atmosphere. A temperature range of 20–380 °C was used with a heating rate of 10 °C/min. Thermal gravimetric analysis (TGA) was performed on a Perkin–Elmer Pyris1 TGA thermogravimetric analyzer between 20 and 750 °C with a 10 °C/min heating rate under a nitrogen flow rate of 20.0 mL/min.

2.5. Thermal-sensitivity properties characterization of CECTS-g-PDMA aqueous solution

Surface-tension measurements were performed on the automatic BZY-1 Model surface-tension apparatus (a product of Hengping Apparatus Co. Ltd., Shanghai, China); the turbidity of the graft copolymer aqueous solutions was measured using a 721 Model spectrophotometer (a product of the 3rd Company of Analytical Apparatus in Shanghai, China) and temperature-variable ^{1}H NMR analysis was performed on a Bruker 400-MHz NMR instrument by using $NaOD/D_2O$ as solvent.

3. Results and discussion

3.1. Characterization of CECTS-g-PDMA

3.1.1. XRD analysis. The XRD curve of the graft copolymer (b) was compared with that of CECTS (a) as shown in Figure 1. The obvious diffraction peak appeared at about 2θ, being 19° for CECTS. It disappeared in the XRD curve of graft copolymer CECT-g-PDMA, showing that the introduction of PDMA into CECTS by graft copolymerization destroyed the crystallinity of the CECTS backbone.

3.1.2. FTIR spectroscopy. Figure 2 shows the FTIR spectra of CECTS (a), PDMA homopolymer (f) and CECTS-g-PDMA graft copolymer with different grafting percentages (GPs) (b–e). The band that appears in (b–e) at $\sim$1730 cm^{-1} was assigned to the carbonyl group of the grafted PDMA. The $\sim$1457 cm^{-1} peak in both PDMA homopolymer and graft copolymer was assigned to the C–N stretching;[18] the peak at $\sim$1565 cm^{-1} attributed to the amino groups in CECTS appeared in the FTIR spectra of the graft copolymers. The ratio of the

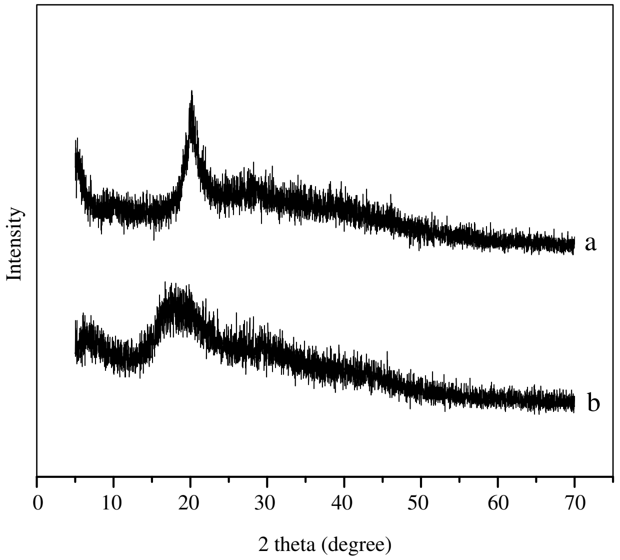

Figure 1. XRD spectra of CECTS (a) and graft copolymer CECTS-g-PDMA (b).

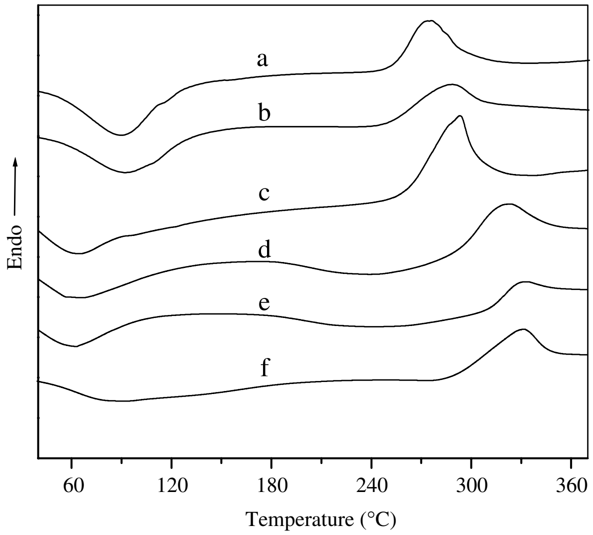

Figure 3. DSC curves of CECTS (a), PDMA homopolymer (f) and graft copolymer CECTS-g-PDMA with various grafting percentages (b–e): GP = 63%, 45%, 38% and 24%, respectively.

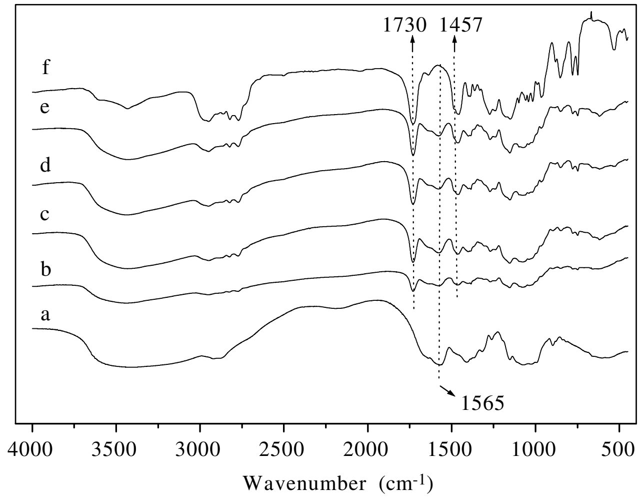

Figure 2. FTIR spectra of CECTS (a), PDMA homopolymer (f) and graft copolymer CECTS-g-PDMA with different grafting percentages (b–e): GP = 24%, 38%, 45% and 63%, respectively.

peak intensity at 1730 to 1565 cm^{-1} increased with an increase of GP, indicating the graft copolymerization of DMA onto CECTS by partial destruction of the amino groups.[13]

3.1.3. DSC analysis. Figure 3 illustrates the DSC analysis of CECTS (a), PDMA homopolymer (f) and graft copolymer CECTS-g-PDMA with different GPs (b–e). DSC traces of (a–e), which includes the CECTS segment, exhibited two obvious ranges: the stage with a temperature lower than 100 °C, which corresponds to the evaporation of water absorbed through a hydrogen bond between the CECTS backbone and water. The enthalpy with a temperature higher than 150 °C was due to the possible esterification of –OH and –COOH, the amidation of $-NH_2$ and –COOH in CECTS, and the decomposition of PDMA, as well as the possible crosslinking between CECTS-g-PDMA, according to report concerning the inter-macromolecular crosslinking of graft copolymers, provided that no monomer, but only initiator exists.[13] For a graft copolymer, the temperature with the most heat flow shifts towards a lower temperature with an increase of GP, indicating that the decomposition of the PDMA side chain plays a significant role in the decomposition of the graft copolymer.

3.1.4. TGA analysis. Figure 4 shows the TGA traces of CECTS (a), PDMA homopolymer (f) and graft copolymer CECTS-g-PDMA with various grafting percentages (b–e). Both CECTS and graft copolymer (a–e) are indicated by two steps of decomposition when the temperature was higher than 150 °C. The weight loss with a temperature under 100 °C was attributed to the evaporation of absorbed water, similar to the results of the DSC characterization, but with a lower weight loss ratio of CECTS-g-PDMA than that of CECTS, probably due to the decreased hydrophilicity of the PDMA side chain. The graft copolymer began its first decomposition at a relatively lower temperature (~180 °C) than of CECTS (~270 °C) because of the partial destruction of strong hydrogen bonds between the inter- and/or intra-molecules of CECTS. For graft copolymers, the value of weight retention decreases with an increase of GP, which is attributed to the fact that the PDMA side chain decomposes to a higher degree than the CECTS backbone under the same conditions, which could be confirmed by comparing the TGA traces of CECTS (a) and PDMA homopolymer (f).

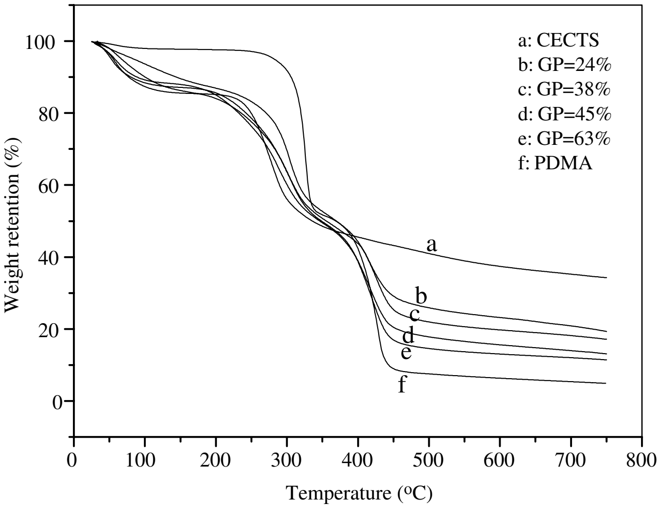

Figure 4. TGA analysis of CECTS (a), PDMA homopolymer (f) and graft copolymer CECTS-g-PDMA with various grafting percentages (b–e).

3.2. Effect of polymerization variables on grafting parameters of CECTS-g-PDMA

3.2.1. Initiator concentration. Figure 5 shows the effect of concentration of ammonium persulfate (APS) on the graft copolymerization of DMA onto CECTS by keeping other reaction variables constant. Both GP and GE showed an increase at first, followed by a decrease with the increase of the initiator concentration. The increase of GP may be ascribed to the increase of macroradicals generated by the attack of more APS for the saccharide unit of CECTS, and therefore, the more active sites of CECTS reacting with DMA. When the concentration of APS was further increased more than 13 mmol/L, the concentration of $^{\bullet}OSO_3H$ and $^{\bullet}OH$ radicals increased and consequently initiated more of the homopolymerization of DMA, which resulted in the decrease of both GP and GE.[19]

3.2.2. Monomer concentration. The effect of monomer concentration on grafting parameters is shown in Figure 6, which indicates an initial increase, followed by a decrease of GP with the increase of monomer charged. A maximum is reached when the weight ratio of DMA to CECTS is 8 (g/g). This increasing tendency is ascribed to the accumulation of monomer molecules in close proximity to the CECTS backbone due to the increase in monomer concentration.[18] As the graft copolymerization progresses, the depletion of the available monomer, as well as a reduction in the active sites on the CECTS backbone, results in a decrease of GP. The continuous decrease of GE with the increase of DMA concentration could be associated with the fact that more monomer molecules are helpful for homopolymerization than for graft copolymerization.[20]

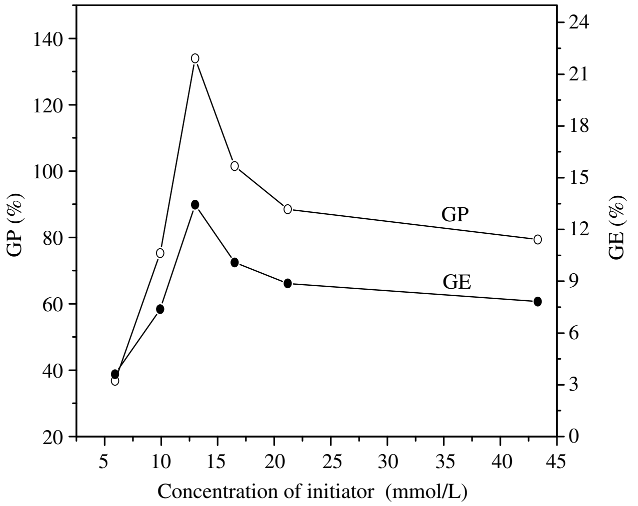

Figure 5. Effect of initiator concentration on GP and GE (m_{DMA}:m_{CECTS} = 10:1 (g/g); T = 60 °C; t = 2 h).

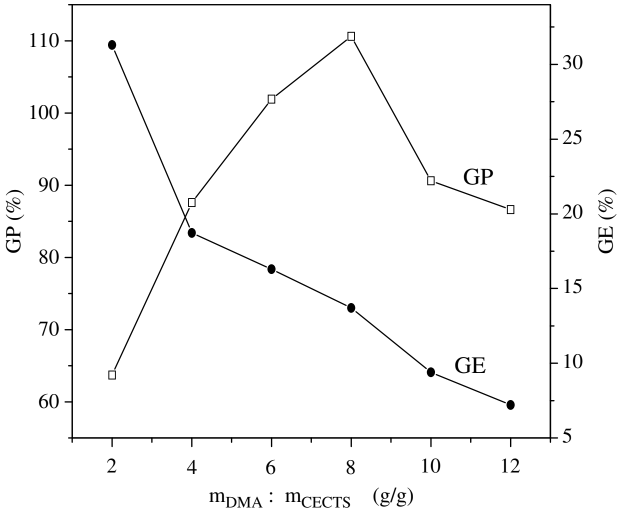

Figure 6. Effect of monomer concentration on GP and GE (T = 60 °C; t = 2 h; [APS] = 13 mmol/L).

3.2.3. Reaction time. As shown in Figure 7, both GP and GE showed a gradual increase with an increase in reaction time and leveled off 2 h later, reaching a saturated grafting value. The reduced monomer and free radicals in the reaction system with an increase in reaction time led to the leveling off of grafting parameters.[21]

3.2.4. Reaction temperature. The effect of reaction temperature on graft copolymerization of DMA onto CECTS was investigated by changing the temperature from 40 to 80 °C, while keeping other reaction variables constant. Figure 8 shows that both GP and GE reached a maximum at 60 °C. The redox reaction between the amino group of CECTS and APS did not progress readily when the reaction temperature was low. A higher temperature was helpful in increasing the chance of collision for APS and CECTS, which led to the increase of CECTS macroradicals, and therefore enhanced the graft copolymerization of DMA onto CECTS. On the other hand, GP and GE decreased with a further increase in temperature, probably due to the greater possibilities of termination and chain transfer at a relatively higher reaction temperature.[22]

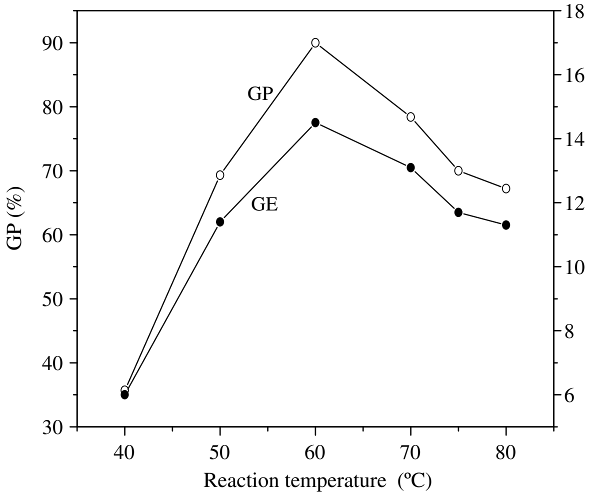

Figure 8. Effect of reaction temperature on GP and GE (m_{DMA}:$m_{\mathrm{CECTS}} = 8{:}1$ (g/g); $t = 2$ h; [APS] = 13 mmol/L).

3.3. Thermally sensitive properties of CECTS-g-PDMA aqueous solution

3.3.1. Surface-tension measurements. Block copolymers containing a PDMA segment were shown to exhibit thermal sensitivity in aqueous solution.[23] Surface-tension measurements illustrated such a thermally responsive property of a graft copolymer containing a PDMA side chain as shown in Figure 9. This showed that in basic medium (solution pH: 10.8), the surface tension of a CECTS-g-PDMA aqueous solution decreased from 45.3 to 40.4 mN/m, showing the partial aggregates formed when the solution temperature increased from 20 to 46 °C. The reason for the formation of aggregates could be attributed to deprotonation of the tertiary amino group in the PDMA side chain. When the solution temperature increased, the interaction between water and the tertiary amino group changed, and the degree of solvation of the PDMA side chain decreased; therefore, PDMA aggregates formed, and the surface tension decreased accordingly. Nevertheless, the surface tension did not decrease very significantly probably due to the relatively low GP (GP = 98%). At the same time, it is noted that the surface tension of the CECTS-g-PDMA aqueous solution was 45.3 mN/m at 20 °C, which is lower than that of pure water (74.2 mN/m) at the same temperature, showing that the solution of the graft copolymer in basic medium

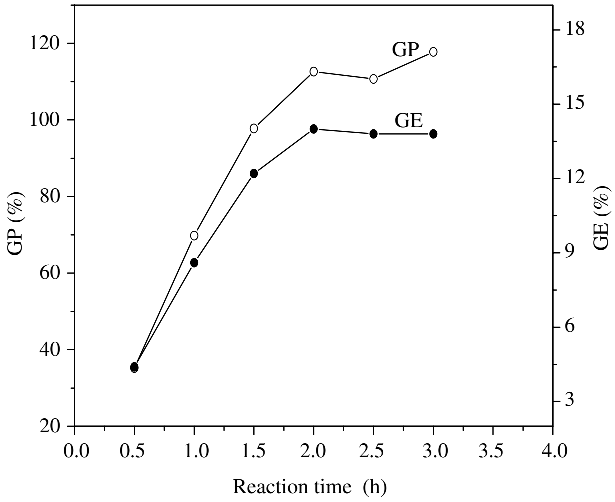

Figure 7. Effect of reaction time on GP and GE ($T = 60$ °C; m_{DMA}:$m_{\mathrm{CECTS}} = 8{:}1$ (g/g); [APS] = 13 mmol/L).

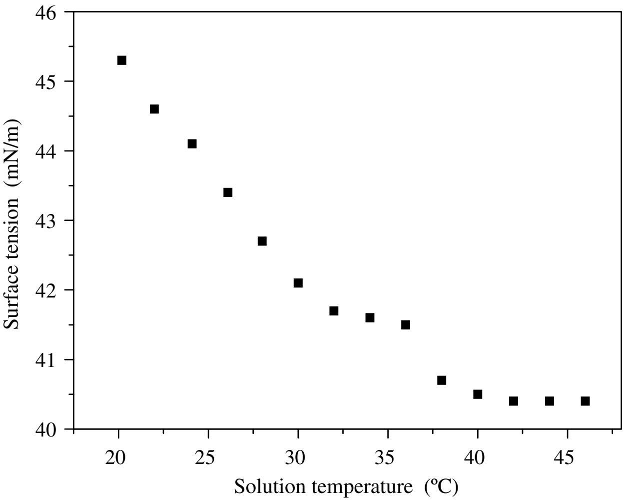

Figure 9. Effect of solution temperature on the surface tension of CECTS-g-PDMA aqueous solution (pH 10.8).

somehow exhibited a surface activity. This phenomenon could be explained by the hydrophobicity of the CECTS backbone due to the lack of protonation of the amino groups at pH 10.8, and the hydrophilicity of both the carboxyl group in the CECTS backbone and PDMA side chain.

3.3.2. Turbidity measurements. Figure 10 shows the turbidity measurements of the CECTS-g-PDMA aqueous solution (solution pH: 10.8) when increasing the solution temperature from 18 to 54 °C, showing that the transmittance of the graft copolymer solution decreased with the increase in temperature, indicating that the scattering of particles was strengthened due to the accumulation of CECTS-g-PDMA. This phenomenon is in accordance with the result of surface-tension measurements as described above. The cloudy point (determined as the midpoint of T_1 and T_2) was about 34 °C as shown in Figure 10.

3.3.3. Temperature-variable ^{1}H NMR analysis. In order to confirm the thermal sensitivity of the CECTS-g-PDMA basic aqueous solution, ^{1}H NMR analysis at various solution temperatures was conducted as shown in Figure 11. The proton signal at δ 2.35 (peak a), ascribed to the methyl proton in the tertiary amino group in the PDMA units, became weaker with an increase of solution temperature from 24 to 40 °C, indicating that the interaction between water and the tertiary amino group weakened, and the degree of solvation of the PDMA side chain decreased. This is similar to results reported in the literature,[24] and provides as well significant supporting evidence for the results acquired by surface-tension and turbidity measurements as described before.

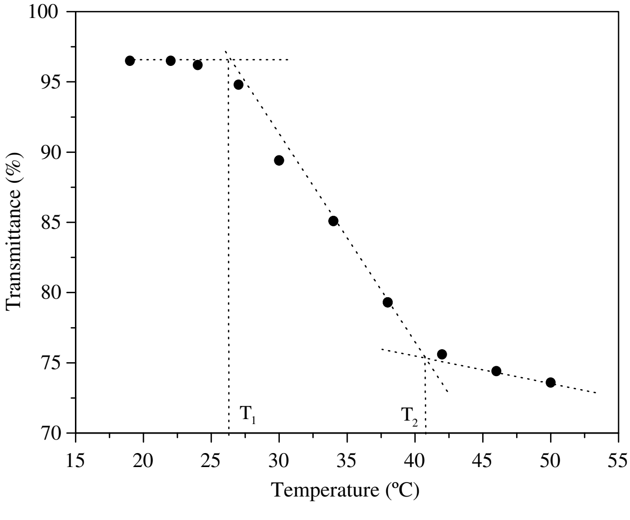

Figure 10. Effect of temperature on transmittance of CECTS-g-PDMA aqueous solution ([CECTS-g-PDMA] = 2.06 mg/mL; GP = 98%; solution pH 10.8).

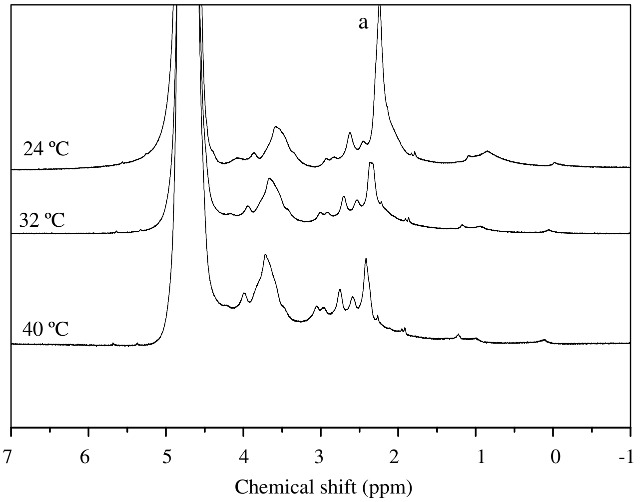

Figure 11. Temperature-variable ^{1}H NMR spectra of CECTS-g-PDMA aqueous solution (solvent: 1 wt % NaOD/D_2O; solution pD 11.0).

4. Conclusions

The graft copolymer of CECTS-g-PDMA was synthesized through homogeneous graft copolymerization of (*N*,*N*-dimethylamino)ethyl methacrylate (DMA) onto *N*-carboxyethylchitosan (CECTS) in aqueous solution using ammonium persulfate (APS) as initiator. The impact of polymerization variables including initiator concentration, monomer concentration, reaction time and temperature on grafting parameters was investigated.

XRD, FTIR, DSC and TGA analyses confirmed the introduction of the PDMA side chain into the CECTS backbone by graft copolymerization.

Surface-tension and turbidity measurements, as well as temperature-variable ^{1}H NMR analysis of the CECTS-g-PDMA aqueous basic solution illustrated the thermal sensitivity of the graft copolymer due to the existence of tertiary amino groups in the PDMA side chain.

Acknowledgements

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