

Synthesis and Characterization of Temperature-sensitive Cellulose-graft-Poly(*N*-isopropylacrylamide) Copolymers^{*}

Li-li Yang^{a,b}, Jin-ming Zhang^a, Jia-song He^a, Jun Zhang^{a**} and Zhi-hua Gan^{c**}

^a Beijing National Laboratory for Molecular Sciences, CAS Key Laboratory of Engineering Plastics, Institute of Chemistry, Chinese Academy of Sciences (CAS), Beijing 100190, China

^b University of Chinese Academy of Sciences, Beijing 100049, China

^c State Key Laboratory of Organic-inorganic Composites, Beijing Laboratory of Biomaterials, College of Life Science and Technology, Beijing University of Chemical Technology, Beijing 100029, China

Abstract A new series of cellulose-graft-poly(*N*-isopropylacrylamide) (cellulose-g-PNIPAM) copolymers were prepared by atom transfer radical polymerization (ATRP) of *N*-isopropylacrylamide monomers from a cellulose-based macro-initiator, which was homogeneously synthesized in an ionic liquid 1-allyl-3-methylimidazolium chloride (AmimCl). The composition of cellulose-g-PNIPAM copolymers could be adjusted by altering the feeding ratio and reaction time. The resultant copolymers with relatively high content of PNIPAM segments (molar substitution of PNIPAM ≥ 18.3) were soluble in water at room temperature. Aqueous solutions of cellulose-g-PNIPAM copolymers exhibited clear temperature-sensitive behavior, and their sol-to-gel phase transition properties were investigated by dynamic light scattering (DLS) and UV measurements. Compared with pure PNIPAM, the cellulose-g-PNIPAM copolymers possessed higher lower critical solution temperatures (LCST) in a range from 36.9 °C to 40.8 °C, which are close to normal human body temperature, and could be tuned by adjusting the content of PNIPAM segments in copolymers. Spherical structure of cellulose-g-PNIPAM copolymers formed at temperatures above LCST and its morphology was observed by TEM and SEM. These novel cellulose-g-PNIPAM copolymers may be attractive substrates for some biomedical applications, such as drug release and tissue engineering.

Keywords: Cellulose-g-PNIPAM; ATRP; Temperature-sensitive; Water soluble.

INTRODUCTION

In recent decades, numerous intelligent materials that respond to specific stimuli, such as temperature^[1], pH^[2, 3], and enzymatic activity^[4], have captured much attention, due to their great potential in biomedical applications, including biosensing, drug delivery, tissue engineering and diagnostics^[5, 6]. Among various stimuli-responsive materials, poly(*N*-isopropylacrylamide) (PNIPAM), a typical temperature-sensitive polymer possessing both hydrophilic amide groups and hydrophobic isopropyl groups in its side chains, has attracted much research interest, because its lower critical solution temperature (LCST) of 30–32 °C is near body temperature. At temperatures below LCST, PNIPAM chains exist as coils, and PNIPAM is soluble in water. When the temperature is above LCST, the polymer chains collapse to globules, and aqueous solutions of PNIPAM become cloudy^[7]. More interestingly, the LCST of PNIPAM-based materials can be tuned by copolymerization with other polymers. When PNIPAM is copolymerized with hydrophobic polymers, the LCST of the resultant copolymers will decrease^[8]; on the contrary, copolymerizing hydrophilic polymers with PNIPAM will lead to

^{*} This work was financially supported by the National Natural Science Foundation of China (No. 51425307).

^{**} Corresponding authors: Jun Zhang (张军), E-mail: jzhang@iccas.ac.cn

Zhi-hua Gan (甘志华), E-mail: zhgan@mail.buct.edu.cn

Received April 29, 2015; Revised May 14, 2015; Accepted May 17, 2015

doi: 10.1007/s10118-015-1703-2

increased LCST^[9–11]. Thermoresponsive micelles or nanoparticles consisting of PNIPAM segments can solubilize hydrophobic compounds and enable triggered release at target sites within the body^[12, 13]. This method has significant advantages in clinical applications, since it is relatively straightforward to implement. Meanwhile, side effects of medicines can be reduced, because the systemic concentration of medicine is decreased, whilst the local concentration resides within the therapeutic window. It should be noted that, to release medicines site-specifically upon local heating requires the medicines to be loaded by thermoresponsive polymer matrix with a LCST between 37 °C and 42 °C^[7]. Therefore, a lot of work has been done about the design of PNIPAM-containing copolymers suitable in drug release system.

As the most abundant natural polymer, cellulose is an almost inexhaustible renewable resource with fascinating structure and properties. It has excellent mechanical and chemical properties combined with the features of a bioresource, *e.g.* biodegradability, biocompatibility and sustainability^[14, 15]. More importantly, cellulose possesses a large amount of hydroxyl groups, thus cellulose is hydrophilic and can be modified easily^[16–18]. Some efforts have been made to introduce PNIPAM segments onto cellulose. Kondo *et al.* photo-grafted PNIPAM onto regenerated cellulose films. The resultant PNIPAM grafted film exhibited a thermoresponsive behavior, that it swelled and shrank in water at lower and higher temperatures than around 32 °C, respectively^[19]. Huang *et al.* synthesized series of hydroxypropyl cellulose-*g*-PNIPAM copolymers with LCST in a range of 27–40 °C. They pointed that the LCST of the copolymers decreased with the decrease of the length of PNIPAM side chains^[20]. However, so far, there are few reports about the homogeneous grafting polymerization of PNIPAM with unmodified cellulose, due to the insolubility of cellulose in common organic solvents.

In this work, a cellulose-based macro-initiator was prepared in an ionic liquid firstly, and then PNIPAM was homogeneously grafted from cellulose skeleton through an atom transfer radical polymerization (ATRP) route. A series of cellulose-based copolymers with different PNIPAM contents were obtained. The temperature-sensitive behaviors of cellulose-*g*-PNIPAM copolymers in the aqueous solutions were investigated in the present work.

EXPERIMENTAL

Materials

Microcrystalline cellulose with a degree of polymerization 220 was purchased from Beijing Fengli Jingqiu Commerce and Trade Company, and dried under vacuum at 50 °C for 12 h before use. 2-Bromoisobutryl bromide (BrBiB), copper(I) bromide (Cu(I)Br) and *N,N,N',N',N''*-pentamethyldiethylenetriamine (PMAETA) were purchased from Alfa. *N*-isopropylacrylamide (NIPAM) was obtained from Acros. Ionic liquid 1-allyl-3-methylimidazolium chloride (AmimCl) was synthesized according to our previous work^[21]. All other chemicals were of analytical grade without further purification, and obtained from Beijing Chemical Reagent Factory.

Synthesis of Cellulose-based Macro-initiator

Cellulose (1.0 g) was dispersed in AmimCl (24.0 g), and then the mixture was heated at 80 °C with mechanical stirring until cellulose was dissolved completely. 10 mL of anhydrous DMF was added to reduce the viscosity of the cellulose/AmimCl solution. The resultant cellulose/AmimCl/DMF solution was soaked in an ice/water bath for 0.5 h. After that, a solution of BrBiB (7.0 g)/DMF (10 mL) was added dropwise with stirring. The mixture was left at room temperature and stirred for 24 h. Then, the crude product was obtained by precipitation with excess deionized water. The crude product was filtered, dried, and then redissolved in DMF and precipitated with water again. The macro-initiator was dried under vacuum at 50 °C until a constant weight.

Synthesis of Cellulose-*g*-PNIPAM Copolymers

Macro-initiator, NIPAM monomer, PMDETA and 1, 4-dioxane were charged to the schlenk tube with a magnetic stirrer in turn. The reaction system was degassed with two freeze-evacuate-thaw cycles to remove dissolved oxygen. After Cu(I)Br was quickly introduced into the flask under argon atmosphere, three additional freeze-evacuate-thaw cycles were carried out. The polymerization was then allowed to proceed at 30 °C for 8 h

under argon atmosphere. After polymerization, the mixture was dialyzed against distilled water for 4 days. The resultant polymer was lyophilized for 24 h.

Characterization

^1H -NMR spectra of cellulose-g-PNIPAM copolymers were measured with a Bruker AV400 NMR apparatus, using DMSO-d_6 as the solvent. A drop of trifluoroacetic acid- d_1 was added to shift active hydrogen to low field area.

FTIR spectra of neat cellulose and cellulose-g-PNIPAM samples were recorded with a Nicolet 6700 FTIR spectrometer from 650 cm^{-1} to 4000 cm^{-1} at room temperature. The samples were scanned 16 times and the resolution was 4 cm^{-1} .

The size of the copolymers in aqueous solutions was measured with Multi Purpose Tittator MPT-2 with a 173° scattering angle. The concentration of all samples was $1\text{ mg}\cdot\text{mL}^{-1}$.

The transmittance of the copolymer aqueous solutions was measured at 500 nm through a quartz cell on a Shimadzu 2450 UV spectrometer, which was equipped with a water-jacketed cell holder and a circulating water bath. The distilled water was used as a reference. The programmed temperature increased from $20\text{ }^\circ\text{C}$ to $60\text{ }^\circ\text{C}$ at a heating rate of $0.5\text{ K}\cdot\text{min}^{-1}$.

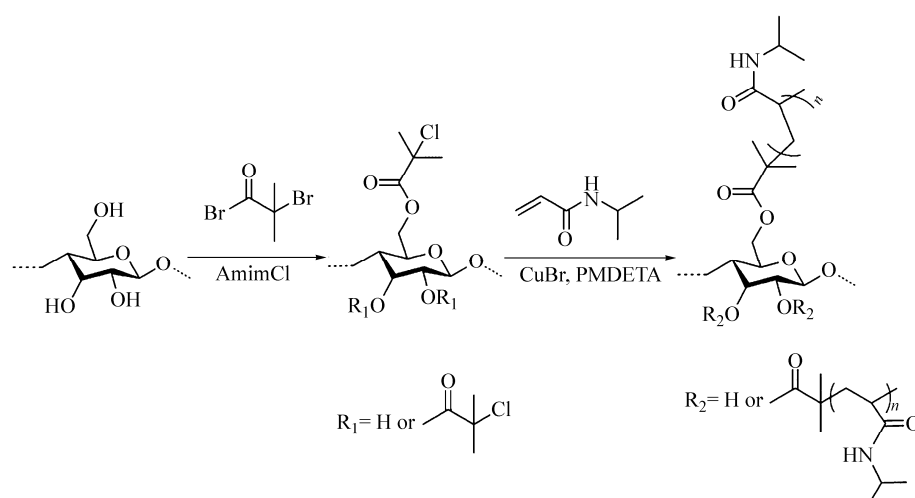
The molecular weight and distribution of copolymers were recorded on a Wyatt DH-002 GPC at room temperature. DMF containing $10\text{ mmol}\cdot\text{L}^{-1}$ LiBr was used as the mobile phase at a flow rate of $1\text{ mL}\cdot\text{min}^{-1}$. Calibration was conducted by using a series of 10 near-monodisperse polystyrene standards.

The aggregation morphology of cellulose-g-PNIPAM in aqueous solutions at $45\text{ }^\circ\text{C}$ was observed with a JEOL JEM-1011 transmission electron microscope (TEM). $10\text{ }\mu\text{L}$ of the copolymer aqueous solution was dropped on carbon-coated copper grids. The excess of copolymer solution was wiped off with a filter paper, and the grids with a thin polymer layer were dried at $45\text{ }^\circ\text{C}$ before measurements.

RESULTS AND DISCUSSION

Synthesis of Cellulose-based Macro-initiator and Cellulose-g-PNIPAM Copolymers

The homogeneous synthesis of cellulose-based macro-initiator was carried out by the esterification of cellulose with 2-bromoisobutyryl bromide in AmimCl (Scheme 1). The FTIR spectrum of macro-initiator (Fig. 1b) showed that the stretching vibration of carbonyl appeared at 1744 cm^{-1} , indicating the ester group was introduced onto cellulose successfully. ^1H -NMR spectrum also confirmed the formation of cellulose-based macro-initiator, as shown in Fig. 2(c). The peaks at $\delta = 1.5\text{--}2.0$ were attributed to methyl protons of isobutyryl



Scheme 1 Synthesis route of cellulose-g-PNIPAM copolymers

group. Besides, peaks at $\delta = 3.0\text{--}5.5$ were the characteristic peaks of cellulose skeleton. According to the integral areas of methyl in isobutyryl group and cellulose skeleton, the degree of substitution (DS) of macro-initiator was calculated to be ~ 0.91 . The reaction is preferred at C-6 and the order of reactivity is $\text{C6-OH} > \text{C2-OH} > \text{C3-OH}$ according to ^{13}C -NMR results (not given). In order to verify the existence of halogen atom, XPS spectrum was studied. Only chlorine instead of bromine was detected, which is in accordance with the previous literature^[8] and explained by replacement of the bromine with chloride anions in AmimCl *via* a nucleophilic substitution reaction. This result indicated that the resultant cellulose-based macro-initiator was cellulose 2-chloroisobutyrylate (cellulose-Cl), as shown in Scheme 1.

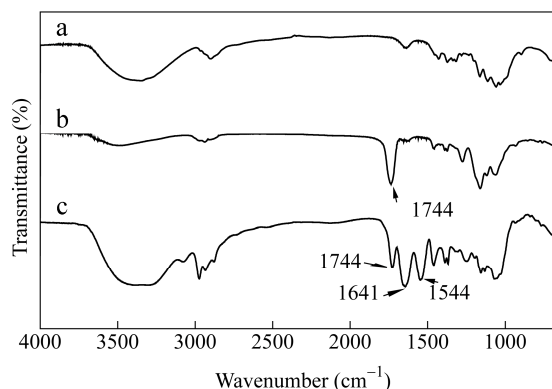


Fig. 1 FTIR spectra of (a) neat cellulose, (b) cellulose-based macro-initiator and (c) cellulose-g-PNIPAM_{4.7} copolymer

The macro-initiator cellulose-Cl can dissolve in some common solvents, such as DMSO, DMF and 1,4-dioxane. The subsequent homogeneous ATRP polymerization of PNIPAM from the macro-initiator was carried out in 1,4-dioxane, yielding to cellulose-g-PNIPAM copolymers. In the FTIR spectrum of cellulose-g-PNIPAM (Fig. 1c), the existence of PNIPAM chains was demonstrated by the appearance of peaks at 1641 cm^{-1} and 1546 cm^{-1} , corresponding to $\text{C}=\text{O}$ stretching and N-H stretching of PNIPAM, respectively. The graft copolymers were also characterized by ^1H -NMR analysis (Fig. 2b). The peak at $\delta = 3.8$ was ascribed to protons of methine group ($-\text{CH}$) in PNIPAM. And, peaks at $\delta = 0.7\text{--}1.1$ were attributed to methyl group ($-\text{CH}_3$) in PNIPAM. These results proved that cellulose-g-PNIPAM copolymers were synthesized successfully.

As the molar ratio of NIPAM to macro-initiator cellulose-Cl increased, the molar substitution of PNIPAM ($\text{MS}_{\text{PNIPAM}}$) increased, as shown in Table 1 and Fig. 3. For example, as the molar ratio of NIPAM/cellulose-Cl increased from 5:1 to 10:1 and 200:1, $\text{MS}_{\text{PNIPAM}}$ value increased from 4.7 to 8.9 and 50.1. But, there was an abnormal phenomenon in GPC spectrum (Fig. 3) that the elution time of cellulose-Cl was shorter than that of the copolymer, which was likely due to the different relationships between the hydrodynamic volume and molecular weight of cellulose-Cl and cellulose-g-PNIPAM. Similar results were also reported by Barner-Kowollik *et al.*^[8]. The kinetics of polymerization rate versus time is depending on the type of polymerization process. As shown in Fig. 4, first-order kinetic plot shows noticeable linear character, especially in the early stage of polymerization, implying the grafting polymerization of NIPAM onto cellulose macro-initiator is controllable.

A series of cellulose-g-PNIPAM copolymers with $\text{MS}_{\text{PNIPAM}}$ of 4.7–50.1 were obtained by ATRP method. Introducing PNIPAM segments into cellulose backbone significantly improved the solubility of the resultant copolymers. As shown in Table 1, all cellulose-g-PNIPAM copolymers can dissolve in dichloromethane and DMF. The solubility of cellulose-g-PNIPAM in water varied with $\text{MS}_{\text{PNIPAM}}$ of copolymers. When $\text{MS}_{\text{PNIPAM}}$ is lower than 18.3, cellulose-g-PNIPAM copolymers cannot dissolve in water, while $\text{MS}_{\text{PNIPAM}}$ is higher than 18.3, the copolymers are water soluble.

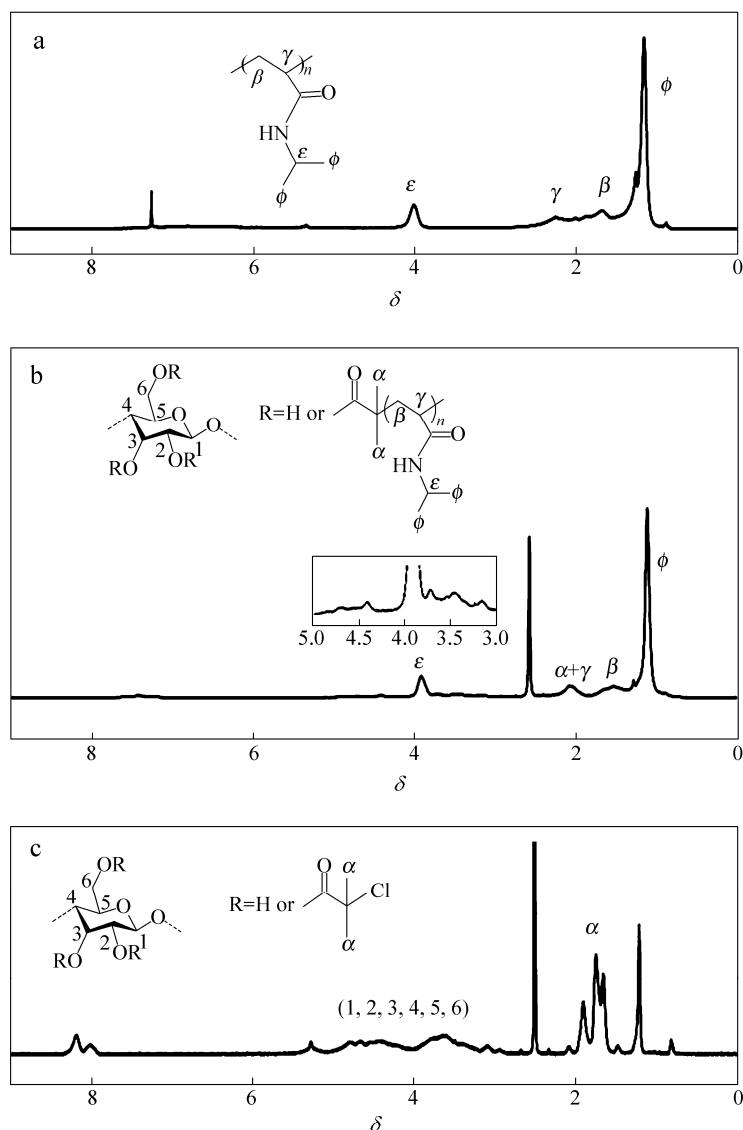


Fig. 2 ^1H -NMR spectra of (a) PNIPAM, (b) cellulose-g-PNIPAM_{4.7} and (c) cellulose-based macro-initiator

Table 1. Conditions and results of homogeneous graft copolymerization of PNIPAM on cellulose in AmimCl, and solubility of cellulose-g-PNIPAM copolymers in common solvents

Sample*	Molar ratio of NIPAM/AGU**	MS _{PNIPAM} ***	PDI	Solubility		
				H ₂ O	DCM	DMF
1	5:1	4.7	1.865	—	+	+
2	10:1	8.9	1.584	—	+	+
3	20:1	16.1	1.524	—	+	+
4	50:1	18.3	1.394	+	+	+
5	100:1	32.1	1.772	+	+	+
6	200:1	50.1	1.660	+	+	+

—: insoluble; +: soluble; * The name of samples is labeled as cellulose-g-PNIPAM_{MS}; ** AGU: anhydroglucose unite; *** MS_{PNIPAM}: molar substitution of PNIPAM, which is defined as the average number of introduced NIPAM units per AGU.

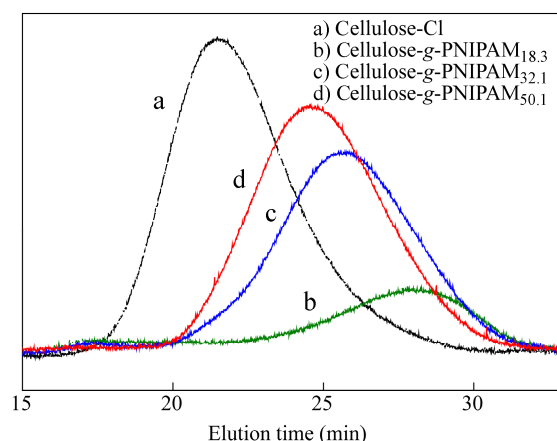


Fig. 3 GPC curves of cellulose-Cl, cellulose-g-PNIPAM_{18.3}, cellulose-g-PNIPAM_{32.1} and cellulose-g-PNIPAM_{50.1}

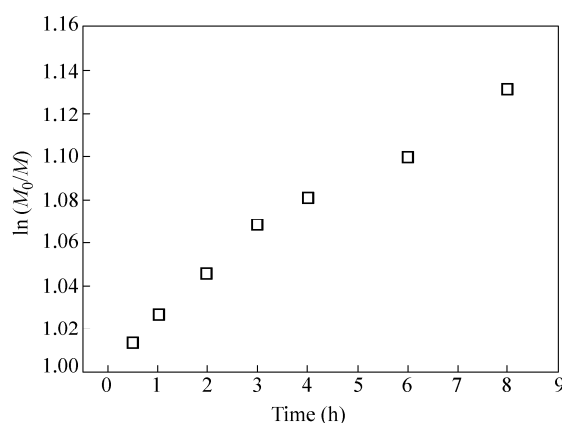


Fig. 4 Kinetic plot for the grafting-from ATRP polymerization of NIPAM using cellulose-Cl

Temperature-sensitive Properties of Cellulose-g-PNIPAM Aqueous Solutions

The transmittance of cellulose-g-PNIPAM aqueous solutions at different temperatures was used to record their temperature-sensitive behaviors, as displayed in Fig. 5. The temperature at which the transmittance changed for 50% was taken as the LCST or cloud point of the copolymers. The LCST of neat PNIPAM in our experiment was appropriately 30.1 °C. It has been accepted that the phase transition of PNIPAM at the temperatures above LCST was attributed to disrupting of hydrogen bonding of water with amide groups and inter-segment interactions between the isopropyl groups dominating as a consequence. It can be seen that the LCST of cellulose-g-PNIPAM copolymers was higher than that of neat PNIPAM, and the LCST decreased with the increase of MS_{PNIPAM} . For example, when MS_{PNIPAM} was 18.3, the LCST of cellulose-g-PNIPAM copolymer was the highest (40.8 °C). When MS_{PNIPAM} increased to 32.1 and 50.1, the LCST decreased to 37.6 °C and 36.9 °C, respectively. This phenomenon was because that the LCST was determined by a balance between PNIPAM segment-water interactions and PNIPAM segment-PNIPAM segment interactions. When PNIPAM was copolymerized with cellulose, the hydrophilic properties of cellulose component would increase segment-water interactions, and hence the LCST would increase.

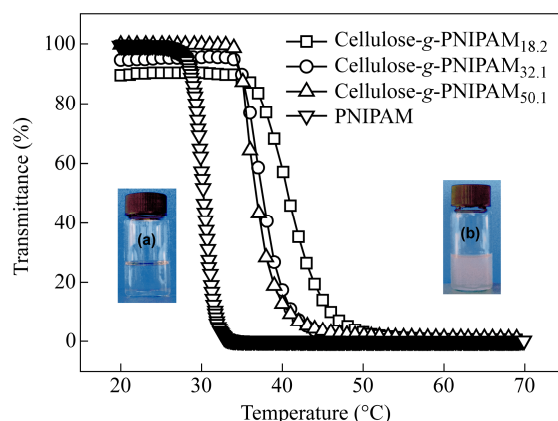


Fig. 5 The transmittances of cellulose-g-PNIPAM_{18.3}, cellulose-g-PNIPAM_{32.1}, cellulose-g-PNIPAM_{50.1} and PNIPAM aqueous solutions; and the photos of cellulose-g-PNIPAM_{50.1} aqueous solution taken at (a) 25 °C and (b) 45 °C, respectively (The concentration of polymers was 1 mg·mL⁻¹.)

DLS measurements were performed to obtain the size distribution of cellulose-g-PNIPAM copolymers in aqueous solutions at different temperatures (Fig. 6). The concentration of all the samples was 1 mg·mL⁻¹. For cellulose-g-PNIPAM_{18.3}, the solution was transparent and the copolymer was fully dissolved at 25 °C. When the temperature was raised up to 33 °C, some large particles around 200 nm formed. When the temperature was further raised up to 40 °C, the size of the copolymer increased to 550 nm abruptly, and the solution turned into opaque. And the size increased by a small degree to 620 nm when the temperature was up to 50 °C. For cellulose-g-PNIPAM_{32.1} and cellulose-g-PNIPAM_{50.1}, their temperature responsive behaviors were similar with that of cellulose-g-PNIPAM_{18.3}, although there were some large particles around 150 nm in aqueous solutions at 25 °C, which was in accordance with the UV results that transmittance of these two aqueous solutions was just 96.3% and 90.5% at 25 °C, respectively. The reason for the existence of large particles was that, as the amount of the grafted PNIPAM increased, the incompatibility between cellulose skeleton and PNIPAM changed. Some of the cellulose-g-PNIPAM associated together to form loose structure containing cellulose-rich domains and PNIPAM-rich domains, which is similar with PEO-g-PNIPAM copolymers^[22, 23].

The morphology of cellulose-g-PNIPAM copolymers at 45 °C, a higher temperature than LCST, was observed by SEM and TEM. From Fig. 7, it can be seen that all of the cellulose-g-PNIPAM copolymers formed spherical particles, when the temperature of their aqueous solutions was above the LCST. For cellulose-g-PNIPAM_{18.3} and cellulose-g-PNIPAM_{32.1} (Figs. 7a and 7b), the spheres were discerned, and their sizes were tens of nanometer and much smaller than the DLS results due to a shrinkage during drying. The SEM photograph of cellulose-g-PNIPAM_{18.3} also proved the spherical morphology. For cellulose-g-PNIPAM_{50.1} (Fig. 7c) with the highest MS_{PNIPAM}, a slight aggregation of spherical particles occurred.

The thermo-responsive behavior of cellulose-g-PNIPAM was further characterized by variable temperature ¹H-NMR analysis, as shown in Fig. 8. It is noted that, as temperature increased from 25 °C to 50 °C, a gradual decrease of typical proton signals of PNIPAM segments was detected. It is well known that, for the pure PNIPAM, as the increase of the temperature, its hydrophobicity became stronger, and PNIPAM segments will converge together at temperatures above its LCST^[24, 25]. In our case, when the temperature was above LCST, cellulose-g-PNIPAM copolymers formed spherical structure, in which hydrophobic PNIPAM segments served as the core and the hydrophilic cellulose segments acted as the shell. Such core-shell spherical structure led to the decrease of proton signals of PNIPAM segments in ¹H-NMR spectra.

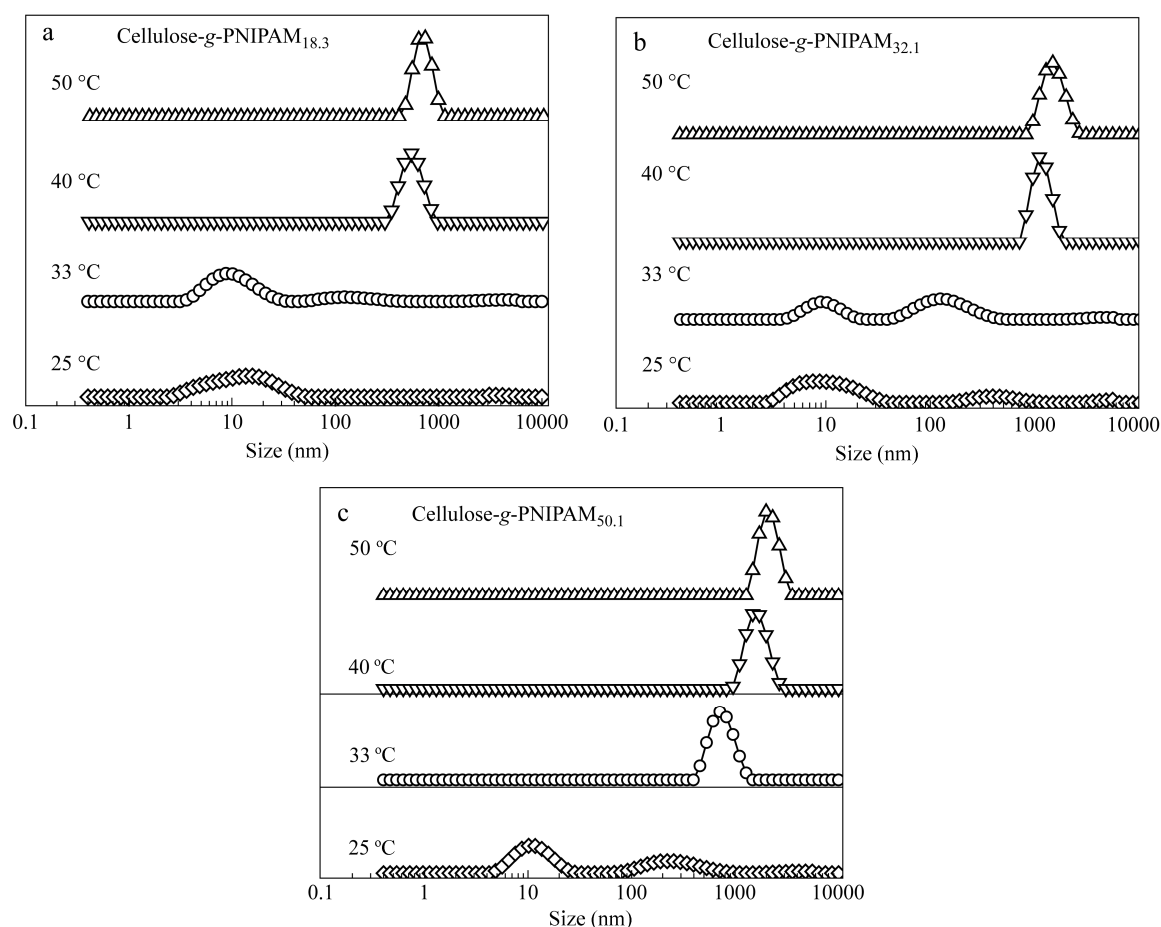


Fig. 6 Hydrodynamic diameter distributions of (a) cellulose-g-PNIPAM_{18.3}, (b) cellulose-g-PNIPAM_{32.1} and (c) cellulose-g-PNIPAM_{50.1} aqueous solutions at different temperatures measured by DLS (The concentration of copolymers was 1 mg·mL⁻¹.)

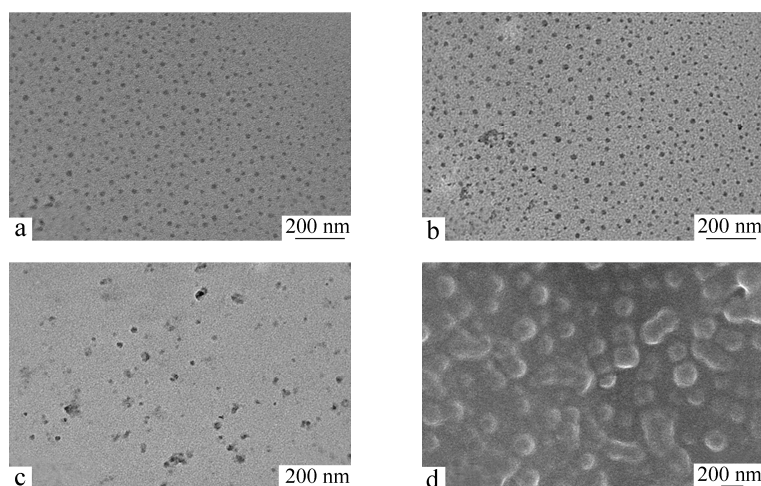


Fig. 7 Photographs of the morphology of micelles prepared from cellulose-g-PNIPAM aqueous solutions at 45 °C: TEM images of (a) cellulose-g-PNIPAM_{18.3}, (b) cellulose-g-PNIPAM_{32.1} and (c) cellulose-g-PNIPAM_{50.1}; (d) SEM image of cellulose-g-PNIPAM_{18.3}

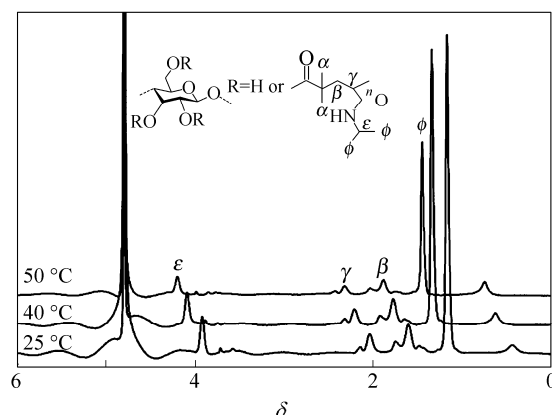


Fig. 8 ^1H -NMR spectra of cellulose-g-PNIPAM_{18.3} in D_2O at different temperatures

CONCLUSIONS

A cellulose-based macro-initiator for ATRP polymerization was prepared in ionic liquid AmimCl. Then, *via* using ATRP method, a series of cellulose-g-PNIPAM copolymers were synthesized and the composition of copolymers can be tuned by adjusting feeding ratio and reaction time. The resultant copolymers with relatively high molar substitution of PNIPAM (molar substitution ≥ 18.3) were soluble in water at room temperature. And, when the temperature was higher than LCST, the copolymers turned into spherical micelles with core-shell structure, in which the hydrophobic PNIPAM segments converged together as the core and the hydrophilic cellulose segments served as the shell encircling PNIPAM. More interestingly, compared with pure PNIPAM, the LCST of cellulose-g-PNIPAM copolymers was in a range from 36.9 °C to 40.8 °C, which is close to normal human body temperature and can be adjusted by controlling the composition of cellulose-g-PNIPAM. This new kind of cellulose-g-PNIPAM copolymers is expected to be used in some biomedical fields, such as drug release and tissue engineering.

REFERENCES

- 1 Zhou, H.Y., Jiang, L.J., Cao, P.P., Li, J.B. and Chen, X.G., *Carbohydr. Polym.*, 2015, 117: 524
- 2 Guo, J.H., Pan, S.J., Yin, X.C., He, Y.F., Li, T. and Wang, R.M., *J. Appl. Polym. Sci.*, 2015, 132(9): 41572
- 3 Araujo, J.V., Davidenko, N., Danner, M., Cameron, R.E. and Best, S.M., *J. Biomed. Mater. Res., Part A*, 2014, 102(12): 4415
- 4 Li, N., Li, N., Yi, Q.Y., Luo, K., Guo, C.H., Pan, D.Y. and Gu, Z.W., *Biomaterials*, 2014, 35(35): 9529
- 5 Abeer, M.M., Amin, M.C.I.M., Lazim, A.M., Pandey, M. and Martin, C., *Carbohydr. Polym.*, 2014, 110: 505
- 6 Kumashiro, Y., Yamato, M. and Okano, T., *Ann. Biomed. Eng.*, 2010, 38(6): 1977
- 7 Liu, R., Fraylich, M. and Saunders, B., *Colloid Polym. Sci.*, 2009, 287(6): 627
- 8 Hufendiek, A., Trouillet, V., Meier, M.A.R. and Barner-Kowollik, C., *Biomacromolecules*, 2014, 15(7): 2563
- 9 Alli, S., Alli, A. and Hazer, B., *J. Appl. Polym. Sci.*, 2012, 124(1): 536
- 10 Xiao, J.J., Li, X.B., Wang, X., Yi, C.W. and Su, S.P., *Chinese J. Polym. Sci.*, 2015, 33(3): 456
- 11 Seidi, F. and Heshmati, P., *Chinese J. Polym. Sci.*, 2015, 33(1): 192
- 12 de Graaf, A.J., dos Santos, I.I.A.P., Pieters, E.H.E., Rijkers, D.T.S., van Nostrum, C.F., Vermonden, T., Kok, R.J., Hennink, W.E. and Mastrobattista, E., *J. Control. Release*, 2012, 162(3): 582
- 13 Cheng, R., Meng, F.H., Ma, S.B., Xu, H.F., Liu, H.Y., Jing, X.B. and Zhong, Z.Y., *J. Mater. Chem.*, 2011, 21(47): 19013
- 14 Domingues, R.M.A., Gomes, M.E. and Reis, R.L., *Biomacromolecules*, 2014, 15(7): 2327
- 15 Rajwade, J.M., Paknikar, K.M. and Kumbhar, J.V., *Appl. Microbiol. Biotechnol.*, 2015, 99(6): 2491

- 16 Yan, C., Zhang, J., Lv, Y., Yu, J., Wu, J., Zhang, J. and He, J., *Biomacromolecules*, 2009, 10(8): 2013
- 17 Wu, J., Zhang, J., Zhang, H., He, J.S., Ren, Q. and Guo, M., *Biomacromolecules*, 2004, 5(2): 266
- 18 Sui, X.F., Yuan, J.Y., Zhou, M., Zhang, J., Yang, H.J., Yuan, W.Z., Wei, Y. and Pan, C.Y., *Biomacromolecules*, 2008, 9(10): 2615
- 19 Pyun, J. and Matyjaszewski, K., *Chem. Mater.*, 2001, 13(10): 3436
- 20 Jin, X., Kang, H.L., Liu, R.G. and Huang, Y., *Carbohydr. Polym.*, 2013, 95(1): 155
- 21 Zhang, H., Wu, J., Zhang, J. and He, J., *Macromolecules*, 2005, 38(20): 8272
- 22 Mei, A.X., Guo, X.L., Ding, Y.W., Zhang, X.H., Xu, J.T., Fan, Z.Q. and Du, B.Y., *Macromolecules*, 2010, 43(17): 7312
- 23 Yan, J., Ji, W., Chen, E., Li, Z. and Liang, D., *Macromolecules*, 2008, 41(13): 4908
- 24 Ma, L., Kang, H.L., Liu, R.G. and Huang, Y., *Langmuir*, 2010, 26(23): 18519
- 25 Wu, C., Ying, A. and Ren, S., *Colloid Polym. Sci.*, 2013, 291(4): 827