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Star Polymers with Both Temperature Sensitivity and Inclusion Functionalities

Yu-Yang Liu,* Yao-Bing Zhong, Jiang-Kun Nan, and Wei Tian

Department of Applied Chemistry, School of Science, Northwestern Polytechnical University, Xi'an, 710072, People's Republic of China

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ABSTRACT: We designed and synthesized novel star poly(*N*-isopropylacrylamide) (star-PNIPAm) and star-PNIPAm with cyclodextrin (CD) end groups (star-PNIPAm-CD) by atom transfer radical polymerization (ATRP). In the synthesis, β-CD-core with 21 initiation sites, heptakis[2,3,6-tri-*O*-(2-chloropropionyl)]-β-cyclodextrin (21Cl-β-CD), was first synthesized by the reaction of β-CD with 2-chloropropionyl chloride (CPC). Then, 21-arm star-PNIPAm (PDI = 1.03) was synthesized by ATRP of *N*-isopropylacrylamide (NIPAm) initiated via 21Cl-β-CD. Finally, a star-PNIPAm-CD (PDI = 1.02) was synthesized by ATRP of a monovinyl β-CD (GMA-EDA-β-CD) initiated via star-PNIPAm. The obtained star-PNIPAm and star-PNIPAm-CD were characterized by means of SEC/MALLS, NMR, IR, and DSC. By using 8-anilino-1-naphthalenesulfonic acid ammonium salt hydrate (ANS), 1-adamantanamine hydrochloride (ADA-NH_3Cl), and ibuprofen sodium salt (ibuprofen-Na) as guest molecules, thermal sensitivity and inclusion behaviors of the star polymers were investigated by fluorescence spectrophotometer and DLS. It is found that the star polymers can combine both thermal sensitivity of PNIPAm and inclusion behavior of β-CD. Interestingly, the star polymers can self-assembly to form nanosized aggregates in aqueous solution above their LCSTs. The self-assembly behavior shows molecular recognition capability. And formation and dissociation of the nanosized aggregation can change reversibly by changing temperature above and below the LCST.

Introduction

Star polymers are three-dimensional hyperbranched structure in which linear arms of the same or different molecular weights emanate from a central core.[1−10] The morphology, physical properties, and functions of star polymers may considerably differ from those of the linear counterparts.[2,3] The existence of numerous functional groups in a small volume makes these polymers important for use in biological and pharmaceutical applications[4−7] and catalyst carriers.[8−10] Active molecules can be immobilized on the surface of a star polymer or included into its inside.[4−10] On the basis of these properties of a star polymer, in this paper, we expected to synthesize novel star polymer architecture based on the combination of thermal sensitivity of poly(*N*-isopropylacrylamide) (PNIPAm) and supramolecular inclusion property of cyclodextrin (CD).

PNIPAm is a widely investigated smart polymer. This is because PNIPAm exhibits a lower critical solution temperature (LCST) of ~32 °C in aqueous solution. It is water-soluble and water-insoluble below and above LCST, respectively.[11] PNIPAm can thus sense temperature variation and has potential application in smart drug delivery field.[12−18] Therefore, different structure and functional PNIPAm-based polymers have investigated widely.[11−28]

A CD molecule is characteristic of a hydrophilic exterior surface and hydrophobic interior cavity, which can accommodate a wide range of molecules as guests.[29−32] Therefore, supramolecular inclusion systems of CDs with guest molecules are of interest.[16−18] These systems have been used in many fields such as mimicking biological recognition,[21,22] controlled drug release,[6,7,16−18,32−36] and polymer synthesis.[37,38] When CD units are incorporated in a polymer, the polymer can combine both the favorable property of CDs to form inclusion complexes and properties of polymers, even their cooperative effect.[29,30] Therefore, studies on novel CDs-based materials attract attention from researchers all the time.[16−18,21−36,39−43]

Both arm-first and core-first methods can be used to prepare star polymers,[2,3,44−53] but we selected the core-first method to prepare star polymer and used β-CD as core for the synthesis. On the one hand, when CD was used as a core, it can still include guest molecules.[44] On the other hand, the core-first method is effective and convenient for preparing well-defined stars with precise numbers of arms.[2,3,27,44−47] On the basis of these, here we report two novel star polymers based on β-CD and PNIPAm. One (star-PNIPAm) was synthesized by the ATRP of NIPAm using β-CD derivative as core initiator. The other one (star-PNIPAm-CD) was synthesized by further ATRP of a monovinyl β-CD monomer using star-PNIPAm as macroinitiator. Obviously, for star-PNIPAm-CD, β-CD was attached on star-PNIPAm arm end. Therefore, star-PNIPAm-CD contains CD core and shell, which can include a guest molecule. The architecture may endow star-PNIPAm-CD unique properties. However, as far as we know, this type of star polymer is not still reported.

On the basis of the consideration above, in the present work, we prepared and characterized novel star-PNIPAm and star-PNIPAm-CD by the core-first method of ATRP as mentioned above. By using 8-anilino-1-naphthalenesulfonic acid ammonium salt hydrate (ANS), 1-adamantanamine hydrochloride (ADA-NH_3Cl), and ibuprofen sodium salt (ibuprofen-Na) as guest molecules, thermal sensitivity and inclusion behaviors of the star polymers were investigated by fluorescence spectrophotometer and DLS. The influence of addition of guest molecules on self-assembly behavior of the star polymers in aqueous solution was tried to be explored.

*Corresponding author. E-mail: liu_yyang1120@nwpu.edu.cn.

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Experimental Section

Materials. *N*-Isopropylacrylamide (NIPAm, 99%), tris(2-aminoethyl)amine (TREN, 96%), 8-anilino-1-naphthalenesulfonic acid ammonium salt hydrate (ANS, 97%), and 2-chloropropionyl chloride (CPC, 95%) were purchased from Acros. 1-Adamantanamine hydrochloride (ADA-NH_3Cl, 99%) was from Alfa Aesar. Glycidyl methacrylate (GMA, >95%) and 2-(4-isobutylphenyl)propionic acid (ibuprofen) were purchased from TCI, Japan. *p*-Toluenesulfonyl chloride (*p*-TsCl, chemical grade) were from Sinopharm Chemical Reagent Co., Ltd., Shanghai, China. *N*,*N*,*N*′,*N*″,*N*″-Pentamethyldiehylenetriamine (PMDETA) was supplied by Yutian Chemical (Liyang, China) and used as received. β-CD was acquired from Bodi Chemical Plant, Tianjing, China. Cuprous Chloride (CuCl, HongYan Chemical Reagent Factory, China) was purified by being stirred in acetic acid overnight. After filtration, it was washed with ethanol and ether and then dried in a vacuum oven at 25 °C. All other reagents including ethylenediamine (EDA) and dimethylformamide (DMF) were analytical grade and made in China. They were used as received without further purification.

Tris[2-(dimethylamino)ethyl]amine (Me_6TREN) was synthesized from TREN according to refs 54 and 55. A monovinyl β-CD monomer (CH_2=C(CH_3)COOCH_2CH(OH)CH_2NHCH_2CH_2NH-β-CD, GMA-EDA-β-CD) was synthesized by the reaction of mono-6-EDA-β-CD with GMA according to our previously reported method.[56]

Synthesis of 21Cl-β-CD Core. 1.13 g of dry β-CD, which was purified thrice by recrystallization from water prior to use, was dissolved in 25 mL of dry *N*-methyl-2-pyrrolidone (NMP). Under the conditions of ice−water bath and magnetic stirring, 9 mL of 2-chloropropionyl chloride was added to the solution. After the reaction mixture was stirred for 10 min in ice bath, it was stirred at room temperature for 4 h and at 40 °C for an additional 44 h. After completion of stirring, the mixture was allowed to cool to room temperature, and 100 mL of dichloromethane was added in the mixture. The organic solution was washed with aqueous sodium bicarbonate solution and distilled water. After the organic solution was dried with anhydrous magnesium sulfate ($MgSO_4$), the dichloromethane was removed by rotary evaporator. The syrup obtained was diluted with acetone and then precipitated into methanol/water (70/30, v/v). The product repeated to be purified by the precipitation. The obtained 21Cl-β-CD was dried in vacuum oven at 50 °C for 2 days. IR (KBr): 2989, 2941 cm^{-1} (w, C−H), 1 754 cm^{-1} (s, C=O), 1 073 cm^{-1} (s, C−O−C). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.5−5.5 (70H, residues of β-CD and −C*H*(CH_3)Cl), 1.73 (63H, −CH_3). ^{13}C NMR ($CDCl_3$, 400 MHz): δ 169.4(*C*=O), 96.5 (*C*(1) of β-CD), 70−74 (*C*(2,3 and 5) of β-CD), 63.8 (*C*(6) of β-CD), 52.1 (−*C*−Cl), 21.1 (−*C*H_3). $C_{105}H_{133}O_{56}Cl_{21}$: Calcd C 41.52, H 4.38. Found C 41.77, H 4.09.

Synthesis of Star PNIPAm (Star-PNIPAm). Synthesis of star-PNIPAm was carried out by ATRP. After a mixture of NIPAm (8.32 g), 21Cl-β-CD (0.152 g), and Me_6TREN in 15 mL of isopropanol/butanone (6/9, v/v) was bubbled with nitrogen gas for 15 min, CuCl was added to the mixture. The mixture was bubbled with nitrogen gas for 30 min, during it was sonicated thrice and each time lasted 1 min. The reaction proceeded at 40 °C for 5.5 h. Then, the reaction mixture was precipitated into the mixed ether/hexane solvent. The precipitate was dissolved in THF and passed through an alumina column to remove the copper complex. The resulting solution was concentrated by rotary evaporator and was then precipitated into diethyl ether. The product repeated to be purified by the precipitation. The obtained star-PNIPAm was dried in vacuum oven at 40 °C for 1 day (yield, 74.9%).

Synthesis of Star PNIPAm with Cyclodextrin End Groups (Star-PNIPAm-CD). Synthesis of star-PNIPAm-CD was carried out by an ATRP. Briefly, after a mixture of DMF (10 mL), star PNIPAm-Cl (1.5 g), GMA-EDA-β-CD (1.5 g), and PMDETA (60 mg) was bubbled with nitrogen gas for 15 min, CuCl was added to the mixture. After the mixture was bubbled with nitrogen gas for an additional 30 min, it was put in oil bath of 85 °C. After 48 h of stirring, the mixture was allowed to cool to room temperature, and it was poured into diethyl ether. The precipitate was dissolved in water and subjected to dialysis (molecular weight cutoff: 12 000−14 000) against water for 10 days. After completion of the dialysis, the aqueous sample of polymer was filtered and star-PNIPAm-CD was obtained by lyophilizing the filtrate.

Analyses. Infrared spectroscopy measurements were preformed on a NICOLET iS10 (USA). NMR measurements were conducted on Varian INOVA-400, using $CDCl_3$ or DMSO-d_6 as solvent. Elemental analysis was carried out on a Vario EL III instrument.

The molecular structure parameters of the star polymers were determined on a DAWN EOS size exclusion chromatography/multiangle laser light scattering (SEC/MALLS). HPLC grade DMF containing LiCl (0.01 mol/L) (at 40 °C) was used as eluent at a flow rate of 0.5 mL/min. The refractive index increment (d*n*/d*c*) value of sample in DMF solution containing LiCl (0.01 mol/L) was determined by an Optilab rEX detector at 25 °C through a batch model.

Zetasizer Nano-ZS dynamic light scattering (DLS) (Malvern, English) was used to characterize sizes and zeta-potentials of the star polymers in aqueous solution. For a DLS measurement, a 1 mg/mL polymer solution was used. All samples were measured directly without any filter. A sample was kept at predetermined temperature for 5 min before data collection and was determined at temperature ranging from 25 to 50 °C.

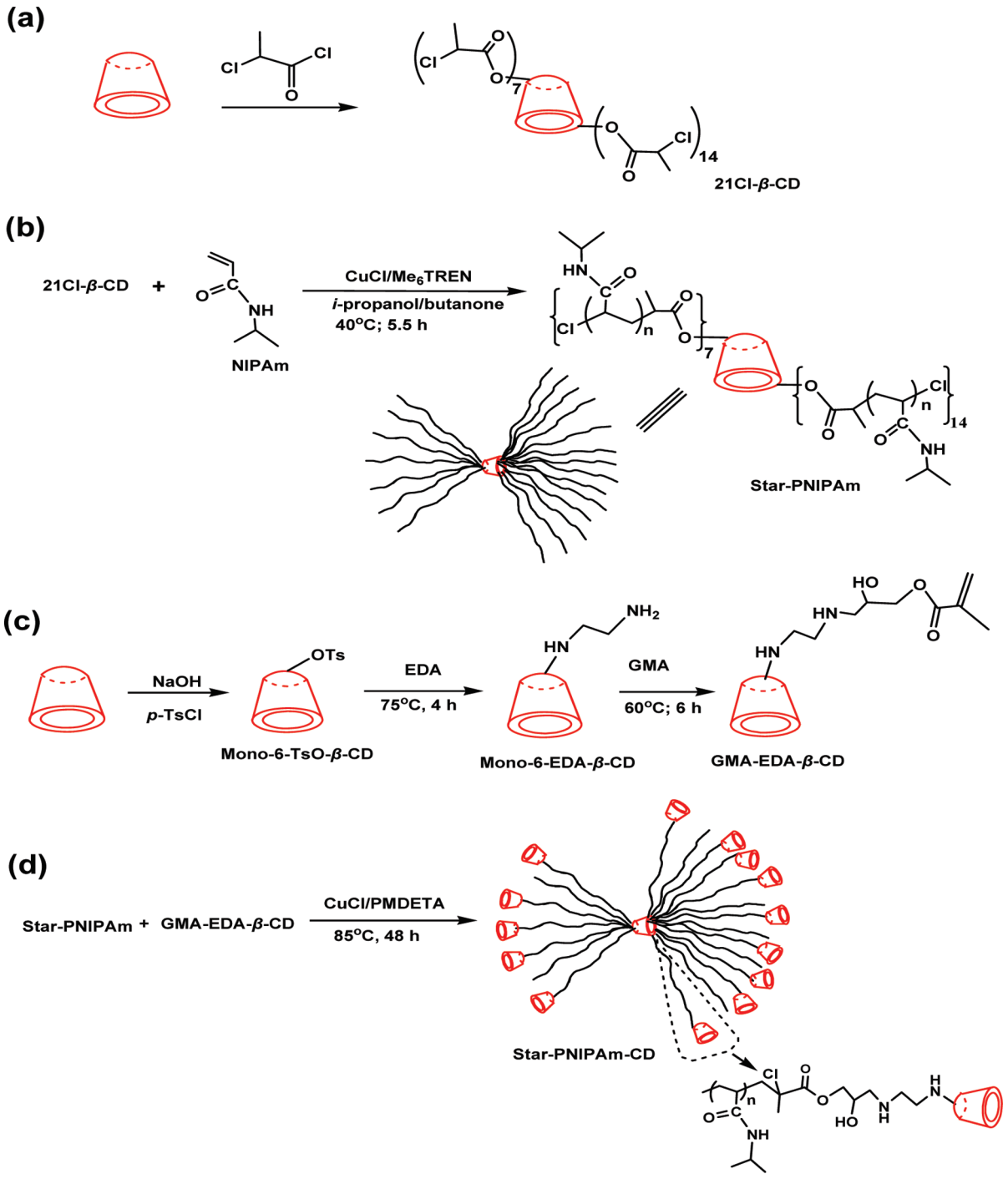
Differential scanning calorimetry (DSC) (model MDSC 2910, TA Instruments) measurements were used to determine glass transition temperature (T_g) and lower critical solution temperature (LCST) of a star polymer. When T_g of a polymer sample was determined by DSC, it was subjected to two times scanning. The sample was first heated from room temperature to 210 °C with the scan rate of 20 K/min to remove thermal history. T_g is obtained by the second scanning from room temperature to 210 °C with the scan rate of 10 K/min, and the value was estimated from the midpoint of the special heat increment. Also, DSC was used to determine related polymers' LCSTs. A range of 10−15 mg of 50 mg/mL aqueous samples was placed in a sealed sample pan and scanned from 0 to 60 °C with a heating rate of 2 K/min. The onset temperature of the thermogram was treated as the LCST.

Fluorescence of aqueous ANS solutions in the presence of star-PNIPAm or star-PNIPAm-CD was observed by using a fluorescence spectrophotometer (F-4600, Hitachi, Japan). The excitation wavelength for the measurement was 350 nm, while the emission wavelength was from 370 to 650 nm. All samples were kept to equilibrium at a predetermined temperature for 10 min before data collection.

Results and Discussion

Synthesis of 21Cl-β-CD. The esterification of CD with acyl halide or anhydride agents was widely used to synthesize ATRP agents.[25,27,44−46,50] By the reaction mechanism, CD cores with functional bromine group for ATRP have been synthesized. For example, Ohno et al.[44] and Li et al.[45,46] have reported heptakis[2,3,6-tri-*O*-(2-bromo-2-methylpropoionyl)]-β-cyclodextrin (21Br-β-CD) by different methods. In this paper, we synthesized a β-CD core with 21 functional chlorine groups, heptakis[2,3,6-tri-*O*-(2-chloropropionyl)]-β-cyclodextrin (21Cl-β-CD). The 21Cl-β-CD was synthesized by the esterification of β-CD with CPC in NMP, and the reaction route is shown in Scheme 1a. Li et al synthesized 21Br-β-CD by the reaction of β-CD with 2-bromoisobutyryl bromide in NMP.[46] They concluded that NMP was good solvent for their reaction because NMP possesses good compatible with the reactants and the resultants. Therefore, in our synthesis, NMP was still selected as solvent. The result shows that NMP is

Scheme 1. Schematic Illustration of the Synthesis Routes of Star-PNIPAm and Star-PNIPAm-CD



suitable for the synthesis. The structure of 21Cl-β-CD was confirmed by IR, NMR, and element analysis, and the specific data are shown in the Experimental Section.

Synthesis and Characterization of Star-PNIPAm and Star-PNIPAm-CD. We used 21Cl-β-CD as a core initiator and synthesized a novel star PNIPAm with β-CD end groups (star-PNIPAm-CD) by ATRP in two steps. The specific synthesis routes of star-PNIPAm-CD are shown in Scheme 1. In the first step, a living 21-arm PNIPAm (star-PNIPAm) was first synthesized in isopropanol/butanone at 40 °C by using CuCl/Me_6TREN as the catalyst (see Scheme 1b).[57−59] Then, using star-PNIPAm as an initiator, a star-PNIPAm-CD was synthesized by ATRP of GMA-EDA-β-CD at 85 °C for 48 h in the presence of catalyst CuCl/PMDETA (see Scheme 1d). That is, the chlorine end groups of star-PNIPAm reacted with vinyl group of GMA-EDA-β-CD to form star-PNIPAm-CD. The used GMA-EDA-β-CD monomer was synthesized according to the method developed by us previously (see Scheme 1c).[56] The obtained star-PNIPAm and star-PNIPAm-CD were characterized by means of SEC/MALLS, NMR, IR, and DSC.

Figure 1 shows differential refractive index (DRI) signals of SEC/MALLS chromatograms of star-PNIPAm and star-PNIPAm-CD. The dn/dc values of the two star polymers were determined to be 0.0673 and 0.0696 mL/g, respectively. The M_n and $PDI_{SEC/MALLS}$ of star-PNIPAm were found to be 2.036×10^5 g/mol and 1.03, respectively, and those of star-PNIPAm-CD were 2.309×10^5 g/mol and 1.02, respectively

(see Table 1). According to the M_n value of star-PNIPAm, the average number of NIPAm units of per star-PNIPAm arm was calculated to be 84.4, whereas the corresponding value of the feed composition was 70. However, the very low $PDI_{SEC/MALLS}$ values of star-PNIPAm indicated that the ATRP could proceed well, suggesting that a star−star coupling during the polymerization seemed to be neglectable. Therefore, the determined M_n of star-PNIPAm may be higher than its actual value. The difference of M_n values of star-PNIPAm-CD and star-PNIPAm was found to be 27 300. According the difference, the mean CD numbers of per star-PANIPAm-CD molecule was calculated to be 20.7, which is close to 21. However, note that there existed weight loss during dialysis of star-PANIPAm-CD (i.e., low molecular weight of star-PNIPAm-CD was removed in the procedure), which also led to an increase in average molecular weight of star-PNIPAm-CD. Therefore, the results did not suggest that all PNIPAm arm ends could be conjugated by a CD unit.

Figure 2 shows ^{1}H NMR spectra of star-PNIPAm and star-PNIPAm-CD using DMSO-d_6 as the solvent. In Figure 2a, δ 7.21, 3.85, and 1.04 are assigned to proton peaks of −CON*H*−, −C*H*$(CH_3)_2$, and −CH(C*H*$_3$) for PNIPAm arms, respectively. This means that star PNIPAm was synthesized. However, it was difficult to detect signals of CD core for star-PNIPAm due to its low content. Therefore, the molecular weight of star-PNIPAm could not be assessed by ^{1}H NMR. In Figure 2b, δ 5.76, 4.83, and 4.4 are assigned to proton peaks of C(2 and 3)−O*H*, C(1)−*H*, and C(6)−O*H* of β-CD end groups, respectively. According to proton peak intensity at δ 7.21 and 4.83 on the NMR curve, the molar ratio of β-CD component to NIPAm units of star-PNIPAm-CD was calculated to be 1:91. This result evidenced that β-CD was conjugated onto PNIPANm arm ends in the second ATRP. However, it was clear that part of PNIPAm arm was not conjugated by β-CD units, in spite of monomer GMA-EDA-β-CD being in excess in the ATRP. Possible reasons are that part of PNIPAm arm ends lost reactivity or the β-CD monomer exhibited low reactivity to PNIPAm-Cl under these reaction conditions. The result also implied that the β-CD monomer possessed low reactivity to itself by ATRP in the reaction condition. This was confirmed in our other experiment; e.g., linear PNIPAm with β-CD end group could be synthesized by the method. This may be caused by the steric hindrance of β-CD. Therefore, there was little possibility of more than one CD unit attached onto a PNIPAm arm end.

Figure 3 shows IR spectra of star-PNIPAm and star-PNIPAm-CD. As seen clearly in Figure 3, there is a C=O stretching vibration peaks around 1640 cm^{-1} and a N−H deformation vibration around 1560 cm^{-1} from PNIPAm component.[56] In comparison with IR characteristic absorptions of star-PNIPAm, it could clearly be observed from IR

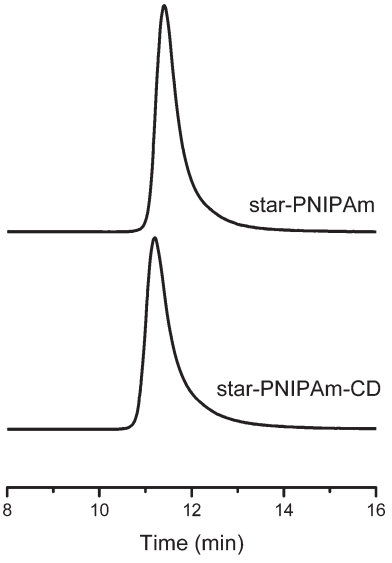


Figure 1. DRI signals of SEC/MALLS chromatograms of star-PNIPAm and star-PNIPAm-CD.

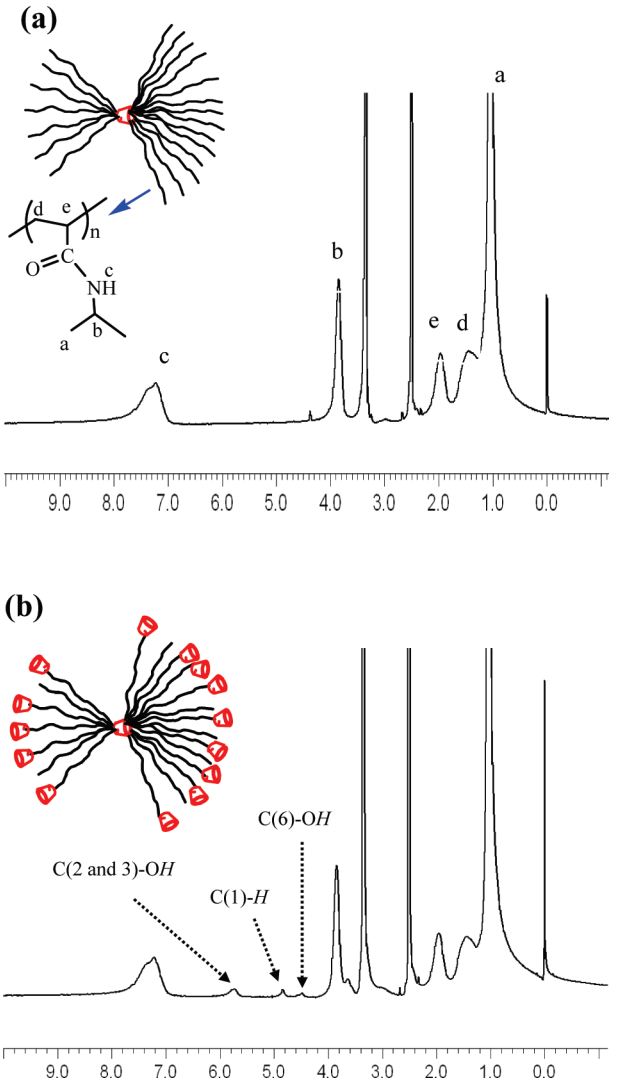


Figure 2. ^{1}H NMR spectra of star-PNIPAm (a) and star-PNIPAm-CD (b).

Table 1. M_ns of Star-PNIPAm and Star-PNIPAm-CD

polymer	yield (wt %)	SEC/MALLS measurement M_n ($PDI_{SEC/MALLS}$)[a]	DLS measurement[b] D_z (PDI_{DLS})
star-PNIPAm	74.9	203 600 (1.03)	16.5 nm (0.102)
star-PNIPAm-CD	93.1[c]	230 900 (1.02)	17.1 nm (0.221)

[a] $PDI_{SEC/MALLS}$ = weight-average molecular weight (M_w)/number-average molecular weight (M_n). [b] *Z*-average diameter (D_z) was determined at star polymer of 1 mg/mL in distilled water by DLS at 25 °C. [c] Weight ratio of the product to feed star-PNIPAm (1.5 g).

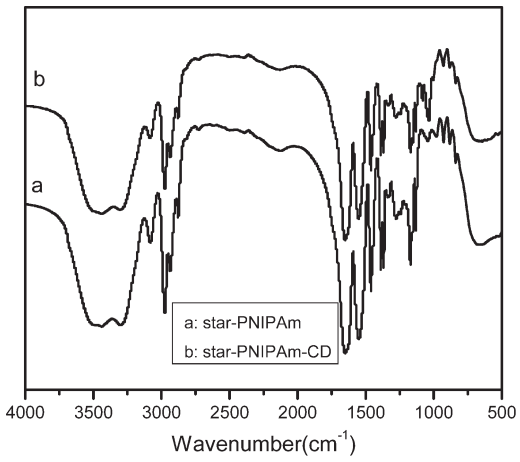


Figure 3. IR spectra for star-PNIPAm (a) and star-PNIPAm-CD (b).

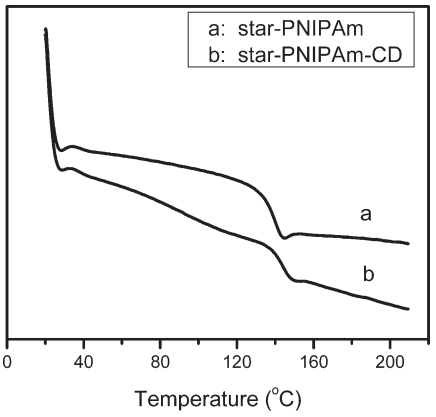


Figure 4. DSC thermograms of star-PNIPAm and star-PNIPAm-CD.

absorption curve of star-PNIPAm-CD that there was a C−O−C characteristic stretching vibration around 1035 cm^{-1} from β-CD units.[33,56,60] This demonstrates that β-CD was conjugated into PNIPAm arms in the second ATRP.

Figure 4 is DSC profiles of star-PNIPAm and star-PNIPAm-CD. As seen in Figure 4, the T_g values of star-PNIPAm and star-PNIPAm-CD are 140.2 and 144.5 °C, respectively. This means that incorporation of β-CD unites onto PNIPAm arm end can increase the T_g of star-PNIPAm. This is due to steric hindrance of β-CD. The influence of the presence of β-CD on T_g of a polymer can be seen in our previous works.[33,60] In summary, the results above exhibit that star-PNIPAm and star-PANIPAm-CD were synthesized successfully.

Thermal Sensitivity and Inclusion of Star-PNIPAm and Star-PNIPAm-CD. It is well-known that potential application of a polymer in biomaterials-related fields was generally related to aqueous media. Therefore, the properties of the star polymers in aqueous solutions were studied in detail by means of DSC, fluorescence spectrometry, and DLS.

A. DSC Measurement. The temperature sensitivity of aqueous star-PNIPAm and star-PNIPAm-CD solutions was first tested by DSC,[12,61,62] and the result is shown in Figure 5. As seen in Figure 5, on the DSC curves, there are evident endothermic peaks, which correspond to phase transition of the aqueous polymer solutions with an increase in temperature.[61,62] This means that star-PNIPAm and star-PNIPAm-CD could sense a change in temperature. However, the DSC peak of star-PNIPAm-CD shifted higher temperature region compared with that of star-PNIPAm. The LCST of star-PNIPAm and star-PNIPAm-CD were

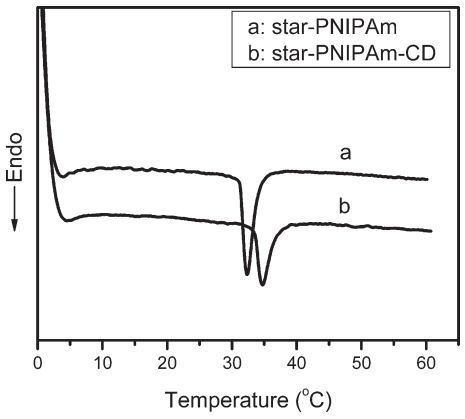


Figure 5. DSC thermograms of star-PNIPAm and star-PNIPA-CD in water (polymer concentration, 50 mg/mL).

31.2 and 32.6 °C, respectively. The corresponding ΔH values of the phase separations of the two star polymer solutions were in turn 36.7 and 30.8 J/g of polymer. This is attributed to the presence of hydrophilic β-CD on the end of PNIPAm arm, which can increase the LCST of star PNIPAm.[58]

B. Fluorescent Spectra. It is well-known that the fluorescent characters of ANS are largely affected by the polarity of the microenvironment, and variation of a microenvironment can thus cause apparent intensity and emission peak wavelength of ANS to change.[21−24,39] Therefore, we investigated inclusion behavior of star-PNIPAm and star-PNIPAm-CD in aqueous solution by using fluorescent probe ANS as a model guest molecules. Figure 6 indicates the fluorescence spectra of ANS (0.05 mM) in the presence of various concentrations of star-PNIPAm and star-PNIPAm-CD at 25 °C. As seen in Figure 6a, at star-PNIPAm contents of 0, 1, 2, 4, and 6 mg/mL, the emission peak wavelengths of ANS were 511, 468.2, 468, 464, and 464.2 nm, respectively, and the fluorescence intensity of ANS increased with an increase in star-PNIPAm concentration; e.g., the addition of 6 mg/mL star-PNIPAm could cause fluorescence intensity of ANS to increase about 8 times. The blue shift of emission peaks and the increase in intensity of ANS mean that the inclusion complex between star-PNIPAm and ANS took place.[21−23] This is because increasing amount of host molecules could cause more amount of inclusion complexes to be formed.[21−23,26,39] Although at this temperature PNIPAm chains are hydrophilic, the CD molecule attached by PNIPAm have a more hydrophobic microenvironment, which could include ANS molecules.[21]

In Figure 6b, we can observe that addition of star-PNIPAm-CD into aqueous ANS solution can still lead to blue shift the emission peaks and the increase in fluorescence intensity of ANS; e.g., the addition of 6 mg/mL star-PNIPAm-CD could change emission peaks of ANS from 511 to 471 nm. Compared with star-PNIPAm solutions with the same concentration, star-PNIPAm-CD could cause the fluorescence intensity of ANS to increase higher. This means that inclusion interaction between star-PNIPAm-CD and ANS seemed to be stronger. This ascribes to the presence of β-CD at star-PNIPAm-CD arm ends, which could include ANS molecules (see Scheme 2a). To confirm this, we added monomer GMA-EDA-β-CD to aqueous ANS solution, and it was found that GMA-EDA-β-CD could indeed lead to a blue shift of emission peaks and an increase in intensity of ANS. The emission peak of GMA-EDA-β-CD/ANS (molar ratio, 10:1) system was at 479 nm. This result suggests that β-CD on star-PNIPAm-CD arm end could include ANS. As we expected, it is very interesting that the inclusion behaviors of star-PNIPAm-CD

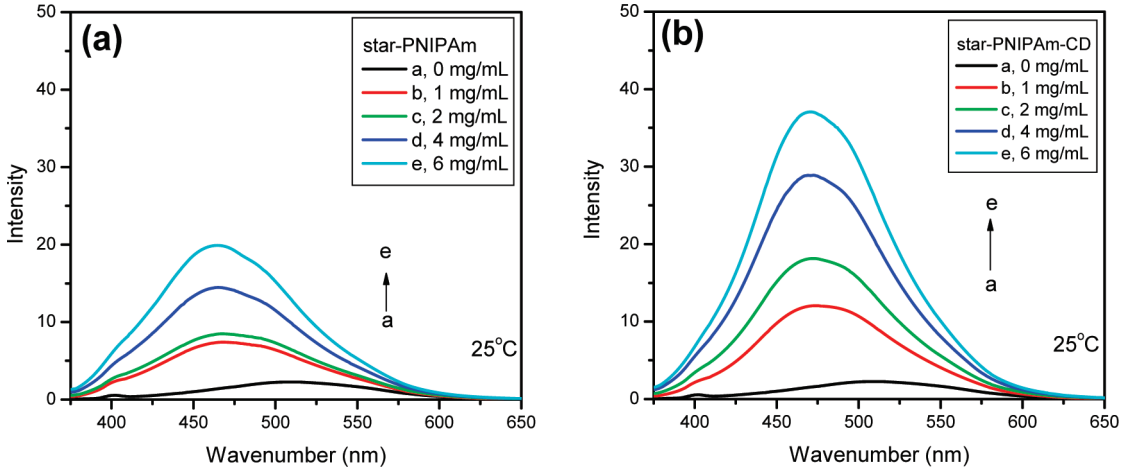


Figure 6. Fluorescence spectra of ANS (0.05 mM) in the presence of various concentrations of star-PNIPAm (a) and star-PNIPAm-CD (b) at 25 °C.

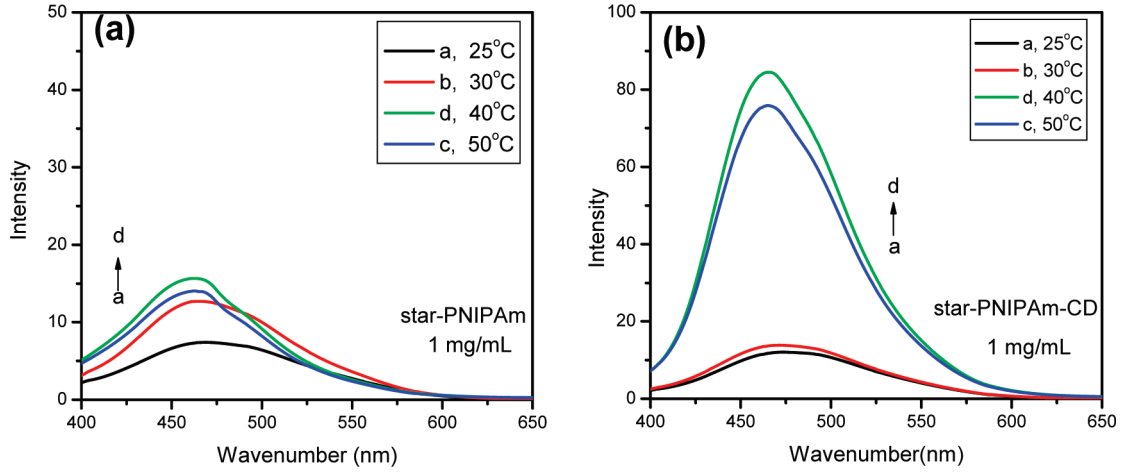
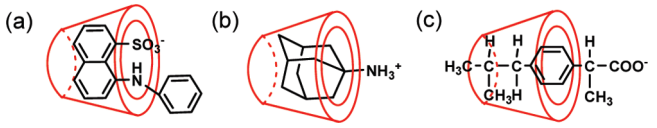


Figure 7. Influence of temperature variation on fluorescence spectra of ANS/star-PNIPAm system (a) and ANS/star-PNIPAm-CD system (b) ([ANS] = 0.05 mM; [polymer] = 1 mg/mL).

Scheme 2. Schematic Illustration of Inclusion Complex of β-CD Unit with ANS (a), ADA-NH_3^+ (b), and Ibuprofen Anion (c)[f]



[f] Association constants (M^{-1}): (a) 20 °C, 83 ± 10; 41 °C, 44 ± 10.[21] (b) 25 °C, 8.43×10^3.[65] (c) 25 °C, $(8.7 \pm 0.4) \times 10^3$; 40 °C, $(6.2 \pm 0.8) \times 10^3$.[66]

were from both β-CD core and end groups. Thus, a star-PNIPAm-CD molecule could be more sensitive to ANS.

Figure 7 shows influence of temperature variation on fluorescence spectra of ANS. In the measurement, four temperatures were studied, i.e., 25, 30, 40, and 50 °C. As seen in Figure 7a, the fluorescence intensity of the ANS/star-PNIPAm system increased with the increase in temperature ranging from 25 to 40 °C. However, at 50 °C the fluorescence intensity of polymer/ANS was lower than that at 40 °C but was higher than that at 30 °C. It may be attributed to temperature sensitivity of PNIPAm arms, which could induces the increase in fluorescence intensity.[21] Moreover, noteworthy is the fact that temperature variation could affect star-PNIPAm-CD/ANS system more dramatically (see Figure 7b). When temperature was elevated from 25 to 40 °C, the fluorescence intensity of star-PNIPAm-CD/ANS increased about 7 times. This means that the interaction of star-polymer-CD with ANS seems to be stronger at 40 °C (> LCST). This result suggests that temperature rise could evidently affect the interaction between star-PNIPAm-CD and ANS.

The above-mentioned results can be interpreted by thermal sensitivity of PNIPAm and inclusion character of CD. Below the LCST, PNIPAm is hydrophilic, whereas above the LCST, it becomes hydrophobic. Therefore, at 25 °C (< LCST), hydrophobic cavity of CD includes ANS molecules; above the LCST, hydrophobic PNIPAm could also include ANS.[24] Ohashi et al. investigated linear PNIPAm with β-CD side groups, and they concluded that at lower temperature ANS is in CD and at higher temperature (> LCST) ANS was in hydrophobic PNIPAm moieties.[24] Ohani also drew a similar conclusion.[21] Therefore, in Figure 7, at 40 °C the higher intensity of polymer/ANS may be due to the interaction of hydrophobic PNIPAm arms with ANS. However, for star-PNIPAm-CD, it may not simply be attributed to the interaction of hydrophobic PNIPAm chains with ANS above the LCST. It may be cooperativity of all factors such as the presence of β-CD end groups, hydrophobic PNIPAm, and the aggregate size of star-PNIPAm-CD.

C. Aggregation Behaviors of Star Polymers in Aqueous Solutions. Figure 8 shows temperature dependence of the D_zs of star-PNIPAm and star-PNIPAm-CD at a concentration of 1 mg/mL. At 25 °C, the D_zs of star-PNIPAm and star-PNIPAm-CD were 16.5 and 17.1 nm, respectively, indicating

that they dissolved in water molecularly. For star-PNIPAm, its D_zs showed less change at temperature ranging from 25 to 31 °C. At 33 °C, the D_z of star-PNIPAm increased to 1330 nm, but the value showed poor stability. This means that star-PNIPAm is easy to form larger size aggregate above its LCST, even precipitation. However, for star-PNIPAm-CD, it could form nanosized particles above the LCST. At 35 °C, its D_z increased to about 84 nm. Afterward, the D_z of star-PNIPAm-CD aggregates decreased somewhat with temperature rise, and the change in D_z showed good reversibility upon cooling. The result means that above the LCST star-PNIPAm-CD could form nanosized aggregates; below the LCST, it could molecularly dissolve in water again. The behavior of the star polymers can be interpreted by their molecular structures. For star-PNIPAm, there is strong a hydrophobic intermolecular interaction above the LCST, and this caused the formed particles not to be stabilized well. The phenomenon can be seen in micelles with PNIPAm shell layers.[63,64] For example, Chung et al. found that size of micelle formed by PNIPAm-$C_{18}H_{35}$ increased drastically above the LCST of the polymer.[63] However, the incorporation of CD onto PNIPAm arm ends could cause star-PINPAm-CD to self-assembly to form nanosized particles above the LCST. Obviously, the self-assembled nanoparticles could be stabilized by the hydrophilic CD groups. When PNIPAm become hydrophobic chains, the external hydrophilic groups can effectively prevent strongly hydrophobic aggregation of intermacromolecules, and as a result, a stable nanosized particle could be formed. This was confirmed by the zeta-potential of 16.4 mV for star-PANIPAm-CD aggregates at 35 °C (see Table 2). This indicates the presence of positive charges at the surfaces of the aggregates. The positive charge should be from the protonated amine groups of GMA-EDA-β-CD units in aqueous solution.[60] The result suggests that CD units should locate at the surface of the star-PNIPAm-CD aggregates.

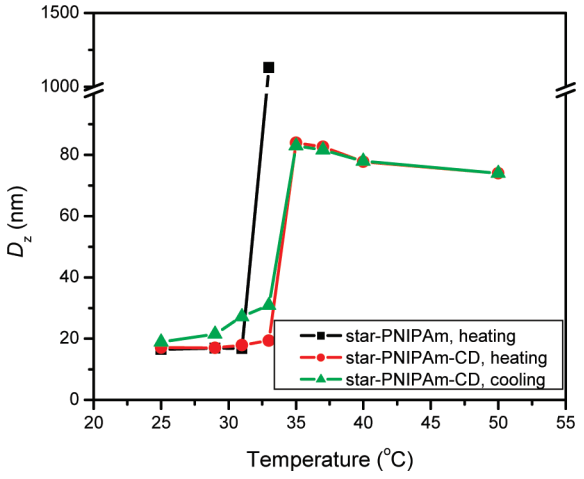


Figure 8. *Z*-average diameter (D_z) of star-PNIPAm and star-PNIPAm-CD in distilled water as a function of temperature (polymer concentration, 1 mg/mL).

D. Influence of Addition of Guest Molecules on Aggregation Behavior of Star Polymer in Aqueous Solutions. For a potential drug carrier, it is related to the interaction of guest molecules with polymer carrier in aqueous solution. Therefore, here using ANS, ADA-NH_3Cl, and ibuprofen-Na as guest molecules, we investigated influence of their addition on self-assembly aggregation behavior of the star polymers. A schematic illustration of the inclusion complex of β-CD unit with the guest molecules is shown in Scheme 2.

Because ANS can interact with both CD and hydrophobic PNIPAm chains,[21−24] its influence on aggregation of star polymers was first studied. Below the LCST of polymer, the presence of fluorescence molecule ANS could perturb D_z measurement of the star polymers by DLS. Therefore, Figure 9 shows D_z of star-PNIPAm and star-PNIPAm-CD above the LCSTs in the presence of ANS. However, the procedure of elevating temperature for the experiment was the same with that in distilled water (see Figure 8), namely to begin from 25 °C. As seen in Figure 9a, the addition of guest ANS molecules could exert an evident impact on D_z of star-PNIPAm above its LCST. At ANS concentration of 0.05, 0.1, and 0.2 mM, star-PNIPAm could form a nanosized particles; e.g., at 35 °C, the D_zs at three ANS concentrations were 191, 180, and 172 nm. The result suggests that the addition of guest molecules like ANS could change aggregation behavior of star-PNIPAm above its LCST. This should be related to the interaction of hydrophobic PNIPAm chains with ANS. As discussed in the Fluorescent Spectra section, above the LCST, ANS could be included in PNIPAm moieties due to their strong hydrophobic interaction.[21−24] The sulfonic acid group of ANS may stabilize the aggregates of star-PNIPAm. To confirm this, zeta-potentials of star-PNIPAm aggregates in the presence of ANS were determined. It was found that at ANS of 0, 0.05, 0.1, and 0.2 mM zeta-potentials of the self-assembled star-PNIPAm aggregates were −2.63, −17.1, −21.6, and −22.4 mV at 35 °C (see Table 2), respectively. This means that addition of ANS molecules could indeed increase charges of the surfaces of the star-PNIPAm aggregates. This again suggests that there indeed exist the interaction of PNIPAm with ANS in the star-PNIPAm/ANS system above the LCST. The conclusion is in agreement with that from fluorescence measurement.

However, we can see clearly from Figure 9b that the addition of ANS gave a contrary influence on aggregation behavior of star-PNIPAm-CD compared with that of star-PNIPAm. Increasing ANS concentration could evidently increase size of star-PNIPAm-CD aggregate. At ANS concentrations of 0, 0.05, 0.1, and 0.2 mM, the D_zs of the self-assembled aggregates of star-PNIPAm-CD at 35 °C were 83.9, 91.9, 109, and 192 nm, respectively. This can be interpreted by inclusion complex of CD attached on the PNIPAm arm ends with ANS. The formation of inclusion

Table 2. Zeta-Potentials (mV) of Samples Star-PNIPAm and Star-PNIPAm-CD at 35 °C[a]

solution	zeta-potential (mV)	
	star-PNIPAm	star-PNIPAm-CD
water	−2.63	16.4
ANS (0.2 mM)	−22.4	7.68
ANS (0.1 mM)	−21.6	8.85
ANS (0.05 mM)	−17.1	11.7
ANS (0.1 mM) + ADA-NH_3^+ (0.1 mM)	−17.2	10.4
ADA-NH_3^+ (0.2 mM)	7.0	14.2
ibuprofen-Na (2.06 mM)	−14.0	−11.9

[a] Polymer concentration, 1 mg/mL.

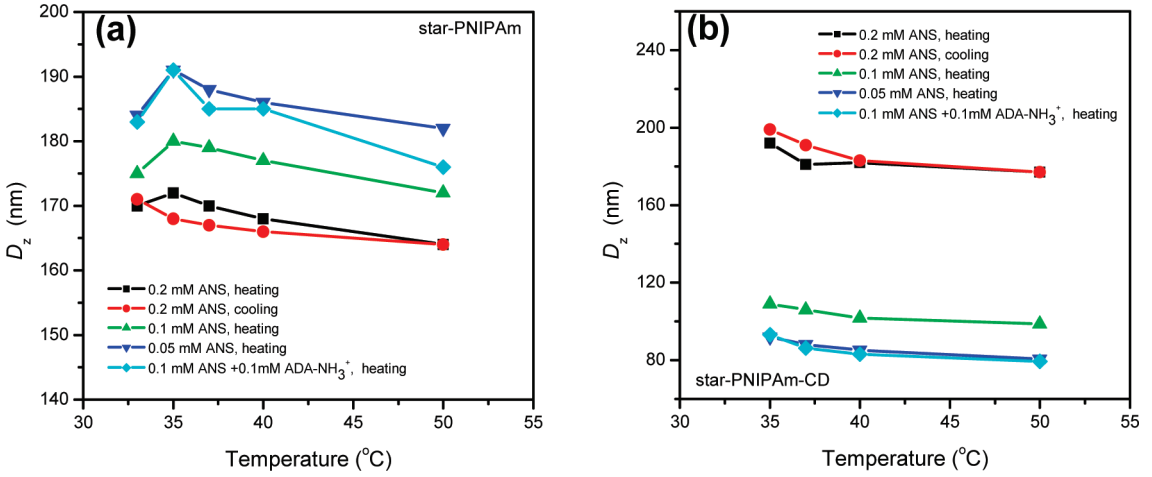


Figure 9. *Z*-average diameter (D_z) of star-PNIPAm (a) and star-PNIPAm-CD (b) in aqueous solution with guest molecules as a function of temperature (polymer concentration, 1 mg/mL).

complex between CD unit and ANS can increase hydrophobicity of CD end groups due to the protrusion of the ANS hydrophobic phenyl groups from the CD cavity (see Scheme 2a).[24,26] The phenomenon can be seen in linear PNIPAm with CD side groups.[24,26] Therefore, the formation of inclusion complex of CD with ANS increased hydrophobic intermolecular interaction of star-PNIPAm-CD and caused the aggregate to have larger size. So we can say that above polymer LCSTs, regardless of star-PNIPAm or star-PNIPAm-CD, the sizes of their aggregations can be modulated by variation of ANS concentrations. Also, as seen in Figure 9, the change in D_z of aggregates of the star polymers in aqueous ANS solution of 0.2 mM showed good reversibility upon cooling.

Figure 10 shows temperature dependence of the D_zs of star-PNIPAm and star-PNIPAm-CD in the presence of ADA-NH_3Cl, which is hydrophilic compound. The ADA groups can form inclusion complex with CD,[20,25,39,43,65] and the inclusion constant of the CD/ADA group is higher than that of the CD/ANS system (see Scheme 2).[21,39,65] Therefore, ADA-NH_3Cl was used as guest molecules in this study. When the addition of ADA-NH_3^+ (0.2 mM), above the LCST star-PNAIPAm did not form a stable nanosized aggregate like in aqueous ANS solution, whereas its aggregation behavior was similar to that in distilled water. This means the interaction of hydrophobic PNIPAm with ADA-NH_3^+ may be weak. As a result, the charge of ADA-NH_3^+ did not stabilize star-PNIPAm aggregate. However, after the addition of ADA-NH_3^+ into aqueous star-PNIPAm-CD solution, the D_z of star-PNIPAm-CD aggregates was similar to that in water. This is due to the formation of inclusion complex of ADA groups with CDs on the PNIPAm arm ends. The charge from of $-NH_3^+$ groups of the inclusion complex could stabilize star-PNIPAm-CD aggregates (see Scheme 2b). When we used the mixed guest molecules of ANS (0.1 mM) and ADA-NH_3^+ (0.1 mM), at 35 °C, the D_zs of star-PNIPAm and star-PNIPAm-CD aggregates were 191 and 93.1 nm, respectively. Comparison of the results from Figures 9 and 10 suggests that ANS of the mixed guest molecules played an important role in star-PNIPAm aggregation, whereas ADA-NH_3^+ worked in star-PNIPAm-CD aggregation because of its higher association constant with β-CD. This means that the star polymers could selectively interact with guest molecules. That is, self-assembly behaviors of the two star polymers possess molecular recognition capability.

Figure 11 shows the temperature dependence of the D_zs of star-PNIPAm and star-PNIPAm-CD in aqueous solution

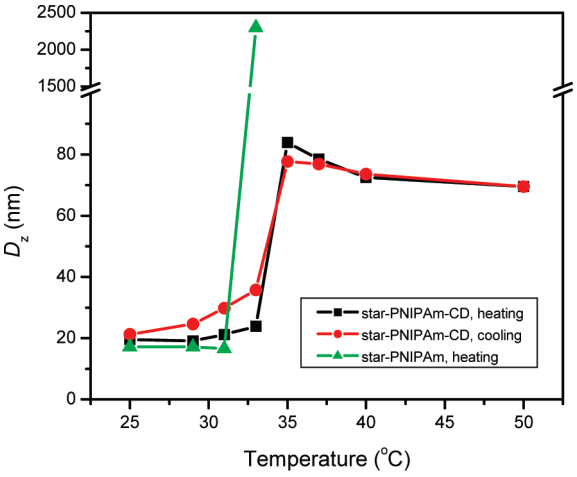


Figure 10. *Z*-average diameter (D_z) of star-PNIPAm and star-PNIPAm-CD in aqueous solution with ADA-NH_3^+ of 0.2 mM as a function of temperature (polymer concentration, 1 mg/mL).

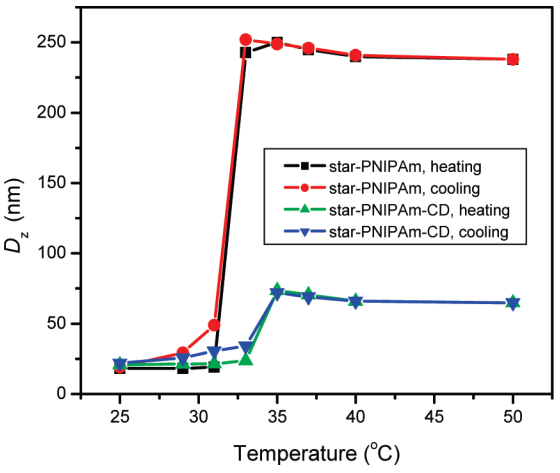


Figure 11. *Z*-average diameter (D_z) of star-PNIPAm and star-PNIPAm-CD in aqueous solution with ibuprofen sodium of 2.06 mM as a function of temperature (polymer concentration, 1 mg/mL).

with ibuprofen sodium salt. Ibuprofen sodium salt was prepared by the reaction of ibuprofen with sodium hydroxide. As seen in Figure 11, star-PNIPAm could form nanosized particles

with D_z of 250 nm at 35 °C (see heating curve). This means that there may be the interaction of hydrophobic groups of ibuprofen with hydrophobic PNIPAm chains. This was supported by zeta-potentials of −14.0 mV. Above the LCST the D_z of star-PNIPAm-CD aggregates in ibuprofen sodium solution were smaller than those in distilled water and aqueous ADA-NH_3^+ solution, but the change in its D_z with temperature is similar to those in the two solutions. This is because inclusion complex of CD with ibuprofen can lead to a protrusion of the ibuprofen hydrophilic $-COO^-$ groups from the CD cavity,[66,67] causing the PNIPAm arm ends carry negative charge (see Scheme 2c). This charge could stabilize the formed star-PNIPAm-CD nanosized particles. This was confirmed by the zeta-potentials of −11.9 mV (see Table 2). This means that CD end groups have ability of inclusion complex with guest molecules. In addition, as shown in Figure 11, the formation and dissociation of the nanosized aggregation of star-PNIPAm and star-PNIPAm-CD in aqueous solution of Ibuprofen sodium salt can change reversibly by changing temperature above and below the LCST.

The results above mean that aggregation of the star polymers in aqueous solution shows molecular recognition capability. For star-PNIPAm, its molecular recognition was mainly carried out by the interaction of PNIPAm arms with guest molecules, whereas for star-PNIPAm-CD, its molecular recognition was from the formation of inclusion complex of CD end groups with guest molecules.

Conclusion

Novel star-PNIPAm and star-PNIPAm-CD have been synthesized by core-first method successfully. In the synthesis, CD-core with 21 initiation sites could be synthesized by the reaction of CD with CPC. Star-PNIPAm could be synthesized by ATRP of NIPAm using 21Cl-β-CD as initiator. Star-PNIPAm-CD could be synthesized by ATRP of GMA-EDA-β-CD initiated via star-PNIPAm. The molecular weight distributions of the star polymers are narrow. By using ANS, ADA-NH_3Cl, and ibuprofen-Na as guest molecules, thermal sensitivity and inclusion behaviors of the star polymers were investigated by fluorescence spectrophotometer and DLS. It was found that the star polymers can combine both thermal sensitivity of PNIPAm and inclusion behavior of β-CD. Interestingly, the star polymers can self-assemble to form nanosized aggregation above LCSTs of the star polymers in aqueous solution. Below the LCSTs, the formed nanoparticles could molecularly dissolve in aqueous solution again.

The aggregation of the star polymers in aqueous solution shows molecular recognition capability. It is concluded that (1) the size of self-assembled star-PNIPAm aggregates depends on the interaction of PNIPAm arms with guest molecules above the LCST and (2) the size of self-assembled star-PNIPAm-CD aggregates depends on the property of inclusion complex of CD end groups with guest molecules. Therefore, the selection of appropriate guest molecule can modulate the aggregation behavior of the star polymer in aqueous solution. These are very useful for their application.

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