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Synthesis of poly(*N*-isopropylacrylamide) by ATRP using a fluorescein-based initiator

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Summary

A new fluorescent initiator fluorescein 2-bromoisobutyrate (Flu-Br) was synthesized. The use of Flu-Br as initiator, Me_6TREN as ligand and CuCl as catalyst by atom transfer radical polymerization allowed for chain-end fluoresceined poly(*N*-isopropylacrylamide) (Flu-PNIPAM) in one step. The polymerization reached high conversion (65 %) and low polydispersity (PDI) (1.15~1.28). The linearity plot of the $M_{n,\ GPC}$ and $M_{n,\ NMR}$ against conversion and the low PDI revealed the well-controlled polymerization by ATRP. In addition, *N*-isopropylacrylamide (NIPAM) was copolymerized with hydrophilic *N, N*-dimethylacrylamide (DMAM) using Flu-Br initiator by ATRP. By changing the feed ratio of NIPAM to DMAM, it was very easy to obtain thermo-sensitive fluorescent copolymers with proper lower critical solution temperature (36.0 ± 0.2°C, 38.0 ± 0.2°C). The pH dependence on fluorescence intensity of Flu-PNIPAM displayed a similar behavior to the parent fluorescein.

Introduction

Exploration of synthetic methods to obtain polymers with special ending functionality has been an active research area for many years. Post-polymerization modification of polymer chain ends was undertaken to form the reactive end site, requiring numerous reaction and purification steps, and the degree of substitution still need to be improved [1-2]. Recently, the development of the controlled/living free radical polymerization techniques, such as reversible addition-fragmentation chain transfer (RAFT) [3-4] polymerization and atom transfer radical polymerization (ATRP) [5-7], has made it easy to directly synthesize polymers with desired ending functionality and low polydispersity.

Conjugation of stimuli-responsive polymers to biomolecules yield "smart" bioconjugates that can respond to external stimuli, such as temperature, ionic strength and pH, and these bioconjugates can be used for biosensors, enzyme recovery, triggered drug release, and affinity separations [8-10]. Poly(*N*-isopropylacrylamide) (PNIPAM) exhibits a lower critical solution temperature (LCST~32°C) in water. A great deal of attention has been focused on the thermo-responsive property of

various copolymers consisting of *N*-isopropylacrylamide (NIPAM) from the view point of advanced materials for their extensive applications, such as a temperature-dependent controlled release system [11] and nanotechnology [12].

Fluorescein is widely employed as a platform for various florescence probes and fluorescence labels because of its high fluorescence quantum efficiency in aqueous media. And both its excitation wavelength and emission wavelength are in the range of visible region, which is beneficial for its detection. Since it was reported that fluorescein was relatively non-toxic [13], among the most versatile of chromophores, they were used as fluorescence probes in aqueous system of biological molecules. For example, fluorescein labeled DNA sequences and bovine serum albumin (BSA) have been used as fluorescent marker in biological chemistry field [14-15].

ATRP has been applied to a wide variety of functional monomers including water-insoluble monomers such as styrene, (meth)acrylates and water-soluble monomers such as *N,N*-dimethylacrylamide (DMAM), and *N*-isopropylacrylamide. In general, the living nature of polymerization affords well-defined macromolecular architectures; therefore, it is interesting to prepare poly(*N*-isopropylacrylamide) (PNIPAM) with specific structures and properties. As a multicomponent system, the reactive system of ATRP is mainly composed of the monomer, the initiator, the catalyst, and some additives. In general, initiator is considered as a very important component, because it determines the molecular weight of polymers and structures of end groups. On the basis of this opinion, some functional units can be located in the polymeric chain as end group. For example, Maynard's group has reported biotinylated PNIPAM synthesized by ATRP from biotinylated initiator [16]. Polymerization of NIPAM from the protein macroinitiators resulted in thermosensitive BSA-PNIPAM and lysozyme-PNIPAM was also demonstrated [17].

Recently, considerable effort has been devoted to the fluorescence technique because of its high sensitivity. The fluorescence technique has been widely used in the fields of biochemistry and polymer chemistry [17-18]. Kim *et al*. [19] synthesized anthracene labeled poly(methyl methacrylate) using 9,10-bis(chloromethyl)anthracene as an initiator, via ATRP. Cheng *et al*. [20] synthesized well defined naphthalene-labeled polystyrene, through ATRP. Pekcan's [21] group obtained pyrene-labeled polystyrene using 1-pyrenylmethyl 2-bromopropanoate as an initiator by ATRP. PNIPAM with pyrenyl group as an initiator by ATRP was also achieved [22].

In our case, we preferred fluorescent initiator that had a low toxicity and high fluorescence quantum efficiency in biological systems to minimize any problems that could arise during experiments. From this point of view, fluorescein and its derivatives are suitable labels. To the best of our knowledge, preparation of fluorescent polymers with fluorescein by ATRP has not yet been reported. Here, we synthesized functional fluorescein 2-bromoisobutyrate (Flu-Br) and using it as an initiator we prepared fluorescent PNIPAM by ATRP polymerization without post-polymerization modification. Fluorescent PNIPAM displayed a similar fluorescent behavior compared with the parent fluorescein (λ_{ex} = 490 nm, λ_{em} = 515 nm). These water soluble polymer precursors should have potential applications in the field of biotechnology.

Experimental section

Materials

N-isopropylacrylamide (NIPAM; Acros, 99 %) was recrystallized from mixtures of toluene and hexane (1/2 by volume). *N, N*-dimethylacrylamide (DMAM; Fluka Chemie; >98.0 %) was distilled before used. CuCl (Aldrich, 98 %) was purified by stirring in acetic acid, washed with methanol, and then dried in vacuum. Tris(2-aminoethyl)amine (Acros, 96 %) was used as received and Me_6TREN was prepared as described in the literature [23]. Fluorescein (Acros, 99 %) and 2-bromoisobutyryl bromide (Aldrich, 99 %) were used as received without further purification. All the other reagents were from Shanghai Chemical Reagent Factory and used as received without further purification.

Instruments

Molecular weight and polydispersity (PDI) data for the polymer samples were obtained with an Agilent 1100 gel permeation chromatograph with an Eclipse XDB-C18 column (4.6 mm × 150 mm), using PMMA as standards. The solvent used was THF at 20°C. The monomer conversion was determined by gravimetric measurement. All ^{1}H NMR spectra were recorded on a Varian Mercury VX-300 MHz spectrometer (USA) in DMSO-d_6.

Synthesis of the Flu-Br Initiator

The preparation of the Flu-Br initiator was carried out according to the procedure showed in Scheme 1. In a 250-mL three-necked, round-bottom flask, fluorescein (1.66g, 5 mmol) was dissolved in 50 mL of dry THF and 1 mL of EtN_3 with magnetic stirring. Then, 2-bromoisobutyryl bromide (0.76 mL, 6 mmol) was slowly dropped into the solution at 0°C in an ice-water bath. The reaction mixture was further stirred at room temperature overnight. Then the mixture was filtrated and the solvent was removed under reduced pressure. A light yellow crystalline product was obtained. The product was puried by column chromatography with $CHCl_3$ as the mobile phase. Yield = 72 %; ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 10.1 (1H, s, COOH), 6.58-8.10 (10H, m, ArH), 2.05 (6H, s, CH_3). ESI-MS (in CH_3CN): *m/z* (*RI*): 481 (MH^+, 100).

Preparation of poly(N-isopropylacrylamide) (Flu-PNIPAM)

Poly(*N*-isopropylacrylamide) (Flu-PNIPAM) was prepared by ATRP. Polymerization of NIPAM was polymerized with Me_6TREN as ligand as well as CuCl as catalysts and Flu-Br as initiator. Typically CuCl (9.99 mg, 0.1 mmol) and Flu-Br (48.3 mg, 0.1 mmol) were added to a round-bottom flask that was fitted with a rubber septum and pump-filled with nitrogen three times. The mixture was subsequently deoxygenated, and inhibitor-free NIPAM (2.26 g, 0.02 mol), DMSO and isopropanol (4 mL/4 mL), and ligand (23.5 mg, 0.1 mmol) were added to the round-bottom flask under nitrogen. The solution was further deoxygenated by three freeze-pump-thaw cycles before being kept in 20°C, and the solution was maintained at this temperature for 6 h. Samples were taken out periodically for conversion and GPC analysis via syringe. The polymer was purified by the passage of the solution over a basic alumina

column and was isolated by precipitation in cold diethyl ether and drying in vacuum. The conversion of monomer was determined by gravimetric method.

Preparation of poly(N-isopropylacrylamide)-co- poly(N,N-dimethylacrylamide) (Flu-PNIPAM-co-PDMAM)

Flu-PNIPAM-co-PDMAM was prepared by ATRP. Polymerization of Flu-PNIPAM-co-PDMAM was polymerized with Me_6TREN as ligand as well as CuCl as catalysts and Flu-Br as initiator. Typically CuCl (9.99 mg, 0. 1 mmol) and Flu-Br (48.3 mg, 0.1 mmol) were added to a round-bottom flask that was fitted with a rubber septum and pump-filled with nitrogen three times. The mixture was subsequently deoxygenated and inhibitor-free NIPAM (1.02 g, 9 mmol) and DMAM (0.99 g, 1 mmol), DMSO and isopropanol (3 mL/3 mL), and ligand (23.5 mg, 0.1 mmol) were added to the round-bottom flask under nitrogen. The solution was further deoxygenated by three freeze-pump-thaw cycles before at 20°C, and the solution was maintained at this temperature for 6 h. The following was the same as the above.

LCST Measurement

The LCST values of thermo-sensitive (co)polymers were estimated by cloud points (CP) measurements, which were done visually by following the variation of the turbidity of polymeric aqueous solutions with temperature. Polymeric aqueous solution (1.0 wt %) in a sample tube was immersed in a thermostated cell with a circulating water bath. The heating rate was regulated around 0.1°C/min and the cp was defined as the temperature at which the solution started to turn cloudy. The reproducibility of the determination was ± 0.2°C.

Fluorescence Spectroscopy

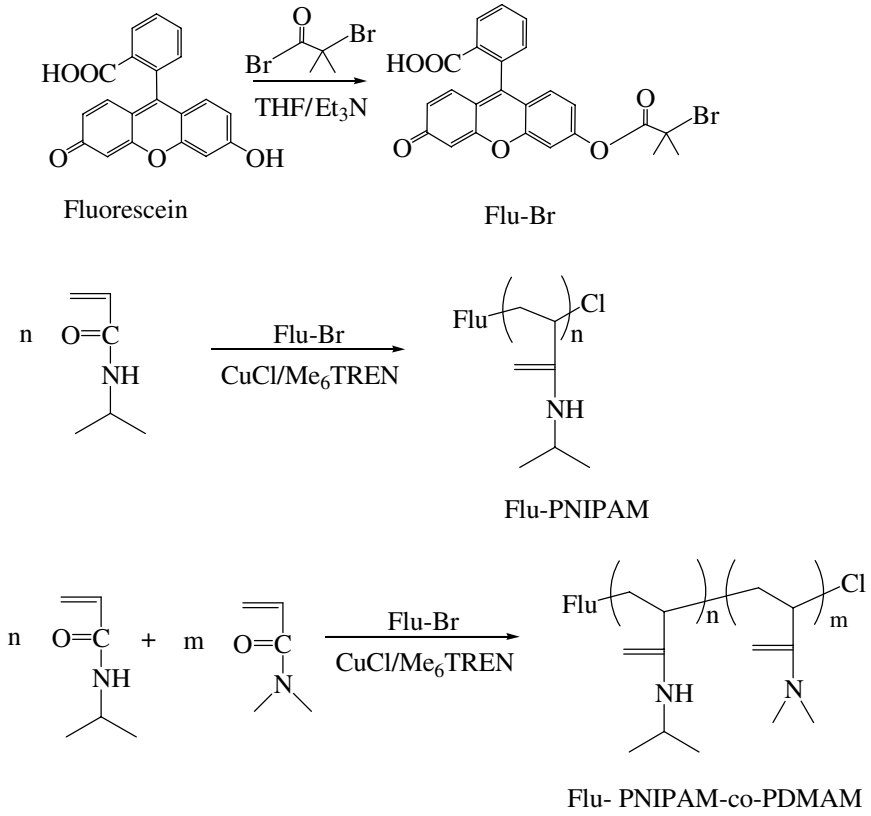
Fluorescence spectra were recorded in Shimadzu RF-5301PC spectrometer (Japan). A quartz cuvette with 3 cm path length was used. Fluorescence spectra of Flu-PNIPAM were obtained by recording the emission in aqueous solution with different pH at fixed excitation wavelength (λ_{ex} = 494 nm). During the measurement, a little concentrated HCl and NaOH were used to adjust pH to the desired value and samples were carried out at room temperature. The pH range of the sample was changed from 2 to 12 by a digital pH controller.

Results and discussion

ATRP of NIPAM using Flu-Br as initiator

The polymerization of NIPAM was undertaken in mixture solvent of isopropanol and DMSO to allow for solubilization of all components (Scheme 1). The mixture solvent was chosen as the solvent for ATRP on the basis that isopropanol was a hydrogen-bonding solvent which could bind to the amide groups of monomer and polymer, thus reduced their interaction with both catalyst and propagating chain end.

The polymerization solution was diluted with THF and filtered over alumina using $CHCl_3$ as eluent to remove the catalyst. The solvent was then evaporated under vacuum, and the polymer subjected to precipitation cycles into cold diethyl ether.



Scheme1. Synthetic pathway for the Flu-Br initiator and ATRP polymerization of Flu-PNIPAM and Flu- PNIPAM-co-PDMAM.

Conversions for polymer were determined gravimetrically. Polymer molecular weights (M_n) and polydispersity (PDI) were determined by a relative method to measure polymer molecular weight. Therefore, the M_n values determined by GPC in our work were only used to reveal trends. More precise molecular weights were determined using ^{1}H NMR spectroscopy. The number-average molecular weight (M_n) of the polymer was calculated by comparison of the signals from the fluorescein chain-end at 7.0 to 7.80 ppm and the polymer *iso*-propyl groups (C*H*Me$_2$) signal at δ 3.90 ppm.

Figure 1(a) showed the reaction conversion calculated from gravimetric analysis of Flu-PNIPAM as a function of time. Using Flu-Br as initiator, the polymerization of NIPAM gave a conversion of approximately 65 % in about 7 hours. The conversion increased with time, but it increased faster in the beginning 4 h. If the number of active sites is constant over time, the reaction kinetics in ATRP is expected to follow a first-order behavior [7].

In Figure 1(a), First-order kinetic plot of $\ln([M]_0/[M])$ against time showed a nonlinear curvature in the latter 3 h polymerization. The plot is not linear in time but exhibits a slight downward curved behavior, corresponding to a decrease in the

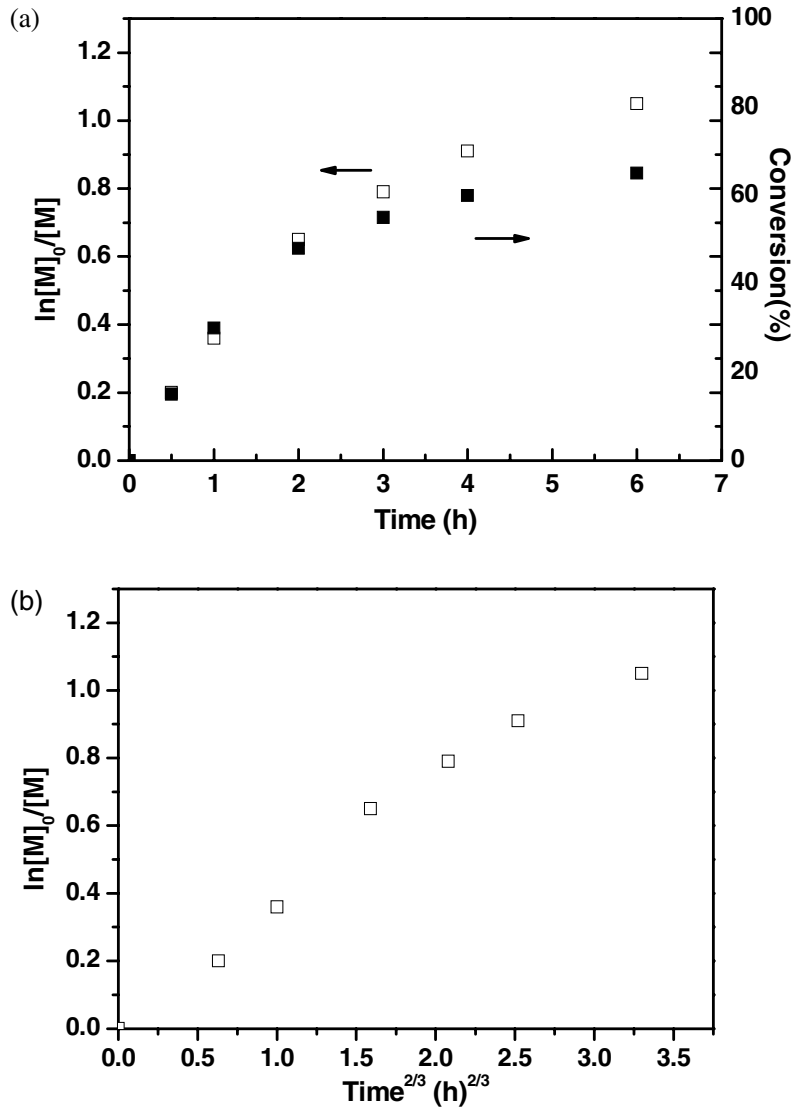


Figure 1. Kinetic plot of the reaction ($[NIPAM]_0$:$[I]_0$:$[CuCl]_0$:$[Me_6TREN]$=200:1:1:2) by ATRP in mixture solvent of isopropanol and DMSO at 20°C. (a) Monomer consumption $\ln([M]_0/[M])$ and conversion as a function of time, (b) Monomer consumption $\ln([M]_0/[M])$ as a function of $time^{2/3}$.

polymerization rate. These results were similar to the biotinylated poly(N-isopropylacrylamide) by ATRP [15]. Biesalski and his group had reported that $\ln([M]_0/[M])$ was not linear in *t*, but followed a $time^{2/3}$ dependency for NIPAM by ATRP using peptide-polymer nanotubes as initiator [24]. With respect to kinetics introduced by Fisher [25], the plot of $\ln([M]_0/[M])$ against $time^{2/3}$ was also used to analyze the kinetic plot. In Figure 1(b), the linear plot of $\ln([M]_0/[M])$ as a function of $time^{2/3}$ was observed.

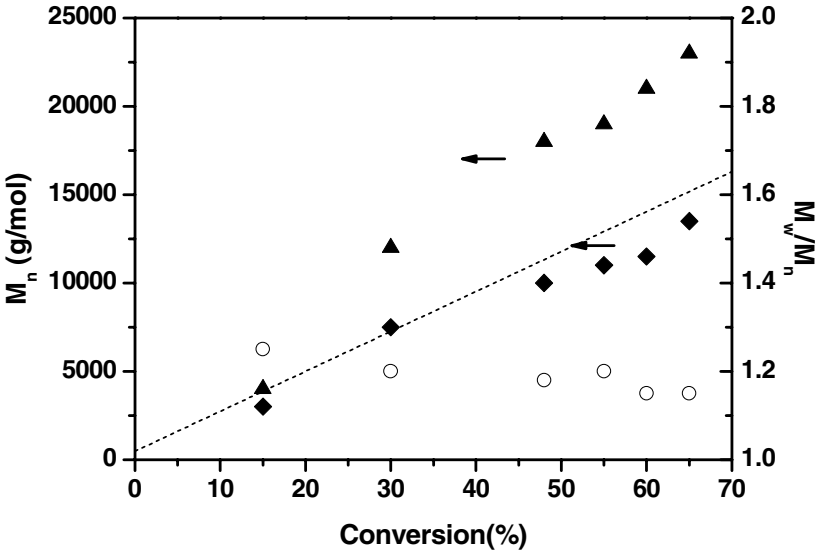


Figure 2. PNIPAM molecular weight and polydispersity as a function of conversion in NIPAM polymerization mediated with $[NIPAM]_0:[I]_0:[CuCl]_0:[Me_6TREN]=200:1:1:2$ in the mixture solvent of isopropanol and DMSO at 20°C, $M_{n,GPC}$ (▲), $M_{n,NMR}$ (◆), and PDI (○). The dotted line represents the theoretical molecular weight.

Figure 2 gave a linear increase of M_n with conversion and the polydispersity (PDI) remained low throughout the polymerization. Müller and his group [26] have reported that the apparent M_n values measured by GPC were about twice as high as expected. In our case, the M_n values of Flu-PNIPAM were assessed by GPC analysis of the polymers in THF against PMMA standards. As there is no salt added to the mobile phase to break H-bonds between polymers, the aggregation of Flu-PNIPAM molecules in THF solution may occur. This will cause an overestimation of the M_n values and an underestimation of the PDI. The M_n values of Flu-PNIPAM determined by NMR were slightly lower than the target molecular weight. It is due to catalyst

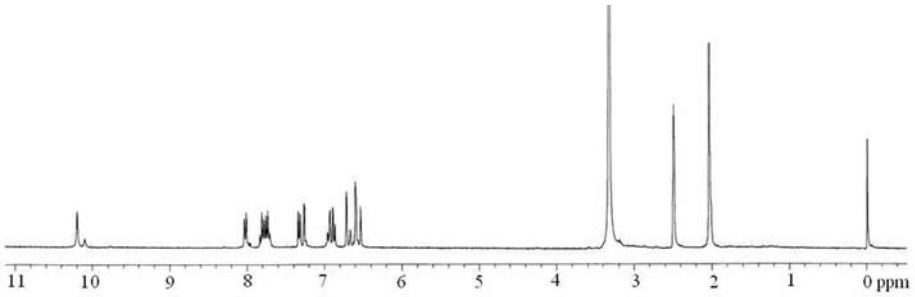


Figure 3. ^{1}H NMR spectrum of Flu-Br initiator in DMSO-d_6.

deactivation and/or termination reactions during the polymerization [7], and some error associated with the integration of the weak fluorescein signals in NMR. In Figure 2, the M_n values determined by either GPC ($M_{n,GPC}$) or NMR ($M_{n,NMR}$) increased in proportion to the ratio of monomer to initiator ([M] : [I]), and $M_{n,\ NMR}$ values were in reasonably agreement with theoretical values. The linearity of the $M_{n,GPC}$ and $M_{n,NMR}$ vs conversion plot and the low PDI reveals that the initiator is efficient and the polymerization is well-controlled.

The effect of copolymer chain architecture on LCST of Flu-PNIPAM-co-PDMAM

The LCST of such thermally sensitive polymers can be tuned to a desired temperature range by copolymerization with a more hydrophilic comonomer (which raises the LCST) or a more hydrophobic comonomer (which lowers the LCST) [27].

The copolymer chain architecture of PNIPAM affected the copolymer LCST. Wu and his group showed that the NIPAM-co-*N*-vinylpyrrolidone copolymer chains prepared by different copolymerization method exhibited different lower critical solution temperature (LCST) in water [28-29]. To NIPAM-co-*N*-vinylpyrrolidone, the LCST of random copolymer is higher than the block copolymer, and the LCST of the latter is close to PNIPAM. The studies of the effect of comonomer distribution on the coil-to-globule transition (folding) of individual copolymer chains were reported by Khokhlov's group and Timoshenko's group [30-31]. The folding of an AB copolymer chain with a segmented comonomer distribution was easier, and the resultant mesoglobular phase was more stable in comparison with a random copolymer chain under the same condition. The LCST of random copolymers was tuned easily than block copolymers.

In order to obtain fluorescent PNIPAM with higher LCST, NIPAM was copolymerized with hydrophilic DMAM using the same CuCl catalyst system and Flu-Br initiator by ATRP. The polymerization of NIPAM with DMAM by ATRP would lead to a random distribution [32] of DMAM on the PNIPAM chain. Flu-PNIPAM-co-PDMAM-I and Flu-PNIPAM-co-PDMAM-II were synthesized with different ratio of NIPAM to DMAM. The ^{1}H NMR spectra of Flu-PNIPAM and Flu-PNIPAM-co-PDMAM-II were shown in Figure 4. Seen in Figure 4(A), the signals from the *iso*-propyl groups (C*H*Me$_2$ at δ 3.90 and CH*Me*$_2$ at δ 1.0, signal d and signal e, respectively) and N*Me*$_2$ groups at δ 2.81- 2.92 (signal f) were observed. In addition, broad peaks at δ 1.48 and 1.92 (signal b and signal c) were observed for the methylene protons (CHC*H*$_2$) and the methine protons (CH$_2$C*H*CO) of both polymerized monomers. The broad peaks around 7.0-7.80 ppm (signal a) derived from the protons of fluorescein group in the polymer chain. The ^{1}H NMR spectra of the fluorescein end-capped copolymers proved the successful conjugation of NIPAM and DMAM moiety to the initiator. The feed monomer ratio of NIPAM to DMAM was 95:5 and 85:15, respectively. After polymerization, the molar ratio of NIPAM to DMAM in copolymer chain estimated by ^{1}H NMR spectra was 88:12 and 73:27, respectively. The ratio value of copolymer was approximately lower to the feed ratio of the two monomers. The two monomers exhibited different reactivity in the polymerization reactions. To the polymerization system of NIPAM and DMAM by ATRP, it indicates higher reaction activity of DMAM than NIPAM during the polymerization. The result was similar to the free radical polymerization of DMAM and NIPAM in THF [33].

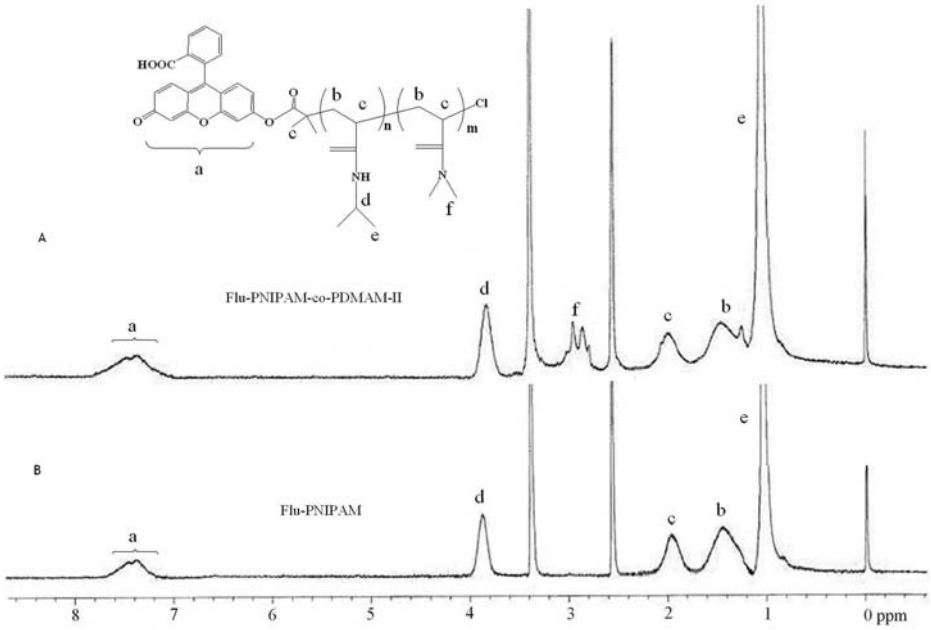


Figure 4. ^{1}H NMR spectra of Flu-PNIPAM and Flu-PNIPAM-co-PDMAM-II in DMSO-d_6.

Table 1. Copolymer composition, molar mass, polydispersity, LCST and conversion data for ATRP of NIPAM and DMAM using Flu-Br as initiator in the mixture of isopropanol and DMSO.

Entry	Comp in Feed[a]	Comp in Copolym[b]	GPC[c]	PDI[c]	LCST[d] (°C)	Con[e] %
Flu-PNIPAM	100:0	100:0	17,500	1.15	32.0 ± 0.1	65
Flu-PNIPAM-co-PDMAM-I	95:5	88:12	21,000	1.23	36.0 ± 0.2	68
Flu-PNIPAM-co-PDMAM-II	85:15	73:27	23,000	1.26	38.0 ± 0.2	72

[a] Feed molar ratio of NIPAM:DMAM
[b] Composition in copolymer estimated from ^{1}H NMR spectra based on the peak integral ratios of the methine protons of NIPAM segments [-C*H*Me$_2$, signal d, δ=3.90], the dimethyl protons of DMAM segments [-N*Me*$_2$, signal f, δ=2.81- 2.92]
[c] Determined by GPC
[d] Estimated by optical transmittance for three times, and the errors of the LCST values were determined by standard derivation
[e] Determined by gravimetric method

The chemical structures of DMAM and NIPAM differ just by one methane group (Scheme 1). PDMAM is more hydrophilic than PNIPAM and it does not present any LCST behavior in water. So the incorporation of DMAM raised the LCST of copolymer. Table 1 showed the LCST of Flu-PNIPAM, Flu-PNIPAM-co-PDMAM-I and II. The LCST values were determined by optical transmittance. When the feed ratio of NIPAM to DMAM changed from 100:0, 95:5 to 85:15, the LCST increased from 32.0 ± 0.1°C, 36.0 ± 0.2°C, to 38.0 ± 0.2°C. With the increase of DMAM/NIPAM ratio, the effect from hydrophilic DMAM chains became stronger and the coil-to-

globule transitions of these copolymer chains were more difficult. Thus Flu-PNIPAM-co-PDMAM polymers become more soluble in water and their LCST grow higher.

Fluorescent PNIPAM with proper LCST was synthesized by ATRP. This method provides an easy and effective polymerization method to obtain fluorescent and thermo-sensitive copolymer with good water-solubility.

Fluorescent properties of Flu-Br and Flu-PNIPAM

Fluorescence spectra of Flu-Br and Flu-PNIPAM in DMF were shown in Figure 5. The excitation at 494 nm and emission at about 519 nm were observed. A red shift and spectral broadening were observed for Flu-Br in DMF. The spectra revealed that Flu-PNIPAM displayed a similar fluorescent behavior, compared with the parent fluorescein (λ_{ex} = 490 nm, λ_{em} = 515 nm) in DMF. Fluorescent intensities of Flu-PNIPAM dissolved in different solvents were changeable while the fluorescent emission wavelengths showed no difference. It was generally observed that hydrogen bonding between one conjugates molecule and one non-conjugated molecule to an enhancement of the fluorescence yield [34]. The hydrogen power between water and Flu-PNIPAM was stronger than DMF and Flu-PNIPAM, so the fluorescent intensity of Flu-PNIPAM in water was higher than in DMF.

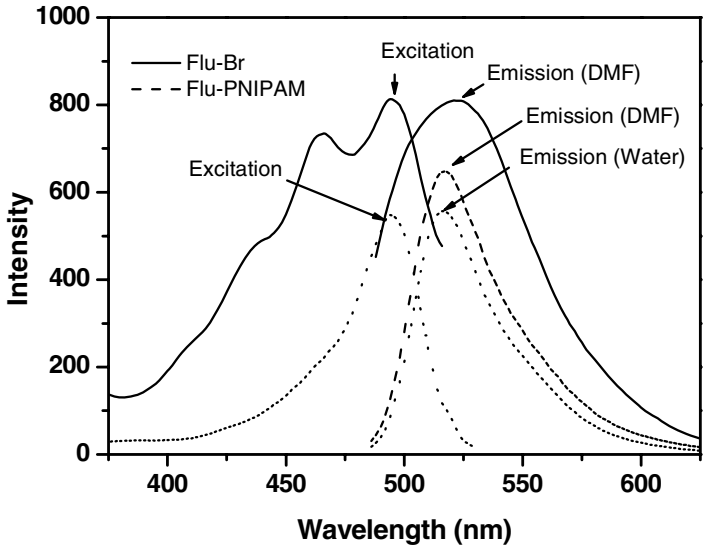


Figure 5. Fluorescence spectra of Flu-Br in DMF, Flu-PNIPAM in DMF and in aqueous solution (2.0 mM phosphate buffer, pH=7.2) with the equal absorbance at room temperature (λ_{ex} = 490 nm, λ_{em} = 515 nm).

pH dependence on the fluorescence intensity of Flu-PNIPAM

As showed in figure 6, the fluorescence intensity of Flu-PNIPAM increased with further basification when excitated at 494 nm. The polymer showed nearly complete loss of emission at acidic pH (< 5.5). The fluorescence intensity increased with the increase of pH (> 6). The fluorescence intensity rapidly increased when the pH

between 8.0 and 9.0. The results show that Flu-PNIPAM largely preserves the well known pH dependence on the fluorescence intensity of fluorescein [35] which is an advantage to probe pH.

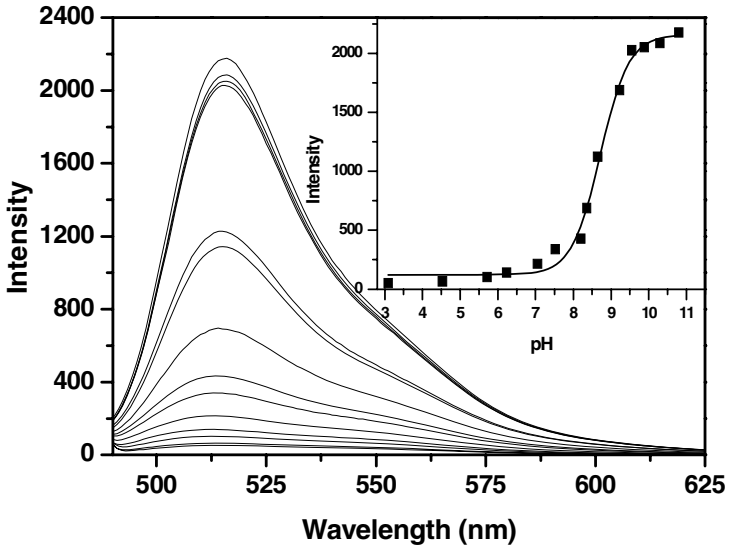


Figure 6. Fluorescence spectra for Flu-PNIPAM in aqueous solution at different pH (λ_{ex}=494 nm).

Conclusion

ATRP of NIPAM using new fluorescent Flu-Br initiator leaded to Flu-PNIPAM with good molecular weight control and low polydispersity. NIPAM was also copolymerized with hydrophilic DMAM with different feed ratio using Flu-Br initiator by ATRP. The structure of Flu-PNIPAM-co-PDMAM was confirmed by ^{1}H NMR. When the feed ratio of NIPAM to DMAM varied from 100:0, 95:5 to 85:15, the copolymer ratio of NIPAM to DMAM changed from 100:0, 88:12 to 73:27 and the LCST increased from 32.0 ± 0.1°C, 36.0 ± 0.2°C, to 38.0 ± 0.2°C. Fluorescent measurements analysis confirmed that Flu-PNIPAM displayed a pH dependence on fluorescence intensity which was similar to the parent fluorescein. These exploitations represented a significant step toward the facile synthesis of fluorescent water-soluble polymer materials with thermo-sensitivity and pH-sensitivity.

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References

1. Velonia K, Rowan AE, Nolte RJ (2002) Am Chem Soc 124:4224
2. Ding Z, Fong RB, Long CJ, Stayton PS, Hoffman AS (2001) Nature 411:59
3. Chiefari J, Chong YK, Ercole F, Krstina J, Jeffery J, Le TP, Mayadunne RT, Meijs GF, Moad CL, Moad G, Rizzardo E, Thang SH (1998) Macromolecules 31:5559

4. Chiefari J, Mayadunne RT, Moad CL, Moad G, Rizzardo E, Postma A, Skidmore, MA, Thang, SH (2003) Macromolecules 36:2273
5. Wang JS, Matyjaszewski K (1995) J Am Chem Soc 117:5614
6. Kamigaito M, Ando T, Sawamoto M (2001) Chem Rev 101:3689
7. Matyjaszewski K, Xia J (2001) Chem Rev 101:292
8. Hoffman AS, Stayton PS (2004) Macromol Symp 207:139
9. Stayton PS, Shimoboji T, Long C, Chilkoti A, Chen GH, Harris JM, Hoffman AS (1995) Nature 378:472
10. Ding Z, Fong RB, Long CJ, Stayton PS, Hoffman AS (2001) Nature 411:59
11. Chilkoti A, Dreher MR, Meyer DE, Raucher D (2002) Adv Drug Delivery Rev 54:613
12. Zhu MQ, Wang LQ, Exarhos GJ, Li AD (2004) J Am Chem Soc 126:2656
13. DiLoreto Jr DA, Das T, del Cerro C, Cox C, del Cerro M (1997) Curr Eye Res 16:1159.
14. Wang S, Gaylord BS, Bazan GC (2003) J Am Chem Soc 126:5446
15. Wischke C, Borchert HH (2006) Pharmazie 61:770
16. Bontempo D, Li RC, Ly T, Brubaker CE, Maynard HD (2005) Chem Commun 37:4702
17. Disney M, Zheng J, Swager T, Seeberger P (2004) J Am Chem Soc 126:13343
18. Morawetz H, Allen, NS, Rabek JF, In New Trends in the Photochemistry of Polymers; Elsevier: London, 1985
19. Kim CS, Oh SM, Kim S, Cho CG (1998) Macromol Rapid Commun 19:91
20. Cheng ZP, Zhu XL, Zhu J, Lu JM, Yu JH (2005), J Macromol Sci Pure Appl Chem 42:34
21. Erdogan M, Hepuzer Y, Cianga I, Yagci Y, Pekcan O (2003) J Phys Chem 107:8363
22. Duan Q, Miura Y, Narumi A, Shen X, Sato S, Satoh T, Kakuchi T (2006) J Polym Sci Part- Polym Chem 44:1117
23. Ciampolini M, Nardi N (1966) Inorg Chem 5:41
24. Couet J, Biesalski M (2006) Macromolecules 39:7258
25. Fischer H (2001) Chem Rev 101:3581
26. Schilli C, Lanzendörfer MG, Müller AH (2002) Macromolecules 35:6819
27. Feil H, Bae YH, Feijen J, Kim SW (1993) Macromolecules 26:2496
28. Siu MH, Zhang G, Wu C (2002) Macromolecules 35:2723
29. Zhang G, Wu C, Jiang M (2005) Polym Bull 4:82
30. Khokhlov AR, Khalatur PG (1999) Phys Rev Lett 82:3456
31. Timoshenko EG, Kuznetsov YA (2000) Chem Phys 112:8163
32. Lee SB, Russell AJ, Matyjaszewski K (2003) Biomacromolecules 4:1386
33. Liu SQ, Tong YW, Yang Y (2005) Biomaterials 26:5064
34. Guan X, Liu X, Su Z, Liu P (2006) React & Func Polym 66:1227
35. Martin MM, Lindqvist L (1975), J Lumin 10:381