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Synthesis and characterization of Eu(III) complexes of modified cellulose and poly(*N*-isopropylacrylamide)

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ABSTRACT

A series of thermo-responsive copolymers of poly(*N*-isopropylacrylamide) (PNIPAM) and cellulose were synthesized via atom transfer radical polymerization (ATRP) using *N*-isopropylacrylamide as the monomer, cellulose acetate as the initiator, and CuCl/tris(2-dimethylaminoethyl)amine (Me_6TREN) as a catalytic system. The resulting polymers had a narrow range of polydispersity indexes 1.27–1.37, and molecular weights of 8600–17,300 g mol^{-1}. Novel functional complexes of cellulose-g-PNIPAM/Eu(III) with excellent thermosensitive and fluorescent properties were then formed by the chelation of copolymers and europium(III) ions. The maximum emission intensity of the complexes at 613 nm was enhanced by a factor of approximately 10 relative to that of the corresponding Eu(III) complexes. Additionally, the lower critical solution temperatures (LCSTs) of cellulose-g-PNIPAM/Eu(III) were slightly greater than those of the copolymers.

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1. Introduction

Fluorescent molecules are important in intracellular sensing and imaging. Among these molecules, lanthanide complexes are often used as probes and labels for the direct determination of organic analytes and nucleic acids in immunodiagnostic assays (Huhtinen et al., 2005; Zheng et al., 2002). For example, europium(III) has been used extensively as a probe due to the well-documented sensitivity of its fluorescence (Lujan-upton, Okamoto, & Walser, 1997; Zhen & Liu, 2012). However, the cytotoxicity, chemical perturbation effects, water dispersibility, cell permeability and signal stability of the europium(III) complexes often interfere with cellular processes.

Cellulose, a naturally occurring polysaccharide, has received a great deal of attention in the past several decades, due to its applications in biology (Brown & Laborie, 2007; Tabuchi, Kobayashi, Fujimoto, & Baba, 2005; Wu & Lia, 2008; Yu & Zhou, 2007), medicine (Bodin, Backdahl, & Risberg, 2007; Gisela et al., 2006; Millon and Wan, 2006; Millon, Guhados, & Wan, 2008; Schumann et al., 2008), paper manufacturing (Jung et al., 2008; Li, Xiu, Wang, & Shanxi, 2007; Shah & Brown, 2005; Song, Zhang, & Guo, 2004), industrial purification processes (Krystynowicz, Bielecki, Czaja, & Rzyska, 2000; Suetsugu, Oshima, Ohe, & Baba, 2007; Tabuchi & Baba, 2005; Xu & Sun, 2008) and the food industry (Fu & Chi, 2008; Lin & Lin, 2004; Xue, Yang, & Li, 2004; Zhou, Dong, & Jiang, 2003). However, the key drawback of cellulose is its lack of solubility. Grafting is an effective way to improve such properties as solubility, chelation and biocompatibility. The use of a "living" polymerization technique had led to better control of the formation of grafted copolymers with well-defined structures, thus providing information on the structure–property relationships. For example, methyl methacrylate (Carlmark & Malmstrom, 2002) and 2-(dimethylamino)ethyl methacrylate (Sui et al., 2008) have been successfully grafted to cellulose particles by atom transfer radical polymerization (ATRP). The resulting copolymers could form stable micelles in aqueous solution and exhibited good environmental responses.

Poly(*N*-isopropylacrylamide) (PNIPAM) is a well-known thermoresponsive polymer which that can changes its appearance from a clear solution to a turbid suspension in water at a relatively lower

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critical solution temperature (LCST) of 32 °C (near that of the human body) (Gil & Hudson, 2004; Schild, 1992). Investigations on the phase transition of PNIPAM have revealed that its macromolecules experience dehydration, collapsing from a hydrated, extended coil to a hydrophobic globule and raising the temperature above the cloud point, which ultimately results in intermolecular aggregation (Yamazaki, Song, Winnik, & Brash, 1998). Functional PNIPAMs have been synthesized and combined with various hydrophobic polymer blocks, such as azobenzene (Tao & Qian, 2011), chitosan (Bao et al., 2010), and β-cyclodextrin (Gao, 2011) by different methods, including microfluidic emulsification (Yu & Chu, 2012).

In this study, a series of well-defined thermoresponsive copolymers containing PNIPAM and cellulose were synthesized by ATRP. These copolymers had a low polydispersity index and could chelate with europium. The cellulose-g-PNIPAM/Eu(III) complexes had important thermoresponsive and fluorescence properties. Our research is expected to provide a new fluorescent probe for use in the biomedical field.

2. Materials and instrumentation

N-isopropylacrylamide (Aldrich, 98%) was recrystallized twice from a hexane/benzene mixture (3/2, v/v). Tris(2-(dimethylamino)ethyl)amine (Me_6TREN) was synthesized from tris(2-amino) ethyl amine (TREN, Aldrich, 99%) according to the literature (Ciampolini & Nardi, 1966). CuCl (Aldrich, 99%) was washed successively with acetic acid and ether and then dried and stored under nitrogen. 2-Chloropropionyl chloride (Acros, 97%) and cellulose were obtained commercially and were used as received unless otherwise stated.

The ^{1}H nuclear magnetic resonance (NMR) spectra of monomers and polymers in $CDCl_3$ were obtained on a Varian Unity 400 NMR spectrometer. The molecular weights (M_n) and polydispersity (M_w/M_n) were measured by a gel permeation chromatograph (GPC) using a Waters 510 pump and a Model 410 differential refractometer at 25 °C. THF was used as a mobile phase at a flow rate of 1.0 ml min^{-1}. The LCSTs of the polymer solutions were determined by turbidimetry, using Shimadzu-1240 UV–Vis spectrophotometer with a heating rate of 0.1 °C min^{-1}. FT-IR spectra were recorded on a Shimadzu IR-8400S spectrometer. A Shimadzu RF-5301PC fluorescence spectrophotometer was used to obtain fluorescence spectra. The XPS spectra (Mg Kα) were recorded with a VG Scientific ESCALAB instrument.

2.1. General procedure for cellulose-g-PNIPAM synthesis

Cellulose-g-PNIPAM was synthesized as follows (Scheme 1). A mixture of CuCl and Me_6TREN in 1:1 (v/v) DMF/H_2O (1.0 ml) was placed on one side of an H-shaped ampoule glass and stirred at room temperature. NIPAM and initiator (cellulose-Cl, which was synthesized by using 4-dimethylaminopyridine as a catalyst, M_n = 1600 g mol^{-1}, PDI = 1.24) in DMF (1.5 ml) were placed on the other side of the ampoule. Nitrogen was bubbled through both mixtures for 5 min to remove any oxygen. Three freeze–pump–thaw cycles were performed to degas the solution. Both mixtures were placed in an oil bath and thermostated at 80 °C for several hours. The polymerization was terminated by exposing the mixture to air. The reaction mixture was diluted with DMF and purified using a neutral Al_2O_3 column. Next, the solvent was evaporated, and the remainder was dialyzed in DMF using a cellophane tube (Spectra/Por6, Membrane). Finally, the solvent was evaporated and a white product was collected by filtration and dried in a vacuum oven overnight.

Table 1
Polymerization of cellulose-g-PNIPAM.

Sample	Time (h)	Conv. (%)[a]	M_{nGPC} [b]	PDI_{GPC} [b]
P1	24	50.6	8600	1.34
P2	30	59.8	9500	1.29
P3	36	65.4	10,700	1.27
P4	42	71.3	12,100	1.31
P5	48	78.6	17,300	1.37

[a] Determined by gravimetric measurement.
[b] Determined by GPC using polystyrene standards.

2.2. Synthesis of cellulose-g-PNIPAM/Eu(III) complexes

A solution of $EuCl_3$ and PNIPAM (W_{Eu}^{3+} : W_{PNIPAM} = 0.08 : 1) in ethanol was added to a flask. The mixture was stirred with a magnetic stirring bar for 24 h. The product was purified and then dried under vacuum at room temperature, yielding the cellulose-g-PNIPAM/Eu(III) complexes.

3. Results and discussion

3.1. Synthesis and characterization

The data in Table 1 showed that all the samples had narrow molecular weight distributions in the range of 1.27–1.37. Using an NIPAM/initiator/CuCl/Me_6TREN feed ratio of 100/1/1.2/1.2, we could achieve different conversion rates and products with different M_n values.

Fig. 1 shows the ^{1}HNMR spectra of cellulose acetate and the product. In Fig. 1A, the peaks located at 4.47 ppm and 1.67 ppm corresponded to the protons next to the secondary and primary carbons of the chloride group, respectively. The signal at 6.4 ppm in Fig. 1B was attributed to the protons adjacent to nitrogen atoms of NIPAM group, and the signals at 4.0 and 1.23 ppm were characteristic of the isopropyl.

The IR spectrum of cellulose revealed the characteristic hydroxyl absorption band 3000–3450 cm^{-1}, as shown in Fig. 2a. The intensity of this band decreased significantly after esterification (Fig. 2b),

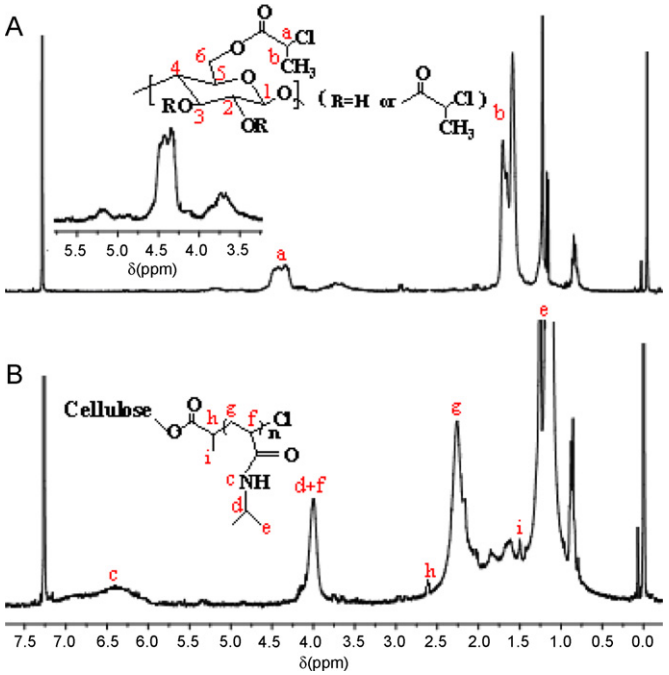
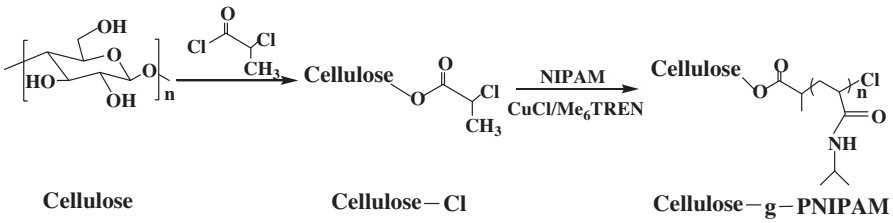


Fig. 1. ^{1}HNMR spectra in $CDCl_3$ of the cellulose acetate (A) and cellulose-g-PNIPAM (B) polymers.



Scheme 1. Cellulose-g-PNIPAM synthesis by ATRP.

the peaks at 1750 cm^{-1} and 1256 cm^{-1} correspond to ester group absorption. The band at 3446 cm^{-1} in Fig. 2c was assigned to the stretching vibration (ν_{N-H}) of the acylamino group. The band at 1653 cm^{-1} was ascribed to amide I [mainly the carbonyl stretching vibration ($\nu_{C=O}$)] and the band at 1558 cm^{-1} was ascribed to amide II [mainly the N—H bending vibration (δ_{N-H})]. In Fig. 2d, the stretching vibration (ν_{N-H}) of the acylamino group was shifted to 3436 cm^{-1}. The bands of amide I and amide II were shifted to 1629 cm^{-1} and 1544 cm^{-1}, respectively. The redshift of the above-mentioned characteristic bands was attributed to the bonding of Eu(III) to the O and N atoms of the acylamino group. The electron density and vibrational frequency of N—H and C=O decreased (Cai, Chen, Ji, Huang, & Shen, 2003).

3.2. Thermoresponsivity and fluorescence characterization

The excitation and emission spectra of the $EuCl_3$ and cellulose-g-PNIPAM/Eu(III) complexes are shown in Fig. 3. The spectrum obtained under excitation at 613 nm is shown in Fig. 3A. $EuCl_3$ showed negligible ultraviolet absorption. Cellulose-g-PNIPAM/Eu(III) complexes exhibited a different spectral shape, and the absorption peak intensity increased sharply to 355 nm. This phenomena was attributed to the π–π^* transition by exciting

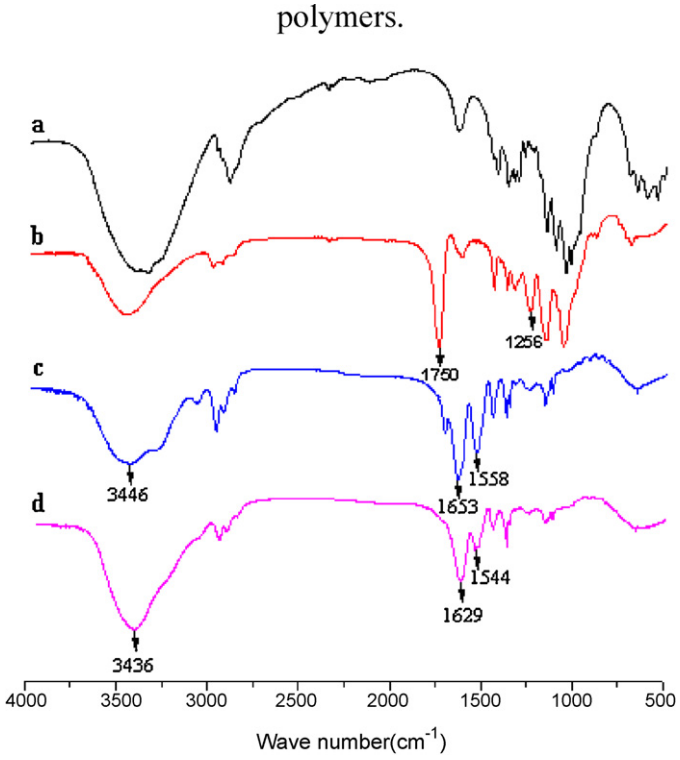


Fig. 2. FT-IR spectra for (a) cellulose, (b) cellulose acetate, (c) cellulose-g-PNIPAM and (d) cellulose-g-PNIPAM/Eu(III) complexes.

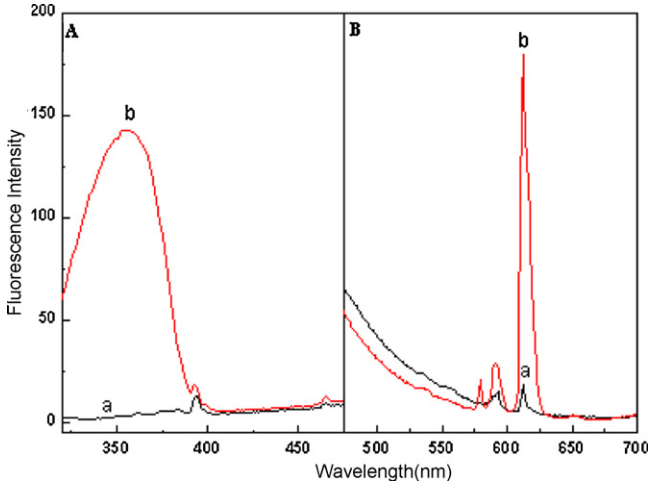


Fig. 3. Excitation (A) and emission (B) spectra of (a) $EuCl_3$ and (b) cellulose-g-PNIPAM/Eu(III) (Ex/Em slit: 5/5 nm).

the carbonyl and acylamino group of cellulose-g-PNIPAM/Eu(III). In Fig. 3B, curve (a) exhibited very weak emission peaks characteristic of Eu(III). Curve (b) displayed four strong, narrow emission peaks at 579, 591, 613 and 650 nm, corresponding to the $^5D_0 \rightarrow {}^7F_J$, ($J$ = 0, 1, 2, 3) electronic transitions, respectively, which occurred from the excited state D to the multiplet F. The most pronounced peak was situated at 613 nm ($^5D_0 \rightarrow {}^7F_2$). Owing to the shielding of the 4f orbital from the environment by an outer shell of 5s and 5p orbitals, the f–f absorption bands were very narrow (Li, Li, & Wu, 2001). The maximum emission intensity of the complexes at 613 nm was enhanced by a factor of approximately 10 relative to that of the corresponding Eu(III) complex. Meanwhile, the Eu(III) coordination number has been achieved due to the introduction of PNIPAM. Fluorescence decay was not observed over 6 months of observation.

The XPS spectra of the cellulose-g-PNIPAM/Eu(III) and copolymer are shown in Fig. 4. The average binding energies of O_{1s}, N_{1s} and Eu_{4d} are listed in Table 2. The binding energy of C_{1s} at 284.6 eV was used as the reference. Table 2 shows that the average binding energy of O_{1s} and N_{1s} in the Eu(III) complexes increased by 1.15 eV and 0.37 eV, respectively, relative to that of cellulose-g-PNIPAM, which indicated a decrease in the electron density of O_{1s} and N_{1s} atoms in the Eu(III) complexes. Meanwhile, the average binding energy of Eu_{4d} decreased by 5.09 eV, indicating an increase in the electron density of Eu(III) in the cellulose-g-PNIPAM/Eu(III)

Table 2
Binding energy of O_{1s}, N_{1s} and Eu $_{4d}$ for $EuCl_3$, cellulose-g-PNIPAM and cellulose-g-PNIPAM-Eu(III) complexes.

Compound	O_{1s} (eV)	N_{1s} (eV)	Eu_{4d} (eV)
Cellulose-g-PNIPAM	531.09	399.06	
Cellulose-g-PNIPAM/Eu(III)	532.24	399.43	136.87
$EuCl_3$			141.96

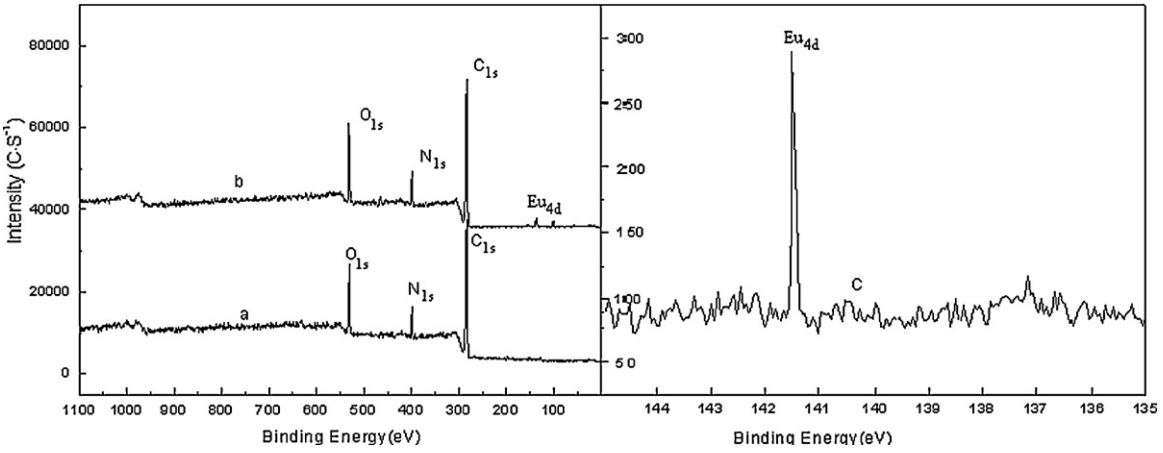


Fig. 4. XPS spectra of the (a) cellulose-g-PNIPAM and (b) cellulose-g-PNIPAM/Eu(III) complexes and (c) $EuCl_3$.

Table 3
LCSTs of cellulose-g-PNIPAM copolymers and cellulose-g-PNIPAM/Eu(III) complexes.

Copolymers	LCST (°C)	Complexes	LCST (°C)
P1	30.8	P1–Eu(III)	30.9
P2	31.1	P2–Eu(III)	31.3
P3	31.9	P3–Eu(III)	32.1
P4	32.2	P4–Eu(III)	32.7
P5	32.9	P5–Eu(III)	33.3

complexes. Based on these results, the Eu(III) complexes were formed by the coordination among Eu(III) and the O and N atoms of the acylamino group. (Drolet, Manuta, Lees, Katnani, & Dand Coyle, 1988). This coordination shifted the electron density from the oxygen and nitrogen of the acylamino groups to the outer orbitals of europium(III), increasing the outer layer charge density and the shielding effect, which in turn decreased the internal electron binding energy. Additionally, the decrease in the electronic cloud density of the nitrogen atom after coordination was caused by the inductive effect.

LCST is a basic physical property of thermoresponsive water-soluble polymers. For linear PNIPAM homopolymers, Stover and co-workers (Xia, Burke, & Stover, 2006) had recently elucidated the effects of end-group hydrophobicity and molecular weight on LCST. In the current case, the copolymers and complexes possessed the same sequences of NIPAM, but the latter possessed Eu^{3+} coordinated with PNIPAM. Thus, this system was suitable for the investigation of the Eu^{3+} end group on the thermal phase transition behavior of PNIPAM. The LCSTs of the P1, P2, P3, P4 and P5 copolymers and their europium(III) ions complexes are shown in Table 3, The LCSTs of the cellulose-g-PNIPAM/Eu(III) complexes were slightly higher than those of cellulose-g-PNIPAM. This phenomenon might be due to Eu^{3+} in complexes coordinating with water molecules in solution, as the coordination bond energy was greater than that of water molecule hydrogen bonds. When the temperature of the solution was near the LCST, more energy was needed to destruct the coordination bonds between Eu^{3+} and the water molecules, therefore, a higher temperature was required for the phase transition. These findings also proved the formation of complexes between Eu^{3+} and PNIPAM.

4. Conclusion

In this paper, narrow-disperse PNIPAM copolymers with cellulose were successfully prepared by ATRP. Cellulose-g-PNIPAM/Eu(III) complexes were formed by the interaction between PNIPAM and Eu(III) ions. The coordination among the oxygen and nitrogen of the acylamino group and Eu^{3+} provided the complexes with the intensive characteristic fluorescence of Eu(III), the maximum emission intensity of cellulose-g-PNIPAM/Eu(III) at 613 nm was greater than that of the corresponding Eu(III). The LCSTs of the complexes were higher than those of the corresponding cellulose-g-PNIPAM. The complex might be able to be used to probe functional polymers, and broaden the application of temperature-sensitive PNIPAM.

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