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

Guihua Cui[a,b], Yanhui Li[a], Tiantian Shi[c], Zhengguo Gao[d], Nannan Qiu[a], Toshifumi Satoh[e], Toyoji Kakuchi[e], Qian Duan[a,*]

[a] Department of Materials Science and Engineering, Changchun University of Science and Technology, Changchun, Jilin 130022, China
[b] Department of Chemistry, Jilin Medical College, Jilin 132013, China
[c] Department of Chemistry, No. 1 Middle School Jining, Jining, Shandong 272000, China
[d] Chemical and Engineering College, Yantai University, Yantai, Shandong 264005, China
[e] Division of Biotechnology and Macromolecular Chemistry, Graduate School of Engineering, Hokkaido University Sapporo 060-8628, Japan

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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 ($Me_6$TREN) 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.



## 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

* Corresponding author. Tel.: +86 431 85583105; fax: +86 431 85583015.
*E-mail address:* duanqian88@hotmail.com (Q. Duan).

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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 ($Me_6$TREN) 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 $Me_6$TREN 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/$Me_6$TREN 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),

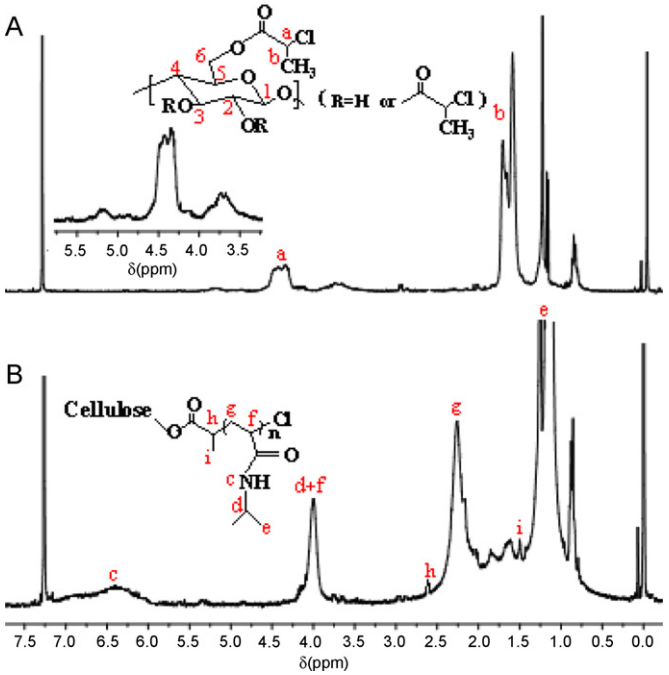


**Fig. 1.** $^1$HNMR spectra in $CDCl_3$ of the cellulose acetate (A) and cellulose-g-PNIPAM (B) polymers.