Available online at www.sciencedirect.com
ScienceDirect
Carbohydrate Research 341 (2006) 2414–2419
Carbohydrate
RESEARCH

Phase-transition and aggregation characteristics of a thermoresponsive dextran derivative in aqueous solutions

Huan-Ying Shi and Li-Ming Zhang*

Laboratory for Polymer Composite and Functional Materials, Institute of Optoelectronic and Functional Composite Materials, School of Chemistry and Chemical Engineering, Sun Yat-Sen (Zhongshan) University, Guangzhou 510275, China

Received 3 February 2006; received in revised form 18 June 2006; accepted 22 June 2006
Available online 18 July 2006

Abstract—Grafting of poly(*N*-vinylcaprolactam) side chains onto a hydrophilic dextran backbone was found to provide the dextran with new, thermoresponsive properties in aqueous solutions. Depending on its solution concentration, the resulting dextran derivative could exhibit a temperature-induced phase-transition and critical transition temperature (T_c). Different anions and cations of added salts, including five potassium salts and five alkali-metal chlorides, were observed to influence the T_c value of its aqueous solution. Except for potassium iodide, all added salts were found to lower the T_c value. The addition of the surfactant, cationic cetyltrimethylammonium bromide or anionic sodium dodecyl sulfate, resulted in an increase of the T_c value. With the help of the Coomassie Brilliant Blue dye as a polarity probe, the formation of hydrophobic aggregates above the T_c was revealed for this new dextran derivative in aqueous solution.

© 2006 Elsevier Ltd. All rights reserved.

Keywords: Dextran; Thermoresponsive behavior; Poly(*N*-vinylcaprolactam); Critical transition temperature; Hydrophobic aggregates

1. Introduction

Dextran is a water-soluble glucose polymer consisting predominantly of a linear α-(1→6)-glucosidic linkage with some degree of branching via a (1→3)-linkage, which could be produced by bacteria from sucrose or by chemical synthesis.[1–3] Due to its biocompatibility and biodegradability, this polysaccharide and its derivatives have been widely used and investigated for various biomedical applications, especially their uses for the delivery of drugs, proteins, enzymes, and imaging agents.[4]

In recent years, water-soluble polymers showing a thermoresponsive property have attracted much attention due to their potential applications in biomedicine and biotechnology.[5] Once a critical temperature is surpassed in such polymer solutions, spontaneous phase separation or precipitation of the polymer will take place. At the molecular level, this phenomenon corresponds to a transition from the well-dissolved coil to the less-soluble globular state of the macromolecule.[6] It is well known that thermally induced phase separations of such polymer solutions are very attractive because they require no additives and are usually reversible, especially for a temperature-responsive drug release system.[7,8] Among various temperature-sensitive polymers reported in the literature, poly(*N*-vinylcaprolactam) (PNVCL), which shows a critical transition temperature (T_c) in water around 32 °C, is especially interesting due to the fact that it is nontoxic, stable, and biocompatible.[9]

In the present work, a dextran derivative was prepared for the first time by grafting PNVCL chains onto a dextran backbone. It is expected that such a chemical modification could provide a dextran with new, temperature-responsive properties in aqueous solutions. Described herein are its phase-transition and aggregation characteristics in aqueous media.

---

* Corresponding author. Tel./fax: +86 20 84112354; e-mail: ceszhlm@mail.sysu.edu.cn

0008-6215/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2006.06.015

2. Results and discussion

Figure 1 gives the optical transmittances as a function of temperature for aqueous solutions of pure dextran and its derivative with the same concentration of 0.87 g/L. As shown, the aqueous solution of pure dextran is transparent in the whole temperature range studied due to its high hydrophilicity, and its optical transmittance was found to be fully independent of temperature. For the dextran derivative, however, its aqueous solution showed a temperature-dependent transmittance change due to the introduction of thermosensitive PNVCL graft chains, and the critical transition temperature (T_c) value was determined to be 31.9 °C. Moreover, the solution concentration of the dextran derivative was found to influence the optical transmittance and phase-transition temperature, as shown in Figure 2. For example, the T_c value changed from 31.9 to 35.4 °C when the solution concentration decreased from 0.87 to 0.10 g/L, and a dilute concentration of 0.01 g/L is too low to induce the transmittance change.

For 0.50 g/L aqueous solutions of the dextran derivative, the effects of various salts and their concentrations on the T_c were investigated. Figures 3 and 4 demonstrate how the T_c value changes as a function of different salts, including five potassium salts and five alkali-metal chlorides, and their concentrations. In all cases except for potassium iodide, the T_c was lowered by the addition of the salt and was shown to decrease almost linearly with increasing salt concentration ('salt out' effect). The shift in the phase-transition temperature depends strongly on the counterions used, and significant differences were obtained. For the potassium salts investigated, the extent of lowering the T_c was found to be in the following order: $K_2SO_4 > KCl > KNO_3 > KBr$. For alkali-metal chlorides studied, the extent of lowering the T_c was found to be in the following order: $KCl > NaCl > BaCl_2 > CaCl_2 > LiCl$. These results may be explained by the difference in the salting-out capacity of these added salts. According to Freitag et al.,[10] the phase separation of the thermoresponsive polymer solutions stems from two effects. One is the disruption of hydrogen bonds, which form at lower temperature between groups of the polymer chain and the water molecules, and the other is a tendency to reduce the volume of the layer with a deformed water structure that forms around the more hydrophobic groups of the polymer units. Therefore, the effects of added salts on the T_c may be considered to result mainly from the influence of a particular salt on the water structure.

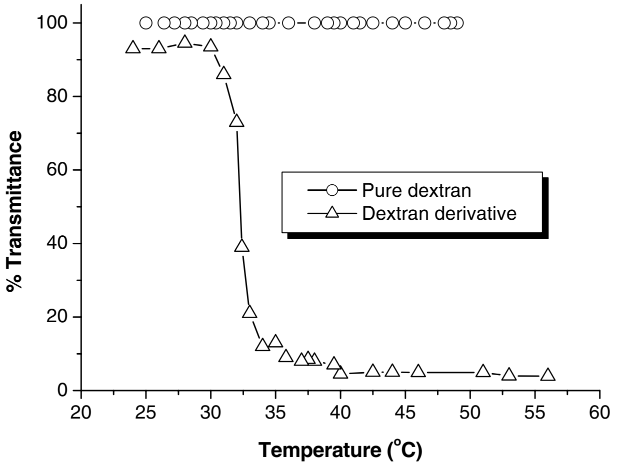

Figure 1. The optical transmittances as a function of temperature for aqueous solutions of pure dextran and its derivative with the same concentration of 0.87 g/L.

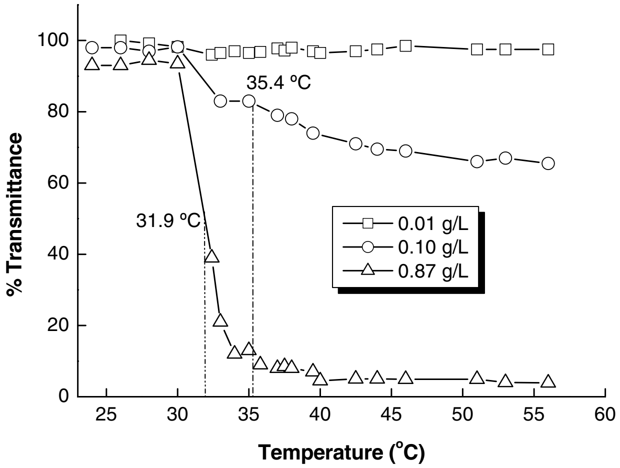

Figure 2. The influence of the solution concentration of the dextran derivative on the optical transmittance.

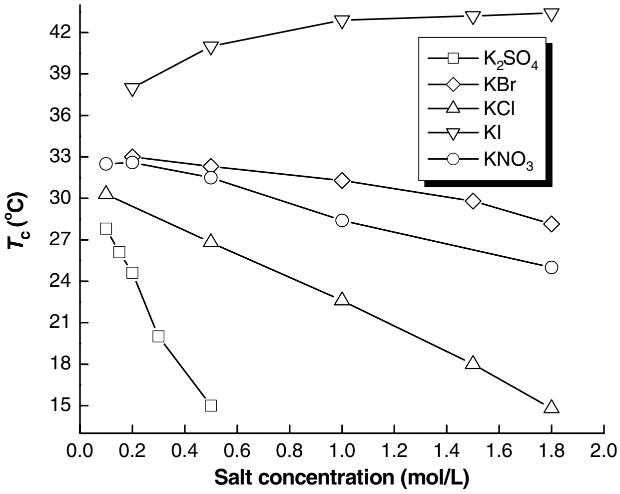

Figure 3. The changes of T_c value with the concentrations of different potassium salts (dextran concentration, 0.50 g/L).

Figure 5 shows the influences of two ionic surfactants, cationic cetyltrimethylammonium bromide (CTAB) and anionic sodium dodecyl sulfate (SDS), on the T_c for

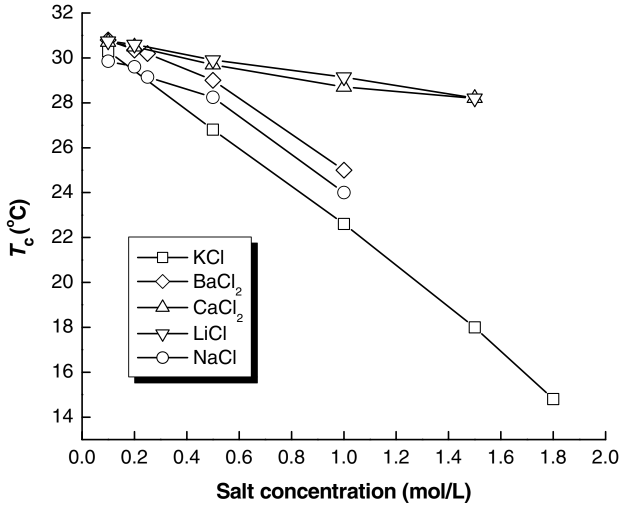

Figure 4. The changes of T_c value with the concentrations of different alkali-metal chlorides (dextran concentration, 0.50 g/L).

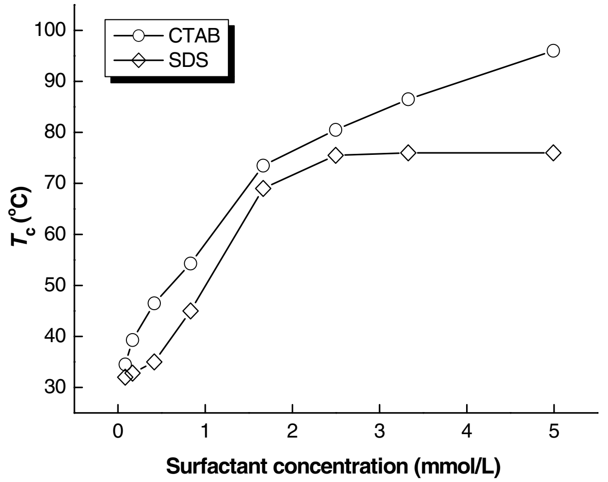

Figure 5. The effects of added surfactants on the T_c for aqueous solution of the dextran derivative (dextran derivative, 0.50 g/L).

0.50 g/L aqueous solutions of the dextran derivative. It was found that the addition of CTAB or SDS resulted in an increase of the T_c. Moreover, the T_c increases with the increase of CTAB or SDS concentration. In contrast, cationic CTAB increases the T_c more obviously than anionic SDS at the same concentration. Similar results were also observed for other temperature-responsive polymer solutions.[11–13] A possible explanation is that the presence of ionic surfactants prevent the temperature-induced coil–globule transition and macromolecular aggregation.[12,13]

The Coomassie Brilliant Blue (CBB) method was used to investigate the aggregation behavior of the thermosensitive dextran derivative in aqueous solution as a function of temperature. It is known that CBB is highly soluble in water and possesses a marked hydrophobic character due to the presence of six aromatic rings.[14,15] Moreover, its maximum absorption could be shifted toward higher wavelength when its microenvironment changes from polar media to apolar media.[14,15]

Figure 6 shows the absorption spectra of CBB in the dextran derivative solution with the concentration of 0.50 g/L under various temperatures. As seen, the maximum absorption peak of CBB in the solution of the dextran derivative is different below and above the T_c of the dextran derivative. Below the T_c, corresponding to 26.0 and 32.0 °C, the maximum absorption peaks of CBB appeared at almost the same wavelength of 598 nm. However, the maximum absorption peak of CBB was shifted toward higher wavelengths (616 nm) when the temperature was above the T_c, corresponding to 37.0, 40.0, or 49.0 °C. Moreover, the absorption intensity at 616 nm increased with an increase in temperature. These results indicate that the environment of CBB becomes apolar.

For a comparison study, the absorption spectra of CBB in water as well as the adsorption spectra of the dextran derivative solution with the concentration of 0.50 g/L were also measured at 26.0 and 49 °C, as shown in Figure 7. In the wavelength range studied, there is no maximum absorption peak for an aqueous solution of the dextran derivative. In the case of CBB in pure water, its wavelength of maximum absorption was found to be 588 nm, which is close to the value (584 nm) reported for the CBB in polar media,[16] and shown to be independent of the temperature investigated. Figure 8 shows the absorption spectra of CBB in the dextran solution with the concentration of 0.50 g/L under various temperatures. As seen, the wavelength corresponding to the maximum absorption peak of CBB in the dextran solution is also 588 nm, regardless of the T_c. In contrast, the

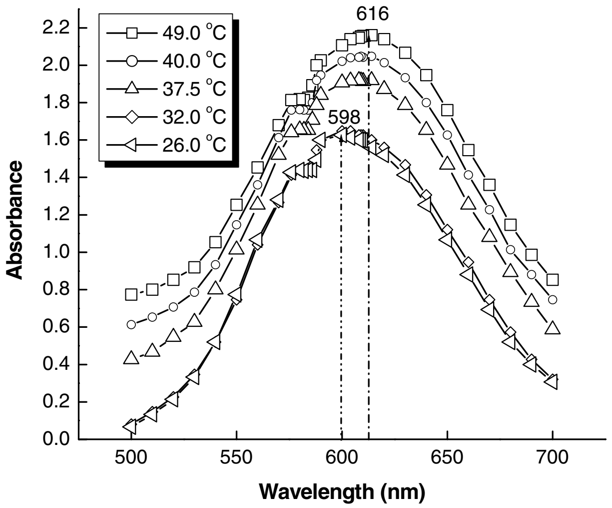

Figure 6. The absorption spectra of CBB in the dextran derivative solution under various temperatures (dextran derivative, 0.50 g/L; CBB, 0.047 g/L).

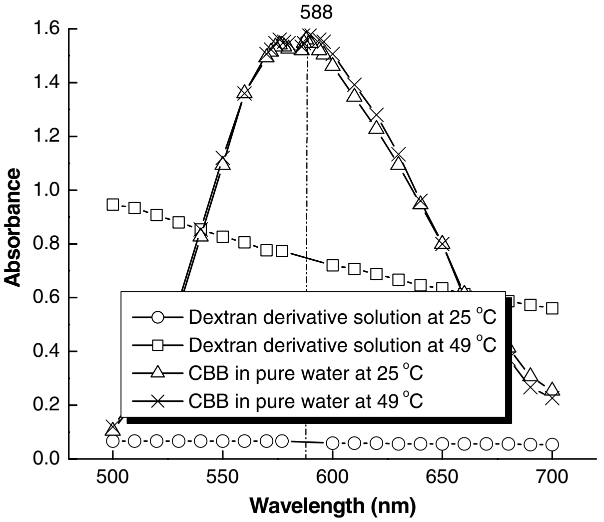

Figure 7. The absorption spectra of CBB in water and aqueous solution of the dextran derivative at 26.0 and 49 °C (dextran derivative, 0.50 g/L; CBB, 0.047 g/L).

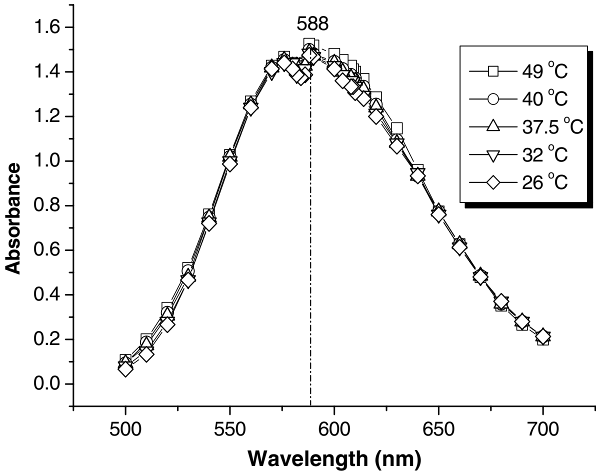

Figure 8. The absorption spectra of CBB in the dextran solution under various temperatures (dextran, 0.50 g/L; CBB, 0.047 g/L).

maximum absorption of CBB in the dextran derivative solution appeared at higher wavelength (Fig. 6), demonstrating that there is a microenvironment polarity that is different from that of water. In particular, the wavelength of 616 nm, corresponding to the maximum absorption of CBB in the dextran derivative solution above the T_c, was found to be very close to the value (618 nm) reported for the CBB in apolar media.[16] These facts confirmed the formation of hydrophobic aggregates in aqueous solution of the thermoresponsive dextran derivative when the temperature was raised above the T_c.

In conclusion, the dextran derivative incorporating poly(*N*-vinylcaprolactam) graft chains could exhibit a temperature-induced phase-transition and critical transition temperature in aqueous solutions, and its T_c value may be modulated by changing the concentration of the derivative solution. The presence of some co-solutes such as added salts (K_2SO_4, KCl, KNO_3, KBr, NaCl, $BaCl_2$, $CaCl_2$, LiCl) and surfactants (CTAB, SDS) affected the temperature-induced phase-transition behavior of this dextran derivative in aqueous media. By measuring the absorption spectra of CBB as a polarity probe in the dextran derivative solution as a function of temperature, the formation of hydrophobic aggregates above the T_c was revealed.

3. Materials and methods

3.1. Materials

Dextran was purchased from ACROS, and its molecular weight was determined to be 6.3×10^{-4} g/mol by a static light-scattering method. The monomer, *N*-vinylcaprolactam (NVCL) was purchased from Aldrich Chemical Co., and used as received. The free-radical initiator, 2,2′-azodiisobutynitrile (AIBN), was recrystallized from MeOH three times and dried in vacuum at room temperature. Other reagents and solvents were obtained from commercial sources and used without further purification.

3.2. Chemical modification of dextran

In a three-necked flask equipped with a reflux condenser and a magnetic stirrer, 0.5 g of the dextran was dissolved in a mixed solvent system composed of 5 mL of dimethyl sulfoxide (DMSO) and 5 mL of deionized water. The flask was purged with nitrogen for 30 min, and the temperature was adjusted to 75 °C with a water bath. Then, 0.005 g of AIBN was added. After stirring for 10 min, 1.0 g of NVCL was added to the flask. The reaction was continued for 6 h under the nitrogen atmosphere. The reaction mixture was subsequently precipitated in 90 mL of EtOH and filtered. In order to remove the homopolymer (PNVCL) of NVCL, the resulting white precipitate was further dissolved in water, and the purification process was repeated twice. The product was then dried at 50 °C for 12 h. The IR spectra of pure dextran, PNVCL(homopolymer), and the dextran derivative, measured on a Nicolet Nexus 670 Fourier-transform infrared (FTIR) instrument and shown in Figure 9, confirmed the existence of the modification reaction. As seen, the spectrum of the graft copolymer shows not only the characteristic stretching vibrations of the hydroxyl groups from the dextran at 3413 and 1016 cm^{-1}, but also the characteristic absorption bands of the amide I band (1625 cm^{-1}) and C–N stretching (1480 cm^{-1}) from PNVCL. Although the spectrum of pure dextran also has an absorption band at 1625 cm^{-1}, its intensity

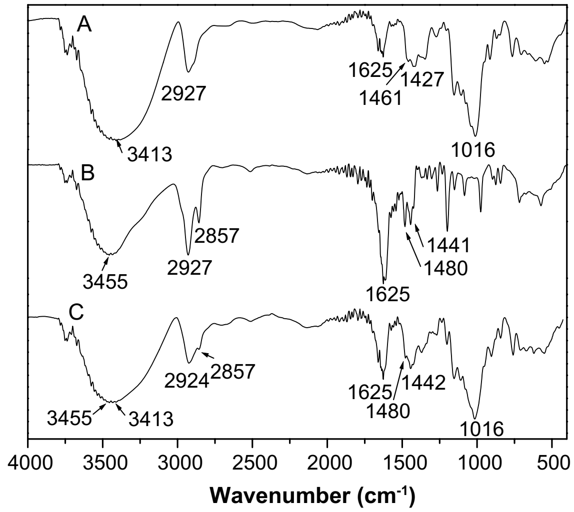

Figure 9. The infrared spectra of dextran (A), PNVCL (B), and the dextran derivative (C).

is much weaker than that of PNVCL. In addition, the characteristic absorption bands of C–H stretching (2857 cm^{-1}) and C–H deformational band (1442 cm^{-1}) from the dextran derivative demonstrate also the existence of PNVCL units. Further evidence for the modification reaction is from ^{1}H NMR analyses by means of a Varian Mercury-Plus 300-MHz spectrometer, as illustrated in Figure 10. The ^{1}H NMR spectrum (Fig. 10C) of the dextran derivative shows not only the characteristic resonance peaks at 3.4–3.9 and 4.8 ppm, corresponding to the protons in methylene groups and other five methine groups of dextran (Fig. 10A), but also the proton signals at 4.2 ppm (1H, –NCH– of the α position), 3.2 ppm (2H, –NCH_2–), 2.3 ppm (2H, –COCH_2–), and 0.9–2.0 ppm (6H, –CH_2– of the caprolactam ring, and 2H, –CH_2– of the backbone) for the PNVCL units (Fig. 10B). From the measurement of the nitrogen content of the modified dextran by using an Elementar Elemental Analyzer, the grafting percentage for the above sample was 71.0%.

3.3. Measurement of optical transmittance

The optical transmittance for the sample solution was monitored under various conditions as a function of the temperature at 540 nm by using a Shanghai Instrument Company model 721 spectrophotometer (China). The temperature of the sample cell was thermostatically controlled using a circulator system. At each temperature, the sample solution was allowed to equilibrate for about 10 min before the measurement. Pure water was used as a reference. The value of the critical transition temperature (T_c) of the sample solution was determined as the temperature at which the transmittance is 50% of the value under various conditions.[16,17]

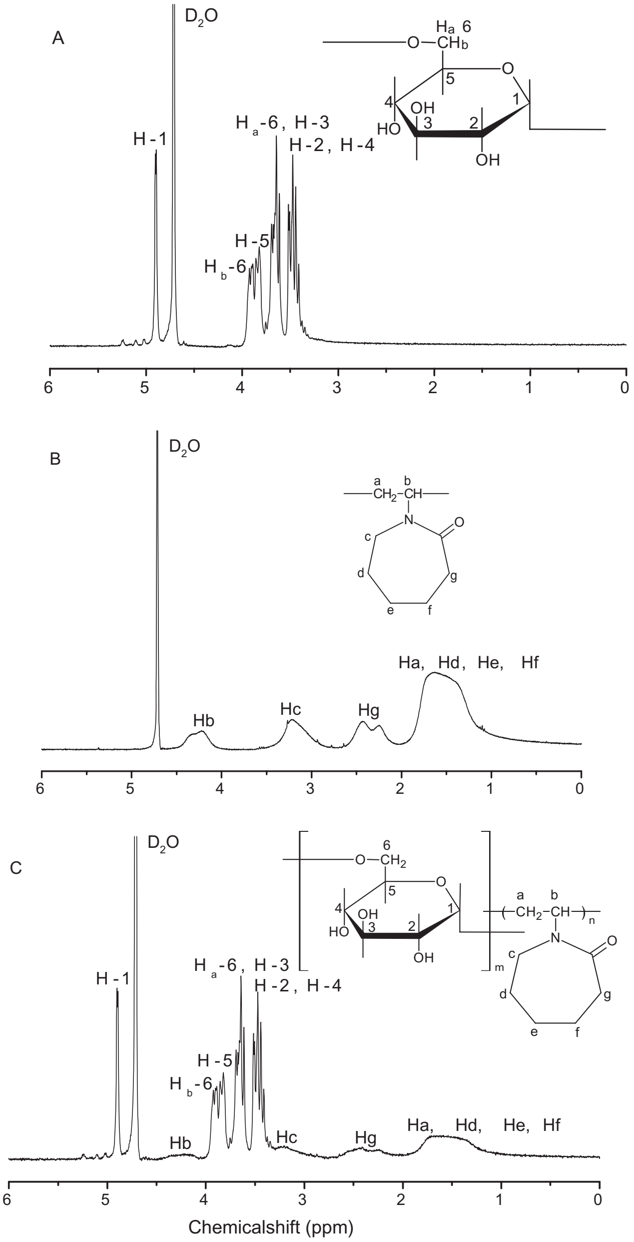

Figure 10. The ^{1}H NMR spectra of dextran (A), PNVCL (B), and dextran derivative (C) in D_2O.

3.4. Detection of hydrophobic aggregates

According to the protocol previously described by Samsonoff et al.[15] for the measurement of the critical micelle concentration of molecular surfactants, the hydrophobic microdomain in the sample solution was evidenced with the Coomassie Brilliant Blue (CBB) dye. About 1.0 g/L aqueous sample solution was diluted to a set of solutions with different concentrations. To 2.5 mL of those solutions, 2.5 mL of 0.094 g/L CBB was added. The mixed solution was homogenized and incubated for 10 min at a predetermined temperature. The absorption of CBB in aqueous media was recorded between 500 and 700 nm (Shimadzu UV-240 UV–vis recording spectrophotometer).

Acknowledgments

This work was supported by NSFC (20273086; 30470476), NSFG (021769; 039184), Department of Science and Technology of Guangdong Province (2004B33101003), and NCET Program in Universities as well as SRF for ROCS, SEM, China.

References

1. Larsen, C. *Adv. Drug Delivery Rev.* **1989**, *3*, 103–154.
2. Walker, G. J. Dextrans. In *Biochemistry of Carbohydrates II*; Manners, D. J., Ed.; University Park Press: Baltimore, 1978; Vol. 16, p 75.
3. Reese, E. T.; Parrish, F. W. *Biopolymers* **1966**, *4*, 1043–1045.
4. Mehvar, R. *J. Controlled Release* **2000**, *69*, 1–25.
5. Hoffman, A. S. *Macromol. Symp.* **1995**, *98*, 645–664.
6. Kubota, K.; Fujishihe, S.; Ando, I. *J. Phys. Chem.* **1990**, *94*, 5154–5158.
7. Yin, X.; Stover, H. D. H. *Macromolecules* **2003**, *36*, 9817–9822.
8. Bae, Y. H.; Okano, T.; Hsu, R.; Kim, S. W. *Macromol. Chem. Rapid Commun.* **1987**, *8*, 481–485.
9. Galaev, I. Y.; Mattiasson, B. *Trends Biotechnol.* **1999**, *17*, 335–340.
10. Freitag, R.; Garret-Flaudy, F. *Langmuir* **2002**, *18*, 3434–3440.
11. Makhaeva, E. E.; Tenhu, H.; Khokhlov, A. R. *Polymer* **2000**, *41*, 9139–9145.
12. Makhaeva, E. E.; Tenhu, H.; Khokhlov, A. R. *Macromolecules* **1998**, *31*, 6112–6118.
13. Faes, H.; De Schryver, F. C.; Sein, A.; Bijma, K.; Kevelam, J.; Engberts, J. B. F. N. *Macromolecules* **1996**, *29*, 3875–3880.
14. Duval-Terrie, C.; Hugust, J.; Muller, G. *Colloids Surf., A* **2003**, *220*, 105–115.
15. Samsonoff, C.; Daily, J.; Almog, R.; Berns, D. S. *J. Colloid Interface Sci.* **1986**, *109*, 325–329.
16. Chaw, C. S.; Chooi, K. W.; Liu, X. M.; Tan, C. W.; Wang, L.; Yang, Y. Y. *Biomaterials* **2004**, *25*, 4297–4308.
17. Shtanko, N.; Lequieu, W.; Goethals, E. J.; Prez, F. E. D. *Polym. Int.* **2003**, *52*, 1605–1610.