Journal of Macromolecular Science, Part A
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597274

Synthesis and Characterization of Thermo-responsive Poly(*N*-vinyl-2-pyrrolidinone)-*block*-poly(*N*-isopropylacrylamide) Micelles

Wei Tang[ab]; Jing Chen[a]; Zhao-Tie Liu[ac]; Zhong-Wen Liu[ac]
[a] Key Laboratory of Applied Surface and Colloid Chemistry, Shaanxi Normal University, Ministry of Education, Xi'an, P.R. China [b] Beijing Institute of Aerospace Testing Technology, Beijing Rate Technology Corp, Beijing, P.R. China [c] School of Chemistry & Materials Science, Shaanxi Normal University, Xi'an, P.R. China

Online publication date: 30 August 2010

To cite this Article Tang, Wei , Chen, Jing , Liu, Zhao-Tie and Liu, Zhong-Wen(2010) 'Synthesis and Characterization of Thermo-responsive Poly(*N*-vinyl-2-pyrrolidinone)-*block*-poly(*N*-isopropylacrylamide) Micelles', Journal of Macromolecular Science, Part A, 47: 10, 1026 — 1031
To link to this Article: DOI: 10.1080/10601325.2010.508012
URL: http://dx.doi.org/10.1080/10601325.2010.508012

Journal of Macromolecular Science, Part A: Pure and Applied Chemistry (2010) **47**, 1026–1031

ISSN: 1060-1325 print / 1520-5738 online
DOI: 10.1080/10601325.2010.508012

Taylor & Francis
Taylor & Francis Group

Synthesis and Characterization of Thermo-responsive Poly(*N*-vinyl-2-pyrrolidinone)-*block*-poly (*N*-isopropylacrylamide) Micelles

WEI TANG[1,2], JING CHEN[1], ZHAO-TIE LIU[1,3,*] and ZHONG-WEN LIU[1,3]

[1]*Key Laboratory of Applied Surface and Colloid Chemistry, Shaanxi Normal University, Ministry of Education, Xi'an, P.R. China*
[2]*Beijing Institute of Aerospace Testing Technology, Beijing Rate Technology Corp, Beijing, P.R. China*
[3]*School of Chemistry & Materials Science, Shaanxi Normal University, Xi'an, P.R. China*

Received February 2010, Accepted April 2010

A novel thermo-responsive diblock copolymer of poly(*N*-vinyl-2-pyrrolidinone)-*block*-poly(*N*-isopropylacrylamide) (PNVP-*b*-PNIPAM) was synthesized. FT-IR, ^{1}H-NMR and SEC results confirmed the successful synthesis of PNVP-*b*-PNIPAM diblock copolymer via anionic polymerization. The polymeric micelles formed from PNVP-*b*-PNIPAM copolymer in aqueous solution were developed and characterized as a potential thermo-responsive and biocompatible drug delivery system. Micellization of the diblock copolymer in aqueous solution was characterized by dynamic laser scattering (DLS), turbidity measurement, tension measurement and transmission electron microscopy (TEM). The thermo-responsive polymeric micelles with the size ranges of 200 to 260 nm and thickness of 30 nm are localized, selected and targeted for drug release, having a great potential in response to external-stimulus such as temperatures from 35 to 39°C. The critical micellization concentration (cmc) of PNVP-*b*-PNIPAM in aqueous solution is 0.0026 wt% determined by turbidity measurement. The size of micelles determined by DLS increased from 163 to 329 nm with increasing concentration of PNVP-*b*-PNIPAM from 0.25 to 0.5 wt% in aqueous solution at 40°C, which is determined by DLS.

Keywords: Poly(*N*-vinyl-2-pyrrolidinone), poly(*N*-isopropylacrylamide), anionic polymerization, self-assembling, micelle

1 Introduction

Developing stable micelles as intelligent drug carriers that could respond to external physical- stimulus such as heat, light and sound can be applied to localized release of drugs (1). Recently, growing research shows that many kinds of nano-scale carriers including environment-responsive micelle, microsphere and crosslinked hydrogel will have a significant impact on a wide range of polymeric materials. Scientists have focused on the self-assembling behavior in water of stimuli-responsive block copolymers (2, 3) since their physical and chemical properties can be adjusted by the change of external environment (4). The thermo-responsive polymeric micelles have attracted extensive attention because their properties could change sharply in response to a small change of environment, which would be applied to biomaterials, environmental engineering, molecular switch etc (5). Stimuli-responsive copolymers as drug carriers have been investigated recently (6). The environmental stimuli-sensitivity of these smart polymers could realize both site and temporal drug delivery, which will have a great potential in targeted drug delivery system. The dynamic behavior of these smart polymers in aqueous solution is important on both theory and practice. Because the local heating depends on get-up was realized easily, a variety of strategies have been employed to tailor the temperature-induced phase transition in order to adjust appropriate temperatures for intelligent drug carriers (7).

Currently, the self-assembling properties of poly(*N*-isopropylacrylamide) (PNIPAM) in dilute solutions are studied most frequently (8). PNIPAM is a typical thermo-responsive polymer which has a lower critical solution temperature (LCST) at around 32°C. When the temperature goes up to 32°C, the chains of PNIPAM will shrink several hundred folds within a narrow range of temperature. As a typical example of "smart" polymers, due to the exhibition of the well-known LCST in water, the self-assembling of PNIPAM-based block polymers has been

*Address correspondence to: Zhao-Tie Liu, Key Laboratory of Applied Surface and Colloid Chemistry, Shaanxi Normal University, Ministry of Education, Xi'an 710062, P.R. China and School of Chemistry & Materials Science, Shaanxi Normal University, Xi'an 710062, P.R. China. Tel.: +86 29 85303682; Fax: +86 29 85307774; E-mail: ztliu@snnu.edu.cn

widely used in the field of different applications like environmentally responsive micelles, drug delivery, smart surfaces (9), catalysis (10), temperature-controlled carrier for reaction of biomacromolecules and so on. The volume phase transition temperature of PNIPAM can be adjusted when it copolymerizes with hydrophilic or hydrophobic monomers such as poly(ethylene glycol) (PEG) (11, 12), poly(methyl methacrylate) (PMMA) (13, 14), poly(*N*, *N*-dimethylacrylamide) (PDMAAM) (15), and polystyrene (PS) (16).

Poly(*N*-vinyl-2-pyrrolidinone) (PNVP) is a nitrogen-containing and water-soluble polymer which has been proven to be biocompatible and extensively used in pharmaceutical industry. In physiology, PNVP agrees well with human blood. Nitrogen atom of PNVP can easily form coordinate bonds with lots of drugs, especially protein and polypeptides, which can be used as carrier of drugs. Recently, Ranucci et al. (17–20)reported the synthesis of hydroxyl-terminated PNVP oligmers via free radical polymerization in isopropyl alcohol (IPA), using cumene hydroperoxide as the initiator.

In this paper, we focus on the synthesis of PNVP-*b*-PNIPAM through anion polymerization and its thermal behavior and micellization behavior in aqueous solution by self-assembly in order to design a well-defined and thermo-responsive diblock copolymer to be used as drug carrier with a narrower polydispersity index (PDI).

2 Experimental

2.1 Materials

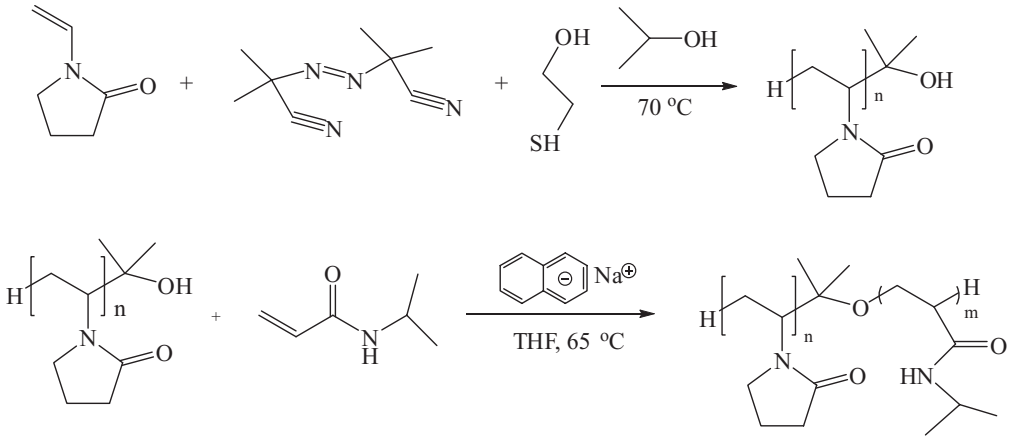
N-vinyl-2-pyrrolidinone (Aldrich, 99%) was dried over anhydrous magnesium sulfate and purified by distillation under reduced pressure. 2-Mercaptoethanol (MCE) purchased from Aldrich was purified by distillation. *N*-isopropylacrylamide (Kohjin, 99%) was recrystallized twice in hexane/toluene. Tetrahydrofuran (THF) (Kermel, AR-grade) was distilled just before use over benzophenone/sodium couple as a drying agent. 2,2'-Azobis(isobutyronitrile) (AIBN) (Aldrich, 99%) was purified by recrystallization for three times from ethanol. Sodium naphthalene was synthesized by naphthalene/sodium in THF before use, and its concentration was determined by titration. Isopropyl alcohol (Kermel, HPLC-grade) was purified by distillation under reduced pressure. Methylene chloride and diethyl ether (Kermel, AR-grade) were used without further purification.

2.2 Synthesis of Macroinitiator PNVP-OH

The hydroxyl-terminated PNVP-OH macroinitiator was synthesized by free radical polymerization via appropriate mixing proportion according to the procedure available in the literature (21). IPA is chosen as solvent for NVP, AIBN as initiator and MCE as chain-transfer reagent. The polymerization was performed at 70°C for 24 h under argon. The excessive IPA was distilled under reduced pressure after polymerization. The product was dissolved in methylene chloride, and then precipitated in diethyl ether. This process was repeated at least three times. Finally, the product was purified by diethyl ether in Soxhlet extractor for 24 h, and then dried at 40°C for 24 h under vacuum.

2.3 Synthesis of PNVP-*b*-PNIPAM

PNVP-*b*-PNIPAM was synthesized by anionic polymerization of PNVP-OH followed by NIPAM initiated with sodium naphthalene under argon (Sch. 1). The PNVP-OH (0.75 g, 0.3 mmol end hydroxyl group) was dried in toluene as the azeotropic solvent, and then transferred into a dried round bottom flask under argon atmosphere, pumping vacuum and purging with argon for three times. The PNVP-OH was dissolved under stirring by adding the freshly distilled anhydrous THF (6 mL), and then sodium naphthalene (1.5 mL, 1 mmol) was added. When the solution turned



Sch. 1. Synthesis of PNVP-OH Homopolymer and PNVP-*b*-PNIPAM Diblock Copolymer.

to green, the NIPAM solution (c=1.10 mol/L) which was prepared by dissolving dried NIPAM (1.5 g, 10.5 mmol) in 10 mL of THF was introduced, and the polymerization was carried out at 65°C for 24 h. Finally, the polymer was precipitated in cold diethyl ether, purified by diethyl ether in Soxhlet extractor for 24 h, and freeze-dried for 48 h.

2.4 Characterization of PNVP-*b*-PNIPAM

FT-IR spectrum was recorded on EQUINX55 (Brucher, Germany, KBr disc method was used). ^{1}H-NMR spectra were recorded on a superconducting Fourier digital NMR spectrometer (AVANCF 300 MHz, Bruker, Swiss) at room temperature by using tetramethylsilane (TMS) as internal reference and D_2O as a solvent. The molecular weights of both PNVP-OH and PNVP-*b*-PNIPAM were determined using size exclusion chromatography (SEC) with a Waters-Breeze systems using a refractometer Waters 2414 (Waters Corporation, Massachusetts). SEC was performed in THF with a flow rate of 1.0 mL/min at 35°C, and narrowed disperse polystyrene was used as a calibration standard.

2.5 Preparation of Micelle

The PNVP-*b*-PNIPAM diblock copolymer was self-assembled by stirring in deionized water for 12 h, in which water is a good solvent for both PNVP and PNIPAM blocks at room temperature. PNIPAM has the LCST around 32°C in aqueous solution. When the temperature is up to the LCST, phase separation takes place due to the breaking of hydrogen bonds with water. The property of PNIPAM changes from hydrophilic to hydrophobic, and then micellization was achieved by increasing temperature to form a micelle solution.

2.6 Dynamic Laser Scattering (DLS)

DLS was used to determine the particle size distribution and the hydrodynamic diameter of the micelle in aqueous solution. DLS analysis of the PNVP-*b*-PNIPAM diblock copolymer micelles was carried out on a BI-90 Plus (Brookhaven Instruments Corp., Holtsville, New York) equipped with a He-Ne laser (633 nm). All measurements were made at a scattering angle of 90°. The temperature-dependence DLS experiments were run with a heating program of 25 to 50°C and a step size of 2°C, equilibrating the polymer solution for 10 min at each step. The particle size distribution and the intensity of the mean particle size were recorded.

2.7 Turbidity Measurement (UV)

Turbidity measurements were performed on a TU-1901 UV-Vis (Beijing Purkinje General Instrument Co., Ltd., Beijing, China) spectrophotometer. The turbidity experiment was operated at temperature-control device with an external thermostatic controller. The temperature of the polymer solutions (0.1 wt%) was increased from 20 to 50°C with an increment of 0.5°C, and the transmittance was monitored at 500 nm. All measurements were performed after each temperature was settled for 10 min, in order to ensure thermal equilibrium. The LCST of PNVP-*b*-PNIPAM was determined as the onset of the decrease in transmittance plots as a function of temperature in aqueous solution.

2.8 Tension Measurement

A Dataphysics DCAT 21 (Dataphysics Corporation, Stuttgart) tensiometer equipped with a wilhelmy plate was used to determine the critical micellization concentration (cmc) of the aqueous diblock copolymer solutions. The surface tensions at different polymer concentrations were recorded and analyzed using the SCAT program. The micellization of thermo-responsive PNVP-*b*-PNIPAM diblock copolymer in deionized water was determined at the concentration range from 0.001 to 0.5 wt% under various temperatures. The cmc of the copolymer was determined from curves of the surface tension versus logrithm concentrations.

2.9 Transmission Electron Microscopy (TEM)

TEM observations were performed on H-600 instrument (Hitachi, Ltd., Japan), operated at an accelerating voltage of 80 kV. The PNVP-*b*-PNIPAM aqueous solutions at different concentrations were prepared at various temperatures. Above its LCST, the copper grid with a carbon film was immersed in a drop of the aqueous polymer solution for 2 min and then removed and dried. A drop of phosphotungstic acid was placed on the copper grid for 2 min. The samples were then kept and dried overnight at this temperature under vacuum fleetly prior to measurement.

3 Results and Discussion

3.1 Synthesis of PNVP-OH and PNVP-*b*-PNIPAM

The FT-IR spectrum of PNVP-OH (not given) shows characteristic infrared absorption peaks of amine group stretching vibration band at 1662 cm^{-1} and hydroxyl stretching band at 3454 cm^{-1}. Therefore, hydroxyl-terminated PNVP oligmers via free radical polymerization in IPA, using AIBN as the initiator and MCE as the chain-transfer reagent, was synthesized successfully from the FT-IR and ^{1}H-NMR results.

The FT-IR spectrum of PNVP-*b*-PNIPAM was shown in Figure 1. The infrared absorption bands of PNIPAM with characteristic bands at 1662 cm^{-1} (amide) and 1547 cm^{-1} (amide) were observed. The characteristic

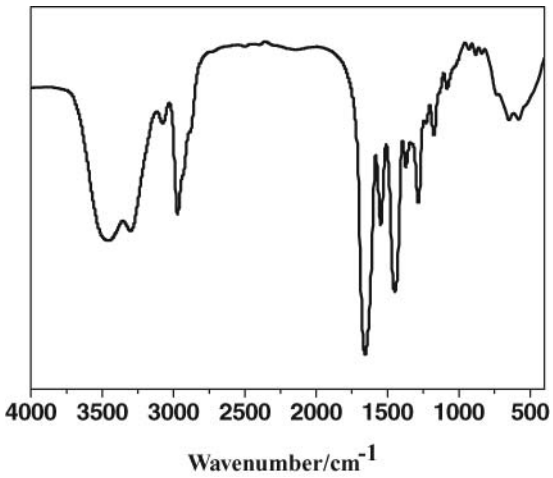


Fig. 1. FT-IR spectrum of PNVP-*b*-PNIPAM.

infrared absorption bands of -OH bonds and N-H bonds at 3468 cm^{-1} and 3313 cm^{-1} were indicated. The characteristic infrared absorption frequency for C-N bonds at 1284 cm^{-1} was higher than the C-O bands at 1079 cm^{-1}.

The ^{1}H-NMR spectrum of PNVP-*b*-PNIPAM is shown in Figure 2. The peaks at 3.75 ppm and 1.00 ppm are attributed to the –N-CH and -$(CH_3)_2$ groups, respectively. The ratio of integral areas is in accordance with the theoretical value of 1:6. Signals for other –CH and –CH_2 groups are assigned in Figure 2. The content of PNIPAM block calculated by ^{1}H-NMR is about 75 wt%.

3.2 SEC Measurement

The M_n and PDI of PNVP-OH were characterized by SEC, done in DMF/LiBr. As can be seen from Table 1 the PDI of diblock copolymer is 1.30, which illustrates the success of synthesis of PNVP-b-PNIPAM.

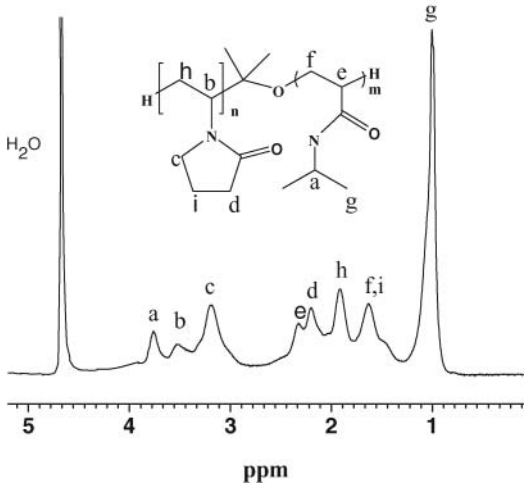


Fig. 2. ^{1}H=NMR spectrum of PNVP-*b*-PNIPAM in D_2O with TMS as internal standard.

Table 1. Molecular weight data for PNVP-OH and PNVP-b-PNIPAM

Name	M_n *(g/mol)*	*PDI*
PNVP-OH	3958	1.18
PNVP-b-PNIPAM	16458	1.30

3.3 Thermal Properties of PNVP-*b*-PNIPAM in Aqueous Solution

At a low temperature, the main interaction between the PNIPAM and water molecules is the hydrogen-bonding interactions between water and amide groups. Below the LCST, PNIPAM can dissolve in water due to the effect of hydrogen bonding and Van Der Waals force, which make water molecules, are around the macromolecular chains and thus form highly ordered "water cages" connected by hydrogen bonding. Moreover, the polymer chains exist in an extended "coil" conformation. Hydrogen-bonding interactions and the solvate layer of hydrophobic part of the macromolecular chain can be disrupted when the temperature of local solution increased. The association interaction of the hydrophobic moiety enhanced as the temperature increased. So the particle of PNIPAM undergoes morphological transition from a loosely swollen network to rigid spheres. At the same time, water can be expelled from the matrix of the "water cage" and a large-magnitude volume change occurs, which generates the temperature-sensitivity.

3.4 Micelles Formation

The well-defined PNVP-*b*-PNIPAM diblock copolymers can self-assemble to form micelles at different concentrations above LCST. To obtain information on the size and shape of the polymerized micelle, DLS analysis was carried out. DLS analysis of the aqueous solutions indicates the presence of micellar aggregates in nanometer scale. The dynamic laser scattering instrument was used to measure the effective diameter of the micelles and the population distribution of the micelles in terms of their size. Figure 3 shows the effect of temperature on size of micelle of PNVP-*b*-PNIPAM at different concentrations. Because the polymers have a remarkable temperature-sensitivity, when the temperature increased from 37 to 47°C, the size of the polymerized micelle increased largely from 40 to 442 nm for 0.25 wt% PNVP-*b*-PNIPAM solution. When the concentration of PNVP-*b*-PNIPAM increased from 0.25 to 0.5 wt%, the micelle size increased from 40 to 263 nm.

3.5 Turbidity Measurement

Cloud points of 0.1 wt% of PNVP-*b*-PNIPAM) in aqueous solution were measured by turbidimetry. Transmittance of the solution was recorded with the increase of temperature. The temperature of the polymers solutions was increased from 30 to 42°C in increments of 0.5°C and

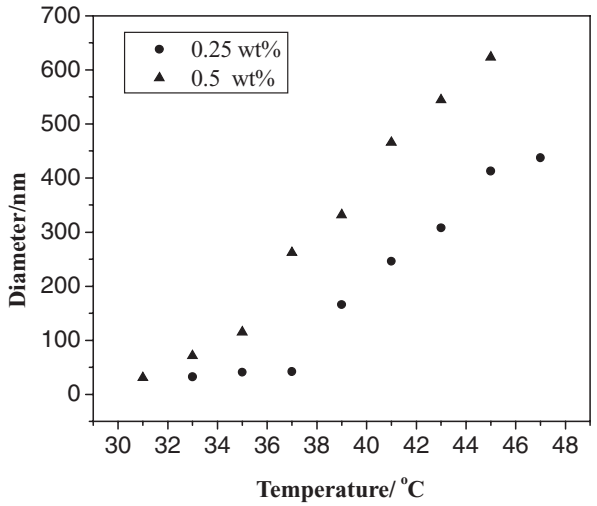


Fig. 3. DLS of PNVP-*b*-PNIPAM in aqueous solution at different concentrations.

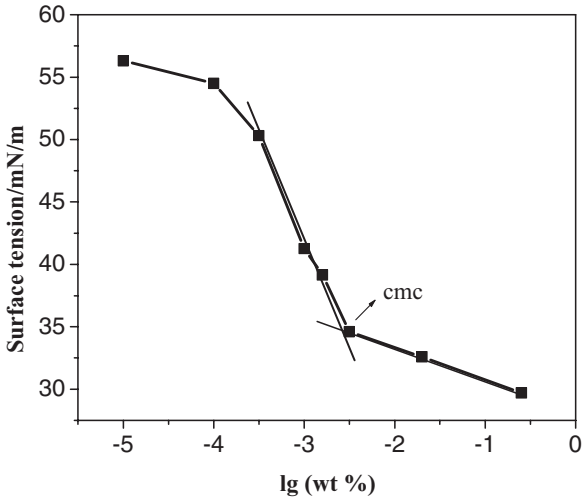


Fig. 5. The surface tension of PNVP-*b*-PNIPAM above LCST in aqueous solution.

operated at temperature-control device with external thermostatic controller. Figure 4 shows the transmittance as a function of temperature for PNVP-*b*-PNIPAM in aqueous solution under the turbidity at 500 nm. The LCST of PNVP-*b*-PNIPAM that determined from the onset of the decrease in transmittance plots is 37°C, which is higher than that of PNIPAM solution (32°C). In addition, a relatively broad temperature of transition was observed, which can be explained by the introduction of hydrophilic groups of PNVP block.

3.6 Surface Tension

The micellization of thermo-responsive PNVP-*b*-PNIPAM diblock copolymer in deionized water was determined over the concentration range from 0.0001 to 0.5 wt% at various temperatures. For the diblock copolymer, the molecules presented a tendency to be enriched on water-air interface. When the temperature of the aqueous solution is higher than the LCST, the solution of copolymer presented as a typical amphiphilic copolymer. When the concentration is higher than that of cmc, micelles with hydrophobic cores and hydrophilic shells will form. The effect of PNVP-*b*-PNIPAM content on the surface tension of the block copolymer solution is shown in Figure 5. The cmc of the copolymers can be determined from curve of the surface tension vs. concentration, which is 0.0026 wt% for PNVP-*b*-PNIPAM in aqueous solution.

Figure 6 shows the surface tension of block copolymer solution as a function of time. With the increase of temperature, molecular motions is quicker than that of the lower temperature, which effectively improves molecular adsorption velocity on the surface as shown in Figure 6. The surface tension of block copolymer solution at 13°C

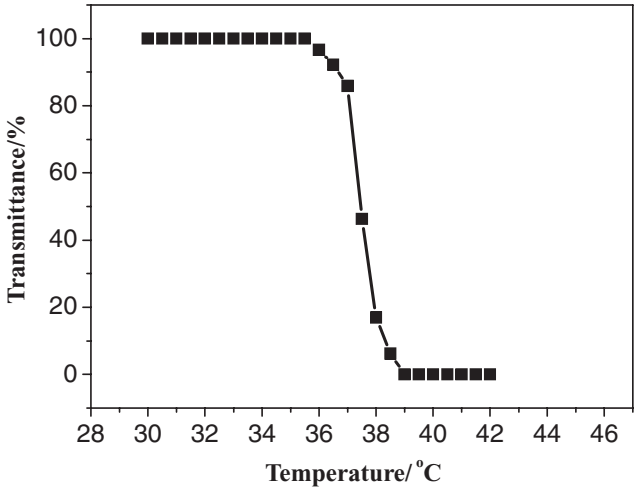


Fig. 4. Temperature-dependent transmittance of 0.1 wt% PNVP-*b*-PNIPAM in aqueous solution.

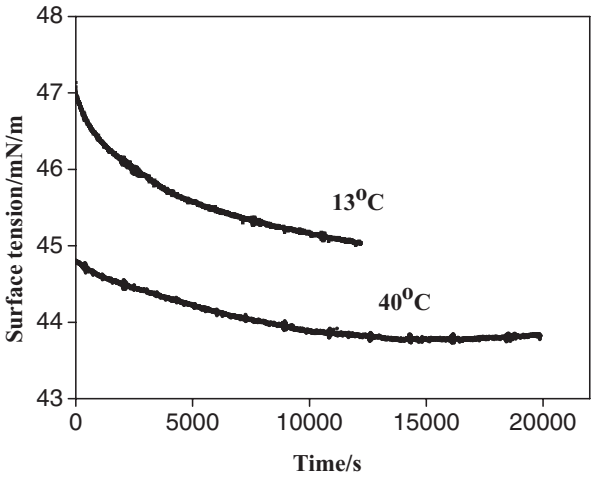


Fig. 6. Dependence of the surface tension of the aqueous solution (0.01 wt%) of PNVP-*b*-PNIPAM on the temperature.

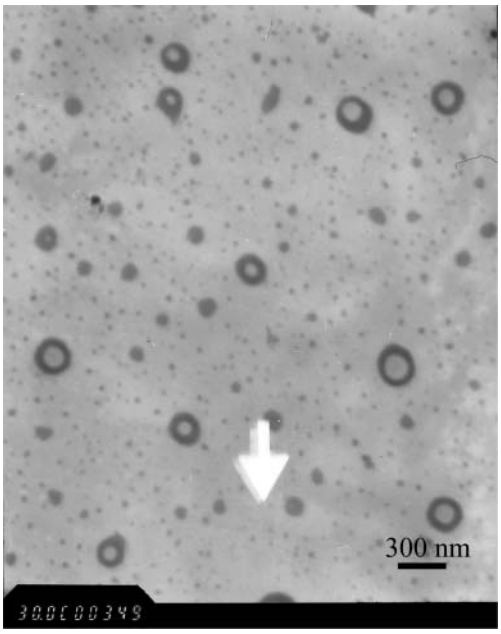


Fig. 7. TEM micrograph of associated structure of PNVP-*b*-NIPAM in the solution of 0.2 wt %.

was more rapid than that at 40°C to reach equilibrium. The equilibrium surface tension at 40°C was significantly lower than that at 13°C. Due to the thermo-responsive PNIPAM block of copolymer, when the temperature increased above LCST, the PNIPAM chains became hydrophobic, which results in aggregation and precipitation of PNVP-*b*-PNIPAM in solution.

3.7 TEM

Self-assembly of block copolymers in selective solvent can be used to prepare nano-scale aggregates with different morphologies. Figure 7 shows the micromorphology and size at the concentration of 0.2 wt% of PNVP-*b*-PNIPAM. The well-defined PNVP-*b*-PNIPAM block copolymers can self-assemble in aqueous solution into well-defined micelles with relatively small size and high homogeneity above LCST. The TEM shows that the nanoparticles mainly form spherical micelles with a core-shell structure, and the average diameter of the formed micelles is in the range of 200–260 nm and the thickness of the shell is about 30 nm.

4 Conclusions

Developing stable micelles which can be responsive to external physical stimulus has great potential in localized, selective and targeted release. The thermo-responsive diblock copolymer PNVP-*b*-PNIPAM was successfully synthesized through free radical polymerization and anion polymerization. The diblock copolymers can self-assemble into well-defined micelles in aqueous solution with a relatively small size of 200 to 260 nm and high homogeneity above LCST, which is suitable and has potential for high-efficient drug delivery system.

Acknowledgments

The authors gratefully acknowledge the financial support from the 973 Program of China (Program/Grant No. 2009CB226105).

References

1. Rapoport, N. (2007) *Prog. Polym. Sci.*, 32, 962.
2. Sugihara, S., Kanaoka, S. and Aoshima, S. (2005) *Macromolecules*, 38, 1919.
3. Mertoglu, M., Garnier, S., Laschewsky, A., Skrabania, K. and Storsberg, J. (2005) *Polym.*, 46, 7726.
4. Xia, Y., Burke, N.A.D., and Stöver, H.D.H. (2006) *Macromolecules*, 39, 2275.
5. Tiera, M.J., dos Santos, G.R., de Oliveira Tiera, V.A., Vieira, N.A.B., Frolini, E., da Silva, R.C. and Loh, W. (2005) *Colloid Polym. Sci.*, 283, 662.
6. Yoshino, K., Kadowaki, A., Takagishi, T. and Kono, K. (2004) *Bioconjugate Chem.*, 15, 1102.
7. Pagonis, K., and Bokias, G. (2007) *Polym. Bull.*, 58, 289.
8. Kujawa, P., Watanabe, H., Tanaka, F. and , Winnik, F.M. (2005) *Eur. Phys. J. E,* 17, 129.
9. Bergbreiter, D.E. (2002) *Chem. Rev.*, 102, 3345.
10. Neradovic, D., Soga, O., Van Nostrum, C.F. and Hennink, W.E. (2004) *Biomater.,* 25, 2409.
11. Wei, H., Zhang, X.Z., Zhou, Y., Cheng, S.X. and Zhuo, R.X. (2006) *Biomater.,* 27, 2028.
12. Kelarakis, A., Tang, T., Havredaki, V., Viras, K. and Hamley, I.W. (2008) *J. Colloid Interf. Sci.*, 320, 70.
13. Nakayama, M. and Okano, T. (2005) *Biomacromolecules*, 6, 2320.
14. Zhou, X.C., Ye, X.D. and Zhang, G.Z. (2007) *J. Phys. Chem. B*, 111, 5111.
15. Liu, Z.F. and Rimmer, S. (2002) *Macromolecules*, 35, 1200.
16. Luo, L.B., Ranger M., Lessard, D.G., Le Garrec, D., Gorl, S., Leroux, J.C., Rimmer, S. and Smith, D. (2004) *Macromolecules,* 37, 4008.
17. Ranucci, E., Spagnoli, G., Sartore, L., Bignotti, F. and Ferruti, P. (1995) *Macromol. Chem. Phys.*, 196, 763.
18. Ranucci, E., Tarabic, M., Gilberti, M. and Albertsson, A.C. (2000) *Macromol. Chem. Phys.*, 201, 1219.
19. Ranucci, E., Macchi, L., Annunziata, R., Ferruti, P. and Chiellini, F. (2004) *Macromol. Biosci.*, 4, 706.
20. Ranucci, E., Ferruti, P., Annunziata, R., Gerges, I. and Spinelli, G. (2006) *Macromol. Biosci.*, 6, 216.
21. Burke, S.E. and Eisenberg, A. (2001) *Langmuir,* 17, 8341.