Available online at www.sciencedirect.com
ScienceDirect
ELSEVIER
Polymer 49 (2008) 263–267
polymer
www.elsevier.com/locate/polymer

Self-dissociation of water-soluble PANa/ETC nano-aggregates

Rui Liu, Feng Qiu*, Daoyong Chen*

The Key Laboratory of Molecular Engineering of Polymers, Department of Macromolecular Science, Fudan University, Shanghai 200433, China

Received 14 May 2007; received in revised form 2 November 2007; accepted 9 November 2007
Available online 19 November 2007

Abstract

Complexation between poly(sodium acrylate) (PANa) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide methiodide (ETC) in water, which leads to the formation of vesicle-like aggregates, was studied. Different from reported nano-aggregates of polymer complexes that may dissociate when being stimulated, the nano-aggregates of PANa/ETC complexes dissociate automatically in the aqueous solution at ambient environment, exhibiting a behavior similar to that of the nano-aggregates composed of degradable polymers. In the newly formed nano-aggregates, there are hydrophobic domains with a hydrophobicity close to that of hexane. Along with the dissociation, the hydrophobic domains vanish gradually.

Keywords: Nano-aggregates; Self-dissociation; Complexation

1. Introduction

Polymeric nano-aggregates are much in demand for the use as nano-carriers for catalytic particles, molecules with electronic and photonic functions, as well as biological and medical species, and so forth [1]. Recently, the polymeric micelles [2–5] or nano-aggregates formed due to the complexation between two polymers [6] or the two blocks of a zwitterionic diblock copolymer [7] are especially interesting, since they are sensitive to pH value, ionic intensity and temperature of environment and promising for the preparation of controlled drug delivery systems. Generally speaking, in the structure of the nano-aggregates formed due to the complexation, the polymer chains should be partially complexed. The un-complexed block or repeating units solubilize the aggregates while the complexed ones bind different polymer chains together and drive the association into nano-aggregates. It is imaginable that when the complexes are decomplexed, the polymeric nano-aggregates dissociate. Actually, the complexes are stabilized by the electrostatic interaction or hydrogen bonding, which is sensitive to pH value or ionic intensity of the medium. This makes the association and dissociation of the nano-aggregates controllable by controlling the nature of the medium. Ionic intensity, pH and temperature sensitive polymeric nano-aggregates resulting from the complexation are largely reported [8,9]. In the present study, we report on the preparation of polymeric nano-aggregates due to the interaction between poly(sodium acrylate) (*PANa*) and a small molecular compound 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide methiodide (*ETC*) in water. Different from the reported nano-aggregates of polymer complexes which dissociate only when being stimulated, nano-aggregates of PANa/ETC complexes dissociate automatically. Furthermore, existing studies realized the dissociation by using bio-degradable polymer to construct polymeric micelles. The present study realizes the dissociation by the hydrolysis of the chemical structure, which is seldom reported.

2. Experimental section

2.1. Materials

PAA (Aldrich, M_w of 5100), pyrene (Aldrich, analytical purity) and ETC (J & K Chemical Ltd, analytical purity) were used as obtained.

---

* Corresponding authors. Tel.: +86 21 65643989; fax: +86 21 65640293.
E-mail addresses: fengqiu@fudan.edu.cn (F. Qiu), chendy@fudan.edu.cn (D. Chen).

0032-3861/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.polymer.2007.11.027

2.2. Formation of PANa/ETC nano-aggregates

ETC (in H_2O) was added dropwise into the aqueous solutions of PANa. In the resultant 3 mixture solutions, the final concentration of PANa was 1 mg/mL, whereas the MRs were 0.05, 0.07, 0.09, respectively.

2.3. Laser light scattering (LLS)

A commercial laser light scattering (LLS) spectrometer (Malvern Autosizer 4700) equipped with a multi-τ digital time correlation (Malvern PCS7132) and a solid-state laser (ILT5500QSL, output power 100 mW at $\lambda = 514.5$ nm) as light source was used. All the dynamic light scattering (DLS) measurements were performed at 25 ± 0.1 °C and at a scattering angle 90° as only a little of scattering angle dependence of the $\langle D_h \rangle$ of the nano-aggregates was observed. All the nano-aggregate solutions were measured at different concentrations directly without dilution. The light scattering intensity measurements for different samples were carried out at a fixed slit.

2.4. Transmission electron microscopy (TEM)

The TEM observations were performed with a Philips EM400 microscope. The accelerating voltage is 80 kV. The samples for TEM observations were prepared by depositing a drop of the solution (without dilution) on copper grids and were observed without being stained.

2.5. Fluorescence measurements

The Fluorescence measurements were performed on a FLS920 microscope using the pyrene as a fluorescence probe. The encapsulation of pyrene and their release from the as-prepared micelles and the measurements to track the processes are carried out based on the procedures well documented in literature [10].

2.6. FT-IR measurements

The solutions were transferred onto aluminum foils and quickly dried under an infrared lamp to form very thin transparent films. Since the hydrolysis reaction occurs very slowly in the core of the aggregates, this operation should have little effect on the kinetics of the hydrolysis reaction. The aluminum foils were chosen here as infrared reflective substrates and fixed on glass sheets before introduced into the infrared beam path. These samples were detected directly by the regular infrared reflection-adsorption accessory. Background spectrum was collected using a clean aluminum foil. All FT-IR spectra were obtained at 20 °C by a magna 550 spectrometer with a resolution of 2 cm^{-1} and the wavenumber range was 400–4000 cm^{-1}.

2.7. ^1H NMR measurements

The ^1H NMR measurements were conducted on a Bruker DMX500 spectrometer in D_2O using DMSO as an internal reference.

3. Results and discussion

PANa can be molecularly solubilized in water. ETC is water-soluble as well. This is evidenced by the dynamic light scattering measurements, in which no aggregates in the solutions of PANa and ETC at concentrations of either 0.1% or 1% are detected. After the mixing of ETC with PANa, when the MRs were between 0.05 and 0.09, the resultant mixture solutions were with a blue opalescence. When the MR was greater than 0.09, precipitates appeared in the solution. When the MR was lower than 0.05, for example 0.03, the solution was clear, and very weak signal is detected by dynamic light scattering (DLS) in the same conditions of other samples. Therefore, DLS measurements demonstrated the formation of nano-aggregates in the solutions at MR between 0.05 and 0.09, as shown in Fig. 1.

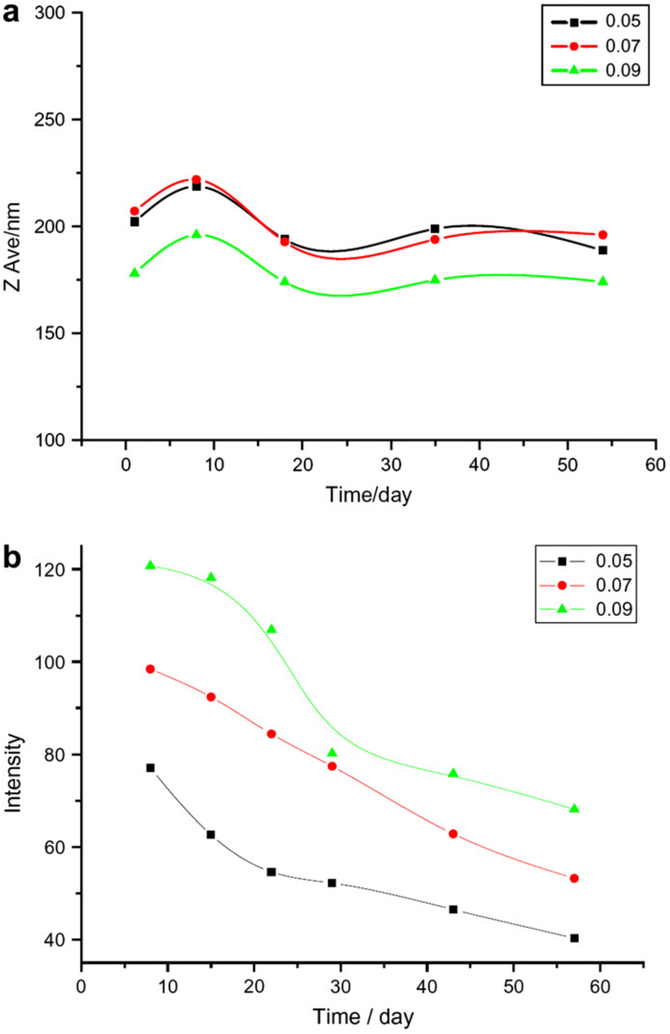


Fig. 1. (a) The variation of the average hydrodynamic diameter $\langle D_h \rangle$ of the nano-aggregates with time; (b) the variation of light scattering intensity (I_s) of the aggregate solution with time. The timing begins upon the mixing of ETC with PANa. The concentration of PANa is 1.0 mg/mL and the MRs are 0.05 (red line), 0.07 (green line) and 0.09 (black line), respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

However, we found that the blue opalescence of the solutions dims out gradually. We tracked the aggregate solutions by dynamic light scattering (DLS) measurements and found that the light scattering intensities (I_s) of the aggregate solutions decreased monotonously with time (Fig. 1b), whereas the average hydrodynamic diameters ($\langle D_h \rangle$) of the aggregates remain almost constant at the $\langle D_h \rangle$ value of the nano-aggregates formed upon the mixing of ETC with PANa (Fig. 1a). It is known that the nano-aggregates may experience fusion and fission [11,12] in the solutions. For examples, when the primary nano-aggregates are not sufficiently solubilized, the fusion will be dominant and I_s increases due to the aggregation between the primary ones into larger ones; when the nano-aggregates formed at the early stage are kinetically entangled with each other, they may dissociate with each other during the storing, i.e., the fission is dominant, resulting in decrease in I_s. In all these cases, the variation in I_s takes no more than several days to complete and is accompanied with the remarkable change in the $\langle D_h \rangle$. Differently, in the present study, the sizes of the nano-aggregates formed at all the MRs changes slightly whereas I_s decreases monotonously, and the decrease in I_s last for even months. In principle, I_s is proportional to $n \times M^2$ (where n denotes the number of the aggregates in a unit volume and M the average mass of a nano-aggregates) [13]. The decrease in I_s, in the present study, should result from the decrease in n or M or the both. As evidenced by our TEM observations, during the storing of the nano-aggregates, the number of the nano-aggregates decreases apparently and the nano-aggregates disappear in 30 days after their formation.

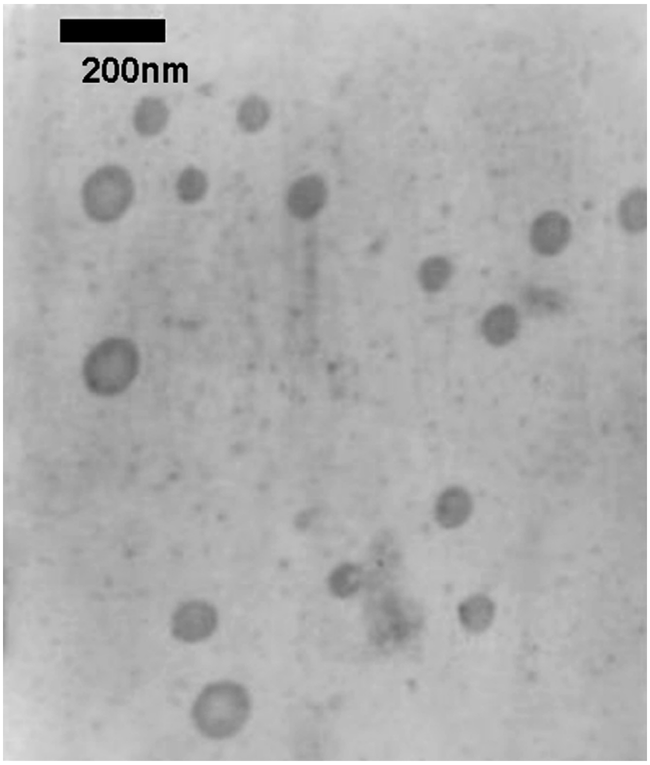
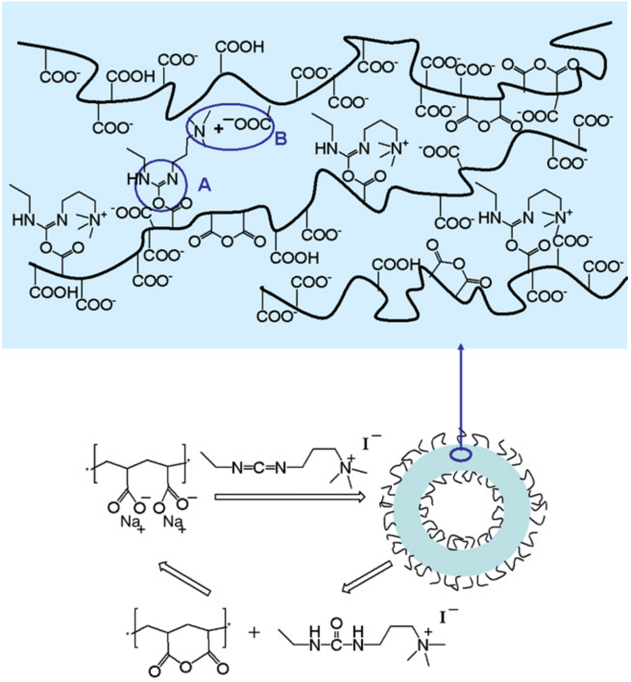


Fig. 2. TEM image of the nano-aggregates formed by the complexation between ETC and PANa, the scale bar represents 200 nm.

We tracked the changes of the nano-aggregates with time by TEM observations. The nano-aggregates observed several hours after the mixing of ETC with PANa are quite regular with a vesicle-like morphology (Fig. 2). The average sizes of the aggregates observed by TEM are less than those measured by DLS due to the contraction during the drying of the TEM samples. Our further TEM observations demonstrate that the number of the vesicle-like nano-aggregates decreases with time. Thirty days after the mixing, the vesicle-like aggregates disappear completely, and only a small number of irregular aggregates with a high contrast were observed to be sparsely dispersed on the copper grid (data not shown). These irregular aggregates with a high contrast in the TEM images should be iodine particles resulting from the oxidation of I^{-1} that is the counterion of ammonium cation of ETC. The iodine particles remained in the solution should be responsible for the fact that the I_s is not zero when the regular nano-aggregates disappear (Fig. 1b).

Obviously, the disappearance of the vesicle-like nano-aggregates indicates their self-dissociation. A detailed scheme is proposed as follows (Scheme 1): Since each of the two ends of ETC can interact with carboxylic anions of PANa, ETC acts as a cross-linker to bind different PANa chains together, forming polymeric nano-aggregates at low molar ratios of ETC to carboxylic anions of PANa. Among the two connection points of ETC with PANa (A and B, Scheme 1), A is *O*-acylisourea resulting from the reaction between N=C=N group of ETC



Compound 2

Compound 3

Scheme 1. ETC cross-link PANa in the way that the reaction between a carboxylic anion and an N=C=N group forms *O*-acylisourea (the connection point A) and the ion–ion interaction between ammonium and COO^{-1} leads to the formation of the connection point B. The cross-linking leads to the association of the complexes, forming vesicle-like nano-aggregates when the MRs are low. The subsequent hydrolysis of *O*-acylisourea leads to breakage of A, resulting in dissociation of the as-formed nano-aggregates.

and COO^- group of PANa; B is an ion pair of ammonium and COO^-. The gradual hydrolysis of *O*-acylisourea leads to the breakage of A, resulting in the dissociation and the change in the hydrophobicity of the nano-aggregates with time, which exhibit a behavior like the nano-aggregates composed of degradable polymers. The processes of the self-association and the self-dissociation of the nano-aggregates are schematically described in Scheme 1. Considering that PANa is an undegradable polymer, the connection point B (Scheme 1) due to the ion–ion interaction is stable when the pH and ionic intensity of the medium are unchanged; the self-dissociation should be due to the breakage of the connection point A (Scheme 1). It is reported that the *O*-acylisourea, the reaction product of N═C═N and COO^{-1}, is unstable in water since it will be hydrolyzed in aqueous solution [14]. As indicated in Scheme 1, after the hydrolysis, the connection point A is broken and the complexes change to compound **2** and PANa via an active intermediate anhydride (compound **3**). Apparently, compound **2** can no longer interact with PANa to form aggregates (as evidenced by our ^1H NMR characterization, described below) so that the nano-aggregates dissociate.

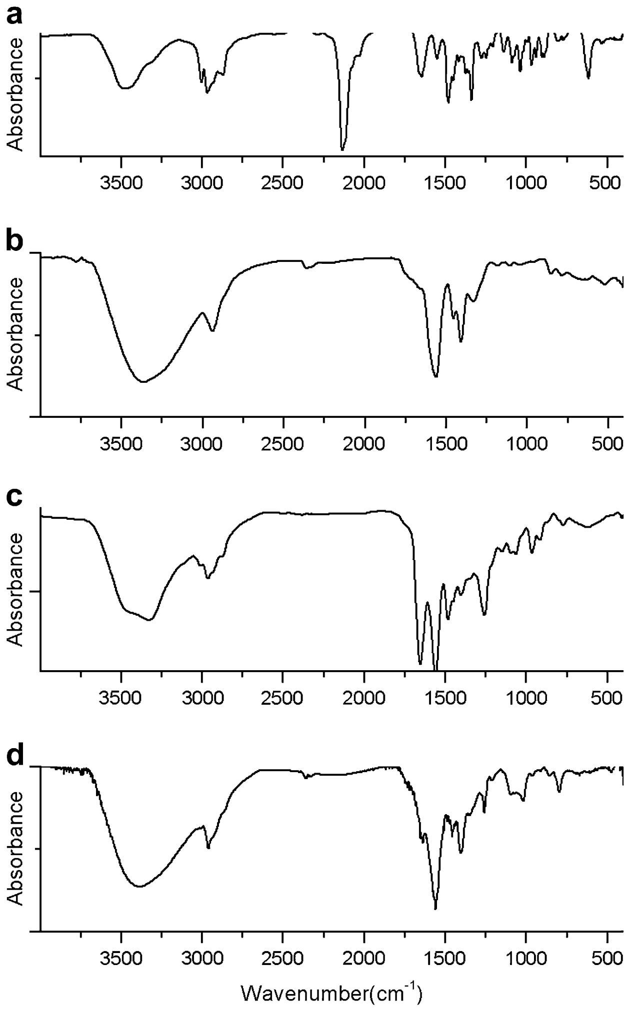


Fig. 3. FT-IR spectra of: (a) pure ETC, (b) pure PANa, (c) ETC/PANa complex measured upon its formation (the MR is 0.07) and (d) ETC/PANa complex measured 30 days after its formation.

The FT-IR spectra of pure ETC, pure PANa and newly formed ETC/PANa complex at MR of 0.07 are presented in Fig. 3 as spectra a–c, respectively. The FT-IR spectra of these samples were measured after they were quickly dried on aluminum foils by heating. Since the hydrolysis reaction occurs very slowly in the core of the micelles, this operation should have little effect on the kinetic of the hydrolysis reaction. The peaks at 2100 cm^{-1} and 1680 cm^{-1} in spectrum a are assigned to the stretching vibration of N═C═N and C═N bonds of ETC. The peak at 1560 cm^{-1} in spectrum b is assigned to the stretching vibration of C═O bonds in $COO^{-1}$of PANa. It is shown in spectrum c that upon the mixing of ETC with PANa, the peak at 2100 cm^{-1} disappears and meanwhile, a new peak at 1685 cm^{-1} appears, coexisting with the C═O signal of the COO^{-1} at 1560 cm^{-1}. This is consistent with the fact that ETC will react with the COO^{-1} groups to form *O*-acylisourea. With the hydrolysis of *O*-acylisourea, the relative intensity of the signal at 1685 cm^{-1} decreases steadily. Thirty days after the mixing, the peak at 1685 cm^{-1} became very weak (Fig. 3d), demonstrating that most of the *O*-acylisourea structure disappeared in 30 days. According to literature [10], the final products of the hydrolysis are compound **2** and PANa. This is consistent with the results obtained from our liquid ^1H NMR characterization of the ETC/PANa complex 30 days after the mixing. The ^1H NMR spectrum presents all the signals of compound **2** and PANa (the ^1H NMR spectrum and the assignments of the H atoms are presented in Fig. 4). The fact that nearly all the signals of compound **2** are detected in the ^1H NMR spectrum indicates that the molecules of compound **2** are in a soluble state [15], i.e., it can no longer interact with PANa to form aggregates.

The pH of PANa solution itself is 8.27. When the solution pH value is changed, for example, to 4.3, or 9.3, further aggregation among the nano-aggregates takes place and thus

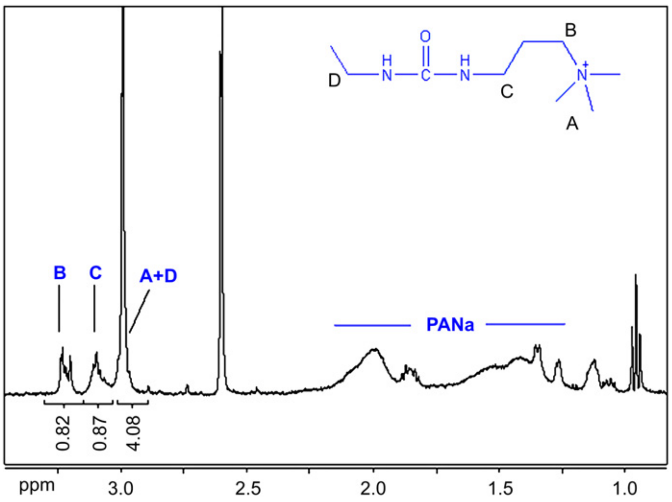


Fig. 4. The ^1H NMR spectrum of ETC/PANa complex at MR of 0.07 measured 30 days after the mixing. The relative intensity ratios of peak B:peak C:peak (A + D) are about 2:2:11, which are the number ratios of respective H atoms in compound **2**. The peaks between 1 ppm and 2.25 ppm were the signals of PANa and the peak at 2.6 ppm was the signal of DMSO that was used as an internal reference.

precipitates appear. However, very interestingly, when NaCl is added in order to change the ionic strength, the fresh aggregates will quickly dissociate in a few hours; when NaCl is added before the formation of the nano-aggregates, no aggregates can form. Obviously, the increase in the ionic strength destroys the connection between the carboxylic anion and the quaternary ammonium.

It is significant that during the dissociation, the hydrophobicity of the nano-aggregates changes with time. We used pyrene as the probe to track the variation in the hydrophobicity of the nano-aggregates. It is known that the intensity ratio of I_{373}/I_{383} in the fluorescence is highly dependent on the polarity of the environment surrounding pyrene molecules [16]. We measured the fluorescence spectra of pyrene in the nano-aggregate solutions at MR of 0.05, 0.07 and 0.09 during the storing. In the spectra of pyrene in the solutions of the newly formed nano-aggregates (several hours after the mixing of PANa with ETC), the values of I_{373}/I_{383} are between 0.6 and 0.7. This indicates that the newly formed nano-aggregates provide hydrophobic domains with a hydrophobicity close to that of hexane [12]. During the storing of the nano-aggregate solutions, the value of I_{373}/I_{383} increases gradually. In the spectra measured 30 days after the formation of the nano-aggregates, the values increase to ca. 1.5, close to that in methanol [16]. That is to say, 30 days after the formation of the nano-aggregates, the hydrophobic domains vanish.

4. Conclusion

In conclusion, we prepared self-dissociable polymeric nano-aggregates via the complexation between ETC and PANa. The formation of *O*-acylisourea due to the reaction between the N=C=N group and a carboxylic anion of PANa leads to the connection between one end of the ETC molecules with a PANa chain. The ammonium cations at another end of ETC molecules complex with the carboxylic anions of other PAN chains, resulting in the aggregation among PANa chains. When MR is below 0.09, regular nano-aggregates with a vesicle-like morphology were formed. Due to the gradual hydrolysis of *O*-acylisourea structure, ETC molecules lose their connection with PANa chains and the nano-aggregates dissociate. Along with the dissociation process, the hydrophobic domains existing in the newly formed nano-aggregates gradually vanish.

References

[1] Duan H, Chen D, Jiang M, Gan W, Li S, Wang M, et al. J Am Chem Soc 2001;123(48):12097–8.

[2] Lysenko EA, Bronich TK, Slonkina EV, Eisenberg A, Kabanov VA, Kabanov AV. Macromolecules 2002;35(16):6351–61.

[3] Lee CT, Huang CP, Lee YD. Biomacromolecules 2006;7(4):1179–86.

[4] Liu XY, Wu J, Kim JS, Eisenberg A. Langmuir 2006;22(1):419–24.

[5] Qian JW, Wang X, Qi GR, Wu C. Macromolecules 1997;30(11):3283–7.

[6] Kakizawa Y, Harada A, Kataoka K. J Am Chem Soc 1999;121(48):11247–8.

[7] Liu S, Armes SP. Langmuir 2003;19(10):4432–8.

[8] Wang J, Kuang M, Duan H, Chen D, Jiang M. Eur Phys J E 2004;15:211–5.

[9] Chung JE, Yokoyama M, Okano T. J Controlled Release 2000;65:93–103.

[10] Li M, Jiang M, Zhang YX, Qin F. Macromolecules 1997;30(2):470–8.

[11] Li Z, Hillmyer MA, Lodge TP. Macromolecules 2006;39(2):765–71.

[12] Rharbi Y, Winnik MA. J Phys Chem B 2003;107(7):1491–501.

[13] Wan X, Tu Y, Zhang D, Zhou Q, Wu C. J Am Chem Soc 2000;122(41):10201–5.

[14] Ma QG, Remsen EE, Clark CG, Kowalewski T, Wooley KL. Proc Natl Acad Sci USA 2002;99:5058–63.

[15] Chen D, Peng H, Jiang M. Macromolecules 2003;36:2576–8.

[16] Kalyanasundaram K, Thomas J. J Am Chem Soc 1977;99:2039–44.