

THERMAL-SENSITIVE BETA-CYCLODEXTRIN-CONTAINING POLY(*N*-ISOPROPYLACRYLAMIDE) HYDROGELS CROSSLINKED BY Si—O—Si BONDS —SYNTHESIS, CHARACTERIZATION AND PROLONGING *IN VITRO* RELEASE OF 5-FLUOROURACIL*

Jian-tao Zhang, Shi-wen Huang, Ya-nan Xue, Ji Liu and Ren-xi Zhuo**

Key Laboratory of Biomedical Polymers, Ministry of Education; Department of Chemistry,
Wuhan University, Wuhan 430072, China

Abstract A series of novel p(*N*-isopropylacrylamide) (PNIPAM) hydrogels were synthesized by radical copolymerization of *N*-isopropylacrylamide (NIPAM) and 3-methacryloxypropyltrimethoxysilane (MPTMS). The copolymers were then crosslinked through hydrolysis of the siloxane in acetic acid/water mixed solvent. Beta-cyclodextrin (Beta-CD) was introduced into the polymeric networks by condensation of 3-glycidioxypropyltrimethoxysilane derived beta-cyclodextrin (KH560-beta-CD) with MPTMS under acidic condition. These gels were heterogeneous, porous and exhibited fast deswelling kinetics when the temperature was elevated to above lower critical solution temperature (LCST). The swelling ratios of the gels containing beta-CD at room temperature were higher than that of the normal PNIPAM hydrogel, which was caused by the lower crosslinking density in beta-CD contained gels. In comparison to that of the normal PNIPAM gel, the amount of loaded-drug in the hydrogel containing beta-CD was higher, and the release time of 5-fluorouracil (5-Fu) was prolonged, which was attributed to the formation of inclusion compounds between 5-Fu and beta-CD in gel network.

Keywords: Hydrogel; Thermal-sensitive; Poly(*N*-isopropylacrylamide); Beta-Cyclodextrin; Fast responsive.

INTRODUCTION

Chemically or physically crosslinked hydrophilic polymeric hydrogel networks can absorb and retain large amounts of water in their scaffolds. The hydrogels, which can change their chemical or physical properties in response to the environmental stimuli including temperature, pH, ion and additives, *etc.*, are regarded as “intelligent” materials. As a typical thermal-sensitive representative, PNIPAM hydrogel has attracted more and more attentions in the past three decades^[1–6]. The special phase transition property of PNIPAM hydrogel has been applied in many biomedical and biotechnological fields, such as drug delivery^[7, 8], tissue engineering^[9], gene carrier^[10] and immobilization of enzyme^[11], *etc.*

The utilizations of PNIPAM hydrogel as biomaterials, especially drug delivery systems, have been well investigated; however, some limitations still exist. For example, the response rate of conventional PNIPAM gel to the temperature change is so slow that it is not suitable for some specific applications, such as the on-off valve and artificial muscle. Moreover, other disadvantages, such as fast release of small molecule drug from PNIPAM gel and low drug loading in PNIPAM matrix also need to be overcome urgently. Many efforts have been made to improve the response properties of PNIPAM hydrogel. Okano's group have prepared rapid-responsive thermal-sensitive PNIPAM hydrogel by introducing pendent chains into the homogenous PNIPAM network to form water release channels^[12–14]. Phase-separated PNIPAM hydrogel has been synthesized by performing the polymerization at a temperature above LCST^[15, 16]. This hydrogel is heterogeneous and porous, which allows

* This work was financially supported by the National Key Basic Research Program of China (973 Program) (No. G1999064703).

** Corresponding author: Ren-xi Zhuo (卓仁禧), E-mail: pibmp@public.wh.hb.cn

Received October 8, 2004; Revised December 10, 2004; Accepted December 20, 2004

water molecules to be squeezed out quickly. We have previously prepared heterogeneous, macroporous PNIPAM hydrogel with fast-responsive deswelling kinetics by carrying out the polymerization in the salt^[17] or sarcosinate^[18, 19] aqueous solutions. We also found that both interpenetrating hydrophilic polymer chains into the polymeric network^[20] and using mixed solvent as reaction media^[21–23] could significantly increase the response rate of the formed PNIPAM hydrogels.

However, to our knowledge, few efforts have been made to improve the drug loading and prolong the release rate of small-molecule drugs from PNIPAM hydrogel. It is well known that beta-CD is a cyclic oligosaccharide with a polar hydrophilic outer and a relatively hydrophobic cavity^[24]. Because of its special ability to form inclusion compounds with many drug molecules, it is an ideal carrier material to design advanced drug delivery systems with the time-controlled or rate-controlled release characteristics^[25, 26]. In this work, a series of novel thermal-sensitive PNIPAM hydrogels containing beta-CD were prepared by carrying out the radical copolymerization of vinyl monomers, followed by the hydrolysis of Si—OMe and crosslinking of Si—OH to form Si—O—Si in water/acetic acid. The formed hydrogels exhibit fast deswelling kinetics when the temperature is increased above LCST. 5-Fu, as a model drug, was loaded into the novel hydrogel. The results suggested that the amount of loaded 5-Fu in the gel containing beta-CD is higher and the release rate is also slower than those of the PNIPAM hydrogel.

EXPERIMENTAL

Materials

N-isopropylacrylamide (NIPAM, Aldrich Chemical Co., Inc., USA) was purified by recrystallization in benzene/*n*-hexane mixed solvent. 3-Glycidioxypropyl-trimethoxysilane derived beta-CD (KH560-beta-CD) was prepared according to the Ref. [27]. 3-Methacryloxypropyltrimethoxysilane (MPTMS, Chemical Plant of Wuhan University, Wuhan, China) was distilled under vacuum before use. Ammonium persulfate (APS), sodium bisulfite (SBS), and glacial acetic acid (HAc) were analytical grade and used as received from Shanghai Chemical Co. (China). 5-Fu (Wuhan Drug Plant, Wuhan, China) was purified by recrystallization with distilled water three times.

Synthesis of Thermal-Sensitive Hydrogels Containing Beta-Cyclodextrin

The monomers NIPAM, MPTMS and KH560-beta-CD were dissolved in water/acetic acid after adding APS and SBS; the reaction mixture was incubated at 25°C for 24 h. The obtained hydrogels were cut into discs (2.5 cm in diameter and 2 mm in thickness) and then immersed in distilled water for several days, during which the water was replaced repeatedly to leach out the unreacted monomers and water-soluble chemicals. The feed composition and sample codes of hydrogels are listed in Table 1.

Table 1. Feed composition and code numbers of hydrogels

Component	Sample code			
	SiG0	SiG1	SiG2	SiG3
NIPAM (mg)	200	200	200	200
KH560-beta-CD (mg)	0	10	20	30
MPTMS (μL)	20	20	20	20
33 wt% APS (μL)	30	30	30	30
33 wt% SBS (μL)	30	30	30	30

Scanning Electron Microscopy Analysis

The swollen hydrogels were dried in a freeze dry system (LABCONCO). After being sputtered by gold, the surface morphologies of the dried gels were observed on a scanning electron microscope (SEM, Hitachi-X650, Japan).

Swelling Ratio Measurement

The swelling ratio (*SR*) of the hydrogels was measured gravimetrically at a temperature range from 22 to 41°C. *SR* is defined as $SR = W_s/W_d$, where W_s is the weight of the water within the hydrogels at a certain temperature

and was determined after wiping off the excess water on the gel surface with moistened filter paper. Before measurement, the gels were conditioned in distilled water for at least 24 h at a particular temperature. W_d is the weight of the dried hydrogels.

Deswelling Kinetics Measurement

The deswelling kinetics of the hydrogels was measured gravimetrically at 37°C. The gels were allowed to swell to equilibrium in distilled water at 25°C, and then the swollen gels were transferred into 37°C distilled water to record the weight at different time. Water retention (WR) was calculated as follows: $WR = 100 \times (W_t - W_d)/W_s$, where W_t is the weight of hydrogels at a certain time during deswelling, W_s and W_d are the same as above.

Loading and Release of 5-Fluorouracil

The swollen hydrogels were immersed into 5-Fu aqueous solution (0.4 wt%) for five days to reach absorption equilibrium. Then, the gels were initially dried in air and then in vacuum overnight. The release of 5-Fu was measured at 25°C, the dried, drug-loaded gel samples were transferred into a beaker, into which 20 mL phosphate buffer saline (PBS) was added. The solution was replaced by 20 mL fresh PBS periodically and the released drug was measured and calculated by the absorption of light at 266 nm using a UV-spectrometer (PERKIN ELMER, Lambda Bio40).

RESULTS AND DISCUSSION

Hydrogel Synthesis

Two kinds of reactions occurred during the formation of hydrogels. The first one is the radical polymerization of NIPAM and MPTMS, which is initiated by the redox system composed of APS and SBS as the initiator and the accelerator, respectively. Another is the hydrolysis of the methoxyl groups in MPTMS to produce silanol and the condensation of the silanols among different linear polymer chains to form Si—O—Si bonds and generate crosslinked polymeric networks. At the same time, beta-CD components as pendant groups were introduced by the hydrolysis of the methoxyl groups in the KH560-beta-CD and condensation of the formed beta-CD-contained silanols with the silanols on the polymer network. The synthesis scheme of the gels is shown in Fig. 1. Because the monomer, MPTMS, is insoluble in water, water/acetic acid as the mixed solvent was used for the gel preparation to allow the monomers to be dissolved adequately. Additionally, acetic acid was used as an organic acid for the hydrolysis of Si—OMe, the condensation and cross-linkage of formed Si—OH to produce Si—O—Si crosslinked hydrogels.

Surface Morphology

The surface morphologies of SiG0 and SiG2 hydrogels are shown in Fig. 2. We can observe that the structures of both gels are heterogeneous and porous. It is known that added chemicals^[28–31] affect the LCST of PNIPAM and phase separation is the prerequisite to prepare the porous materials^[32]. In this work, the mixture of water and acetic acid were poor solvents for the produced polymers. Therefore, in the process of gel formation, phase separation occurred and the heterogeneous structure formed.

Swelling Ratio

The temperature dependence of swelling ratios of the hydrogels at the temperature range from 22 to 41°C is displayed in Fig. 3. It is found that at lower temperature the swelling ratios (SRs) of the hydrogels containing beta-CD (SiG1–SiG3) are higher than that of the PNIPAM gel without beta-CD (SiG0), however, there are no significant differences among SiG1, SiG2 and SiG3. KH560-beta-CD in the polymerization system was introduced into gels (SiG1–SiG3) as pendant groups via the condensation of Si—OMe in KH560-beta-CD with Si—OMe in P(NIPAM-co-MPTMS) chains. The condensation and crosslinking reactions are competitive, which results in that crosslinking density in the gels decreasing with the addition of KH560-beta-CD into the polymerization system. Therefore, the crosslinking density of SiG0 is the highest, while that of SiG3 is the lowest. Taking this ingredient into consideration, the SR of SiG0 is lower than those of gels containing beta-CD. With further increase in the content of KH560-beta-CD in gels, the polymer fraction became higher and SRs of gels decreased. The combination of two opposite factors resulted in that SiG1, SiG2 and SiG3 had almost the same SRs at the temperature below LCST. When the temperature is increased to 34°C, the SRs of all samples decrease sharply, which is regarded as the LCST.

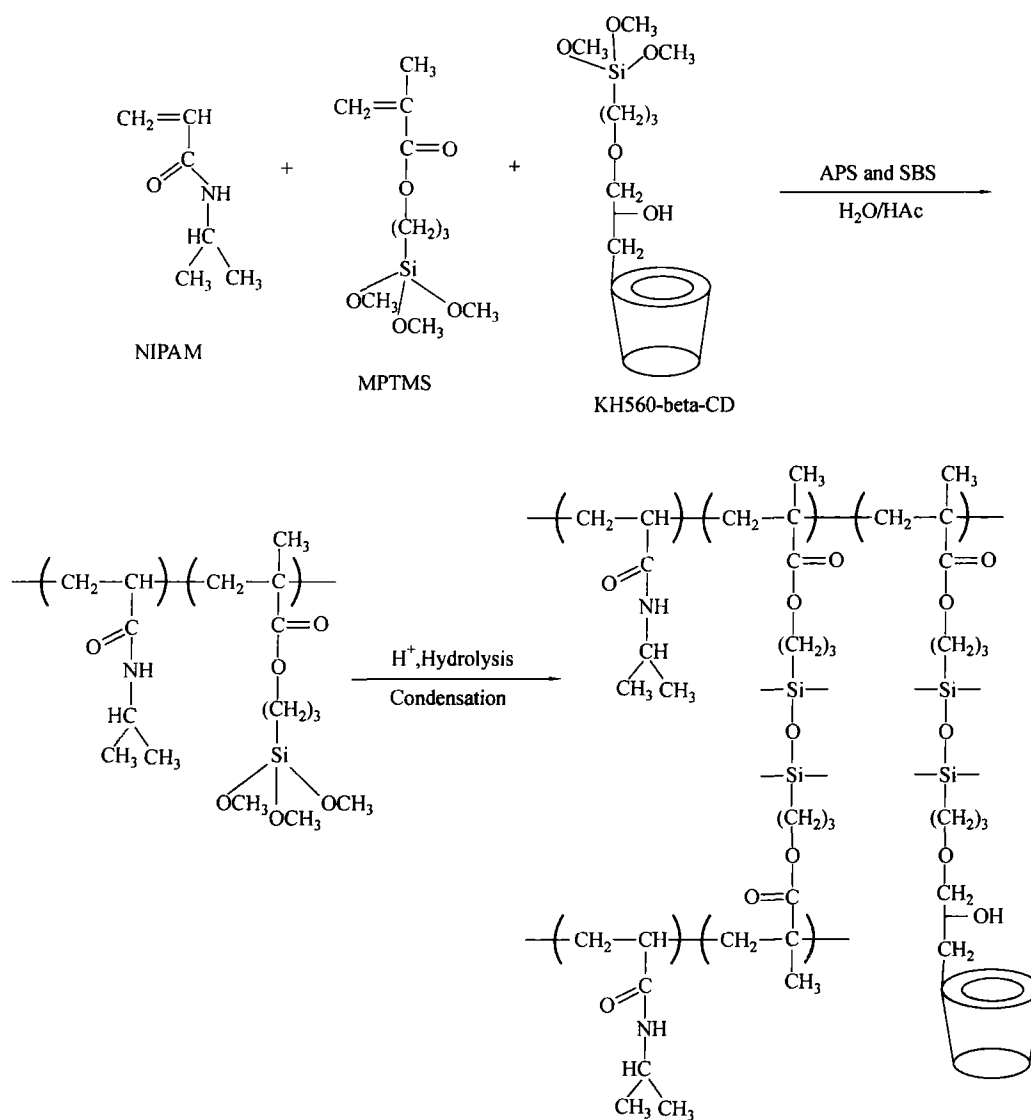


Fig. 1 The synthesis scheme of the PNIPAM hydrogels containing beta-CD

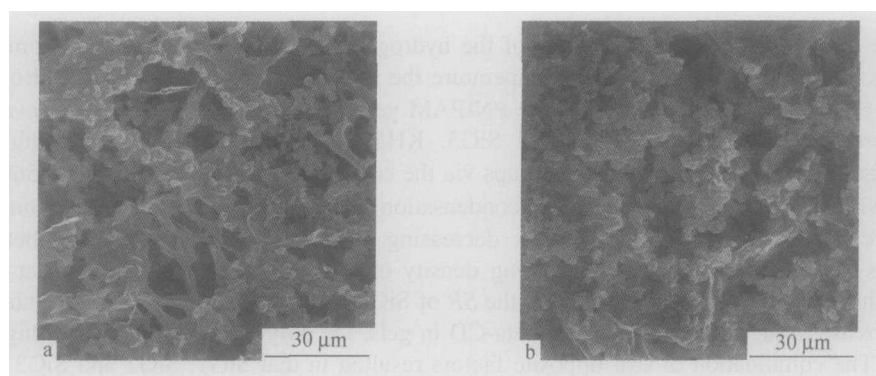


Fig. 2 SEM microphotograph of normal PNIPAM (a, SiG0) and beta-CD incorporated hydrogel (b, SiG2)

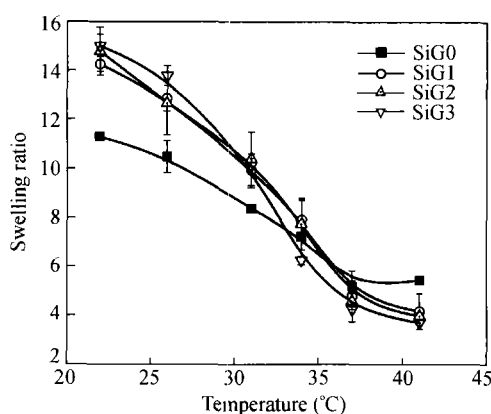


Fig. 3 Temperature dependence of equilibrium swelling ratio of the hydrogels at the temperature range from 22 to 41°C

Deswelling Kinetics

When the swollen hydrogels were transferred into 37°C water, the gels began to shrink. The deswelling kinetics of the hydrogels containing beta-CD (SiG1, SiG2 and SiG3) and the PNIPAM hydrogel (SiG0) is illustrated in Fig. 4, from which it can be observed that all the gels shrink and lose water rapidly at the temperature above LCST and reach their stable equilibrium within several minutes.

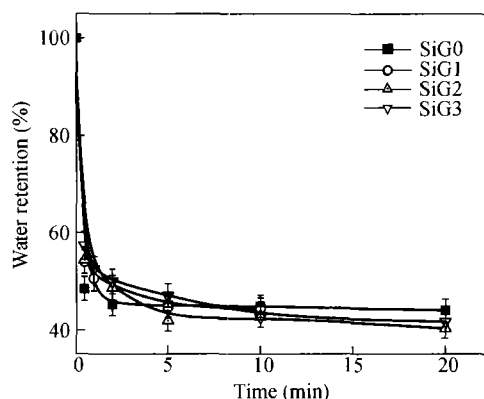


Fig. 4 Deswelling kinetics of the hydrogels containing beta-CD (SiG1, SiG2 and SiG3) and normal PNIPAM hydrogel (SiG0) at 37°C

It is well known that the homogeneous PNIPAM hydrogel prepared by radical polymerization of NIPAM in water deswells slowly, and it needs 24 h to reach equilibrium. This phenomenon is caused by the thick dense layer appearing on the surface of homogeneous PNIPAM hydrogel^[33–35]. However, in this investigation, the hydrogels prepared in acetic acid/water mixed solvent possess heterogeneous porous structures, which allow water molecules to be quickly squeezed out of the gel matrix when the environment temperature is higher than LCST. Therefore, the deswelling kinetics of SiG gels is fast.

5-Fu Release Behaviors

5-Fluorouracil (5-Fu), as a model drug, was loaded into the gels by absorption method. The dried drug-loaded hydrogels SiG0 and SiG2 were immersed in PBS at 25°C to study their release behaviors. The release kinetics of 5-Fu from the hydrogels is displayed in Fig. 5. We can find that there is an ‘explosion’ release for both hydrogels at the first stage. Then, drug molecules diffuse out steadily afterwards and almost all the loaded drug is released within several hours. We also find that the release rate of 5-Fu from SiG0 is faster than that of SiG2. The reasonable explanation is that the beta-CD groups in SiG2 hydrogel network may form complex compounds with

part of 5-Fu molecules by host-guest interactions, which results in a slower release rate of 5-Fu from SiG2 hydrogel. Additionally, the amount of loaded 5-Fu in SiG2 gel (0.0478 mg/mg dried gel) is higher than that in SiG0 (0.0427 mg/mg dried gel), which can partially confirm the above presumption.

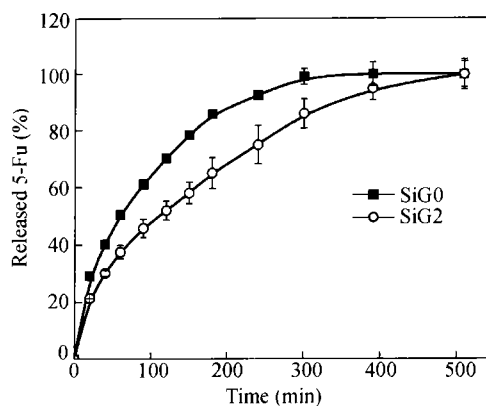


Fig. 5 Cumulative release of 5-Fu from the normal PNIPAM gel (SiG0) and beta-CD contained hydrogel (SiG2) as a function of time in PBS at 25°C

CONCLUSION

A series of novel thermal-sensitive PNIPAM hydrogels containing beta-CD were prepared by carrying out the polymerization in acetic acid/water mixed solvent. Due to the phase separation in the process of gel formation, the gels have heterogeneous and porous microstructure. When the temperature was raised to above LCST, all the hydrogels can rapidly shrink to equilibrium. Compared to that of PNIPAM hydrogel, the release time of 5-Fu from beta-CD-containing hydrogel was greatly prolonged, which may probably be ascribed to the formation of inclusion complexes between CD and drug molecules.

REFERENCES

- 1 Hu, Z.B., Chen, Y.Y., Wang, C.J., Zheng, Y.D. and Li, Y., *Nature*, 1998, 393: 194
- 2 Soga, O., van Nostrum, C.F., Ramzi, A., Visser, T., Soulimani, F., Frederik, P.M., Bomans, P.H.H. and Hennink, W.F., *Langmuir*, 2004, 20: 9388
- 3 Shibayama, M. and Nagai, K., *Macromolecules*, 1999, 32: 7461
- 4 Zhang, X.Z. and Zhuo, R.X., *Langmuir*, 2001, 17: 12
- 5 Zhang, J. and Peppas, N.A., *Macromolecules*, 2000, 33: 102
- 6 Yusa, S., Shimada, Y., Mitsukami, Y., Yamamoto, T. and Morishima, Y., *Macromolecules*, 2004, 37: 7507
- 7 Zhang, X.Z., Zhuo, R.X., Cui, J.Z. and Zhang, J.T., *Int. J. Pharm.*, 2002, 235: 43
- 8 Chung, J.E., Yokoyama, M. and Okano, T., *J. Control. Rel.*, 2000, 65: 93
- 9 Lee, K.Y. and Mooney, D.J., *Chem. Revs.*, 2001, 101: 1869
- 10 Kurisawa, M., Yokoyama, M. and Okano, T., *J. Control. Rel.*, 2000, 69: 127
- 11 Liu, F., Tao, G.L. and Zhuo, R.X., *Polym. J.*, 1993, 25: 561
- 12 Yoshida, R., Uchida, K., Kaneko, Y., Sakai, K., Kikuchi, A., Sakurai, Y. and Okano, T., *Nature*, 1995, 374: 240
- 13 Kaneko, Y., Sakai, K., Kikuchi, A., Yoshida, R., Sakurai, Y. and Okano, T., *Macromolecules*, 1995, 28: 7717
- 14 Kaneko, Y., Nakamura, S., Sakai, K., Aoyagi, T., Kikuchi, A., Sakurai, Y. and Okano, T., *Macromolecules*, 1998, 31: 6099
- 15 Kabra, B.G. and Gerhrke, S.H., *Polym. Commun.*, 1991, 32: 322
- 16 Wu, X.S., Hoffman, A.S. and Yager, P., *J. Polym. Sci., Part A: Polym. Chem.*, 1992, 30: 2121
- 17 Cheng, S.X., Zhang, J.T. and Zhuo, R.X., *J. Biomed. Mater. Res.*, 2003, 67A: 96
- 18 Zhang, J.T., Cheng, S.X. and Zhuo, R.X., *J. Polym. Sci., Part A: Polym. Chem.*, 2003, 41: 2390

- 19 Zhang, J.T., Cheng, S.X., Huang, S.W. and Zhuo, R.X., *Macromol. Rapid. Commun.*, 2003, 24: 447
- 20 Zhang, J. T., Cheng, S.X. and Zhuo, R.X., *Colloid Polym. Sci.*, 2003, 281: 580
- 21 Zhang, X.Z., Zhuo, R.X. and Yang, Y.Y., *Biomaterials*, 2002, 23: 1313
- 22 Zhang, X.Z. and Zhuo, R.X., *Eur. Polym. J.*, 2000, 36: 643
- 23 Zhang, J.T., Huang, S.W. and Zhuo, R.X., *Macromol. Chem. Phys.*, 2004, 205: 107
- 24 Ritter, H. and Tabatabai, M., *Prog. Polym. Sci.*, 2002, 27: 1713
- 25 Hirayama, F. and Uekama, K., *Adv. Drug Deliv. Rev.*, 1999, 36: 125
- 26 Redenti, E., Pietra, C., Gerloczy, A. and Szente, L., *Adv. Drug Deliv. Rev.*, 2001, 53: 235
- 27 Feng, Y.Q., Xie, M.J. and Da, S.L., *Anal. Chim. Acta*, 2000, 403: 187
- 28 Annaka, M., Motokawa, K., Sasaki, S., Nakahira, T., Kawasaki, H., Maeda, H., Amo, Y. and Tominaga, Y., *J. Chem. Phys.*, 2000, 113: 5980
- 29 Kawasaki, H., Sasaki, S., Maeda, H., Mihara, S., Tokita, M. and Komai, T., *J. Chem. Phys.*, 1996, 100: 16282
- 30 Sasaki, S., Koga, S. and Annaka, M., *J. Phys. Chem. B*, 2003, 107: 6893
- 31 Takigawa, T., Araki, H., Takahashi, K. and Masuda, T., *J. Chem. Phys.*, 2000, 113: 7640
- 32 Okay, O., *Prog. Polym. Sci.*, 2000, 25: 711
- 33 Nakamoto, C., Motonaga, T. and Shibayama, M., *Macromolecules*, 2001, 34: 911
- 34 Zhang, J.T., Huang, S.W. and Zhuo, R.X., *Macromol. Biosci.*, 2004, 4: 575
- 35 Hirose, H. and Shibayama, M., *Macromolecules*, 1998, 31: 5336