ACS APPLIED MATERIALS & INTERFACES

Letter

www.acsami.org

New Monolithic Capillary Columns with Well-Defined Macropores Based on Poly(styrene-*co*-divinylbenzene)

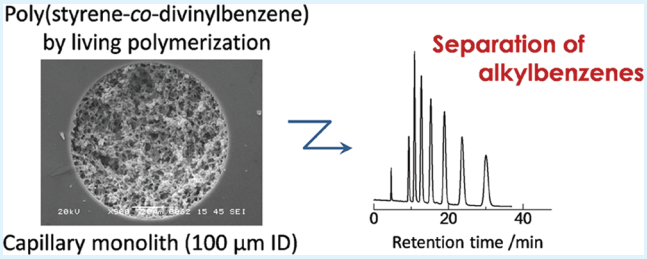
George Hasegawa,[†] Kazuyoshi Kanamori,[†,*] Norio Ishizuka,[‡] and Kazuki Nakanishi[†]

[†]Department of Chemistry, Graduate School of Science, Kyoto University, Kitashirakawa, Sakyo-ku, Kyoto 606-8502, Japan

[‡]Emaus Kyoto, Inc., 26 Saiin Nishida-cho, Ukyo-ku, Kyoto 615-0055, Japan

Supporting Information

ABSTRACT: Macroporous polymer monoliths based on poly(styrene-*co*-divinylbenzene) with varied styrene/divinylbenzene ratios have been prepared by organotellurium-mediated living radical polymerization. The well-defined cocontinuous macroporous structure can be obtained by polymerization-induced spinodal decomposition, and the pore structures are controlled by adjusting the starting composition. The separation efficiency of small molecules (alkylbenzenes) in the obtained monoliths has been evaluated in the capillary format by high-performance liquid chromatography (HPLC) under the isocratic reversed-phase mode. Baseline separations of these molecules with a low pressure drop (~2 MPa) have been achieved because of the well-defined macropores and to the less-heterogeneous cross-linked networks.



KEYWORDS: *polymer monoliths, controlled/living radical polymerization, co-continuous macroporous structure, HPLC separation media, capillary columns, separation of small molecules*

Porous materials based on organic polymers are extensively studied for broad applications such as separation, adsorption, catalysis and filters.[1−4] Fine controls of porosity, pore size and shape are central issues in the research for the effective applications. Among these, monolithic separation media based on porous organic polymers (so-called polymer monoliths) have been playing an important role especially as the stationary phase for separation of large molecules such as peptides, proteins and polynucleotides, both in pressure-driven and electroosmotic modes of high-performance liquid chromatography (HPLC).[5−16] The effective separations of such large molecules are attributed to the absence of micro- and mesopores in each microglobule, which are formed as a result of free radical polymerization of vinyl and multivinyl monomers and consists of the porous structure. Because the diffusional mass transport of large molecules becomes exceedingly slower if the microglobules are porous, the nonporous microglobules are advantageous in large-molecules separations. Meanwhile, the conventional polymer monoliths are not effective for the separation of small molecules.

Recently, several attempts have been made to improve the separation of small molecules with polymer monoliths. Careful structural controls by finely tuning the porosity or by hypercrosslinking[17] have found to be effective in small molecules separations.[18−22] Although the limiting factors for small molecule separations are complicated, the major problem lies in the state of molecular-level (approximately less than 1 nm) micropores, which are inherently formed in the networks during polymerization. These micropores may exist in the networks when swollen by the mobile phase even if no micropores are found by the gas adsorption measurement on dried samples. Too extended diffusion and enhanced retention of analytes in these narrow interstices may considerably broaden the elution bands. In our previous study, a silica-coated monolith without undergoing drying exhibited too strong retentions of alkylbenzenes and elution bands were considerably broadened because of the micropores.[23] To avoid the inherent heterogeneity in the networks, controlled/living radical polymerization (CRP) would become a good candidate. It is well-known that more homogeneous networks form by CRPs as a result of the gradual increase of molecular weight vs reaction time.[24]

Peters et al. and Viklund et al. used CRP to prepare styrene (S)-divinylbenzene (DVB)-based polymer monoliths.[25,26] Macroporous cross-linked polymer networks were obtained as a result of nucleation and growth-type phase separation in the low-molecular-weight solvent. It was shown that the monoliths exhibit a good performance in the size exclusion chromatography (SEC) mode and that a surface modification by grafting from the living growing end is available for functionalization. However, no reports were given on the separation of small molecules. Although some other polymer monoliths prepared by living polymerization have been reported so far, no separation of small molecules has been demonstrated up to now.[27−30]

Received: March 29, 2012
Accepted: April 24, 2012
Published: April 24, 2012

Since 2006, we have reported a new class of polymer monoliths with the well-defined cocontinuous macroporous structure regulated by spinodal decomposition, which is induced in the homogeneous networks derived from CRPs.[31−35] It has also been reported that the independent control of macropore size and volume is possible, though it is difficult in the conventional polymer monoliths. In this study, we have prepared polymer monolithic capillary columns with different cross-linking density from traditional S-DVB comonomers by organotellurium-mediated living radical polymerization (TERP), and for the first time investigated their separation performances of small molecules.

The detailed experimental procedure is provided in the Supporting Information as well as the starting compositions in Table S1. For poly(divinylbenzene) monoliths prepared without styrene, the sample code starts with PDVB followed by the number describing the weight of PDMS (in mg). In the case of poly(styrene-*co*-divinylbenzene), the sample code starts with PSDVB followed by the volume ratio of S/DVB and the weight of PDMS (in mg). A given amount of PDMS (M_w = 9000−10000) was dissolved in a solution mixture of S, DVB (80%, mixture of isomers) and 1,3,5-trimethylbenzene (TMB). To the degassed mixture was added 2,2′-azobis-(isobutyronitrile) (AIBN) followed by purging with nitrogen for 10 min, and subsequently ethyl-2-methyl-2-butyltellanyl propionate (BTEE), a promoter of TERP, was added. The resultant solution was transferred to an ampule or to a fused silica capillary (100 μm ID, methacrylated in advance) and kept at 80 °C for 48 h for polymerization and aging. The originally single-phase transparent solution turns into opaque due to the polymerization-induced phase separation, followed by gelation which can be determined by the loss of fluidity. The obtained gels were washed with tetrahydrofuran (THF), followed by evaporative drying at 60 °C for 24 h. For separation tests, the as-prepared undried monoliths were connected to the HPLC system and pumped with THF to thoroughly wash before the separation tests. A mixture of water and acetonitrile (ACN) (20:80 in volume) were employed as the mobile phase for the isocratic separation of alkylbenzene derivatives ($C_nH_{2n+1}Ph$, n = 0−6). Uracil was used as an unretailed molecule.

Since we only have previously reported PDVB monoliths prepared solely from DVB, first we have investigated the effect of S on the formation of macroporous structure before extending to the capillary columns. The increase in S/DVB ratio in the starting composition naturally results in the decrease in the cross-linking density of the network, which should cause large influences on the macroporous structure. The increase in the amount of S increased the gelation time due to the decrease in the concentration of vinyl groups, when all other components are fixed. Figure S1 in the Supporting Information exhibits the changes in macroporous structures of the PDVB and PSDVB monoliths prepared with varied amounts of PDMS while fixing the amounts of all other components. As discussed in the previous report,[32] the increasing amount of PDMS enlarges the macropore size in each S/DVB ratio because PDMS enhances the thermodynamic instability of the polymerizing system, which will result in the freezing of more coarsened phase-separated structure. It can be seen from Figure S1, the pore structure became coarser and the volume fraction of the network increased with increasing S; as an extreme case, the morphology with isolated pores is observed in the sample PSDVB-3−600 as shown in Figure S1g in the Supporting Information. The macroscopic phase separation into the precipitation and supernatant took place in the samples PSDVB-3−700 and PSDVB-3−800 (Figure S1h, i in the Supporting Information) because the phase separation tendency was too high, and this behavior is more enhanced when S/DVB = 4 (not shown). It is deduced that the lower cross-linking density with increasing S results in the higher swelling capacity predominantly with the solvent. The volume fraction of the network therefore is increased by swollen with TMB, and the lower porosity with the isolated-pores structure was found. With the higher S/DVB ratio, in addition, the phase-separated structures are more extensively coarsened during the longer gelation time; 80−90 min for S/DVB = 0, 110−120 min for S/DVB = 1, 160−170 min for S/DVB = 3 and 170−180 min for S/DVB = 4. This drives the systems into macroscopic phase separation to precipitation and supernatant.

In order to decrease the phase separation tendency and obtain well-defined macroporous structures with higher S/DVB (= 3 and 4), we found the best way is to reduce the concentration of the living radical polymerization promoter, BTEE. The decrease in the amount of BTEE leads to faster gelation and to the decrease in the population of growing polymers. The faster gelation fixes gel morphology earlier, leading to the finer pore strctures. The smaller population of growing polymers increases the molecular weight of each polymer. Since such situation lowers the swelling capacity of the branched/cross-linked polymers with the solvent TMB, the volume fraction of the resultant network is decreased (porosity increased) in the opposite way to the case of increasing S/DVB discussed above. Hence, by decreasing the amount of BTEE with fixing the other compositions, the pore structure becomes finer and changed from the isolated pores to cocontinuous structures as shown in Figure S2. As a result, the well-defined macroporous monoliths suitable for separation media can be successfully obtained even when the volume ratios S/DVB = 3 and 4 simply by decreasing the amount of BTEE.

In the capillaries confined in 100 μm ID, the obtained morphologies tend to become coarser compared to those prepared in the free space because of the wetting-induced coarsening effect.[36] The PDVB (S/DVB = 0) and PSDVB (S/DVB = 1 and 4) capillary columns have been prepared from the fixed monomers and different BTEE concentrations, which ideally leads to the identical porosity. In order for adjusting in the similar macropore size in each S/DVB ratio, the amount of PDMS and TMB was slightly varied. The macroporous structures of the obtained capillary monoliths are shown in Figure 1 with their starting compositions in Table 1. The well-defined cocontinuous macroporous structures similar to the bulk samples are successfully obtained. These capillary monoliths have been evaluated as the stationary phase without drying to avoid the possible shrinkage.

Figure 2 exhibits chromatograms of the obtained PDVB and PSDVB columns, and Figure 3 shows the height equivalent to a theoretical plate (H)−linear velocity (u) curves and pressure drop (Δp)−u curves for each column. Linear velocity in each column is set almost equal in the chromatograms. The alkylbenzenes are successfully separated in the reversed phase mode using water/ACN = 20/80 (in volume ratio) mobile phase with relatively low back pressures. Among PDVB series with S/DVB = 0, the macropore size increases in the order of PDVB-800 < PDVB-820 < PDVB-850, which can also be confirmed by the decreasing pressure drop (Figure 3b). The retention factors (k) for hexylbenzene are 8.0, 9.9, and 6.9, respectively. In fact, the macropore skeletons of the PDVB

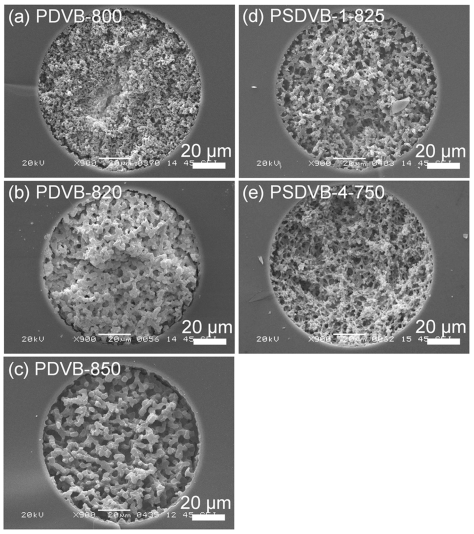


Figure 1. SEM images of PDVB and PSDVB monoliths prepared in a fused silica capillary (100 μm ID). The starting compositions are shown in Table 1.

Table 1. Starting Compositions of the Polymer Monolithic Capillary Columns Investigated in This Study[a]

code	*S* (mL)	DVB (mL)	PDMS (g)	BTEE (μL)
PDVB-800		5.0	0.800	100
PDVB-820		5.0	0.820	100
PDVB-850		5.0	0.850	100
PSDVB-1−825	2.5	2.5	0.825	80
PSDVB-4−750	4.0	1.0	0.750	40

[a]Amounts of TMB and AIBN are fixed in 10 mL and 0.029 g.

monoliths contain smaller pores less than *ca.* 100 nm, and pore volume of these pores and specific surface area decrease with increasing amount of PDMS.[31,32] Hence, the retention factor should become smaller with increasing PDMS. The reason for the exceptionally small retention in PDVB-800 is still unclear but is presumably related with finer and disordered macroporous structures. The highest theoretical plate number $N \approx 34\,300$ m^{-1} for benzene and $N \approx 27\,400$ m^{-1} for the most retained hexylbenzene are observed in the PDVB-820 monolith (100 μm ID and 250 mm long) at $u = 0.50$ mm s^{-1} and $\Delta p = 2.1$ MPa.

The monoliths prepared from the S-DVB mixtures (PSDVB-1−825 and PSDVB-4−750) possess comparable macropore size with PDVB-820, which also can be confirmed by the $\Delta p-u$ curves of these three samples (Figure 3b). However, the retention factors of the PSDVB monoliths are smaller than PDVB-820 ($k = 7.1$ for PSDVB-1−825 and $k = 5.5$ for PSDVB-4−750, compared to $k = 9.9$ for PDVB-820). The smaller pores inside the macropore skeletons are formed by secondary phase separation in the networks,[32] and the lower cross-linking density with increasing S renders the networks more compatible with PDMS and more deformable. The former factor hampers the occurrence of secondary phase separation and makes the resultant skeletons less porous. In addition, since the networks are deduced to be more inherently homogeneous because of the less cross-linking density,[37] the analytes spend less time in the networks. All these factors lead to the smaller *k*. The less porosity in the skeletons can be speculated by the nitrogen adsorption−desorption isotherms as shown in Figure

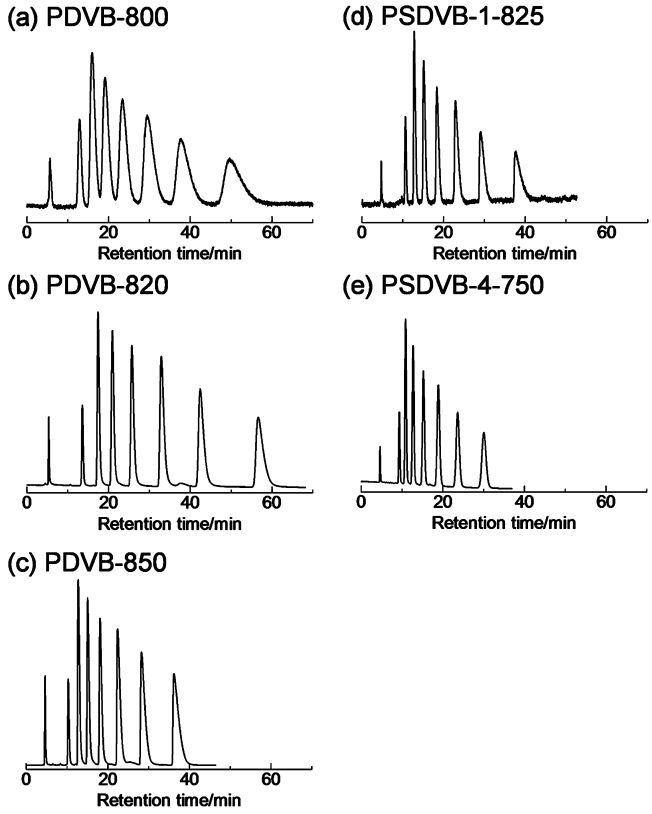


Figure 2. Chromatograms of the isocratic reverse-phase separations of alkylbenzenes using PDVB and PSDVB monolithic capillary columns (100 μm ID and 250 mm length). The sample mixture contains (1) uracil, (2) benzene, (3) toluene, (4) ethylbenzene, (5) propylbenzene, (6) butyl benzene, (7) amylbenzene, and (8) hexylbenzene. Mobile phase, acetonitrile/water = 80/20 (v/v); linear velocity ≈ 1.0 mm s^{-1}; and detection, UV at 254 nm.

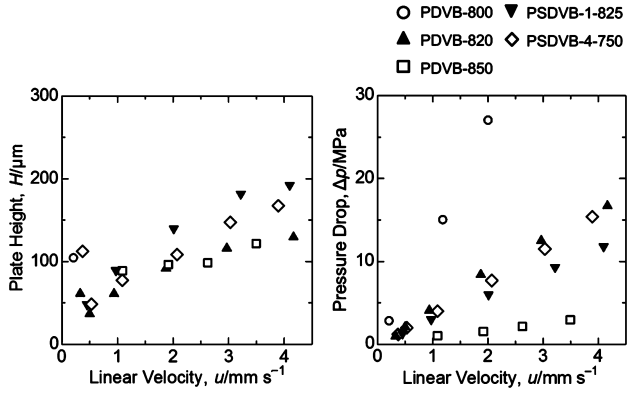


Figure 3. (Left) Van Deemter curves of the capillary monoliths. The minimum plate height (H) was obtained at the linear velocity $u \approx 0.5$ mm s^{-1} in all cases except for PDVB-800. (Right) Relationship between pressure drop (Δp) and linear velocity for all samples. Plate height values for PDVB-800 in the van Deemter plot are out of range at linear velocities higher than 0.25 mm s^{-1}.

S3 in the Supporting Information. Although we should note that the monoliths are wet and weakly swollen by the polar mobile phase during the separation tests while dried when measuring nitrogen adsorption−desorption isotherms, but the considerable reduction in micro- and mesoporosity with increasing S suggests the different state of networks during swollen with the mobile phase. The above discussion is also

supported by the samples dried with supercritical CO_2, which gave the similar results as in Figure S3 in the Supporting Information.

There have been reports on the separations of small molecules by optimizing the network structure and separation conditions (injection amount, column temperature, etc.). We believe the "effective" molecular-level micropores in the swollen state give the critical effect on the separation functions, while the micro- and mesopores enough larger than analyte molecules improve the low retention on polymeric networks. Hypercrosslinking using the Friedel–Crafts reaction after the radical cross-linking of monomers ensures a higher mechanical stability when contacted with the mobile phase,[21] also in which we deduce the molecular-level micropores are reduced by the post-cross-linking, resulting in the good separation performance. Veverka et al. have pointed out that absorption (swelling) inside the networks is no longer important in liquid-phase sorption of aromatic hydrocarbons to the hypercrosslinked sorbents.[38] Adsorption and pore filling in the micro- and mesopores should cause retention in the highly cross-linked networks like hypercrosslinked ones and our PDVB monoliths. The present preliminary results also suggest the better-controlled networks by CRP and the cocontinuous macroporous structure are advantageous in small molecules separations. Further efforts on controling over the molecular-level structure will enhance the separation efficiency, which is currently undergoing.

In conclusion, macroporous poly(divinylbenzene) (PDVB) and poly(styrene-*co*-divinylbenzene) (PSDVB) monoliths with well-defined cocontinuous structure have been prepared by controlled/living radical polymerization (CRP). Monoliths with porous structures suitable for chromatographic separation have been obtained with styrene (S)/divinylbenzene (DVB) volume ratios of 0–4. Monolithic capillary columns based on PDVB and PSDVB networks have been for the first time tested as the separation media for small molecules (alkylbenzenes) in the isocratic reversed phase mode. The separation of these small molecules was successful with a relatively low back pressure owing to the high regularity of macropores and intrinsically less-heterogeneous networks derived from CRP. Further improvement of efficiency is expected by optimizing the homogeneity of network and separation conditions.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Detailed experimental procedure; additional figures and table (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel/Fax: +81-75-753-7673. E-mail: kanamori@kuchem.kyoto-u.ac.jp.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The present work was supported by the Grant-in-Aid for Scientific Research (22·75 for G.H., 22750203 for K.K.) from the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan. Adaptable and Seamless Technology Transfer Program through Target-Drived R&D (A-STEP) from Japan Science and Technology Angency (JST) is also acknowledged.

■ REFERENCES

(1) Okay, O. *Prog. Polym. Sci.* **2000**, *25*, 711–779.
(2) Svec, F.; Tennikova, T. B.; Deyl, Z., Eds. In *Monolithic Materials: Preparation, Properties and Applications*; Elsevier: Amsterdam, 2003.
(3) Hillmyer, M. A. *Adv. Polym. Sci.* **2005**, *190*, 137–181.
(4) Jones, B. H.; Lodge, T. P. *Polym. J.* **2012**, *44*, 131–146.
(5) Svec, F.; Fréchet, J. M. J. *Anal. Chem.* **1992**, *64*, 820–822.
(6) Peters, E. C.; Petro, M.; Svec, F.; Fréchet, J. M. J. *Anal. Chem.* **1997**, *69*, 3646–3649.
(7) Gusev, I.; Huang, X.; Horváth, C. *J. Chromatogr. A* **1999**, *855*, 273–290.
(8) Ericson, C.; Holm, J.; Ericson, T.; Hjertén, S. *Anal. Chem.* **2000**, *72*, 81–87.
(9) Hoegger, D.; Freitag, R. *J. Chromatogr. A* **2001**, *914*, 211–222.
(10) Lee, D.; Svec, F.; Fréchet, J. M. J. *J. Chromatogr. A* **2004**, *1051*, 53–60.
(11) Reichmuth, D. S.; Shepodd, T. J.; Kirby, B. J. *Anal. Chem.* **2005**, *77*, 2997–3000.
(12) Urban, J.; Jandera, P.; Schoenmakers, P. *J. Chromatogr. A* **2007**, *1150*, 279–289.
(13) Li, Y.; Tolley, H. D.; Lee, M. L. *J. Chromatogr. A* **2010**, *1217*, 4934–4945.
(14) Liu, M.; Liu, H.; Liu, Y.; Bai, L.; Yang, G.; Yang, C.; Cheng, J. *J. Chromatogr. A* **2011**, *1218*, 286–292.
(15) Buchmeiser, M. R.; , *Polymer* **207**, *48*, 2187-2198.
(16) Svec, F. *J. Chromatogr. A* **2010**, *1217*, 902–924.
(17) Veverka, P.; Jeřábek, K. *React. Funct. Polym.* **1999**, *41*, 21–25.
(18) Coufal, P.; Čihák, M.; Suchánková, J.; Tesařová, E.; Bosáková, Z.; Štulik, K. *J. Chromatogr. A* **2002**, *946*, 99–106.
(19) Moravcová, D.; Jandera, P.; Urban, J.; Planeta, J. *J. Sep. Sci.* **2003**, *26*, 1005–1016.
(20) Lubbad, S. H.; Buchmeiser, M. R. *J. Chromatogr. A* **2010**, *1217*, 3223–3230.
(21) Urban, J.; Svec, F.; Fréchet, J. M. J. *J. Chromatogr. A* **2010**, *1217*, 8212–8221.
(22) Nischang, I.; Teasdale, I.; Brüggemann, O. *J. Chromatogr. A* **2010**, *1217*, 7514–7522.
(23) Kanamori, K.; Nakanishi, K.; Hanada, T. *J. Sep. Sci.* **2006**, *29*, 2463–2470.
(24) Gao, H.; Matyjaszewski, K. *Prog. Polym. Sci.* **2009**, *34*, 317–350.
(25) Peters, E. C.; Svec, F.; Fréchet, J. M. J. *Macromolecules* **1999**, *32*, 6377–6379.
(26) Viklund, C.; Nordström, A.; Irgum, K. *Macromolecules* **2001**, *34*, 4361–4369.
(27) Buchmeiser, M. R.; Atzl, N.; Bonn, G. K. *J. Am. Chem. Soc.* **1997**, *119*, 9166–9174.
(28) Sinner, F.; Buchmeiser, M. R. *Macromolecules* **2000**, *33*, 5777–5786.
(29) Zhang, R.; Qi, L.; Xin, P.; Yang, G.; Chen, Y. *Polymer* **2010**, *51*, 1703–1708.
(30) Yang, G.; Bai, L.; Yan, C.; Gu, Y.; Ma, J. *Talanta* **2011**, *85*, 2666–2672.
(31) Kanamori, K.; Nakanishi, K.; Hanada, T. *Adv. Mater.* **2006**, *18*, 2407–2411.
(32) Hasegawa, J.; Kanamori, K.; Nakanishi, K.; Hanada, T.; Yamago, S. *Macromolecules* **2009**, *42*, 1270–1277.
(33) Kanamori, K.; Hasegawa, J.; Nakanishi, K.; Hanada, T. *Macromolecules* **2008**, *41*, 7186–7193.
(34) Hasegawa, G.; Kanamori, K.; Nakanishi, K.; Yamago, S. *Polymer* **2011**, *52*, 4644–4647.
(35) Hasegawa, G.; Kanamori, K.; Nakanishi, K.; Hanada, T.; Yamago, S. *Macromol. Rapid Commun.* **2009**, *30*, 986–990.
(36) Kanamori, K.; Yonezawa, H.; Nakanishi, K.; Hirao, K.; Jinnai, H. *J. Sep. Sci.* **2004**, *27*, 874–886.
(37) Yu, Q.; Zhou, M.; Ding, Y.; Jiang, B.; Zhu, S. *Polymer* **2007**, *48*, 7058–7064.

(38) Veverka, P.; Jeřábek, K. *React. Funct. Polym.* **2004**, *59*, 71−79.