Transactions of the Materials Research Society of Japan 29[7] 3179-3182 (2004)

Preparation of Mesoporous Silica Films Filled with Various Amino Acids and Peptides in Their Nanopores

Katsuhiko Ariga,*[a] Qingmin Zhang,[a] Akihiro Okabe,[a] Makiko Niki,[a] Takuzo Aida*[a]
Tomoyuki Koga,[b] Yukihiro Kojima,[b] and Takatoshi Kinoshita[b]
[a] ERATO Nanospace Project, JST
c/o National Museum of Emerging Science and Innovation, 2-41 Aomi, Koto-ku, Tokyo 135-0064, Japan.
Fax: +81-3-3570-9183, e-mail: ariga@nanospace.miraikan.jst.go.jp
[b] Department of Materials Science and Engineering, Nagoya Institute of Technology
Gokiso-cho, Showa-ku, Nagoya 466-8555, Japan.
Fax: +81-52-735-5267, e-mail: kinosita@mse.nitech.ac.jp

Mesoporous silica films densely filled with various amino acids and dipeptides in their nanopores have been successfully synthesized using template surfactants possessing the corresponding residues. The highly regular structure of the prepared films in hexagonal geometry was confirmed by XRD measurement and TEM observation. In addition, we have pioneered preparation of mesoporous silica films filled with an amphiphilic polypeptide that was composed of a hydrophilic poly(ethylene glycol) chain (MW, ca. 3000) and a hydrophobic peptide segment with defined length (leucine 16mer). Structural regularity of the prepared transparent films was again verified by XRD and TEM measurements. Preservation of α-helix structure of the poly(leucine) was confirmed by CD and FT-IR spectroscopies. It is the first example for successful preparation of mesoporous silica film confining polypeptide assemblies with a defined secondary structure.

Key words: Mesoporous Silica, Film, Peptide, Secondary Structure, α-Helix

1. INTRODUCTION

Mesoporous silica is expected to be used in a wide range of applications.[1-5] Unfortunately, the mesoporous composites of silica and biological components have not been fully explored, although they have great potential in applications such as molecular separation, sensing, drug release, chirality-based chemistry, and enzyme-like catalysis with advantageous silica characteristics of possible biocompatibility, huge surface area, easy coating and so on. Peptides are especially attractive candidates as biological partners of the composites because they have well-defined assembling structures and a high density of chiral centers. As related materials, structurally organized silica particle containing polypeptides,[6] bio-inspired silica materials,[7,8] and protein-entrapped mesoporous silica[9-11] were reported so far. However, preparation of mesoporous silica densely confining peptide assemblies in size-controlled nanopores has never been established, although we already presented several preliminary efforts.[12-14] In this paper, we exemplify preparation of mesoporous silica films using various amphiphilic amino acids and peptides as templates, and the first example of α-helix confinement in mesoporous silica medium is demonstrated.

2. RESULTS AND DISCUSSION

2.1 Film structure

Formulae of amphiphilic amino acids and peptides used as templates for mesoporous silica film preparation are shown in Fig. 1. Amino acid residues used in this

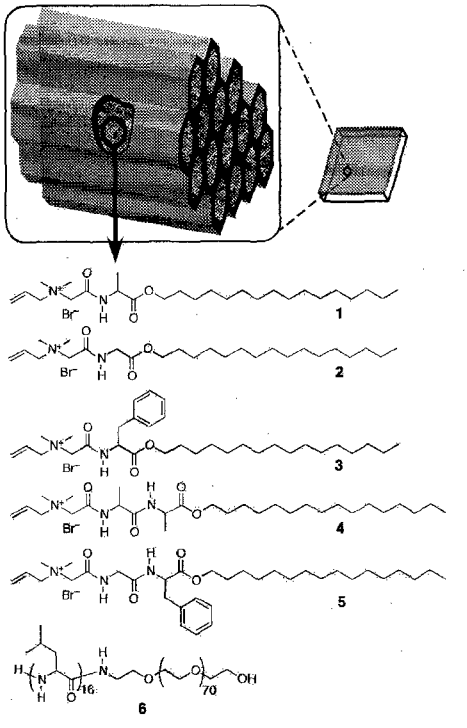

Fig. 1 Schematic image of mesoporous silica film and formulae of template amphiphiles used in this research.

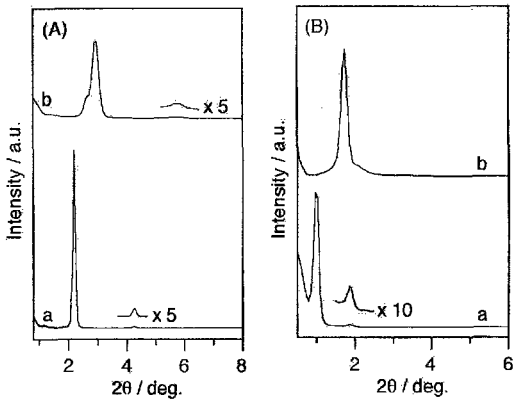

Fig. 2 XRD profiles of the mesoporous silica films templated by **1** (A) and templated by **6** (B): a, before calcination; b, after calcination.

research are all in L configuration. Prior to investigation of peptide-containing mesoporous silica film, synthetic condition for mesoporous silica film was first examined using a simple alanine-based template (**1**) in order to establish standard preparative guideline. Under optimized condition (see EXPERIMENTAL), highly transparent films with very sharp X-ray diffraction (XRD) peaks (Fig. 2A(a)) were obtained. The *d*-spacing values in this XRD pattern appeared at ca. 4.2 nm reasonably reflecting the length of the peptide molecules. Although only (100) and (200) peaks were observable in the XRD profile of the **1**-templated film, XRD patterns of powder scraped from the film included a (110) peak in addition to the former two peaks. These XRD characteristics indicate the formation of hexagonal regular structures that preferentially orient parallel to the substrate surface. Calcination (450 °C, 3h) decreased the *d*-spacing (3.0 nm) upon structural shrinkage but the shape of the patterns basically remain unchanged (Fig. 2A(b)). The latter fact also supports the formation of the hexagonal structure in the spin-coated films.

More direct structural evidence was supplied by transmission electron microscopic (TEM) observation of a cross-section of the mesoporous silica film. As shown in Fig 3A the obtained film showed uniform thickness at the micron level. Highly regular pore structures were observed everywhere in the film cross-section. Part of the cross-sectional view was magnified in Fig. 3B to show a highly regular arrangement of hexagonal pore array. The pore size estimated from the TEM image was consistent with that calculated from the XRD *d*-spacing. In another view, regular line textures with even intervals ran in parallel. The latter image probably reflects a side view of extended and aligned pores. These microscopic evidences indicate that most pores lay in parallel to the substrate surface. Thermogravimetric analysis (TGA) revealed that loss of the organic contents in the range 150–600 °C was ca. 45 wt% in mesoporous composite from **1**, which is comparable to the results previously reported for conventional mesoporous composites.[15]

A similar preparative condition can be successfully applied for preparation mesoporous silica films

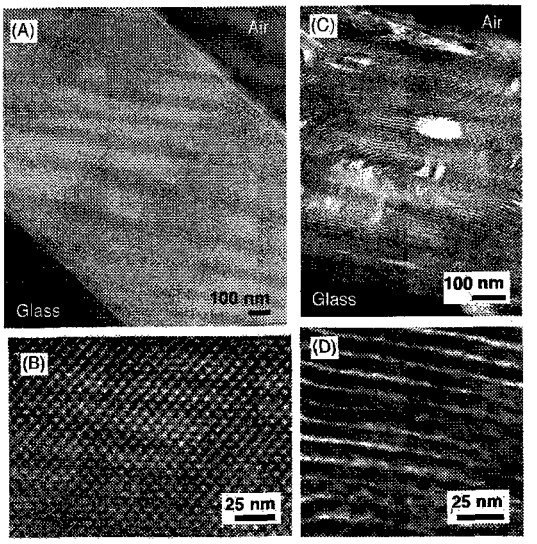

Fig. 3 (A), (B) TEM images of the mesoporous silica film templated by **1**. (C), (D) TEM images of the mesoporous silica film templated by **6**.

templated by the other surfactants containing gylcine (**2**), phenylalanine (**3**), alanylalanine (**4**), and glycylphenylalanine (**5**) residues. In contrast, a change in component ratio of the sol-gel solution (see EXPERIMENTAL) was required for successful preparation of mesoporous silica film templated by **6**, which is composed of a hydrophilic poly(ethylene glycol) chain and a hydrophobic leucine 16mer segment. The XRD profile of the resulting film (Fig. 2B) showed clear sharp peaks with *d*-spacing value of 8.7 nm reflecting larger molecular length of **6** compared to **1**. The XRD (100) peak remained sharp after calcination treatment at 450 °C for 3h, although decrease of the *d*-spacing (5.1 nm) was also detected. The latter fact indicates that the obtained structure is not in lamellar layered geometry because the calcinaton treatment is known to cause collapse of the lamellar structure accompanying disappearance of the XRD peaks.[16]

The film structure was also investigated by TEM observation. A TEM image of the film cross-section (Fig. 3C) shows surface roughness to some extent but the film essentially has constant thickness. Part of the

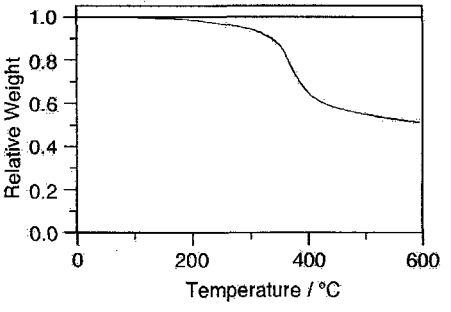

Fig. 4 TGA profile of the mesoporous silica film templated by **6**.

image was magnified where aligned linear patterns can be detected (Fig. 3D). This morphology probably reflects a side view of extended and aligned pores, and in the other parts there are pore cross-sections. However, the pore array is somewhat disordered compared with the corresponding image of **1**-templated mesoporous silica film. These characteristics suggest that the **6**-templated mesoporous silica film has a rather disordered array of one-dimensional pores. The TGA analysis revealed that loss of the organic contents in the **6**-templated mesoporous composite was ca. 48 wt% in the range 150– 600 °C (Fig. 4). This value is also comparable to the results previously reported for conventional mesoporous composites.

2.2 Structure of peptide assembly

Hydrogen bonding structure of amino acids and peptides confined in mesopores were mainly evaluated by FT-IR spectra of amide I and II regions. The peak top frequency of the amide I observed in the **1**-filled mesoporous silica film was 1689 cm^{-1} and was assigned to a non-bonded state. A similar tendency was observed for dipeptides in mesopororous silica. For example, a mesoporous silica film similarly prepared with **4** as a template showed broad amide I peak between 1690 and 1660 cm^{-1}, indicating that the alanylalanine residues were still in a weakly bonded state. When amphiphilic tripeptides and tetrapeptides were used as templates for mesoporous silica film preparation under similar conditions, structural regularity evaluated from XRD profiles of the films was significantly deteriorated. These kinds of peptides are known to form parallel β-sheet type hydrogen bond motif based on strong intermolecular hydrogen bonding.[17–19] Planar β-sheet structure in a template would preclude formation of hexagonal structure.

As described above, formation of overly strong interpeptide hydrogen bonding might be disadvantageous for mesoporous silica structure. In many previous works, effective intermolecular hydrogen bonding is reported to induce extended supramolecular morphologies such as fibril structures[17-21] that are not appropriate for mesoporous silica preparation. Therefore, we selected poly(leucine)-based amphiphile **6** as a template for mesoporous silica formation because these types of peptides are known to form micelles in aqueous media with forming α-helix motif based on intramolecular hydrogen bonding.[22–25] As shown above, the use of template **6** actually resulted in formation of the mesoporous silica film in regular structure. The obtained mesoporous silica films from the sol-gel solution containing **6** were next analyzed by circular dichroism (CD) and FT-IR spectra. The CD spectrum of the **6**-filling film showed peaks at 210 and 222 nm that were typical characteristics of the α-helix structure (Fig. 5A). The FT-IR spectrum of the same sample exhibited strong peaks at 1659 and 1546 cm^{-1} assignable to amide I and amide II of the α-helix motif (Fig. 5B). Therefore, the polypeptide **6** mainly formed the α-helix structure in its mesoporous silica film. It is the first example of successful preparation of the mesoporous silica film densely filled with defined peptide secondary structure.

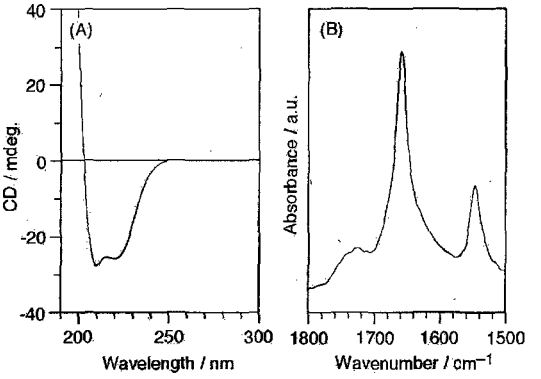

Fig. 5 (A) CD spectrum and (B) FT-IR spectrum of the mesoporous silica film templated by **6**.

3. CONCLUSION

In this research, we demonstrated the first preparation of mesoporous silica film densely filled with various amino acids and peptides. Especially, preservation of the α-helix structure in mesopores is an attractive feature with respect to biological applications. From the viewpoint of fundamental science, conversion of secondary structures in silica mesoporous is also under investigation to clarify fundamental nature of peptide assembling ability in structurally restricted nanospaces.

4. EXPERIMENTAL

The alanine-carrying surfactant (**1**) was synthesized according to the literatures reported for the similar compounds.[19] The C-terminal of L-alanine was first esterified by hexadecanol with *p*-toluenesulphonic acid as catalysts, and then the resulting *N*-terminal was reacted with bromoacetyl bromide. The obtained bromo-terminated peptide was reacted with dimethylallylamine to provide the alanine-containing amphiphile [**1**: ^{1}H NMR (500 MHz, DMSO-d_6): δ= 9.03 (br, 1H), 6.09-6.01 (m, 1H), 5.66 (d, J = 10.0 Hz, 1H), 5.61 (d, J = 16.5 Hz, 1H), 4.31-4.28 (m, 1H), 4.10 (d, J = 6.5 Hz, 2H), 4.08-4.02 (m, 2H), 4.04 (s, 2H), 3.16 (s, 3H), 3.15 (s, 3H), 1.56-1.54 (m, 2H), 1.31 (d, J= 7.5 Hz, 3H), 1.23 (m, 26H), 0.84 (t, J = 7.3 Hz, 3H); MALDI-TOF-MS: 439.33 ([M - Br$^-$]$^+$ calcd for $C_{26}H_{51}N_2O_3^+$: 439.39); Anal: calcd for $C_{26}H_{51}BrN_2O_3$: C, 60.10; H, 9.89; N, 5.39. obsd: C, 60.24; H, 9.90; N, 5.10.]. Amphiphiles **2**, **3**, **4**, and **5** were synthesized by the similar way [**2**: ^{1}H NMR (500 MHz, DMSO-d_6): δ= 9.04 (br, 1H), 6.10-6.02 (m, 1H), 5.66 (d, J = 10.0 Hz, 1H), 5.62 (d, J = 17.0 Hz, 1H), 4.12 (d, J = 7.0 Hz, 2H), 4.09 (s, 2H), 4.05 (t, J = 6.3 Hz, 3H), 3.94 (d, J = 6.0 Hz, 2H), 3.17 (s, 6H), 1.57-1.54 (m, 2H), 1.23 (m, 26H), 0.84 (t, J = 6.8 Hz, 3H); MALDI-TOF-MS: 425.16 ([M - Br$^-$]$^+$ calcd for $C_{25}H_{49}N_2O_3^+$: 425.37); Anal: calcd for $C_{25}H_{49}BrN_2O_3 \cdot 5/4H_2O$: C, 56.38; H, 9.83; N, 5.30. obsd: C, 56.73; H, 9.77; N, 4.95. **3**: ^{1}H NMR (500 MHz, DMSO-d_6): δ = 9.10 (d, J = 7.5 Hz, 1H), 7.30-7.20 (m, 5H), 6.00-5.95 (m, 1H), 5.61 (d, J = 10.0 Hz, 1H), 5.54 (d, J = 17.5 Hz, 1H), 4.60-4.56 (m, 1H), 4.07-3.97 (m, 6H), 3.09-3.06 (m, 1H), 3.08 (s, 3H), 3.06 (s, 3H), 2.95-2.90 (m, 1H), 1.48 (br, 2H), 1.22 (m, 26H),

0.84 (t, J = 6.8 Hz, 3H); MALDI-TOF-MS: 515.16 ([M - Br^-]$^+$ calcd for $C_{32}H_{55}N_2O_3^+$: 515.42); Anal: calcd for $C_{32}H_{55}BrN_2O_3 \cdot 2H_2O$: C, 62.63; H, 9.36; N, 4.56. obsd: C, 62.91; H, 9.50; N, 4.20. **4**: ^{1}H NMR (500 MHz, DMSO-d_6): δ = 8.79 (d, J = 7.0 Hz, 1H), 8.47 (d, J = 7.0 Hz, 1H), 6.06-6.00 (m, 1H), 5.65 (d, J = 8.5 Hz, 1H), 5.60 (d, J = 16.5 Hz, 1H), 4.35-4.33 (m, 1H), 4.25-4.22 (m, 1H), 4.08 (d, J = 7.0 Hz, 2H), 4.06-3.95 (m, 2H), 3.98 (s, 2H), 3.14 (s, 3H), 3.13 (s, 3H), 1.54-1.51 (m, 2H), 1.27 (d, J = 7.5 Hz, 3H), 1.24 (d, J = 7.0 Hz, 3H), 1.23 (m 26H), 0.84 (t, J = 7.0 Hz, 3H); MALDI-TOF-MS: 510.37 ([M - Br^-]$^+$ calcd for $C_{29}H_{56}N_3O_4^+$: 510.43); Anal: calcd for $C_{29}H_{56}BrN_3O_4 \cdot CH_3COOC_2H_5 \cdot 3/2H_2O$: C, 56.16; H, 9.57; N, 5.95, obsd: C, 56.35; H, 9.46; N, 6.17. **5**: ^{1}H NMR (500 MHz, DMSO-d_6): δ = 8.8 (t, J = 5.8 Hz, 1H), 8.54 (d, J = 7.5 Hz, 1H), 7.28-7.19 (m, 5H), 6.06-6.01 (m, 1H), 5.64 (d, J = 10.0 Hz, 1H), 5.60 (d, J = 16.5 Hz, 1H), 4.49-4.45 (m, 1H), 4.09 (d, J = 7.0 Hz, 2H), 4.02 (s, 2H), 3.98-3.95 (m, 2H), 3.84-3.74 (m, 2H), 3.13 (s, 6H), 3.05-2.98 (m, 1H), 2.93-2.89 (m, 1H), 1.47-1.44 (m, 2H), 1.22 (m, 26H), 0.84 (t, J = 6.8 Hz, 3H); MALDI-TOF-MS: 572.17 ([M - Br^-]$^+$ calcd for $C_{34}H_{58}N_3O_4^+$: 572.44); Anal: calcd for $C_{34}H_{58}BrN_3O_4 \cdot H_2O$: C, 60.88; H, 9.02; N, 6.26. obsd: C, 60.91; H, 9.35; N, 5.87]. The poly(leucine)-carrying surfactant (**6**) was prepared through a step-wise solid state synthesis using TentaGel (benzyl ester type) containing amino-terminated poly(ethyleneglycol) side chains (MW, ca. 3000). The length of the peptide moiety of **6** was exactly defined to be 16mer, and GPC analysis provided molecular weight distribution to be M_w / M_n of 1.03. The peptide **6** is well soluble in $CDCl_3$ and characterized by ^{1}H NMR. Its CD spectrum in trifluoroethanol (1 unit mM) showed a typical profile of the α-helix. The details of synthesis and characteristics will be reported in future publication.

Typical procedures for preparation of mesoporous silica films templated by **1** can be described as follows. Tetramethyl orthosilicate (TMOS, 1g) and *conc*-HCl (0.0053 g) were dissolved at 0 °C in a mixture of H_2O (0.204 g) and MeOH (1.68 g), and TMOS was prehydrolyzed at room temperature for 10 min. To part of the resulting solution (0.434 g), **1** (0.052 g) was added, and the reaction mixture (molar ratio: **1** / H_2O / HCl / TMOS / MeOH = 0.1 / 1.73 / 0.0076 / 1 / 8) was stirred at room temperature for additional 20 min. Transparent sol-gel solution was spin-coated on a cover glass at 2000 rpm. Mesoporous silica films templated by **2**, **3**, **4** or **5** were similarly prepared. Amphiphilic polypeptide **6** required modified condition. A mixture of tetraethyl orthosilicate (TEOS), H_2O, EtOH and HCl in the molar ratio, 1 / 1 / 3 / 10^{-5}, respectively, was first refluxed for 1 h. To the prehydrolyzed mixture (0.017 g), 0.0020 g of **6**, 0.040 g of EtOH and 0.0040 g of 0.055 M HCl were added. After stirring at room temperature for 5 min, transparent mesoporous film could be obtained with the similar spin-coating technique.

CD spectra, FT-IR spectra, and XRD patterns were recorded on JASCO model J-820 spectrophotometer, JASCO FT/IR-660 plus spectrophotometer, and Rigaku model RINT2500PC small and wide-angle XRD diffractometer, respectively. The TEM images were obtained on a JEOL JEM-2010 transmission electron microscope at Mitsui Chemical Analysis and Consulting Service Inc. TGA profiles were recorded on a Mettler Toledo TGA/SDTA851 thermogravimetric analyser.

ACKNOWLEDGEMENT

The authors thank Dr. Neale S. McAlpine for helpful discussion.

REFERENCES

[1] A. Stein, *Adv. Mater.*, **15**, 763-775 (2003).
[2] M. E. Davis, *Nature*, **417**, 813-821 (2002).
[3] M. Ogawa, *J. Photochem. Photobiol.*, **3**, 129–146 (2002).
[4] P. Yang, G. Wirnsberger, H. C. Huang, S. R. Cordero, M. D. McGehee, B. Scott, T. Deng, G. M. Whitesides, B. F. Chmelka, S. K. Buratto and G. D. Stucky, *Science*, **287**, 465–467 (2000).
[5] N. K. Mal, M. Fujiwara and Y. Tanaka, *Nature*, **421**, 350-353 (2003).
[6] J. N. Cha, G. D. Sticky, D. E. Morse and T. J. Deming, *Nature*, **403**, 289-292 (2000).
[7] J. N. Cha, K. Shimizu, Y. Zhou, S. C. Christiansen, B. F. Chmelka, G. D. Stucky and D. E. Morse, *Proc. Natl. Acad. Sci. USA*, **96**, 361-365 (1999)
[8] E. Dujardin and S. Mann, *Adv. Mater.*, **14**, 775-788 (2002).
[9] J. Deere, E. Magner, J. G. Wall and B. K. Hodnett, *Chem. Commun*,. 465-466 (2001).
[10] Y.-J. Han, G. D. Stucky and A. Butler, *J. Am. Chem. Soc.*, **121**, 9897-9898 (1999).
[11] R. Bhatia, C. J. Brinker, and A. K. Gupta, and A. K. Singh, *Chem. Mater.*, **12**, 2434-2441 (2000).
[12] K. Ariga, Q. Zhang, M. Niki, A. Okabe and T. Aida, *Stud. Surf. Sci. Cat.*, **146**, 427–430 (2003).
[13] Q. Zhang, K. Ariga, A. Okabe and T. Aida, *Stud. Surf. Sci. Cat.*, **146**, 465–468 (2003).
[14] K. Ariga, T. Aimiya, Q. Zhang, A. Okabe, M. Niki and T. Aida, *Int. J. Nanosci.*, **1**, 521–525 (2002).
[15] A. Okabe, T. Fukushima, K. Ariga and T. Aida, *Angew. Chem. Int. Ed.*, **41**, 3414–3417 (2002).
[16] I. Honma and H. S. Zhou, *Adv. Mater.*, **10**, 1532-1536 (1998).
[17] N. Yamada, K. Ariga, M. Naito, K. Matsubara and E. Koyama, *J. Am. Chem. Soc.*, **120**, 12192-12199 (1998).
[18] K. Ariga, J. Kikuchi, M. Naito, E. Koyama and N. Yamada, *Langmuir*, **16**, 4929-4939 (2000).
[19] N. Yamada and K. Ariga, *Synlett*, 575-586 (2000).
[20] J. D. Hartgerink, E. Beniash and S. I. Stupp, *Science*, **294**, 1684-1688 (2001).
[21] J. H. Jung, H. Kobayashi, M. Masuda, T. Shimizu and S. Shinkai, *J. Am. Chem. Soc.*, **123**, 8785-8789 (2001).
[22] T. Doi, T. Kinoshita, H. Kamiya, S. Washizu. Y. Tsujita, H. Yoshimizu, *Polym. J.*, **33**, 160-164 (2001).
[23] H. Yokoi, S. Hayashi, T. Kinoshita, *Prog. Polym. Sci.*, **28**, 341-357 (2003).
[24] Y. Kojima, T. Kinoshita and T. Yonezawa, *Trans., MRS-J*, **28**, 585-588 (2003).
[25] A. Harada, S. Cammas, and K. Kataoka, *Macromolecules*, **29**, 6183-6188 (1996).

(Received October 11, 2003; Accepted December 22, 2003)