# DNA-Loaded Porous Polyethersulfone Particles for Environmental Applications I. Preparation

**Changsheng Zhao,[1] Shudong Sun,[1] Kaiguang Yang,[1] Motoyoshi Nomizu,[2] Norio Nishi[2]**

[1]*Department of Biopolymer Materials, College of Polymer Science and Engineering, Sichuan University, Chengdu 610065, People's Republic of China*
[2]*Division of Bioscience, Graduate School of Environmental Earth Science, Hokkaido University, Kita-ku, Sapporo 060-0810, Japan*

Received 30 July 2004; accepted 10 December 2004
DOI 10.1002/app.22335
Published online in Wiley InterScience (www.interscience.wiley.com).

**ABSTRACT:** DNA-loaded porous poly(ether sulfone) (PES) particles are prepared for environmental applications by means of a liquid–liquid phase separation technique. The particles prepared by this method have very large porosity and specific surface area, which are useful as absorbents. By manipulating the fabrication conditions, we could control the microsphere structure, including the diameter and porosity of the particles, the pore size, and the specific surface area. Increasing the polymer concentration, which causes lower porosity and smaller pores on the outer surface of the microspheres, led to increased stability of the DNA-loaded particles and high DNA incorporation efficiency. The DNA-loaded porous PES particles could accumulate and remove a DNA intercalating pollutant, ethidium bromide (EB). Both the DNA amount incorporated in the particles and the microsphere structure have great effect on the EB removal ratio. These results indicate that the DNA-loaded PES particles have the potential to be used in environmental applications, which will be further discussed in the following paper. © 2005 Wiley Periodicals, Inc. J Appl Polym Sci 98: 1668–1673, 2005

**Key words:** DNA; poly(ether sulfone); porous particles; preparation; microsphere structure

## INTRODUCTION

DNA, one of the most important materials for the genetic process of living organisms, can also be regarded as a naturally occurring and highly specific functional biopolymer. Water-insoluble DNA films and gels conjugated with alginic acid or collagen and DNA immobilized onto nonwoven cellulose fabric or glass beads by UV irradiation could be prepared, and they were found to adsorb DNA-binding intercalating materials, such as ethidium bromide and dioxin derivatives.[1,2] In our recent research, DNA was used to modify polysulfone membranes; the hydrophilicity of the modified membranes increased, and the membranes showed better blood compatibility.[3,4]

Functional polymer microspheres or particles are widely used in the medical and biochemical fields as absorbents, latex diagnostics, affinity bioseparators, and drug and enzyme carriers. The microspheres are directly prepared by heterogeneous polymerization, e.g., emulsion polymerization, precipitation polymerization, suspension polymerization, and dispersion polymerization.[5,6] Recently, porous polyamide microparticles were prepared by use of a slow crystallization process in the nonequilibrium state. The particles could be used as functional materials.[7] We prepared DNA-loaded polysulfone (PSf) microspheres with a uniform size using a method termed phase inversion technique, which is caused by liquid–liquid phase separation. The microspheres were found to accumulate and remove poisonous organic compounds.[8,9]

Poly(ether sulfone) (PES), a parent material of PSf, is a well-known polymeric material. The PES and PES-based membranes show more outstanding oxidative, thermal, and hydrolytic stabilities as well as good mechanical and film-forming properties. Almost all the PES membranes are prepared using a common phase transition technique. In the present study, DNA-loaded PES porous particles were prepared using the phase inversion method; the particle size, porosity, and the amount of incorporated DNA into the particles were then characterized. The utilization of the porous particles will be investigated in the following paper.

## METHODS

### Preparation of the PES microspheres

PES (Ultrason E 6020P, CAS No.: 25,608–63-3, BASF Aktiengesellschaft) was dissolved in *N*-methyl-2-pyrrolidinone (NMP; HPLC grade, 99+%, Aldrich Chemical) or *N*,*N*-dimethyl acetamide (DMAc; AR, Shen-

*Correspondence to:* C. Zhao (zhaochsh70@yahoo.com.cn).

yang Chemical Regent Factory, China) to obtain the PES solution. Double-stranded DNA from salmon milt (Na salt, molecular weight $5 \times 10^6$) was obtained from the Yuki Fine Chemical (Tokyo, Japan). The DNA was dissolved in distilled water (20 mg/mL) and then dropped into the PES solution to obtain the mixed solution; the weight ratios of DNA to PES were controlled at 0.005, 0.01, and 0.015. All the materials were used without further purification.

The mixed PES solution was injected into water using syringe needles and stirred at about 300 rpm. The injection speed was controlled at 60–100 drop/min. The air gap from the needle to the water was 8–15 cm. The microspheres were then incubated in water for over 24 h to elute the solvent from the particles.

### Scanning electron microscopy (SEM)

For the SEM observation, the microsphere samples were dried at room temperature and then cut with a single-edged razor blade, attached to the sample supports, and coated with a gold layer. The SEM images were recorded using an *S*-2500C microscope (voltage = 20 kV, Hitachi, Japan).

### Calculation of the microsphere diameter and porosity

The diameter and the porosity of the microspheres were calculated from the density of the polymer and the weight change before and after drying,[8] using the formulas

$$\text{Diameter} = \left(\frac{6[W_A/\rho_P + (W_B - W_A)/\rho_W]}{\pi}\right)^{1/3},$$

$$\text{Porosity} = \frac{(W_B - W_A)/\rho_W}{W_A/\rho_P + (W_B - W_A)/\rho_W}$$

where $W_B$ is the weight of the membrane before drying (g); $W_A$ is the weight of the membrane after drying (g); $\rho_W$ is the density of water, $\rho_W = 1.0$ g/cm$^3$; and $\rho_P$ is the density of the poly(ether sulfone), $\rho_P = 1.43$ g/cm$^3$.

### Specific surface measurement

Mercury porosimetry (Micromerities, Model No. 1300) was used to determine the specific surface area in the porous particles. The particles were dried at room temperature; about 0.1 g dried particles was used as samples. The report data are obtained using Quantachrome Poremaster for Windows, Version 4.02.

### Calculation of DNA incorporation efficiency

The amount of DNA incorporated in the PES microspheres was determined by the following procedures: for dried microspheres (10 mg), each particle was cut into four sections and then hydrolyzed by 6*M* HCl solution at 100 °C for 12 h. The amount of the DNA in the solution was quantified by the absorption at 260 nm using a UV–vis spectraphotometer U-200A (Hitachi, Tokyo, Japan). The incorporation efficiency of the DNA in the microspheres was calculated as the mass ratio of the DNA extracted from the microspheres to the DNA input into the process.

### Accumulation and removal of ethidium bromide by the microspheres

Ethidium bromide (EB), a well-studied double-stranded DNA intercalating reagent, was used to test the functional utilization of the DNA-loaded poly(ether sulfone) porous microspheres. The accumulation of this reagent was examined by the following procedures: the PES microspheres (about 20 mg in dry weight) were put in an aqueous EB solution (7 mL) and incubated for 48 h at room temperature. The amount of the EB in the solution was quantified by the absorption at 480 nm using a UV–vis spectrophotometer U-200A (Hitachi).

## RESULTS AND DISCUSSION

### Structure characterization of the porous particles

We first studied the phase diagram for the ternary system (nonsolvent/PES/solvent) and for the quaternary system (nonsolvent/PES/solvent/DNA). Cloud point measurements were carried out by a titration method.[10] The solubility of the solvent was NMP > DMAc > dimethyl formamide > dimethyl sulfoxide and the order of increasing nonsolvent content at the cloud point was water <methanol <ethyl alcohol < propyl alcohol. For preparation of DNA-loaded PES microspheres, the DNA and the PES dissolved in a mixed solvent containing water and solvent. When a large amount of DNA was dissolved in the mixed solvent, a large amount of water was required. However, water is a nonsolvent of PES. A large amount of water caused the precipitation of PES. We chose the NMP and DMAc as the solvent and water as the poor solvent in this study.

A liquid–liquid phase separation technique was employed to fabricate the microspheres. When the PES solution containing DNA was added into water, liquid–liquid phase separation caused by the rapid exchange of the solvent (NMP or DMAC) and water occurred, and a skin layer formed due to the rapid phase separation, as shown in Figure 1. With the completion of the exchange between the solvent and the

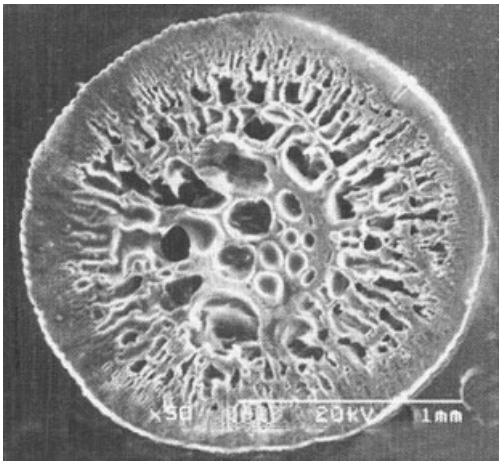


**Figure 1** SEM picture of the cross-section of the DNA-loaded porous PES particles. Voltage, 20 kV; magnification ×50. PES concentration used: 12.5%.

nonsolvent, the porous PES particles were prepared, and many pores existed in the spheres. The DNA was incorporated into the particles with the formation of the particles.

Figure 1 shows that a skin layer was found on the outside of the particles, followed with a finger-like structure, which was regarded as a typical structure for membranes prepared using the liquid–liquid phase separation and precipitation technique. The pore size gradually increased from the outside to the internal side of the particles. This structure is very useful when the particles were used to remove small molecular chemicals. Smaller molecules could pass through the pores on the skin layer into the particles, while larger molecules were separated from the particles.

The diameter and the porosity of the DNA-loaded poly(ether sulfone) particles are calculated from the density of the polymer and the particle weight change before and after drying, as shown in Table I. The diameter of the microspheres was dependent on the PES concentration and the diameter of the syringe needle. With the increase of PES concentration, the microsphere diameter gradually increased. The diameters of the particles (in Table I, particles 1, 4, and 9) prepared using the 10.0, 12.5, and 15% PES concentrations were about 1.62, 1.78, and 1.92 mm, respectively. The solvent (NMP or DMAc) of the PES and the ratio of DNA to PES slightly affected on the diameter. The diameter of the syringe needle used to prepare the particles has a great effect on the microsphere diameter. For 12.5% PES solution, when a 0.4-mm-diameter syringe needle and a 0.7-mm-diameter needle were used, the microsphere diameter (particles 4 and 8) increased from 1.78 to 2.14 mm.

The particle diameter is much larger (about three times larger) than that of the needle diameter used. This is caused by the surface tension of the polymer solution and the viscoelasticity of the polymer. The diameter was calculated from the formula, not directly determined. Also, the microsphere was not a regular sphere; it was an ellipsoid type, so the calculated diameter was the equivalent diameter. The results showed no significant difference between the calculated results and the experimental results; only the standard deviation obtained from the experiments was larger than that from the calculated results.

The porosity of the particles decreased with the increase of PES concentration, and the porosity is volume porosity. It can also be obtained from the mercury porosimetry measurements. There is no significant difference between the calculated results and the measured results.

The specific surface area, which was determined from the mercury porosimetry, is also shown in Table

**TABLE I**
**Diameter, Porosity, Specific Surface, and DNA Incorporation Efficiency for Several Particles Fabricated by the Phase Inversion Method**

| Particle No. | PES concentration (wt %) | Ratios of DNA to PES | Needle diameter (mm) | Diameter[a] (mm) | Porosity[a] (%) | Specific surface ($m^2/g$) | Incorporation efficiency[b] (%) |
|---|---|---|---|---|---|---|---|
| 1 | 10.0 | 0.010 | 0.4 | 1.62 ± 0.05 | 86.2 ± 0.5 | 27.92 | 65.9 ± 6.8 |
| 1d | 10.0 | 0.010 | 0.4 | 1.56 ± 0.06 | 87.0 ± 0.5 | 28.13 | 63.8 ± 6.3 |
| 2 | 12.5 | 0.000 | 0.4 | 1.74 ± 0.06 | 84.7 ± 0.4 | 44.35 | 0 |
| 3 | 12.5 | 0.005 | 0.4 | 1.76 ± 0.06 | 84.6 ± 0.4 | 43.97 | 65.2 ± 5.4 |
| 4 | 12.5 | 0.010 | 0.4 | 1.78 ± 0.06 | 84.5 ± 0.4 | 43.82 | 73.2 ± 5.6 |
| 5 | 12.5 | 0.015 | 0.4 | 1.79 ± 0.06 | 84.3 ± 0.4 | 43.62 | 78.4 ± 7.3 |
| 6 | 12.5 | 0.010 | 0.5 | 1.86 ± 0.07 | 85.2 ± 0.5 | 41.35 | 71.2 ± 5.0 |
| 7 | 12.5 | 0.010 | 0.6 | 2.04 ± 0.08 | 86.1 ± 0.5 | 36.28 | 66.3 ± 5.0 |
| 8 | 12.5 | 0.010 | 0.7 | 2.14 ± 0.06 | 86.4 ± 0.5 | 27.97 | 56.7 ± 5.0 |
| 9 | 15.0 | 0.010 | 0.4 | 1.92 ± 0.09 | 82.4 ± 0.4 | 61.16 | 83.2 ± 5.8 |
| 9d | 15.0 | 0.010 | 0.4 | 1.84 ± 0.08 | 83.4 ± 0.4 | 63.74 | 81.6 ± 6.6 |

*Note*. Data are expressed as the means ± SD of independent measurements: [a]$n = 10$; [b]$n = 3$.
1d and 9d, PES dissolved in DMAC solvent; others, PES dissolved in NMP solvent.

I. The specific surface area is ranged from about 28 to about 64 $m^2/g$ for the different porous particles. It is very interesting that when the PES concentration decreased, the porosity increased, while the specific surface decreased. When the particles were fabricated using a low concentration of PES solution, a large amount of very large pores formed, which had a great contribution to the porosity. However, large pores did not have a great contribution to the specific surface area. When the number of small pores is very large, the contribution to the specific surface area is larger than that of large pores.

### Amount of DNA incorporated into the porous particles

The incorporation efficiency of the DNA encapsulated into the PES microspheres was calculated as the ratio of the quantity of DNA extracted from the microspheres to the initial amount of DNA input into the process. Data are also shown in Table I. The incorporation efficiency depended on the PES concentration, which corresponding to the diameter and the porosity of the porous particles. When the PES concentration was very low, the incorporation efficiency was also very low. As shown in Table I, the incorporation efficiency was about 66% in the particles prepared using 10% PES solution. When the PES concentration increased to 15%, the incorporation efficiency increased to about 83%.

The PES solvent slightly affected the incorporation efficiency, as shown in Table I. The ratio of DNA to PES also affected the incorporation efficiency: with the increase of the ratio, the incorporation efficiency increased. However, the ratio could not increase to a very large value due to the miscibility of DNA and PES in the same solution.

The incorporation efficiency corresponds to the stability of the DNA-loaded porous particles. When the particles were prepared using higher concentration of PES solution, the microsphere porosity decreased and the eluted amount of DNA decreased, while the DNA stability increased; therefore, the incorporation efficiency increased. The DNA incorporation efficiency was higher for the microspheres with lower porosity, when the diameter was the same. This result indicated that the incorporation efficiency depended on the structure of the microspheres.

### Stability of the DNA-loaded particles

DNA is water-soluble. The stability of the DNA-loaded microspheres was very important when the microspheres were used as absorbents. The stability of the DNA encapsulated into the different microspheres was examined in water, and then the amounts of eluted DNA from the microspheres were determined.

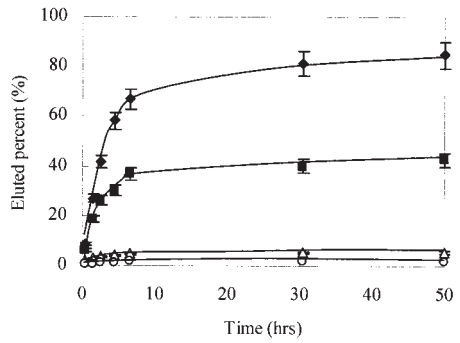


**Figure 2** Amounts of DNA eluted from the porous particles prepared by 12.5% PES solution into different solutions. (○) $H_2O$; (△) 0.9% NaCl solution; (■) 2% SDS solution; (◆) 2*M* HCl solution. Particle (No. 4.) Data are expressed as means ± SD of three independent measurements.

As mentioned above, the DNA encapsulated into the microspheres prepared by a high concentration of PES solution was very stable. However, a large amount of the DNA was eluted from the particles prepared by a very low concentration of PES solution into water. The porosity and the pores on the outer surface of the particles were larger when lower polymer concentration was used; thus, the DNA was easier to elute from the particle. These results indicated that the microsphere structure had a great effect on the stability of the encapsulated DNA.

The stability of the DNA-loaded microspheres was also examined in various solutions, including $H_2O$, 0.9% NaCl solution, 2% SDS solution, and 2*M* HCl solution using the particles prepared by 12.5% PES solution. The amount of DNA eluted from the microspheres was then determined (Fig. 2). The encapsulated DNA in the microspheres was stable in water and in NaCl solution, and not more than 6% of the incorporated DNA was eluted from the spheres, even after being incubated for 48 h. However, a large amount of DNA was eluted into the solution when the microspheres were incubated under acidic conditions. About 80% of the incorporated DNA was eluted into 2*M* HCl solution at room temperature. About 40% of the incorporated DNA was eluted into the SDS solution. A large amount of DNA was eluted into an HCl solution, which suggests that the DNA phosphodiester bonds were hydrolyzed under acidic conditions. A part of the DNA was also eluted into the SDS solution due to the surface activity of the SDS. These results showed that the DNA-loaded PES porous microspheres were stable in water, indicating that they should be amenable for use under neutral and basic conditions.

### Effect of DNA amounts on the EB removal

As we know, DNA is the most important material for the genetic process of living organisms. However,

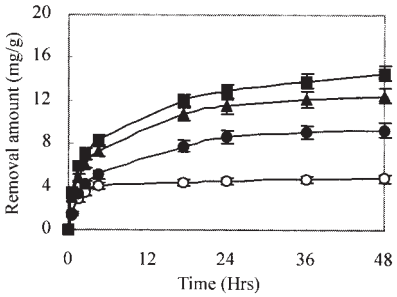


**Figure 3** Effect of DNA amount on the EB removal amount per gram of DNA-loaded PES porous particles. Ratios of DNA to PES: (○) 0; (●) 0.005; (▲) 0.01; (■) 0.015. EB solution; 80 $\mu M$; 7 mL. Porous particles (2, 3, 4, and 5, respectively): 20 mg. Data are expressed as means ± SD of three independent measurements.

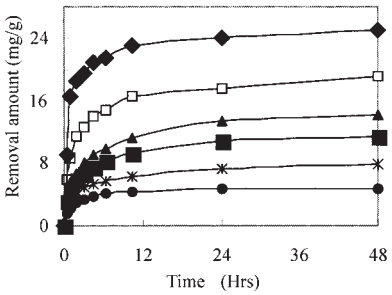


**Figure 4** Effect of EB concentration on the EB removal amount per gram of DNA-loaded PES porous particles. EB concentration: (○) 20 $\mu M$; (×) 40 $\mu M$; (■) 80 $\mu M$; (▲) 120 $\mu M$; (□) 180 $\mu M$; (■) 180 $\mu M$; (◆) 250 $\mu M$. DNA/PES = 0.01. EB solution: 7 mL; Porous particles (No. 4): 20 mg.

DNA is regarded as a naturally occurring and highly specific functional biopolymer in this study. The DNA-loaded porous PES particles can be used for the accumulation and removal of DNA-intercalating materials. EB, a well-studied double-stranded DNA intercalating reagent, was used to test the functional utilization of the DNA-loaded porous particles. When the EB solution was applied to the microspheres for 24 h, the maximum absorption peak of the EB at about 480 nm disappeared, and the white PES microspheres were dyed red by the EB adsorption onto the surface of the microspheres after incubating in EB solution.

Figure 3 shows the effect of the ratios of DNA to PES in the polymer solution on the amount of accumulated EB by the particles per gram. The DNA ratio corresponds to the incorporated DNA amount in the porous microspheres. It was demonstrated that with the increase of encapsulated DNA, the amount of removed EB increased. The amounts of accumulated EB after 48 h were 4.7, 9.3, 12.5, and 14.6 mg/g for the microspheres prepared by the ratios 0, 0.005, 0.01, and 0.015 of DNA to PES solution (the concentration of PES was 12.5%), respectively. The PES porous particles without DNA also accumulated the EB, which was caused by the porosity and high specific surface area of the PES particles.

### Effect of EB concentration on EB removal

We also examined the effect of EB concentration applied to the particles on the amount of accumulated EB per gram of the particles (Fig. 4). With the increase of the EB concentration in solution, the amount of accumulated EB increased. However, the removal ratio, defined as the ratio of the removed amount to the total amount in the solution applied to the particles, decreased due to the large EB amount in the solution.

The amount of accumulated EB increased when the EB concentration increased and reached a constant value for the water-insoluble DNA film when the EB concentration increased to about 200 $\mu$M.[11] The binding constant of EB for DNA was calculated to be about $6.8 \times 10^4\ M^{-1}$. However, there is no such a constant value for the DNA-loaded porous particles in this study. With the increase of the EB concentration from 120 to 250 $\mu M$, the amount of the accumulated EB increased from 14.8 to 24.2 mg/g. This result suggested that the porous structure played a very important role in the EB binding to the DNA-loaded PES particles when the EB concentration was very high, especially for the particles with very high specific surface area.

### 3.6 Effect of microsphere structure on EB removal

As mentioned above, the porous PES particles without DNA also accumulated EB from its aqueous solution due to the porosity and high specific surface. Next, we investigated the effect of the microsphere structure on the EB removal. Figure 5 shows the removal ratios for the DNA-loaded particles prepared by different PES concentration when the ratio of DNA to PES was the same. It was clearly demonstrated that with the in-

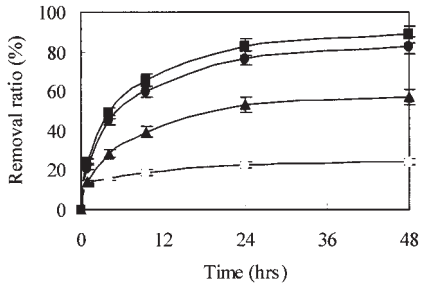


**Figure 5** Effect of PES concentration on the EB removal. PES concentration: (○) 12.5% without DNA; (▲) 10%; (◆) 12.5%; (■) 15%. Particles (20 mg) are Nos. 2, 1, 4, and 9, respectively. EB solution: 80 $\mu M$; 7 mL. Data are expressed as means ± SEM of three independent measurements.

crease of PES concentration with the same ratio of DNA to PES, the removal ratio increased. The removal ratios were 56.8, 83.2, and 88.8% for the particles prepared by the 10, 12.5, and 15% PES solutions with the same ratio of DNA to PES of 0.01, respectively.

With the increase of PES concentration from 10 to 15%, the specific surface area of the particles increased from about 28 to about 61 $m^2/g$, as shown in Table I. The DNA incorporation efficiency also increased, and the DNA-loaded particles show more stability. Thus, a large amount of EB was removed by the particles prepared from high PES concentration. However, it is difficult to prepare the particles with very high PES concentration and very high DNA to PES ratio due to the miscibility of DNA and PES in the polymer solution.

The effect of microsphere diameter on the EB removal was also investigated. With the increase of the diameter of the syringe needle used to prepare the particles, the microsphere diameter increased, the specific surface area decreased, and the DNA incorporation efficiency also decreased. These led to the decrease of EB removal.

## CONCLUSIONS

DNA-loaded porous PES particles could be prepared using a liquid–liquid phase separation technique. By manipulating the fabrication conditions, we could control the microsphere structure, including the diameter and porosity of the particles, the pore size, and the specific surface area. With the increase of DNA amount incorporated into the particles and the specific surface area, the removal ratio of EB increased. These results suggested that the DNA-loaded PES porous particles have the potential to be used in environmental applications.

## References

1. Yamada, M.; Kato, K.; Nomizu, M.; Ohkawa, K.; Yamamoto, H.; Nishi, N. Environ Sci Technol 2002, 36, 949.
2. Yamada, M.; Kato, K.; Shindo, K.; Nomizu, M.; Haruki, M.; Sakairi, K.; Ohkawa, K.; Yamamoto, H.; Nishi, N. Biomaterials 2002, 22, 3121.
3. Zhao, C. S.; Liu, X. D.; Nomizu, M.; Nishi, N. J Membr Sci 2003, 179, 214.
4. Zhao, C. S.; Liu, X. D.; Nomizu, M.; Nishi, N. Biomaterials 2003, 24, 3747.
5. Kawaguchi, H. Pro Polym Sci 2000, 25, 1171.
6. Omi, S.; Senba, T.; Nagai, M.; Ma, G. H. J Appl Polym Sci 2001, 79, 2200.
7. Asano, Y.; Nakayama, K.; Yao, S. J Appl Polym Sci 2003, 90, 2428.
8. Zhao, C. S.; Liu, X. D.; Nomizu, M.; Nishi, N. J Colloid Interface Sci 2004, 275, 470.
9. Zhao, C. S.; Liu, X. D.; Nomizu, M.; Nishi, N. Separation Science and Technique 2004, 39, 3043.
10. Baik, K. J.; Kim J. Y.; Lee, H. K.; Kim, C. K. J Appl Polym Sci 1999, 74, 2113.
11. Yamada, M.; Kato, K.; Nomisu, M.; Sakairi, N.; Ohkawa, K.; Yamamoto, H.; Nishi, N. Chem Eur J 2002, 8, 1407.