Colloid Polym Sci (2004) 282: 965–971
DOI 10.1007/s00396-003-1014-y

ORIGINAL CONTRIBUTION

Hidekazu Yoshizawa
Masa-aki Maruta
Satoshi Ikeda
Yasuo Hatate
Yoshiro Kitamura

Preparation and pore-size control of hydrophilic monodispersed polymer microspheres for size-exclusive separation of biomolecules by the SPG membrane emulsification technique

Received: 17 July 2003
Accepted: 3 October 2003
Published online: 17 February 2004

H. Yoshizawa (✉) · Y. Kitamura
Department of Environmental Chemistry and Materials, Faculty of Environmental Science and Technology,
Okayama University,
3-1-1 Tsushima-Naka,
700-8530 Okayama, Japan
E-mail: yhide@cc.okayama-u.ac.jp
Tel.: +81-86-251-8909
Fax: +81-86-251-8909

M. Maruta · S. Ikeda · Y. Hatate
Department of Applied Chemistry and Chemical Engineering,
Faculty of Engineering,
Kagoshima University,
1-21-40 Korimoto,
890-0065 Kagoshima, Japan

Abstract This paper describes an experiment directed toward the preparation of monodispersed porous polymer microspheres with a diameter of ca. 50 μm, which is applicable to the chromatographic separation of biomolecules such as proteins and peptides by size exclusion. Fairly monodispersed polymer microspheres were successfully prepared by suspension copolymerization of polyethylene glycol monomethacrylate and ethylene glycol dimethacrylate as monomer and cross-linker, respectively. Monodispersed O/W emulsion was prepared by the SPG membrane emulsification technique, and was used in the subsequent droplet-swelling process in which monodispersed seed droplets were swollen by adsorbing the secondary emulsion droplets. The effects of the organic diluent in suspension polymerization and comonomer on the porous structure of the polymer microspheres were investigated by scanning electron microscopy and mercury-intrusion porosimetry, and the separation performances of polystyrene, polyethylene glycol, and various biomolecules by size-exclusion chromatography. As a result, it was evident that benzene, 1-butanol, and butyl acetate worked as nonsolvents for the polymer prepared in this study, and that polymer microspheres prepared with these solvents had larger pores. Size-exclusion chromatography revealed that the exclusive limiting molecular weight was 1.9×10^5 when polystyrene was used as a standard polymer, and 3.5×10^4 when polyethylene glycol was used as a standard polymer. Furthermore, we confirmed that the monodispersed polymer microspheres with defined pores clearly separated the six representative kinds of biomolecules with molecular weights ranging from 75 to 6.4×10^5.

Keywords Polymer microsphere · Monodispersed emulsion · Swelling method · Hydrophilic polymer · Size-exclusion chromatography · Size-exclusive separation

Introduction

Monodispersed polymer microspheres are regarded as sophisticated materials, and demand for these materials has been increasing in such fields as chemicals, medicine and pharmaceutics, and biotechnology. Fixed phases in liquid chromatography, enzyme-immobilized beads, and electrophotographic toner particles all use monodispersed polymer microspheres.

Until now, various techniques to synthesize monodispersed polymer microspheres have been proposed, namely, emulsion and dispersion polymerizations. However, limited investigations have been conducted on the procedure for preparing monodispersed polymeric

microspheres with a 10-μm diameter. In fact, only the following processes have been reported: seeded emulsion polymerization [1], polymerization of monomer-swelled particles [2, 3], and conventional nonaqueous dispersion polymerization [4]. These processes are very complicated, however, and require a long production time, although they promise the preparation of fairly monodispersed polymeric microspheres. Considering conventional suspension polymerization, which was widely adopted as a production process for commercially available polymeric microspheres, the problem of low yield and high cost is unavoidable, because severe classification is necessary to prepare 10-μm diameter polymeric microspheres.

The SPG (Shirasu porous glass) membrane emulsification technique is now utilized in the production of margarine and the spacer in liquid crystalline displays, and is a promising technique for making various types of monodispersed emulsions [5, 6, 7, 8, 9, 10]. So far, we've succeeded in preparing monodispersed polymer microspheres ranging from about 1 to 30 μm in diameter by adopting the SPG membrane emulsification technique [11, 12, 13].

In contrast, monodispersed polymer microspheres with a diameter of 50–100 μm have been used as the stationary phase in size-exclusion chromatography for the determination of molecular weights and their distribution in synthetic and natural polymers, and for the separation of biomolecules such as proteins and peptides.

The preparation procedure for such larger monodispersed polymer microspheres is limited, and suspension polymerization following the sieving process, the two-step swelling method [2], the dynamic swelling method [3], and the emulsion swelling method [14, 15] have been proposed. Suspension polymerization following the sieving process was less effective, due to the reasons mentioned above. The two-step swelling method and the dynamic swelling method are well-established techniques, but require a long operation time in the swelling step, and expertise in the technique. In the emulsion swelling technique proposed by the group led by Prof. Omi, seed emulsion droplets were swollen by absorption of the monomer from secondary emulsion droplets. Therefore, the emulsion swelling method is more effective than the other swelling methods, because of a shorter operation time and because the method is a one-pot operation.

We successfully prepared 50-μm monodispersed polymer microspheres by the combination process employing both the SPG membrane emulsification technique and the emulsion swelling method. In this paper, we discuss polymer microspheres prepared by changing experimental factors such as the organic diluent and comonomer, with attention to the formation of pores of the prepared polymer microspheres. We investigated the separation properties of the polymer microspheres in size-exclusion chromatography by using standard polymers and biomolecules.

Experimental

Reagents

Blemmer PE-200 as a monomer was kindly supplied by the NOF Co., and was used in the experiments without further purification. The chemical formula of Blemmer PE-200 is illustrated in Fig. 1. Blemmer PE-200 is polyethylene glycol monomethacrylate, and works as a macromonomer which has polyethylene glycol as a branched chain. The polymerization degree of ethylene glycol was 4.5. Styrene (St) and ethylene glycol dimethacrylate (EGDMA) were used as a monomer and a cross-linking agent, respectively, and were purchased from Wako Pure Chemicals Co., and then purified by distillation under reduced pressure to remove the polymerization inhibitor. The polymerization initiator was *tert*-butyl peroxypivalate (*t*-BPP), which was a gift from Kayaku Akzo Co., and was used without further purification. The polymerization initiators were stored in a refrigerator prior to use. All other reagents were of analytical grade, and were used without further purification.

Preparation of monodispersed swollen droplets by the emulsion swelling technique

Table 1 represents the preparation conditions of the polymer microspheres in this study. The primary monodispersed emulsion droplets were prepared by the SPG membrane emulsification technique. The SPG membrane emulsifier, POEM-LABO, which was supplied from Reika Kogyo Co., Ltd., was adopted to prepare monodispersed primary emulsion droplets. The detailed procedure for SPG membrane emulsification was described in our previous paper [11]. The continuous solution (150 g) is an aqueous solution of 1.0 wt% PVA and 0.2 wt% SDS. The dispersed phase is benzene solution dissolving *t*-BPP. In part experiments (samples C and D), St was dissolved in the dispersed droplets to prepare copolymer microspheres of St and polyethylene glycol monomethacrylate.

Next, secondary emulsions were prepared with a homogenizer (10,000 rpm, 15 min). Benzene was used as solvent for samples A, C, and D. A mixed solution of butanol (BuOH) and butyl acetate (AcOBu), and ethyl acetate (AcOEt) were used as solvent for samples B and E, respectively.

The primary and secondary emulsions were poured into a reactor under moderate stirring, with special care taken to prevent the break-up and coalescence of the prepared emulsion droplets. The primary emulsion droplets were swollen by the absorption of monomer supplied from the secondary emulsion droplets through the continuous phase. During the swelling process, the primary and secondary emulsion droplets were monitored periodically by optical microscope. After swelling, suspension polymerization was performed at 343 K for 16 h under a nitrogen atmosphere. No aqueous polymerization inhibitor was added in this study, because

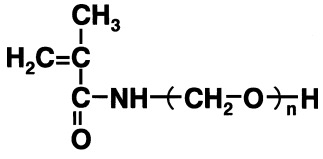

Fig. 1 Chemical formula of polyethylene glycol monomethacrylate used in this study (Blemmer PE-200); the polymerization degree of ethylene glycol was 4.5

Table 1 Recipes for suspension polymerization of polyethylenec glycol monomethacrylate with/without styrene in the presence of organic diluent after preparation of monodispersed emulsion droplets

	Sample A	Sample B	Sample C	Sample D	Sample E
Primary emulsion of seed droplets					
Continuous solution (g)	150	150	150	150	150
PVA (wt%)	1.0	1.0	1.0	1.0	1.0
SDS (wt%)	0.2	0.2	0.2	0.2	0.2
Dispersed solution					
Benzene (g)	5.0	5.0	1.64	5.0	5.0
Styrene (g)	–	–	4.10	12.5	–
t-BPOP (g)	1.37	1.37	0.63	1.92	1.37
Secondary emulsion					
Continuous solution (g)	150	150	150	150	150
PVA (wt%)	1.0	1.0	1.0	1.0	1.0
SDS (wt%)	0.1	0.1	0.1	0.1	0.1
Dispersed solution					
Benzene (g)	25.0	–	25.0	25.0	–
Butyl acetate (g)	–	7,62	–	–	–
Ethyl acetate (g)	–	–	–	–	25.0
Butanol (g)	–	22.9	–	–	–
Blemmer PE-200 (g)	17.8	14.3	17.8	17.8	17.8
EDGDMA (g)	7.60	6.13	7.60	7.60	7.60

no secondary microsphere was formed in the suspension polymerization. After polymerization, the polymer microspheres obtained were dried at room temperature after being washed with methanol and distilled water.

Characterization of the prepared polymer microspheres

Optical microscopic observation was carried out to monitor the swelling process and to determine the average diameter and diameter distribution of the prepared emulsion droplets. The average diameter mentioned in this investigation was number-averaged.

The surface of the prepared polymer microspheres was coated with gold prior to examination of the morphology by scanning electron microscopy (Topcon, SM-300) at the intensity of 15.0 kV under various magnifications.

The porous structure of the prepared polymer microspheres was evaluated by mercury-intrusion porosimetry (Yuasa Ionics Co. Ltd., Autoscan-60 Porosimeter), in which we measured the average pore diameter and pore-diameter distribution. The pore volume was measured from the volume of intruded mercury, and the pore diameter was calculated from the intruded pressure using the Washburn equation under the assumption that the pore shape was a hollow-type cylinder.

The characteristics in size-exclusion chromatography were evaluated by using HPLC equipped with both a refractive index monitor and UV detector, to determine the exclusive limiting molecular weight. Columns packed with the prepared polymer microspheres were used for the size-exclusion chromatographic experiments. Two experiments were conducted; that is, polystyrene and polyethylene glycol were used as the standard polymers in cases in which the eluents were tetrahydrofuran (THF) and distilled water, respectively. The eluent was supplied at the rate of 1.0 ml/min, and the measurement temperature was thermostatically kept at 298 K.

Results and discussion

Preparation of polymer microspheres

Figure 2 shows representative photographs of emulsion droplets before, during, and after the swelling step (sample E). It was clear that SPG membrane emulsification provided fairly monodispersed emulsion droplets, judging from the photograph of the primary emulsion droplets shown in Fig. 2a. The average diameter of the prepared primary emulsion droplets was 29.6 μm, which is about three times larger than the pore size of the SPG membrane used in this study (pore size: 9 μm). It is well known that the ratio of the droplet diameter to the pore size ranges from 2.44 to 6.62, and strongly depends on the SPG membrane itself, from the previous investigations in which SPG membrane emulsification was adopted to make O/W emulsions [9, 10, 11, 12, 14, 16, 17, 18]. The difference in the proportional constant may be due to the difference in the geometrical shape at the opening of the micropores, as described by Omi et al. [14, 16]. As can be seen in Fig. 2, it was observed that the diameter of the primary emulsion droplets clearly increased, and that smaller droplets were gradually eliminated as the swelling process proceeded, owing to the absorption of monomer

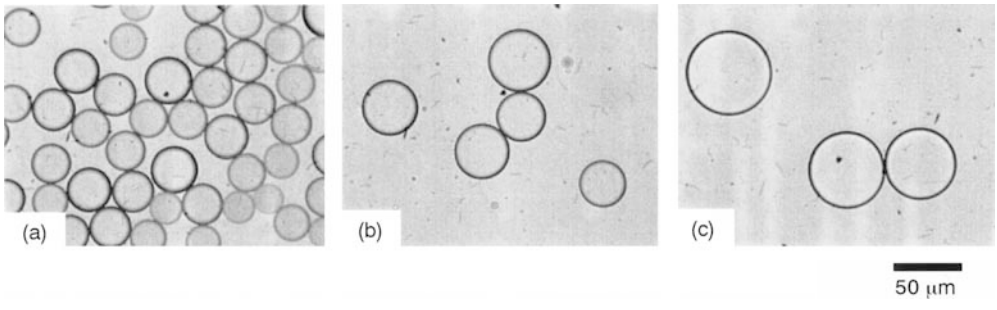

Fig. 2a–c Optical photographs of emulsion droplets **a** just after preparation by the SPG membrane emulsification technique (primary monodispersed droplets), **b** in the swelling method, and **c**)after complete swelling of the primary droplets by the adsorbing secondary emulsion droplets. The pore diameter of the SPG membrane used in this study was 9 μm and the emulsion was prepared under the conditions of sample E

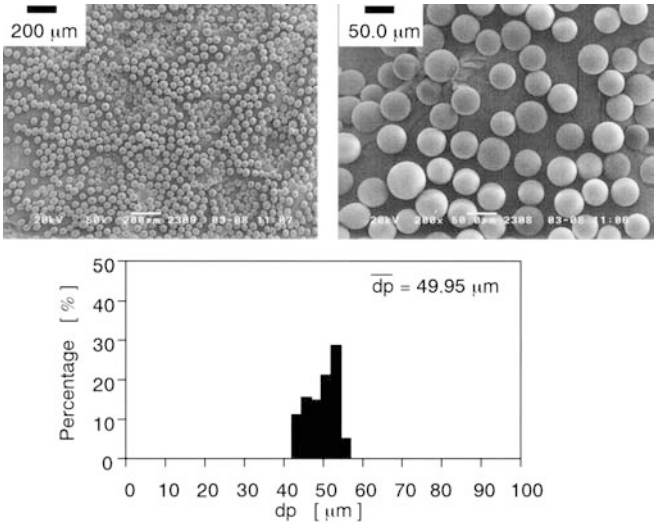

Fig. 3 SEM photographs and diameter distribution of polymer microspheres prepared by suspension polymerization after the droplet-swelling process of primary emulsion droplets. The polymer microspheres in these photographs were prepared under the conditions of sample E

from the secondary emulsion droplets. This phenomenon may presumably progress according to the Ostwald ripening, in which the droplet diameter plays a vital role. Finally, after the complete swelling of the primary emulsion droplets, their average diameter became 60.8 μm. The average diameter of the swollen droplets was 8.7 times that of the primary droplets, meaning a large portion of the secondary emulsion droplets were absorbed into the primary emulsion droplets.

Figure 3 shows representative SEM photographs and the diameter distribution of the prepared polymer microspheres after suspension polymerization. As can be seen in this figure, it was clear that the polymer microspheres prepared in this study were spherical and monodispersed. The average diameter of the polymer microspheres was 50.0 μm, and the CV, 7.64%. This result indicated that the monodispersion was maintained at a lower value even after the swelling and polymerization processes, because neither break-up nor coalescence occurred under the condition of low shear stress (the agitation speed was 120 rpm in polymerization).

SEM photographs of the polymer microspheres with higher magnification to investigate the effects of the organic diluent and comonomer (styrene) on surface morphology are presented in Fig. 4. The effect of the addition of styrene as a comonomer in the dispersed solution was found to be so significant, in connection with pore size measurement, as will be stated below, that an apparent change in surface morphology of samples A, B, and E was observed compared to that of samples C and D. That is, the surfaces of polymer microspheres (samples A, B, and E) were uneven, indicating the formation of pores of a relatively large size. However, no apparent differences in surface morphology were detected from SEM observation. In contrast, samples C and D had smooth and uniform surfaces.

Table 2 shows the results on pore size measurement of polymer microspheres by mercury-intrusion porosimetry. It is obvious from this table that samples A and B had the largest pores, with diameters around 25 nm. The pore size of sample E was smaller than that of samples A and B. However, the polymer microspheres prepared by copolymerization of polyethylene glycol monomethacrylate with St (samples C and D) were found to have no pores. This tendency concerning the addition of comonomer was supported by SEM observation, as described before. From the previous research concerning the pore-size control of polymer microspheres used in gel-permeation chromatography, it was revealed that organic solvent as a diluent during suspension polymerization was one of the most significant parameters controlling the pore size of polymer microspheres [11]. Moore

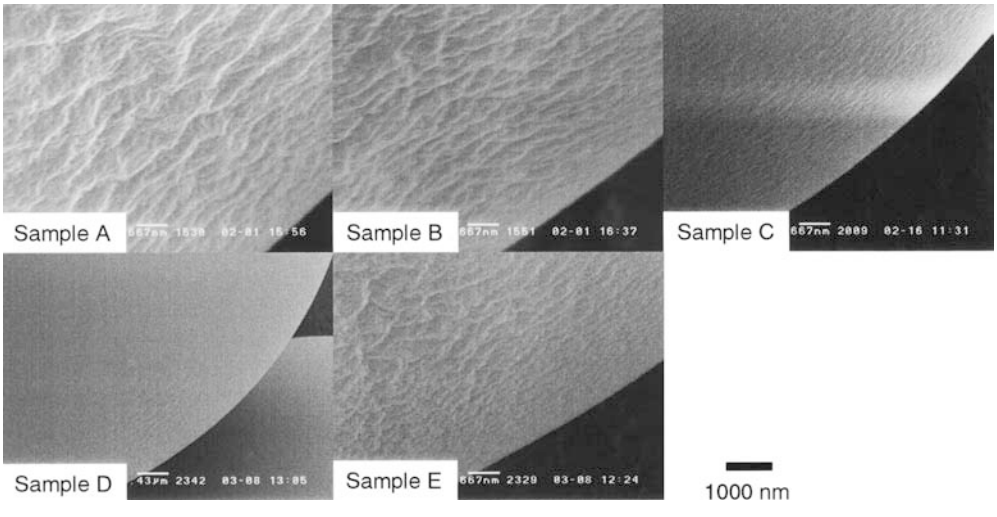

Fig. 4 SEM photographs of polymer microspheres to investigate the effects of organic diluent and comonomer on surface morphology. The organic diluent was benzene (samples A, C, and D), a mixture of benzene, butanol, and butyl acetate (sample B), or a mixture of benzene and ethyl acetate (sample E). Samples C and D were prepared by adding styrene as a comonomer

Table 2 Pore size characterization of polymeric microspheres prepared by suspension polymerization via the SPG membrane emulsification technique with the subsequent droplet-swelling process

	Sample A	Sample B	Sample C	Sample D	Sample E
Cumulative pore size (cc/g)	0.2506	0.1884	0.0191	0.0483	0.1283
Cumulative surface area (m^2/g)	39.92	33.10	14.84	30.50	48.85
Av. pore size (nm)	25.1	22.8	5.1	6.3	10.5
Max. pore size (nm)	42.8	35.2	4.1	4.1	12.0

clarified that the pore diameter increased with an increase in the 1-dodecane fraction in mixed diluent of 1-dodecane and toluene in the case of cross-linked polystyrene microspheres prepared by suspension polymerization with benzoyl peroxide as an oil-soluble initiator [19]. Cheng et al. clarified, in their research on the preparation of porous cross-linked polystyrene microspheres by dispersion polymerization, that the addition of a nonsolvent such as *n*-hexane as an organic diluent led to the formation of a macroporous structure in the polymer microspheres [20]. This may be presumed due to the enhancement of microphase separation on the precipitated polymer. It is likely that using a nonsolvent for the polymer as a diluent induces the formation of larger pores, whereas using a solvent for the polymer as a diluent forms smaller pores. In cases where the diluent is a solvent for the polymer, the precipitated polymer gels formed by microphase separation are swollen and softened in the solvent. Therefore, the dense structure is induced by piling and entanglement of the softened polymer gels as the polymerization proceeds. This leads to the formation of smaller pores and the smooth surface of the prepared microspheres. In the other case where the diluent is a nonsolvent for the polymer, the hard precipitated polymers were stacked up, leading to the formation of the macropores.

From the results mentioned in previous papers and in connection with the electron microscopic observation and pore size measurement mentioned above, it was considered that benzene and butyl acetate worked as a nonsolvent for polyethylene glycol monomethacrylate, leading to a relatively larger pore. Furthermore, the addition of styrene raised the solubility of the prepared copolymer of polyethylene glycol monomethacrylate with styrene, and resulted in the formation of smaller pores.

Characterization of prepared polymer microspheres in size-exclusion chromatography with standard polymer

In order to clarify the performance of the prepared polymer microspheres as gel beads in size-exclusion chromatography, the exclusive limiting molecular weight was estimated by elution curves of the column packing of polymer microspheres which were prepared without styrene, and had relatively larger pores, as shown in the section above.

Figure 5 shows the elution curves of samples A, B, and E, which were measured with THF and polystyrene as the mobile phase and the standard polymer, respectively. The elution curves of samples A and B illustrated in Fig. 6 were measured under the conditions of distilled water and polyethylene glycol used as the mobile phase and the standard polymer, respectively.

From the measurement with THF and polystyrene, it was found that the exclusive limiting molecular weight of samples C and D was below 1,000, which was attributable to the formation of smaller pores, that is, 5.1 nm (sample C) and 6.3 nm (sample D). Furthermore, the measurement of sample C in the water system did not progress, due to the lack of peaks in the chromatogram. This might be because of the adsorption of polyethylene glycol on the packed polymer microspheres.

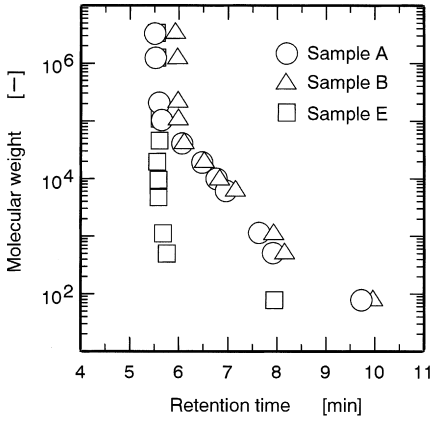

Fig. 5 Elution curves of samples A, B, and E; THF and polystyrene were used as the mobile phase and the standard polymer, respectively

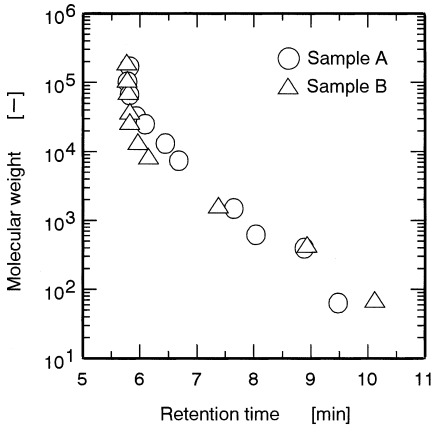

Fig. 6 Elution curves of samples A and B; distilled water and polyethylene glycol were used as the mobile phase and the standard polymer, respectively

It is obvious from Figs. 5 and 6 that different exclusive limiting molecular weights were observed by changing the organic diluent used in the preparation of polymer microspheres both in the THF system and in the water system. Polymer microspheres with benzene (sample A), benzene/1-butanol/butyl acetate (sample B), and benzene/ethyl acetate (sample C) in the THF system had exclusive limiting molecular weights of 1.9×10^5, 4.0×10^4, and 600, respectively, indicating that the exclusive limiting molecular weight corresponds well with the pore size of polymer microspheres, whereas in a water system, polymer microspheres with benzene (sample A) and benzene/1-butanol/butyl acetate (sample B) had exclusive limiting molecular weights of 3.5×10^4 and 1.1×10^4, respectively. This means that the columns of samples A and B have the performance to separate water-soluble polymers in which weight-averaged molecular weight is up to 3.5×10^4 and 1.1×10^4, respectively.

Separation behavior of biomolecules with the prepared polymer microspheres in size-exclusion chromatography

In order to clarify the separation performance of biomolecules, two types of polymer microspheres, samples A and B, were used in an experiment in which the mobile phase was 0.2 M phosphate buffer (pH 7.0), and the absorbance was monitored at 280 or 210 nm (in the case of glycine). In this measurement, we used six kinds of biomolecules, that is, thyroglobulin ($Mw=6.4\times10^5$), bovine serum albumin ($Mw=6.7\times10^4$), ovalbumin ($Mw=4.7\times10^4$), cytochrome c ($Mw=1.24\times10^4$), aprotinin ($Mw=6.5\times10^3$), and glycine ($Mw=75$).

Figure 7 demonstrates the separation abilities of the polymer microspheres (samples A and B) prepared in this study for biomolecular separation in size-exclusion chromatography. It is clearly revealed from this figure that the molecular weight decreased with an increase in the retention time. In particular, the retention times of sample A for each biomolecule became faster than those of sample B. This may be due to the difference in pore size and its distribution, that is, sample A had slightly larger pores than sample B. This result implies that the column packed with polymer microspheres prepared following the proposed recipe and protocol successfully separated the biomolecules corresponding to their molecular weights.

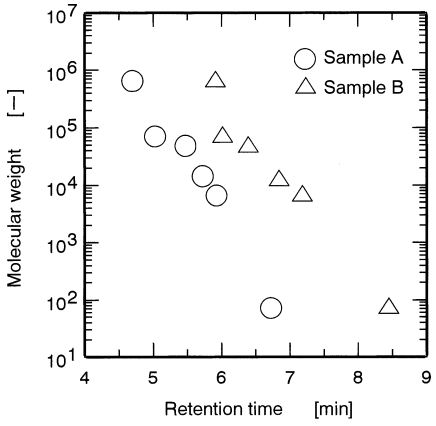

Fig. 7 Separation performance of polymer microspheres (samples A and B) for biomolecular separation in size-exclusion chromatography. The mobile phase was 0.2 M phosphate buffer (pH 7.0), and the absorbance was monitored at 280 or 210 nm (in the case of glycine). The biomolecules used were thyroglobulin ($Mw=6.4\times10^5$), bovine serum albumin ($Mw=6.7\times10^4$), ovalbumin ($Mw=4.7\times10^4$), cytochrome c ($Mw=1.24\times10^4$), aprotinin ($Mw=6.5\times10^3$), and glycine ($Mw=75$)

Conclusion

We attempted to prepare monodispersed porous polymer microspheres of 50-μm diameter by adopting suspension polymerization via SPG membrane emulsification with subsequent swelling of emulsion droplets, and investigated the separation performance of the biomolecules by size-exclusion chromatography with the columns packed with the monodispersed porous polymer microspheres.

Fairly monodispersed porous polymer microspheres of polyethylene glycol monomethacrylate and ethylene glycol dimethacrylate with defined pore sizes were prepared using organic diluent. The diameter of the prepared polymer microspheres was around 50 μm. The characterization of the polymer microspheres by size-exclusion chromatography demonstrated that the exclusive limiting molecular weight was varied by pore size, which was controlled by changing the organic diluent used. The prepared polymer microspheres could separate polymers in which the weight-averaged molecular weight ranges from 75 to 1.9×10^5 in the THF system (polystyrene standard). Furthermore, size-exclusive separation of biomolecules was achieved with the prepared polymer microspheres and six kinds of biomolecules.

Acknowledgments The authors wish to express their sincere thanks to Messrs. Masadumi Hasegawa and Hiroyuki Moriyama, Toso Co., Ltd., Sin-Nanyo, Japan for their valuable discussion and to Mr. Tohru Taniguchi, Reika Kogyo Co., Ltd., Miyazaki, Japan for supplying the SPG membrane emulsifier, POEM-LABO. We would also like to acknowledge the help of Mr. Kazuma Kanaoka, The Ohtsu Tire & Rubber Co., Ltd., Osaka, Japan for supplying the SPG membranes. The authors are indebted to NOF Co. for Blemmer PE-200, and to Kayaku Akzo Co. for *t*-BPP.

References

1. Vanderhoff JW, El-Aasser MS, Sudol ED, Tseng CS, Silwanowicz A, Cornfeld DM, Vicente FA (1984) J Disper Sci Technol 5:231
2. Ugelstad J, Mufutakhamba HR, Mork PC, Ellingsen T, Berge A, Schmid R, Holm L, Jorgedal A, Hansen FK, Nustad K (1985) J Polym Sci Polym Symp 72:225
3. Okubo M, Shiozaki M, Tsujihiro M, Ysukuda Y (1991) Colloid Polym Sci 269:1
4. Ober CK, Hair ML (1987) J Polym Sci Polym Chem 25:1395
5. Nakashima T, Shimizu M (1985) US Patent 4,657,875
6. Tomita M, Sotoyama K, Kato R, Asano Y, Takahashi K (1992) Preprints of the 18th SPG Forum: 34 (in Japanese)
7. Naito M (1994) Preprints of the 22nd SPG Forum: 66 (in Japanese)
8. Nakashima T, Shimizu M, Kukizaki M (1991) Membrane emulsification—operation manual, 1st edn. Industrial Research Institute of Miyazaki Prefecture, Miyazaki, Japan (English version)
9. Nakashima T, Shimizu M (1993) Kagaku Kogaku Ronbunshu 19:984
10. Nakashima T, Shimizu M, Kukizaki M (1993) Kagaku Kogaku Ronbunshu 19:991
11. Hatate Y, Uemura Y, Ijichi K, Kato Y, Hano T, Baba Y, Kawano Y (1995) J Chem Eng Japan 28:656
12. Hatate Y, Ohta H, Uemura Y, Ijichi K, Yoshizawa H (1997) J Appl Polym Sci 64:1107
13. Yoshizawa H, Ohta H, Maruta M, Uemura Y, Ijichi K, Hatate Y (1996) J Chem Eng Japan 29:1027
14. Omi S (1996) Colloids Surf A Physicochem Eng 109:97
15. Omi S, Taguchi T, Nagai M, Ma GH (1997) J Appl Polym Sci 63:931
16. Omi S, Katami K, Yamamoto A, Iso M (1994) J Appl Polym Sci 51:1
17. Omi S, Katami K, Taguchi T, Kaneko K, Iso M (1995) J Appl Polym Sci 57:1013
18. Omi S, Kaneko K, Nakayama A, Katami K, Taguchi T, Iso M, Nagai M, Ma GH (1997) J Appl Polym Sci 65:2655
19. Moore JC (1964) J Polym Sci B 2:835
20. Cheng CM, Micale FJ, Vanderhoff JW, El-Aasser MS (1992) J Polym Sci A 30:235