

Preparation and characterization of spherical polymer packings from polybutadiene for size-exclusion chromatography

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Abstract

Porous polymer spherical particles for column packings in nonaqueous size-exclusion chromatography (SEC) were prepared from 1,2-syndiotactic polybutadiene by suspension and evaporation method. The polymer microbeads obtained were crosslinked by radical reaction between 2-vinyl groups in polybutadiene with ultraviolet radiation, to render them insoluble. These microbeads have wider chromatographic separation width than polystyrene column packings. In addition, the polybutadiene microbeads did not show the excessive retention observed with commercial polystyrene columns for polycyclic aromatic compounds. Therefore, a close correlation between the elution volume and M_n for polycyclic aromatic compounds was observed with polybutadiene microbeads columns.

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1. Introduction

In nonaqueous size-exclusion chromatography (SEC), styrene-divinylbenzene (PS-DVB) microbeads has generally been used. Porous PS-DVB microbeads have been used as effective LC packing materials over a wide range of pH [1–3]. However, PS-DVB microbeads show abnormal adsorptions or excessive retention for solutes such as polycyclic aromatic compounds. This undesirable chromatographic behavior is derived from π -electron effect caused by the aromaticity of the styrene unit and divinylbenzene moiety used as crosslinking monomers [4–10]. These interactions result in peak tailing, long retention time and peak broadening, thus applications of PS-DVB microbeads to SEC have been limited. In addition, the pore size distribution for PS-DVB microbeads obtained by suspension copolymerization is wide, resulting in a lowered chromatographic resolution. This is not favorable

for separation of several compounds with similar molecular weight, for instance oligomers.

On the other hand, polyolefin is as stable as PS-DVB in alkaline and acidic solution. In addition, LC supports prepared from polyolefin are considered to show no excessive retention for polycyclic aromatic compounds due to π -electron effect, since polyolefin has no aromaticity. Araki and coworkers moulded particles by adding polypropylene (PP) or polyethylene (PE) melted under high pressure (13–20 kg/cm²) and high temperature (190–200 °C) into emulsifier aqueous solution [11,12]. The PP or PE particles obtained were spherical and insoluble against common organic solvents, such as tetrahydrofuran, chloroform and dimethylformamide, but had no pores. Therefore, the PP and PE particles could be used for RPLC support, but not as SEC packing materials [13–16].

In order to develop nonaqueous SEC packing materials possessing the characteristics: (1) no abnormal adsorption for aromatic compounds, (2) wide separation width for molecular weight, and (3) high porosity, we focused on

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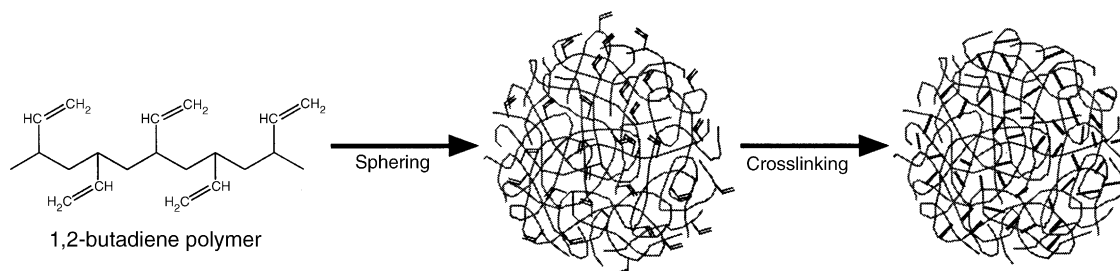


Fig. 1. Preparation of crosslinked PBD microbeads from 1,2-butadiene polymer.

1,2-syndiotactic polybutadiene (PBD). The porous spherical microbeads were prepared from PBD by suspension and evaporation method as previously reported for cellulose [17], pullulan [18], glucomannan [19], chitosan [20] and poly(α -amino acid) [21]. In addition, polyolefin microbeads (crosslinked-PBD) that were insoluble against common organic solvents were obtained by crosslinking radical reaction between 2-vinyl groups in a butadiene unit with ultraviolet radiation (Fig. 1). In this paper, we detail their preparation and SEC properties.

2. Experimental

2.1. Polybutadiene sphering and crosslinking procedure

One hundred and sixty milliliters of 5.0% (w/w) 1,2-syndiotactic polybutadiene (PBD, RB830, JSR, Tokyo, Japan) (molecular weight, i.e., 100,000 for catalogue) solution in chloroform containing diethylbenzene (11.38 ml, 2 ml/g-for the weight of PBD) as diluent was prepared. The solution was mixed with 640 ml of a 2.0% (w/w) solution of aqueous poly(vinyl alcohol) (PVA, Gohsenol GH-20, Nippon Synthetic Chemical Industry, Tokyo, Japan) and suspended by stirring at 1200 rpm at 40 °C for 24 h. During sphering by suspension evaporation method, 152 ml of chloroform was gradually evaporated from the suspension particles, and reduced completely after 24 h. The particles obtained were washed successively with 500 ml each of water, methanol and acetone to form porous PBD microbeads. All the solvents were purchased from nacalai tesque, Tokyo, Japan. The PBD microbeads with diameters 22–44 μ m were collected using 500 mesh sifter and 325 mesh shifter. In order to crosslink 2.0 g of the PBD, ultraviolet radiation was carried out with a 100 W high-pressure mercury vapor lamp (UVL-100HA, Rikokagaku, Chiba, Japan) in a stream of 20 ml/min of N₂ gas, as shown in Fig. 2, for 2–48 h and in 100 ml of solvents such as methanol, 1-propanol, 1-butanol and hexane to render the microbeads insoluble against organic solvents. The PBD microbeads were also crosslinked by radical reaction of vinyl groups using the radical initiator azobisisobutyronitrile (AIBN). All of the PBD microbeads crosslinking-reacted were washed successively with 100 ml each of water, methanol and acetone with filtering. The

PBD microbeads crosslinking-reacted for 24 h were most favorable as SEC column packings. The alcohol such as methanol, 1-propanol and 1-butanol PBD microbeads was suitable for crosslinking solvent. The M_{lim} and porosity increased with decreasing polarity of the solvent (polarity: methanol > 1-propanol > 1-butanol) used for crosslinking reaction. On the other hand, because the PBD microbeads crosslinking-reacted in hexane was too soft, they could not be packed in column.

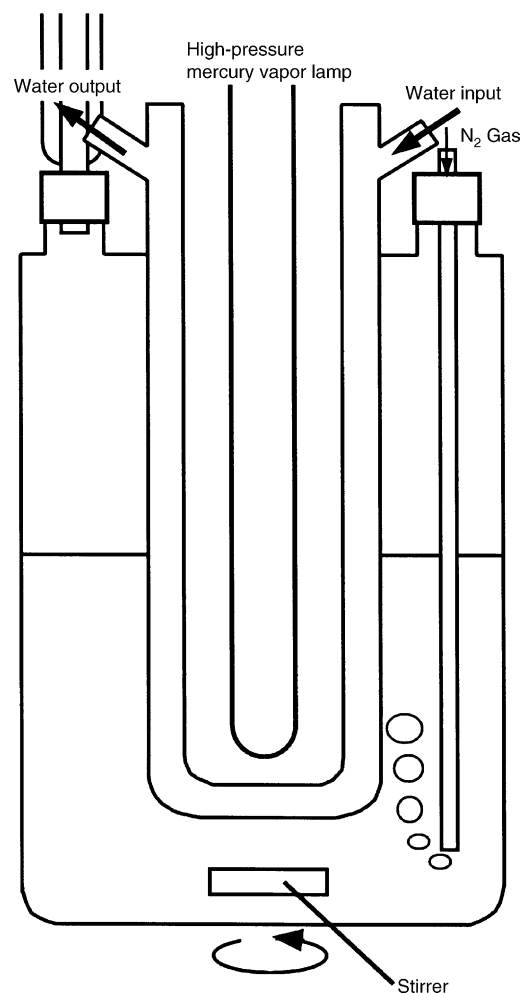


Fig. 2. Reaction apparatus for crosslinking reaction of PBD microbeads using UV radiation.

2.2. Determination of double bonds in crosslinked-PBD microbeads

Double bonds in PBD microbeads were determined by Wijs method [22]. One hundred and fifty milligrams of the PBD microbeads was swelled in 10 ml of carbon tetrachloride in a 300 ml flask. After the dispersion of PBD was added to 25 ml of Wijs reagent, it was allowed to stand in the dark. While washing the stopper with 20 ml of 10% potassium iodide aqueous solution, the solution was poured into the dispersion of PBD microbeads and 100 ml of water was added. Iodometry was continued until the color turned yellow, and 1.0% starch aqueous solution was added. Titrating continued until the blue decolorized. A blank test was carried out in the same manner. The iodine value is given in Eq. (1).

$$\text{iodine value (\%)} = (B - S) \times F \times \frac{1.269}{W} \quad (1)$$

where B , S , F and W are the blank value, the main value of titration, factor of 0.1 N sodium thiosulfate and dry weight of the PBD microbeads, respectively. The amount of vinyl group residue in PBD microbeads was calculated from the iodine value using Eq. (2)

$$\text{vinyl group (mol/g)} = \frac{\text{iodine value}}{126.91 \times 2 \times 100} \quad (2)$$

2.3. Determination of swelling degree

The swelling degree (Sd) of crosslinked-PBD microbeads obtained was determined. After the weighed crosslinked-PBD microbeads were soaked in organic solvents, i.e., THF and benzene at 30 °C for 24 h, the volume in organic solvents was measured. The Sd was estimated as a ratio of volume in organic solvents for the dry weight of crosslinked-PBD microbeads as follows:

$$\text{Sd (wet-ml/dry-g)} = \frac{V_{\text{organic}}}{W_0} \quad (3)$$

where V_{organic} and W_0 are the volume in THF or benzene and dry weight of the crosslinked-PBD microbeads, respectively.

2.4. Chromatography

A slurry of 2.0 g of the microbeads with diameters of 22–44 μm was packed with tetrahydrofuran degassed into a stainless steel column (150 mm \times 4.6 mm I.D.). The chromatograph included a Waters 600 pump, Waters 600 controller, Waters 996 photodiode array and Waters 410 refractive index monitor. A 5 μl portion of the sample dissolved in an eluent (1.0 mg ml^{-1}) was injected through a Rheodyne Model 7125 injector. All chromatography was carried out at 30 °C. As a reference, Shodex KF-801 as polystyrene (PS-DVB) packing was used. Polypropylene spherical microbeads (PP) packing was purchased from Sumitomo Seika Chemicals, Himeji, Japan. The SEC calibration curves

in tetrahydrofuran were prepared using polystyrene standards: (M_n : 1.32×10^3 , 3.25×10^3 , 9.24×10^3 , 2.85×10^4 , 6.60×10^4 , 1.56×10^4 , 5.01×10^5 , 1.13×10^6 , 1.95×10^6 , 3.04×10^6). The elution volume is given by Eq. (4)

$$\text{elution volume (\%)} = \frac{V_c}{V_t} \times 100 \quad (4)$$

where V_t and V_c are the total volume of the column and the elution volume of the polystyrene, respectively.

The elution volume values were obtained by extrapolating the linear part of curve. The porosity is given by Eq. (5). This value corresponds to the elution volume of benzene, which is the smallest molecule permeable within the reticulated matrix of PBD microbeads.

$$\text{porosity (\%)} = \frac{V_{\text{benzene}} - V_0}{V_t - V_0} \times 100 \quad (5)$$

where V_t , V_0 and V_{benzene} , are the total volume of the column, the interstitial volume of the gel bed, and the elution volume of the benzene, respectively. Eq. (5) is transformed into Eq. (6), because the elution volume of permeable substances with molecular weights from M_i to M_{i+1} corresponds to $V_{i+1} - V_i$.

$$\text{porosity (\%)} = \sum_{i=1}^{n-1} \frac{V_{i+1} - V_i}{V_t - V_0} \times 100 \quad (6)$$

where V_1 and V_n correspond to V_0 and V_{benzene} in Eq. (5), respectively. The pore size distribution is estimated by plotting $(V_{i+1} - V_i)/(V_t - V_0) \times 100$ against the molecular weight of permeable substances ($M_i - M_{i+1}$).

The linear part corresponding to the coverage of SEC separation is shown in Eq. (7)

$$\log M = \beta - \alpha_{\text{eff}} \left(\frac{V_c}{V_t} \right) \quad (7)$$

where V_t is the total volume of the gel bed and V_c is the eluting volume of a substance with molecular weight M .

2.5. Other measurements

Scanning electron micrographs of the spherical microbeads were obtained using a field emission scanning electron microscope (FE-SEM) (Hitachi S-4000, Hitachi, Tokyo, Japan). X-ray diffraction was carried out using an X-ray Diffractometer (RINT 2000, Rigaku, Tokyo, Japan).

3. Results and discussion

3.1. Preparation of polybutadiene spherical particles

In general, polyolefin can be obtained by Ziegler-Natta catalyzed polymerization of olefin. However, it is difficult to mould into a spherical shape by means of conventional suspension evaporation or suspension polymerization methods,

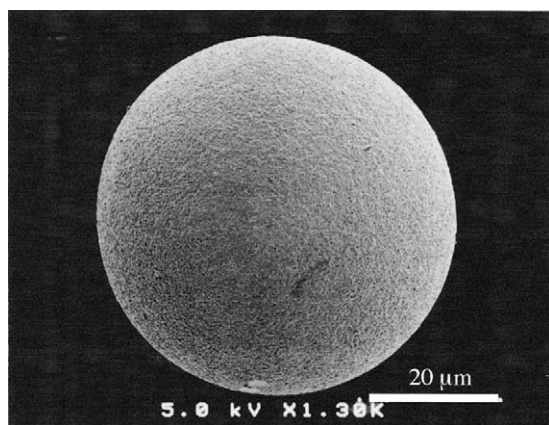


Fig. 3. SEM image of PBD microbeads obtained by suspension evaporation method.

because polyolefins such as polypropylene or polyethylene possess high glass transition temperature and stability against organic solvents. Recently, polypropylene or polyethylene spherical beads have been obtained by emulsion method using surfactants under high temperature and pressure in autoclave [11,12], but it is difficult to obtain porous spherical beads [13,14]. We focused on 1,2-butadiene polymer (PBD) containing 93 mol% of syndiotactic structure, since PBD possesses relatively lower crystallinity and better solubility against organic solvents than polypropylene or polyethylene. Fig. 3 shows the SEM images of the PBD particles obtained. The PBD particles were perfectly spherical. However, PBD microbeads are soluble in organic solvents such as THF, DMF and chloroform. To render them insoluble in these solvents, the PBD microbeads must be crosslinked by radical reaction between the vinyl groups of 1,2-butadiene units. Therefore, radical initiators azobisisobutyronitrile (AIBN) and UV irradiation were used for radical reaction. The swelling degree (Sd) of crosslinked-PBD microbeads against THF was investigated and summarized in Table 1. The Sd value of AIBN-crosslinked-PBD microbeads becomes increasing smaller with addition of AIBN. The Sd value of PBD-5 crosslinked by UV irradiation for 48 h is smaller than that of PBD-4 crosslinked by UV irradiation for 24 h. These results suggest that crosslinking moiety increased with increasing radical reaction between vinyl groups. The PBD microbeads crosslinked using radical initiator AIBN were more swollen than those crosslinked by UV irradiation. Therefore, we adopted the crosslinking method by UV irradiation in order

Table 1
Swelling degree of crosslinked PBD microbeads

Microbeads	AIBN (%)	UV irradiation time (h)	Sd _{for THF} (ml/dry-g)
PBD-1	5	–	11.5
PBD-2	10	–	10.5
PBD-3	20	–	9.1
PBD-4	–	24	6.3
PBD-5	–	48	3.2

Table 2
Iodine number and content of vinyl group in PBD estimated by Wijs method

UV irradiation time (h)	Iodine number	Vinyl group (mmol/g)
0	344.5	13.6
2	316.4	12.5
4	300.1	11.8
6	269.6	10.6
8	251.3	9.90
10	197.4	7.78
12	137.3	5.41
24	83.1	3.27
36	60.3	2.38
48	32.0	1.26

to prepare porous microbeads favorable for SEC packing materials.

Table 2 summarizes the amount of vinyl group in PBD microbeads estimated by Wijs method. The amount of vinyl group decreased rapidly to 1/2.5 in the first 12 h, and then decreased gradually in the next 48 h, as shown in Fig. 4. The amount of vinyl group in PBD microbeads decreased to 1/11 by UV irradiation for 48 h. As a result, the microbeads treated by UV irradiation came to be insoluble in THF, DMF, and chloroform. Fig. 5 shows the X-ray diffraction patterns of UV non-irradiated PBD and irradiated PBD particles. The longer the time of UV irradiation is, the broader the reflection peak is. This indicates that the crystallinity of PBD decreased gradually due to disorderly reactions between vinyl groups with UV irradiation.

The Sd of crosslinked-PBD microbeads was considered to depend on the polarity of solvent used for crosslinking. The Sd for benzene in the PBD microbeads crosslinked by UV irradiation in several organic solvents is summarized in Table 3. The Sd of PBD in benzene increased with decrease of the polarity of solvent used. Especially, if hexane was used as the dispersion solvent for crosslinking reaction, PBD microbeads became soft gels, although they retained their spherical shape. This indicates that crosslinking reaction

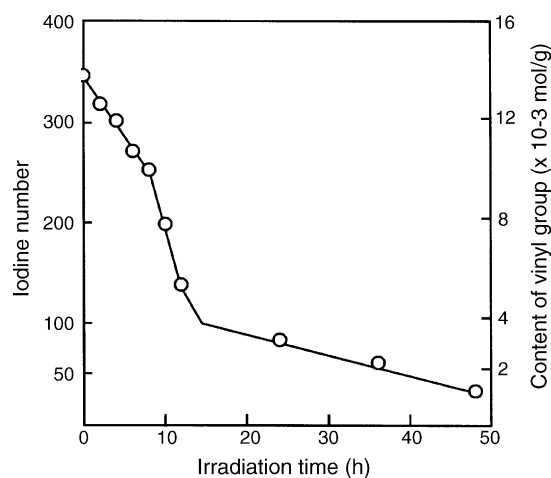


Fig. 4. Relationship among UV irradiation time, iodine number and amount of vinyl group in PBD.

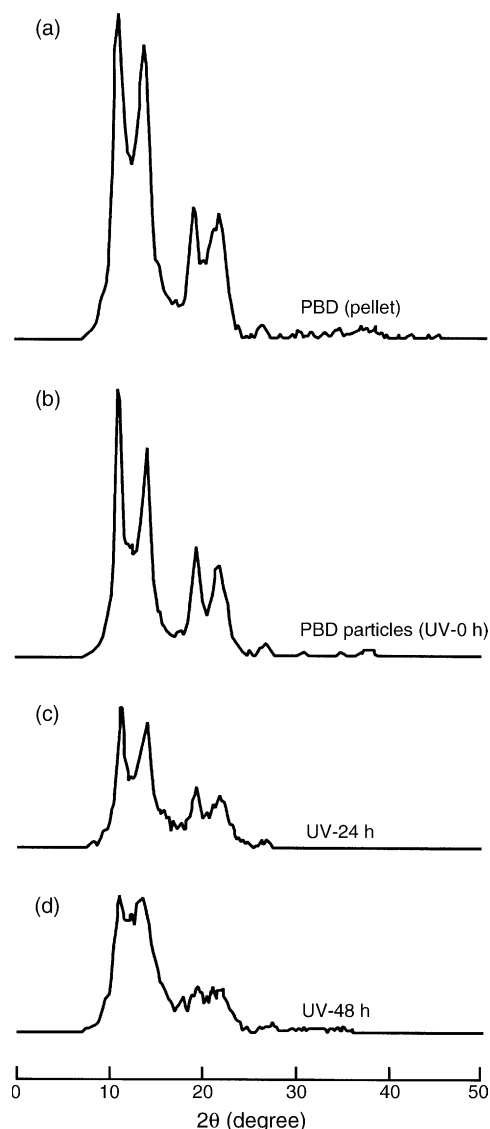


Fig. 5. X-ray diffraction patterns of UV non-irradiated PBD and irradiated PBD particles: (a) PBD (pellet); (b) non-irradiated PBD; (c) PBD UV-irradiated for 24 h PBD; and (d) UV-irradiated for 48 h.

occurred while the hexane molecules expanded their matrices. In contrast, the S_d of PBD microbeads crosslinked in high polar solvents such as methanol was smallest, because the crosslinking reaction progressed while the matrices of the high polar solvents shrank. Fig. 6a–d show the SEM images of the surface of PBD microbeads crosslinked in methanol,

Table 3
Swelling degree in benzene, vinyl group estimated by Wijs method, M_{lim} and porosity of PBD crosslinked by UV irradiation

Microbeads	Solvent	$S_{d\text{for benzene}}$ (ml/dry-g)	Vinyl group (mmol/g)	M_{lim}	Porosity (%)
PBD-4	MeOH	4.6	3.27	1000	58
PBD-6	PrOH	7.0	7.66	5000	85
PBD-7	BuOH	8.2	7.78	5500	92
PBD-8	Hexane	–	–	–	–

1-propanol and 1-butanol. All of the PBD microbeads are spherical and have porous structure. Their surface structure became more markedly uneven with lower polarity of solvent used for crosslinking reaction (polarity: methanol > 1-propanol > 1-butanol).

3.2. Porosity

SEC was carried out using a column packed with PBD microbeads. The exclusion molecular weight (M_{lim}) and porosity were determined from the calibration curve obtained using polystyrene standards. The effects of solvents used in crosslinking were examined in order to increase the porosity in the PBD microbeads. Fig. 7 shows typical calibration curves of nonaqueous SEC for PBD microbeads (PBD-4, PBD-6 and PBD-7) crosslinked by UV-radiation in methanol, 1-propanol and 1-butanol. The M_{lim} values of PBD-4, PBD-6 and PBD-7 were 1000, 5000 and 5500, respectively. The porosities of PBD-4, PBD-6 and PBD-7 were 58, 76 and 92%. The M_{lim} and porosity increased with decreasing polarity of the solvent used for crosslinking reaction. The affinity to PBD increased in the order of 1-butanol, 1-propanol, and methanol, and solvent molecules were able to permeate into networks among polymer main chains in the order of 1-butanol, 1-propanol and methanol. We assumed that larger solvent domains in polymer network produced longer distances of crosslinking points among polymer chains, and thus the degree of crosslinking was lower. Therefore, the residual vinyl group decreased in the order of PBD-4, PBD-6 and PBD-7, as shown in Table 3.

3.3. Comparison with polystyrene and polypropylene column as nonaqueous SEC

Fig. 8 shows typical calibration curves of nonaqueous SEC for the PBD-7 column, PP column and PS-DVB column. Polypropylene microbeads have no porosity, as expected. Although the M_{lim} of PBD-7 was equal to that of PS-DVB, its porosity was two times higher than PS-DVB. The value of α_{eff} is smaller for PBD-7 than for PS-DVB. This indicates that PBD-7 is more applicable for separation of similar molecular weights, for instance the SEC separation of oligomers.

The value of α_{eff} is an indication of pore size homogeneity: the smaller the value, the more homogeneous the pore. As shown in Fig. 9, the pore size distribution of PBD-7 was narrower than PS-DVB. In general, PS-DVB microbeads are obtained by suspension copolymerization of styrene and divinylbenzene. The formation of porosity used for SEC packing is carried out by addition of diluents during copolymerization. The conversion from monomer to polymer occurs heterogeneously, because polymerizing and nonpolymerizing domains mix in the sphering process. Also, the addition of diluents into inhomogeneous radical reactive solution induces partial inhomogenization. Therefore, the pore size distribution of PS-DVB microbeads is large. A large pore size distribution of packing material

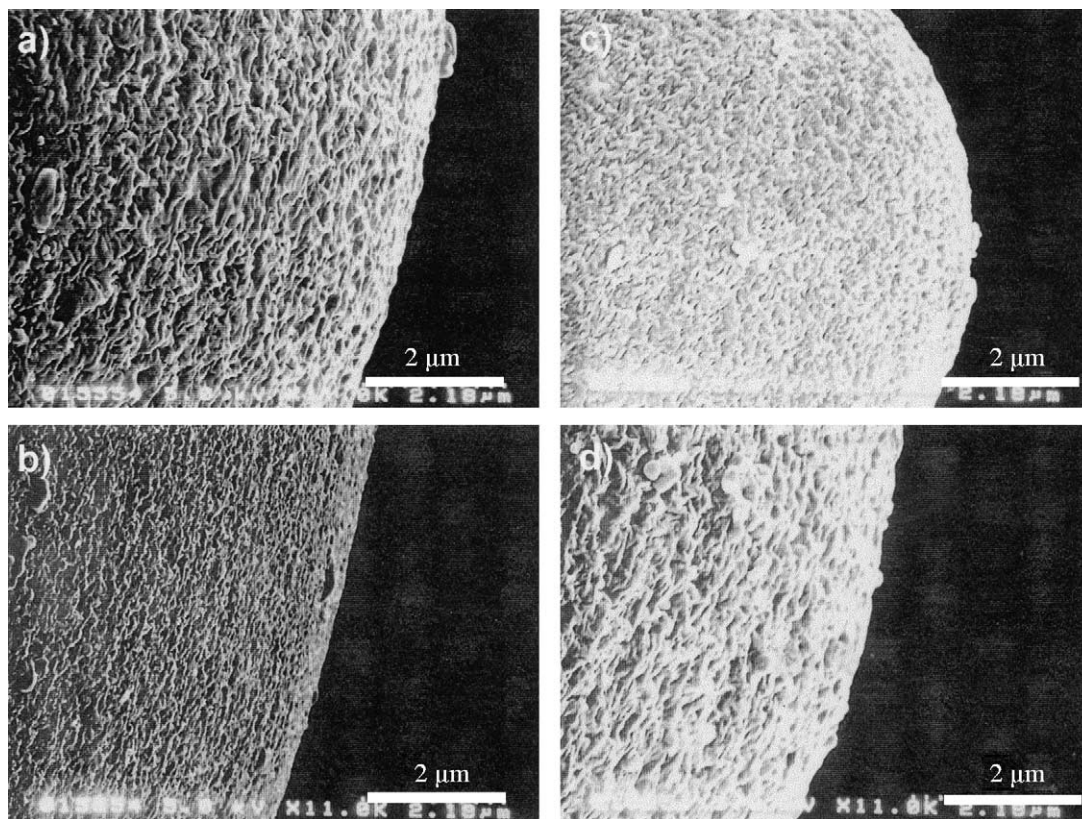


Fig. 6. SEM images of the surface of non-irradiated PBD and PBD microbeads crosslinked: (a) non-irradiated PBD; (b) PBD crosslinked in methanol; (c) PBD crosslinked in 1-propanol; and (d) PBD crosslinked in 1-butanol.

is not suitable for SEC separation of similar molecular weights.

In contrast, using the suspension evaporation process with polymer solution and diluent, the diluent domains were considered to be distributed in the polymer solu-

tion homogeneously. As the chloroform in polymer solution evaporated gradually, the diluent domain remained in the butadiene polymer matrix homogeneously. As a result, spherical particles with narrow pore size distribution were formed.

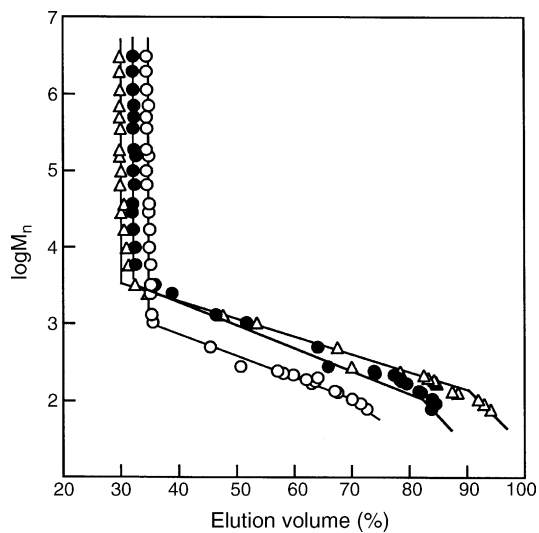


Fig. 7. Calibration curve of nonaqueous SEC for PBD-4 (○), PBD-6 (●) and PBD-7 (△) column. Eluent, THF; flow rate, 0.5 ml/min; and column, 150 mm × 4.6 mm I.D.

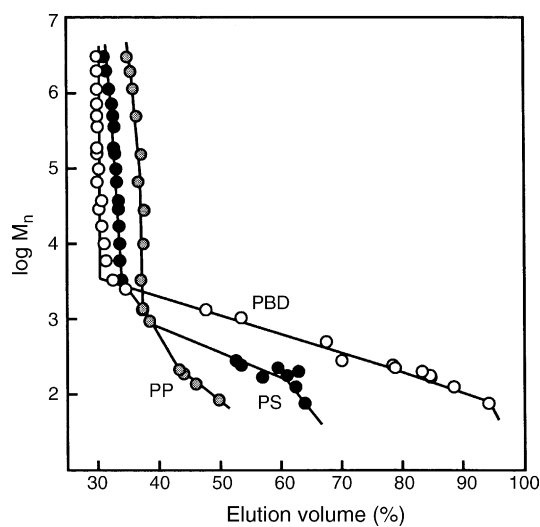


Fig. 8. Calibration curves of nonaqueous SEC for PBD-7 (○), PS (●) and PP (⊙) column. Eluent, THF; flow rate, 0.5 ml/min; and column, 150 mm × 4.6 mm I.D.

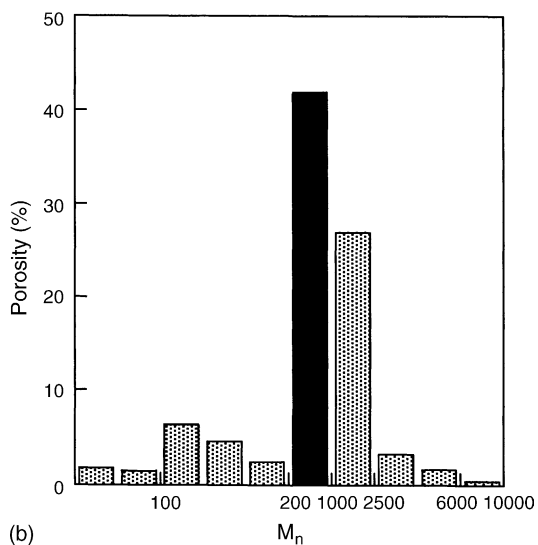
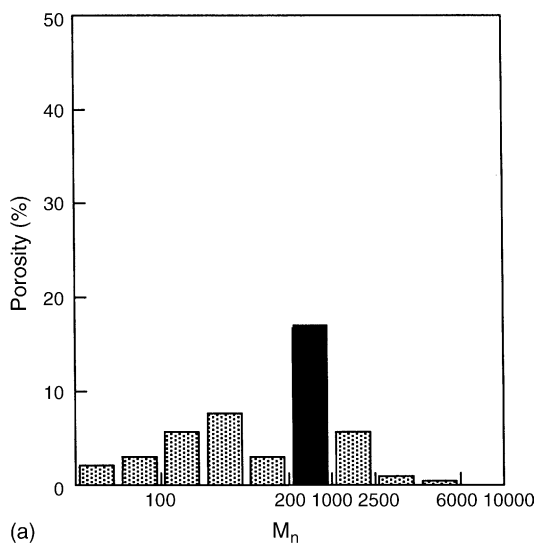


Fig. 9. Pore size distribution for PS (a) and PBD-7 (b) microbeads.

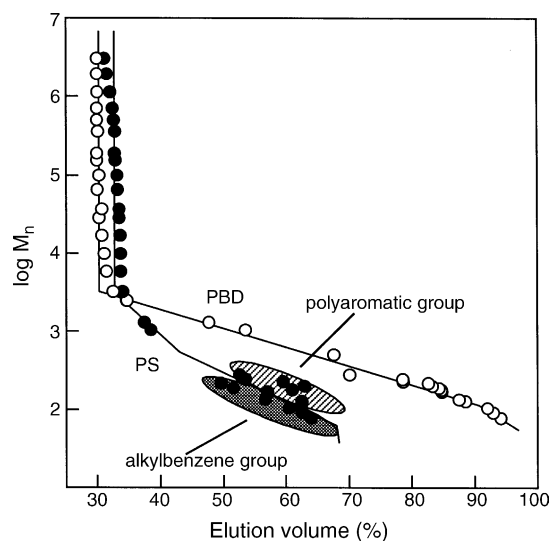


Fig. 10. Calibration curves of nonaqueous SEC for PBD-7 (○) and PS (●) columns, using alkylbenzenes and polycyclic aromatic hydrocarbon as solutes. Eluent, THF; flow rate, 0.5 ml/min; and column, 150 mm × 4.6 mm I.D.

3.4. Elution property for hydrocarbons

Fig. 10 shows calibration curves of nonaqueous SEC for PBD-7 and PS-DVB columns, using alkylbenzenes and polycyclic aromatic hydrocarbon as solutes. The PS-DVB plots divided into two groups: alkylbenzene series and polycyclic aromatics compounds series. However, the plots of PBD connected in a straight line. The separation mechanism for PBD is dependent on size-exclusion effect alone, and is simpler than separation mixed-modes such as π -electron effect and size-exclusion effect with PS-DVB. This shows that PBD is able to separate solutes by size-exclusion effect alone. Fig. 11 shows the plots of elution volume for M_n of aromatic hydrocarbons with PBD-7 and PS-DVB columns. Scattered plots for aromatic compounds of PS-DVB columns were observed. The retention increases with the increase in number of π -electrons

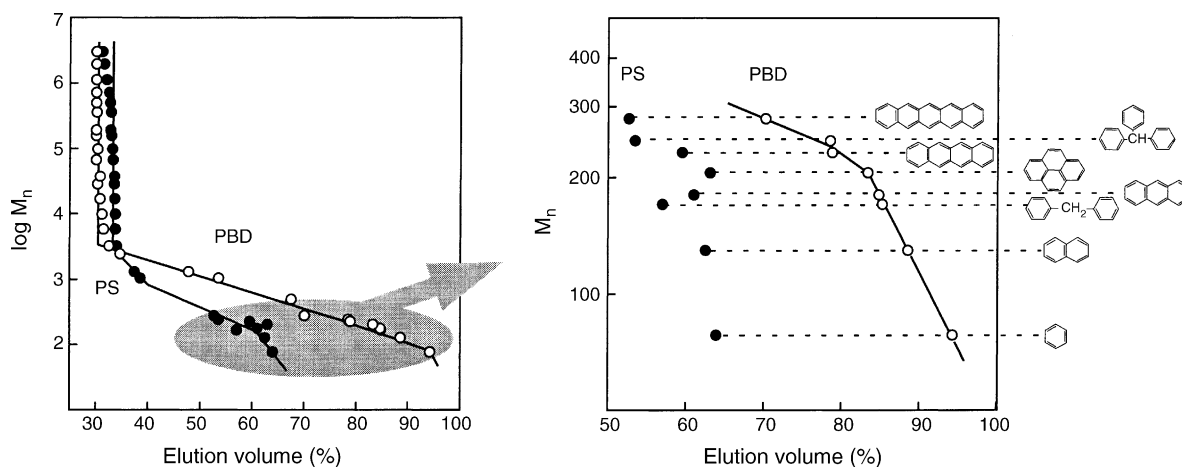


Fig. 11. Elution volume for M_n of polycyclic aromatic hydrocarbons with PBD-7 (○) and PS (●) columns. Eluent, THF; flow rate, 0.5 ml/min; and column, 150 mm × 4.6 mm I.D.

for aromatic hydrocarbons. These attributes are derived from the aromaticity of the styrene unit and divinylbenzene moiety used as crosslinking monomers in PS-DVB microbeads. These results are not satisfactory with respect to the SEC properties. In contrast, separation of aromatic hydrocarbons using PBD columns was eluted in the order of high molecular weight. Close correlation between the elution volume and M_n for polycyclic aromatic compounds was observed with PBD columns.

4. Conclusion

Porous polymer spherical particles for column packings in nonaqueous SEC were prepared from 1,2-syndiotactic polybutadiene by the suspension and evaporation method. The polymer microbeads as SEC packing materials were obtained using crosslinking by radical reaction between 2-vinyl groups in polybutadiene with ultraviolet radiation, to render them insoluble.

The PBD microbeads have the following advantageous SEC chromatographic properties:

- (1) The M_{lim} and porosity of PBD microbeads could be controlled by choosing polarity of dispersion solvents for crosslinking radical reaction of PBD microbeads.
- (2) There was no excessive retention for aromatic compounds. The separation mechanism with PBD is dependent on size-exclusion effect alone. Close correlation between the elution volume and M_n for polycyclic aromatic compounds was observed with polybutadiene microbeads column.
- (3) The porosity is twice as high as commercial polystyrene columns. Thus it is more applicable to separation of oligomers.

These favorable properties of PBD packing will enable other applications for LC support in addition to SEC.

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