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Methacrylate-ester-based Reversed Phase Monolithic Columns for High Speed Separation Prepared by Low Temperature UV Photo-polymerization

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Butyl methacrylate-based reversed phase capillary monolithic columns were prepared using ultraviolet (UV) photo-polymerization. The effects of two photo-polymerization conditions (UV irradiation intensity and polymerization temperature) on the column characteristics were investigated. Both the higher UV irradiation intensity and the lower polymerization temperature lead to the superior column efficiency. The column prepared under the optimized conditions was evaluated through the separation of the uracil and five alkylbenzenes in the linear flow rate range of 1 – 110 mm/s. At 1 mm/s, all analytes were well separated (N = 36000 – 45000 plates/m). The high speed separation within 8 s was performed at 110 mm/s (back pressure, 33 MPa) at room temperature, whereas the peaks eluted earlier were overlapped partially. The relationship between the flow rate and the back pressure indicated that some kind of structural change of the monolith might occur in 50 – 110 mm/s, although no visible or hysteresis changes of the monolith were observed after the measurement.

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Introduction

The demand for high speed separation has been growing in the studies that analyze vast amounts of samples and compounds such as metabolomics and proteomics.[1-4] In order to accomplish high speed separation on high performance liquid chromatography (HPLC), one must solve two problems at high flow rate: decrease in column efficiency and increase in back pressure. One of the recent solutions for high speed separation is the ultrahigh pressure liquid chromatography (UPLC).[5,6] In the UPLC, a column packed with micro particles less than 3 μm in size is used, since the column efficiency increases with decreasing of particle diameter (d_p).[7] Although the column packed with smaller particles results in the preferable column efficiency, the back pressure significantly increases inversely proportional to d_p^2. Therefore, the UPLC needs to use a highly pressure-resistant LC system and the flow rate limit for the UPLC is around 10 mm/s (one order larger than that in the general flow rate in HPLC). However, further reduction in the analytical period is required.

In recent years, monolithic columns have attracted much attention and have been intensively studied as the alternative solution for the high speed separation operated with relatively low back pressure.[8-11] The high speed separation using monolithic column have been widely studied. To the best of our knowledge, almost all studies using monolithic columns performed several to a few ten times faster separation than the conventional packed column. As an instance of extremely high speed separation, Ueki *et al*. achieved 100 times faster separation than that in the conventional HPLC.[12]

The monolithic columns can be divided into two types, *i.e.*, silica- and organic polymer-based monolithic columns.[13,14] The silica monolithic column has some advantages over the polymer-based one, *e.g.* well controlled pore structure, good mechanical strength, and high column efficiency especially for small molecules.[15-18] The techniques for the preparation and modification of silica monolithic columns have been extensively studied, and their users are increasingly widespread.[19-22] On the other hand, polymer-based monolithic columns have unique advantages such as an availability over a wide pH range and simplicity of preparation.[23-27] In recent years, the polymer-based monolithic columns which have high column efficiencies comparable to those of silica-based ones have been developed.[28,29] In addition, their column efficiencies for small molecules have continued to be improved.

General polymer-based monolithic columns are prepared by *in situ* polymerization of a reaction solution containing monomer, crosslinker, porogenic solvent, and initiator; the composition of this solution affects the characteristics of the produced monolithic columns, *i.e.*, backpressure, retention factor, and column efficiency.[30-34] The characteristics of the columns were also affected by the polymerization conditions, such as the temperature and the radical generation rate.[35-37] In other words, the efficiency of the polymer-based monolithic column can be improved by the optimization of polymerization conditions. The usual polymerization methods of the monolithic columns are thermal- and photo-polymerizations.[23,24,26] The thermal-polymerization is more commonly used than the photo-polymerization because of its simplicity. Ueki *et al*. prepared the low flow resistance polymer-based monolithic

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column using the thermal polymerization.[12] They achieved the high speed separation of the alkylbenzenes at a linear flow rate of 100 mm/s (100 times faster than the conventional HPLC) with 33 MPa back pressure, which is available on the conventional HPLC pump.

Meanwhile, the photo-polymerization has some advantages over the thermal-polymerization; the former can be achieved at a localized and targeted region by controlling irradiation area. This advantage is essential to prepare polymer-based monolith in micro fluidic devices.[38,39] In addition, the radical generation rate in the photo-polymerization is able to be controlled independently of the polymerization temperature by varying the light source intensity. In other words, the polymerization temperature can also be changed without considering the radical generation rate. Thus, the photo-polymerization has a wide latitude of the polymerization conditions. For the preparation of the photo-polymerized monolithic columns, however, the effects of the UV irradiation intensity and the polymerization temperature on the column efficiency have not been sufficiently studied. This is the case in particular for the preparations at lower temperatures, at which the thermal polymerization does not efficiently contribute.

In this study, we prepared butyl methacrylate-based reversed phase monolithic columns for the high speed separation, over 100 times faster than that in the conventional HPLC using photo-polymerization. In order to control the column properties, we independently varied two polymerization conditions, the UV irradiation intensity and the polymerization temperature. Their effects on the column efficiency and the back pressure, which are critical for the high speed separation, were investigated. Particularly, we focused on the polymerization at relatively low temperature. The performance and stability of the prepared monolithic column in the high speed separation were evaluated at flow rates up to 110 mm/s.

Experimental

Chemicals

Butyl methacrylate (BMA), ethylene dimethacrylate (EDMA), 1-decanol, cyclohexanol, 2,2-dimethoxyphenyl-2-acetophenone (DMPA), acetone, sodium hydroxide, hydrochloric acid, methanol, acetonitrile, uracil, naphthalene, anthracene, toluene, *n*-propylbenzene, *n*-butylbenzene, and *n*-pentylbenzene were purchased from Wako Pure Chemicals (Osaka, Japan). Ethylbenzene and 3-methacryloxypropyltrimethoxysilane were obtained from Tokyo Chemical Industry (Tokyo, Japan) and Shin-Etsu Chemicals (Tokyo), respectively. All chemicals were used as received. Distilled water was used for all experiments.

Column preparation

First, a UV-transparent fused silica capillary (100 μm i.d., 375 μm o.d., 20 cm long, GL Science, Tokyo, Japan) filled with 1 M NaOH aqueous solution was kept in a water bath at 65°C for 1 h, and then sequentially flushed with distilled water, 1 M HCl aqueous solution, and distilled water. After drying with a N_2 stream for 1 h, the capillary was filled with 33% 3-methacryloxypropyltrimethoxysilane in acetone and then kept in water bath at 65°C for 3 h to introduce the anchor for attaching polymer-based-monolith to the capillary inner wall. After the treatment, the capillary was flushed with methanol and then dried with a N_2 stream for 1 h.

A polymerization mixture consisting of BMA (24 wt%, monomer), EDMA (16 wt%, crosslinker), 1-decanol (34 wt%, porogenic solvent), cyclohexanol (26 wt%, porogenic solvent), DMPA (1 wt% respect to monomers, photoinitiator) was poured into the pretreated capillary. The composition was decided empirically. Then a photo-polymerization was performed using a UV illuminator (3UV Benchtop Trance Illuminator, Upland., CA) as a UV (254 nm) light source in an incubator (MIR-153, Sanyo, Osaka, Japan) under various polymerization conditions shown in Table 1.

Table 1 Photo-polymerization conditions of butyl methacrylate-based monolithic columns

Condition No.	UV lamp tube	Reflector	UV irradiation intensity/ mW cm^{-2}	UV irradiation time/min	Polymerization temperature/°C
1	1	—	0.4	8	0
2	6	—	1.0	8	0
3	6	—	1.0	16	0
4	6	+	2.0[a]	8	0
5	6	+	2.0[a]	8	10
6	6	+	2.0[a]	8	20

a. Estimated value.

The capillary was irradiated at 5 cm distance from the UV illuminator, which has six UV lamp tubes. In the condition No. 1 of Table 1, the capillary was irradiated with the UV light from only one lamp. In the conditions Nos. 2 – 6, the UV light from all of six lamp tubes was irradiated to the capillary. In the conditions Nos. 4 – 6, semi-cylindrical reflector (diameter of 20 cm, length of 20 cm) was additionally placed over the capillary to enhance the UV irradiation intensity and homogeneity. The values of the UV intensities of the conditions Nos. 1 – 3 were measured by a UV meter (UVC-254, AS ONE, Osaka, Japan), and those of Nos. 4 – 6 were values estimated by considering the reflection. As shown in Table 1, the polymerizations were performed under the relatively lower temperature (0 – 20°C, ±2°C). After the polymerization, the capillary was immediately connected to a LC pump and then washed with methanol for at least 6 h at a flow rate of 2 μL/min. Finally, the column was cut to 10 cm long.

Chromatography

The capillary HPLC system used consisting of a pump (LC-10ADvp, Shimadzu, Kyoto, Japan), a sample injector (Model 7520, Rheodyne, Cotati, CA) with a 0.5 μL sample loop, a splitter for split injection (resistance capillary: 30 cm × 50 μm i.d.), and a UV/VIS detector (CE-1575, Jasco, Tokyo, Japan). The split ratio is about 20:1 except in case of the column prepared with the condition No. 6 (60:1) which has about three times higher flow resistance than the other five columns. All of chromatographic experiments were performed by isocratic elution using acetonitrile–water (50:50, v/v) as the mobile phase at room temperature (around 20°C). A linear flow rate was calculated from the elution time of uracil (t_0 marker) and the column length. Theoretical plate numbers were calculated using the half width and the retention time of each peak.

SEM measurement

The cutting planes of the monolithic columns were analyzed with a scanning electron microscope (SEM, JXA-8800, JEOL, Tokyo, Japan). After chromatographic measurements, the columns were washed with methanol at a flow rate of 2 μL for at least 12 h, followed by drying at ambient temperature for 3 days and N_2 purging for 3 h. Each dried capillary was cut into

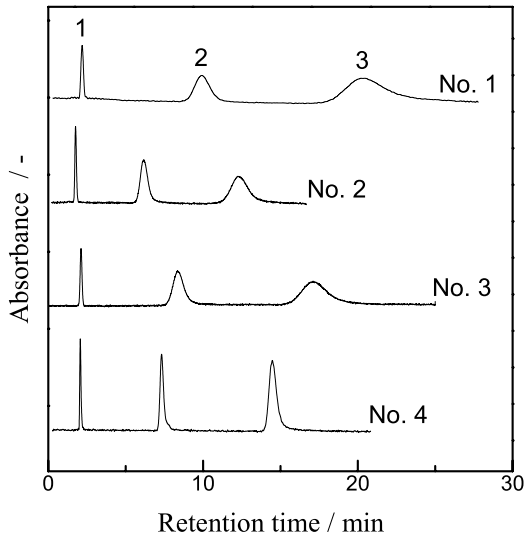

Fig. 1 Separations of polycyclic aromatics on the butyl methacrylate-based monolithic columns prepared with different UV irradiation conditions: (No. 1) UV irradiation intensity, 0.4 mW/cm^2; UV irradiation time, 8 min, (No. 2) 1 mW/cm^2, 8 min, (No. 3) 1 mW/cm^2, 16 min, (No. 4) 2 mW/cm^2, 8 min. Column size, 100 mm × 100 μm i.d.; mobile phase, acetonitrile–water (50:50, v/v); linear flow rate, 1 mm/s; UV detection at 217 nm. Analytes: (1) uracil (t_0 marker), (2) naphthalene, (3) anthracene. Analyte concentration: 1 mM.

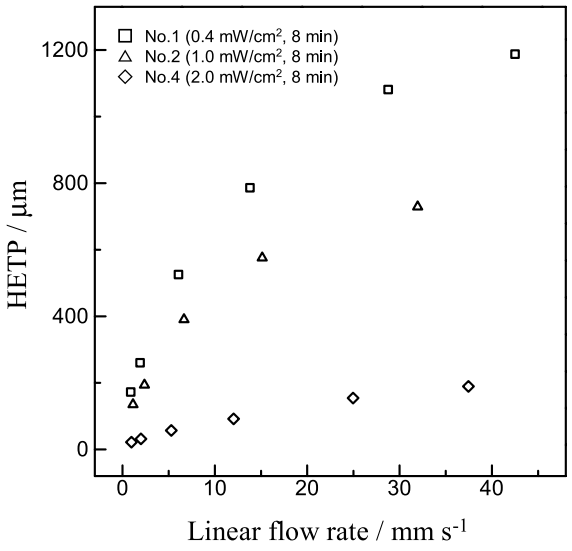

Fig. 2 *H–u* plots of the monolithic columns prepared with 8 min of the UV irradiation (Nos. 1, 2, and 4). Chromatographic conditions are the same as in Fig. 1. HETP was calculated from the naphthalene peak.

Table 2 Chromatographic characteristics of monolithic columns prepared with different conditions

Condition No.	Back pressure[a]/ MPa s mm^{-1}	k[b]	N/L[c]/ m^{-1}
1	0.28	3.5	4000
2	0.25	2.5	9000
3	0.28	3.0	6000
4	0.31	2.5	45000
5	0.28	2.9	32000
6	0.91	4.0	16000

a. Slope value for flow rate-back pressure relation in the back pressure from 0.2 to 11 MPa ($r^2 > 0.999$).
b. The retention factor of naphthalene at a linear flow rate of 1 mm/s.
c. The theoretical plate numbers of the naphthalene peak at a linear flow rate of 1 mm/s.

at least 3 pieces; each piece was spattered with gold and then analyzed by SEM.

Results and Discussion

Effects of the UV irradiation intensity on the column efficiency

In a photo-polymerization, the UV irradiation conditions should directly affect the rate and the uniformity of the radical generation. Therefore, we investigated their effect on the column efficiency of the butyl methacrylate-based monolithic column. At first, several monolithic columns were prepared under various UV irradiation conditions (Nos. 1 – 4 in Table 1) at the polymerization temperature of 0°C. Then separations of a standard mixture (uracil, naphthalene, anthracene) using these columns were evaluated in the flow rate range of about 1 – 40 mm/s.

Figure 1 shows separations of the standards using the columns prepared with various irradiation conditions of Nos. 1 – 4 at the linear flow rate of 1 mm/s. In comparison with the columns prepared at the same irradiation time for 8 min (conditions of Nos. 1, 2, and 4), the column prepared at the higher UV intensity provided better efficiency, *i.e.*, narrower peaks. When the total UV irradiation energies were the same (conditions of Nos. 3 and 4), the column prepared with the higher UV irradiation intensity (No. 4) produced more efficient separation.

The effect of the linear flow rate on the column efficiency was also evaluated. Figure 2 shows the *H–u* plots for naphthalene using the columns prepared with 8 min of the UV irradiation (Nos. 1, 2, and 4). As clearly shown in Fig. 2, the plots shift to the lower (HETP decreased) with increase in the UV irradiation intensity. The improvement of the column efficiency with the higher UV irradiation intensity was observed over the entire linear flow rate range of 1 – 40 mm/s and its magnitude at higher flow rate was larger than that at lower flow rate.

The chromatographic characteristics of the six columns prepared are described in Table 2. In comparison with the conditions Nos. 1 – 4, the UV irradiation conditions did not affect significantly the back pressure (flow resistance of the column), and thus did not affect the through pore structures of the monolithic columns. The SEM images of the monolithic columns prepared are shown in Fig. 3. It is clear that all of the monolithic columns in Fig. 3 have agglomerated structures. Figures 3A – D are the images of the columns prepared under the different UV irradiation conditions. These images show that the monolithic through pore structures, such as the sizes of the monolithic globules and the through pores, are comparable for the four columns. The retention factors are distributed in a range of 2.5 – 3.5 for the four columns, but no specific correlation between the retention factor and the UV irradiation conditions was observed. The theoretical plate numbers of the columns were significantly affected by the UV irradiation intensity, as clearly demonstrated in Fig. 1 and Table 2; the theoretical plate numbers increased with increase in the UV irradiation intensity.

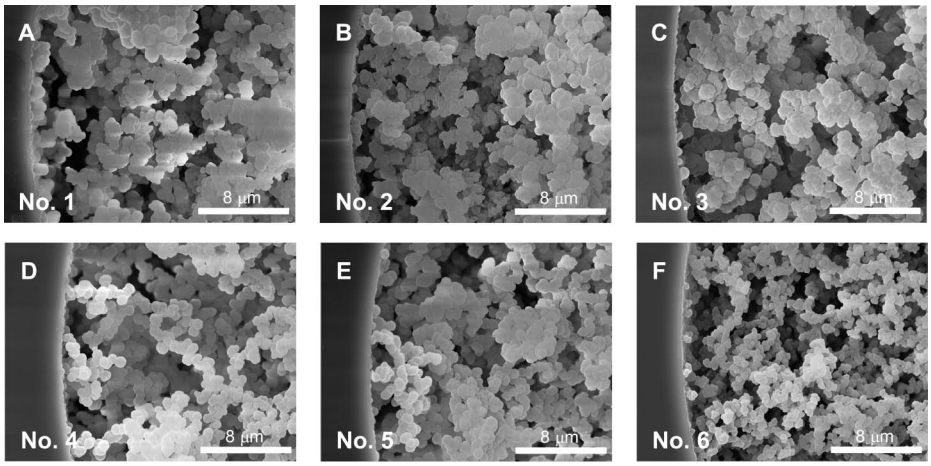

Fig. 3 SEM images of the monolithic columns prepared with different polymerization conditions in Table 1.

The directional uniformity of the UV irradiation also increases in the order of No. 1 < Nos. 2, 3 < No. 4. Not only the stronger UV irradiation but also its higher directional uniformity, therefore, might enhance the column efficiency. However, further studies are required to explain in detail such variations induced by the UV irradiation conditions.

Effects of the polymerization temperature on the column efficiency

The polymerization temperature changes the porogenic solvent's viscosity, the solubility of monolith polymer in the porogenic solvent, and the polymerization rate; such changes could affect the characteristics of the monolithic column. In this section, the effect of the polymerization temperature on the column efficiency with the constant UV irradiation intensity for the photo-polymerization was investigated. Here, we focused on the polymerization at the lower temperature, which the thermal-polymerization could not induce. Therefore, the monolithic columns were prepared in the relatively lower temperature range between 0 and 20°C (conditions of Nos. 4 – 6 in Table 1). Considering the results of the former section, we prepared these columns at the UV irradiation intensity of 2 mW/cm^2 for 8 min. The *H*-*u* plots of the columns prepared with different polymerization temperatures (conditions Nos. 4 – 6) are shown in Fig. 4. With decreasing polymerization temperature, the column efficiency was enhanced. Especially the HETP was much more decreased from 20 to 10°C than from 10 to 0°C. Table 2 also shows the chromatographic characteristics of the columns prepared with the conditions of Nos. 4 – 6. The highest back pressure value was observed for the column prepared at 20°C; those of the other columns (10 and 0°C) were significantly lower and nearly the same. The drastic difference in the back pressure between 20 and 10°C indicates that the polymerization temperature alters the base structure of the monolith, such as the sizes of through pore or the skeleton, which affect the back pressure directly. In fact, Figs. 3D – F revealed that the column prepared at 20°C had smaller through pores and globules than those at lower temperatures (the sizes of the column structure prepared at 10°C were almost the same as those at 0°C). Whereas the temperature-induced changes in the porogenic solvent properties (*i.e.* solvency and viscosity), in the rates of the polymerization reaction, and in the differences in the heat diffusion rate of the reaction heat might affect the column properties, more studies are needed to explain the details.

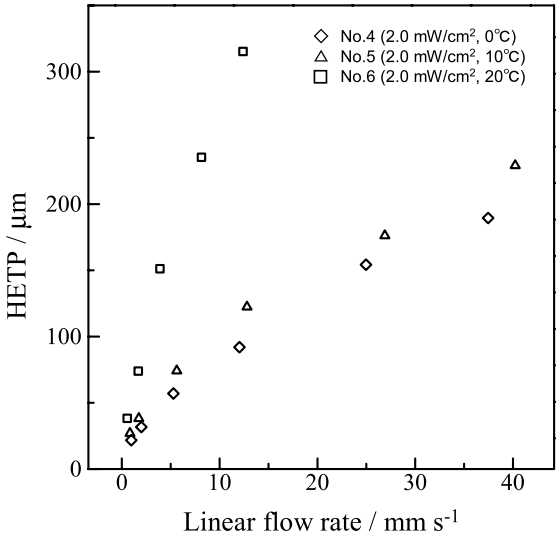

Fig. 4 *H*-*u* plots of the monolithic columns prepared with different polymerization temperatures. HETP values were calculated from naphthalene peak. Chromatographic conditions are the same as those in Fig. 1.

The lower temperature and the higher UV irradiation intensity were found to enhance the column efficiency. Much higher UV irradiation intensity (> 2 mW/cm^2) and lower polymerization temperature (< 0°C) could not be applied because of limitation of our instruments. In this study, the best performance was achieved by the column prepared with the condition of No. 4 (2 mW/cm^2, 8 min, 0°C) in Table 1. The reproducibility (batch-to-batch) of the columns prepared in this condition was good, *i.e.*, the relative standard deviations (RSDs) of back pressure, retention factor, and theoretical plate number were 10, 4, and 6%, respectively (n = 5).

High speed separation of alkylbenzenes

The column prepared with the condition of No. 4 (2 mW/cm^2, 8 min, 0°C) was applied to the high speed separation, in which

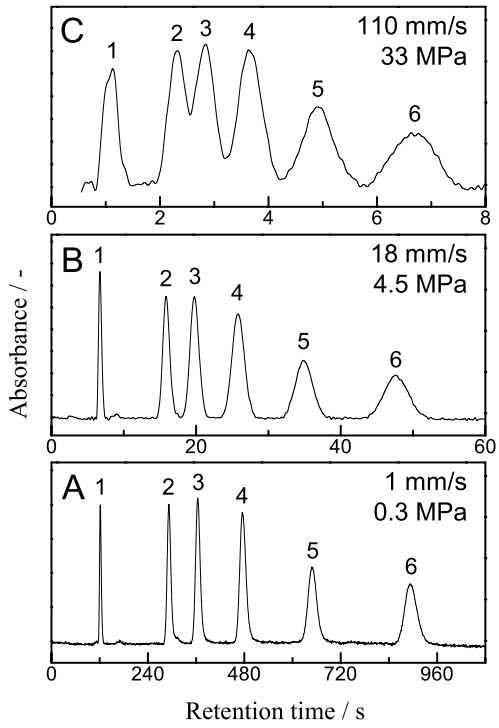

Fig. 5 Separations of alkylbenzenes on the monolithic column prepared with polymerization condition No. 4 (UV irradiation intensity, 2 mW/cm^2; UV irradiation time, 8 min; polymerization temperature, 0°C) at various flow rates. Analytes: (1) uracil (t_0 marker), (2) toluene, (3) ethylbenzene, (4) *n*-propylbenzene, (5) *n*-butylbenzene, (6) *n*-pentylbenzene. Analyte concentration: 0.3 mM (uracil), 1 mM (alkylbenzenes). UV detection at 190 nm. Other conditions are the same as those in Fig. 1.

uracil (t_0 marker) and five alkylbenzenes (toluene, ethylbenzene, *n*-propylbenzene, *n*-butylbenzene, and *n*-pentylbenzene) were separated at the linear flow rate of up to 110 mm/s.

The typical separations of alkylbenzenes with various linear flow rates are shown in Fig. 5. The theoretical plate numbers of alkylbenzenes (toluene, ethylbenzene, *n*-propylbenzene, *n*-butylbenzene, and *n*-pentylbenzene) at the linear flow rate of 1 mm/s were 45000, 45000, 42000, 40000, and 36000 plates/m, respectively. Figure 5A shows the separation of alkylbenzenes at the linear flow rate of 1 mm/s, the standard linear flow rate for the conventional packed column, in which the back pressure was quite low (0.3 MPa). According to the increase in the flow rate, both the analytical period and the separation efficiency decreased. The baseline separations of the analytes were kept at the linear flow rate up to 18 mm/s, as shown in Fig. 5B. At this flow rate, the separation was completed within 60 s. With further increase in the linear flow rate, the peaks that eluted earlier were partially overlapped. Figure 5C shows the separation of the analytes at the linear flow rate of 110 mm/s. At this extremely fast flow rate, the separation of toluene and ethylbenzene (peaks 2 and 3) was insufficient, but the peak top separation of the six analytes including t_0 marker was achieved within 8 s. Furthermore, the back pressure at the linear flow rate of 110 mm/s is 33 MPa, which can be achieved using a normal LC pump. The high speed separation at the linear flow rate of 110 mm/s could be performed at moderate back pressure, 33 MPa, but in order to achieve more efficient separation, we need more improvements of the column efficiency.

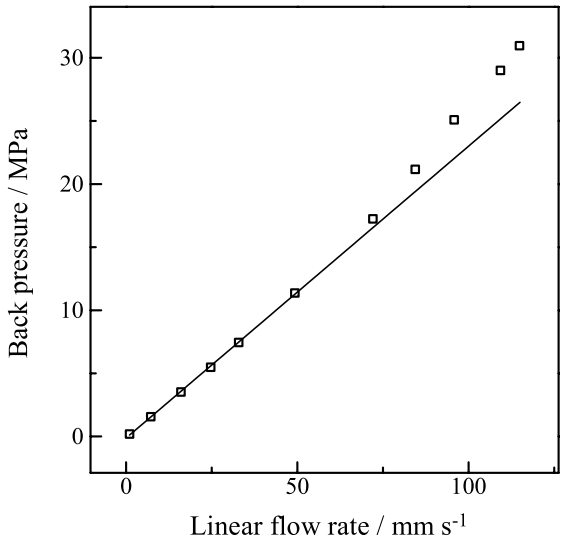

Fig. 6 Relationship between linear flow rate and back pressure (*P*–*u* plot) of the monolithic column prepared with condition No. 4 (UV intensity, 2 mW/cm^2; irradiation time, 8 min; polymerization temperature, 0°C). The line on the plot is the fitting line for six points in 1 – 50 mm/s. Chromatographic conditions are the same as those in Fig. 5.

Stability of monolithic column on high back pressure

Generally, the mechanical strength of polymer-based monoliths with the agglomerated structures is relatively lower than those of silica-based ones. Under the higher back pressure, polymer-based monoliths would be compressed, which causes a decrease in the column efficiency.[40] Therefore the stability of the polymer-based monolithic column in the higher pressure region is an important factor to keep column efficiency. In this section, we present the stability values of the column prepared with condition No. 4 at a linear flow rate of 1 – 110 mm/s (0.3 – 33 MPa).

Figure 6 shows a relationship between the back pressure and the linear flow rate (*P*–*u* curve). The *P*–*u* curve in Fig. 6 shows good linearity until a flow rate of 50 mm/s (11 MPa), and then the slope of the *P*–*u* curve gradually increases. This *P*–*u* curve shows that the through pore structure changed at 50 mm/s (11 MPa). Figure 7 shows the *H*–*u* plots of three alkylbenzenes (toluene, *n*-propylbenzene, and *n*-pentylbenzene) in a linear flow rate range of 1 – 110 mm/s. In the linear flow rate range of 1 - 50 mm/s (0.3 - 11 MPa), all three *H*–*u* plots showed similar behaviors. Beyond 50 mm/s (11 MPa), the slope of the *H*–*u* plot of toluene gradually increased. On the other hands the values, those of *n*-propylbenzene and *n*-pentylbenzene hardly changed throughout the flow rate range tested. The relationships between the retention factors and the linear flow rate are shown in Fig. 8. The retention factors of all alkylbenzenes decreased with increases in the linear flow rate. The decrement was almost the same for every analyte, *i.e.*, the retention factor at 110 mm/s was about 75% of that at 1 mm/s. As is interesting to note, a decrease in the retention factor was observed over the flow rate of 20 mm/s. This value differed from the turning point of the *P*–*u* and *H*–*u* plot shown in Figs. 6 and 7. The mechanism for this difference is under study.

These *P*–*u*, *H*–*u*, and *k*–*u* plots show that the structure of monolith gradually altered according to the increase in the back pressure. However, no visible change of the monolithic structure, such as compression, was observed after measurements

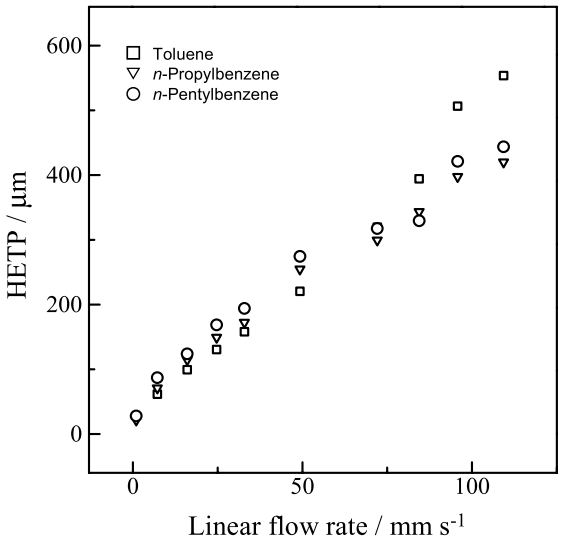

Fig. 7 *H–u* plots of the monolithic column prepared with condition No. 4 (UV intensity, 2 mW/cm^2; irradiation time, 8 min; polymerization temperature, 0°C). Chromatographic conditions are the same as those in Fig. 5.

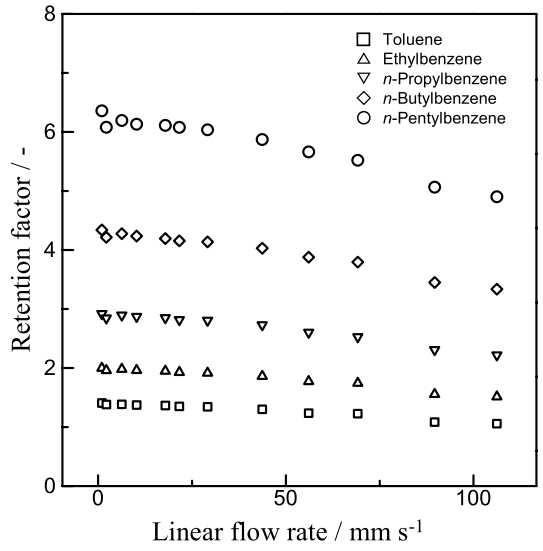

Fig. 8 Relationships between linear flow rate and retention factor of alkylbenzenes of the monolithic column prepared with condition No. 4 (UV intensity, 2 mW/cm^2; irradiation time, 8 min; polymerization temperature, 0°C). Chromatographic conditions are the same as those in Fig. 5.

at 33 MPa. Moreover, these chromatographic behaviors (*P–u*, *H–u*, and *k–u* relations) in the region of 1 – 110 mm/s were reproduced in multi-cycle measurements. The structural changes would return to the original state after the high pressure was released.

Conclusion

We prepared the monolithic columns for the high speed separation using low temperature UV photo-polymerization. We demonstrated that their separation performances at the flow rates over 100 mm/s are the same as or even better than that of the column prepared by thermal polymerization reported previously. The structural change under the high pressure is one of the possible disadvantages for the polymer-based monolithic column. Unfortunately, the structure of the column prepared in this study would be deformed under the high pressure caused by the fast flow rate. The suppression of the structural change is necessary to achieve more efficient high speed separation. Needless to say, the increase in the separation efficiency is also important for the high speed separation. The higher UV irradiation intensity and the lower polymerization temperature were found to be effective to enhance the column efficiency. Although our experimental system could not to be adjusted to the conditions for the column preparation at lower temperature and higher UV irradiation intensity, the photo-polymerization with such conditions would be suitable to enhance the separation efficiency of polymer-based monolithic columns.

References

1. O. Fiehn, *Plant Mol. Biol.*, **2002**, *48*, 155.
2. D. Figeys, *Anal. Chem.*, **2003**, *75*, 2891.
3. D. Ryan and K. Robards, *Anal. Chem.*, **2006**, *78*, 7954.
4. J. C. Smith, J.-P. Lambert, F. Elisma, and D. Figeys, *Anal. Chem.*, **2007**, *79*, 4325.
5. J. R. Mazzeo, U. D. Neue, M. Kele, and R. S. Plumb, *Anal. Chem.*, **2005**, *77*, 460A.
6. D. T.-T. Nguyen, D. Guillarme, S. Rudaz, and J.-L. Veuthey, *J. Sep. Sci.*, **2006**, *29*, 1836.
7. E. Katz, K. L. Ogan, and R. P. W. Scott, *J. Chromatogr.*, **1983**, *270*, 51.
8. C. Legido-Quigley, N. D. Marlin, V. Melin, A. Manz, and N. W. Smith, *Electrophoresis*, **2003**, *24*, 917.
9. G. Desmet, D. Cabooter, P. Gzil, H. Verelst, D. Mangelings, Y. V. Heyden, and D. Clicq, *J. Chromatogr., A*, **2006**, *1130*, 158.
10. K. K. Unger, R. Skudas, and M. M. Schulte, *J. Chromatogr., A*, **2008**, *1184*, 393.
11. R. Wu, L. Hu, F. Wang, M. Ye, and H. Zou, *J. Chromatogr., A*, **2008**, *1184*, 369.
12. Y. Ueki, T. Umemura, Y. Iwashita, T. Odake, H. Haraguchi, and K. Tsunoda, *J. Chromatogr., A*, **2006**, *1106*, 106.
13. H. Minakuchi, K. Nakanishi, N. Soga, N. Ishizuka, and N. Tanaka, *Anal. Chem.*, **1996**, *68*, 3498.
14. F. Svec and J. M. J. Fréchet, *Anal. Chem.*, **1992**, *64*, 820.
15. L. Rieux, H. Niederländer, E. Verpoorte, and R. Bischoff, *J. Sep. Sci.*, **2005**, *28*, 1628.
16. M. Kato, K. Sakai-Kato, and T. Toyo'oka, *J. Sep. Sci.*, **2005**, *28*, 1893.
17. O. Núñez, K. Nakanishi, and N. Tanaka, *J. Chromatogr., A*, **2008**, *1191*, 231.
18. A.-M. Siouffi, *J. Chromatogr., A*, **2006**, *1126*, 86.
19. K. Cbrera, *J. Sep. Sci.*, **2004**, *27*, 843.
20. T. Ikegami, E. Dicks, H. Kobayashi, H. Morisaka, D. Tokuda, K. Cabrera, K. Hosoya, and N. Tanaka, *J. Sep. Sci.*, **2004**, *27*, 1292.
21. K. Nakanishi and N. Tanaka, *Acc. Chem. Res.*, **2007**, *40*, 863
22. K. Nakanishi, T. Amatani, S. Yano, and T. Kodaira, *Chem. Mater.*, **2008**, *20*, 1108
23. F. Svec, *J. Sep. Sci.*, **2004**, *27*, 1419.
24. S. Eeltink and F. Svec, *Electrophoresis*, **2007**, *28*, 137.
25. Y. Ueki, T. Umemura, J. Li, T. Odake, and K. Tsunoda, *Anal. Chem.*, **2004**, *76*, 7007.

26. N. W. Smith and Z. Jiang, *J. Chromatogr., A*, **2008**, *1184*, 416.
27. J. Urban and P. Jandera, *J. Sep. Sci*., **2008**, *31*, 2521.
28. K. Hosoya, N. Hira, K. Yamamoto, M. Nishimura, and N. Tanaka, *Anal. Chem*., **2006**, *78*, 5792.
29. K. Hosoya, M. Sakamoto, K. Akai, T. Mori, T. Kubo, K. Kaya, K. Okada, N. Tsujioka, and N. Tanaka, *Anal. Sci*., **2008**, *24*, 149.
30. D. Lee, F. Svec, and J. M. J. Fréchet, *J. Chromatogr., A*, **2004**, *1051*, 53.
31. J. Grafnetter, P. Coufal, E. Tesařová, J. Suchánková, Z. Bosáková, and J. Ševčík, *J. Chromatogr., A*, **2004**, *1049*, 43.
32. S. Eeltink, J. M. Herrero-Martinez, G. P. Rozing, P. J. Schoenmakers, and W. Th. Kok, *Anal. Chem*., **2005**, *77*, 7342.
33. S. Eeltink, G. P. Rozing, P. J. Schoenmakers, and W. Th. Kok, *J. Chromatogr., A*, **2006**, *1109*, 74.
34. S. Eeltink, L. Geiser, F. Svec, and J. M. J. Fréchet, *J. Sep. Sci*., **2007**, *30*, 2814.
35. F. Svec and J. M. J. Fréchet, *Chem. Mater*., **1995**, *7*, 707.
36. F. Svec and J. M. J. Fréchet, *Macromolecules*, **1995**, *28*, 7580.
37. C. Viklund, F. Svec, J. M. J. Fréchet, and K. Irgum, *Chem. Mater*., **1996**, *8*, 744.
38. C. Yu, F. Svec, and J. M. J. Fréchet, *Electrophoresis*, **2000**, *21*, 120.
39. D. S. Peterson, T. Rohr, F. Svec, and J. M. J. Fréchet, *Anal. Chem*., **2002**, *74*, 4081.
40. T. Jiang, J. Jiskra, H. A. Claessens, and C. A. Cramers, *J. Chromatogr., A*, **2001**, *923*, 215.