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# Split flow and bypass flow systems for monolithic capillary columns in liquid chromatography

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**Abstract**

Split flow and bypass flow systems were assembled using Nano Y Connectors with low dead volume commercially available for capillary liquid chromatography (LC). The split ratio could be controlled by changing the dimension of restriction tubing and applied back pressure to the restriction tubing. The split flow system allowed us to use valve injectors and pumps commercially available for capillary LC. The reproducibility of the present split flow system was acceptable. The relative standard deviation for six successive measurements was 0.4% for the retention time, whereas that for the peak height and peak area was 1–3% depending on the analytes. The bypass flow system uses two Nano Y Connectors, where the eluent split at the first Nano Y Connector, which is located in the inlet of the separation column, is merged again into the effluent from the column at the second Nano Y Connector. The bypass flow system could avoid on-column detection and allowed us to use flow cells, leading to an approximate three times improvement in signal-to-noise. The present flow systems were evaluated by using aromatic hydrocarbons and alkylbenzenes as test analytes.


*Keywords:* Split flow system; Bypass flow system; Gradient elution; Monolithic columns

## 1. Introduction

Although use of microcolumns has been investigated for many years in LC [1–10], microcolumn-LC (μLC) has not been become very popular so far in spite of its features and advantages. The reasons for such a slow popularization may lie in the fact that ancillary techniques such as gradient elution, post-column derivatization and switching valve techniques have not been matured in μLC as well as in its undistinguished column efficiency as long as particle-packed columns are used.

Since monolithic silica capillary columns prepared by using a sol–gel process provide much lower separation impedance than particle-packed columns in LC, they therefore have a potential to achieve higher column efficiency than conventional packed columns because of their unique pore structures and permeability, viz. micrometer order of through pore and silica domain [11–16]. The micrometer order of through pore structure increases the permeability, allowing longer column lengths, whereas the micrometer order of the silica domain size maintains its higher efficiency. Monolithic silica capillary columns have been prepared in 50–100 μm i.d. fused-silica tubing, and split injection and on-column detection have been usually adopted for the operation of such narrow capillary columns. This is because these injection and detection methods are indispensable in order not to cause deterioration of column efficiency of

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capillary columns. However, the on-column detection leads to poor sensitivity due to the limited pathlength. The stationary phase itself also deteriorates the sensitivity as it may reflect or scatter the incident light, leading to an increase in the noise.

Split flow techniques have been developed for pressure-driven and electroosmotically-driven system in μLC [17–19]. Berloni et al. used a conventional cross with a gradient LC system [17]. Although the split ratio is usually altered by changing the dimension of restriction tubing in the split injection, it is not convenient to adjust the split ratio.

The present paper describes split flow and bypass flow systems for monolithic capillary columns in LC. These two flow systems were assembled by using Nano Y Connectors with low dead volume commercially available. In the present split flow system, we apply the back pressure to alter the split ratio. This provides a more convenient and more easily adjustable split method, compared with conventional split method using a restricting valve or capillary tubing. On contrary, the bypass flow system will allow us to use a flow cell for detection because make-up flow temporally reduces the extra-column band broadening. On-column detection is not convenient in some cases.

## 2. Experimental

### 2.1. Apparatus

The split flow and bypass flow systems were assembled from a MicroPro pump equipped with two syringes (Eldex, Napa, CA, USA) or a Model MF2 Microfeeder (Azumadenki Kogyo, Tokyo, Japan) equipped with a 0.5 ml MS-GAN050 or a 1 ml MS-GAN100 gas-tight syringe (Ito, Fuji, Japan), a Model CE-1570 or UV-2070 Plus Intelligent UV-Vis detector (Jasco, Tokyo, Japan), a Model ML-522 microvalve injector with an injection volume of 0.2 μl (Jasco), a monolithic column with 0.1 mm i.d., and one or two Nano Y Connectors (Upchurch Scientific, Oak Harbor, WA, USA), as shown in Fig. 1. According to the manufacturer's technical information, the Nano Y Connector has a 0.1 mm through hole and a dead volume of 17 nl. The chromatographic data were collected by using a Chromatopac C-R7Ae plus data processor (Shimadzu, Kyoto, Japan). The CE-1570 UV detector was used for on-column detection, whereas the UV-2070 UV detector with a 50 μm i.d. capillary flow cell (Jasco) was used for bypass flow system. Constant nitrogen pressure was also utilized to supply the eluent into the capillary column.

In order to adjust the split ratio, the pressure was applied to the restriction tube from a nitrogen gas cylinder, as illustrated in Fig. 1A. The pressure of nitrogen was regulated by a model CFC14PP flow controller (Shimadzu). Fused-silica tubing with 25–150 μm i.d. (GL Sciences, Tokyo, Japan) was used for the restriction tube. The length of the restriction tube was adjusted the split ratio. Fused-silica tubing with

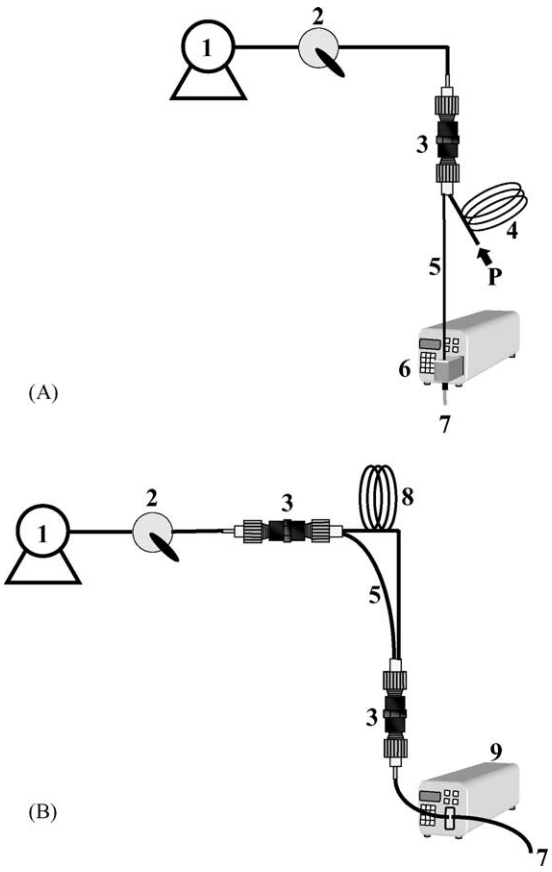


Fig. 1. Split flow (A) and bypass flow system (B) for monolithic capillary columns. (1) MicroPro pump or microfeeder; (2) ML-522 microvalve injector; (3) Nano Y Connector; (4) restriction tube; (5) monolithic capillary column; (6) CE-1570 UV detector; (7) drain; (8) capillary tube for bypass; (9) UV-2070 Plus Intelligent UV-Vis detector.

50 μm i.d. × 0.9 m length was used for the bypass flow line as illustrated in Fig. 1B.

### 2.2. Reagents

Acetonitrile and distilled water were of HPLC grade and obtained from Nacalai Tesque (Kyoto, Japan). Other reagents were of reagent grade and were obtained from Nacalai Tesque, unless otherwise noted. The reagents were used as received. Aromatic hydrocarbons and alkylbenzenes were obtained from Tokyo Chemical Industry (Tokyo, Japan) or Nacalai Tesque. Tetramethoxysilane (TMOS) and tetrachlorosilane were purchased from Tokyo Chemical Industry. Poly(ethylene glycol) with average molecular weight 10,000 (PEG 10,000) were obtained from Aldrich (USA).

### 2.3. Preparation of monolithic capillary columns

Monolithic silica capillary columns were prepared according to the sol–gel method reported by Fujimoto [13] with some modifications. Fused-silica capillary tubes with 0.1 mm i.d. (GL Sciences, Tokyo, Japan) were treated

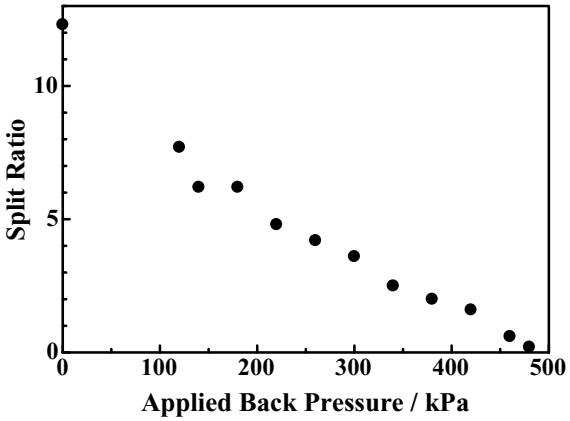


Fig. 2. Split ratio vs. the applied back pressure under constant inlet pressure. Column: monolithic silica ODS, 375 mm × 0.10 mm i.d. Eluent: acetonitrile:water (70:30). Inlet pressure: 500 kPa. Restrictor for split: 74.6 cm × 50 μm i.d.

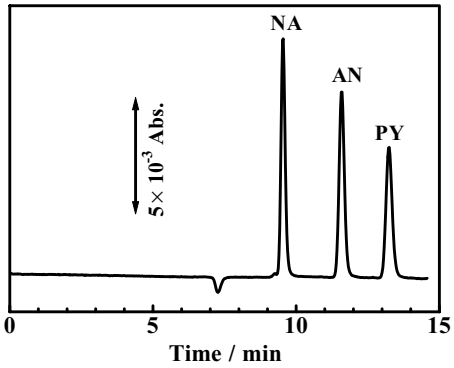


Fig. 3. Isocratic separation of naphthalene (NA), anthracene (AN) and pyrene (PY) by split injection and on-column detection. Column: monolithic silica ODS, 410 mm × 0.10 mm i.d. Eluent: acetonitrile:water (70:30). Pump: MicroPro pump. Flow rate of the pump: 4.0 μl/min. Injection volume before split: 0.2 μl. Restrictor for split: 9 cm × 25 μm i.d. Split ratio: 9.8:1. Wavelength of UV detection: 254 nm.

with 1 M sodium hydroxide at 60 °C for 2 h, followed by washing with 1 M hydrochloric acid and methanol. The fused-silica capillary tubes were then dried at 120 °C in a stream of nitrogen or argon for 1 h, and filled with 1% tetrachlorosilane toluene solution, followed by heating at 60 °C for 3 h. The fused-silica capillary tubes were finally washed with methanol. Sol–gel solution was prepared by dissolving 0.53 g PEG 10,000 in a mixture of 2 ml TMOS and 5 ml 0.01 M acetic acid, followed by agitation in an ice water-bath for 30 min. The solution was then degassed under vacuum for 10 min before filling the solution into the above pretreated capillary. The fused-silica capillary tubes filled with the tetramethoxysilane solution were kept at 40 °C for 20 hrs, followed by washing with water and 0.1 M aqueous ammonia solution. The monolithic silica capillary columns filled with 0.1 M ammonia aqueous solution was kept at 60 °C for 48 h, followed by washing with 60% ethanol aqueous solution. The capillary columns were then heated at 330 °C for 5 h. After drying in a stream of nitrogen at 110 °C for 1 h, 10% dimethyloctadecylchlorosilane toluene solution was passed into the monolithic silica capillary columns by using a microfeeder at 140 °C overnight for preparation of hydrophobic stationary phases, followed by washing with toluene, tetrahydrofuran, methanol and mobile phase.

## 3. Results and discussion

### 3.1. Split flow system

Fig. 2 shows the relationship between the split ratio and the applied back pressure, where the eluent is supplied in the constant inlet pressure mode at 500 kPa. It can be seen that the split ratio can be altered up to 12 by changing the applied back pressure. The restricting tubing employed is 74.6 cm × 50 μm i.d. Split ratio larger than 12 can be achieved if more permeable restricting tubing is attached with the Nano Y Connector (see No. 4 of Fig. 1A). In addition, the split ratio cannot be regulated without any restring tubing, and the split ratio can be affected by the viscosity of the eluent. The above split flow method is good for isocratic elution.

The theoretical plate number was measured as a function of the split ratio in Table 1, where 0.2 μl of naphthalene as the test analyte is injected into the split system. The retention factor of naphthalene was 0.27 under the conditions in Table 1. It can be seen that larger theoretical plate number is obtained with increasing split ratio. The result in Table 1 indicates that the sample volume loaded onto the column should be less than 20 nl in terms of the column efficiency. In addition, the theoretical plate number ($N$) was calculated from peak area ($A$), peak height ($H$) and retention time ($R_t$) of naphthalene as $N = 2\pi(H \times R_t/A)^2$.

Fig. 3 demonstrates the isocratic separation of naphthalene (NA), anthracene (AN) and pyrene (PY) on a monolithic ODS capillary column, where split injection and on-column

Table 1
Plate number and split ratio as a function of applied back pressure

| Back pressure (kPa) | Plate number | Split ratio |
|---|---|---|
| 0 | 13600 | 12.3 |
| 120 | 12700 | 7.7 |
| 140 | 12000 | 6.2 |
| 180 | 12200 | 6.2 |
| 220 | 12000 | 4.8 |
| 260 | 12200 | 4.2 |
| 300 | 11600 | 3.6 |
| 340 | 11100 | 2.5 |
| 380 | 10800 | 2.0 |
| 420 | 10300 | 1.6 |
| 460 | 9470 | 0.6 |
| 480 | 9130 | 0.2 |

Sample: 0.02% (w/v) naphthalene. Injection volume before split: 0.2 μl. Other operating conditions as in Fig. 2.

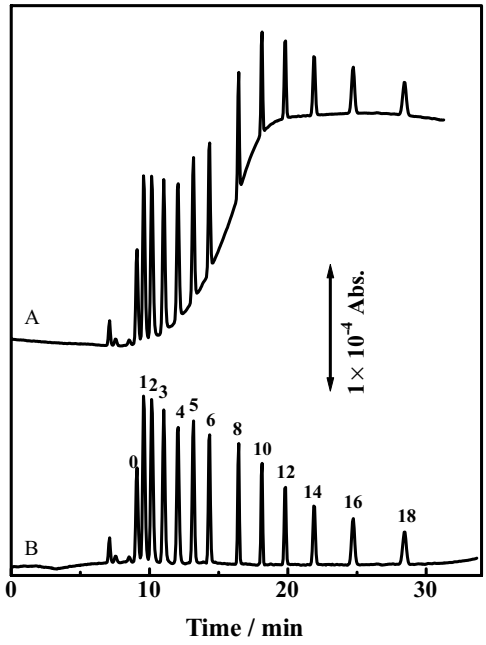


Fig. 4. Row (A) and subtracted (B) chromatograms for gradient separation of alkylbenzenes. Column: monolithic silica ODS, 410 mm × 0.10 mm i.d. Eluent: acetonitrile–water, acetonitrile concentration was linearly changed from 70 to 100% in 8 min and kept at 100% for the rest of the analysis. Pump flow rate: 4.0 μl/min. Split ratio: 9.5:1. Restrictor for split: 8.5 cm × 25 μm i.d. Sample loaded volume: 19 nl. Analytes: benzene (0) and alkylbenzenes (1–18), the number refers to the carbon number of the alkyl group. Wavelength of UV detection: 210 nm.

detection at 254 nm are adopted. The flow rate of the pump was set at 4.0 μl/min and the split ratio was determined to be 9.8:1. As observed in Fig. 3 the peak shape of the analytes is symmetric without any tailing. In addition, the noise level achieved in Fig. 3 is $1.3 \times 10^{-5}$ Abs.

The reproducibility of the retention time and the signal intensity under the conditions in Fig. 3 was estimated for six successive measurements. The relative standard deviations (R.S.D.s) for the retention time were 0.4%, whereas those for the peak height and peak area were 1–3% depending on the analytes.

### 3.2. Gradient elution of alkylbenzenes

Fig. 4 demonstrates the gradient separation of benzene and alkylbenzenes on a monolithic ODS column. The chromatogram in Fig. 4A is a raw chromatogram, whereas the one in Fig. 4B is obtained by subtracting the baseline. The flow rate of the pump was operated at 4.0 μl/min, and the split ratio was adjusted to 9.5:1 by using fused-silica capillary tubing with 8.5 cm × 25 μm i.d. as the restrictor. The actual flow rate of the eluent through the column was, therefore, 0.38 μl/min. The acetonitrile concentration was linearly changed from 70 to 100% in 8 min and kept at 100% for the rest of the analysis. These concentrations refer to the ones at the inlet of the capillary column. It takes ca. 7 min for non-retained solvent to pass through the column under the conditions in Fig. 4. The delay time of the gradient, which is attributed to the part between the pump and the injector as well as between the injector and the capillary column, is also involved, e.g. the delay time is expected to be ca. 3 min considering the baseline drift observed in Fig. 4A.

Since the analytes were monitored by the on-column detection method, the baseline was severely drifted owing to the variation in the acetonitrile concentration, as demonstrated in Fig. 4A. The chromatogram demonstrated in Fig. 4B is, therefore, obtained by subtracting the baseline drift due to variation in the mobile phase composition. For the on-column absorbance detection, such a severe baseline drift is possibly due to a refractive index (RI) effect, namely the light intensity reaching the detector changes as the mobile phase RI changes due to the gradient. The capillary may serve as a lens.

It is found that the baseline demonstrated in Fig. 4B is flat, which reveals that the reproducibility of the present system is excellent. The reproducibility of the retention time and the signal intensity were estimated by conducting six successive measurements under the conditions in Fig. 4A. The R.S.D.s for the retention time of each analyte were 0.26–1.3%, whereas those for the peak height and peak area were 0.9–3.2% except for ethylbenzene, viz. ca. 7%.

### 3.3. Bypass flow system

Since the inner volume of the separation columns employed in μLC is so small that the dead volume should be reduced so as not to cause additional band broadening. This situation often forces us to adopt on-column detection in μLC, where a part of the separation column is subjected for the detection. Flow cells cannot be utilized in some cases owing to the above reason. However, when additional flow is merged at an appropriate flow rate into the effluent from the column, the dispersion in terms of time occurring in the connecting tube between the separation column and the flow cell of the detector can be neglected. In this article, this flow system is called the bypass flow system. The bypass flow system allows us to use flow cells in μLC.

The separation of aromatic hydrocarbons in Fig. 5 was carried out by using the bypass flow system illustrated in Fig. 1B. The single separation is shown in different scales in Fig. 5. The eluent and the sample were split through the first Nano Y Connector and then merged into the effluent from the column at the second Nano Y Connector. One-eleventh of the original sample was loaded onto the column and separated in the isocratic mode. The capillary flow cell (Jasco) is composed of 50 μm i.d. fused-silica tubing and the analytes are detected parallel to the flow direction. The pathlength of the detection is estimated to be 1.5–2 mm. This means that the pathlength, i.e. the signal intensity observed, is around 20 times larger than that in the case of the on-column detection for the column with 0.1 mm i.d. On the other hand, the noise level achieved in Fig. 5 is $1.0 \times 10^{-5}$ Abs, which

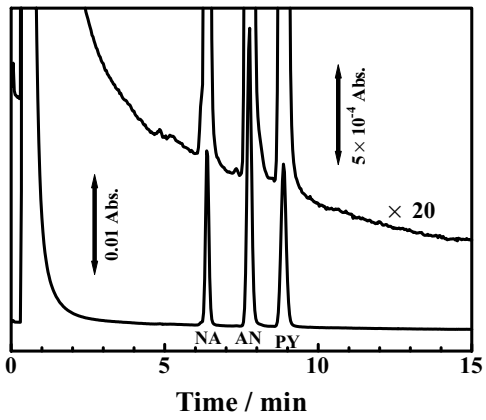


Fig. 5. Isocratic separation of aromatic hydrocarbons by bypass flow system. Column: monolithic silica ODS, 460 mm × 0.10 mm i.d. Eluent: acetonitrile:water (70:30). Pump flow rate: 8.0 μl/min. Bypass ratio: 10:1. Bypass tubing: 0.9 m × 50 μm i.d. Sample loaded volume: 18 nl. Analytes: naphthalene (NA), anthracene (AN) and pyrene (PY). Wavelength of UV detection: 254 nm.

is slightly better than that achieved by on-column detection, as shown in Fig. 3. This is because of the presence of the stationary phase for the latter detection. Consequently, the signal-to-noise ratio is improved by a factor of ca. 3, compared to the on-column detection although the sample is diluted in the bypass flow system. The bypass flow system allows us to use a gradient pump, an injection valve and a flow cell for detection. This situation is very friendly to users. In addition, it should be noted that the bypass ratio can be easily estimated from the peak areas of bypassed analyte and column-loaded analyte.

## 4. Conclusion

It was proved that the Nano Y Connector could be used for split as well as for bypass flow systems in μLC without any deterioration of column efficiencies. The present system allows us to use injectors and flow cells commercially available for μLC. Gradient elution can also be applied for monolithic capillary columns.

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## References

[1] D. Ishii, Jasco Rep. 11 (6) (1974) 1.
[2] R.P.W. Scott, P. Kucera, J. Chromatogr. 125 (1976) 251.
[3] K. Hibi, D. Ishii, I. Fujishima, T. Takeuchi, T. Nakanishi, J. High Resolut. Chromatogr., Chromatogr. Commun. 1 (1978) 21.
[4] T. Tsuda, M.V. Novotny, Anal. Chem. 50 (1978) 632.
[5] J.W. Jorgenson, E.J. Guthrie, J. Chromatogr. 255 (1983) 335.
[6] K. Jinno, Chromatographia 25 (1988) 1004.
[7] J.E. MacNair, K.C. Lewis, J.W. Jorgenson, Anal. Chem. 69 (1997) 983.
[8] T.F. Hooker, J.W. Jorgenson, Anal. Chem. 69 (1997) 4134.
[9] F.E. Regnier, J. High Resolut. Chromatogr. 23 (2000) 19.
[10] T. Takeuchi, Anal. Bioanal. Chem. 375 (2003) 26.
[11] N. Tanaka, H. Kobayashi, H. Nakanishi, H. Minakuchi, N. Ishizuka, Anal. Chem. 73 (2001) 420A.
[12] N. Tanaka, H. Nagayama, H. Kobayashi, T. Ikegami, K. Hosoya, N. Ishizuka, H. Minakuchi, K. Nakanishi, K. Cabrera, D. Lubda, J. High Resolut. Chromatogr. 23 (2000) 111.
[13] C. Fujimoto, J. High Resolut. Chromatogr. 23 (2000) 89.
[14] N. Ishizuka, H. Minakuchi, K. Nakanishi, N. Soga, H. Nagayama, K. Hosoya, N. Tanaka, Anal. Chem. 72 (2000) 1275.
[15] J.D. Hayes, A. Malik, Anal. Chem. 72 (2000) 4090.
[16] M. Motokawa, H. Kobayashi, N. Ishizuka, H. Minakuchi, K. Nakanishi, H. Jinnai, K. Hosoya, T. Ikegami, N. Tanaka, J. Chromatogr. A 961 (2002) 53.
[17] A. Berloni, A. Cappiello, G. Famiglini, P. Palma, Chromatographia 39 (1994) 79.
[18] M.P. Balogh, C.C. Stacey, J. Chromatogr. 562 (1991) 73.
[19] J.N. Alexander IV, J.B. Jannet, K.E. Markides, Anal. Chem. 71 (1999) 2398.