Original

Development of a Solvent–Saving Direct–Pumping Recycle Chromatographic System and Its Application to the Separation of Deuterated Benzenes in Liquid Chromatography

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Abstract

A closed–loop direct–pumping recycle chromatography (RC) system was examined for analytical scale to achieve high resolution in high–performance liquid chromatography (HPLC), where a 6–way switching valve was switched after the system had been stabilized, resulting in no more consumption of additional mobile phase. The extra–column band broadening effect was minimized by using an HPLC pump equipped with small–discharging–volume plungers, low–dead–volume devices and connecting tubes. The plate number achieved was linear to the number of cycle, and it increased by more than 10,000 per cycle using a 25–cm separation column packed with 5–μm reversed–phase materials. The mobile phase conditions were optimized in terms of analysis time, and the present RC system was applied to the separation of benzene and deuterated benzene isomers.

Keywords : HPLC, Recycle chromatography, Solvent–saving direct–pumping system, High resolution, Deuterated benzenes

1. Introduction

When a difficult separation cannot be accomplished by changing the column and mobile phase conditions, two alternate methods are available. The most common approach is to increase the column length with identical packing material, and the second approach is to recycle the sample through the column. However, the former approach might lead to a limitation to the separation efficiency due to an increased inlet pressure whereas in the latter case, the dead volume in the recycle lines causes band broadening and peaks can only be recycled until they begin to overlap. Thus, the recycle technique has been limited to preparative chromatography where larger diameter columns are employed [1–7]. In preparative chromatography, the recycle technique is well–known as a useful tool to improve enantioseparation, production rate and recovery yield.

Trone et al. [6] have developed a peak trapping recycle chromatography (RC) system and optimized it for peak purity assessment of active pharmaceutical ingredients. After being analyzed using a reversed phase analytical column, peaks of interest were trapped and were subsequently introduced to an RC system. They have achieved over 227,000 theoretical plates for some compounds.

Zhang and McConnell [7] have described the development of a novel column–switching technique called "simulated moving columns" (SMC) to quickly achieve complete chiral resolution on columns with limited enantioselectivity. SMC helped to achieve chiral resolution by virtually multiplying the column length, thus enhancing separation efficiency and resolution, without increasing back-pressure. The results clearly indicated that SMC eliminated the significant band broadening that was inevitable in the closed–loop re-

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cycling techniques currently used in preparative chromatography.

For analytical purposes, RC has been attempted to improve the resolution of analytes of interest [8, 9]. The separation of oxygen isotopic compounds was attempted by Tanaka et al. [8] in reversed–phase LC, based on the small differences in the dissociation constants of organic acids. Oxygen isotopes of benzoic acid as well as *p*–chlorobenzoic acid and *p*–nitrophenol were separated by ionization control using two pairs of reversed–phase columns and a six–way switching valve [8].

RC is an approach where the sample is being recycled through the column(s), thus producing the same separation effect as when a relatively long column is being used. The advantages of RC are that it allows the separation of compounds with very near retention time, no increase in inlet pressure, and it also solves the problem of having difficulties in making long columns. On the other hand, once the analytes are being separated, it would be mixed again during recycle process, and thus peaks can only be recycled until they begin to overlap.

In our previous study, we designed an alternate–pumping (AP) recycle system for capillary LC using highly permeable monolithic silica columns [9]. The developed system was proven to be suitable for the separation of isotopic molecules, either partially or completely substituted with deuterium atoms. For difficult isotopic separation that requires long analysis time, the recycle system utilizing capillary columns reduces the consumption of mobile phase. However, this AP system requires two columns and the switching valves needed to be monitored and switched accordingly, either manually or automatically, so that the samples could be recycled through the columns without being drained away.

The resolution, R_s, of two chromatographic peaks is defined as :

$$R_s = \frac{\alpha-1}{2(\alpha+1)} \cdot \frac{k_{av}}{k_{av}+1} \cdot (N)^{1/2} \qquad (1)$$

where N is the number of theoretical plates, α is the separation factor and k_{av} is the average retention factor of the two peaks. Thus, the R_s is proportional to the square root of N and hence to the column length. So, a 4–fold increase in the length of the column would double the resolution, and this is accompanied by an increase in the retention time. The recycle technique, which practically increases the length of the column, would theoretically increase the resolution of a separation.

Therefore, the main aim of this work was to develop a direct–pumping (DP) recycle system in such a way that the eluent was recycled within the line in a closed–loop, i.e. once the sample is being injected into the system, the valve will be switched and there will be no additional eluent supplied to the system. This is possible by placing a 6–way switching valve between the eluent reservoir and the detector. The only concern of this DP system was severe extra–column band broadening that might be caused by the pump and thus it can only be applied to a conventional or preparative LC system. This DP recycle LC system was made possible by using a double–plunger type HPLC pump and other low–dead–volume devices. The system was then applied to the separation of benzene and deuterated benzenes.

2. Experimental

Apparatus

The DP recycle system, as illustrated in Figure 1, consisted of a pump, a 6–way switching valve, an injector, a separation column and a UV detector. The eluent was first supplied by a double–plunger type HPLC pump model LC–20 AD (Shimadzu, Kyoto, Japan). When the system has been stabilized, the 6–way switching valve is switched and the effluent from the detector is directly supplied to the pump and thus avoids substantial consumption of the eluent. The diameter of the separation column was 4.6 mm (L–column ODS, 5 μm ; Chemicals Evaluation and Research Institute, Tokyo, Japan), and sample injection volume was 20 μL. Both the injector and the 6–way switching valve were from Rheodyne (Cotati, CA, USA) and the connecting tubes between the detector and pump used were of 1/16" O. D. PEEK tubes. A model UV–970 UV detector (Jasco, Tokyo, Japan) was used and the analytes were detected at the wavelength of 210 nm. All data were collected by a

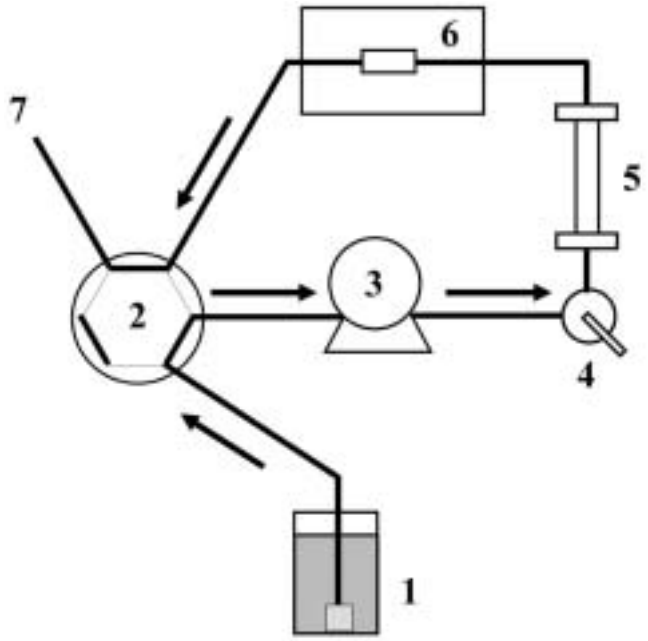

Figure 1. Schematic diagram of the DP recycle separation system employed in this work.
1=mobile phase, 2=6–way switching valve, 3=HPLC pump, 4=loop injector, 5=separation column, 6=UV detector, 7=drain.

C–R 7 Ae plus Chromatopac data processor (Shimadzu).

Reagents

Acetonitrile (ACN) and distilled water were of HPLC grade and obtained from Wako Pure Chemical Industries (Osaka, Japan). Special grade benzene was also purchased from Wako Pure Chemical Industry while other deuterated benzenes were obtained from Aldrich Chemical Co. (Milwaukee, WI, USA). Other reagents were of reagent grade and obtained from Nacalai Tesque (Kyoto, Japan). The reagents were used as received, unless otherwise noted.

3. Results and Discussion

N versus number of cycle

The N obtained per cycle was measured by using benzene as the analyte. When using a 25–cm column packed with 5 μm ODS, N values obtained for the first cycle (N_1) depended on the ACN concentration in the eluent, and N_1 values between 13,800 and 17,200 were achieved for 30–60% ACN aqueous solutions as the eluent, as shown in Table 1. It can be seen that the largest N_1 is achieved for 40% ACN. Table 1 also shows average additional N values (ΔN) obtained for the second to fifth cycle. The largest ΔN is also achieved for the 40% ACN. Smaller ΔN values are achieved for 50 and 60% ACN, which is due to additional extra–column band broadening.

Figure 2 demonstrates N values obtained for the first eighteen cycles using 50, 55 and 60% ACN using a 25–cm column packed with 5–μm ODS. It can be seen from Figure 2 that the N increases with increasing number of cycle. It was found that when using lower concentration of ACN, the increase of N was larger than those obtained using higher concentration of ACN although the total analysis time increases with decreasing ACN concentration. In other words, the larger the retention time, the larger N was achieved at each cycle. This means that the additional band broadening due to the recycle cannot be neglected under the conditions in Table 1. The discharging volume of each plunger of the pump employed in this work is 10 μL. The tubing between the outlet of the column and the 6–way switching valve as well as between the 6–way switching valve and the pump was PEEK tubing with 0.25 or 0.50 mm ID. The total length of these connecting tubes is ca. 1 m. It is presumed that band broadening caused in the above parts as well as in the check valves may not be neglected. The cell volume of the UV detector can be another potential contribution to additional band broadening. It should be noted that the pumps employed in this research do not equip any dampener, which could greatly contribute to extra–column band broadening.

Although 15–cm columns were also examined in this work, the results were not satisfactory due to the effect of extra–column band broadening. Two columns connected in tandem were also examined, but we have not found any advantage over a single 25–cm column. In addition, use of a single 25–cm column packed with 3–μm ODS did not provide any advantage over the 25–cm column packed with 5–μm ODS. Considering these results, a single 25–cm column packed with 5–μm ODS is used as the separation column for RC in the following examination.

Table 1. Theoretical plate number obtained for the first cycle (N_1) and average values obtained for the second to the fifth cycle (ΔN).

ACN(%)	N_1	ΔN
60	13,200	6,700
50	15,700	9,100
40	17,200	12,700
30	15,600	11,000

Separation column : L–column ODS (250×4.6 mm I.D.). Eluent : as indicated. Flow–rate : 1.0 mL min^{-1}.Wavelength of UV detection : 210 nm. Analyte : 0.01% benzene. Injection volume : 20 μL.

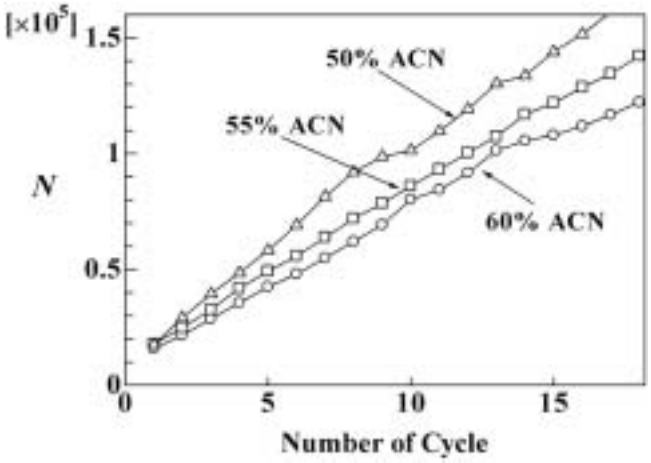

Figure 2. Theoretical plate number versus number of cycle in RC. Separation column : L–column ODS (250×4.6 mm I.D.). Eluent : as indicated. Flow rate : 1.0 mL min^{-1}. Wavelength of UV detection : 210 nm. Analyte : 0.01% benzene. Injection volume : 20 μL.

Separation of benzene and deuterated benzenes

Figure 3 demonstrates the separations of benzene and hexadeuterated benzene (benzene–D6) using different concentration of ACN as the eluent, where baseline separations are achieved at different analysis time, as indicated by the arrows. It can be seen from the figure that faster baseline separation could be achieved for lower ACN concentration. This result can be expected from the data in Figure 2.

Considering the above data, 40% ACN aqueous solution was chosen for the separation of benzene, 1,3,5–trideuterated benzene

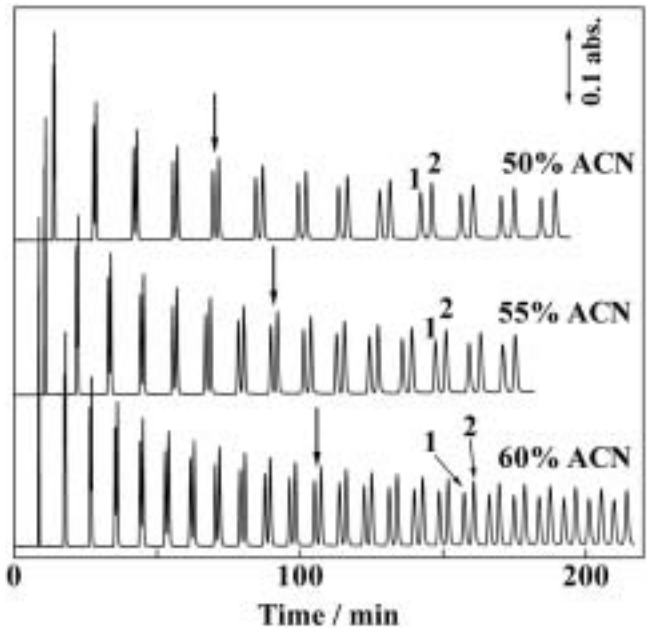

Figure 3. Recycle separation of benzene and benzene–D6.
Operating conditions are as in Figure 2, except for sample. Sample : 0.01% each for each benzene–D6 (1) and benzene (2) dissolved in the eluent.

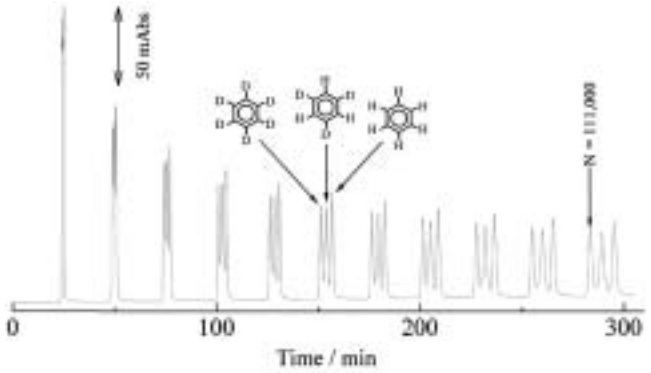

Figure 4. Recycle separation of benzene, benzene–D3 and benzene–D6.
Operating conditions are as in Figure 2, except for the eluent and the sample. Eluent : ACN : water (40 : 60). Sample : 0.01% each for benzene, benzene–D3 and benzene–D6 dissolved in the eluent.

(benzene–D3) and benzene–D6. The separation is demonstrated in Figure 4. Baseline separation (i.e., R_s=1.5) was achieved after the 10th cycle and the separation took less than 280 min using a commercially available conventional column. The calculated N achieved for the first resolved peak, i.e. benzene–D6, after the 10th cycle was approximately 111,000.

The system was also applied for the separation of benzene and monodeuterated benzene (benzene–D1), which needs much larger N values. The present closed–line RC system managed to achieve a fairly good separation after 800–min operation, and the chromatogram is shown in Figure 5. The upper trace is the enlargement of the lower trace from 200 to 870 min.

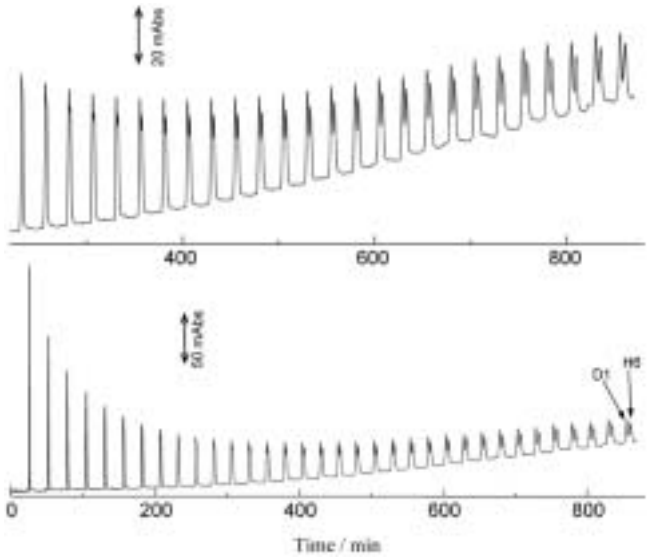

Figure 5. Recycle separation of benzene–d1 and benzene.
Operating conditions are as in Figure 4, except for the sample ; Sample : 0.01% each for benzene (H 6) and benzene–D 1(D 1).

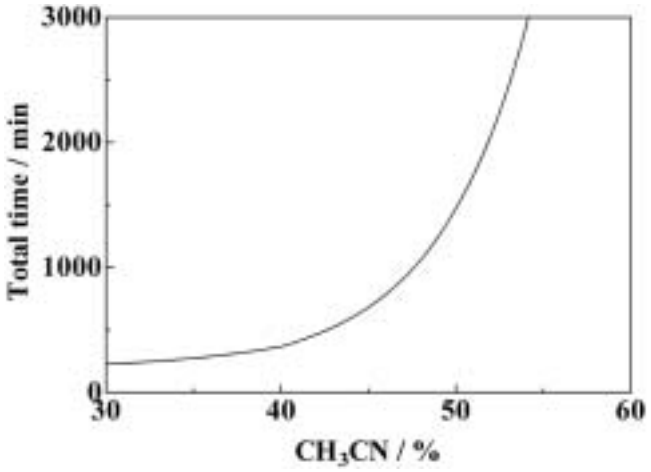

Figure 6. Simulation of required analysis time to achieve R_s=1 for benzene and benzene–D1.
Simulated conditions are as in Tables 1 and 2.

For difficult isotopic separation that requires long analysis time, the closed–line DP–RC system reduces the consumption of mobile phase. This system also provides a relatively simple and inexpensive mean of attaining higher separation efficiency, i.e. any difficult separation could possibly be achieved by recycling the sample through the column in the closed–line until sufficient resolution is attained. This would reduce the turnaround time by optimization of the separation condition on a conventional chromatographic system.

Table 2. Retention data of benzene–D1 and benzene for simulation.
The operating conditions are the same as in Table 1 except for the analytes.

[ACN] % (v/v)	t_M min	k		α
		Benzene–D1	Benzene	
60	2.240	2.639	2.646	1.0024
50	2.220	4.558	4.575	1.0036
40	2.269	8.264	8.352	1.0106
30	2.353	16.033	16.294	1.0163

Simulation of required analysis time

Equation (1) allows the simulation of analysis time required for achieving an adequate R_s value when we could measure accurate retention factors of analytes and the void volume (t_M). Table 2 shows the data obtained for benzene–D1 and benzene. It is seen from the table that the separation factor increases with decreasing ACN concentration. By using the data shown in Tables 1 and 2, the required analysis time was calculated for various ACN concentrations. The result is illustrated in Figure 6. The simulation indicates that the lower ACN concentration is more favorable for shorter analysis. From the simulation in Figure 6, it takes ca. 6 h to achieve R_s=1 for benzene–D1 and benzene. In practical, it took more time to achieve the separation with R_s=1. This may be caused by the fact that the effect of extra–column band broadening increases with increasing numbers of cycles. A more precise measurement of the retention factors is needed in order to improve the accuracy of the simulation result.

4. Conclusion

A DP–RC system was designed and applied to the separation of isotopic compounds. After the system was stabilized, the 6–way switching valve was switched and thus resulting in no extra consumption of eluent. The analyte could be recycled in a closed–line and only minimum amount of eluent that remained in the line is sufficient to resolve the sample. It was observed that the N increased with increasing number of cycle. The optimization of the present system in terms of analysis time and resolution is being investigated.

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References

[1] Yoshida, T. ; Shu, C–K. ; Theimer, E. T. *J. Chromatogr.* **1977**, *137*, 461–464.

[2] Johnson, W. M. P. ; O' Keefe, D.F. ; Rihs, K. J. *Chromatogr.* **1984**, *291*, 449–452.

[3] Okano,T. ; Masuda, S. ; Kusunose, S. ; Komatsu, M. ; Kobayashi, T. *J. Chromatogr.* **1984**, *294*, 460–465.

[4] Crary, J. R. ; Cain–Janicki, K. ; Wijayaratne, R. *J. Chromatogr.* **1989**, *462*, 85–94.

[5] Letter, W. S. *J. Chromatogr*. **1992**, *590*, 169–173.

[6] Trone, M. D. ; Vaughn, M. S. ; Cole, S. R. *J. Chromatogr. A* **2006**, *1133*, 104–111.

[7] Zhang, Y. ; McConnell, O. *J. Chromatogr. A* **2004**, *1133*, 227–238.

[8] Tanaka, N. ; Araki, M. ; Kimata, K. *J. Chromatogr.* **1986**, *352*, 307–312.

[9] Lim, L.W. ; Uzu, H. ; Takeuchi, T. *J. Sep. Sci*. **2004**, *27,* 1339–1344.