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Sample enrichment by using monolithic precolumns in microcolumn liquid chromatography

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Abstract

An on-line sample enrichment system was designed using monolithic precolumns in microcolumn LC. The monolithic ODS capillary columns were prepared via in situ sol–gel processes. The enrichment efficiency of the monolithic columns was tested by using phthalates as the analytes. The relative standard deviations ($n = 6$) for the retention time, peak area and peak height were between 0.4 and 1.2%, 0.9 and 5.5% and 0.4 and 3.9%, respectively. The system was linear ($R^2 > 0.99$) within the working sample concentration and sample volume ranges. Comparing to 0.2 μl injection with a typical sample injector, the theoretical plate number of a same separation column was increased by 3–6-fold when the precolumn unit was used for sample injection. The recoveries of the analytes were between 88 and 120%, and the sample volume that could be injected into the system was increased up to 5000-fold. The limits of detection were improved by more than 2000-fold and were between 0.21 and 0.87 ng ml^{-1} even with a UV absorbance detector. This system was applied to the determination of phthalates contained in laboratory distilled water and tap water samples.

Keywords: Sample enrichment; Monolithic precolumns; Water analysis; Phthalates

1. Introduction

Various efforts have been made to miniaturize separation columns in LC since the 1970s. This is because the microcolumn LC possesses advantages such as increased mass sensitivity (due to a decrease in column's cross-sectional area), low consumption of solvent, reagents and packing materials, use of exotic mobile phase and mobile phase additives, etc. However, it has not been widespread due to the fact that the column efficiency and detection sensitivity of microcolumn LC at this point are not vividly higher than those of conventional LC [1,2].

One of the most essential tasks in modern analysis is trace-level determination of organic and inorganic constituents in complex environmental and biological samples. In such cases, on-line sample pretreatment [3–6] and post-column derivatization [7] have been proven to be useful as to enhance the sensitivity and selectivity of the detections [8–10]. Supported liquid membranes were used to enrich metals for the determination with atomic absorption spectrometric detection [3]. C_{18} columns were used to concentrate and determine Cr (VI) in water samples for direct spectrophotometric detection [4]. Bromate and perchlorate in drinking water were concentrated 20-fold using a PTFE beaker in a microwave oven and were determined with conductivity detection [5]. Visser et al. combined on-line trace enrichment with a post-column addition of a make-up liquid in order to improve the detectability of the GC-infrared spectroscopy (IR) and LC-IR to the μg l^{-1} level [8,9]. While recently, Bruzzoniti et al. reported an on-line preconcentration and spectrophotometric determination of Pd in ion chromatography after a post-column reaction [10]. The separation and sensitivity of Pd were influenced by the eluent concentration and the post-column reagent's composition; the detection limit of this method was within 300 ng l^{-1}. All these methods allowed large injection volumes of the samples and thus resulted in improved detection limits down to the μg l^{-1} level.

Recently, a new type of continuous porous polymer and silica-based monolithic columns has gained much attention

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[11–15]. The double-pore structure of the monolithic column allows the use of a higher flow-rate due to the low column pressure drop. The use of the monolithic column also eliminates the problems in micro-particulate packing and frit-making procedures in packed columns. Since there are no limitations relating to a packing procedure, long columns can be prepared in order to obtain high plate numbers.

Quirino et al. [16] demonstrated the possibility of preconcentration using a photopolymerized sol–gel monolith in capillary electrochromatography (CEC). When the sample plug length was increased by 3965-fold, improvements of more than 1100 times in peak heights were achieved even with a 10-fold lower concentration samples. These values were claimed to be the highest reported sensitivity improvements using a simple on-line preconcentration technique in CEC. Nevertheless, this technique also had shown a loss of resolution with increasing sample plug length. And thus, an injection of sample plugs of more than 356% of the total capillary length was not performed.

The present paper describes an on-line sample enrichment system using laboratory-made monolithic precolumns in microcolumn LC to overcome shortcomings, such as limited sample loadability and restricted concentration detectability, encountered in most capillary systems. The on-line enrichment system allows an increment in sample injection volume without any loss in resolution.

2. Experimental

2.1. Apparatus

The eluent was supplied by a model MF-2 Microfeeder syringe pump (Azumadenki Kogyo, Tokyo, Japan), which was equipped with an MS-GAN 050 gas-tight syringe (0.5 ml; Ito, Fuji, Japan), at the flow-rate of 4.2 μl min^{-1}. A laboratory-made microcolumn was used as the separation column, while monolithic precolumns were connected directly to an M435 micro injection valve (Upchurch Scientific, Oak Harbor, WA, USA) via fused-silica capillaries (50 μm i.d. × 375 μm o.d.; GL Sciences, Tokyo, Japan) and were used as the concentration columns. A UV-970 UV-Vis detector (Jasco, Tokyo, Japan) was operated at 204 nm and all of the data were collected by a C-R4AX Chromatopac processor (Shimadzu, Kyoto, Japan).

The laboratory-made separation column was prepared by using fused silica capillary (100 × 0.32 mm i.d. × 0.45 mm o.d.; GL Sciences) as reported previously [17]. Since the M435 micro injection valve has a swept volume of ca. 0.05 μl, a 75 × 0.05 mm i.d. fused-silica capillary (0.15 μl; GL Sciences) was connected to the valve to make an effective injection volume of 0.2 μl, and it was used as a replacement to the commercially available sample injector due to its ability to produce better peak shape.

2.2. Reagents and materials

HPLC-grade acetonitrile and distilled water were obtained from Nacalai Tesque (Kyoto, Japan). Diethyl phthalate (DEP), benzyl *n*-butyl phthalate (BBP) and di-*n*-butyl phthalate (DBP) were purchased from Tokyo Chemical Industry (Tokyo, Japan). Other reagents were of guaranteed reagent grade and were obtained from Nacalai Tesque. All reagents were used without any further treatment, unless otherwise noted.

The stationary phase of the separation column employed in this work was L-column ODS (5 μm particle diameter; Chemicals Evaluation and Research Institute, Tokyo, Japan), while Develosil C30-UG beads (30 μm; Nomura Chemical, Seto, Japan) were packed into a 10 × 0.25 mm i.d. PTFE tubing and was used as the enrichment column when making a comparison with the monolithic precolumn in terms of relative recovery and relative intensity.

2.3. Enrichment unit

Fig. 1 shows the diagram of the enrichment unit when loading (A), or injecting (B) the sample. The flow of the eluent, which also acts as the desorption solvent, indicated that the enriched sample was back-eluted into the separation column. The connection capillary tubes attached to the M-435 micro injection valve were prepared from fused-silica tubing with 50 or 75 μm i.d. × 375 μm o.d. (GL Sciences). The sample was loaded via a hand-made rubber band-driven pumping device during all concentrations.

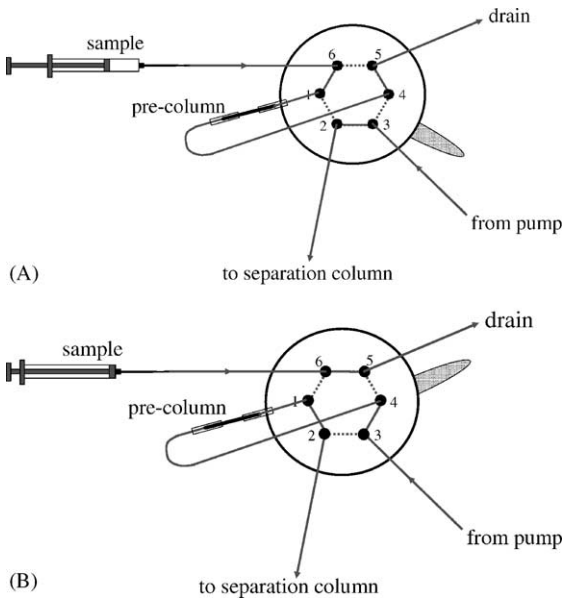

Fig. 1. Diagrams of the enrichment system when loading the sample (A), or when injecting the sample (B).

2.4. Monolithic precolumns

The monolithic precolumns (20 × 0.1–0.32 mm i.d.) were prepared via in situ sol–gel process in a manner similar to that given in ref. [14] with some modifications on the preparation conditions. In order to minimize the effects of shrinkage during gelation in the 200 and 320 μm i.d. capillaries, 2.0 ml of a hybrid of tetramethoxysilane and methyltrimethoxysilane (39:1), together with 0.53 g PEG (MW = 10,000), were dissolved in 5.0 ml of 0.01 M acetic acid by stirring the solution for 30 min at 0 °C. The solution was degassed for 10 min before it was filled into the pretreated fused-silica capillaries. After leaving the capillaries at 40 °C for 24 h, they were washed with water and 0.1 M aqueous ammonia, and were kept at 60 °C for 66 h. After washing with 60% ethanol, the capillaries were heated at 330 °C for 5 h, followed by purging with nitrogen gas at 110 °C for 1 h. The capillaries were then reacted with 10% dimethyloctadecylchlorosilane in toluene solvent at 140 °C for 24 h. After that, they were washed with toluene, THF, methanol and the eluent prior to use.

3. Results and discussion

3.1. Signal enhancement of phthalates

A separation of an authentic mixture of DEP (peak 1), BBP (peak 2) and DBP (peak 3) was shown in Fig. 2. Acetonitrile–water (75:25) solution was used as the eluent and the above three phthalates were detected at 204 nm with a UV absorbance detector. Fig. 2a was obtained when a 0.2 μl volume of 20 μg ml^{-1} of DEP, BBP and DBP was injected into the column, while Fig. 2b was obtained when 0.4 ml of 20 ng ml^{-1} of the same mixture was concentrated on-line into a monolithic precolumn before it was injected. It is interesting to note that though the injection volume was increased by 2000-fold in Fig. 2b, there is not any band broadening noticed; in fact the peak shape was improved as can be seen. Compare to Fig. 2a, the theoretical plate number of the same separation column was improved by 4-fold in Fig. 2b. Even though the sample concentration was reduced by a factor of 1000 and the injection volume was increased by a factor of 2000, the peak heights of DEP, BBP and DBP were increased by 7.6, 5.5 and 7.5 times, respectively. It should be noted that these values are larger than two. The detection limits at a signal-to-noise ratio (S/N) equal to 3 were improved from 0.17, 0.26 and 0.36 μg ml^{-1} (Fig. 2a) to 0.06, 0.13 and 0.13 ng ml^{-1} (Fig. 2b) for DEP, BBP and DBP, respectively. Lower detection limits could be obtained by simply increasing the enrichment volume. In addition, the peak before peak 1 appeared in Fig. 2b is due to impurities contained in purified water and acetonitrile.

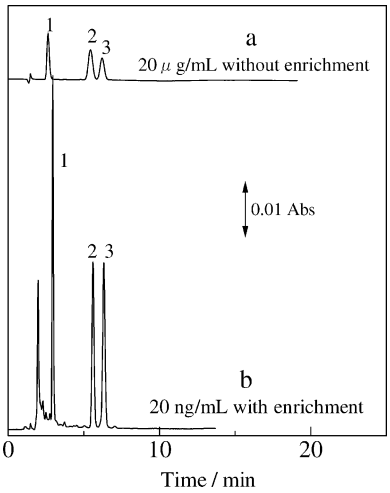

Fig. 2. Chromatograms of the separation of phthalates. Column: L-column ODS, 100 × 0.32 mm i.d. Injector: fused silica (75 × 0.05 mm i.d.) connected to the M435 micro injection valve (a). Precolumn: laboratory-made monolith, 20 × 0.2 mm i.d. (b). Mobile phase: acetonitrile–water (75:25). Flow-rate: 4.2 μl min^{-1}. Analytes: DEP (1), BBP (2) and DBP (3). Sample volume and concentration: 0.2 μl containing 20 μg ml^{-1} for each phthalate (a), and 0.4 ml containing 20 ng ml^{-1} for each phthalate (b). Wavelength of UV detection: 204 nm.

3.2. Enrichment efficiency: monolithic versus C30 precolumns

The enrichment efficiency of the monolithic precolumn was tested by using phthalates as the samples. A 0.1 ml volume of an authentic mixture of DEP, BBP and DBP was loaded into a monolithic precolumn. Effects of the direction of the eluent passing through the enrichment column on recoveries and band broadening were examined. The flow direction was either in the normal-eluted, which is the same direction as when loading the sample, or in the back-flush mode (see Fig. 1). The same experiment was carried out by replacing the monolithic precolumn with a packed C30 precolumn. The relative recoveries and intensities as well as the peak signals are compared in Table 1. The relative signal intensities and recoveries of the phthalates show differences more than 53% between the normal-eluted mode and the back-flush mode when the C30 precolumn was used. On the contrary, the signal intensities and recoveries of those were similar to one another when the monolithic precolumn was used. These results show that the overall enrichment efficiency of the monolithic precolumn was better than the C30 precolumn as long as the relative recoveries and intensities are concerned. One possible explanation is that the porous monolith has a higher permeability that promotes preconcentration of the dilute samples. Besides, the minute gaps between the C30 particles with 30 μm average particle diameter also cause the dispersion of the samples when the eluent was normal-eluted to pass through the entire precolumn.

3.3. Effects of monolithic precolumn i.d.

Monolithic precolumns with 0.1, 0.15, 0.2 and 0.32 mm i.d. were prepared and were used to enrich DEP, BBP and

Table 1
Comparison of the signal recoveries and intensities for the C30 and monolith precolumns when the eluent was supplied in the normal- or back-eluted mode

Analyte	Precolumn	Eluent flow	Peak area (V s)	Relative recovery	Peak height (mV)	Relative intensity
DEP	C30	Normal	0.255	38.2	34.7	31.9
		Back	0.611	91.5	96.6	89.0
	Monolith	Normal	0.673	101	106	97.3
		Back	0.668	100	109	100
BBP	C30	Normal	0.162	26.1	10.6	21.7
		Back	0.544	87.5	42.1	86.3
	Monolith	Normal	0.590	94.8	46.7	95.6
		Back	0.622	100	48.8	100
DBP	C30	Normal	0.191	28.3	12.2	24.1
		Back	0.596	87.9	43.8	86.3
	Monolith	Normal	0.670	98.8	48.6	95.7
		Back	0.678	100	50.8	100

Separation column: L-column ODS, 100 × 0.32 mm i.d. Precolumn: laboratory-made C30 (10 × 0.25 mm i.d.) or monolith (20 × 0.2 mm i.d.). Mobile phase: acetonitrile–water (75:25). Flow-rate: 4.2 μl min^{-1}. Sample: 0.1 ml containing 200 ng ml^{-1} for each phthalate. Wavelength of UV detection: 204 nm.

DBP aqueous solution. In the preparation of the 0.1 and 0.15 mm i.d. monolithic columns, tetramethoxysilane was used, while the 0.2 and 0.32 mm i.d. monolithic columns were prepared by using a hybrid of tetramethoxysilane and methyltrimethoxysilane. This is because the use of tetramethoxysilane alone failed to produce excellent monolithic columns with 0.2–0.32 mm i.d. and gap or void along the inner surface was frequently observed.

The SEM photos of the two types of the monolith are shown in Fig. 3. Fig. 4 shows the chromatograms of an authentic mixture of DEP, BBP and DBP when monolithic precolumns with different i.d. were used. No outrageous differences were noticed except the observation that there was a little delay in the retention time with increasing precolumn i.d. For example, the retention times of DBP were 6.27, 6.46, 6.45 and 6.53 min for 0.1, 0.15, 0.2 and 0.32 mm i.d. precolumn, respectively. This is due to an increase in the total dead volume when the four precolumns used had a different i.d. but the same length.

The ratio of tetramethoxysilane to methyltrimethoxysilane has been examined from 19:1 to 79:1. Since the monolithic columns prepared from the ratio of methyltrimethoxysilane to tetramethoxysilane 39:1 gave the best results in terms of the permeability and the column efficiency, monolithic precolumns prepared from the ratio of 39:1 have been used for monolithic columns with 0.2 and 0.32 mm i.d.

Although the methyl-containing monolith should be more hydrophobic than the purely inorganic monolith, we have not observed any distinct difference between the both monolithic columns.

3.3.1. Reproducibility and recovery

Table 2 summarizes all the repeatability data for the retention time and peak signals for DEP, BBP and DBP when using precolumns with different i.d. The recoveries are also compared in Table 2. For six successive chromatographic runs, the relative standard deviations (R.S.D.) for the

Table 2
Repeatability of the retention time and signals for DEP, BBP and DBP ($n = 6$), together with the recoveries and LODs

Precolumn i.d. (mm)	Analyte	Retention time		Peak area		Peak height		Recovery (%)	LOD (S/N = 3) (ng ml^{-1})
		Mean (min)	R.S.D. (%)	Mean (V s)	R.S.D. (%)	Mean (mV)	R.S.D. (%)		
0.1	DEP	2.86	0.798	0.609	1.38	110	0.411	93.0	0.21
	BBP	5.54	0.467	0.605	0.915	59.2	1.94	87.9	0.39
	DBP	6.27	0.431	0.610	1.54	56.0	1.10	95.3	0.41
0.15	DEP	2.97	0.776	0.638	2.13	113	1.10	97.3	0.27
	BBP	5.72	0.439	0.644	5.48	60.9	2.55	93.7	0.52
	DBP	6.46	0.401	0.649	3.96	57.6	2.61	101	0.54
0.2	DEP	2.99	0.873	0.770	3.48	117	2.40	118	0.33
	BBP	5.73	1.15	0.740	5.41	60.9	3.62	108	0.63
	DBP	6.45	1.07	0.767	5.25	57.9	3.61	120	0.64
0.32	DEP	3.14	0.797	0.618	2.68	110	1.21	94.3	0.45
	BBP	5.81	0.422	0.622	3.54	60.2	2.55	90.4	0.82
	DBP	6.53	0.433	0.636	3.46	57.3	3.90	99.3	0.87

Operating conditions as in Fig. 4.

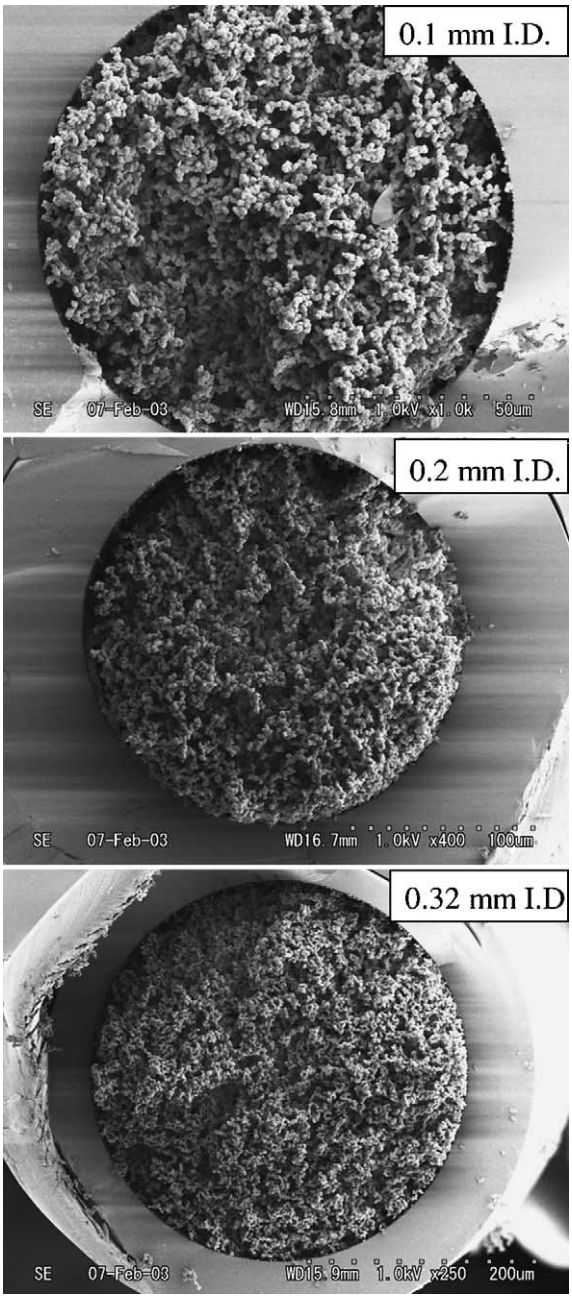

Fig. 3. Cross-sectional SEM photos of the monolith columns with 0.1, 0.2 and 0.32 mm i.d.

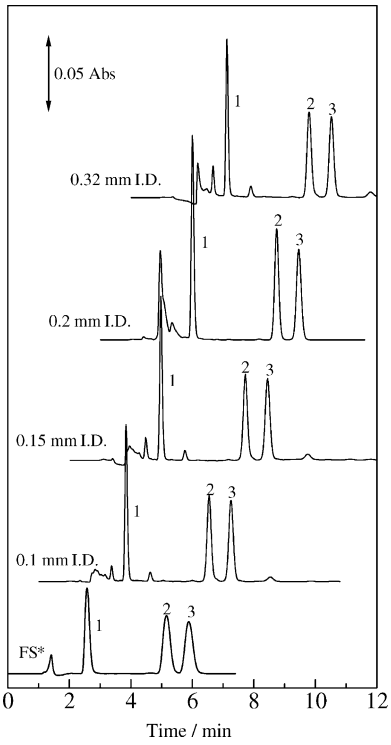

Fig. 4. Separation of the standard DEP (peak 1), BBP (peak 2) and DBP (peak 3) by using precolumns with different i.d. Column: L-column ODS, 100 × 0.32 mm i.d. Injector: fused silica (75 × 0.05 mm i.d.) connected to the M435 micro injection valve (FS*). Precolumn: laboratory-made monolith, 20 × 0.1–0.32 mm i.d. Mobile phase: acetonitrile–water (75:25). Flow-rate: 4.2 μl min^{-1}. Sample volume and concentration: 0.2 μl containing 100 μg ml^{-1} for each phthalate (FS*), and 0.1 ml containing 200 ng ml^{-1} for each phthalate (0.1–0.32 mm i.d.). Wavelength of UV detection: 204 nm.

retention time, peak area and peak height were between 0.40 and 1.2%, 0.92 and 5.5% and 0.41 and 3.9%, respectively. It is found that good repeatability (R.S.D. < 1.9%) can be achieved when concentrating 0.1 ml of 0.2 μg ml^{-1} of the aqueous samples using a 0.1 mm i.d. monolithic precolumn. R.S.D. for the peak area obtained using the 0.15 and 0.2 mm i.d. precolumns were relatively higher. However, when 50 μl of 10 ng ml^{-1} of the aqueous samples was concentrated, the R.S.D. of the peak area obtained with the 0.15 mm i.d. monolithic precolumn were improved to between 2.26 and 3.07%. This shows that the 0.15 mm i.d. monolithic precolumn has the potential to analyze phthalates at low ppb level. For comparison, a 0.2 μl injection of 100 μg ml^{-1} of the same mixture using a commercially available valve was carried out, and the R.S.D. for the retention time, peak area and peak height were between 0.38 and 0.59%, 1.6 and 2.6% and 0.50 and 0.64%, respectively.

Recoveries are normally calculated based on the calibration of the standard samples. However, in this study, the recoveries were calculated based on a comparison with a commercially available sample injector. The recoveries of the analytes were between 88 and 120%. The 0.2 mm i.d. precolumn had the highest recovery with an average of 115%, whereas the lowest was obtained with the 0.1 mm i.d. precolumn, with an average of 92.1%. The ideal recoveries were obtained with the 0.15 mm i.d. precolumn with an average of 97.3%. This system solved the problems of having low recoveries of the analytes (33 and 65% for BBP and DEP, respectively) with the packed ODS precolumn as reported previously [18].

3.3.2. Signal intensity and limit of detection (LOD)

Under the operating conditions as in Fig. 4, the theoretical plate numbers, *N*, of the signals obtained via the M435

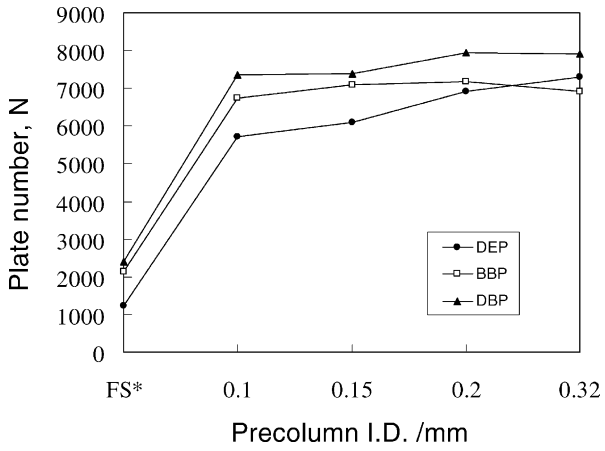

Fig. 5. Theoretical plate numbers of the phthalates vs. the precolumn i.d. Operating conditions as in Fig. 4.

micro injection valve (indicated as FS*) and the monolithic precolumns were calculated based on the following equation:

$$N = 2\pi \left(\frac{R_t \times \mathrm{PH}}{\mathrm{PA}} \right)^2 \quad (1)$$

where R_t is the retention time, PH is peak height, and PA is peak area. For the peaks obtained via FS* in Fig. 4, the N values calculated for peaks 1–3 were 1165, 2096 and 2452, respectively, based on the conventional formula:

$$N = 16 \left(\frac{R_t}{w} \right)^2 \quad (2)$$

where w is the peak width measured in the same unit as R_t. While N for these peaks obtained via Eq. (1) were 1236, 2153 and 2401, respectively, indicating that nearly the same values as those calculated with Eq. (2) were obtained. The N values showed a small deviation for enriched sample volumes of up to 0.3 ml. And it dented to approximately 30% when the amount of the sample was increased to 0.6 ml.

Comparing to FS*, the theoretical plate numbers of the same separation column was increased by 3–6-fold when the precolumn unit was used as the sample injector, as shown in Fig. 5. This shows that the enrichment unit using the in-house made monolithic precolumns improved the resolution, because the resolution is proportional to the square root of N.

The LODs for DEP, BBP and DBP when using precolumns with different i.d. is also shown in Table 2. The highest sensitivity of the analytes was achieved with a 0.1 mm i.d. precolumn, while the lowest was obtained with the 0.32 mm i.d. precolumn. The LODs of the system were improved by more than 2000-fold and were between 0.21 and 0.87 ng ml^{-1} even with a UV absorbance detector. It is found that the LODs of the analytes increased with increasing i.d. of the precolumn. It should be noted that when we used a lower concentration of the analytes and increased the enrichment volume, the LODs were improved (see Section 3.1).

Table 3
Summarized calibration data for the phthalates

Analyte	Precolumn i.d. (mm)	Concentration range (ng ml^{-1})	R^2	
			Peak area	Peak height
DEP	0.1	0–20	0.991	0.990
	0.15	0–30	0.990	0.991
	0.2	0–30	0.992	0.990
	0.32	0–20	0.992	0.990
BBP	0.1	0–40	0.990	0.990
	0.15	0–40	0.998	0.997
	0.2	0–40	0.992	0.992
	0.32	0–40	0.991	0.993
DBP	0.1	0–40	0.998	0.997
	0.15	0–40	0.995	0.997
	0.2	0–40	0.996	0.995
	0.32	0–40	0.994	0.995

Operating conditions as in Fig. 4, except for the sample. Sample volume: 50 μl. Sample concentration: as indicated.

3.4. Linearity

3.4.1. Signal intensity versus sample concentration

Table 3 gives the linear concentration ranges investigated and the resulting linear-regression coefficients (R^2) of the calibration graphs. For BBP and DBP, all the four different-i.d. precolumns showed linear relationships between the peak signals (peak area or peak height) and the sample concentration, ranged from 0 to 40 ng ml^{-1}, with R^2 = 0.990–0.998. On the contrary, the linear ranges for the DEP were between 0 and 20 ng ml^{-1} for the 0.1 and 0.32 mm i.d. precolumns and between 0 and 30 ng ml^{-1} for the 0.15 and 0.2 mm i.d. precolumns.

3.4.2. Signal intensity versus enrichment volume

When the sample concentration was fixed at 20 ng ml^{-1}, the relationships between the peak signals and the enriched sample volume were investigated. For all the four precolumns with different i.d., the signals of BBP and DBP were proportional to the enriched sample volume. Alternatively, there was no linear increment in DEP peak signals with increasing sample volumes except for 0.2 and 0.32 mm i.d. precolumns, where the calibration graphs were almost linear, R^2 = 0.922–0.991, between the sample volume range of 0–300 μl. The 0.15 and 0.2 mm i.d. precolumns had the best linear relationships for both BBP and DBP between the sample volumes from 0 to 500 μl with R^2 = 0.993–0.998 and 0.995–0.999, respectively. Equally good linear relationships were obtained with the 0.1 mm i.d. precolumn; R^2 = 0.991–0.994 was obtained for DBP between a sample volume range of 0–300 μl, and a linear sample volume range was obtained between 0 and 500 μl for the BBP.

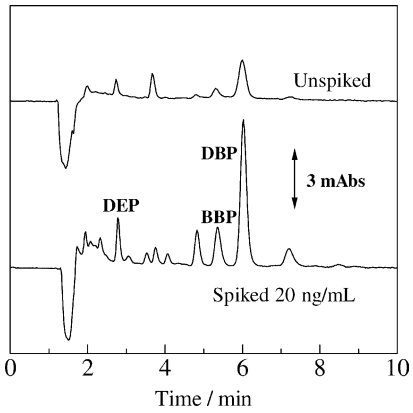

Fig. 6. Determination of phthalates in laboratory distilled water. Column: L-column ODS, 100 × 0.32 mm i.d. Precolumn: laboratory-made monolith, 20 × 0.15 mm i.d. Mobile phase: acetonitrile–water (75:25). Flow-rate: 4.2 μl min^{-1}. Sample: 0.4 ml of laboratory distilled water. Wavelength of UV detection: 204 nm.

When the sample concentration was increased and fixed at 0.2 μg ml^{-1}, only the 0.32 mm i.d. precolumn achieved considerably good linear calibration graphs for both BBP and DBP between the sample volumes of 0–600 μl. For both analytes, the R^2 = 0.922–0.938 and 0.957–0.992 for the peak area and peak height, respectively. The results are due to larger amounts of the stationary phase existing in the 0.32 mm i.d. precolumn.

3.5. Application to water analysis

The on-line concentration system using a monolithic precolumn was applied to the trace analysis of phthalates contained in laboratory distilled water and tap water samples. The 0.15 mm i.d. precolumn was used since it possesses good recovery and higher sensitivity for phthalates at low ppb level. Figs. 6 and 7 illustrate the chromatograms for the laboratory distilled water and tap water samples. The concentrations of phthalates were determined by using the standard addition method, and the enriched sample volumes were maximized up to 1.0 ml. The DEP, BBP and DBP concentrations in the laboratory distilled water were determined to be 7.0, 4.6 and 5.9 ng ml^{-1}, respectively. On the other hand, the tap water contained 5.6 and 15.3 ng ml^{-1} of BBP and DBP, respectively. In addition, the broadening of the peaks in Fig. 5 is due to the 1.0 ml injection volume.

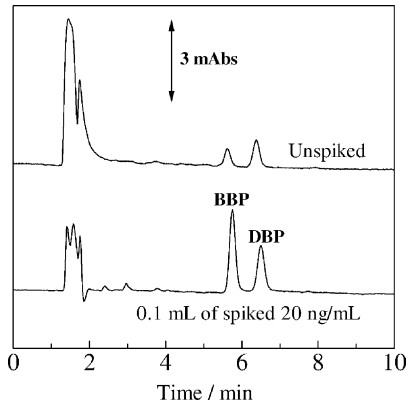

Fig. 7. Determination of phthalates in tap water. Operating conditions as in Fig. 6, except for the sample. Sample: 1.0 ml of tap water (upper trace), and 0.1 ml of spiked 20 ng ml^{-1}, as indicated.

4. Conclusions

A novel on-line sample concentration system using monolithic precolumns in microcolumn LC was designed. The double-pore structure of the monolithic precolumns allows enrichment of aqueous samples of up to 1 ml without any loss in resolution; in fact the theoretical plate number was increased, together with an improvement in the LODs. Thus, the precolumn enrichment unit could be used as an alternative sample injection method to the typical syringe-injection method in order to improve the concentration sensitivity in microcolumn LC. Since the phthalates in the laboratory distilled water and tap water samples could be determined simultaneously, the precolumn focusing system could also serve as a pretreatment device in the analysis of most real samples.

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