Preconcentration of Aromatic Compounds in Aqueous Samples with a Polymer-coated Fiber-packed Capillary and Subsequent Temperature-programmed Elution with Water for Pseudo-2D LC Separations

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A bundle of polymer-coated filaments was successfully introduced as an extraction medium for the preconcentration of an aqueous solution of aromatic compounds. The extraction was simply carried out with pumping the aqueous sample solution to the extraction capillary at ambient temperature. The extracted analytes were sequentially eluted with a flow of pure water using temperature-programmed heating of the extraction capillary in an oven. The results clearly suggest that the polymer-coated fiber-packed capillary could be employed in the sample preparation process for the analysis of various aqueous samples. Introducing the fractions eluted from the fiber-packed capillary to a conventional microcolumn liquid chromatography (micro-LC) system via a home-made valve-based modulator, an on-line coupled extraction/separation system was developed and a possibility to a pseudo-two-dimensional (pseudo-2D) LC separation of aromatic compounds in aqueous matrices has also been demonstrated.

Keywords Pseudo-2D separation, fiber, sample preparation, liquid chromatography, modulator, spline interpolation

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Introduction

Taking advantage of an excellent resistance to typical organic solvents and high temperatures, fine synthetic fibrous materials have been introduced in separation science, especially as an extraction medium in sample preparation and a stationary phase in chromatography.1–10 The miniaturized sample preparation cartridge packed with a bundle of synthetic filaments showed a good extraction performance, including a high preconcentration factor with simple and easy operation.11–14 By introducing a polymer-coating onto the surface of the filaments, an enhanced retentivity was accomplished as a stationary phase in gas chromatography (GC),15 and the resulting short polymer-coated fiber-packed capillary columns were employed in a high-temperature GC separation of complex sample mixtures.16,17 The selectivity of the polymer-coated fibrous media has been regarded as mainly depending on the type of coating material, while a contribution from the fibrous support material could be observed, suggesting possible optimization for the combination of fibrous supports and polymer coatings.7,15 The possibility to the trap medium in an interface between liquid chromatography (LC) and GC was studied,18 where a successful coupling of LC and GC was confirmed.

Packed into a needle-shaped capillary, fibrous materials also showed some successful applications to the sample preparation of analytes in aqueous samples as well as gaseous analytes in air samples. Down-sizing of the extraction cartridge enables a direct introduction of the extracted analytes to GC, which allows an improved recovery of target analytes along with an easy and quick sampling, but without any specially-trained skill for on-site sampling.19,20 An in-needle derivatization reaction of several classes of target analytes significantly improved the sensitivity.21–24

On the basis of our previous investigations, as typically described above, a short extraction capillary was prepared with a bundle of polymer-coated filaments as the extraction medium. The extraction of aromatic compounds from aqueous solutions was studied along with a sequential elution of the extracted analytes with a flow of pure water at elevated temperatures. With a home-made modulator, the analytes eluted from the extraction capillary were modulated for subsequent injections to microcolumn LC (micro-LC). Pseudo-two-dimensional (pseudo-2D) separation of several aqueous sample mixtures was established with the modulator having a set of two polymer-coated fiber-packed capillaries therein.

Experimental

Materials and reagents

Diethyl phthalate (DEP), dipropyl phthalate (DPP), dibutyl phthalate (DBP), propyl benzoate (3B), pentyl benzoate (5B),

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hexyl benzoate (6B), hexyl 4-hydroxybenzoate (6PB), and heptyl 4-hydroxybenzoate (7PB) were obtained from Tokyo Chemical Industries (Tokyo, Japan). All other reagents and solvents were of analytical reagent grade and purchased from either Kishida Chemical (Osaka, Japan) or Wako Pure Chemical Industries (Osaka, Japan). Water was purified by a Milli-Q Water Purification System (Merck-Millipore, Tokyo, Japan).

As the synthetic fibrous material, Zylon, poly(p-phenylene-2,6-benzobisoxazole), fiber of ca. 11.5 μm o.d. was obtained from Toyobo (Otsu, Japan). The bundle of Zylon originally consisted of 166 fine filaments, and the number of the filaments per bundle was adjusted as described below. On the basis of preliminary results, an HR-17 (50%-phenyl-50%-methylpolysiloxane, Shinwa Chemical Industries, Kyoto, Japan) was employed as the material of polymer-coating onto Zylon filaments.

Preparation of polymer-coated filaments and the extraction capillary

For the preparation of the polymer-coated filaments, the following procedure was carried out. First, a short (1.0 m) fused-silica capillary of 0.32 mm i.d. was packed with 332 Zylon filaments. The capillary was connected to a pressure-proof vessel containing 10 mL of acetone, and washed with the solvent pumped by N2 gas at a pressure of 500 kPa; and it was then, sequentially washed with water, acetone and chloroform in a similar manner. The capillary was allowed to dry with N2 flow at ambient temperature for 2 h. Next, the capillary was subjected to heating in a GC oven with a flow of N2 gas. The oven temperature was programmed from 30 to 300 °C at 2 °C min⁻¹, and then held at the final temperature for about 10 h. After the above-mentioned washing process, a solution of the polymeric coating material (HR-17) in n-hexane containing a cross-linking reagent was pumped through the packed capillary. The concentration of the polymer solution was optimized as 0.5 wt% on the basis of preliminary experiments, as the first concentration of the polymer solution was optimized as 2.0 wt% HR-17, as the first result, to evaluate the fundamental extraction/retention characteristics. Next to the pumping of a polymer solution of 5 mL, N2 flow through the capillary was maintained for more than 5 h. The capillary was then installed in the GC oven again, and the temperature was elevated with a program from 40 to 300°C at 0.5 °C min⁻¹, followed by extended heating for more than 60 h to make sure of successful coating.

To prepare the polymer-coated fiber-packed capillary, a bundle of 3320 polymer-coated Zylon filaments synthesized as mentioned above was longitudinally packed into a stainless-steel tube of 0.8 mm i.d., 150 mm length using a method as described previously, while two trap capillaries for a modulation valve were prepared by packing 1328 filaments of polymer-coated Zylon filaments into two stainless-steel capillaries having 0.5 mm i.d., 100 mm length in a similar manner. For the coating to the filaments to be packed to these trap capillaries, a polymer solution of 2.0 wt% HR-17 was employed so as to avoid any undesirable bubble formation that may cause an unstable baseline, especially for elution from the extraction capillary at high temperatures. As mobile phase solvents, a mixture of acetonitrile/water was used at a typical flowrate of 200 μL min⁻¹, unless otherwise specified.

Data acquisition and processing

All data collection was made using ChromNAV data handling/analysis software (Jasco) running on a personal computer, where an appropriate baseline correction was made using the software, if needed. For 2D data analysis, a home-made data analysis program was developed. Spline interpolation was employed to produce the 2D elution profile by combining a set of consecutive elution profiles from the extraction capillary i.e. the 1st dimension. Details of the home-made 2D data analysis program are described in the following section.

Results and Discussion

An overview of the separation system is illustrated in Fig. 1. An aqueous sample was pumped with one of the syringe pumps, and aromatic compounds were extracted in the polymer-coated fiber-packed capillary in oven 1 maintained at near to room temperature. Next, pure water was delivered to the extraction capillary with another syringe pump, where the temperature of oven 1 was elevated for thermal desorption. The eluted analytes were sequentially delivered to one of the polymer-coated fiber-
packed trap capillary in the modulation valve maintained at room temperature. By switching the modulation valve as well as two 6-port valves connected to the modulation valve, all of the fractions modulated in the valve were sequentially injected to the micro-LC for subsequent quick separations, typically within 2 to 3 min.

The absorbance data from the UV/Vis detector were continuously monitored as a single chromatogram. Then, the chromatogram was divided into individual short chromatograms obtained in the 2nd dimension. The starting signal in the 2nd dimension was generated from the modulator valve. A set of typical chromatograms for the separation of phthalates is illustrated in Fig. 2, where fractions of odd and even numbers were modulated with the first trap capillary and another trap capillary, respectively, in the modulation valve. As can be seen in Fig. 2, fractions #3 to #6, #7 to #10 and #11 to #14 mainly consisted of DEP, DPP and DBP, respectively, in the 2nd dimension. Since the sequential elution of analytes is not comparable to typical chromatographic elution from a modern LC column in terms of the resolution, the pseudo-2D separation could offer a quite satisfactory separation of these three compounds in this system.

A similar trend was also observed for the separation of benzoates (Fig. 3). For a better resolution of all peaks, the modulation interval was set at 3 min, allowing for a careful sequential elution from the extraction capillary and a relatively mild LC separation in the 2nd dimension. Taking advantage of an improved separation performance, a more complex aqueous sample containing five aromatic compounds could be analyzed with satisfactory resolution for all of the analytes, as shown in Fig. 4. Despite of the additional one component (6PB) to the sample mixture analyzed (Fig. 3), all of the analytes were well separated by pseudo-2D separation. In this case, an overlap problem of 3B and 6PB in the 2nd dimension was solved by an advantage of the pseudo-2D separation. However, as can be seen in the array of the chromatograms depicted above, additional data processing is needed to produce a 2D presentation of these data.

As illustrated in Fig. 5, a spline interpolation was employed for data smoothing between fractions. A set of UV absorbance data at a certain retention time in the 2nd LC separation for all

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**Fig. 2** An array of chromatograms obtained with the pseudo-2D separation system for the separation of phthalates. Extraction and elution conditions: extraction capillary, polymer-coated (HR-17, 0.5 wt%) fiber-packed; extraction flowrate, 16.6 μL min⁻¹; extraction time, 10 min; water flowrate for elution, 50 μL min⁻¹; elution temperature program, 25 °C (2 min) to 200 °C at the rate of 7 °C min⁻¹; modulation interval, 2.0 min. Micro-LC conditions: column, Superiorex ODS (1.0 mm i.d., 150 mm, 5-μm particle size); mobile phase, ACN/water = (80/20); column temperature, 30 °C; detection, UV at 240 nm; flowrate, 200 μL min⁻¹. Other conditions are given in the text. Peaks: (a) DEP; (b) DPP; (c) DBP.

**Fig. 3** Chromatograms obtained with the pseudo-2D separation system for the separation of benzoates. Extraction and elution conditions: elution temperature program, 30 to 240 °C at the rate of 4 °C min⁻¹; modulation interval, 3.0 min. Micro-LC conditions: column, Capcell Pak ODS UG 120 (1.0 mm i.d., 150 mm, 5-μm particle size); mobile phase, ACN/water = (72/28). Other conditions are the same as in Fig. 2. Peaks: (a) 3B; (b) 7PB; (c) 5B; (d) 6B.

**Fig. 4** Typical pseudo-2D separation of a mixture of five aromatic compounds. The conditions are the same as in Fig. 3. Peaks: (a) 3B; (b) 6PB; (c) 7PB; (d) 5B; (e) 6B.
of the chromatograms was extracted, as shown in Fig. 5a. If these data points are linearly linked, as illustrated in Fig. 5b, linear spline interpolation can be done. However, a cubic interpolation was employed to produce a smoother drawing of the 2D chromatogram in this work. In the cubic interpolation calculation, the blank section between two adjacent data points was approximated by a cubic function, as depicted in Fig. 5c, showing a pseudo-chromatogram over the fractions at a desired retention time in the 2nd LC separation. The cubic spline interpolation was processed for every 0.2 s over the total analysis time in the 2nd dimension. The resulting typical 2D contour plot and the corresponding 3D chromatograms are illustrated in Figs. 6 and 7, respectively. To construct these 2D and 3D chromatograms, the data in Fig. 4 were processed by a home-made data-processing software that was developed with Microsoft Visual Studio 2010.

Although the entire separation efficiencies in these chromatograms could not be comparable with that of a modern 2D separation system based on commercially available silica based stationary phases for high performance separations, a satisfactory pseudo-2D LC separation in the developed on-line coupled system was confirmed. The obtained recovery for 5B and 6PB are typically more than 95%, although the recovery could be further improved by an additional optimization in the modulation process. Taking advantage of the home-made back-pressure column at the exit from the flow-cell of the UV/Vis detector, no significant baseline disturbance was observed in those temperature-programmed separations up to about 250°C.

Conclusions

By introducing polymer-coated filaments as the extraction
medium for the sample preparation of aqueous solution of aromatic compounds, not only a preconcentration of these compounds, but also a successful partial separation of these compounds was demonstrated with a temperature-programmed elution of the extraction capillary. A pseudo-2D separation was also demonstrated with an on-line coupled system consisting of the extraction capillary and a micro-LC using a home-made valve-based modulator. With the home-made data-processing software, the separation could be successfully expressed as 2D and 3D chromatograms. More extensive studies, including the more precise control of the trap temperature, and the development of other polymer-based materials, are currently progressing in our laboratory, including the employment of these fibrous materials as an extraction medium in a miniaturized sample preparation technique.

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