High Throughput On-line Preconcentration Using "Spongy-monolith" Prepared by Pore Templates

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We developed novel spongy-monolith which had several dozen micron pore size and is suitable for high throughput analyses. The results of liquid chromatographic evaluations suggested that the spongy-monolith showed excellent performance as preconcentration column for on-line liquid chromatographic system with low pressure drop.

Recently, high throughput analyses have become very important in the fields of biochemistry, environmental chemistry, and industrial chemistry because of increasing demand of analytes. In most cases of quantitative analysis, consequently the pretreatment procedure is ineluctably required to remove contamination and concentrate the objective compounds. During pretreatment using solid-phase extraction and/or on-line column switching liquid chromatography,[1,2] the most serious problem is increasing pressure drop.

To achieve high throughput analyses and/or preconcentration, several materials containing uniform sized particles[3–5] and monolithic type materials[6–8] have been applied. Especially, monolithic materials are suitable for sample preparation and purification[9,10] because of their high porosity and domain size. However, it is not easy to accomplish quality assurance and reproducibility in either case, particles or monoliths. For example, the control of size and uniformity of particles is much more difficult. Moreover, there are some limitations of pore size distribution in monolithic materials. In other words, we have to develop novel material which can be prepared with easy procedures and high reproducibility. Also, the material must have large domain size (skeleton and through pore) and be suitable for high throughput analyses.

In this study, we utilized a "spongy-monolith" as an analysis or preconcentration column of liquid chromatography. The spongy-monolith was prepared as follows: polyolefin chips, consisting of polyethylene and poly(vinyl acetate)/EVA resin, and pore templates (water-soluble compounds) were melted at 130 °C and stirred to combine. The combined material was extruded in columnar style at 130 °C. Then, the columnar material was cooled in water immediately and the stick-shaped material was obtained. After cooling, the stick material was washed with water using ultrasonication to remove the water-soluble compounds. Here, the water-soluble compounds worked as pore templates. Finally, spongy-monolith was obtained and the porosity of this material was 74%. The physical appearances of the materials are shown in Figure 1. As shown in the SEM image, the spongy-monolith had large pores, more than 10 µm in diameter whereas the through pore size of usual monolithic materials are less than a few µm. And, we can easily control the pore size by changing the composition of the kind of EVA and water-soluble compounds. Thus, formability, flexibility, and operationality of novel spongy-monolith were much superior to the commonly used particles or monolithic materials. By means of these advantages, there was the hope that the spongy-monolith was applicable for practical high throughput evaluations.

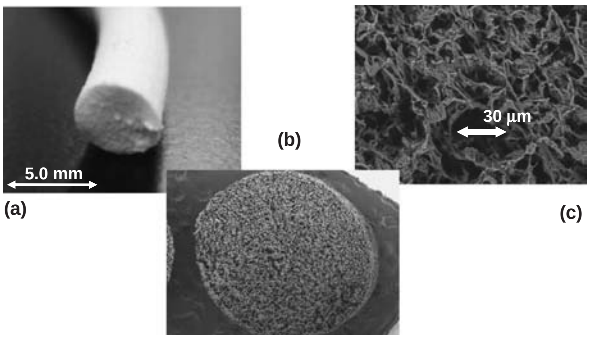

Figure 1. Physical appearance and SEM image of spongy-monolith, (a): physical appearance (4.7-mm o.d.), (b): SEM image (×60), (c): SEM image (×500).

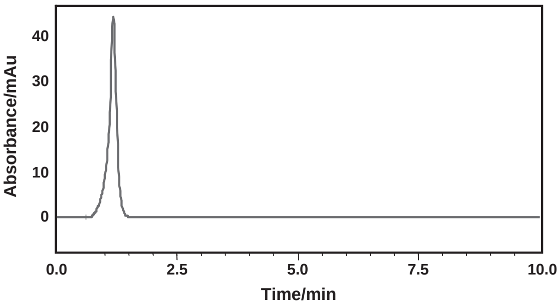

Figure 2. The chromatogram on the spongy-monolithic column LC conditions; column size: 100 × 4.6-mm i.d., Flow rate: 1.0 mL min^{-1}, pressure: 1.1 MPa, detection: UV 254 nm, sample: 1.0-µL uracil (0.1 mg mL^{-1}), mobile phase: MeCN/H_2O = 7/3 (w/w).

Therefore, the spongy-monolith (4.7-mm o.d.) was packed into a stainless steel column (4.6-mm i.d.) to investigate the performance as column of liquid chromatography. Then, we tried to analyze the column using a commonly used liquid chromatography system. Figure 2 shows the chromatogram on the prepared column using uracil as a solute. We obtained an unexpectedly good asymmetric peak. Additionally, according to calculation of void volume of the column, the spongy-monolith could be packed into a column without space between the spongy-monolith and inner wall of column. In fact, the leaking peak was not observed as shown in Figure 2.

Furthermore, the results of analyses for alkylbenzenes are shown in Figure 3, where the retention factor k' was defined as follows:

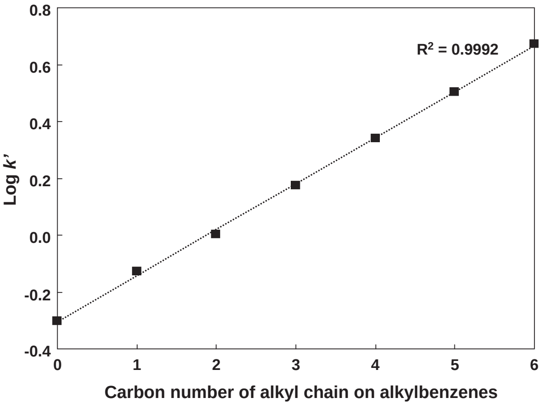

Figure 3. Log k' of alkylbenzenes on the spongy-monolithic column LC conditions; column size: 100 × 4.6-mm i.d., Flow rate: 1.0 mL min^{-1}. pressure: 1.1 MPa, detection: UV 254 nm, sample: 1.0-µL alkylbenzenes (1.0 mg mL^{-1}), mobile phase: MeCN/H_2O = 7/3 (w/w).

$$k' = [V(\text{retention volume of solute}) - V(\text{void volume})]/V(\text{void volume}) \tag{1}$$

These results clearly indicated that the column was hydrophobic. Next, we also investigated about pressure drop of the column on chromatographic evaluation. The column could be used at negligibly low pressure drop with very high flow rate; notably the pressure drop was 9.2 MPa at 9.9 mL min^{-1}. Actually, in the case of commonly used columns having similar size, the particulate type can be used at not more than 2.0 mL min^{-1} because of limitations of pressure drop, and the pressure drop was 19.1 MPa at 6.0 mL min^{-1} on even a typical silica-based monolithic column (the upper limitation of the column is 20.0 MPa). Accordingly, we can easily understand that the novel column was suitable for high throughput operations. Furthermore, the durability for buffer (pH: 3.0 to 10.0) and organic solvent such as methanol and acetonitrile, which are commonly used for reverse phase analyses, was stable.

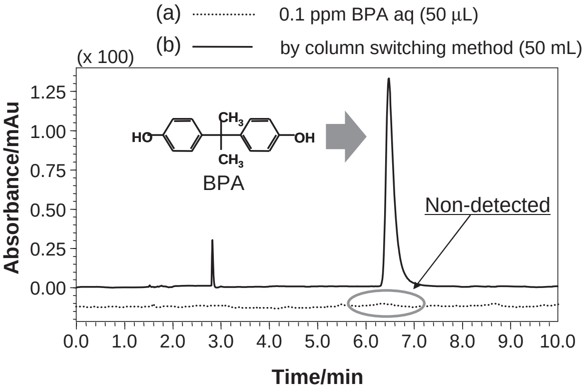

Figure 4. The chromatograms of BPA (a) before concentration, (b) after concentration LC conditions; analytical column: mightysil (150 × 4.6-mm i.d.), concentration column: novel porous polymer column (100 × 4.6-mm i.d.), analysis flow rate: 1.0 mL min^{-1}, concentration: 5.0 mL min^{-1}, pressure drop on concentration: 3.8 MPa, detection: UV 274 nm, mobile phase on analysis: MeOH/H_2O = 60/40 (w/w).

Finally, we applied the column as preconcentration of hydrophobic compound (bisphenol A: BPA) to make active use of high porosity and hydrophobicity of the column. BPA is one of the important environmental pollutants and often detected in environmental water samples. In this evaluation, the prepared column was utilized as pretreatment of an on-line column switching system.[1,2] In the column switching system, 50–100 mL of sample solution can be concentrated using pretreatment column, and the concentrated compounds easily detected in analysis part even if much lower concentration compounds.[11] Several concentrations of authentic aqueous BPA solution was concentrated with this system, and the recovery was observed on high throughput preconcentration. As a result, quantitative recovery was completely achieved in each flow rate at 1.0 ppb to 0.50 ppm (over 95%). According to Figure 4, BPA could be detected clearly after preconcentration though the peak was not observed on direct sample injection. Here, we utilized the long column (100 mm), which is much longer as a preconcentration column, because we could not obtain quantitative recovery when we used a shorter column (30 mm). However, we completely achieved high throughput preconcentration at low pressure drop. Consequently, the spongy-monolithic column effectively worked for on-line high throughput concentration.

In conclusion, we developed a novel spongy-monolithic column which had large skeleton and through pore size. The results of chromatographic evaluations suggested that the spongy-monolith was suitable for high throughput analysis and preconcentration. We expected that this column will be used for on-line analysis systems for environmental and/or biological samples.

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