Rapid Reversed-phase Separation Using Methacrylate-based C18 Monolithic Capillary Columns at High Flow Rates and Elevated Temperatures

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Rapid reversed-phase separation in the time-scale of seconds was conducted using methacrylate-based C18 monolithic capillary columns at high flow rates and elevated temperatures. The reversed-phase monolithic columns were prepared by in situ polymerization of octadecyl methacrylate (ODMA) and ethylene dimethacrylate (EDMA) within the confines of a polyimide-coated fused silica capillary. When a mixture of isoamyl alcohol and 1,4-butandiol was employed as a porogenic solvent, highly permeable monolithic stationary phases with adequate separation efficiency were reproducibly produced. The pressure drops of 20 cm-long columns were typically below 0.4 MPa at a normal flow rate of 3 μL/min (linear velocity of 1 mm/s), and the theoretical plate numbers mostly reached 3000 plates/20 cm. These monolithic columns exhibited excellent mechanical strength as well as thermal stability, and they were properly operated even at a temperature of 80 °C and a high linear velocity of 100 mm/s. At 80 °C, the theoretical plate numbers exceeded 6000 plates/20 cm due to the enhanced mass transfer. Consequently, a series of alkylbenzenes, from toluene to amylbenzene, could be efficiently separated within 30 s on the 20 cm-long column by carrying out a ultra-high flow rate gradient at 80 °C.

Well-ordered macroporous materials have attracted much attention in the fields of catalysis, adsorption, and separation science. In the early 1990's, Svec and Frechet introduced a simple "molding" process for the preparation of rigid organic polymer monoliths possessing micrometer-sized flow-through pores and nanometer-sized micropores as interaction spaces.[1] Since then, the monolithic structures have steadily gained acceptance and importance as an alternative to traditional particulate-type products, due to the novel characteristics such as low flow resistance, high reaction efficiency, and enhanced mass transfer.[2,3] In 1996, practically useful inorganic silica-based monoliths were also produced by a sol–gel process,[4] and in the last decade, the needs and expectations for monoliths have further grown, especially in separation science.[5–10]

Silica-based and organic polymer-based monoliths as separation media have their own advantages and disadvantages. Silica monoliths produced by sol–gel technology have more regularly controlled monolithic structures, allowing highly efficient separations of small molecules, but generally silica monoliths cannot be used with alkaline eluents. In contrast, organic polymer monoliths tolerate wide pH ranges, but have a problem of limited range of usable organic solvents due to the swelling and shrinking. In addition, the existence of micropores at the surface is often pointed out to be critical drawback of organic polymer monoliths, which have deleterious effects on the separation of small molecules. Meanwhile, the polymerization techniques have continued to be improved, and recently organic polymer monoliths have been shown also to have a potential for highly efficient separation of small analytes, comparable to the performance of silica-based monoliths.[11,12]

Generally, organic polymer monoliths have potential advantages over silica monoliths, including wide pH stability, absence of deleterious silanol effect, inertness to biomolecules, and facile preparation and modification. These characteristics may be beneficial especially in the analysis of biological samples containing high levels of matrix. In the present paper, thus, the preparation of polymer-based reversed-phase monolithic column possessing low hydraulic resistance and high separation efficiency even for small analytes, which are fundamentally conflicting requirements, was attempted, and the chromatographic performance was evaluated through the separation of a series of alkylbenzenes, from toluene to amylbenzene.

The capillary liquid chromatography system used consisted of a model LC-10ADVP pump (Shimadzu, Kyoto, Japan), a nano-injector (Chemco, Osaka, Japan) with a 20 nL sample reservoir, a fully home-made column thermostat, a model CE-1575 UV–vis detector (Jasco, Tokyo, Japan) equipped with an on-capillary flow cell, a model C-R8A data integrator (Shimadzu). Methacrylate-based C18 monolithic column was prepared by thermally initiated polymerization of octadecyl methacrylate (ODMA) and ethylene dimethacrylate (EDMA) within the confines of a polyimide-coated fused silica capillary (250 μm i.d., 350 μm o.d., GL Science, Tokyo). The detailed procedure for the preparation is described in our previous paper.[12] As a result of trial and error, a binary porogenic solvent consisting of isoamyl alcohol and 1,4-butanediol (5:1, v/v) was found to reproducibly produce low-flow resistant monolithic columns. As for the other parameters for producing monoliths as permeable as possible, total monomer concentration (%T) was examined in the range of 10–40%. The cross linking degree (%C) was fixed at 25%. Taking into account the separation efficiency and low flow resistance, throughout the present work, 25%T column was mainly used.

Figure 1 shows the separations of five alkylbenzenes on a 20 cm-long C18 monolithic column at different temperatures. The eluent was an acetonitrile–water (50:50, v/v) mixture, and the flow rate was set at 3 μL/min (corresponding to a linear velocity of 1 mm/s). As can be seen in Figure 1, the separation time was reduced to one third at 80 °C, compared to that at 20 °C. As a result of raising the column temperature, the pressure drop was also reduced to half because of the reduced mobile phase viscosity. It can also be noticed that the numbers of theoretical plates are improved from 3000 to over 6000 plates/20 cm by raising the column temperature due to the enhanced mass transfer. In terms of thermal stability, no significant degradations in
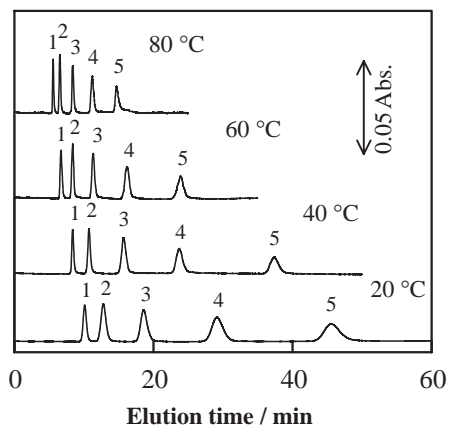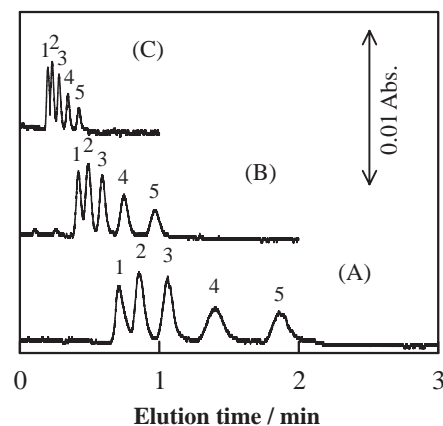
Figure 1. Separations of five alkylbenzenes at different temperatures. Stationary phase: ODMA-based monolith prepared in a capillary of 0.25 mm i.d. ×200 mm long, mobile phase: acetonitrile–water (50:50, v/v), flow rate: 3 μL/min, injection volume: 20 nL, detection: indirect UV absorption at 215 nm (on-column type UV detector), sample: (1) toluene, (2) ethylbenzene, (3) propylbenzene, (4) butylbenzene, and (5) amylbenzene.

the selectivity and resolution were observed after the continuous use for 24 h at 80 °C, although the retention times were reduced by about 5%.

In Figure 1, the pressure drops of the 20 cm-long column were 0.4 MPa at 20 °C and 0.2 MPa at 80 °C. These values correspond to one several tenth of that of conventional particle-packed analytical columns. The low flow resistance of the constructed monolithic column makes it possible to use high flow rate, allowing high-speed separations within the pressure drop constraints. Thus, the durability for high-pressure operation and the separation efficiency at high flow rate were subsequently examined in the linear velocity range of 1–100 mm/s. Figures 2a and 2b show typical chromatograms obtained under the conditions of high flow rates. The column temperature was fixed at 80 °C. As expected, the separation time was reduced along with an increase in the flow rate, although the separation efficiency was deteriorated to some extent. As for the mechanical strength, no significant changes in the retention were observed even after a continuous use at high pressures over 30 MPa. To improve the loss in separation efficiency for weakly retained analytes and to accelerate the elution of strongly retained analytes, a linear flow rate gradient was also carried out. Figure 2c shows high-speed separation of five alkylbenzenes on the 20 cm-long C18 monolithic column, where a ultra-high flow rate gradient from 20 to 100 mm/s in 30 s was run at 80 °C. Due to the novel characteristics of the produced monolithic column, as can be seen in Figure 2c, five alkylbenzenes were nearly baseline-separated within 30 s with separation efficiency on the order of 1000 plates/20 cm.

It was confirmed that methacrylate-based C18 monolithic columns could be properly operated even under the harsh flow

Figure 2. High-speed separations of five alkylbenzenes on a 20 cm-long ODMA-based monolithic capillary column under high flow rate conditions. Flow rate: (A) 30 μL/min, (B) 60 μL/min, and (C) a liner flow rate gradient from 60 to 300 μL/min in 30 s, temperature: 80 °C. Other experimental conditions are the same as in Figure 1.

rate conditions, with maintaining adequate separation efficiency even for small hydrocarbons. With taking into account the pH stability and the inertness to biomolecules, the present methacrylate-based reversed-phase columns would find many applications in post-genomic research, especially in the competitive drug process development and in metallomics research.[13]

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