Notes

Rod-Type Affinity Media for Liquid Chromatography Prepared by *in-situ*-Molecular Imprinting

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Biomolecular host-guest systems have been employed in various selective chromatographic separation techniques, such as affinity chromatography. Over the past decade, molecularly imprinted polymers (MIPs)[1-3] have come to be recognized as stable and easy-to-handle materials in affinity-type separations. The molecular imprinting technique can be summarized as follows: 1) formation of complex(es) between a template and polymerizable functionalized monomer(s), 2) polymerization in the presence of crosslinking agents, and 3) extraction of the template from the polymer matrix, yielding recognition sites complementary to the template. MIPs with a selective affinity for amino acid derivatives[4], sugars[5,6], drugs[7,8] and herbicides[9,10] have been used as chromatographic stationary phases.

Although the polymers can be readily prepared, the conventional approach for the preparation of molecularly imprinted chromatographic columns includes several tedious and time-consuming procedures, such as bulk polymer grinding, sieving and sizing the resulting polymer particles, and column packing. These procedures not only make the molecular imprinting technique less facile, but are generally problematic in terms of controlling the shape and size of polymer particles. Recently, efforts have been made to prepare uniform-size molecularly imprinted beads.[11,12] Also *in-situ*-methods[13,14] have been developed whereby polymerization is carried out in a column tube. These new strategies are expected to make the molecular imprinting technique an easier and more versatile method for preparing tailor-made affinity media.

We previously reported on the *in-situ*-preparation of molecularly imprinted continuous polymer rods for immediate use as chromatographic separation media without the polymer work-up steps described above.[13] In order to explore potential applications of this technique, rod-type MIPs were prepared for xanthine derivatives using both crosslinkers, a conventional ethyleneglycol dimethacrylate (EGDMA) and a styrene-divinylbenzene (St-DVB) system which was the first use in the *in-situ*-method.

Experimental

Theophylline **1** was used as a template for preparation of molecularly imprinted polymer rods by the *in-situ*-method (see Fig. 1 and Table 1). For P(A) and P(C): into cyclohexanol (12.0 g, 120 mmol) and 1-dodecanol (3.0 g, 16 mmol), used as porogenic solvents, were added theophylline (252 mg, 1.4 mmol), methacrylic acid (482 mg, 5.6 mmol) as a functional monomer, EGDMA (8.5 g, 43 mmol), as a crosslinking agent and 2,2′-azobisisobutyronitrile (85 mg) as an initiator. The reaction mixture was degassed by sonication and sparged with nitrogen gas. Stainless-steel columns (50 mm×4.6 mm i.d. or 150 mm×4.6 mm i.d.) were filled with this reaction mixture and placed in a water bath at 45°C for 12 h. After polymerization, the columns were connected to an LC pump and washed exhaustively with methanol/acetic acid (4 : 1, v/v). The columns were then washed with acetonitrile until a stable baseline was obtained. Control polymer rods, P(B) and P(D), were prepared identically, though without the addition of the template species. Styrene-divinylbenzene (St-DVB) based polymer rods, P(E) and P(F), were prepared with theophylline (252 mg, 1.4 mmol), methacrylic acid (482 mg, 5.6 mmol), styrene (5.9 g, 57 mmol) and divinylbenzene (6.1 g, 47 mmol) as crosslinking monomers, and cyclohexanol (6.1 g, 61 mmol) and 1-dodecanol (10.5 g, 56 mmol). Chromatographic tests were performed by isocratic elution with acetonitrile at a flow rate of 0.5 ml/min or 0.25 ml/min. The concentration and volume of samples injected were 50 μM and 20 μl, respectively. Detection was carried out at 260 nm.

Results and Discussion

The capacity factors obtained for the template **1** and

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Table 1 Capacity factors for xanthine derivatives in rod-type separation media

Column	Template	Polymer	Column length/mm	Capacity factor		
				1	2	3
P(A)	1	EGDMA	50	1.2	1.0	0.16
P(B)	none	EGDMA	50	0.47	0.61	0.12
P(C)	1	EGDMA	150	1.2	1.1	0.28
P(D)	none	EGDMA	150	1.1	1.6	0.34
P(E)	1	ST-DVB	50	1.9	1.3	0.37
P(F)	none	ST-DVB	50	1.4	1.3	0.36

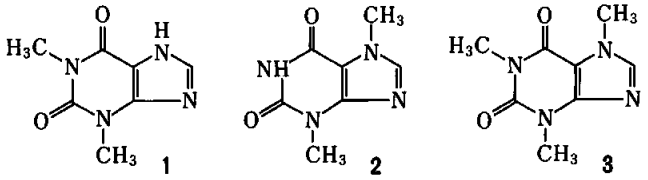

Fig. 1 Structures of the xanthine derivatives used in this study: **1**, theophylline; **2**, theobromine; **3**, caffeine. Theophylline **1** was used as a template molecule to prepare molecularly imprinted polymer rods.

reference xanthine derivatives theobromine **2** and caffeine **3** are shown in Table 1. In all of the polymer rods, **1** and **2** consistently displayed longer retention times than **3**. Both **1** and **2** possess three *tert*-amino groups and one *sec*-amino group, while **3** bears four *tert*-amino groups. Although the *tert*-amino group has a higher electron density than does the *sec*-amino group due to the electron-donating methyl substituent, access to the *tert*-amino group by functional monomer carboxyl moieties is sterically hindered. These chromatographic results therefore suggest that the retention is based upon ionic or hydrogen bonding interactions of the amino groups with carboxyl residues in the polymer rod. The effect of theophylline imprinting is clearly observed upon comparing the capacity factors for P(A) and those for P(B). Theophylline **1** was retained longer than the other two by the P(A) column, whereas **2** was retained longer than **1** by the control polymer P(B). It can be concluded that P(A) recognizes the number and position of the *sec*-amino groups of the xanthine derivatives. Similar results were obtained with the 150 mm long column systems, P(C) and P(D). A typical chromatogram in P(C) is shown in Fig. 2.

The *in-situ*-molecular imprinting of theophylline **1** using the St-DVB crosslinking system successfully produced a theophylline-selective polymer rod column. The control polymer P(F) showed little difference in affinity for **1** and **2**. The imprinted polymer P(E) gave a capacity factor for **1** that was 1.5-times larger than that observed for **2**. Therefore, it appears that rod-type molecularly imprinted polymers can be prepared using the St-DVB system as well as the EGDMA system.

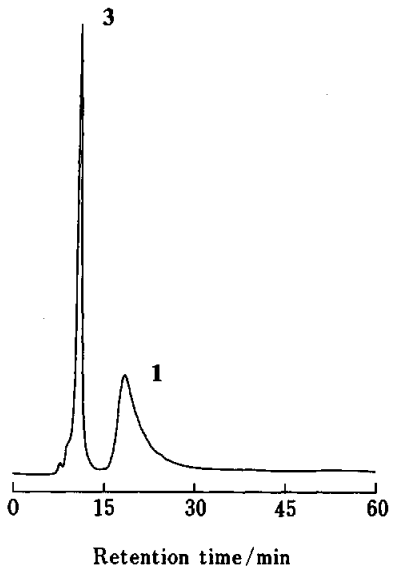

Fig. 2 Typical chromatogram of the theophylline-imprinted polymer rod, P(C). A mixture of theophylline (50 μM) and caffeine (50 μM) was injected as a sample. The eluent was acetonitrile at a flow rate of 0.25 ml/min.

In conclusion, the rod-type MIPs prepared by the *in-situ*-molecular imprinting technique was found to recognize xanthine derivatives. A crosslinking system including styrene and divinylbenzene was successfully introduced to the *in-situ*-technique, demonstrating that the *in-situ*-technique can be expected to be expanded to wide applications. Currently, further studies using various templates are ongoing.

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