Uniformly Sized Polymer Based Stationary Phase Having Multi-Chiral Selectors

Kimihiro Yoshizako, Yuichi Shirasu, Ken Hosoya,* Kazuhiro Kimata, Takeo Araki, and Nobuo Tanaka
Department of Polymer Science & Engineering, Kyoto Institute of Technology, Matsugasaki, Sakyo-ku, Kyoto 606

(Received May 10, 1996)

Uniformly sized polymer based HPLC stationary phase having multi-chiral selectors was prepared through a combination of molecular imprinting technique and in situ surface modification technique. Interestingly, a simple additivity of chiral recognition abilities of each chiral selectors was observed. Size exclusion chromatography revealed a possible cause for the selectivity.

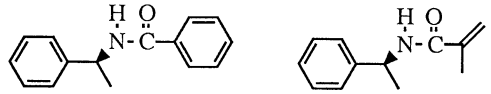
Molecular imprinting technique is a simple but extremely effective technique to afford chromatographic stationary phase having specific retentivity toward the template molecule.[1] Especially, in chiral separation, molecularly imprinted stationary phase shows excellent chiral recognition ability only to the template enantiomer.[2]

Molecularly imprinted polymer has been mostly prepared through bulk polymerization technique.[3] In this method, monomers including high content of cross-linking agent and host functional monomer, and a template molecule as well as porogenic solvent are mixed at once to afford molecularly imprinted polymer block. In some special case, the macroporous polymer block was reported to be directly utilized as a separation medium (polymer rod)[4] but mostly mashed and sieved to get packing material.

We have recently reported preparation of uniformly sized molecularly imprinted stationary phase for HPLC.[5] Using this technique, time-consuming mashing and sieving processes can be avoided and good column efficiency is also obtained due to excellent size monodispersity of packing material. In addition to the technical advantage, in situ surface modification can be made to introduce another surface functionality to the uniformly sized stationary phase.[6] This kind of in situ surface modification to the polymer block is obviously more difficult than that to the polymer particle. Using the in situ surface modification technique, we have reported chiral stationary phase,[7] temperature-responsive stationary phase,[8] and surface hydrophilic stationary phase having hydrophobic internal surface.[9]

In this report, we wish to explore possibility to combine molecularly imprinted technique and in situ surface modification technique using chiral monomer. This combination will show a possibility to afford uniformly sized molecularly imprinted stationary phase having additional chiral selector, namely multi-chiral selectors.

We selected a chiral molecule **1** and a chiral monomer **2** that were used for molecular imprinting and in situ surface modification, respectively.



1 **2**

Figure 1. Template and additional chiral monomer.

Table 1. Chiral recognition ability

Entry	Template **1**	Monomer **2**	DNB (α)	B (α)	BN (α)
1	No	No	1.00	1.00	1.00
2	No	Yes	1.04	1.04	1.28
3	Yes	No	1.06	1.17	1.04
4	Yes	Yes	1.09	1.18	1.31

Mobile phase: Hexane : Ethyl acetate = 1 : 1 (v/v).

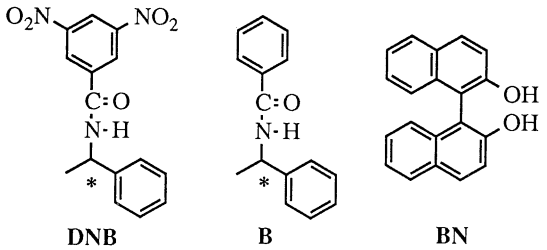
Uniformly sized molecularly imprinted stationary phase was prepared through a typical two-step swelling and polymerization method from polystyrene seed polymer prepared using an emulsifier-free emulsion polymerization that was reported elsewhere.[10] In this case, any host functional monomers were not used to make role of additional chiral selector clear.

The chiral monomer **2** was added during the polymerization step of uniformly sized molecularly imprinted polymer particles with **1**. This in situ surface modification technique is reported to afford a polymeric chiral surface functionality which shows good chiral recognition ability to axis chirality.[7] As was described, this preparation method is just a one-pot procedure.

The obtained polymeric stationary phase was washed with methanol, tetrahydrofuran, and acetone to remove the template molecule and another unbound impurities. Size uniformity of the obtained polymer particles was excellent where CV value was around 3%, and the chemical yield was also quantitative.

For the references, the base stationary phase prepared without either the template or the additional monomer, only morecularly imprinted stationary phase with **1**, and only surface modified stationary phase by the in situ surface modification method with **2** were also prepared.

We utilized three kinds of solutes as shown in Figure 2 for evaluation in HPLC. Chiral recognition ability to these solutes on the prepared stationary phases is summarized in Table 1.



DNB **B** **BN**

Figure. 2. Solutes utilized and abbreviations.

The base stationary phase (Entry 1) did not show any chiral recognition ability (α = 1.00) due to the lack of any chiral selectors. The surface modified stationary phase (Entry 2) afforded chiral recognition ability to binaphthol (**BN**) having an axis chirality, while relatively low chiral recognition ability to amide solutes **DNB** and **B** was observed. The observed chiral recognition ability is just compatible to the results reported in our

Table 2. Pore volume of the prepared stationary phases

Entry	Eva / ml [a]	Evb / ml [b]	Evc / ml [c]	EV tot / ml [d]
1	0.13	0.17	0.65	0.95
2	0.13	0.19	0.47	0.79
3	0.20	0.18	0.56	0.94

a: Elution volume (benzene) - elution volume (hexylbenzene). b: Elution volume (hexylbenzene) - elution volume (polystyrene MW = 760). c: Elution volume (polystyrene MW = 760) - elution volume (polystyrene MW = 20,000,000). d: a + b + c.

previous work dealing with chiral stationary phase having polymethacrylamide as a chiral selector.[7]

Molecularly imprinted stationary phase with the template molecule **(*S*)-B** slowed the largest separation factor to the enantiomer of **B**. As was mentioned, any host functional monomers were not used, therefore the observed α value is not so high, but this is clearly molecularly imprinted effect, because the solute with larger retentivity, **DNB** showed smaller α value.

The stationary phase prepared with both of the template **2** and the additional monomer **1** (Entry 4) showed mixed chiral recognition abilities, where the α values on the stationary phase 4 are thought to be just summation of those obtained on the stationary phases 2 and 3. These facts suggest that molecularly imprinted sites and additional polymeric chiral selectors might work independently.

Size exclusion chromatography was utilized to determine pore volume of the prepared stationary phases (Table 2). The total pore volume of base stationary phase 1 was 0.95 ml / g and the molecularly imprinted stationary phase 3 had 0.94 ml / g which is almost equal to that on the base stationary phase. On the other hand, the stationary phase prepared using the additional monomer 2 resulted in 0.79 ml / g. This is clearly reduced than that of the base stationary phase.[11]

In detail, the reduced pore volume of the surface modified stationary phase 2 is due to a reduced pore volume in relatively large pore size region (EVc), while pore volume in small pore size region is affected on the molecularly imprinted stationary phase 3. These observations suggest that the additional chiral monomer forms bulky polymeric chiral selectors on macropore region, on the other hand, molecular imprinting technique affords imprinted sites in micropore region that is formed by highly cross-linked polymer network. This might be a reason why stationary phase 4 shows the mixed chiral recognition ability of stationary phases 2 and 3.

Traditional molecular imprinting technique has been done by just bulk polymerization, therefore additional modification can hardly made. On the other hand, the method reported here can afford uniformly sized stationary phase having not only molecularly imprinted selector but also another chromatographic selector. In this report, we just report a possibility of molecularly imprinted chiral selector and additional polymeric chiral selector, which is called as multi-chiral selectors, but another combinations have further possibilities to afford functionalized molecularly imprinted stationary phases such as surface hydrophilic stationary phase having molecularly imprinted recognition sites. [9]

This work is partly supported by the fund from the Japanese Ministry of Education.

References and Notes

1 G. Wulff, *Angew. Chem. Int. Ed. Engl.*, **34**, 1812 (1995).
2 M. Kempe and K. Mosbach, *J. Chromatogr. A*, **664**, 276 (1994).
3 D. Kriz, C. B. Kriz, L. I. Andersson, and K. Mosbach, *Anal. Chem.*, **66**, 2636 (1994).
4 J. Matsui, T. Kato, T. Takeuchi, M. Suzuki, K. Yokoyama, E. Tamiya, and I. Karube, *Anal. Chem.*, **65**, 2223 (1993).
5 K. Hosoya, K. Yoshizako, N. Tanaka, K. Kimata, T. Araki, and J. Haginaka, *Chem. Lett.*, **1994**, 1437.
6 K. Hosoya, E. Sawada, K. Kimata, T. Araki, N. Tanaka, and J. M. J. Fréchet, *Macromolecules*, **27**, 3937 (1994).
7 K. Hosoya, K. Yoshizako, N. Tanaka, K. Kimata, T. Araki, and J. M. J. Fréchet, *J. Chromatogr. A*, **666**, 449 (1994).
8 K. Hosoya, K. Kimata, T. Araki, N. Tanaka, and J. M. J. Fréchet, *Anal. Chem.*, **67**, 1907 (1995).
9 K. Hosoya, Y. Kishii, K. Kimata, T. Araki, N. Tanaka, F. Svec, and J. M. J. Fréchet, *J. Chromatogr. A*, **690**, 21 (1995).
10 V. Smigol, F. Svec, K. Hosoya, Q. Wang, and J. M. J. Fréchet, *Angew. Makromol. Chem.*, **195**, 151 (1992).
11 K. Hosoya, S. Maruya, K. Kimata, H. Kinoshita, T. Araki, and N. Tanaka, *J. Chromatogr.*, **625**, 121 (1992).