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One-pot preparation method for a uniform-sized polymer-based chiral stationary phase for high-performance liquid chromatography with polymethacrylamide as a chiral selector

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ABSTRACT

A simple one-pot method for the preparation of a uniform-sized polymer-based chiral stationary phase (PCSP) for high-performance liquid chromatography was investigated with polymethacrylamide as a chiral selector. During the polymerization process of uniform-sized base particles prepared by a two-step swelling method, solid chiral methacrylamide monomer was added directly to the aqueous polymerization medium of the base particles. The methacrylamide polymerized and was incorporated on the surface of the base polymer particles quantitatively without losing the size uniformity of the base particles. When the methacrylamide was added stepwise, the polymer-based chiral stationary phase obtained gave complete resolution of 2,2'-dihydroxy-1,1'-binaphthyl, whereas lower resolution was observed with PCSPs prepared by the traditional copolymerization method.

INTRODUCTION

In 1979, Blaschke and co-workers reported the direct chromatographic resolution of some racemic drugs [1] utilizing chiral polyacrylamide stationary phases [2,3]. The best known example of this work was the resolution of thalidomide, which made a great contribution to the elucidation of the different medicinal actions of each of the enantiomers.

Blaschke and co-workers' chiral polyacrylamide stationary phases were prepared by a very simple procedure (Fig. 1, path A). Chiral acrylamide or methacrylamide monomers were prepared by condensation reactions of acryloyl chloride or methacryloyl chloride with commercially available chiral amines, while chiral polymer gels were prepared by copolymerization of these chiral acrylamide monomers with a cross-linking agent such as ethylene diacrylate [4]. They utilized the prepared chiral polymer gels as stationary phases for the chromatographic resolution of racemic drugs, but the polymer gels were soft gels owing to the low extent of cross-linking (*e.g.*, chiral monomer to cross-linking agent ratio = 10:1 [4]). Therefore, their polymer gels could not be used as stationary phases for HPLC.

To solve this problem, the authors prepared a corresponding stationary phase by a condensa-

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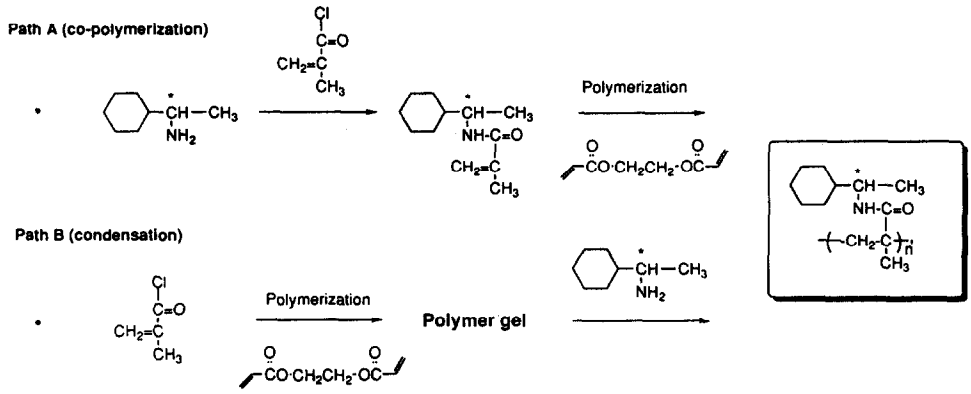

Fig. 1. Preparation of chiral stationary phases including chiral acrylamides.

tion reaction in which a ready-made cross-linked polymer gel including acryloyl chlorides was further modified with the corresponding chiral amines (Fig. 1, path B). However, this stationary phase showed a lower resolution than that obtained by copolymerization of the chiral monomer with the cross-linking agent described earlier [5]. This contrasting observations suggested that polymerization of the chiral acrylamide was important for preparing an effective chiral stationary phase.

Thus chiral recognition by a polymeric chiral selector can hardly be expected from the chemical structure of the corresponding monomer unit, because the three-dimensional structure of a chiral polymer is reported to play an important role in chiral recognition and a small difference in the monomer unit and/or polymer structure unexpectedly makes a large difference in chiral recognition [6]. On the basis of this observation, immobilization of a polymeric chiral selector on an achiral support material by covalent bonding should lower or change its chiral resolution because covalent bonding may somehow disturb the effective three-dimensional structure of the polymeric chiral selector [7]. Moreover, as in this immobilization reaction between a solid (support material) and chiral polymer it can be expected that all of the reactive functional groups of both materials cannot be consumed in the immobilization reaction owing to steric hindrance, the modified particle still leaves reactive functional groups that should affect the selectivity in HPLC and the chemical stability of the stationary phase.

A problem in the preparation of highly cross-linked polymer-based chiral stationary phases for HPLC by a traditional copolymerization method is that part of the chiral monomer should be incorporated into a chromatographically ineffective polymer structure, which means a waste of relatively expensive chiral monomer.

These are the reasons why most stationary phases with polymeric chiral selectors for HPLC have been prepared by adsorption of the polymeric chiral selector on a support material such as silica gel or poly(styrene–divinylbenzene) particles. However, it is clearly a problem that these stationary phases cannot be used in mobile phases that dissolve the polymeric chiral selectors. In addition, physical exfoliation of the chiral selector also cannot be avoided.

Later, Blaschke *et al.* [8] reported the preparation of silica-based chiral polyacrylamide stationary phases by graft polymerization of the chiral acrylamides from commercially available silica gel substituted with diol groups (LiChrosorb Diol) that was further esterified with methacrylic acid. These silica-based chiral stationary phases gave good resolution of drugs in HPLC, but the preparation procedure was relatively complicated and the reaction conditions needed to be carefully controlled in order to achieve good reproducibility. Moreover, silica-based stationary phases are chemically unstable at both high and low pH.

Here, we report a simple one-pot method for the preparation of a uniform-sized polymer-based chiral stationary phase (PCSP) for HPLC containing chiral polymethacrylamide which is

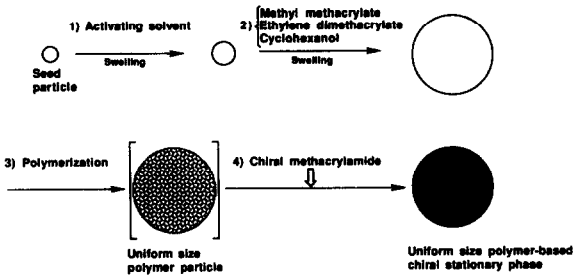

Fig. 2. Procedure for preparation of uniform-sized polymer-based chiral stationary phase.

incorporated by a graft-type copolymerization on the surface of base particles. In this method, solid chiral methacrylamide is added directly to an aqueous polymerization medium of uniform-sized base particles prepared by a two-step swelling method (Fig. 2). The added methacrylamide is expected to be adsorbed according to a molecular diffusion mechanism and polymerized by radical species on the surface of the partly polymerized base particles. We compared the chiral resolution ability of the particles with that of material prepared by a traditional copolymerization method.

EXPERIMENTAL

Reagents

L-(−)-Phenyethylamine and D-(−)-α-phenylglycine were purchased from Nacalai Tesque (Kyoto, Japan) and methacryloyl chloride from Aldrich (Milwaukee, WI, USA). Other reagents for the preparation of chiral monomers were purchased from Nacalai Tesque.

Methyl methacrylate and ethylene dimethacrylate were purchased from Wako (Osaka, Japan) and the stabilizer was removed by a standard technique before use. Initiators such as benzoyl peroxide (BPO) and potassium peroxodisulphate (PPS) were purchased from Nacalai Tesque and used after recrystallization by a reported method [9].

Preparation of chiral methacrylamide

Two different methacrylamides were prepared and their structures and abbreviations are shown in Fig. 3. *N*-Methacryloyl-L-phenylethylamine (PEA) was prepared by condensation of L-phenylethylamine with methacryloyl chloride [2], while the bifunctional methacrylamide PEA-X was prepared by condensation from the enantiomerically pure D-(−)-α-phenylglycinol, which was prepared from D-(+)-α-phenylglycine by a reported method [10].

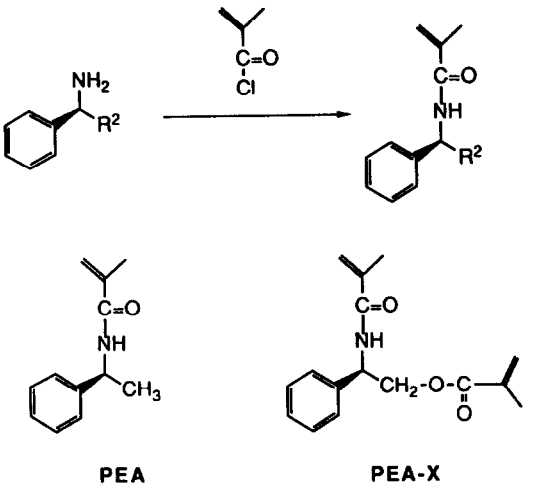

Fig. 3. Structures of methacrylamide monomers prepared for chiral selectors.

Preparation of polymer particles

Polystyrene seed particles 1 μm in diameter was prepared by emulsifier-free emulsion polymerization and purified by a reported method [11].

Uniform-sized base particles were prepared by a typical two-step swelling method as follows. In the first step, 3 ml of an aqueous dispersion of purified polystyrene seed particles ($3.3 \cdot 10^{-2}$ ml/ml of polystyrene particles) was admixed with an emulsion of dibutyl phthalate (activating solvent), which was prepared from 0.18 ml of dibutyl phthalate in which was dissolved 0.05 g of BPO (radical initiator) and 8 ml of water containing 0.02 g of sodium dodecyl sulphate (SDS) by sonication. This swelling step was completed in 3 h at room temperature with stirring at 125 rpm. To this dispersion of swollen particles was added an aqueous dispersion of monomers and porogenic solvent which was prepared from 1.4 ml of methyl methacrylate, 2.1 ml of ethylene dimethacrylate, 3.5 ml of cyclohexanol as porogenic solvent and 14 ml of

water in which was dissolved 0.29 g of poly(vinyl alcohol) (Nacalai Tesque, $d_p = 500$, saponification value = 88) by sonication. This second swelling step was carried out for 10 h at room temperature with slow stirring.

For the polymerization of the swollen base particles, the aqueous dispersion was stirred at 70°C for 24 h under a nitrogen atmosphere with 10 mg of sodium nitrite as a water-soluble radical inhibitor. The polymer particles obtained were washed with methanol, tetrahydrofuran (THF) and acetone to remove the porogenic solvent and other impurities [12].

For the preparation of polymer-based chiral stationary phases, the following preparation procedures were examined.

(1) A 0.35-g amount of PEA was dissolved in the mixture of methylmethacrylate, ethylene dimethacrylate and cyclohexanol in the second step swelling for the preparation of base particles. The particles obtained were washed with methanol and THF (PEA-MIX).

(2) After 1 h from the start of polymerization of the base particles, a one-tenth portion of 0.35 g of PEA was added directly to the polymerization medium of the base particles every 30 min. The total amount of the PEA added was the same as that in PEA-MIX. The particles obtained were washed with methanol and THF (PEA-ADD).

(3) The same method for PEA-ADD was employed using the cross-linking monomer PEA-X (Fig. 3) instead of PEA. The particles obtained were washed with methanol and THF (PEA-X-ADD).

The yields, which were calculated based on the amounts of the monomers utilized, were between 80% and 98%.

Chromatography

All the stationary phases were packed into stainless-steel columns (150 mm × 4.6 mm I.D.) by a slurry technique. HPLC was performed with a Jasco Model 880-PU intelligent HPLC pump equipped with a Rheodyne Model 7125 valve loop injector and a Waters Model 440 UV detector set at 254 nm. Chromatography was carried out at 30 ± 1°C and a Shimadzu C-R4A integrator was utilized.

All the chromatographic solvents were purchased from Nacalai Tesque and used as received. 2,2′-Dihydroxy-1,1′-binaphthyl was gift from Nacalai Tesque.

RESULTS AND DISCUSSION

The amount of methacrylamide incorporated in the base polymer particles can be calculated based on nitrogen elemental analysis. The relationship between the theoretical content of PEA and the experimentally obtained content of PEA is depicted in Fig. 4. The experimentally obtained content was found to agree well with the theoretical content of PEA up to 20% (w/w).

As a separate experiment on the thermal polymerization of PEA dispersed in aqueous media without any radical initiator resulted in only a negligible yield of PEA polymer at the polymerization temperature, PEA should be initiated for polymerization by radical species derived from polymerizing base particles. In addition, as the added water-soluble radical inhibitor, sodium nitrite, prohibited chain transfer and migration of radial species from the base particles to the PEA monomer through the water medium, the polymerization of PEA took place on and/or in the polymerizing base particles after adsorption or migration of PEA to the base particles.

Fig. 5a and b show scanning electron micrographs of the base particles and PEA-ADD, respectively. The good size uniformity of the

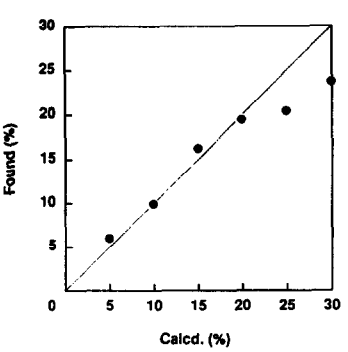

Fig. 4. Relationship between added amount of PEA and amount of combined PEA on base particles. The line is the line for $y = x$.

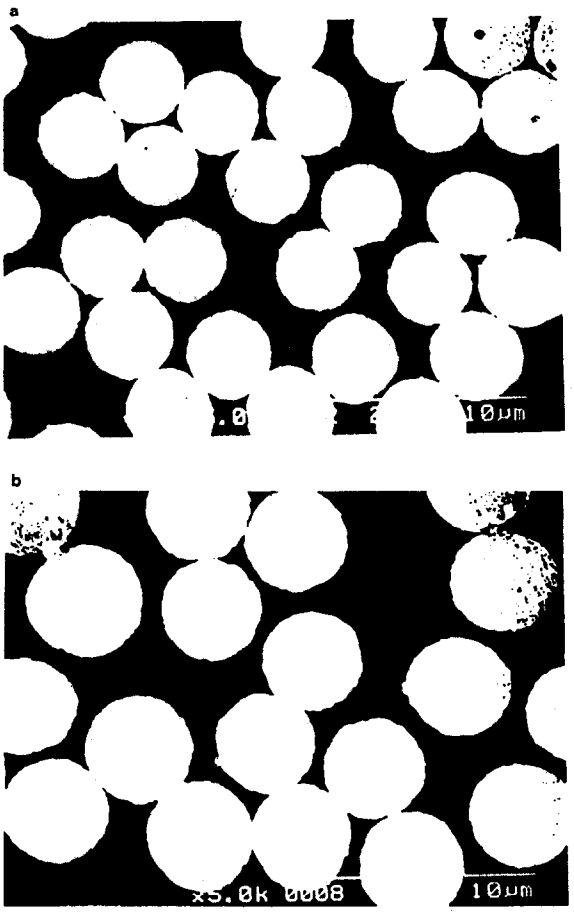

Fig. 5. Scanning electron micrographs of (a) base particles and (b) PEA-ADD.

base particles was not corrupted by the addition to PEA. Moreover, the outward appearances of both particles were relatively similar to each other. These observations strongly suggest that the added PEA did not form any new homopolymer particles (new generation), but was adsorbed and incorporated on the surface of the uniform-sized base particles.

The chromatographic properties of the prepared particles are summarized in Table I. The pore volume of PEA-MIX was not changed from that of the base particles, whereas that of PEA-ADD was decreased. As the contents of incorporated PEA units in both PEA-MIX and PEA-ADD were almost identical, this decrease in the pore volume of PEA-ADD suggested that the added PEA was incorporated on not only the external surface but also the internal surface of the base particles, whereas in PEA-ADD the PEA was thought to be incorporated as a unit of copolymer.

The $\alpha(CH_2)$ values, describing the hydrophobicity of the particles in the reversed-phase mode, were decreased by the addition of PEA, which was an effect of the relatively hydrophilic PEA on the base particles. PEA-MIX was found to be more hydrophilic than PEA-ADD, which indicated that PEA was incorporated more effectively, and the steric selectivity towards planar aromatic compounds described by α(T/O) and α(P/TP) was higher with PEA-ADD than with PEA-MIX, for which the values were similar to

TABLE I

CHROMATOGRAPHIC PROPERTIES OF THE PREPARED PARTICLES

Particle	Pore volume[a] (ml)	$\alpha(CH_2)$[b]	α(T/O)[c]	α(A/TP)[d]
Base	0.95	1.32	1.21	1.07
PEA-MIX	0.94	1.22	1.25	1.00
PEA-ADD	0.79	1.25	1.39	1.27

[a] Determined by size-exclusion chromatography in tetrahydrofuran.
[b] k'(amylbenzene)/k'(butylbenzene) in acetonitrile–water (60:40).
[c] k'(triphenylene)/k'(*o*-terphenyl) in acetonitrile–water (60:40).
[d] k'(anthracene)/k'(triptycene) in acetonitrile–water (60:40).

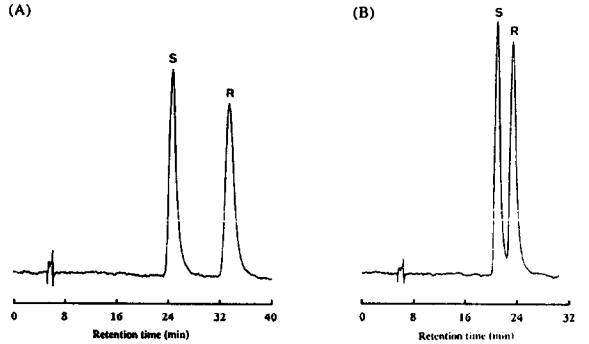

Fig. 6. Chiral separation of 2,2′-dihydroxy-1,1′-binaphthyl with (A) PEA-ADD and (B) PEA-MIX. Chromatographic conditions: column, 300 mm × 4.6 mm I.D.; mobile phase, hexane–ethyl acetate (1:1, v/v); flow-rate, 1 ml/min; detection, UV at 254 nm.

those with the base particles. These findings imply that the form of incorporated PEA in the base particles was different between PEA-MIX and PEA-ADD.

Chiral separations of 2,2′-dihydroxy-1,1′-binaphthyl (DHB) examined with PEA-MIX and PEA-ADD are shown in Fig. 6. The chiral resolutions of DHB showed significant differences. With PEA-ADD, baseline separation could be achieved with a longer retention time in the normal phase mode, whereas the resolution was much worse with PEA-MIX, which indicates that the added PEA worked more effectively as a chiral selector with PEA-ADD than with PEA-MIX.

Table II shows chiral separations using PEA-ADD and PEA-X-ADD, which was prepared utilizing the chiral cross-linking agent as indicated in Fig. 3. If only chirality derived from phenylethylamine influences the chiral recognition, this PEA-X should be as effective as PEA and, moreover, as on polymer-based packing materials micropores usually tend to determine the separation selectivity toward small molecules [13], the cross-linking chiral selector has been thought to be more effective; however, PEA-X-ADD gave a low chiral recognition in spite of the quantitative introduction of PEA-X into the base particles. This result also demonstrates that the chiral form constructed by the addition of monofunctional chiral monomer PEA is very important in this polymer-based chiral stationary phase, which agrees with the chiral recognition of polymeric chiral selectors reported by Blaschke [4,5].

TABLE II

OPTICAL RESOLUTION WITH PCSP MATERIALS

PCSP	Amide (%)[a]	k'	α	R_s
PEA-ADD	12.5	2.70 (*R*)		
			1.29	1.53
		2.10 (*S*)		
PEA-X-ADD	9.8	1.39 (*R*)		
			1.01	0.03
		1.37 (*S*)		

[a] Combined methacrylamide calculated based on nitrogen elemental analysis. Column, 150 mm × 4.6 mm I.D.; mobile phase, hexane–ethyl acetate (1:1); flow-rate, 1 ml/min.

CONCLUSIONS

A uniform-sized polymer-based chiral stationary phase having polymethacrylamide as a chiral selector can be prepared by a very simple one-pot procedure. All the preparation conditions have not yet been optimized and the detailed separation mechanism has not been clarified, but this method should be very effective for preparing chemically stable polymer-based chiral stationary phases for HPLC with a polymeric chiral selector. Optimization of the base particles, chiral monomer, chromatographic conditions and chromatographic applications is in progress.

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