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Short communication

Molecularly imprinted monolithic stationary phases for liquid chromatographic separation of enantiomers and diastereomers

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Abstract

The method for preparation of molecularly imprinted monolithic stationary phase has been improved to achieve liquid chromatographic separation of enantiomers and diastereomers. By adopting low polar porogenic solvents of toluene and dodecanol and optimal polymerization conditions, the molecularly imprinted monolithic stationary phases with good flow-through properties and high resolution were prepared. Enantiomers of amino acid derivatives and diastereomers of cinchona alkaloids were completely resolved using the monolithic stationary phases. The influence of porogenic composition, monomer–template ratio and polymerization conditions on the chromatographic performance was investigated. Some chromatographic conditions such as the composition of the mobile phase and the temperature were characterized. Scanning electron microscopy showed that the molecularly imprinted monolithic stationary phase has a large through-pore structure to allow the mobile phase to flow through the column at very low backpressure. Accelerated separations of enantiomers and diastereomers were therefore achieved at elevated flow rates. Finally, the chiral recognition performance of the prepared stationary phase in aqueous media was investigated. Hydrophobic interaction, and ionic and/or hydrogen bonding interactions were proposed to be responsible for the recognition mechanism.

Keywords: Molecular imprinting; Stationary phases, monolithic, LC; Enantiomer separation; Diastereomer separation

1. Introduction

Molecular imprinting, a technique for the creation of artificial receptor-like binding sites with a ''memory'' for the shape and functional group positions of the template molecule, has become increasingly attractive in many fields of analytical chemistry in recent years, particularly as an affinity material for sensors, binding assays, solid-phase extraction and chromatographic stationary phases [1–3]. Molecularly imprinted polymers (MIPs) as HPLC and CEC stationary phases have been applied to enantio-separation of racemic mixtures due to their unique predetermined selectivity [4,5]. Traditionally, the molecularly imprinted stationary phases have been prepared by bulk polymerization. However, the resulting polymer blocks must be crushed and ground into particles, which in turn have to be sieved to desired size ranges (<25 μm) for practical use. Although the process of bulk polymerization is

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simple, the rest of the preparation steps are tedious, time-consuming and not cost-efficient, as only a portion of the resultant polymer can be recovered as useful packing material, resulting in high consumption of template molecules. In addition, the resulting particles are polydisperse both in shape and size, which also has a negative impact on the chromatographic performance. In another approach, regular molecularly imprinted microspheres have been prepared by suspension polymerization and multi-step swelling polymerization methods [6–9]. However, fairly complicated procedures and reaction conditions are required, and the aqueous suspensions used in this technique could interfere with the imprinting and thus lead to a decrease in selectivity. Recently, precipitation polymerization has been employed for the production of molecularly imprinted microspheres by Ye et al. [10,11]. Despite the higher yields, a large amount of template molecules is needed for the preparation process, because of the high dilution factor. In order to simplify the preparation procedure, Matsui et al. [12] employed the in-situ polymerization technique to prepare molecularly imprinted monolithic polymer rods in 1993. This type of MIPs exhibited recognition ability for some imprint molecules such as theophylline, nicotine, diaminonaphthalene, cinchona alkaloid and enantiomers of phenylalanine anilide [12–16]. Subsequently, the same approach was applied to prepare the molecularly imprinted stationary phases for the separation of racemic mixtures in CEC [17–20]. Using this technique, MIPs can be synthesized directly inside stainless steel columns or capillary columns without the tedious procedures of grinding, sieving and column packing. Furthermore, the preparation of this type of MIPs is more cost-efficient, as the required amount of template molecules is much lower. However, the prepared MIPs often suffer from high backpressures and low efficiencies [4,15], which limit their application in practical separations. Several methods were used, such as increasing the amount of cyclohexanol and adding the latex beads in polymerization mixture, to increase the permeability of rods, but the results were unsatisfactory [15].

In this work, we present an improved method for the preparation of molecularly imprinted monolithic stationary phases with both good flow-through properties and high resolution for liquid chromatographic separation of chiral compounds. Selected enantiomers of amino acid derivatives and diastereomers of cinchona alkaloids are completely resolved on the monolithic stationary phases.

2. Experimental

2.1. Materials

N-(Carbobenzyloxy)-L-tryptophan (Cbz-L-Trp) and *N*-(carbobenzyloxy)-DL-tryptophan (Cbz-DL-Trp) were obtained from Sigma (St Louis, MO, USA). Fmoc-L-tryptophan (Fmoc-L-Trp) and Fmoc-D-tryptophan (Fmoc-D-Trp) were obtained from Fluka (Buchs, Switzerland). Cinchonine (CN), cinchonidine (CD), quinidine and quinine were purchased from Acros (Geel, Belgium). Their structures are shown in Fig. 1. 4-Vinylpyridine (4-VP) and methacrylic acid (MAA) from Acros were distilled under vacuum. Ethylene dimethacrylate (EDMA) from Sigma was extracted with 10% aqueous sodium hydroxide and water, and dried over anhydrous magnesium sulfate. 2,2′-azo-bis(isobutyronitrile) (AIBN) and toluene were dried prior to use. All other chemicals and solvents were of analytical or HPLC grade.

2.2. Preparation of molecularly imprinted monolithic stationary phases

The stationary phase was directly prepared by in-situ polymerization within the confines of a stainless-steel chromatographic column tube of 150× 4 mm I.D. The template molecule, free-radical initiator (AIBN), monomer and cross-linker (EDMA) were dissolved in porogenic solvents (toluene and dodecanol), in the compositions as indicated in Table 1. The solution was sonicated for 5 min and deoxygenated with a stream of nitrogen gas for 5 min. The stainless-steel tube sealed at the bottom was filled with the above polymerization mixture and then sealed at the top. The polymerization was allowed to proceed at 45 °C for 12 h. The seals were removed, the column was provided with fittings, and connected to an HPLC pump and washed exhaustively with methanol/acetic acid (4:1 v/v) to remove the

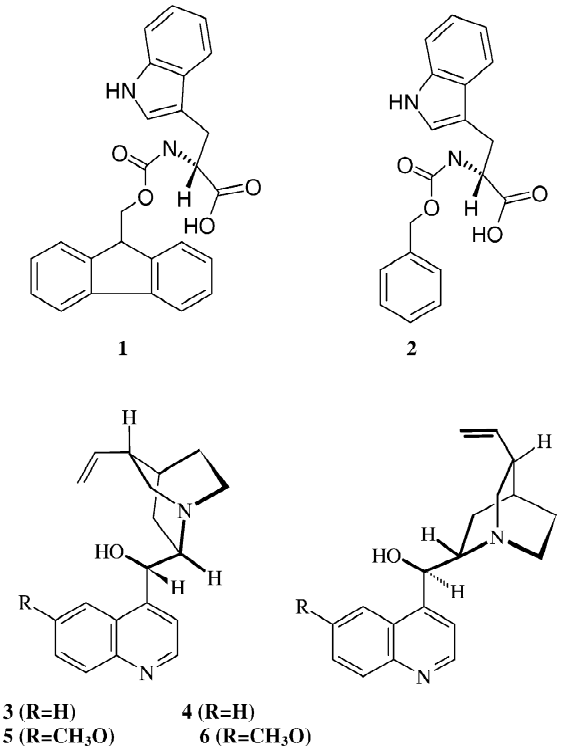


Fig. 1. Structures of template molecules: *N*-(carbobenzyloxy)-L-tryptophan (Cbz-L-Trp, **1**), Fmoc-L-tryptophan (Fmoc-L-Trp, **2**), cinchonine (CN, **3**), cinchonidine (CD, **4**), quinidine (**5**) and quinine (**6**).

porogenic solvents and the template molecules. A non-imprinted blank monolithic column (in the absence of template) was prepared and treated in an identical manner.

2.3. Characterization of monolithic molecular imprinting stationary phases

After the chromatographic experiments had been completed, the column was washed with methanol/acetic acid (4:1 v/v) for 1 h. The bottom column fitting was removed and the monolith inside the column was pushed out of the tube using the pressure of the methanol mobile phase at a flow-rate of 5 ml/min. The cylindrical monolith was dried under vacuum at 40 °C for 24 h and cut into pieces with a razor blade. The pore properties were determined by mercury intrusion porosimetry and its specific surface area was calculated from nitrogen adsorption/desorption isotherms using a combined BET sorptometer and mercury porosimeter (9310 Mercury Porosimeter, USA). Microscopic analysis of the monolith was carried out in an Amary Scanning Electron Microscope (Model 1000B, USA) at 20 keV.

2.4. High-performance liquid chromatography

A Shimadzu LC-10A HPLC system (Shimadzu, Kyoto, Japan) consisting of two LC-10ATvp HPLC pumps and an SPD-10Avp UV–Vis detector was used for all the chromatographic experiments. The data were acquired and processed with a WDL-95 chromatographic workstation (National Chromatographic R&A Center, Dalian, China). An AT-130 temperature controller (Autoscience, Tianjin, China) was used to control the column temperature. The column was washed with mobile phase until a stable baseline was obtained before injection. Acetone was injected as a void marker under corresponding mobile phase. All separations were carried out at

Table 1
The optimized mixture composition for preparation of molecularly imprinted monolithic stationary phases

Stationary phase	Template molecule	Amount of template molecule (mmol)	Monomer	Amount of monomer (mmol)	Amount of EDMA (mmol)	Porogen mixture (toluene/dodecanol, (mmol)	Amount of AIBN (mg)
M1	**1**	0.278	4-VP	0.835	4.19	1.49:6.51	8.75
M2	**2**	0.417	4-VP	0.835	4.19	1.49:6.51	8.75
M3	**3**	0.265	MAA	1.06	4.19	2.71:5.98	8.75
M4	**4**	0.265	MAA	1.06	4.19	2.71:5.98	8.75
M5	**5**	0.265	MAA	1.06	4.19	2.71:5.98	8.75
M6	**6**	0.265	MAA	1.06	4.19	2.71:5.98	8.75

ambient temperature, except for the studies of the temperature effect on the separation. UV detection was carried out at 280 nm.

The retention times were determined by injection of 10 μg of racemates or diastereomer mixtures (dissolved in 4 μl of the eluent). The retention factors, k', were calculated using the equation $k' = (t_R - t_o)/t_o$, where t_R is the retention time of an analyte and t_o is the elution time of the void marker. The separation factor (α) was defined as the ratio of the retention factors of enantiomers or diastereomers and used for the evaluation of the selectivity. The resolution (R_s) was calculated according to the method proposed by Wulff et al. [21]. The number of theoretical plates (N) was calculated by the equation $N = 16(t_R/w)^2$, where w is the baseline peak width of eluted enantiomers.

3. Results and discussion

3.1. Preparation conditions affecting the performance of molecularly imprinted monolithic stationary phases

The preparation process of the molecularly imprinted monolithic stationary phases is quite simple. However, a number of factors have to be taken into account. Among these factors, the selection of the porogenic solvents is crucial for the preparation of the molecularly imprinted monolithic stationary phases. At least three requirements are imposed on the selection of the right mixture: (1) Template molecule, initiator, monomer and cross-linker have to be soluble in the porogenic solvents. (2) The porogen should produce large pores, in order to assure good flow-through properties of the resulting polymer. (3) The porogenic solvents should be of relatively low polarity, in order to reduce the interferences during complex formation between the imprint molecule and the monomer, as the latter is very important to obtain high selectivity MIPs. Several porogenic solvents, i.e. cyclohexanol and dodecanol, toluene and dimethyl sulfoxide, toluene and dodecanol, toluene and isooctane, which have frequently been used in the preparation of monolithic stationary phases, were tested for their compatibility. These investigations revealed that the molecularly imprinted monolithic stationary phases using cyclohexanol and dodecanol or toluene and dimethyl sulfoxide as porogenic solvents showed no selectivity for the template molecule **1**, possibly due to the relatively high polarity of these solvent mixtures. Although the apolar porogenic mixture of toluene and isooctane was ideal for imprinting, the template molecule **1** could not be dissolved in it. Finally, only the low polar porogenic solvents of toluene and dodecanol could fulfil all three requirements. Molecularly imprinted monolithic stationary phases with high selectivity and low backpressure could be obtained using this porogenic mixture.

As shown in Table 2, the ratio of toluene and dodecanol also affects the separation performance through the change in pore structure of the monolithic stationary phases. With increasing proportion of the good solvent toluene, the mean pore size decreased and the specific area and resolution factor increased. However, when the toluene reached 15% in the porogenic mixture, the resulting stationary phase had too small a pore diameter to allow the mobile phase to flow through. Thus, a balance had to be found between the requirements of low flow resistance and large surface area. Finally, 10% of toluene was found to be optimal, as a sufficient solubility of the template molecule **1**, a high specific surface area (111 m^2/g) and low backpressure of the column (0.78 MPa at 1.0 ml/min) were simultaneously obtained. However, the optimum ratio may be different with regard to other template molecules, e.g. the appropriate percent of toluene was just 18% with regard to the template molecules of cinchona alkaloids. Therefore, the ratio of toluene and dodecanol has to be adjusted for different template molecules.

The influence of the template–monomer ratio on the selectivity has been well-documented [22,23]. Only a proper template–monomer ratio can afford high selectivity. As seen in Table 2, a ratio of 1:3 was most appropriate for the template molecule **1**, as higher or lower ratios provided lower selectivity. However, analogous to the optimization of the porogen mixture, the template–monomer ratio may also be different with other template molecules, e.g. the appropriate ratio was 1:4 with regard to the template molecules of cinchona alkaloids and 1:2 for molecule **2** (Table 1).

Table 2
Effect of polymerization conditions on separation performance

Polymer[a]	Template–monomer ratio[b]	Crosslinker content in monomer mixture[c] (v%)	Toluene content in porogen[d] (v%)	α[e]	R_s[e]	N_D[f]	N_L[f]	S[g] (m^2/g)	d_p[h] (nm)
P1	1:3	90	0	1.00	0.00	–	–	37.1	3213
P2	1:3	90	5	1.66	0.70	–	–	45.0	1680
P3	1:3	90	10	2.36	1.67	347	98	111.0	1081
P4	1:3	90	15	[i]	[i]				
P5	1:1	90	10	1.53[j]	0.21	–	–		
P6	1:2	90	10	1.64[j]	0.90	149	100		
P7	1:4	90	10	1.80	0.71	–	–		
P8	1:6	90	10	1.78	0.32	–	–		
P9	1:3	80	10	1.70[k]	0.91	137	79		
P10	1:3	75	10	1.31[k]	0.41	–	–		
P11	1:3	70	10	1.00[j]	0.00	–	–		

[a] The molecule **1** is used as the template molecule to prepare the polymers.
[b] The template–monomer ratio is mol ratio.
[c] The monomer mixture is composed of monomer and cross-linker.
[d] Porogen is composed of toluene and dodecanol.
[e] Mobile phase is acetonitrile containing 0.1% acetic acid (v%) at a flow-rate of 0.5 ml/min.
[f] The number of theoretical plates of enantiomers.
[g] Specific area.
[h] Average pore diameter.
[i] The polymer is too rigid to allow mobile phase to flow through.
[j] The mobile phase of acetonitrile containing 0.3% acetic acid (v%) was used because the retention of enantiomers is too long in the mobile phase of acetonitrile containing 0.1% acetic acid at a flow-rate of 0.5 ml/min.
[k] The mobile phase of acetonitrile containing 0.5% acetic acid (v%) was used because the retention of enantiomers is too long in the mobile phase of acetonitrile containing 0.1% acetic acid at a flow-rate of 0.5 ml/min.

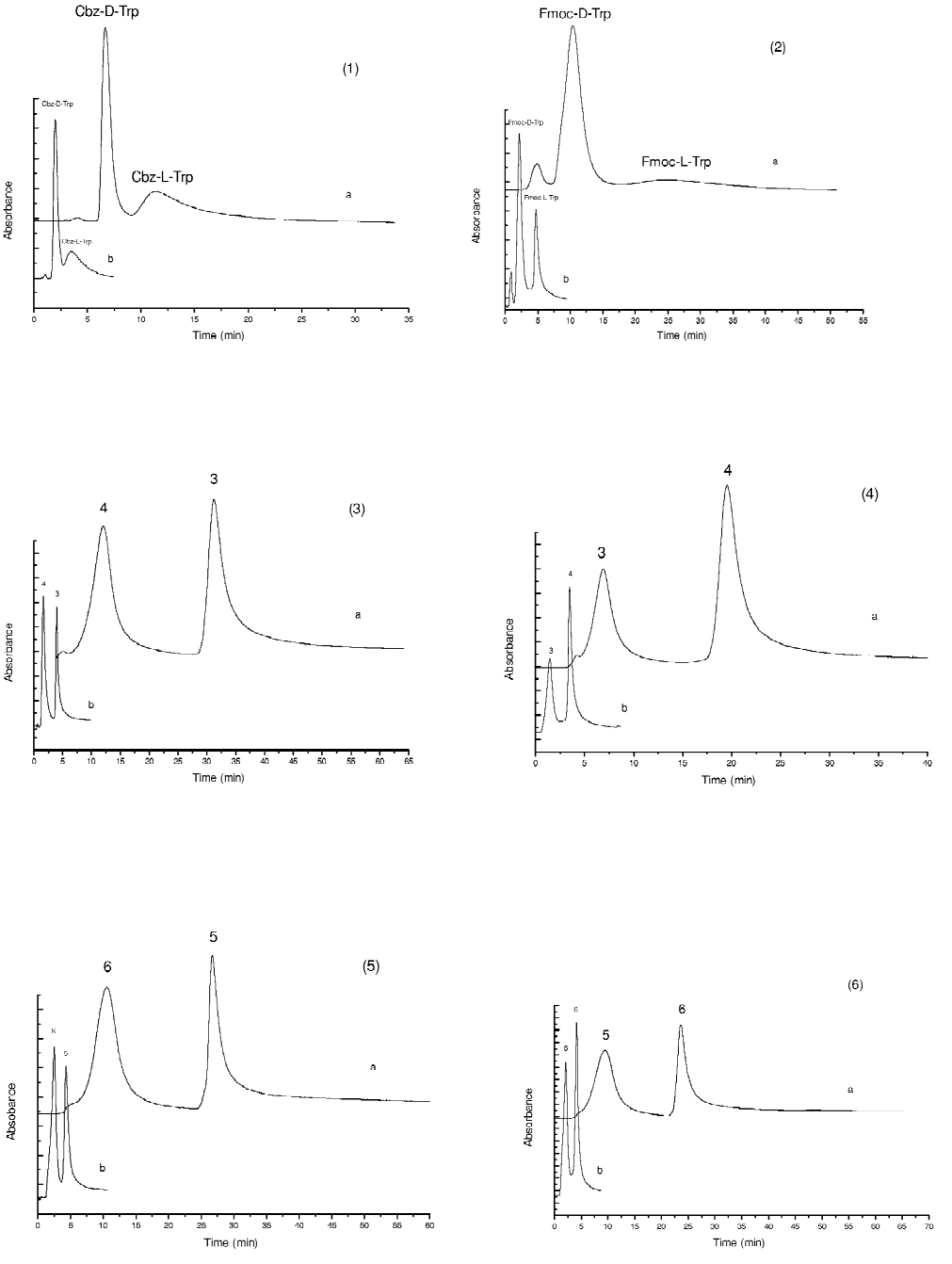
It has been reported previously that the amount of cross-linker should be high enough to maintain the stability of the recognition sites [1]. As shown in Table 2, the percentage of crosslinking agent should be even higher than 80%. When the percentage was lower than 70%, the MIPs showed no recognition ability. The best selectivity was provided at a crosslinking percentage of 90%.

Finally, although the polymerization conditions, such as polymerization temperature and polymerization time affect the efficiency and selectivity of the resultant polymeric stationary phases, it was observed that the polymerization could be finished in 2 h at 60 °C. However, at this temperature the initiation of the polymerization reaction was very fast and therefore hard to control. This resulted in low reproducibility of molecularly imprinted monolithic stationary phases. Furthermore, the relatively high temperature had a negative impact on the complex stability between the imprint molecule and the functional monomer. Thus, the relatively low temperature of 45 °C was selected, in order to yield a more reproducible polymerization, and the reaction time prolonged to 12 h. This was because the polymerization was not complete in less than 10 h, and on the other hand, no difference in the chromatographic performance could be determined, when the polymers were prepared during 12–18 h.

3.2. Chromatographic separation of enantiomers and diastereomers

Six types of molecularly imprinted monolithic stationary phases have been successfully prepared for the separation of enantiomers and diastereomers, and the chromatograms obtained are shown in Fig. 2. The elution of the imprint molecule, as expected, was more retarded than that of its optical antipode. Isocratic elution was used for the separation of enantiomers of the amino acid derivatives, but gradient elution was used to shorten the elution rate as well as improve the peak shape of the retained

diastereomers of cinchona alkaloids. In isocratic elution, these diastereomers exhibited excessively long retention times and peak tailing. Furthermore, no discrimination between enantiomers or diastereomers was observed on the non-imprinted blank column, which proved that the chiral recognition



sites in the molecularly imprinted monolithic stationary phases originated from the molecular imprinting process. Although successful separation of enantiomers and diastereomers had been achieved on the molecularly imprinted monolithic stationary phases, regrettably the efficiency was still not improved compared to the MIPs prepared by bulk polymerization (Table 2).

The recognition of analytes in organic mobile phase is most likely due to hydrogen bonding or ionic interactions between the nitrogen atom of 4-VP or the carboxy group of MAA in the polymer and the carboxy functionalities of the amino acid derivatives or the nitrogen atom of the cinchona alkaloids. A proton donor present in the mobile phase should therefore influence the recognition and separation. As expected, the retention factor and the separation factor decreased with increasing acetic acid concentration in the mobile phase. The best resolution was achieved at 0.1% acetic acid concentration. This condition may not be appropriate for other molecularly imprinted monolithic stationary phases, and thus, the mobile phase composition should be optimized in each case.

The effect of temperature on the separation has been investigated in the range of 30–60 °C. With increasing temperature, the retention factors of the enantiomers decreased, whereas the separation factor and resolution increased. This result was in agreement with previous studies carried out by Sellergren et al. [24,25], which was explained by an endothermic process during solute desolvation on formation of electrostatic interaction between the site and the solute in an acetic acid-containing mobile phase.

3.3. Accelerated separation of enantiomers and diastereomers

The SEM images and the pore size distribution profile of the molecularly imprinted monolithic stationary phase prepared in the presence of the template molecule **1** under optimized conditions are shown in Fig. 3, and its pore parameters are listed in Table 2 (P3). This clearly reveals that large throughpores are present in this type of stationary phase and should allow the mobile phases to flow though with low flow resistance at high flow rates [26,27].

A flow-rate of 2.0 ml/min applied on the column with molecularly imprinted monolithic stationary phase led to a back pressure of only 1.76 MPa, and a linear relationship between backpressure and flow-rate in the range of 0–2.0 ml/min could be maintained. This confirmed that the structure of the monolithic MIP was not compressed under the high flow-rate. Although a maximum separation factor of 2.62 was obtained at a flow-rate of 1.0 ml/min, very little variation in those factors was observed in the flow-rate range between 0.25 and 2 ml/min. This indicated that good separations could be achieved at even higher flow-rates.

Fig. 2. Chromatographic resolution of enantiomers of amino acid derivatives and diastereomers of cinchona alkaloids and chromatograms of accelerated separation. (1): a, Isocratic elution was carried out on the M1 stationary phase at 0.5 ml/min with acetonitrile–acetic acid (99.9:0.1, v/v) mobile phase; b, Isocratic elution was carried out on the M1 stationary phase at 2.0 ml/min with acetonitrile–acetic acid (99.9:0.1, v/v) mobile phase. (2): a, Isocratic elution was carried out on the M2 stationary phase at 0.5 ml/min with acetonitrile–acetic acid (99.9:0.1, v/v) mobile phase; b, Stepwise gradient elution was carried out on the M2 stationary phase at 3.0 ml/min: 0–3 min, acetonitrile–acetic acid (99.9:0.1, v/v); 3–10 min, acetonitrile–acetic acid (90:10, v/v). (3): a, Stepwise gradient elution was carried out on the M3 stationary phase at 0.5 ml/min: 0–20 min, acetonitrile–acetic acid (97:3, v/v); 20–60 min, acetonitrile–acetic acid (90:10, v/v); b, Stepwise gradient elution was carried out on the M3 stationary phase at 2.5 ml/min: 0–2 min, acetonitrile–acetic acid (97:3, v/v); 2–10 min, acetonitrile–acetic acid (90:10, v/v). (4): a, Stepwise gradient elution was carried out on the M4 stationary phase at 0.5 ml/min: 0–10 min, acetonitrile–acetic acid (97:3, v/v); 10–45 min, acetonitrile–acetic acid (90:10, v/v); b, Stepwise gradient elution was carried out on the M4 stationary phase at 2.5 ml/min: 0–1.5 min, acetonitrile–acetic acid (97:3, v/v); 1.5–10 min, acetonitrile–acetic acid (90:10, v/v). (5): a, Stepwise gradient elution was carried out on the M5 stationary phase at 0.5 ml/min: 0–17 min, acetonitrile–acetic acid (98:2, v/v); 17–60 min, acetonitrile–acetic acid (90:10, v/v); b, Stepwise gradient elution was carried out on the M5 stationary phase at 1.5 ml/min: 0–0.7 min, acetonitrile–acetic acid (97:3, v/v); 0.7–10 min, acetonitrile–acetic acid (90:10, v/v). (6): a, Stepwise gradient elution was carried out on the M6 stationary phase at 0.5 ml/min: 0–14 min, acetonitrile–acetic acid (98:2, v/v); 14–60 min, acetonitrile–acetic acid (90:10, v/v); b, Stepwise gradient elution was carried out on the M6 stationary phase at 1.5 ml/min: 0–1 min, acetonitrile–acetic acid (97:3, v/v); 1–10 min, acetonitrile–acetic acid (90:10, v/v).

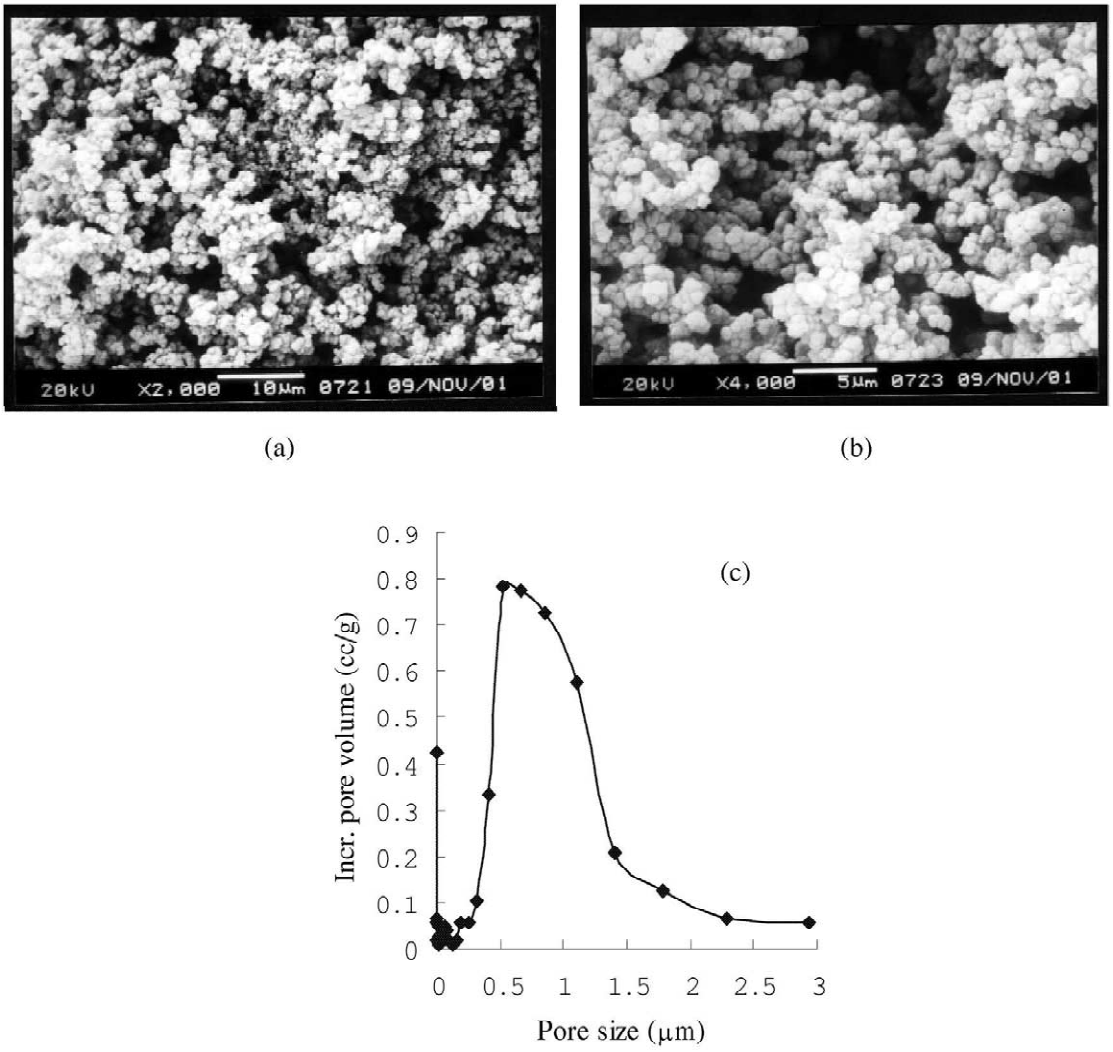


Fig. 3. The SEM images and pore size distribution profile of the chiral monolithic stationary phase (M1). (a) The SEM image magnified 2000-fold; (b) SEM image magnified 4000-fold; (c) pore size distribution profile.

Accelerated separations of enantiomers and diastereomers on the molecularly imprinted monolithic stationary phases have been carried out at the elevated flow-rate, and the chromatograms obtained are shown in Fig. 2. Almost all the separations could be carried out within 6 min.

3.4. Chiral recognition in aqueous media

Although up to now most separations based on molecularly imprinted stationary phases have been carried out in organic solution, the study on chiral recognition in aqueous media may be more important as it would reflect the natural recognition events more closely. The effects of pH and buffer content in the mobile phase on separation of enantiomers were studied using the molecularly imprinted monolithic stationary phase.

At a fixed pH of 4.0, it had been observed that an increasing buffer content in the mobile phase led to an increase in the retention factors of both enantiomers, ranging from 20 to 100%. Furthermore, the

selectivity also increased with increasing buffer content, when the buffer content was at least 40%. On the other hand, buffer contents over 80% resulted in exceedingly long retention times. The best separation was obtained at 60% buffer content. The influence of the pH value on the retention factor and the separation factor at a fixed buffer content of 50% is shown in Fig. 4. With increasing pH, the retention factors of the enantiomers as well as the separation factor and the resolution factor decreased, and when the pH value was higher than 5.0 the selectivity disappeared. A parallel experiment was carried out on the non-imprinted blank column, however no selectivity was observed at any pH. These results indicated that the enantioselective recognition of the prepared stationary phase in aqueous solution was mainly due to hydrophobic interactions in conjunction with ionic and/or hydrogen bonding interactions [28,9]. As described previously, in organic solution, the hydrogen bonding and ionic interaction between analytes and the polymer is dominant for the chiral recognition process, but some weaker interactions such as dipolar and van der Waals interactions may also come into play. However, when polar aqueous media are applied, many of the polarity-dependent interaction types become weak, leaving ionic bonds and/or hydrogen bonding as the main interaction

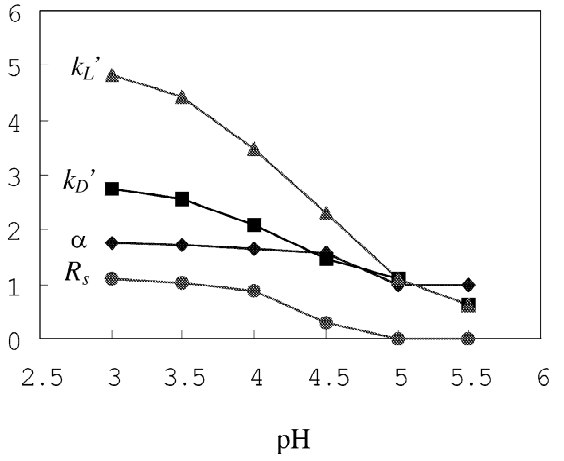


Fig. 4. Effect of pH of aqueous mobile phase on retention factors (▲, k_L'; ■, k_D'), separation factor (♦) and resolution (●) of enantiomers. Isocratic elution was carried out for resolution of Cbz-DL-Trp on the M1 stationary phase at 0.5 ml/min by keeping acetonitrile/10 m*M* acetate buffer at 50:50 (v/v).

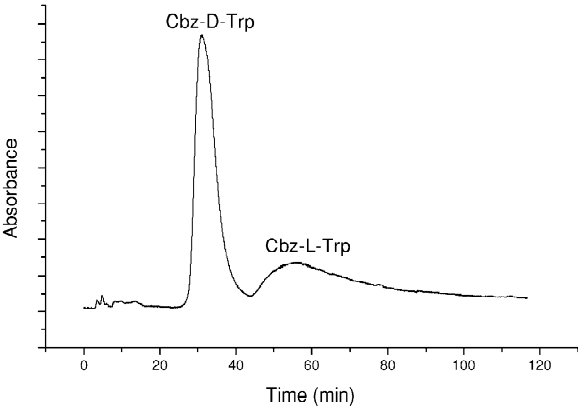


Fig. 5. Chromatographic resolution of Cbz-DL-Trp in aqueous media. Isocratic elution was carried out on the M1 stationary phase at 0.5 ml/min with mobile phase of acetonitrile/10 m*M* acetate buffer (pH 3.5) (40:60, v/v).

mechanism. On the other hand, with increasing aqueous content hydrophobic interactions between analyte and MIP rod become more noticeable, resulting in long retention times as observed at buffer contents above 80%. At a moderate buffer content, pH could be used as a tool for adjusting the selectivity through changing the strength of ionic and/or hydrogen bonding interactions [29]. By the way, an optimized separation of Cbz-DL-Trp was obtained at 60% buffer content and pH 3.5 as shown in Fig. 5.

4. Conclusion

Molecularly imprinted monolithic stationary phases have been prepared and successfully used for liquid chromatographic separation of enantiomers and diastereomers. It integrates the high enantioselectivity of molecularly imprinted polymer and the merits of the monolithic stationary phase. Compared to other types of MIPs, its preparation is quite simple and cost-effective. Good performance of the stationary phase can be achieved by selection of the appropriate porogenic solvents and careful optimization of the polymerization conditions. Enantiomers of amino acid derivatives and diastereomers of cinchona alkaloids have been completely separated on the molecularly imprinted monolithic stationary phase prepared in this way. Some chromatographic

conditions such as the composition of the mobile phase and the temperature were also optimized. Furthermore, the molecularly imprinted monolithic stationary phases possess large through-pores and therefore exhibit very low backpressure. Thus accelerated separation of enantiomers and diastereomers can be achieved at elevated flow-rates. This result is particularly attractive, as long separation times usually represented a serious drawback for separations of chiral compounds on molecularly imprinted stationary phases. Finally, enantio-separation in aqueous media has also been achieved. Hydrophobic interactions in conjunction with ionic and/or hydrogen bonding interactions were determined to be responsible for the chiral recognition in aqueous media.

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