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Research Article

(*S*)-Ibuprofen-imprinted polymers incorporating γ-methacryloxypropyltrimethoxysilane for CEC separation of ibuprofen enantiomers

In this report, a novel preparation method of molecularly imprinted polymers (MIPs) for CEC was developed. Molecularly imprinted monolithic columns for (*S*)-ibuprofen were prepared and evaluated, in which charged entities responsible for establishing EOF have been derived from γ-methacryloxypropyltrimethoxysilane (γ-MAPS), which was hydrolyzed following copolymerization with 4-vinylpyridine (4-VPY) and ethylene glycol dimethacrylate (EDMA). The EOF and molecular recognition of the stationary phase were investigated in aqueous and nonaqueous media, respectively. The experimental results indicated that the material showed a reasonably stable EOF and the prepared separation materials were capable of separating racemic ibuprofen, a task that could not be accomplished by MIPs prepared in parallel, using methacrylic acid (MAA) as a functional monomer. The efficiency at pH 3.2 for the first-eluted enantiomer and the last-eluted enantiomer (the imprinted analyte) were 128 700 and 2100 plates/m, respectively.

Keywords: Capillary electrochromatography / Chiral recognition / Ibuprofen / Molecularly imprinted polymer
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1 Introduction

Molecularly imprinted polymers (MIPs) are tailor-made synthetic polymers with a predetermined selectivity for a given analyte, or group of structurally related compounds, making them ideal materials to be used in separation processes. In applications of MIPs as stationary phases in HPLC, most studies have focused on chiral separation problems and many different racemic compounds have been successfully resolved, including amino acids, peptides, drugs, carboxylic acids, and amines, among others [1, 2].

CEC has been regarded as a very promising analytical separation technique that combines the efficiency of CZE and the selectivity of HPLC. The movement of the mobile phase occurs through EOF. To take advantage of the prominent selectivity of MIP, CEC is thought to be an ideal technique since it provides a high degree of separation efficiency, short separation times, and a minimal consumption of materials and chemicals. MIP-CEC systems have been recently developed through different approaches, namely monolithic capillaries prepared by an *in situ* polymerization [3–7], open-tubular capillaries [8–11], entrapped MIPs [12, 13], and partial filling techniques based on MIPs micro- or nanoparticles [14, 15]. The superporous monoliths are currently the most successfully MIP columns with respect to high selectivity and resolution. But the bulk of the literatures about CEC-MIP focused on the use of methacrylic acid (MAA) for the preparation MIPs [16]. However, MAA is not a universal monomer. In recent report, MAA-MIP filled capillaries failed to separate steroid isomers in CEC mode [17]. Sibrian-Vazquez and Spivak [18] reported that when MAA was added to the imprinted system, the performance of the prepared MIPs decreased. Many studies have also indicated that the 2- or 4-vinylpyridine (VPY) was particularly more suited for the imprinting of carboxylic acid templates and provided higher selectivity than MAA [19–22]. Besides MAA, others functional monomers such as

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Abbreviations: EDMA, ethylene glycol dimethacrylate; **MAA**, methacrylic acid; **γ-MAPS**, γ-methacryloxypropyltrimethoxysilane; **MIP**, molecularly imprinted polymer; **VPY**, vinylpyridine

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2- or 4-VPY should be taken into account to prepare MIPs for CEC in order to enlarge the scope of chemistry that can be imprinted.

In this paper, we reported a novel method for the *in situ* preparation of chiral MIP capillary columns that use γ-methacryloxypropyltrimethoxysilane (γ-MAPS) as an ionizable precusor, which was responsible for establishing EOF. (*S*)-Ibuprofen was chosen as the template molecule, 4-VPY as the functional monomer and ethylene glycol dimethacrylate (EDMA) as the cross-linker, respectively. The chiral MIP stationary phases were evaluated in aqueous and nonaqueous media.

2 Materials and methods

2.1 Materials

Fused-silica capillaries of 100 μm id and 375 μm od were purchased from Yongnian Optical Fiber Factory (Hebei, China). γ-MAPS and 4-VPY were obtained from Sigma-Aldrich (St. Louis, MO, USA). MAA was from Beijing Donghuan Chemical Reagent Factory (Beijing, China). EDMA was obtained from Suzhou Anli Chemical & Engineering Co. Ltd. (Suzhou, China). 4-VPY, MAA and EDMA were distilled under vacuum and collected over type 4 Å molecular sieves. Azobisisobutyronitrile (AIBN) was obtained from Special Chemical Reagent Factory, Nankai University (Tianjin, China), and recrystallized from ethanol before use. (*S*)-Ibuprofen and racemic ibuprofen were gifts from Zhejiang Xianju Charioteer Pharmaceutical Factory (Zhejiang, China). Other analytical reagents such as thiourea were purchased from Tianjin Chemical Reagent Co. Ltd. (Tianjin, China). Analytical-grade phosphoric acid, sodium dihydrogen phosphate, and HPLC-grade ACN were purchased from Tianjin Kermel Chemical Reagent Co. Ltd. (Tianjin, China). All solvents and solutions for CEC analysis were filtered through a 0.22 μm cellulose ester membrane. Samples were dissolved in ACN to a concentration of 10 mM and were diluted in the electrolyte to the desired concentration. Phosphate buffers were prepared by titrating 20 mM sodium dihydrogen phosphate with 20 mM phosphoric acid to desired pH value.

2.2 Instrument

A Beckman P/ACE MDQ system (Beckman, Fullerton, CA, USA) equipped with a UV detector was used for all experiments and a pressure of 5 bar was applied to both vials during the separation. In this paper, since some of analytes were eluted prior to EOF, the enantioseparation factor was evaluated using α', which was calculated from the equation $\alpha' = t_{R2}/t_{R1}$, where t_{R1} and t_{R2} are the retention times of the first- and the second-eluted enantiomers, respectively. Resolution (R_s) was calculated by $R_s = 2\,(t_{R2}-t_{R1})/(w_2 + w_1)$, where w_1 and w_2 are the baseline peak widths of the first- and the second-eluted enantiomers, respectively. The number of the theoretical plates (N) was calculated from the equation $N = 16\,(t_R/w)^2$.

2.3 Preparation of MIP monoliths

Prior to the polymerization, the fused-silica capillary was silanized with γ-MAPS according to the procedure published by Hjertén [23]. The capillary was flushed with 1 M NaOH for 3 h, washed by water until pH reached 7.0, followed by 0.1 M HCl for 30 min and water until pH 7.0 before it was dried with a flow of nitrogen at room temperature. The capillary was filled with 4 μL γ-MAPS in 1 mL of 6 mM acetic acid and kept in the capillary for 5 h. Finally, the capillary was flushed with methanol for several times to remove unreacted γ-MAPS and dried with a flow of nitrogen. A prepolymerization mixture containing (*S*)-ibuprofen, MAA or 4-VPY, EDMA and 1% initiator AIBN were dissolved in 0.96 mL toluene-isooctane (5/3, v/v) was prepared. The mixtures were composed as described in Table 1. The mixture was degassed for 3 min by ultrasonication. A 40 cm long capillary was attached to a syringe and filled with the degassed prepolymerization mixture to a length of 25 cm. After each end of the column was plugged with a piece of rubber, the capillary was submerged in a 50°C bath for 5 h. Subsequently, the column was moved out of the water bath and immediately flushed with ACN as well as 6 mL of 10% v/v acetic acid in methanol in order to hydrolyze the ester bonds (Si–O–C) of γ-MAPS and wash the imprint molecules, the unreacted monomers and the remainder of the initiator out of the capillary column. A detection window was created near the end of the continuous polymer bed by burning off a 2-mm segment of the polyimide outer coating. The final capillary column had a total length of 35 cm and the effective length with MIP-based stationary phase was 20 cm, which was then stored at room temperature

Table 1. Composition of the prepolymerization mixtures

Columns	(*S*)-Ibuprofen (mmoL)	Functional monomer (mmoL)	Cross-linker EDMA (mmoL)	γ-MAPS (μL)
MIP1	0.1	0.3 (MAA)	1.25	0
MIP2	0.1	0.3 (4-VPY)	1.25	0
MIP3	0.1	0.3 (4-VPY)	1.25	10
MIP4	0.1	0.3 (4-VPY)	1.25	5
MIP5	0.1	0.3 (4-VPY)	1.25	3

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until use. A nonimprinted polymer monolithic column (in the absence of template) was prepared and treated in an identical manner.

3 Results and discussion

3.1 MIP monolith synthesis

A molecularly imprinted monolithic stationary phase in CEC must fulfill two functions: one is to provide selective recognition sites for the template molecule, and another is to provide fixed charges for EOF generation. In this study, two frequently used functional monomers (MAA and 4-VPY) were selected to prepare molecularly imprinted columns. MAA could, in principle, be able to interact through hydrogen bonding with (*S*)-ibuprofen. This reagent has an acidic proton, thus providing the fixed charged sites for generation of EOF under CEC conditions. As can be seen from Fig. 1, MIP1 prepared utilizing MAA as the functional monomer only gave 1.05 as the α' values for ibuprofen with poor resolutions. MAA, which has been widely employed to produce molecularly imprinted monolithic columns for CEC, is not actually an effective monomer for the preparation of MIPs for (*S*)-ibuprofen.

4-VPY, a protophilic monomer, will also interact with (*S*)-ibuprofen by hydrogen bonding and π-donor/acceptor interactions. Considering the fact that molecularly imprinted stationary phase prepared by using 4-VPY as a functional monomer is a neutral structure, it may not generate EOF. We measured the velocity of the EOF on MIP2 by using thiourea as the neutral marker and 20 mM phosphate (pH 3.2) ACN (20/80 v/v) as the mobile phase at -20 kV. However, EOF was too weak to be determined, since thiourea was not observed within 2 h. When the pH of the mobile phases was changed, EOF still could not be determined. The causes of this phenomenon may be: (i) the extent of a pyridyl group is low in the 4-VPY-EDMA materials; (ii) the extent of the protonation of a pyridyl group is not large enough during the pH range tested.

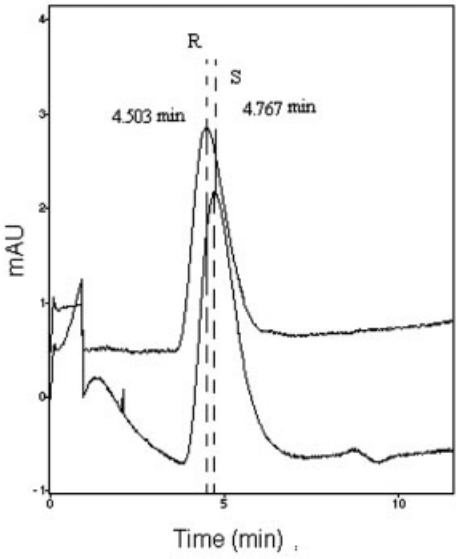

Figure 1. Chiral recognition of racemic ibuprofen on MIP1 (100 μm id $\times$ 20 cm) at 15 kV. The sample was injected electrokinetically at 1 kV for 3 s. The capillary was thermostated to 25°C. Detection wavelength was set at 254 nm. The electrolyte used was composed of ACN-phosphate buffer (pH 3.2, 20 mM) (50:50 v/v).

The success of molecule imprinting is generally associated with the use of inert, apolar porogens such as chloroform and toluene [24]. 2-Acrylamido-2-methyl-1-propanesulfonic acid, which has been widely utilized as ionizable monomer to produce polymer monoliths (nonimprinted) for CEC, is not soluble in apolar solvents. Thus, an ionizable functional monomer should not interfere with the ability of the polymerization system to form well-defined imprints and be soluble in porogens (toluene-isooctane) in this study. γ-MAPS is expected to be specifically suitable for this application. The structural design of this precursor contains two important features: (i) it is soluble in apolar solvents; (ii) the ester bonds (Si-O-C) of γ-MAPS on the surface of the MIP is easily and efficiently cleaved hydrolytically with the loss of methanol to give silanol groups to sustain EOF. An additional advantage of this reagent is that nonspecific absorption can be reduced by interaction between silanol groups and pyridine rings that are located outside the imprinted cavities. In the following experiments, γ-MAPS was incorporated into a mixture of (*S*)-ibuprofen, 4-VPY, and EDMA to develop the MIP monolithic columns. Schematic representation of the synthesis route is shown in Fig. 2.

A different amount of γ-MAPS was introduced to prepare the imprinted stationary phases (Table 1). The scanning-electron micrographs of the end of the MIPs columns are shown in Fig. 3. When 10 μL of γ-MAPS was added to the prepolymerization mixtures, a dense structure was obtained (Fig. 3A). Even after trying to evacuate the solvent with a syringe pump at 40 bar for 20 h, the MIP3 column was still in the occluded state and no solvent flow was found. MIP4 and MIP5 were made with 5 and 3 μL γ-MAPS, respectively; good permeability was obtained (Figs. 3B and C).

3.2 EOF of the MIPcolumns

In CEC, an important evaluation criterion for stationary phases is their ability to generate EOF. This EOF is generated due to an electric double layer at the interface of the

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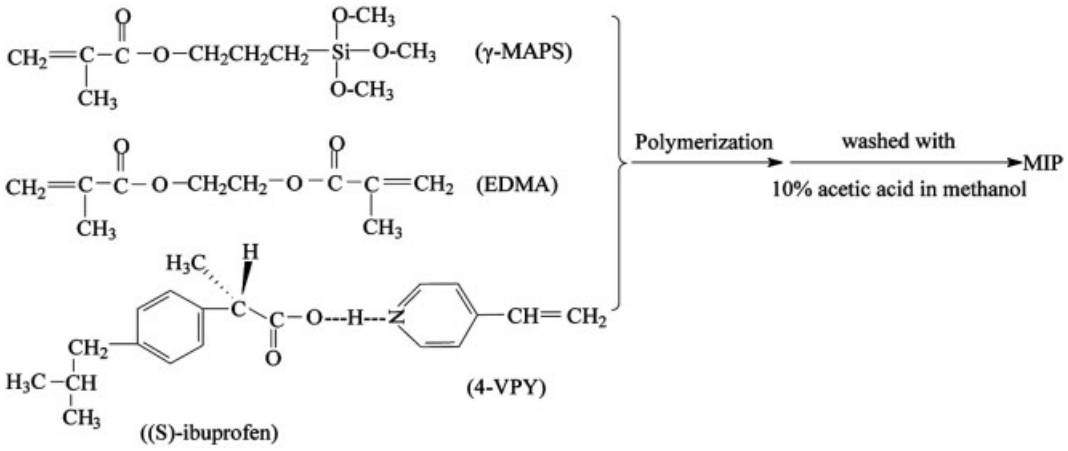

Figure 2. Schematic representation of the synthesis process.

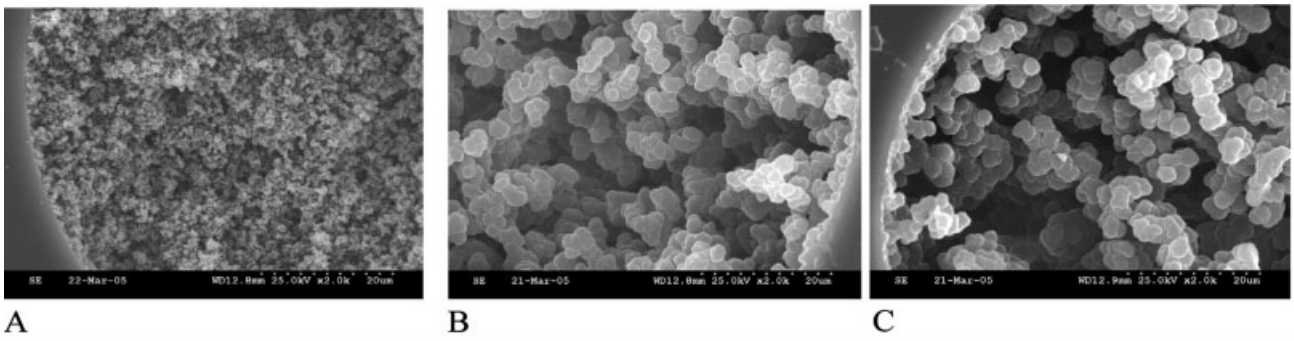

Figure 3. Scanning-electron micrographs of the ends of the MIP3, MIP4, and MIP5.

solid support with the liquid mobile phase. In this study, the deprotonation of the silanol groups on the surface of MIP provided a negative surface charge under CEC running conditions. This negatively charged substrate attracted cations from the electrolyte in the mobile phase forming the electrical double layer and thereby generating a cathode EOF from anode to cathode. Under the experimental conditions used, a strong cathode EOF was observed, suggesting that the surface negative charge due to the deprotonation of the silanol groups that were provided by the hydrolysis of the ester bonds (Si–O–C) was the EOF-determining factor in the prepared MIP monolithic columns.

First, the effect of γ-MAPS content in the prepolymerization mixture on the EOF was investigated at 15 kV, using the mobile phase containing 50% ACN in 20 mM phosphate buffer with pH 3.2. The EOFs of the capillary columns were $1.19 \times 10^{-8}\ m^2 \cdot V^{-1} \cdot s^{-1}$ obtained on MIP4 and $1.08 \times 10^{-8}\ m^2 \cdot V^{-1} \cdot s^{-1}$ MIP5. MIP4 was chosen for further experiments with respect to analysis times.

Consequently, the composition of mobile phase was varied in order to understand the EOF well. The measurements obtained using the MIP4 monolithic capillary were evaluations of the effect of pH on EOF at 15 kV. Phosphate (20 mM)/ACN (50/50 v/v) was used as mobile phase and thiourea as the neutral EOF marker. An EOF of $1.17 \times 10^{-8}\ m^2 \cdot V^{-1} \cdot s^{-1}$ was obtained using the mobile phase at pH 2.5. With the increase of pH values of the mobile phases, the EOF increased slowly and steadily. As the pH reached 5.0, the EOF reached $1.25 \times 10^{-8}\ m^2 \cdot V^{-1} \cdot s^{-1}$.

The effect of ACN concentration on the EOF was also investigated by keeping the phosphate concentration at 20 mM, pH 3.2 and an applied voltage of 15 kV. The EOF slightly increased from 8.02×10^{-9} to $2.25 \times 10^{-8}\ m^2 \cdot V^{-1} s^{-1}$, as the ACN concentration increased from 40 to 70% v/v.

The EOF was also investigated in nonaqueous media on MIP4 at 20 kV. The influence of mobile phase composition on EOF was investigated (Fig. 4). Acetic acid–ACN (1% v/v) based mobile phase gave an EOF of $1.90 \times 10^{-8}\ m^2 \cdot V^{-1} s^{-1}$. EOF was decreased, ($\mu_{EOF} = 8.71 \times 10^{-9}\ m^2 \cdot V^{-1} s^{-1}$), when the mobile phase was changed to 1% v/v acetic acid in methanol. When the ratio of ACN/methanol increased from 30/70 to

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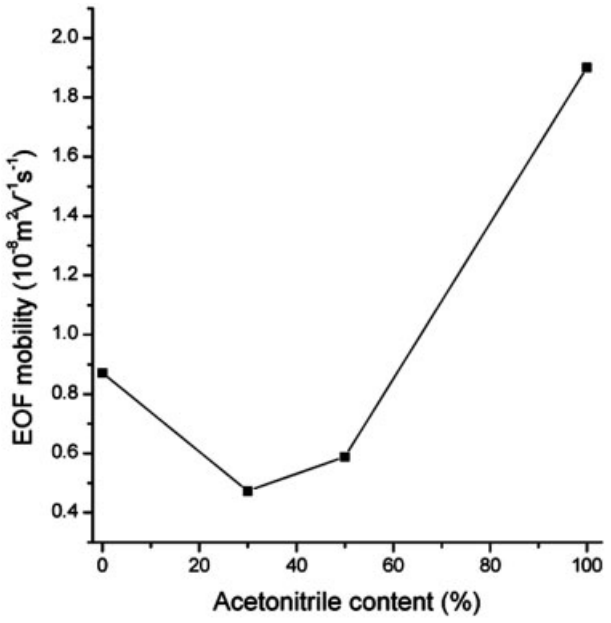

Figure 4. Effect of ACN content on the EOF mobility on MIP4 in nonaqueous mode. Experimental conditions: mobile phase, ACN/methanol (v/v) in different ratios in 1% acetic acid; applied voltage, 20 kV.

50/50 v/v, the EOF increased slightly from 4.72×10^{-9} to 5.88×10^{-9} $m^2 \cdot V^{-1}s^{-1}$. The higher dielectric constant and the lower viscosity of ACN than methanol may explain the reason of this phenomenon. 1% v/v Acetic acid in THF was used as the mobile phase; EOF was very weak ($\mu_{EOF} = 7.41 \times 10^{-10}$ $m^2 \cdot V^{-1}s^{-1}$).

From the above discussion it can be concluded that the prepared MIP monolithic columns exhibited the reasonable EOF required for efficient CEC separations in aqueous and nonaqueous media.

3.3 Chiral recognition on MIP4 and MIP5 columns

MIP4 and MIP5 were evaluated by separation of the enantiomers of ibuprofen, where the eluent used is 20 mM phosphate buffer (pH 3.2)–ACN (50:50 v/v); separation voltage is 15 kV; column temperature is kept at 25°C. As shown in Fig. 5, the resolutions on MIP4 and MIP5 were 1.3 and 2.2, respectively. The calculated plate numbers for the first-eluted enantiomer were 128 700 plates/m for MIP4 and 116 600 plates/m for

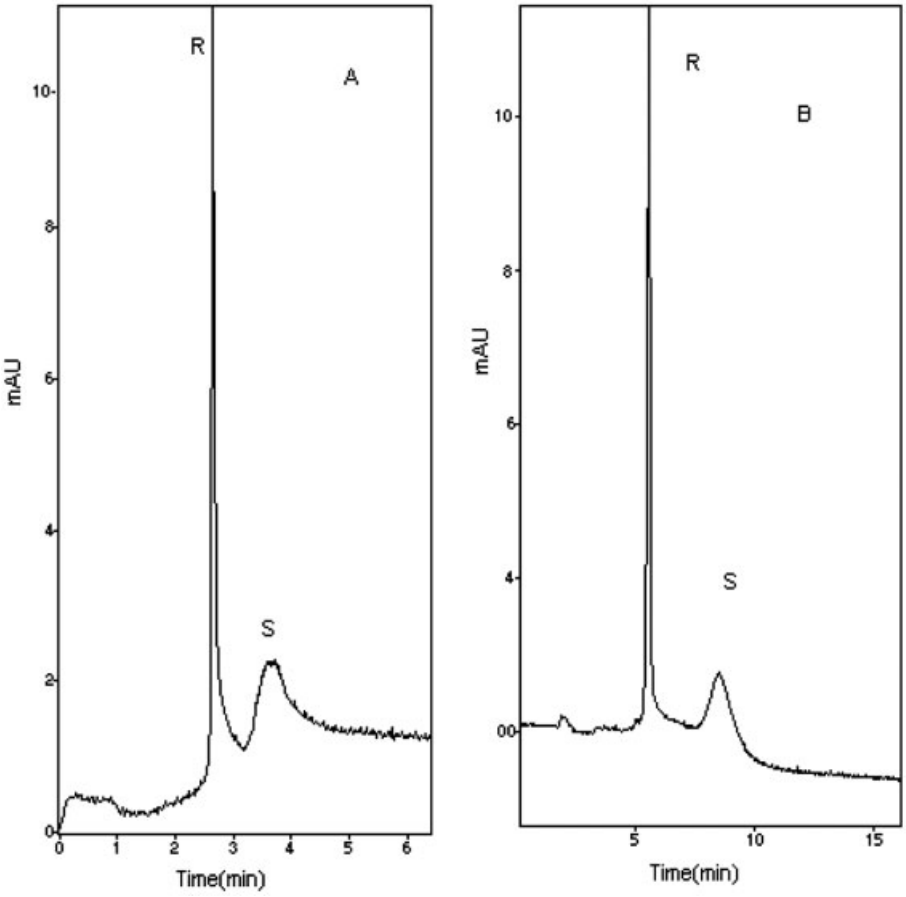

Figure 5. Chiral recognition of racemic ibuprofen on MIP4 (A) and MIP5 (B) at 15 kV. The sample was injected electrokinetically at 1 kV for 3 s. The capillary was thermostated to 25°C. Detection wavelength was set at 254 nm. The electrolyte used was composed of ACN-phosphate buffer (pH 3.2, 20 mM) (50:50 v/v).

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MIP5. The efficiency (plate numbers) is comparable or superior to those which have been reported for non-imprinted CEC separations [25, 26]. This may be explained that the nonspecific sites on the surface of the polymers were masked by electrostatic interaction between the silanol groups and pyridine moieties that were scattered in the bulk of the polymers, reducing (*R*)-ibuprofen nonspecific absorption. However, the plate numbers for the last-eluted enantiomer (the imprinted analyte) were in the same range for both columns (2100, 3400 plates/m, respectively). In the present experiments, (*S*)-ibuprofen was employed as the template molecule to produce these MIP stationary phases; the binding sites in the stationary phase were complementary to the template in shape and chemical functionality. Thus, the higher retention of (*S*)-ibuprofen was observed during separation. MIP4 was chosen for further experiments due to the short separation times. From the comparison of Figs. 1 and 5 it could be concluded that the 4-VPY-MIP resolved the enantiomers of ibuprofen more efficiently than the MAA-MIP (*i.e.*, separation factors (α') on MIP4 and MIP5 were 1.31 and 1.42, respectively). This may be attributed to the difference of interaction prior to polymerization that MAA could only interact through hydrogen bonding with the (*S*)-ibuprofen molecule, whereas 4-VPY, interacted with (*S*)-ibuprofen by hydrogen bonding and π-donor/acceptor interactions. Nonracemic mixtures of ibuprofen (*R*:*S* of 1:6) were also separated on MIP4 (Fig. 6).

3.4 Chiral recognition in aqueous media

In order to understand well the recognition mechanism, a preliminary research on CEC in aqueous media was carried out on MIP4. At a fixed pH of 3.2, it had been observed that an increase in ACN content in the mobile phase leads to a decrease in the retention factors of both enantiomers, the ACN content is ranging from 40 to 80% (Table 2). Furthermore, the selectivity also decreased with the increasing ACN content, when the ACN content was at least 50%. An increase in resolution was seen when the ACN content was increased from 40 to 50%, the fact might be that the higher ACN content was favorable for the formation of hydrogen bond between the template and the imprinting sites. When ACN content was decreased to 30%, the template molecule could not be found due to serious adsorption. On the other hand, ACN contents over 70% resulted chiral recognition ability disappeared. The best separation was obtained at 50% ACN content.

The influence of pH value on the retention factor and the separation factor at a fixed buffer content of 50% is shown in Table 3. With increasing the pH value, the retention factor 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 was disappeared. It must be pointed out that the column effi-

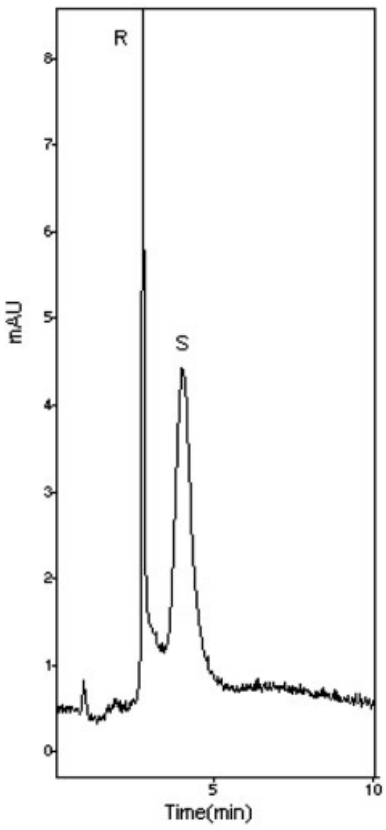

Figure 6. Chiral separation of an ibuprofen sample enriched in the *S*-form (enantiomeric purity *R*:*S* = 1:6). Experiment was performed at 15 kV. The sample was injected electrokinetically at 1 kV for 3 s. The capillary was thermostated to 25°C. Detection wavelength was set at 254 nm. The electrolyte used was composed of ACN-phosphate buffer (pH 3.2, 20 mM) (50:50 v/v).

Table 2. Effect of ACN content on the separation of ibuprofen enantiomers

Concentration of ACN (%)	t_{R1}	t_{R2}	α'	R_s
40	6.7	9.3	1.4	1.61
50	3.4	4.8	1.4	2.12
60	3.3	3.8	1.2	1.18
70	2.3	2.5	1.1	0.78
80	1.9	1.9	1.00	0.00

Experimental conditions: mobile phase, a mixture of phosphate and ACN; separation column, MIP4. Other conditions are the same as in Fig. 1.

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Table 3. Effect of mobile phase pH on the retention time of enantiomers

pH	t_{R1}	t_{R2}	α'	R_s
2.5	3.7	6.7	1.82	2.80
3.2	3.4	4.8	1.42	2.12
4.0	2.9	3.1	1.04	0.38
5.0	2.1	2.1	1.00	0.00

Experimental conditions: mobile phase, 50% v/v ACN in phosphate; separation column, MIP4. Other conditions are the same as in the Fig. 1.

ciency was poor due to the peak tailing at pH 2.5, although the resolution reached 2.8. A parallel experiment was carried on the nonimprinted blank column, however, no enantioselectivity was observed at any pH. A pH 3.2 of the buffer solution was chosen for further experiments with respect to the column efficiency and the resolution.

3.5 Chiral recognition in nonaqueous media

A preliminary research on CEC in nonaqueous media was also carried out on MIP4. Solvents used included methanol, ACN, and THF.

The influence of the nature of organic solvents on chiral recognition was studied. 1% v/v Acetic acid/methanol-based mobile phase gave a good separation (Fig. 7A). When the mobile phase consisted of 1% v/v acetic acid, 30/70 v/v ACN/methanol, the resolution was decreased and a long retention time was observed (Fig. 7B). As the ACN content was increased to 50% v/v, the resolution was increased and the band greatly broadened (data not shown). When the mobile phase was changed to 1% v/v acetic acid in ACN, enantiomer separation was not observed. The cause of the loss of enantioselectivity can be due to a too fast elution. Acetic acid (1% v/v) in THF-based mobile phase gave server broaden peaks as well as a longer retention time of more than 75 min, although the separation factor reached 1.41. In nonaqueous media, the calculated plate numbers for the first-eluted enantiomer were 750 plates/m in Fig. 7A and 1024 plates/m in Fig. 7B. The efficiencies (in plates *per* meter) in nonaqueous media were lower than that in aqueous media. We speculated that nonspecific sites, which could not be masked sufficiently by electrostatic interaction between the silanol groups and pyridine moieties in nonaqueous media, might be responsible for the low efficiency.

4 Concluding remarks

In this study, chiral MIPs were developed using (*S*)-ibuprofen as the template molecule, γ-MAPS as the ionizable precusor, 4-VPY as the functional monomer and EDMA as the cross-linker. By incorporating γ-MAPS into the prepolymerization mixture, the charged groups were successfully coupled to the surface of 4-VPY-MIPs to generate a stable EOF for CEC separation. The MIP made without γ-MAPS showed a very weak EOF that cannot be determined. We also found that the MIP made with 4-VPY yielded better separation than that made with MAA.

The resulting MIP stationary phases showed a good chiral discrimination toward ibuprofen in aqueous and nonaqueous media. From the above discussion, we knew that the chiral separation was governed by both hydrophobic interaction and hydrogen bonding interactions. In

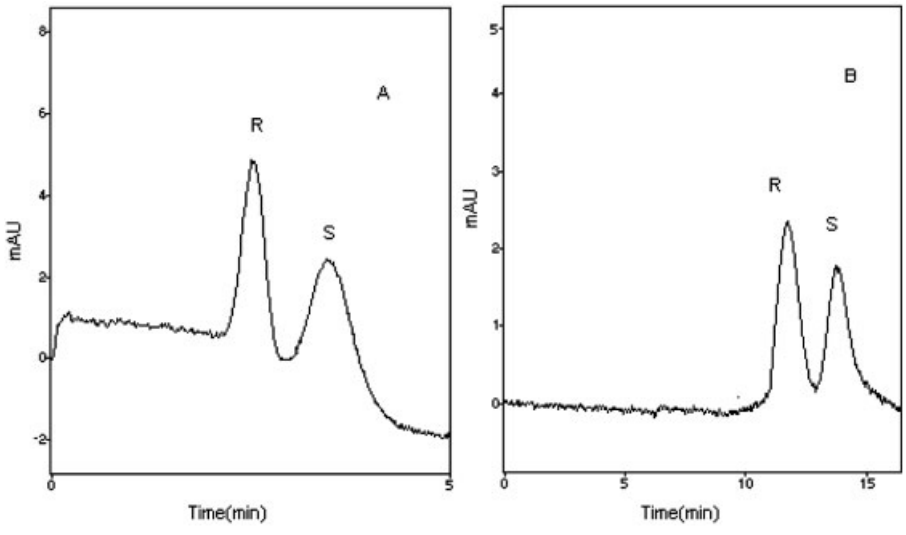

Figure 7. Chiral recognition of racemic ibuprofen on MIP4 at 20 kV. The electrolyte used was composed of methanol/acetic acid (99:1 v/v) (A) and 1% acetaic acid in methanol/ACN (70/30 v/v) (B). The sample was injected electrokinetically at 1 kV for 3 s. The capillary was thermostated to 25°C. Detection wavelength was set at 254 nm.

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aqueous media, hydrophobic interaction plays an important role in the retention and enantioselectivity, whereas in nonaqueous media, polar interactions (hydrogen bonding, ionic interactions, *etc.*) as well as the mobile phase elution ability were mainly responsible for binding and recognition. This method may be extended to imprint neutral or acidic templates utilization functional monomers that cannot provide enough EOF in CEC systems.

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