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Research Article

A molecularly imprinted monolith for the fast chiral separation of antiparasitic drugs by pressurized CEC

Molecularly imprinted polymer (MIP) monoliths with (*S*)-ornidazole ((*S*)-ONZ) as the template molecule have been designed and prepared by the simple thermal polymerization of methacrylic acid, 4-vinylpyridine, and ethylene dimethacrylate in the presence of a binary porogenic mixture of toluene and dodecanol. The influences of polymerization mixture composition on the chiral recognition of ONZ have been evaluated, and the imprint effect in the optimized MIP monolith has been clearly demonstrated. The new monolithic stationary phase with optimized porous property and good selectivity was used for the chiral separation of ONZ by pressurized CEC. The pressurized CEC conditions were also optimized to obtain the good chiral separation. The enantiomers were rapidly separated within 9 min on the MIP-based chiral stationary phase, whereas the chiral separation was not obtained on the nonimprinted polymer. Additionally, the proposed method has been successfully applied to the chiral separation of ONZ in tablet samples by injection of the crude sample. The cross-selectivity for similar antiparasitic drug was investigated. The results indicated that the chiral separation of secnidazole could also be obtained on the optimized MIP monolith within 14 min.

Keywords: Chiral separation / Molecularly imprinted polymer monolith / Ornidazole / Pressurized CEC / Secnidazole
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1 Introduction

An antiparasitic drug, namely ornidazole (ONZ) (Fig. 1), like many other nitroheterocycles, has proved to be effective against anaerobic bacterial infections, intestinal and hepatic amebiasis, giardiasis, and vaginal trichomoniasis [1, 2]. At present, *rac*-ONZ has been widely used in human and poultry industry. However, in many cases, a single enantiomer of a racemic drug has different therapeutic effects and sometimes it even has adverse action. Regulators still insist on full profiling of the role of each enantiomer and its pathway in the body [3]. Hence, there is a great need to develop the technology for analysis and separation of racemic drugs. So far, two ways have been adopted to resolve the enantiomers of ONZ. One way is to use capillary electrophoresis with various cyclodextrin-type chiral selectors in the run buffer. However, the enantiomers of ONZ due to the absence of hydrophobic phenyl moiety and the presence of hydrophilic hydroxyl group could not be resolved using any of the chiral selectors [4]. Another way is to use a packed chiral stationary phase (cellulose tribenzoate, 5 μm) with three different mobile phases [5–7]. The chiral separation of ONZ could be resolved on this packed chiral column, but only within 60 [5, 6] and 30 min [7], respectively.

Compared with packed capillary columns, monoliths have attracted considerable attention as a well-established stationary-phase format in the field of miniaturized separation techniques such as CEC and nano-LC because of ease of preparation, small internal diameters (in the range of 10–500 μm), enhancement in separation efficiency and peak capacity, and easy control over their porous properties and surface chemistries [8–10]. Molecularly imprinted polymers (MIPs) are tailor-made synthetic materials possessing specific recognition sites complementary in shape, size and functional groups to the template molecule, which mimic the binding sites of antibodies and receptors. Molecularly imprinted materials offer attractive properties such as stability, ease of preparation, environment friendly, and low cost for demanding or special analytical tasks [11–13]. Hence, CEC-based MIP monoliths have the potential to be a powerful tool in the chiral separation field, possessing not only predictable recognition ability but also the elution order [14–18]. Traditionally, MIPs are fabricated using one func-

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Abbreviations: EDMA, ethylene dimethacrylate; **MAA,** methacrylic acid; **MIP,** molecularly imprinted polymer; **NIP,** nonimprinted polymer; **(*S*)-ONZ,** (*S*)-ornidazole; **pCEC,** pressurized CEC; **SNZ,** secnidazole; **4-VP,** 4-vinylpyridine

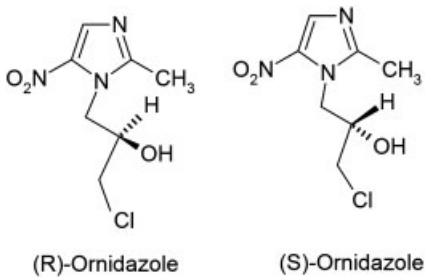

Figure 1. Stereochemistry of the enantiomers of ONZ.

tional monomer, namely methacrylic acid (MAA). However, by using a combination of MAA and 4-vinylpyridine (4-VP) functional monomers, the template recognition of the MIPs would be improved [19]. MIP monolith was fabricated using the two functional monomers and its application to the separation of drugs in pressurized CEC (pCEC) is relatively rare in the literature [20, 21]. Deng *et al.* [20] described the preparation of MIP monolith using felodipine as the template molecule and the MIP monolith as tools for the screening of felodipine from dihydropyridine calcium antagonists by pCEC. Recently, Li *et al.* [21] reported the preparation of MIP monolith using the L-enantiomer of amino acid as the template molecule and investigated the chiral recognition of MIP monolith in pCEC.

In this study, using molecularly imprinted technology, a new monolithic stationary phase for the rapid chiral separation of antiparasitic drugs by pCEC was designed and prepared through a single-step copolymerization. The poly(MAA-co-ethylene dimethacrylate (EDMA)-co-4-VP) monolith was fabricated with (*S*)-ONZ acting as the template molecule. Optimal porous properties and good selectivities were gained, and the chiral recognition mechanism on the MIP monolith was also investigated. Then, the optimized monolith was applied to the fast chiral separation of ONZ and the cross-selectivity investigation of a similar drug secnidazole (SNZ), which was used as a new generation of antiparasitic drugs.

2 Materials and methods

2.1 Chemicals and materials

(*S*)-ONZ was purchased from Xin An Medicine (Xi'an, China). ONZ was purchased from the National Institute for the Control of Pharmaceutical and Biological Products of China (Beijing, China). SNZ was purchased from Xi'an Ziguang Institute of Biochemistry (China). 4-VP, MAA, EDMA, and 3-trimethoxysilyl propyl methacrylate (γ-MAPS) were purchased from Aldrich (Milwaukee, WI, USA). Azobisisobutyronitrile was obtained from the Forth Chemical Reagent Plant (Shanghai, China). Toluene and dodecanol were purchased from Tianjin Chemical Reagent Plant (Tianjin, China). HPLC-grade methanol and ACN were purchased from Chemical Reagent (Shanghai, China). Other analytical reagents were purchased from Chemical Reagent. The water used throughout all the experiments was purified with a Millipore Milli-Q purification system (Milford, MA, USA). All the reagents used were of analytical reagent grade or better. The fused-silica capillaries (100-μm id, 375-μm od) were purchased from the Yongnian Optic Fiber Plant (Hebei, China).

2.2 Instrumentation

pCEC was carried out on a Trisep 2100GV pCEC system (Unimicro Technologies, Pleasanton, CA, USA) which was composed of a solvent gradient delivery module, a high voltage power supply (+30 and −30 kV), a variable wavelength UV/Vis detector (190–800 nm), a microfluid manipulation module (including a six-port injector), and a data acquisition module, as described in detail in the literature [22]. A continuous mobile-phase flow was generated by a solvent gradient delivery module and which then enters a six-port injection valve with microelectric actuators. The samples injected were delivered to the injection valve and introduced in the internal 0.8-μL sample loop, and then carried to the four-port split valve by the mobile phase flow. After splitting in the four-port valve, the flow entered the capillary column under constant supplementary pressure controlled by a back-pressure regulator. A supplementary pressure was applied to the column inlet during the separation. Voltage was applied to the outlet of column, and the inlet of column was connected to the split valve and grounded. In these studies, the isocratic elution system was used. An HPLC pump (Unimicro Technologies) was applied to flush the monolithic columns. SEM images of the monolithic columns with a magnification of 5000 × were obtained by a Philips XL30 E SEM using 20 kV accelerating voltage and 10-mm working distance with zero tilt.

2.3 Single-step preparation of molecularly imprinted monolith

Before stationary-phase introduction, the inner wall of a capillary was treated with γ-MAPS, according to the procedure described elsewhere [23]. A prepolymerization mixture containing (*S*)-ONZ, MAA, 4-VP, EDMA, and azobisisobutyronitrile was dissolved in a binary porogenic solvent mixture consisting of toluene and dodecanol in various ratios (Table 1). Then, the solution was sonicated for 10 min to obtain a homogeneous solution, purged with nitrogen for 5 min, and introduced into the pretreated capillary. The ends of the capillary were sealed with rubber stoppers and submerged in a 56°C water bath for 20 h. After polymerization, the capillary was washed with methanol/acetic acid (9:1, v/v) by an HPLC pump to remove the template and the residual reagents. A 1–2 mm detection

Table 1. Composition of the copolymerization mixture[a)]

Column	MAA to 4-VP ratio (mol/mol)	Toluene[b)] (wt%)	MAA + 4-VP to EDMA ratio (mol/mol)	Template to MAA + 4-VP ratio (mol/mol)	α
A	0:4	10	1:4	1:2	1.12
B	1:3	10	1:4	1:2	1.48
C	2:2	10	1:4	1:2	1.65
D	3:1	10	1:4	1:2	1.53
E	4:0	10	1:4	1:2	1.42
F	2:2	6	1:4	1:2	1
G	2:2	8	1:4	1:2	1.16
H	2:2	14	1:4	1:2	No[c)]
I	2:2	10	1:2	1:2	No[c)]
J	2:2	10	1:3	1:2	1
K	2:2	10	1:4.5	1:2	No[c)]
L	2:2	10	1:4	1:4	1.49
M	2:2	10	1:4	1:2.5	1.52
N	2:2	10	1:4	1:1.5	1.78

a) Conditions: 52 cm column length, 30 cm effective length; mobile phase: ACN/50 mM NH_4Ac-HOAc buffer (98:2, v/v, pH 6.0); template: (*S*)-ONZ; flow rate: 0.05 mL/min; voltage: 20 kV; applied pressure: 20 psi.
b) Percentage of toluene in the porogenic solvent.
c) No: The measurements could not be made because the columns were not applicable.

window was created at the end of the polymer bed by using a thermal wire stripper. A 2-cm length of the capillary containing the monolith inside was cut for SEM analysis.

2.4 Sample preparation

ONZ tablets were purchased from Xi'an Bodyguard Pharmaceutical (China). After removal of film coating, the six tablets were finely powdered and homogenized, and then a portion of the powder (equivalent to the weight of 1/50 tablet) was dissolved with ACN. The mixture was filtered through a 0.45-μm membrane, and the filtrate was proper diluted with ACN in a calibrated 50-mL volumetric flask for further sample analysis by pCEC.

2.5 CEC

Mobile phases were prepared by mixing appropriate volumes of ACN, stock NH_4Ac-HOAc buffer solution, and water. Electrolyte solutions were filtered with a 0.22-μm membrane. Before use, the mobile-phase solution was degassed in an ultrasonic bath for 20 min. Applied voltage was firstly ramped from 0 to −10 kV (or −20 kV) and then operated at −10 kV (or −20 kV). UV detection was operated at 310 nm. Before pCEC experiments, the monolithic column was conditioned on the instrument with the mobile phase for 1 h and equilibrated for about 30 min after every mobile phase changed. Retention factor (k) was calculated by the equation $k = (t–t_0)/t_0$, where t is the retention time of the analyte and t_0 the retention time of the void marker (acetone). The separation factor (α) was calculated by the equation $\alpha = k_S/k_R$, where k_R and k_S are the retention factor of the (*R*)-ONZ and (*S*)-ONZ, respectively. The resolution is calculated from the equation $Rs = 2(t_S–t_R)/(w_S+w_R)$, where t_R and t_S are the retention times of (*R*)-ONZ and (*S*)-ONZ, respectively, and w is the width at the baseline between the tangents drawn to the inflection points for the peaks.

3 Results and discussion

3.1 Concentration and ratio of polymerization mixture

3.1.1 Effect of comonomer

The choice of monomer can be divided into functionality type: either neutral or electrostatic (acidic or basic) but with hydrogen bond donors. Most of the MIPs were synthesized by noncovalent imprinting method using one functional monomer such as MAA, but MIPs prepared using a combination of MAA with VP as functional monomer have been demonstrated to improve the recognition performance of MIPs [19]. In our laboratory, a series of MIP monoliths (columns A–E) using different molar ratio of MAA to 4-VP was prepared and their abilities to separate the enantiomers of ONZ by pCEC are recorded in Table 1. Obviously, the MIP monoliths prepared using two functional monomers, namely MAA and 4-VP (columns B–D), showed an increased selectivity than those made of one functional monomer, namely MAA (column E) or 4-VP (column A). The same phenomena were also reported in the literatures [20, 21]. MAA can act as a hydrogen bond donor or acceptor and 4-VP, a protophilic monomer, can also act as hydrogen

bond acceptor. In this poly(MAA-co-EDMA-co-4-VP) MIP monolithic column, the recognition of analytes in mobile phase is mostly likely due to electrostatic or hydrogen-bond interactions between the carboxy group of MAA in the polymer and the slightly basic imidazole ring and between the nitrogen atom of 4-VP in the polymer and 2-hydroxy group of print molecule. The optimum molar ratio of MAA to 4-VP was 1:1.

3.1.2 Effect of the porogenic mixture

The porogenic solvent plays a dual role in the preparation of MIP monolith. It not only governs the strength of noncovalent interactions but also influences the polymer morphology. In general, inert and apolar porogenic mixture such as toluene–isooctane are preferred for the preparation of MIP monoliths, not only in favor of imprinting process but also easy to control the porosity of the monoliths [24]. However, in this study, (*S*)-ONZ is a polar template molecule and can hardly be dissolved in toluene–isooctane porogenic solvents. To overcome this difficulty, a low polar porogenic mixture of toluene–dodecanol was chosen to prepare the MIP monolith. In order to allow capillary flushing without significant back pressures, it is necessary to control the porosity of the MIP monoliths. Svec has confirmed that the porous properties of the monolithic columns can easily be controlled through changes in the composition of the binary porogenic solvent [25]. The columns F, G, C, H synthesized by *in situ* thermal polymerization, which differed only in the ratio of dodecanol and toluene in the porogenic mixture, showed significant differences in the porous properties (Table 1, Fig. 2). It can be seen from Fig. 2 that micrometer-sized globular particles were aggregated extending throughout the capillary. The aggregates were surrounded by interconnected large through pores which permitted bulk flow to occur throughout the entire capillary. Obviously, with the increase in the wt% of toluene, smaller pores and globular particles were seen in SEM (Fig. 2). In general, larger pores were obtained if poorer solvents were used because of an earlier onset of polymer phase separation [26]. With increasing amounts of toluene in a polymerization mixture, the MIP monolith showed an increase in chiral separation factor due to the increase in strength and amount of noncovalent imprinting interactions (Table 1) and a longer separation time due to the decrease in the porosity of stationary phase and the increase in strength of noncovalent interactions. When the wt% of toluene reached 14% (column H) in the porogenic mixture, it was found that the permeability of stationary phase became so poor that it was impossible to allow the mobile phase to flow through. Taking into account the chiral separation factor, retention time and low flow resistance, only the monolith prepared using a toluene proportion of 10% had better selectivity, appropriate analysis time, and appropriate porosity to allow capillary flushing without significant back pressure.

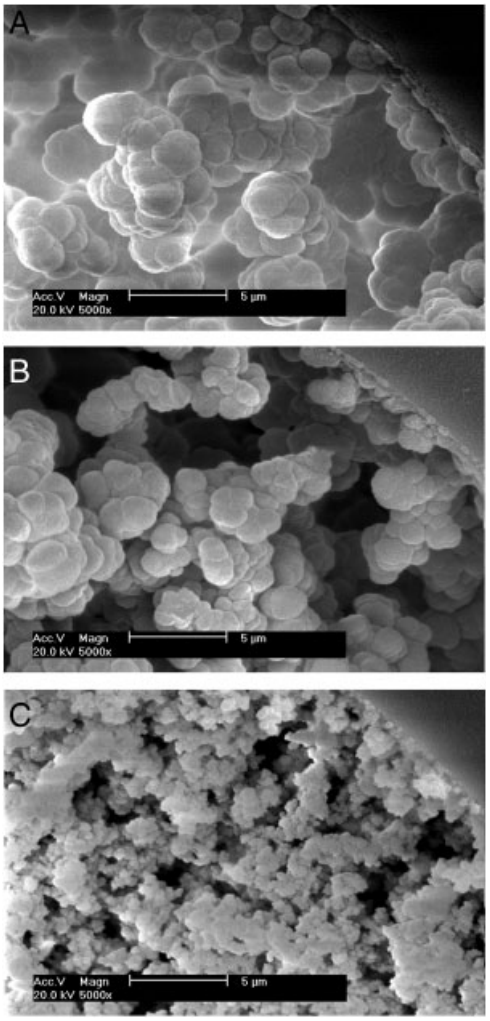

Figure 2. Scanning electron microphotographs of monolithic column with different toluene weight fractions in porogenic solvent: (A) column G, 8% toluene; (B) column C, 10% toluene; and (C) column H, 14 % toluene.

3.1.3 Effect of cross-linking agent

The influence of cross-linking agent on the preparation of MIP monolith has been well-documented. First, it ''freezes'' the template-monomer complex upon polymerization to form the imprinted pocket. Second, it provides the polymeric backbone lending to the polymer mechanical stability. Therefore, the amount of cross-linking was related to not only the recognition ability of MIP columns but also to the flow characteristics in MIP columns. A comparison of columns I, J, C, and K, which differed only in the ratio of functional monomers (MAA + 4-VP) and cross-linking (EDMA) in the polymerization mixture, was investigated in detail (Table 1). Obviously, the MIP monolith showed an increase of flow resistance and recognition ability due to the increase of imprinting cavities with increasing amounts of cross-linking in a polymerization mixture (Table 1). Very little cross-linking may lead to a larger and less recognitive

pore (column J, 1:3) or a soft gel-like appearance (column I, 1:2), and then recognition of the enantiomers is negligible due to very little material and imprint sites. However, if too much cross-linking (column K, 1:4.5) is used, the permeability of column becomes very poor. Taking into account the requirements of low flow resistance and high chiral separation factor, 1:4 (MAA + 4-VP to EDMA ratio mol/mol) was found to be an optimal condition.

3.1.4 Effect of the amount of template molecule

The molar ratio of imprinted molecule to functional monomer is one of the crucial factors in the planning of a molecular imprinting experiment. A series of MIP monoliths (columns L, M, C, N) was prepared, which differed only in the molar ratio of (*S*)-ONZ to MAA+4-VP in the polymerization mixture (Table 1). It can be seen from Table 1, an increase of molar ratio of (*S*)-ONZ to MAA+4-VP in the polymerization mixture resulted in an increase of chiral separation factor of the enantiomers on the resultant MIP monolith. It may be ascribed to that the amount of imprinting cavities increases with the increase in content of templates. The optimization of the template–monomer ratio was 1:1.5 (column N). A nonimprinted polymer (NIP) monolith (in the absence of template) was prepared and treated in an identical manner with column N (Table 1). In this study, it was found that the NIP monolith had less flow resistance than the MIP monolith, which indicated an influence of imprint molecule on the polymer morphology and structure. Under the same electrochromatographic conditions, the enantiomers of ONZ could be resolved on the column N due to strong imprinting effect between (*S*)-ONZ and the MIP monolith, whereas the NIP monolith showed no recognition ability due to the absence of chiral recognition sites complementary to the spatial structure of (*S*)-ONZ.

3.2 Column reproducibility

Therefore, column N had not only a good flow-through property but also a good selectivity. The EOF of column N and the retention times of analytes were measured in the experiments to evaluate the repeatability of the MIP monolithic column through the RSD. The RSD from day to day for EOF and retention times of analytes were $<3.3\%$ ($n = 5$), which indicated the good stability of the obtained monolith. Additionally, both column-to-column and batch-to-batch reproducibilities were also evaluated by preparing three batches and every batch contained three columns, and the RSD for EOF and retention times of analytes were $<6\%$.

3.3 Chromatographic conditions on the chiral separations

The chromatographic conditions are important factors to determine the chiral recognition ability of the MIP monolith besides the conditions of column preparation. Therefore, the influences of electrochromatographic conditions on chiral recognition ability were systematically investigated.

3.3.1 Effect of concentration of organic solvent

In pCEC, a mobile phase is driven by both a pressurized flow and an EOF. The ACN content in the mobile phase had great impact on the EOF and chiral recognition. The effect of ACN content in running buffer on the EOF and separation factors of ONZ on (*S*)-ONZ imprinted monoliths was investigated, respectively. The ACN content was varied from 85 to 98% v/v, while the NH_4Ac-HOAc concentration was kept constant at 50 mmol/L (pH 6.0). These results are shown in Fig. 3. It can be seen that the EOF increased from 0.23 to 0.57 mm/s. The reason is that the viscosity of the solution decreases with the increase of ACN content, at the same time, the ionic strength of the buffer decreases and the zeta potential increases, and then the EOF increases accordingly. It also can be seen from Fig. 3 that the separation factors of ONZ increase with the increase of ACN content. The resolution of ONZ had the same phenomenon with the increase of ACN content. It may be because the hydrogen-bonding interactions between solute and MIP in the process of molecular recognition are stronger with the increase of ACN concentration in hydro-organic mobile phase. Hence, 98% v/v ACN content was used in the following experiments.

3.3.2 Effect of pH values of buffers

The EOF velocity depends on not only the density of charges on the polymer matrix but also the properties of the electrolyte solution. In this study, the EOF was measured with acetone as the unretained marker and calculated by the method in literature [22, 27–29]. The effect of the electrolyte composition on EOF and the degree of chiral separation

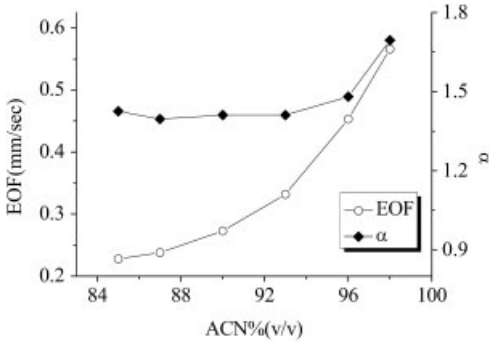

Figure 3. Effect of content of ACN on the EOF and separation factor (α) of ONZ on the (*S*)-ONZ imprinted monolithic column. Electrochromatographic conditions: capillary column: column N, 52-cm column length, 30-cm effective length; mobile phase: ACN/50 mM NH_4Ac-HOAc buffer (85/98%, v/v) pH 6.0; flow rate: 0.05 mL/min; supplementary pressure: 75 psi; and voltage: −20 kV.

were investigated. The results are shown in Fig. 4. The separation factors of ONZ on (*S*)-ONZ imprinted monoliths almost leveled off with the increase of pH values of the buffers from 2.1 to 6.0 and slightly decreased from 6.0 to 6.5, and the resolution of ONZ had the same phenomenon in the same range of pH. However, with the increase of pH values, the EOF increased due to the degree of ionization of MAA increasing and the degree of protonation of 4-VP decreasing. According to the EOF and the degree of chiral separation, pH 6.0 was chosen as optimized condition.

3.3.3 Effect of salt concentration of the buffers

The effect of salt concentration on the recognition of the enantiomers of ONZ and EOF was investigated, respectively, using different ionic strength of electrolyte from 2 to 200 mmol/L NH_4Ac-HOAc (pH 6.0)/ACN (2:98 v/v). The results are shown in Fig. 5. Initially, the EOF increased as the concentration increased from 2 to 50 mmol/L, and then it decreased as the concentration further increased to 200 mmol/L. In this study, the polar poly(MAA-co-EDMA-co-4-VP) monolithic stationary phase and ACN/NH_4Ac-HOAc mobile phase (98:2, v/v) were employed. When a high percentage of ACN is added to the mobile phase, a water-enriched layer is formed on the polar stationary phase [30]. At low electrolyte concentrations, a greater salt concentration would drive more solvated ions into the water-enriched liquid layer and part of them might be immobilized on the monolithic stationary phase, resulting in the increase of the zeta potential [28], and thus the EOF increased initially with the increase in the concentration of electrolyte. With a further increase in the concentration of electrolyte (from 50 to 200 mmol/L), the water-enriched layer on the surface of more polar stationary phases could behave similar to the classic electrical double layer. Because of the increase in the concentration of electrolyte, the thickness thinned out, then the chances of formation of ion pairs increased, the effective charges decreased, and thus the EOF declined. The separation factors of racemic ONZ were slightly decreased with increasing salt concentration in buffer solution (Fig. 5). Hence, 50 mmol/L NH_4Ac-HOAc content was used in the following experiments.

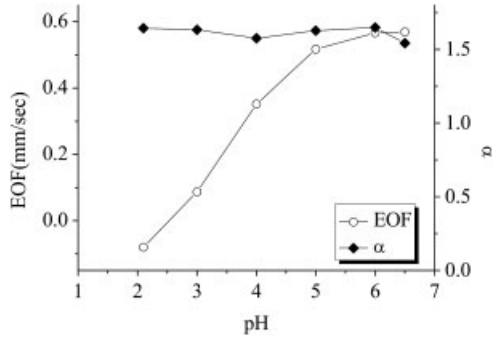

Figure 4. Effect of pH of buffer solution on the EOF and separation factor (α) of ONZ on the (*S*)-ONZ imprinted monolithic column. Electrochromatographic conditions: ACN/50 mM NH_4Ac-HOAc buffer (98:2, v/v), the value of pH from 2.1 to 6.5; other conditions are the same as in Fig. 3.

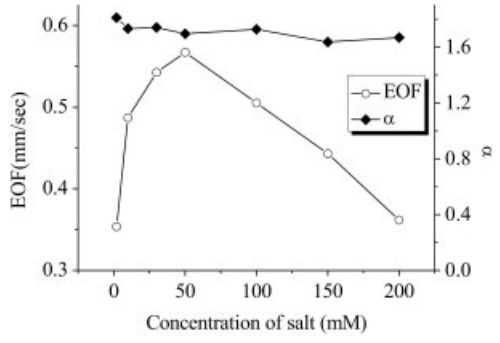

Figure 5. Effect of content of salt on the EOF and separation factor (α) of ONZ on the (*S*)-ONZ imprinted monolithic column. Electrochromatographic conditions: ACN/NH_4Ac-HOAc buffer (98:2, v/v), the content of salt from 2 to 200 mM; other conditions are the same as in Fig. 3.

3.3.4 Effect of applied voltage and supplementary pressure

The pK_a of ONZ is 2.3. Hence, the molecule becomes completely uncharged at around pH 6. Under these conditions, the analytes were driven by the EOF and pressure. Therefore, the effects of applied voltage and supplementary pressure on the chiral separation of ONZ were investigated, respectively. The effect of applied voltage on the chiral separation of ONZ was investigated by applying voltages of 0, −3, −6, −10, −15, and −20 kV, using the mobile phase containing 98% ACN in 50 mM NH_4Ac-HOAc buffer with pH 6.0 under 75 psi. With the increase of applied voltage from 0 to −20 kV, the separation time was greatly shortened, but the resolution of ONZ slightly decreased (Fig. 6). However, very high voltage would cause large current and lead to Joule heating. Hence,

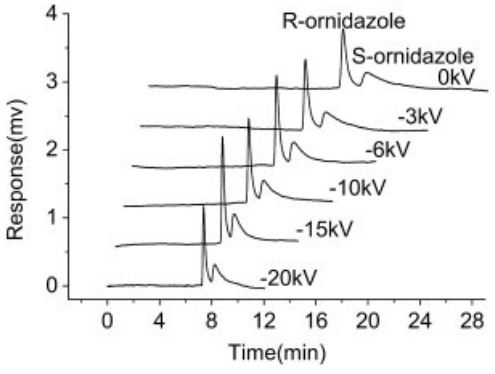

Figure 6. Effect of the applied voltage on the chiral separation of ONZ on the (*S*)-ONZ imprinted monolithic column. Capillary column: 64-cm column length, 43-cm effective length. Electrochromatographic conditions: mobile phase: ACN/50 mM NH_4Ac-HOAc buffer (98:2, v/v, pH 6.00); flow rate 0.05 mL/min, supplementary pressure 75 psi.

−20 kV was the optimized voltage. The effect of supplementary pressure on the chiral separation of ONZ was also investigated by applying supplementary pressures of 20, 60, 75, 100, 175, and 250 psi, using the above-mentioned mobile phase under −20 kV voltage. It was found that the migration times and the resolution of the enantiomers of ONZ decreased with the increase of supplementary pressure (Fig. 7). Hence, 75 psi was chosen because it provided the appropriate resolution and analysis time.

3.4 Method application and validation

3.4.1 Application to analysis of crude tablet sample

The proposed method was applied to the chiral separation of ONZ in locally available pharmaceutical tablets. The samples were prepared as described in Section 2.4. Figure 8 shows the recognition of enantiomers of ONZ on MIP and NIP monoliths. It can be seen from it that the developed method for the fast chiral separation of real sample is feasible (curves 1, 3). It can also be seen from it that the enantiomers of ONZ cannot be resolved on the NIP monolith (curve 2).

3.4.2 Cross-selectivity

SNZ was chosen for the selectivity study. The similar structures of ONZ and SNZ should provide a good test of the selectivity of polymers. They had the same functional group orientation and had only differed in the size and shape of the side chain. Under the same electrochromatographic conditions, the SNZ could only be separated partly, with a smaller separation factor ($\alpha = 1.45$) and shorter retention than that of ONZ. When the $-CH_2Cl$ group (ONZ) is substituted by $-CH_3$ group (SNZ), the structure of SNZ is smaller than the imprint cavity, there are reduced contacts between the SNZ and the (*S*)-ONZ imprinted monolith that makes up the binding site cavity. The decrease of van der Waals volume due to poor shape complementarity and the increase of hydrophobic interactions result in the decrease of the retention and selectivity [21]. Compared with (*R*)-enantiomers, the (*S*)-enantiomers, with a functional group match to the imprint cavity, were found to be retained longer by the MIP stationary phase. Therefore, ONZ, with a functional group or analyte size match to the imprint cavity, could achieve better chiral separation than that of SNZ. The chiral recognition ability of a MIP chiral stationary phase is influenced by not only the composition of polymer but also the conditions of pCEC. Therefore, through the change of electrochromatographic conditions in pCEC, the retention times of the enantiomers of SNZ increased and the separation of the enantiomers of SNZ was realized (Fig. 9).

4 Concluding remarks

A (*S*)-ONZ molecularly imprinted monolithic column, which was prepared by a single-step thermal copolymer of MAA and 4-VP monomers and EDMA cross-linker, has

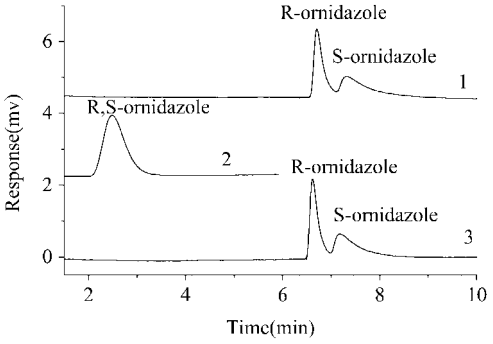

Figure 8. Analytical applications of (*S*)-ONZ imprinted monolith. Electrochromatographic conditions: capillary column: 62-cm column length, 41-cm effective length; mobile phase: ACN/50 mM NH_4Ac-HOAc buffer (98:2, v/v), other conditions are the same as in Fig. 3. MIP monolith: curve 1, ONZ in ACN; curve 3, ONZ pharmaceutical tablets in ACN. NIP monolith: curve 2, ONZ in ACN.

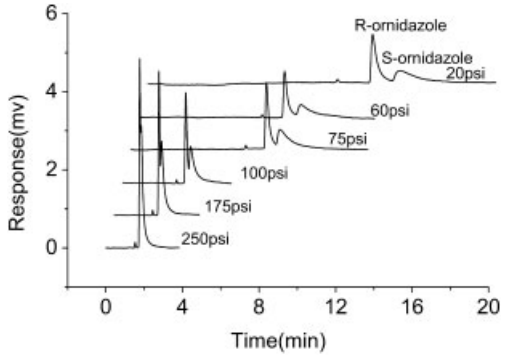

Figure 7. Effect of supplementary pressure on the chiral separation of ONZ on the (*S*)-ONZ imprinted monolithic column. Electrochromatographic conditions: the applied voltage, −20 kV; other conditions are the same as in Fig. 6.

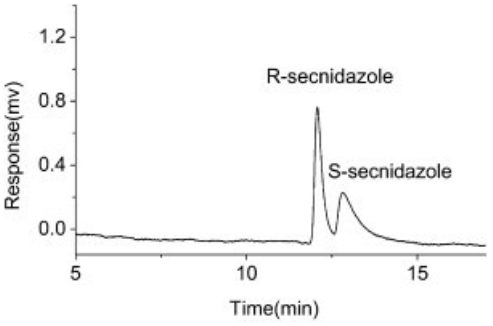

Figure 9. Chiral separation of SNZ on (*S*)-ONZ imprinted monolith. Electrochromatographic conditions: ACN/50 mM NH_4Ac-HOAc buffer (98:2, v/v), applied pressure 40 psi, voltage −10 kV; other conditions are the same as in Fig. 3.

been successfully used as a stationary phase for the rapid chiral separation of antiparasitic drugs by pCEC. In this first study of the MIP materials of ONZ, it was found that the MIP monolithic column was prepared using 4-VP and MAA as mixed functional monomers yielded better chiral recognition ability than that made of MAA or 4-VP. In this investigation on the conditions of column preparation and electrochromatography, cross-selectivity study, and comparison with NIP monolithic column, it was demonstrated that the recognition selectivity was mainly derived from the imprinting cavities on (*S*)-ONZ imprinted monolith and electrostatic and hydrogen-bonding interaction between antiparasitic drug and MIP. The fast chiral separations were achieved on the optimized MIP monolith within 9 min for ONZ and 14 min for SNZ which was firstly resolved by chromatogram method. Furthermore, this method has been successfully used in the chiral separation of ONZ in locally available pharmaceuticals. All these can provide reference for the preparation of MIP monolithic column which have MAA and 4-VP as functional monomers in pCEC.

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