Solid-Phase Extraction of *S*-(-)-Amlodipine from Plasma with a Uniformly Sized Molecularly Imprinted Polymer

Kaixia Luo, Meng Liu, Qiang Fu, Elijan Amut, Aiguo Zeng, Chun Chang

Faculty of Pharmacy, School of Medicine, Xi'an Jiaotong University, Xi'an 710061, China

ABSTRACT: To specifically extract *S*-(-)-amlodipine from plasma, uniformly sized molecularly imprinted polymers (MIPs) for *S*-(-)-amlodipine were prepared in an aqueous system by multistep swelling and polymerization with methacrylic acid as a functional monomer, ethylene glycol dimethacrylate as a crosslinker, and toluene as a porogen. Scanning electron microscopy was used to identify the structural features of the obtained polymers. ^{1}H-NMR and high-performance liquid chromatographic analysis were performed to explore the possible recognition mechanism. The results reveal that spherical polymer beads with uniform size and good monodispersity were obtained, and the MIPs showed specific recognition ability for the template molecule. The ionic hydrophobic and hydrogen-bonding interactions were inferred to play an important role in the recognition mechanism. The results indicate that the MIPs could be used as a solid-phase extraction sorbent for the concentration and purification of *S*-(-)-amlodipine from plasma with a high efficiency. The linear range was 0.25–8.00 μg/mL with a correlation coefficient of 0.9948. The average recovery was 98.3% with relative standard deviation (RSD) less than 9.1%. © 2012 Wiley Periodicals, Inc. J Appl Polym Sci 000: 000–000, 2012

Key words: high performance liquid chromatography; molecular imprinting; polymer synthesis and characterization

INTRODUCTION

Amlodipine, as a dihydropyridine calcium antagonist, is widely used for the treatment of hypertension. Because of its low concentration (typically < 1 ng/mL) in blood and the complexity of the matrices, tedious liquid–liquid extraction or solid-phase extraction (SPE) is routinely needed before analysis. The molecular imprinting technique, introduced by Wulff and Sarhan in 1972[1] and much advanced by Mosbach and Arshady in the 1980s,[2] is a technique used to produce molecularly imprinted polymers (MIPs), which can recognize target molecules specifically. With predetermined selectivity, recognition, feasibility, and chemical stability,[3] MIPs have been increasingly used as chromatographic media, including in the resolution of enantiomers,[4,5] SPE,[6,7] sensors,[8,9] artificial antibodies, and catalysts.[10,11] MIPs can be prepared by covalent or noncovalent methods. Noncovalent methods that are now frequently used include bulk polymerization,[12] suspension polymerization,[13,14] *in situ* polymerization,[15,16] precipitation polymerization,[17,18] and multistep swelling and polymerization.[19,20] Among these methods, the multistep swelling and polymerization method can be used to synthesize monodispersed and uniformly sized MIPs, which are suitable for chromatographic packing materials.[21]

It might be very useful to extract materials for the separation of dihydropyridine calcium antagonists from biological matrices based on MIPs.[19,22] In our previous study, to separate amlodipine enantiomers, a chiral MIPs monolithic column was prepared by an *in situ* polymerization method.[23] Although the column showed specific recognition ability, column collapse and template leaking were observed during its application. In this study, we tried to use another preparation method to obtain uniformly sized MIPs for *S*-(-)-amlodipine with the expectation of avoiding the disadvantages of the monolithic column. Then, the obtained MIPs were used as an SPE sorbent to purify and concentrate *S*-(-)-amlodipine from plasma after method validation. The results indicate that the MIPs could be used as an SPE sorbent for the concentration and purification of *S*-(-)-amlodipine from plasma with a high efficiency.

EXPERIMENTAL

Reagents and materials

Racemic amlodipine, *S*-(-)-amlodipine, and nimodipine were purchased from Pharmaceutical Chemical Reagent Plant (Ruicheng, People's Republic of China). Nitrendipine and nicardipine were obtained

TABLE I
Preparation Conditions for S-(-)-Amlodipine MIPs and NIPs

	Polymer no.	Template (mg)	Functional monomer (μL)	Porogen (mL)	Crosslinker (mL)	Initiator (g)
P_1	MIP_1	817.8	MAA/592	Toluene/5	EDMA/5	AIBN/0.20
P_2	NIP_1		MAA/592	Toluene/5	EDMA/5	AIBN/0.20
P_3	MIP_2	817.8	MAA/592	Cyclohexanol/5	EDMA/5	AIBN/0.20
P_4	NIP_2		MAA/592	Cyclohexanol/5	EDMA/5	AIBN/0.20
P_5	MIP_3	817.8	2-VPY/750	Cyclohexanol/5	EDMA/5	AIBN/0.20
P_6	NIP_3		2-VPY/750	Cyclohexanol/5	EDMA/5	AIBN/0.20

from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, People's Republic of China). Nilvadipine was obtained from Fujisawa Pharmaceutical Co. (Osaka, Japan). Ethylene glycol dimethacrylate (EDMA) and methacrylic acid (MAA) were obtained from Sigma-Aldrich (Milwaukee, WI). 2-Vinylpyridine (2-VPY) was also obtained from Sigma-Aldrich. 2,2′-Azobisisobutyronitrile (AIBN) was purchased from Shanghai No. 4 Reagent Factory (Shanghai, People's Republic of China). Sodium dodecyl sulfate was purchased from Xi'an Chemical Reagent Factory (Xi'an, People's Republic of China). Poly(vinyl alcohol) 400 (degree of polymerization (dp) = 400, saponification value = 87–89%) was purchased from Beijing Organic Chemical Reagent Factory (Beijing, People's Republic of China). EDMA was purified with a 10% sodium hydroxide solution and dried over anhydrous magnesium sulfate before use. MAA and 2-VPY were redistilled before use to remove polymerization inhibitor. Acetonitrile and methanol were chromatographic grade. All other reagents were analytical grade. Water was freshly distilled three times before use. Heparinized plasma, separated by blood centrifugation at 3000 rpm for 10 min, was obtained from Sprague-Dawley (SD) rats supplied by Xi'an Jiaotong University Experimental Animal Center (Xi'an, People's Republic of China).

Preparation of MIPs

The MIPs for S-(-)-amlodipine were prepared by the multistep swelling and polymerization method.[24] In each condition, the MIPs and nonimprinted polymers (NIPs) preparations were conducted twice, and the brief procedure was as follows: 1.5 mL of a polystyrene seed particle emulsion was swollen with the microemulsion prepared from dibutylphthalate (0.48 mL) and sodium dodecyl sulfate (0.02 g), both as activating solvents, and distilled water (10 mL) by sonication. This first-step swelling was carried out at room temperature for 15 h with stirring at 125 rpm until the micro-oil droplets completely disappeared. To the swollen particles, a dispersion of AIBN (0.20 g), toluene or cyclohexanol (5.0 mL), 4.8% poly(vinyl alcohol) 400 solution (10 mL), and water

(12.5 mL) were added. The second-step swelling proceeded at room temperature for 2 h with stirring at 125 rpm. According to the preparation scheme in Table I, S-(-)-amlodipine (0.817 g), EDMA (5.0 mL), and MAA (0.59 mL) or 2-VPY (0.75 mL) were added in sequence. Then, 10 mL of 4.8% poly(vinyl alcohol) solution and 12.5 mL of water were added to the dispersion of swollen particles. The third-step swelling was implemented at room temperature for 2 h with stirring at 125 rpm. After the third-step swelling was completed, the polymerization procedure was carried on with degassing by a stream of nitrogen gas for 24 h and stirring at 125 rpm at 50°C. Afterward, the polymerized polymer particles were dispersed in methanol, and the supernatant was discarded after sedimentation. This process was repeated three times in methanol, once in a mixture of methanol and water, and twice in tetrahydrofuran, and then, the microspheres were filtered and washed with acetone and then dried at room temperature. Comparatively, NIPs were synthesized simultaneously in accordance with the same procedure except for the addition of the template in the preparation.

Morphologies of MIPs

The morphologies of the prepared polymers were analyzed with a Hitachi S-570 scanning electron microscope (Kyoto, Japan) at 15 or 20 keV.

^{1}H-NMR analysis

Deuterated chloroform was used as the solvent. MAA, S-(-)-amlodipine, and the prepolymerization solutions were scanned and recorded with a Bruker Avance 300-MHz NMR apparatus (Zurich, Switzerland).

Chromatographic analysis

Preparation of the MIP stationary high performance liquid chromatography (HPLC) column

The prepared polymers were packed into a stainless steel column (50 mm × 4.6 mm i.d.) by a slurry packing method with a mixture of methanol and 2-propanol (2 : 1, v/v) as the slurry medium and with

methanol as the packing solvent. The column was washed online extensively by a mixture of methanol and acetic acid (4 : 1, v/v) to remove the templates and unreacted monomers, and then, it was eluted by methanol until a stable baseline was achieved.

Chromatographic conditions and evaluation parameters

The chromatographic evaluation experiments were carried out with a Shimadzu HPLC system (Kyoto, Japan) consisting of an LC-20 A pump, an SPD-20 A UV detector, and CS-Light Real Time Analysis Chromatographic software. The concentration of samples was about 1 mg/mL.

The retention factors (k) were calculated by the equation

$$k = (t_R - t_0)/t_0$$

where t_R and t_0 are the retention time and the void time, respectively. t_0 was measured by acetone. The separation factors (α) were measured as the ratio of the k:

$$\alpha = k_1/k_2$$

where k_1 and k_2 were the retention factors of given species. The selectivity factors (S) were calculated by the equation

$$S = k_{MIPs}/k_{NIPs}$$

where the k_{MIPs} and k_{NIPs} are the k of given species on the molecularly imprinted and nonimprinted polymers, respectively.

Extraction of *S*-(-)-amlodipine from plasma by MIP–SPE

The MIP–SPE cartridge was prepared under the optimized conditions, and the preparation procedure was similar to that described previously, except that the polymerization was carried out in a 2.5-mL, one-side sealed polypropylene syringe barrel. The obtained cartridge was washed with 20 mL of methanol–acetic acid (80 : 20, v/v) under negative pressure and sequentially with about 20 mL of methanol.

A 1.0 mg/mL stock solution of *S*-(-)-amlodipine was diluted to various concentrations with methanol. An aliquot of 1.0 mL of the plasma sample was spiked with 25 μL of the *S*-(-)-amlodipine standard solution at different concentrations, and then, 1.0 mL of acetonitrile was added to remove protein by precipitation and centrifugation. Then, a series of quality-control plasma samples with concentrations 0.25, 0.50, 1.00, 2.00, 4.00, and 8.00 μg/mL were obtained.

TABLE II
Chromatographic Retention Behavior of Amlodipine on the MIP Columns

| | k | | |
	R-(-)-amlodipine	*S*-(-)-amlodipine	α
P$_1$	10.13	14.46	1.43
P$_3$	21.84	31.74	1.45
P$_5$	0.45	0.45	1.00

HPLC conditions: mobile phase: methanol–PBS (20 mmol/L, pH 5.0, 70 : 30 v/v), detection wavelength = 238 nm, flow rate = 0.2 mL/min, column temperature = 50°C, loaded amount = 5 μL.

The spiked plasma samples (200 μL) were loaded onto the MIP–SPE column.

The MIP–SPE column was first washed with acetonitrile and then eluted with acetonitrile–acetic acid (9 : 1 v/v). The effluent liquid in each washing step was collected and evaporated to dryness with a nitrogen stream and then reconstituted in 50 μL of methanol before HPLC analysis.

RESULTS AND DISCUSSION

Selection of the functional monomer and porogen

MIPs for *S*-(-)-amlodipine were synthesized with different functional monomers and porogens. The prepared MIPs were evaluated by chromatographic experiments to examine the effects of the functional monomer and porogen on the separation of the amlodipine enantiomers. The results, listed in Table II, show that the MIPs functionalized by the acidic monomer MAA (P$_1$ and P$_3$) showed selectivity to *S*-(-)-amlodipine, whereas there was no selectivity to *S*-(-)-amlodipine in the MIPs functionalized by the basic monomer 2-VPY (P$_5$). It could be explained that the carboxyl groups of MAA could have interacted with the amine groups of *S*-(-)-amlodipine via ionic interaction, whereas hydrogen-bonding interaction existed between the amine groups of 2-VPY and the amine groups of *S*-(-)-amlodipine, which were not as strong as the ionic interactions. P$_1$ and P$_3$, which were prepared with toluene and cyclohexanol as porogens, respectively, both showed enantioselectivity. However, amlodipine had a much stronger retention on P$_3$ than on P$_1$. Because there appeared to be no obvious differences in α between P$_1$ and P$_3$, the P$_1$ column was chosen for further evaluation and application study. The enantioseparation of the amlodipine racemate, shown in Figure 1, illuminated a successful imprinting effect.

^{1}H-NMR spectroscopy

The ^{1}H-NMR spectra of *S*-(-)-amlodipine, MAA, and their prepolymerization solutions are shown in Figure 2. It was clear that the addition of *S*-(-)-
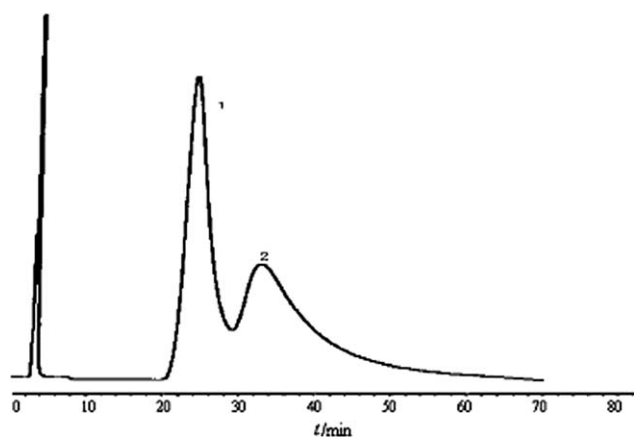
Figure 1 Chromatogram of amlodipine racemate on P_1: (1) R-(+)-amlodipine and (2) S-(-)-amlodipine. HPLC conditions: mobile phase: methanol–PBS (20 mmol/L, pH 5.0, 70 : 30 v/v), detection wavelength = 238 nm, flow rate = 0.2 mL/min, column temperature = 50°C, and loaded volume = 5 μL. t = time.

amlodipine to the functional monomer MAA resulted in a upfield shift of the carboxyl (—COOH) proton. When the molar ratio of MAA to S-(-)-amlodipine (T/M) was 1 : 4, the shift change of the carboxyl (—COOH) proton in MAA was 0.8 ppm, which was much higher than the shift change when the molar ratio was 1 : 2. This result indicates that there existed ionic interactions between MAA and S-(-)-amlodipine, which may have been the main force in making S-(-)-amlodipine and MAA form a complex in the prepolymerization solution.[22] At the same time, it might also have been one of the driving forces of the specific recognition for the template molecule, as shown in Figure 3.

Morphologies of the MIPs

The SEM of polymers prepared when MAA was adopted as the functional monomer and toluene was adopted as the porogen is shown in Figure 4. The diameters of the obtained spherical polymer beads were 3–5 μm, and many cavities were created on the surface of the beads, which might have been formed by the removal of the porogen from the particles during the washing cycle. Such a porous structure provided recognition sites and large surface areas.

Selectivity of the MIPs

The retention properties of the template, its structural analogues, and some other structurally unrelated compounds on the MIP and NIP columns manifested that the S for S-(-)-amlodipine was much higher than that for the other tested compounds on the MIP column. As shown in Figure 5, S for S-(-)-amlodipine was 13.8, almost two times higher than that in a previous report.[23] It was noticed that the MIPs were capable of specifically recognizing the

template in aqueous media and, consequently, could hopefully be applied to the concentration and purification of S-(-)-amlodipine from complex media.

Recognition mechanism of the polymers

Retention properties of the MIP column in an organic mobile phase

The organic solvent acetonitrile was employed as the mobile phase to determine the retention behaviors of the template and its structural analogues on the MIP and NIP columns (P_1 and P_2). It is obvious from
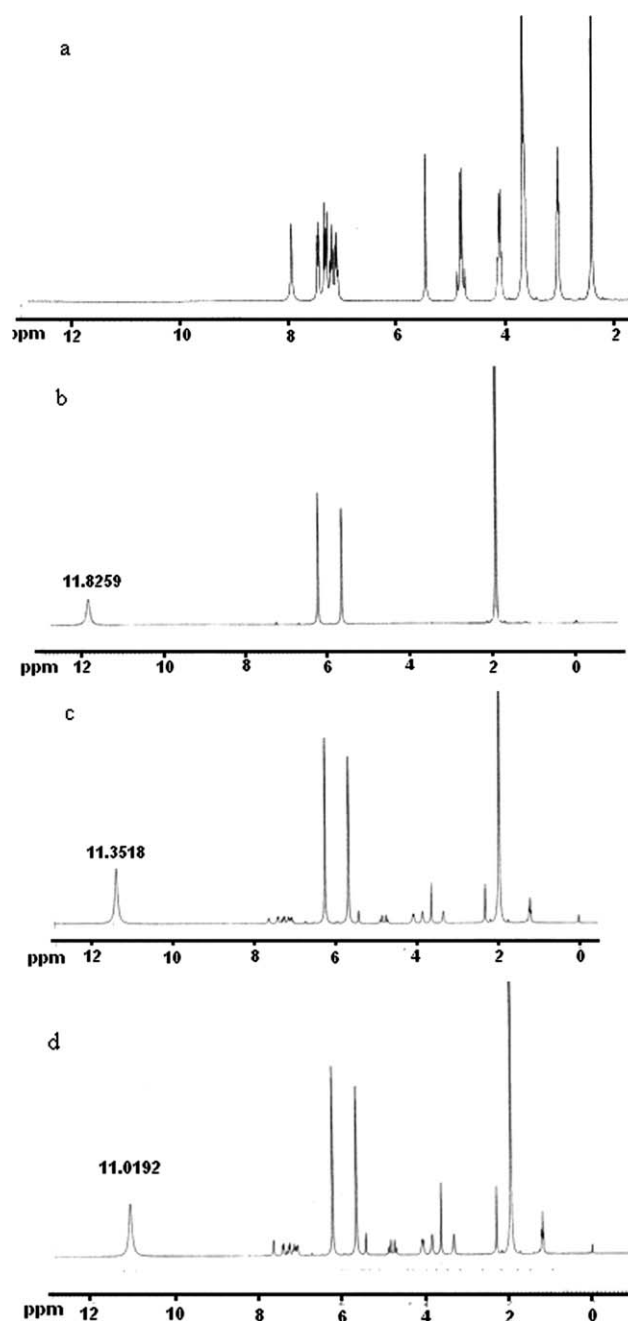

Figure 2 ^{1}H-NMR scanning spectra: (a) S-(-)-amlodipine, (b) MAA, (c) prepolymerization solution (T/M = 1 : 1), and (d) prepolymerization solution (T/M = 1 : 4).
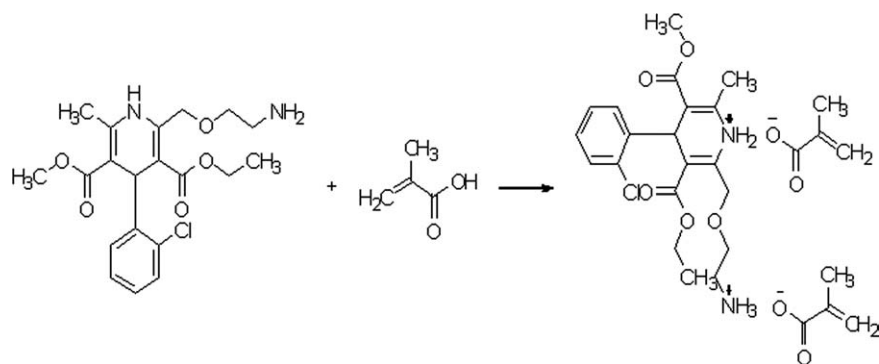
Figure 3 Schematic diagram of the template–monomer complex formation.

Table III that S-(-)-amlodipine was hardly eluted on P_1, whereas the other tested structural analogues were hardly retained, except for nicardipine. This result may be explained by the fact that nicardipine possessed amine groups ($-NH_2$ or $-NH-$), which could form ionic interactions with MAA. S-(-)-amlodipine had a much stronger retention on P_1 than on P_2, with $S = 5.16$ on P_1. However, there were no significant differences in the retention behaviors of the structural analogues on P_1 and P_2. All of these clarified that the specific selectivity and affinity for the template came from the imprinting process, whereas the other tested structural analogues was not specifically recognized by P_1.

The influence of acetic acid on k of S-(-)-amlodipine is shown in Figure 6. With increasing amount of acetic acid, k decreased greatly. When the amount of acetic acid was less than 1%, S-(-)-amlodipine

could hardly be eluted, whereas S-(-)-amlodipine was not retained on the MIPs when the amount of acetic acid was beyond 6%. Considering the results, we deduced that the addition of acetic acid increased the polarity of the solution and destroyed the interaction force between the MIPs and the analytes; this resulted in a decreased k.

Retention properties of the MIP column in the aqueous mobile phase

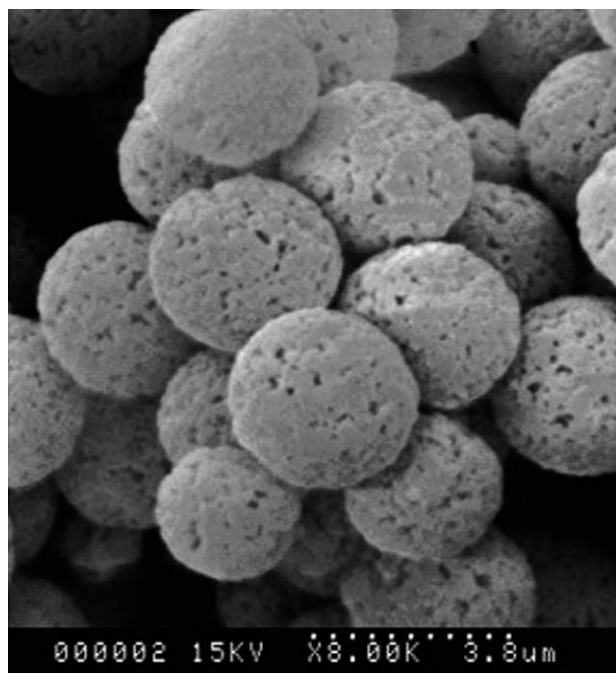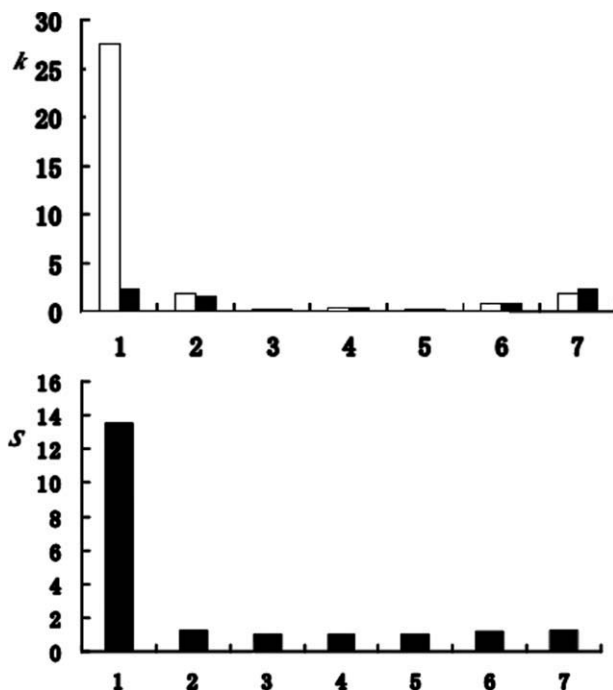The influence of the mobile phase pH on the k of the amlodipine enantiomers was studied when

Figure 5 Selectivity of MIP columns. MIPs NIPs: (1) S-(-)-amlodipine, (2) nicardipine, (3) nimodipine, (4) nitredipine, (5) nilvadipine, (6) matrine, and (7) ephedrine. HPLC conditions: columns: P_1 and P_2, mobile phase: acetonitrile–sodium phosphate buffer (20 mmol/mL, pH 5.5, 70 : 30 v/v), detection wavelength = 238 nm, flow rate = 0.5 mL/min, column temperature = 25°C, and injected amount = 5 µL.

Figure 4 Scanning electron micrographs of MIPs prepared by multistep swelling and polymerization.

TABLE III
Retention of S-(-)-Amlodipine and its Structural Analogues with Acetonitrile as the
Mobile Phase

			k		
	Nimodipine	Nitrendipine	Nilvadipine	Nicardipine	S-(-)-Amlodipine
P_1	0.1	0.1	0.1	9.3	62.5
P_2	0.2	0.2	0.3	10.4	12.1

methanol–sodium phosphate buffer solution (PBS; 20 mmol/L, 70 : 30, v/v) at different pHs was used as the mobile phase. As shown in Figure 7, the k of amlodipine enantiomers increased when the pH value of PBS changed from 3.0 to 6.0. The highest k was reached around pH 6.0, and the k decreased as the pH value changed to be above 6.0. The pK_a of MAA and amlodipine were 4.58 and 8.97, respectively. The outcomes could be explained by the ionization levels of MAA and amlodipine in the polymers. When the pH value of PBS changed from pH 3.0 to 6.0, the ionization degrees of the amine group in amlodipine and the carboxyl group of MAA became larger; this led to a stronger ionic interaction. When the pH value of PBS changed to be above 6.0, the ionization level of the amine group in amlodipine decreased gradually and gave rise to a smaller ionic interaction.

In study, a mixture of methanol–PBS (pH 4.0) was used as the mobile phase to evaluate the influence effect of the methanol content in the mobile phase on the retention characteristics of amlodipine enantiomers. As shown in Figure 8, the retention of S-(-)-amlodipine decreased as the methanol content increased from 30 to 70%, whereas it increased slowly as the methanol content increased from 70 to
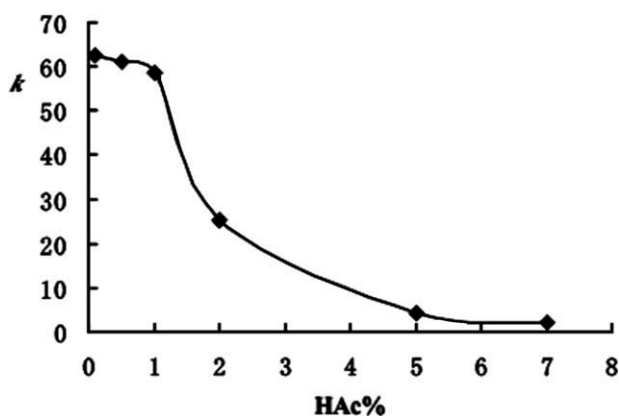
90%. These results could be explained by the fact that hydrophobic interactions were dominant when the methanol content was less than 70%, whereas the prevailing interactions changed into electrostatic force as the methanol content reached beyond 70%. It could be inferred that the solutes was retained on the MIP column mainly because of hydrophobic interactions, in addition to electrostatic interactions of the compounds with MAA in aqueous mobile phases.

Extraction of S-(-)-amlodipine from plasma with an MIP–SPE column

The washing solution and the final eluents from the MIP–SPE column were monitored by HPLC. The extraction conditions were optimized on the basis of the retention characteristics of S-(-)-amlodipine, and the HPLC conditions were established on the basis of the previous report.[23] As shown in Figure 9, the plasma samples ($n = 3$) were purified and
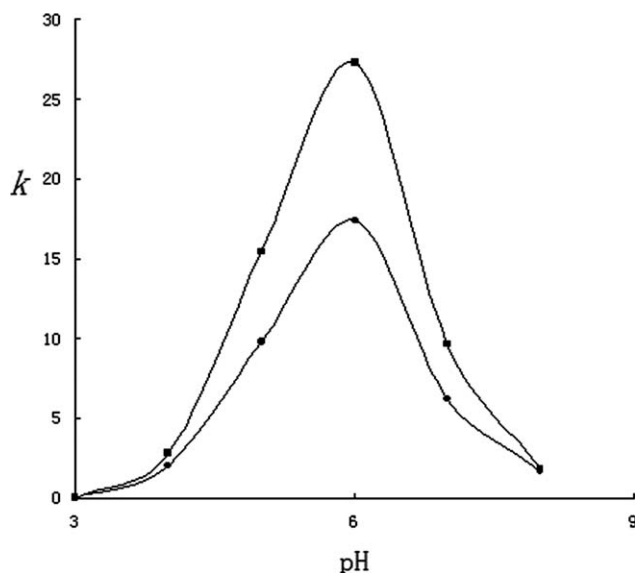
Figure 6 Influence of acetic acid (HAc) in the mobile phase on the retention of S-(-)-amlodipine. HPLC conditions: column: MIP–HPLC column (P_1), mobile phase: acetonitrile–acetic acid (HAc) (v/v), detection wavelength = 238 nm, flow rate = 0.5 mL/min, column temperature: room temperature, and loaded amount = 5 μL.

Figure 7 Effect of the pH of the mobile phase on the capacity factor of amlodipine enantiomers on P_1. S-(-)-Amlodipine R-(-)-amlodipine HPLC conditions: mobile phase: methanol–20 mmol/L PBS (70 : 30 v/v), detection wavelength = 238 nm, flow rate = 0.2 mL/min, column temperature = 50°C, and loaded amount = 5 μL.
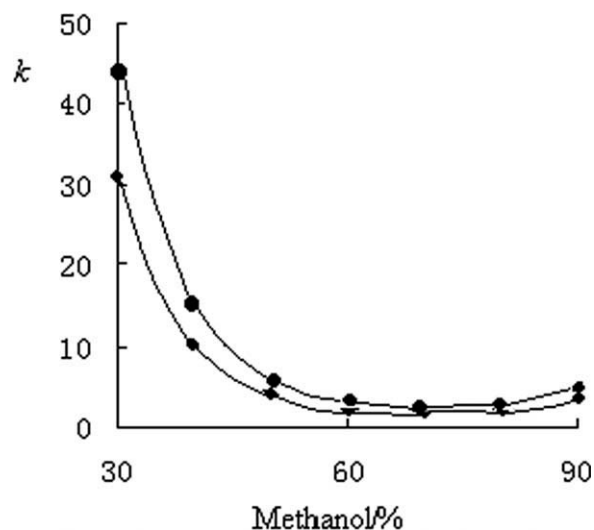
Figure 8 Effect of the proportion of methanol in the mobile phase on the capacity factor of the amlodipine enantiomers on P_1. S-(-)-Amlodipine R-(-)-amlodipine HPLC conditions: mobile phase: methanol–PBS (20 mmol/L, pH 4.0, v/v), detection wavelength = 238 nm, flow rate = 0.2 mL/min, column temperature = 50°C, and loaded amount = 5 μL.

Figure 9 Chromatograms of the S-(-)-amlodipine plasma sample: (a) S-(-)-amlodipine standard solution, (b) S-(-)-amlodipine plasma sample without MIP–SPE treatment, and (c) S-(-)-amlodipine plasma sample with MIP–SPE treatment. HPLC conditions: column: Shimadzu VP-Octadecylsilyl (VP-ODS) column (250 × 4.6 mm², i.d. = 5 μm), mobile phase: methanol–sodium dihydrogen phosphate solution (30 mmol/L, 70 : 30, v/v), detection wavelength = 238 nm, flow rate = 1.0 mL/min, column temperature: room temperature, and load volume = 20 μL. t = time.

concentrated after extraction by the MIP–SPE column. The chromatogram was significantly cleaner after the MIP–SPE cleanup protocol. A standard calibration curve was plotted by the concentration of quality-control plasma [x axis (μg/mL)] and the peak area (y axis). The linear equation was $y = 161{,}918x + 124{,}438$, with a correlation coefficient of 0.9948. On this basis, we perceived that a linear correlation relationship existed between the peak area and the concentration of S-(-)-amlodipine over the range 0.25–8 μg/mL. The results of recovery are presented in Table IV, and the average recovery of S-(-)-amlodipine was 98.3% with relative standard deviation (RSD) less than 9.1% ($n = 5$). Compared with the monolithic column,[23] during the MIP–SPE column application, no column collapse was observed with satisfactory average recovery and stability. The MIP–SPE cartridge was used 25 times without significant deterioration in separation efficiency. In view of these results, it is hoped that the established method could be applied to S-(-)-amlodipine analysis in plasma.

CONCLUSIONS

Spherical MIPs for S-(-)-amlodipine were synthesized by a multistep swelling polymerization method in an aqueous system. The MIPs had a specific binding ability and high selectivity for S-(-)-amlodipine. The MIP cartridge had good workability for the concentration and purification of S-(-)-amlodipine from plasma; this suggests that it might be a
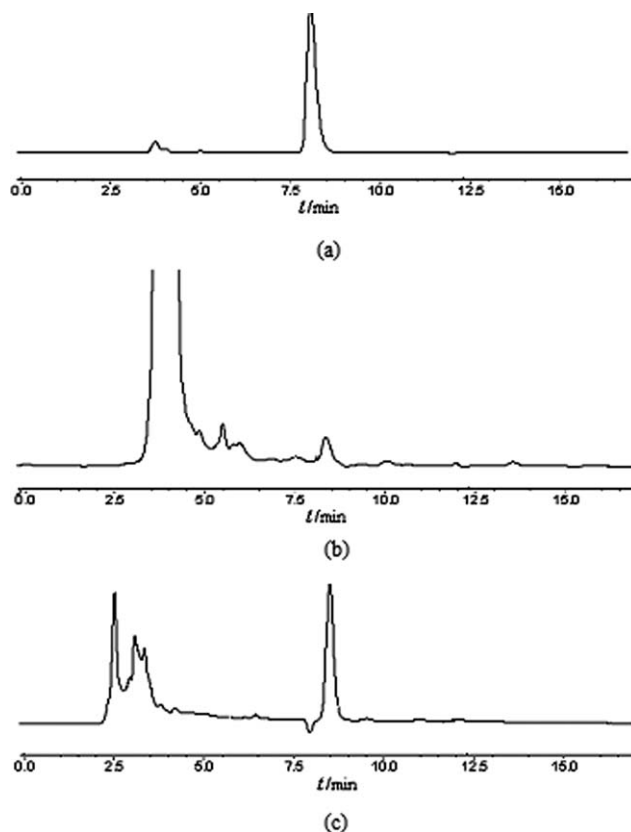
TABLE IV
Accuracy and Precision Results of the Method

Spiked amount (μg/mL)	Measured amount (μg/mL)	Method recovery (%)	RSD (%)
0.25	0.27	106.1	9.1
2.00	1.81	90.6	3.0
8.00	7.87	98.3	3.0

$n = 5$.

promising separation material for S-(-)-amlodipine analysis.

References

1. Wulff, G.; Sarhan, A. Angew Chem Int Ed 1972, 11, 341.
2. Arshady, R.; Mosbach, K. Macromol Chem Phys 1981, 182, 687.

3. Beltran, A.; Caro, E.; Marce, R. M.; Cormack, P. A. G.; Sherrington, D. C.; Borrull, F. Anal Chim Acta 2007, 597, 6.

4. Birnbaumer, G. M.; Lieberzeit, P. A.; Richter, L.; Schirhagl, R.; Milnera, M.; Dickert, F. L.; Bailey, A.; Ertl, P. Lab Chip 2009, 9, 3549.

5. Fu, Q.; Sanbe, H.; Kagawa, C.; Kunimoto, K. K.; Haginaka, J. Anal Chem 2003, 75, 191.

6. Bravo, J. C.; Garcinuno, R. M.; Fernandez, P.; Durand, J. S. Anal Bioanal Chem 2007, 388, 1039.

7. Gonzalez, M. L.; Quintana, J. B.; Rodriguez, I.; Rodil, R.; Gonzalez, P. J.; Cela, R. J Chromatogr A 2009, 1216, 8435.

8. Sadowska, M.; Wandelt, B. Mol Cryst Liq Cryst 2008, 486, 1245.

9. Belmont, A. S.; Jaeger, S.; Knopp, D.; Niessner, R.; Gauglitz, G.; Haupt, K. Biosens Bioelectron 2007, 22, 3267.

10. Skudar, K.; Bruggemann, O.; Wittelsberger, A.; Ramstrom, O. Anal Commun 1999, 36, 327.

11. Visnjevski, A.; Schomacker, R.; Yilmaz, E.; Bruggemann, O. Catal Commun 2005, 6, 601.

12. Brueggemann, O.; Haupt, K.; Ye, L.; Yilmaz, E.; Mosbach, K. J Chromatogr A 2000, 889, 15.

13. Lai, J. P.; Lu, C. Y.; He, X. W. Chem J Chin Univ 2003, 24, 1175.

14. Khan, H. U.; Goel, P. K.; Gupta, V. K.; Wadhera, B. M. L.; Bhattacharyya, K. K. J Appl Polym Sci 1996, 61, 1.

15. Lin, L.; Li, Y.; Fu, Q.; He, L.; Zhang, J.; Zhang, Q. Polymer 2006, 47, 3792.

16. Zhuang, Y.; Luo, H. P.; Duan, D. L.; Chen, L. R.; Xu, X. J. Anal Bioanal Chem 2007, 389, 1177.

17. Liu, Y.; Hoshina, K.; Haginaka, J. Talanta 2010, 80, 1713.

18. Yoshimatsu, K.; Reimhult, K.; Krozer, A.; Mosbach, K.; Sode, K.; Ye, L. Anal Chim Acta 2007, 584, 112.

19. Fu, Q.; He, L.; Zhang, Q. Q.; Amut, E.; Fang, Q.; Chang, C. J Appl Polym Sci 2009, 111, 2830.

20. Haginaka, J.; Tabo, H.; Ichitani, M.; Takihara, T.; Sugimoto, A.; Sambe, H. J Chromatogr A 2007, 1156, 45.

21. Sambe, H.; Hoshina, K.; Haginaka, J. J Chromatogr A 2007, 1152, 130.

22. Fu, Q.; He, L. C. Chin J Clin Pharm 2006, 15, 76.

23. Amut, E.; Fu, Q.; Fang, Q.; Liu, R.; Xiao, A. P.; Zeng, A. G.; Chang, C. J Polym Res 2010, 17, 401.

24. Li, Y. C.; Fu, Q.; Zhang, Q. Q.; He, L. C. Anal Sci 2006, 22, 1355.