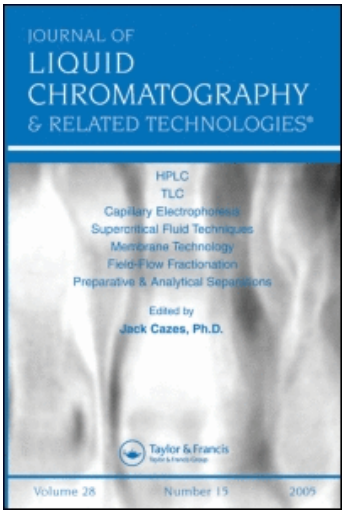

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Chiral Separation of Mandelic Acid and Its Derivatives by Thin-Layer Chromatography Using Molecularly Imprinted Stationary Phases

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Abstract: Molecularly imprinted polymers (MIPs) of *L*-mandelic acid (*L*-MDA) and its derivatives *L*-2-chloromandelic acid (*L*-2-MDA), *L*-4-chloromandelic acid (*L*-4-MDA) were prepared. The thin-layer chromatograms were constructed with these MIPs as chiral stationary phases (CSPs) and the mixture of acetonitrile and acetic acid as mobile phase. The effects of acetic acid content on separation were investigated. With the development system of 5% acetic acid in acetonitrile, the MIPs made CSPs separate the template molecules from their racemates with the chiral separation factor α of 1.45, 1.62, and 1.56 for MDA, 2-MDA, and 4-MDA, respectively. In relation to the template, its structural analogues have a lower separation factors on corresponding CSPs. This was attributed to the best compatibility of template molecules with the cavities of MIPs.

Keywords: Molecularly imprinted polymers, Mandelic acid, Derivatives, Chiral stationary phases, Thin-layer chromatography, Chiral recognition

INTRODUCTION

A reliable, sensitive, and fast method for separation of an enantiomer is very practical. For example, the optical isomers of chiral drugs and drug

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intermediates should be separated from drugs since they cannot be accepted by the target section, and have side effects or even toxic effects.[1,2] Molecular imprinting is an attractive tool for producing polymers to be applied in separating or detecting chiral compounds and analogs.[3–5] With this technology, the appropriate monomers were chosen to form a stable complex around the template molecules, and then the complexes were solidified by forming a high crossed polymer. After the removal of templates, the imprinted cavities left in the MIPs could distinguish the templates from other analytes, even analogues. This technique is schematically shown in Fig. 1. On account of its special recognition capacity, the MIP has been used as a chiral chromatographic stationary phase of high performance liquid chromatography (HPLC) for a number of amino acids, saccharides, and drugs.[6–8] Thin-layer chromatography (TLC) is a practical, widely used, and well developed analytical technique. It has a number of advantages compared to HPLC, such as convenience, rapidity, economic feasibility, and high productivity.[9,10] Using MIPs as stationary phases in TLC will largely increase its ability in enantioseparation, and this has been demonstrated first by Kriz et al.[11] in separating racemic phenylalanine derivatives. The earliest application of MIP based TLC on the separation of chiral drugs was made by Suedee et al.[12,13] who separated a number of adrenergic drug racemates. Recently, this method has also been used in separating a series of flavonoids using morin imprinted TLC plates by Xu et al.[14] These previous studies revealed that MIP based TLC may be an alternative technology in the resolution of racemic drugs through a simple process.

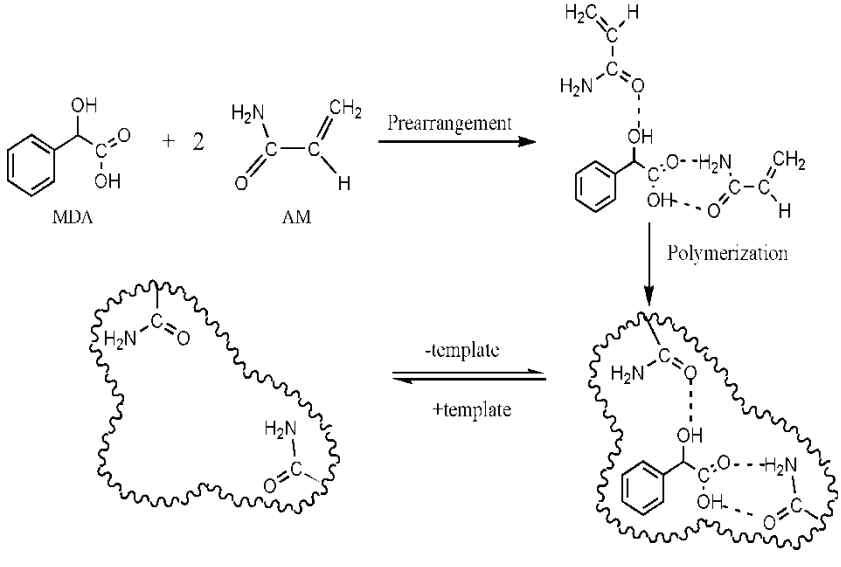

Figure 1. Schematic representation of the molecular imprinting procedures.

L-MDA and its derivatives (Fig. 2) are multifunctional precursors for synthesis of many optical pure amino acids, angiotensin converting enzyme inhibitors, and coenzyme A, etc.[15] For this reason, a facile and effective method for control of optical purity of this class of compounds was highly desirable. Racemic MDA has been resolved into its enantiomers using many different methods, such as HPLC using β-cyclodextrin or Kromasil CHI-TBB as chiral stationary phase,[16,17] and TLC performed with the use of ligand exchange plates based on reversed phase silica gels impregnated with copper salts and optically active amino acid derivatives,[18] which possess lower stability of CSP; desorbed metal ion may contaminate the sample.

In this paper, we use *L*-MDA and its derivates as templates to prepare MIP and illustrate the direct TLC separation of racemates of MDA and its derivatives by MIP type of CSP. The chromatographic condition for chiral separation was discussed, and the results showed that MIP based TLC plates could separate not only the racemic template but also the racemates of template's analogues.

EXPERIMENTAL

Reagents and Instruments

L-mandelic acid (*L*-MDA), *D*-mandelic acid (*D*-MDA), *L*,*D*-mandelic acid (*L*, *D*-MDA), *L*-2-chloromandelic acid (*L*-2-MDA), *L*-4-chloromandelic acid (*L*-4-MDA), *L*,*D*-2-chloromandelic acid (*L*,*D*-2-MDA), *L*,*D*-4-chloromandelic acid (*L*,*D*-4-MDA), Ethylene glycol dimethacrylate (EGDMA), 2,4,6-trimethylbenzoylphenylphosphinic acid ethyl ester (Lucirin LR 8893), fluorescent indicator (Zinc orthosilicate activated by manganese), $CaSO_4 \cdot 1/2H_2O$, Acrylamide (AM), acetonitrile, methanol, and acetic acid are all commercial compounds and used without further purification. A UV-spectrophotometer (UV-8500), scanning electron microscope (SEM) (Srion, FEI), N_2 adsorption instrument (100CX, OMNISORP), and UV analyzer (ZF-I) were used in the experiment.

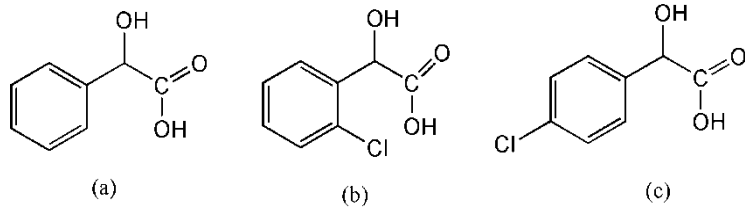

Figure 2. The chemical structures of (a) Mandelic acid, (b) 2-chloromandelic acid, and (c) 4-chloromandelic acid.

UV Spectroscopy Analysis

A series of template monomer solutions were prepared by adding various amounts of acrylamide into *L*-MDA acetonitrile solution. The concentration of *L*-MDA was 0.5 mmol/L while the concentration of acrylamide varied from 0 to 2.0 mmol/L. The solutions were put in a constant temperature bath oscillator for 5 h, and then the absorption of *L*-MDA in samples was monitored by a dual channel UV-spectrophotometer with an acrylamide solution as reference. The concentration of acrylamide in reference solution was same as that in the sample.

Preparation of Polymers

Four kinds of molecularly imprinted polymers were prepared using L-MDA, *D*-MDA, *L*-2-MDA, and *L*-4-MDA as the templates, respectively. Acrylamide was selected as the functional monomer using EGDMA as the crosslinker and LR as initiator. In the preparation of polymers, 0.5 mmol of template and 2 mmol of acrylamide were completely dissolved in 7 mL of acetonitrile, then was added 10 mmol of EGDMA and 12 mg LR. After 10 min purging with N_2, the system was irradiated under UV light (365 nm) at a constant 4°C for 24 h. The resultant bulk polymers were finely ground to pass through a 100 μm sieve. Polymer particles were allowed to sediment several times in acetone to remove the fine particles and placed in a Soxhlet extractor to wash out the templates by methanol and acetic acid (8:2, V/V) until no template molecule could be detected by UV analysis in the elution, followed by elution with methanol to remove the retained acetic acid and dried under vacuum overnight. The non-imprinted polymer (NIP) was prepared as the above steps in the absence of a template.

Chromatographic Procedures

Polymer particles (1 g), $CaSO_4 \cdot 1/2H_2O$ (1 g) and fluorescent indicator (0.2 g) were mixed carefully with distilled water (20 mL) and 20 μL of ethanol. The slurry was cast onto a glass microscope slides (76 × 26 mm) and allowed to dry at room temperature for 24 h. A uniform thin layer was formed with the thickness of about 0.3 mm. Development was performed with the mobile phases composed of acetonitrile and different concentrations of acetic acid (1, 5, 10%, v/v). The samples to be analyzed were dissolved in acetonitrile (4 g/L). After the equilibration of the mobile phase in the development chamber for 30 min, the plates with the adjacent spots from 1 μL volumes of the racemic solution and *L*-enantiomer solution were developed to a distance of 5 cm in about 6 min at ambient temperature. Then, the plates were air dried and the spots were detected by a UV analyzer

(254 nm) and identified by comparison of R_f values of standards developed simultaneously. The separation factor (α) for the racemate was defined as the ratio of the higher R_f value to the lower *Rf* value for the two separated spots.[10] Two parallel experiments were performed for each sample.

Morphologic Analysis

Pore volume and surface area of MIP and NIP were determined by nitrogen adsorption measurements. The morphologic images were obtained by SEM.

RESULTS AND DISCUSSION

UV Analysis

It is now commonly accepted that the principle of molecular imprinting lies in the preservation of the host guest structure in the polymerization solution into a polymer matrix. Therefore, it is important to investigate the interaction between the template and functional monomers at the prearrangement stage. To determine the ratio of the template molecule and the functional monomer, we use UV spectra to study the binding interaction of MDA and acrylamide in acetonitrile.[5]

As shown in Fig. 3, the λ_{max} of MDA absorption band was red shifted and the adsorption intensity was decreased with increased acrylamide concentration. This is typically due to hydrogen bonding formation as depicted in Fig. 1, which changes the electron density of the conjugated system and charge distribution on the whole molecule.[19] When the concentration of acrylamide was increased to 2.0 mmol/L (i.e., the mole ration of template to monomer was 1:4), a large difference was observed indicating the strong interactions achieved between the functional monomer and template with better recognizing ability. The higher concentration would not always bring about better results on enantioseparation because the more excessive acrylamide would result in the nonselective binding sites formed outside the imprinted cavities. The ratio was, therefore, set at 1:4 to synthesize the MDA series imprinted polymer in this paper.

Morphologies of MIP

Pore volume and surface area of the L-2-MDA imprinted polymer were determined to be 0.1715 cm^3/g and 151.35 m^2/g in contrast to 0.0051 cm^3/g and 0.5564 m^2/g of NIP, indicating that MIP is more porous than NIP. Figure 4 shows the scanning electron micrographs of MIP materials prepared in this work. It can be observed, that there are many cavities and channels in the

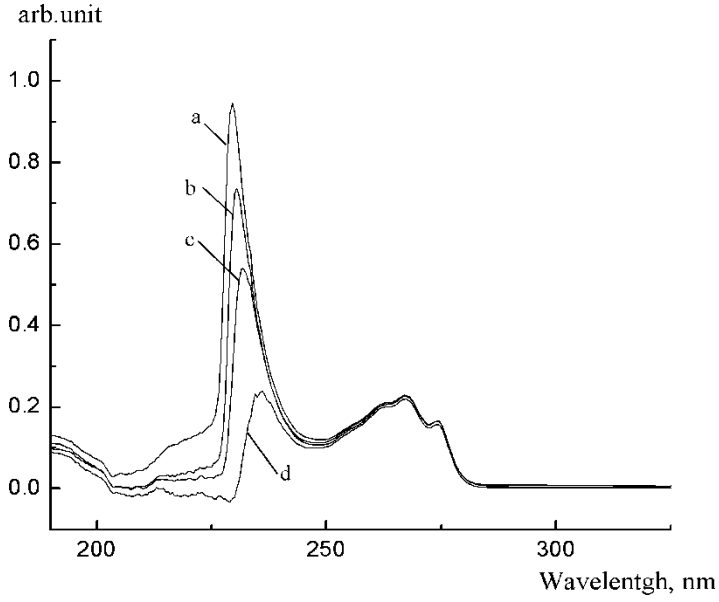

Figure 3. Concentrations influence of acrylamide on UV absorption of *L*-MDA in acetonitrile *L*-MDA: 0.5 mmol/L; Acrylamide/(mmol/L): *a*, 0; *b*, 0.5; *c*, 1.0; *d*, 2.0.

network skeleton of the polymer particles. These cavities and channels enable fast mass transfer of the solutes and provide high adsorption capacity.

The imprinted cavities in the MIP and the template induced functional groups on the cavities determine the template rebound to the polymer with high affinity and make it possible to separate the racemates of the templates, while the NIP can not afford the chiral resolution for any analyte.

Chiral Separation

Separation of *L*,*D*-MDA was first examined on the CSP made from *L*-MDA imprinted polymer, with acetonitrile as mobile phases containing various concentrations of acetic acid (1, 5, 10%). Table 1 shows the R_f values and the separation factors (α) for racemic MDA and its derivatives. On *L*-MDA specific CSP, both R_f value and separation factor of *L*- and *D*-MDA increased with the increased acetic acid content, the maximum α value of 1.45 was achieved in a mobile phase containing 5% acetic acid. The reason lies in the competition between acetic acid and racemic molecules in the hydrogen bond formation with binding sites on MIP, this would result in a quicker adsorption-desorption equilibrium and increase the affinity difference between two enantiomers. However, an exorbitant acid concentration would reduce the amount of effective binding sites and the possibility of hydrogen

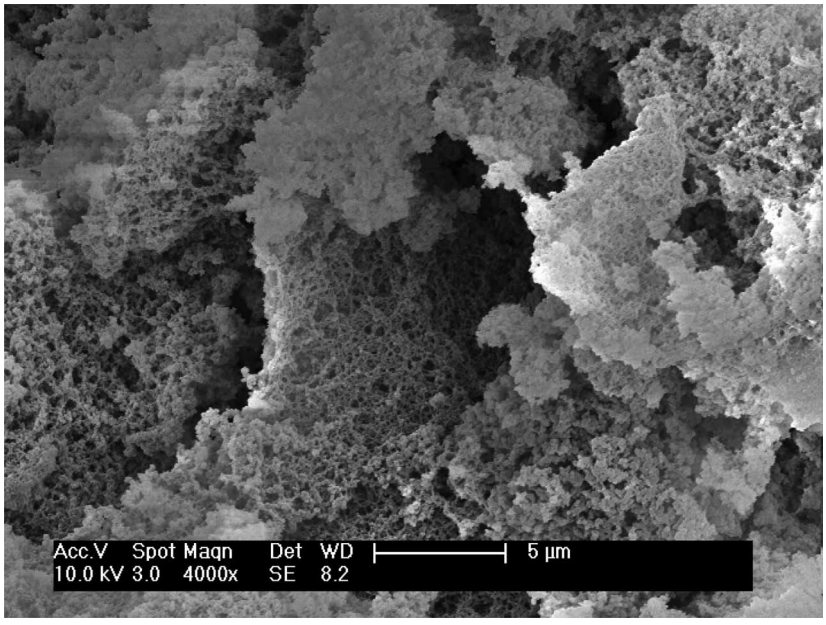

Figure 4. Scanning electron micrographs (SEM) of molecularly imprinted polymers.

bond interactions between the sample molecule and the binding sites. When the acetic acid concentration was as high as 10%, the separation factor decreased to 1.12. Therefore, a development system of 5% acetic acid in acetonitrile was used in the subsequent separations. Also shown in Table 1, the R_f value of *L*-MDA is lower than that of *D*-MDA on *L*-MDA specific CSP, indicating that *L*-MDA was more retained than *D*-MDA on the *L*-specific plates. This result is anticipated since the stereo structure of *D*-MDA molecule is not complementary to the cavities in the *L*-MDA imprinted polymer, which induces the weaker interaction of *D*-MDA

Table 1. Separation of racemates of templates on stationary phase based on MIPs and NIP

Template	$c_{(\text{Acetic acid})}/\%$	$R_f(L)$	$R_f(D)$	α
	1	0.27	0.35	1.30
L-MDA	5	0.40	0.58	1.45
	10	0.67	0.75	1.12
L-2-MDA	5	0.29	0.47	1.62
L-4-MDA	5	0.34	0.53	1.56
D-MDA	5	0.60	0.42	1.43
NIP	5	0.85	0.85	1.00

molecules with binding sites on the cavities. There was specific recognition of MIP to the template molecules, which was also verified by R_f value and separation factor of *L*,*D*-MDA, obtained when using *D*-MDA imprinted plates, on which *D*-MDA was more retained than the *L*-isomer. The R_f values and separation factors were also investigated on *L*-2-MDA and *L*-4-MDA imprinted plates. It was found that *L*,*D*-2-MDA and *L*,*D*-4-MDA could be separated with the separation factor of 1.62 and 1.56, respectively, on their own *L*-isomer imprinted plates. No separation and much less retention of the analyte was observed on plates based on NIP.

Chiral Recognition

The performance of MIP based TLC was further examined by separating the racemates of MDA series derivates. The result was shown in Table 2, where the symbols $P_{L\text{-MDA}}$, $P_{L\text{-2-MDA}}$, $P_{L\text{-4-MDA}}$, and P_{NIP} were defined as *L*-MDA, *L*-2-MDA, *L*-4-MDA imprinted plates, and NIP made plates, respectively.

As seen in Table 2, template imprinted plates have the best resolution to the template and its enantiomer. In all cases, the separation factor, α, is larger than 1.40. This fact confirms the formation of super molecules between template and functional monomers and the keeping of these super molecular structures during synthesis of the imprinting polymers, which enable the cavities in MIP to have enough recognition to template molecules. The data list in Table 2 also shows the feasibility of MIP based TLC in separating racemates of template's analogs. For example, *L*,*D*-2-MDA and *L*,*D*-4-MDA could be resolved on *L*-MDA imprinted plate ($P_{L\text{-MDA}}$), but with lower separation factors (1.20 and 1.10, respectively) than that of *L*,*D*-MDA. Similar results were found in the separation of *L*,D-MDA ($\alpha = 1.25$), *L*,*D*-4-MDA ($\alpha = 1.09$) on *L*-2-MDA imprinted plate ($P_{L\text{-2-MDA}}$) or separation of *L*,*D*-MDA ($\alpha = 1.19$), *L*,*D*-2-MDA ($\alpha = 1.10$) on *L*-4-MDA imprinted plate ($P_{L\text{-4-MDA}}$). In comparison, no enantiomeric resolution was observed on plates prepared by NIP for all racemates.

Table 2. Separation factor of MDA, 2-MDA and 4-MDA on *L*-isomers imprinted polymers and NIP

	Separation factor (α)			
Sample	$P_{L\text{-MDA}}$	$P_{L\text{-2-MDA}}$	$P_{L\text{-4-MDA}}$	P_{NIP}
L,*D*-MDA	1.45	1.25	1.19	1.00
L,*D*-2-MDA	1.21	1.62	1.10	1.00
L,*D*-4-MDA	1.10	1.09	1.56	1.00

Note: Analyses were performed with the mobile phase of acetonitrile containing 5% acetic acid.

When separating racemates of *L*,*D*-MDA, *L*,*D*-2-MDA, and *L*,*D*-4-MDA by various MIP plates, it has been found that *L*,*D*-4-MDA has the lowest separation factor, except on the *L*-4-MDA imprinted plate ($P_{L\text{-4-MDA}}$). The reason may arise from a stereo effect, i.e., larger volume of 4-MDA will obstruct the entrance of *L*-4-MDA into cavities induced by *L*-MDA or *L*-2-MDA with functional monomers. These results indicate that besides the binding sites in the imprinted cavities, the stereo structure of the cavities also plays an important role on separation.

CONCLUSIONS

Molecularly imprinted polymers employed as chiral stationary phases of thin-layer chromatography for separation of *L*-MDA and its derivatives was studied in this research. The racemates of MDA series compounds were resolved successfully by their *L*-isomer imprinted polymers with a separation factor over 1.40. These results suggest that the use of MIP for the TLC is a potentially useful method in separation, analysis, and determination of enantiomers.

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