

Chiral Separation of R,S Clopidogrel with Monolithic Molecularly Imprinted Polymers

Zhiwei Li

College of Chemical & Pharmaceutical Engineering
Hebei University of Science and Technology
Shijiazhuang, China
e-mail: lizhiwei@hebust.edu.cn

Jin xia

College of Chemical & Pharmaceutical Engineering
Hebei University of Science and Technology
Shijiazhuang, China
e-mail: lizhiwei@hebust.edu.cn

Yanling Gao

College of Chemical & Pharmaceutical Engineering
Hebei University of Science and Technology
Shijiazhuang, China
e-mail: lizhiwei@hebust.edu.cn

Qingguo He

Shijiazhuang Zhitong Pharmaceutical Technology Co.Ltd
Shijiazhuang, China
e-mail: lizhiwei@hebust.edu.cn

Abstract—A monolithic column of molecularly imprinted polymer was prepared by an in situ polymerization method with a compound clopidogrel as the template. The molecular recognition capabilities of the prepared monolithic MIP were evaluated by separating the enantiomers. The characteristics of the column and the influences of mobile phases were studied. The results showed that the monolithic column has chiral recognition capability for the template and the enantiomers were baseline separated even in aqueous solution.

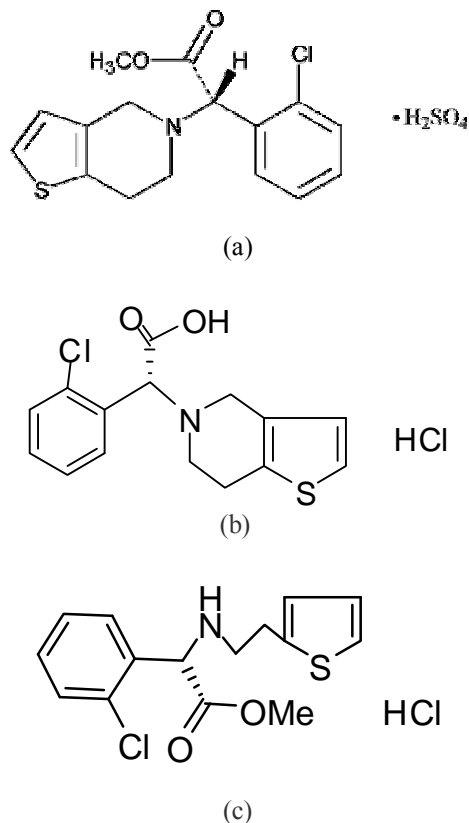
Keywords—monolithic column; molecularly imprinted polymers; chiral separation

I. INTRODUCTION

Molecularly imprinted polymers (MIPs) have been becoming attractive as effective materials for functional separations due to their high selectivity for the objective compounds, namely, the imprinted molecules [1-6]. Because MIP can recognize a specific molecule (template), the molecular imprinting technique has been proven to be an effective tool for chiral separation, solid-phase extraction (SPE), and enrichment of analytes and for use in sensors, immunoanalysis, and catalysts. In recent years, monolithic supports as stationary phases in high performance liquid chromatography (HPLC) and capillary electrochromatography (CEC) have gained significant interest due to their ease of preparation, high reproducibility, versatile surface chemistries and fast mass transport. This type of MIPs exhibited recognition ability for some imprint molecules such as theophylline, nicotine, diamionaphthalene, cinchona alkaloid and enantiomers[7-10]. Because more than half of all drugs on the market are asymmetric and almost 90% of these are administered as racemates, it is very important to separate the racemates and isolate the pharmacodynamically active enantiomer. The advantage of monolithic technique is its easy handling; a single-step procedure which enables us to prepare a column packed with molecular imprinted polymers without any tedious steps because the polymerization is carried out in the column.

Clopidogrel (CL) is a potent anti-platelet and anti-thrombotic drug. Figure 1 shows the structures of Clopidogrel and the impurity A, B and C. Clopidogrel and its impurities were determined by LC using a chiral column and CZE using chiral additive[11-13].

In this paper, a molecularly imprinted monolithic column was prepared and used to separate the enantiomers in solutions containing aqueous.



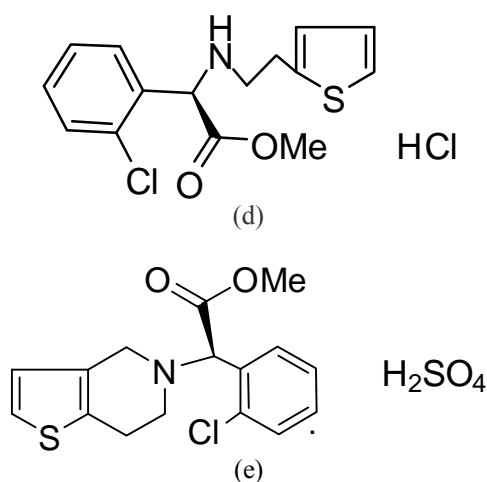


Figure 1. Chemical structures of (a) clopidogrel (S-enantiomer), (b) impurity A, (c,d) impurity B (racemic mixture of isomers), and (e) impurity C (R-enantiomer).

II. EXPERIMENTS

A. Materials

Clopidogrel and its enantiomers were synthesized in our Laboratory. Methanol, acetonitrile of HPLC grade were purchased from Fisher (New Jersey, USA), ethylene dimethacrylate (EDMA) was from Acros (New Jersey, USA), 2,2'-Azobisisobutyronitrile (AIBN) was purchased from Shanghai Chemical Plant (Shanghai, China). Methacrylic acid (MAA), 1-dodecanol and tetrahydrofuran were purchased from Tianjin No.1 Chemical Reagent Factory (Tianjin, China). AIBN was recrystallized. EDMA and MAA were distilled to remove the inhibitors before the polymerization. All solutions and samples were filtered through a 0.45 μ m membrane filter (Millipore-Q) before use.

B. Apparatus

Chromatographic systems were consisted of a LC-10ADvp pump and a variable-wavelength SPD-10Avp detector (Shimadu, Japan). Data processing was performed with an HW-2000 chromatography workstation (Nanjing Qianpu Software, China). Tridimensional structures of the compound and the interaction of the template with functional monomer were simulated with Soft ChemOffice 2006.

C. Preparation of MIP and NIP materials

CL, crosslinking monomer EDMA, functional monomer MAA, 1-dodecanol, AIBN and toluene were dissolved in mixture. After degassing the mixture by sonication, a stainless steel tube was filled with the mixture. The tube was sealed. Polymerization was performed by heating the mixture at 60°C in water bath for 24 hours. After the polymerization, the polymerized monolith was washed with tetrahydrofuran to remove the porogenic agents and other unreacted reagents. Non-imprinted blank polymer (NIP) in the absence of template was prepared and treated in the same manner. Then a solution of methanol-acetic acid (8:2) was used to remove the template. The columns were washed with

mobile phase until a stable baseline was obtained before injection.

D. High performance liquid chromatography

The retention time was determined by injection of 10 μ l of racemates dissolved in acetonitrile. Triple injections were carried out and the average acted as the final data. Capacity factors, k' , were calculated by using the equation $k' = (t_R - t_0)/t_0$, where t_R is the retention time of an analyte and t_0 is the dead time of the void marker. Imprinting factor (I) was defined as the ratio of the equation $I = KMIP/KNIP$ of the enantiomers. NaNO_2 was used to determine the dead volume marker.

III. RESULTS AND DISCUSSION

A. Preparation and Characterization of the MIP

The molecularly imprinted polymer materials were prepared by in-suit and thermal polymerization technique. In the polymer monolith preparations, the phase separation between growing polymer chains and porogenic solvent proceeds so fast and the coarsening of monolithic structure inherently leads to heterogeneous macroporous structures composed of tiny micron size globular particles. Toluene and 1-dodecanol were used to prepare pore and macroporous in the monolith that will result in lower backpressure than the traditional method. The lower backpressure benefits from the macropores. Experiments showed that the back pressure of the monolith was about tenth of the packed particles in traditional method. EDMA and MAA were used as cross-linker and host monomers, respectively. The template was washed out leaving a cavity with appropriately oriented functional groups and the obtained molecularly imprinted polymer was evaluated using an HPLC analysis. For non-covalent molecular imprinting, the most commonly used acidic functional monomer is MAA. MAA was used as the functional monomer in the imprinting of CL which has a fluorine atom and an amide group. In present study, MAA can interact with both fluorine and amide group of template. The specificity of MIPs for CL and its enantiomer was studied by comparing the interaction with the MIPs.

B. Relative retention of the enantiomers on MIP and NIP columns

The capacity factor k' can be used to show the relative retention of CL and its enantiomer on the MIP and NIP monolithic columns. Acetonitrile is commonly used to perform a chromatographic test of hydrogen bond of molecular imprinted polymers. The monolithic MIP polymer obtained using in suit polymerization showed high affinity and selectivity in acetonitrile. As shown in table 1 the retention of the template was longer than its isomer. A mixture of the enantiomers was injected to determine the separation capability of the prepared MIP. Baseline separation was achieved between the enantiomers with R_s about 2.3 on CL-imprinted monolithic polymer column. But there was no separation for the enantiomers on NIP-monolithic polymer column.

Table 1 Retention factors and imprinting factors of the enantiomers of MIP and NIP monolithic column*

	<i>K</i> _{MIP}	<i>K</i> _{NIP}	<i>I</i>
CL	17.21	2.81	6.12
R-enantiomer	2.75	2.83	0.97

*HPLC conditions: column size, 50×4.6 mm i.d.; mobile phase, acetonitrile; flow rate, 1.0 mL/min; detection wavelength, UV 254 nm; temperature, 25°C

C. Retention properties of template on MIP monolithic column in aqueous media

A mixture of acetonitrile and water was used as eluent to evaluate the influence of water on the retention and selectivity of the imprinted polymers. Figure 2 showed the changes of retention factor of the template and its enantiomer molecule with water concentration in the mobile phase. From Figure 2, one can see that the resolution of the enantiomers is larger in pure acetonitrile than other conditions and monolithic column still had selectivity aqueous solution.

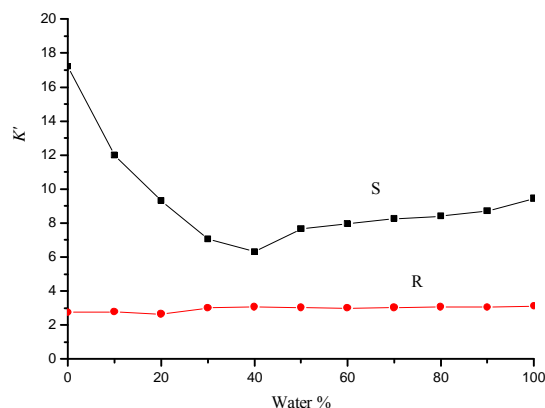


Figure 2. Effect of water concentration in mobile phase on monolithic MIP column. (R) template; (S) -enantiomer.

D. The effect of pH and flow rate of the mobile phase

As we know, the interactions of the template with the monomer were hydrogen bond, hydrophobic, π - π et al. In this experiment, the MAA was selected as the functional monomer and there were amide and fluorine atom. Hydrogen bond was the majority of these interactions, which was approved by the experiment results. The enantiomers will not be separated when the acetic acid was added to the mobile phase even though the concentration of acid was only 0.1%. The resolution of the enantiomers was affected a little by the flow rate when it was near 1.0 mL/min. The peaks of the template and its isomer were wider when the flow rate was lower than 0.5 mL/min and the analysis time was too long to use. The resolution of the enantiomers began to decrease when the flow rate was more than 3.0 mL/min.

E. Separation of the CL enantiomers

By taking the above experiment in account, the optimum separation conditions were as follow: mobile phase, acetonitrile:water = 7:3 (V:V), flow rate, 1.0 mL/min and the detection wavelength was 254 nm. The enantiomers can be baseline separated within 50 minutes (Figure 3). The NIP was tested by the same time and the enantiomers could not be separated.

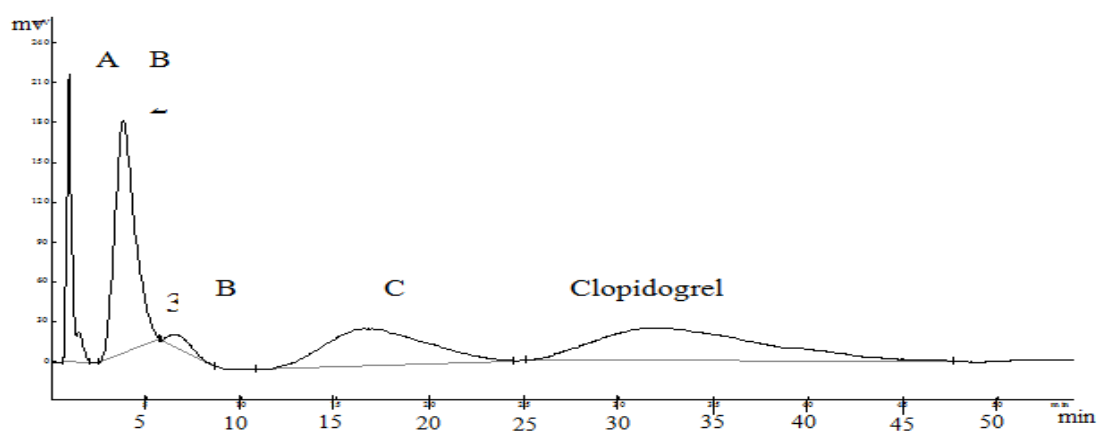


Figure 3 Chromatogram of the Chiral separation CL and its impurities. The experiments were performed under following conditions: mobile phase, Acetonitrile:Water=7:3; flow rate, 1.0 mL min⁻¹; wavelength, 254 nm.

IV. CONCLUSION

The Clopidogrel molecularly imprinted monolithic column was prepared and was used to separated the template and its impurities. The enantiomers were baseline separated within 30 minutes on the MIP monolith column. The monolithic MIP column had lower backpressure and benefit for rapid analysis. This technique provides a very easy way to make chiral stationary phase.

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