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Rapid and efficient chiral separation of nateglinide and its L-enantiomer on monolithic molecularly imprinted polymers

Junfa Yin [a], Gengliang Yang [a,b,*], Yi Chen [a]

[a] *Center for Molecular Science, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100080, PR China*
[b] *College of Pharmacy, Hebei University, Baoding 071002, PR China*

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Abstract

A monolithic molecularly imprinted polymer (monolithic MIP) was designed and prepared for chiral separation of nateglinide and its L-enantiomer. The enantiomers were rapidly separated on this novel monolithic MIP based chiral stationary phase (MIP-CSP), whereas the enantioseparation was not obtained on the non-imprinted polymer (NIP). Chiral recognition was found to be dependent on the stereo structures and the arrangement of functional groups of the imprinted molecule and the cavities on MIP. Thermodynamic data ($\Delta\Delta H$ and $\Delta\Delta S$) obtained by Van't Hoff plots revealed an enthalpy-controlled enantioseparation. The binding capacity was evaluated by frontal analysis. Monolithic nateglinide-MIP had an effective number of binding sites $B_t = 41.15\ \mu mol\ g^{-1}$ with a dissociation constant of $K_d = 7.40$ m*M*. The morphological characteristics of the monolithic MIP were investigated by pore analysis and scanning electron microscope (SEM). Results showed that both mesopores and macropores were formed in the monolith. Over all, this study presents a new and practical possibility for providing high rates of mass transfer, fast separations and high efficiencies without the pressure constraints of the traditional bulk molecularly imprinted polymers, through the monolithic MIPs.

Keywords: Molecularly imprinted polymer; Monolithic column; Chiral separation; Chiral stationary phases; Nateglinide

1. Introduction

The molecular imprinting technique, first proposed by Wulff et al. in 1972 [1], has recently been developed and shown appropriate for preparation of tailor-made chiral stationary phases (CSPs) [2–7]. Molecularly imprinted polymer (MIP) based CSPs are synthesized by the polymerization of cross-linker and the assembling complex of the templates (optically active molecules) and the functional monomers. Once the polymer is obtained, the imprinted molecules are removed leaving cavities complementary in size, shape and matrix of functional groups to the analyte, allowing it to selectively rebind the template molecules. As a consequence, the final MIP-CSPs are able to discriminate the optically active template from its enantiomer.

The traditional bulk MIP has to be grounded and sieved to the desired particles with appropriate size. This tedious and time-consuming process often produces particles that are irregular in size and shape, resulting in minimal chiral separation with low column effect. In addition, some interaction sites are destroyed during grinding, and thus lead to lower MIP loading capacity with respect to theoretical values. Due to these limitations, the traditional bulk MIPs are not absolutely accepted and used in analytical laboratories. In order to overcome these problems, various strategies have been proposed for direct preparation of MIPs. Uniformed spherical particles have been obtained by using multi-step swelling method [8], suspension polymerization [9], imprinting on surfaces of spherical polymer or silica [10,11], etc.

Monolithic MIPs are expected to improve the separation and enable direct analysis with high-speed and high-performance. In recent years, the uses of monolithic media for superior chromatographic separation in high-performance

* Corresponding author. Tel.: +86 10 82627290.
E-mail address: glyang@iccas.ac.cn (G. Yang).

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liquid chromatography (HPLC) and capillary electrochromatography (CEC) have attracted considerable attention [12–14]. Moreover, both monolithic silica columns and monolithic polymer columns are currently commercially available. Their greater porosity, and hence good permeability, and high surface area are well suited for both small molecules and large biopolymers. MIP monoliths have been designed and prepared by in situ polymerization in the 1990s. Matsui et al. [15,16] synthesized continuous rods of molecularly imprinted polymers for chromatographic separations using an in situ method to make the preparation procedure simple and easy to perform. Subsequently, monolithic MIP stationary phases used for CEC have intensively studied [17,18]. Huang et al. [19,20] have developed an improved method for the preparation of short and disk monolithic MIP columns with both satisfactory flow-through properties and high resolution for separation of enantiomers and diastereomers. More recently, Kim and Guiochon [21] compared the thermodynamic properties of monolithic and bulk MIPs against Fmoc-L-tryptophan. Examination of the thermodynamic properties on these two different MIPs showed there were at least three types of binding sites (i.e. the high, the intermediate and the low energy sites) that coexist on the surface. The results suggested that from a thermodynamic point of view, monolithic MIPs could provide a better enantioseparation through the reduction of the density of nonselective interactions of the template with the polymer matrix.

This study describes the use of monolithic MIP-CSPs for chiral separation of nateglinide and its L-enantiomer. Nateglinide [*N*-(*trans*-4-isopropyl-cyclohexylcarbonyl)-D-phenylalanine] (Fig. 1) is a novel non-sulfonylurea oral antidiabetic drug for the treatment of type II diabetes mellitus. Nateglinide can reportedly stimulate a rapid, transient secretion of insulin from the pancreatic β-cells which is dependent on ambient glucose concentrations [22]. An oral dose of 1.6 mg kg^{-1} nateglinide can induce a 20% decrease in blood glucose, whereas approximately 100 mg kg^{-1} of L-enantiomer is required for equal potency. Therefore, developing a more efficient chiral separation procedure for nateglinide and its enantiomer is worthwhile [23]. Our previous work investigates the separations of nateglinide and L-enantiomer using conventional chiral column (SUMICHIRAL-OA-3300) and bulk nateglinide-MIP column [24,25]. The peaks in chromatograms were commonly asymmetric and broad owing to both non-linear adsorption or desorption and slow mass transfer process. The irregular size and shape, the low surface area together with low mesoporosity, lead to low template recognition in the subsequent binding experiments owing to slow analyte diffusion to sites located in micropores. The present study develops a promising and simple method for the preparation of the monolithic MIP-CSP for chiral separation of nateglinide and L-enantiomer. Furthermore, the monolithic nateglinide-MIP exhibited good properties for rapid and efficient enantioseparation over the bulk MIP.

2. Experimental

2.1. Chemicals

Nateglinide, *N*-(*trans*-4-isopropyl-cyclohexylcarbonyl)-L-phenylalanine (L-enantiomer), *N*-(*cis*-4-isopropyl-cyclohexylcarbonyl)-D-phenylalanine (*cis*-isomer) and *trans*-4-isopropylcyclohexanecarboxylic acid (*trans*-acid) were donated by Jiheng Pharmaceutical Co. (Hebei, China). Methanol and acetonitrile (HPLC grade) were purchased from Fisher (New Jersey, USA). Ethylene dimethacrylate (EDMA) from Acros (New Jersey, USA) was extracted with 2 mol l^{-1} NaOH solution and water and dried over anhydroxide magnesium sulfate. Acrylamide (AM) and methacrylic acid (MAA) of analytical grade were purchased from

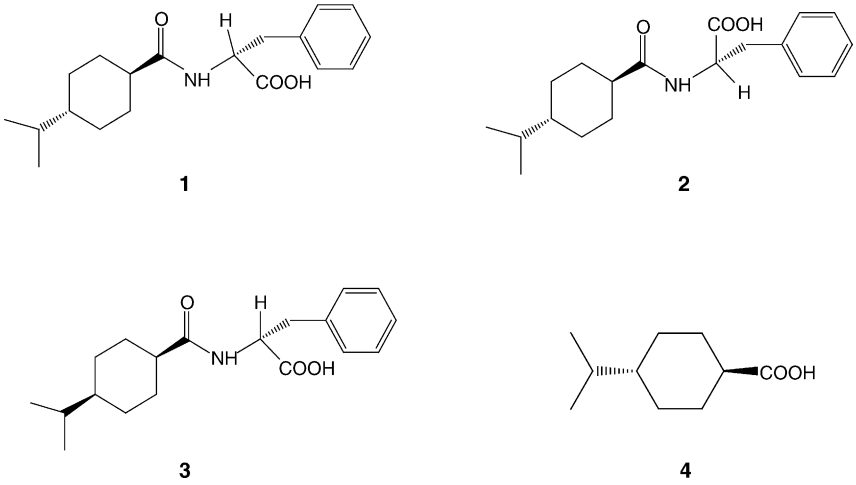

Fig. 1. Structures of nateglinide and related compounds. **1**: *N*-(*trans*-4-isopropyl-cyclohexylcarbonyl)-D-phenylalanine (nateglinide), **2**: *N*-(*trans*-4-isopropyl-cyclohexylcarbonyl)-L-phenylalanine (L-enantiomer), **3**: *N*-(*cis*-4-isopropyl-cyclohexylcarbonyl)-D-phenylalanine (*cis*-isomer), **4**: *trans*-4-isopropylcyclohexanecarboxylic acid (*trans*-acid).