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Enantioseparation by HPLC Using an Inorganic Chiral Mesoporous Silica with Highly-ordered Structure

PENG Bo, FU Shiguo, LI Yanxia, ZHANG Junhui, XIE Shengming, LI Li, LYU Yun, DUAN Aihong, CHEN Xuexian and YUAN Liming*

Department of Chemistry, Yunnan Normal University, Kunming 650500, P. R. China

Abstract Highly-ordered inorganic chiral mesoporous silica(HOCMS) has attracted substantial interest in recent decades. High performance liquid chromatography(HPLC) is the most important approach for the separation of enantiomers and herein reported an HPLC chiral stationary phase composed of HOCMS. The column was fabricated by conventional high pressure slurry packing. Eighteen racemates, including alcohols, ketones, amines, aldehydes and organic acids, were resolved on the column. Good chiral separations of hydrobenzoin, metoprolol, propranolol hydrochloride, 4-methyl-2-pentanol, omeprazole, 2,2′-furoin and ketoprofen were obtained. The relative standard deviations for five replicate separations of racemates were 0.1%—0.16% for retention time and 1.73%—2.64% for peak areas. The results suggest that HOCMS is a promising candidate for preparation of chiral stationary phases for HPLC.

Keywords Highly-ordered inorganic chiral mesoporous silica; Chiral stationary phase; High performance liquid chromatography; Enantioseparation

1 Introduction

Since Kresge and Beck *et al*.[1] published their report on Si-MCM-41, similar materials with ordered mesoporous structure have attracted substantial attention. Ordered mesoporous silicas have advantageous properties including outstanding thermal and chemical stability[2], large pore volume[3] and large surface area[4]. Consequently, these materials have potential applications in many fields, such as stereoselective synthesis[5], tissue engineering[6], catalysis[7], separation[8], and drug delivery[9]. Mesoporous silica can be obtained by self-assembly of silica precursors and surfactants through alkaline or acidic routes[10,11]. Moreover, highly-ordered chiral mesoporous silicas(HOCMS) can be synthesized using chiral or achiral surfactants[12]. Chiral porous materials have been proposed as promising candidates for preparation of chromatographic separation media because of their unique structures[13].

Generally, chirality is frequently in organic materials, but rarely in inorganic materials. The synthesis of chiral inorganic porous materials has posed a substantial research challenge[14]. When presented in an achiral environment, enantiomers have identical physical and chemical properties, but differ in their optical activity. There is substantial interest in the separation of racemates, because chiral recognition is omnipresent in the fields of chemistry and biology, and the separation of chiral compounds is crucially important in the field of analytical chemistry[15]. Moreover, chiral recognition and separation are usually indispensable in research on natural product synthesis, fragrances, food additives, agrochemicals and particularly pharmaceuticals[15—18]. Furthermore, high performance liquid chromatography(HPLC) and high-resolution gas chromatography[19] are effective and convenient methods for the separation and determination of enantiomers[20]. Generally, chiral stationary phases have been obtained by grafting chiral organic molecules onto supports(usually achiral porous silicas) for liquid chromatographic separation. Comparatively few studies have focused on pure highly-ordered inorganic chiral mesoporous silicas, synthesized with chiral surfactant as a template, for HPLC enantioseparation. For example, Jacobs *et al*.[21] have used (*R*)-naphthylethylamine as the chiral selector with amorphous silica, Si-MCM-41 and Si-MCM-48 as the support materials, to synthesize three chiral stationary phases through covalent linking for chiral separation. Zhu *et al*.[22] have obtained a chiral stationary phase for HPLC from functionalized mesoporous organosilica spheres by using trans-(1*R*,2*R*)-diaminocyclohexane as the chiral component. In addition, Ai *et al*.[23] have synthesized modified mesoporous silica *via* a *β*-cyclodextrin derivative as the chiral resource to achieve size control in the 1 μm range.

As mentioned above, the grafting of chiral organic molecules confers chirality on these mesoporous silicas. In contrast, the pore structure of the mesoporous silicas can confer chirality through the backbone. Such mesoporous silicas have been used as liquid chromatography stationary phases for chiral separation. However, to the best of our knowledge, the direct use of pure inorganic chiral mesoporous silica with highly-ordered structure for HPLC enantioseparation has not been reported as yet. We previously described the use of HOCMS as the stationary phase for GC separations[24]. HOCMS was first synthesized by Che *et al*.[25] through a templating route to prepare

*Corresponding author. Email: yuan_limingpd@126.com

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well-ordered mesoporous silicas. The synthesis was based on the self-assembly of chiral anionic surfactants and inorganic precursors using aminosilane or quaternized aminosilane as a co-structure-directing agent. This method enabled the synthesis of mesoporous materials with inherent chirality. Herein, we conducted a report on the novel highly-ordered inorganic HOCMS as the stationary phase for HPLC enantioseparations. An HOCMS-packed column was used to separate 18 chiral compounds representing different chiral compound types. This work demonstrates the great significance of the research on chromatographic enantioseparation through highly-ordered inorganic chiral mesoporous silicas.

2 Experimental

2.1 Chemical Solvents and Reagents

Solvents and reagents of analytical grade or higher purity were used without further purification. *L*-Alanine(99%), myristoyl chloride(98%) and tetraethoxylsilane(TEOS, 99%) were obtained from Adamas-Beta(Shanghai, China). Acetone was purchased from Shandian Pharmaceutical(Yunnan, China) and petroleum ether was purchased from Chengdu Kelong Chemical Co., Ltd.(Sichuan, China). *N*-Trimethoxysilylpropyl-*N,N,N*-trimethylammonium chloride(TMAPS, 50% in methanol) was obtained from J & K Scientific Co., Ltd.(Beijing China). The tested racemates were purchased from Sigma-Aldrich(St. Louis, MO, USA), TCI(Tokyo, Japan) or Adamas-Beta.

2.2 Analytical Techniques

All chromatographic separations were performed on an HPLC system consisting of an Elite P 1201 pump and an Elite UV 1201 detector(Dalian, China). Chromatographic data were acquired and processed with an EC 2006 chromatography data system. The HPLC slurry packer was obtained from Alltech (Connecticut, USA). The stainless steel column(250 mm, 2.0 mm i.d.) was also obtained from Alltech. Column temperature was controlled by an auto Science AT-330 column heater. Small angle powder X-ray diffraction(SA-PXRD) was conducted on a Rigaku D/max-3B diffractometer(Tokyo, Japan), using Cu $K\alpha$ radiation(40 kV, 20 mA). The scanning speed was 1.0°/min, and the scanning range 2θ=1.5°—8.0°. An S-3000N scanning electron microscope(Tokyo, Japan) and a JEM-2100 transmission electron microscope(Tokyo, Japan) were used to characterize the HOCMS. The circular dichroism(CD) spectrum was obtained on a Chirascan Circular Dichroism Spectrometer (Leatherhead, UK).

2.3 Synthesis of Chiral Surfactant(*N*-Myristoyl-*L*-Alanine, C14-*L*-AlaA)

The C14-*L*-AlaA was synthesized through a method reported by Tracey *et al*.[26] and Acharya *et al*.[27]. Briefly, a 1:1 molar mixture of *L*-alanine and sodium hydroxide was dissolved in 30% acetone solution and stirred at 5 °C. Aqueous sodium hydroxide(4.0 mol/L) was added dropwise to adjust the pH to 12.0—12.5. Myristoyl chloride was then added dropwise at 5 °C whilst sodium hydroxide solution(4.0 mol/L) was concurrently added to maintain the system pH at 12.0—12.5. The pH was then adjusted to 1.0 by the dropwise addition of hydrochloric acid. The reaction product was washed several times with water at pH 7.0, and then washed four or five times with petroleum ether to remove residual organic material. Finally, the solid was dried at 50 °C under vacuum.

2.4 Preparation of HOCMS

The HOCMS was synthesized according to the reported method[25]. Tetraethoxylsilane(TEOS) was the silica source, *N*-trimethoxysilylpropyl-*N,N,N*-trimethylammonium chloride (TMAPS, 50% in methanol) was used as the structure-directing agent, and C14-*L*-AlaA was used as the template. C14-*L*-AlaA(0.3 g) was added to deionized water(20.5 mL) and stirred at room temperature. Aqueous NaOH solution(10 mL, 0.1 mol/L) was dripped into the clear solution with stirring at 25 °C over the course of 60 min. TMAPS(0.23 g, 50% in methanol) and TEOS(1.46 g) were added to the stirred solution. After 1 h of stirring, the reaction system was kept static at 20 °C for 2 h. The precipitate was collected by centrifugation, dried at 60 °C, and then calcined at 550 °C for 6 h to remove the template molecules.

2.5 Preparation of the HOCMS-packed Column

The HOCMS was roughly manually milled and suspended in ethanol. The suspension was filtered and dried before the column was packed. To control the packing quality, we used a conventional high pressure slurry packer from Alltech. The HOCMS(1.3 g) was added to *n*-hexane/isopropanol(9:1, volume ratio) and dispersed by ultrasonication for 5 min. The suspension was slurry-packed into a stainless steel column (250 mm, 2.0 mm i.d.) at 40—50 MPa[28]. The packed HOCMS column was purged with *n*-hexane/isopropanol(9:1, volume ratio) at 0.1 mL/min until the baseline was flat before enantioselective separation.

3 Results and Discussion

3.1 Characterization of Synthesized HOCMS

The preparation of HOCMS was confirmed by comparison of its small angle powder X-ray diffraction(SA-PXRD) pattern with that reported in the literature[25]. The obtained SA-PXRD pattern[Fig.1(A)] was in good agreement with the published data[25]. In addition, scanning electron microscopy(SEM) and transmission electron microscopy(TEM) were conducted to investigate the morphology and mesoporous structure of the obtained HOCMS. The SEM image revealed that the HOCMS had a twisted hexagonal rod-like external morphology [Fig.1(C)]. The mesoporous structure of HOCMS was demonstrated by TEM imaging[Fig.1(D)]. The chirality of HOCMS was established by circular dichroism(CD) of a suspension dispersed in ethanol. The spectrum exhibited a CD band with a positive sign[Fig.1(B)], thus indicating that one-handed helixes of rods accounted for a substantial part of the synthesized HOCMS. These results confirmed the synthesis of the highly-ordered chiral mesoporous silica[25].

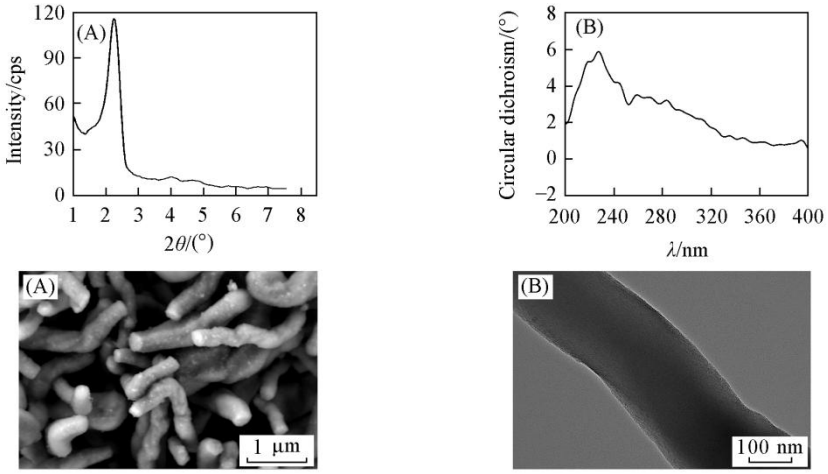

Fig.1 SA-PXRD pattern(A), CD spectrum(B), SEM image(C) and TEM image(D) of the HOCMS

3.2 Separation of the Chiral Compounds

To investigate the enantioseparation capability of the HOCMS-packed column, we separated example racemates on the column using *n*-hexane/isopropanol as the mobile phase. Table 1 shows the retention factors(k_1, k_2), separation factor(α), and resolution(R_s) of the analytes.

Table 1 Separation of racemates on the HOCMS-packed column*

Compound type	Racemate	k_1	k_2	α	R_s
Alcohol	Hydrobenzoin	1.25	2.18	1.74	1.48
	4-Methyl-2-pentanol	0.14	0.54	3.86	1.13
	1-Methoxy-2-propanol	0.18	0.53	2.94	0.86
	1-Phenyl-1-propanol	0.47	0.74	1.57	0.78
	2-Phenyl-1-propanol	0.73	1.05	1.44	0.56
	1-(3-Methylphenyl) ethanol	0.64	0.86	1.34	0.31
Amine	Metoprolol	1.52	2.39	1.57	1.45
	Propranolol hydrochloride	0.14	0.53	3.80	1.43
	Salbutamol	0.17	0.52	3.06	0.65
	Omeprazole	0.15	0.54	3.60	1.24
	sec-Butylamine	0.17	0.56	3.29	0.78
	Zopiclone	0.11	0.35	3.18	0.59
	1-Phenylethylamine	0.49	0.73	1.49	0.55
Ketone	2,2′-Furoin	2.01	3.03	1.51	1.35
	3-Hydroxy-2-butanone	0.18	0.55	3.05	0.80
Aldehyde	2-Phenylpropionaldehyde	0.44	0.72	1.64	0.67
Epoxy acid	Styrene oxide	0.46	0.73	1.58	0.33
	Ketoprofen	0.48	1.07	2.23	1.32

* Chromatographic conditions: mobile phase: *n*-hexane/isopropanol(9:1, *v*/*v*); flow rate: 0.1 mL/min; detection at 254 or 210 nm; temperature: 25 °C.

The HOCMS-packed column exhibited different enantioselectivity towards various racemates when *n*-hexane/isopropanol was used as the mobile phase. Representative HPLC chromatograms are shown in Fig.2. According to the experimental results, the HOCMS-packed column had good chiral recognition, especially for alcohols, ketones and amines.

The chiral microenvironment has a complex influence on the chromatographic separation of chiral compounds. The molecular packing of the chiral surfactant is crucial. After molecular imprinting, numerous chiral grooves derived from the anionic surfactant are imprinted in the pores of the mesoporous silica. Thus, mesoporous silicas with chiral inner-surfaces are obtained. The spatial chiral steric fit between the solute molecule and the chiral inner surface of the HOCMS has a key role in chiral recognition. The internal mesoporous size of the HOCMS is also essential for chiral recognition[25]. The external morphology of the HOCMS is a twisted hexagonal rod-like structure. The Brunauer-Emmett-Teller(BET) surface area and pore volume of HOCMS were determined to be 672 m^2/g and 380 mm^3/g, respectively. The calculated Barrett-Joyner-Halenda(BJH) pore diameter was approximately 2.3 nm, thus permitting easy access of solute molecules. In addition, factors, such as hydrogen-bonding interactions, dispersion forces and van der Waals forces may influence chiral recognition and separation.

The composition of the mobile phase affects HPLC separation[28]. In this work, the influence of *n*-hexane/isopropanol, methanol, methanol/water and acetonitrile/water on separation was investigated. Unfortunately, most of the tested chiral compounds could not be resolved on the HOCMS column by these solvent systems, with the exception of *n*-hexane/isopropanol. Some of the racemates(propranolol hydrochloride,

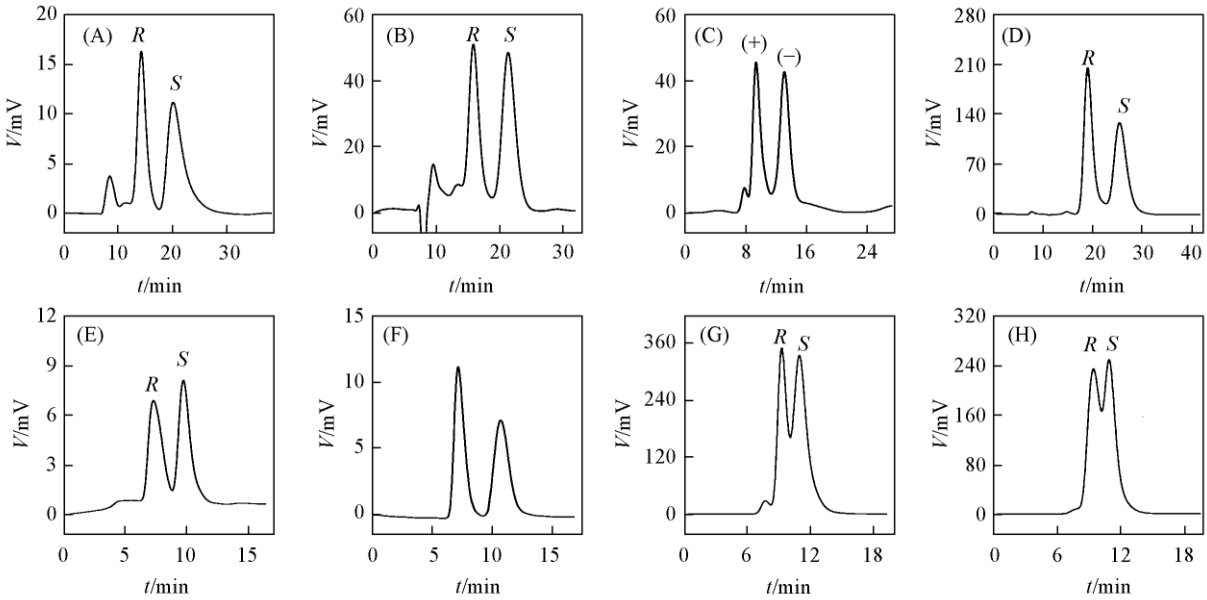

Fig.2 Representative HPLC chromatograms obtained with the HOCMS-packed column for the separation of racemates

(A) Hydrobenzoin; (B) metoprolol; (C) ketoprofen; (D) 2,2′-furoin; (E) omeprazole; (F) propranolol hydrochloride; (G) 1-phenyl-1-propanol; (H) 1-phenylethylamine.

metoprolol and 1-phenylethylamine) were resolved, but their R_s values were poorer than those obtained when the normal phase solvent system(*n*-hexane/isopropanol, Table 2) was used. For example, the separation of metoprolol was better when using *n*-hexane/isopropanol(9:1, volume ratio) was used as the mobile phase rather than methanol, methanol/water or acetonitrile/water(Fig.3). The results suggested that the *n*-hexane/isopropanol system was more favorable than other mobile phases for the separation of these enantiomers on the column. The differing effects of the mobile phase on separation of the same analyte were due to the interactions between the solute molecules and the solvents of different polarity. In polar solvents, hydrogen-bond donors may decrease the hydrogen-bonding interactions between the analyte and stationary phase. In contrast, normal phase solvents favor such hydrogen-bonding[29]. Hydrogen-bonding therefore has an important effect on HPLC separation. Other interactions(such as dispersion forces and van der Waals forces) between the solute molecules and stationary phase may also play important roles in the chiral separation.

Table 2 Effects of different mobile phases on retention and enantioselectivity in the separations of propranolol hydrochloride, metoprolol and 1-phenylethylamine*

Mobile phase(volume ratio)	Propranolol hydrochloride			Metoprolol			1-Phenylethylamine		
	k_1	α	R_s	k_1	α	R_s	k_1	α	R_s
n-Hexane/isopropanol(9:1)	0.14	3.80	1.43	1.52	1.57	1.45	0.49	1.49	0.55
Methanol	0.28	1.57	0.49	0.24	1.79	0.52	0.34	——	——
Methanol/water(9:1)	0.44	——	——	0.38	1.34	0.34	0.43	——	——
Acetonitrile/water(9:1)	0.33	1.64	0.46	0.23	——	——	0.22	1.55	0.41

* Chromatographic conditions were the same as those in Table 1 except for the mobile phase. —— : could not be separated.

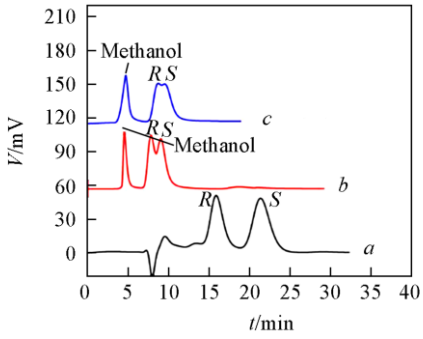

Fig.3 Resolution of metoprolol on the HOCMS-packed column using different mobile phases

a. *n*-Hexane/isopropanol(9:1); *b*. methanol; *c*. methanol/water(9:1).

3.3 Repeatability of the HOCMS-Packed Column Separation

The replicability of the HOCMS-packed column separation was evaluated with hydrobenzoin and ketoprofen as the analytes for five replicate separations using *n*-hexane/isopropanol(9:1) as the binary mobile phase system(Fig.4). The relative standard deviations(RSDs) for the five replicate separations of hydrobenzoin were 0.10% for the retention time and 1.73% for the peak area. For ketoprofen, the relative standard deviations were 0.16% for the retention time and 2.64% for the peak area. No clear changes in retention time or recognition ability were observed. HOCMS, compared with organic chiral stationary phases, had greater resistance to dissolution and erosion by solvents. The obtained data thus indicated that an

HOCMS stationary phase is more practical for HPLC.

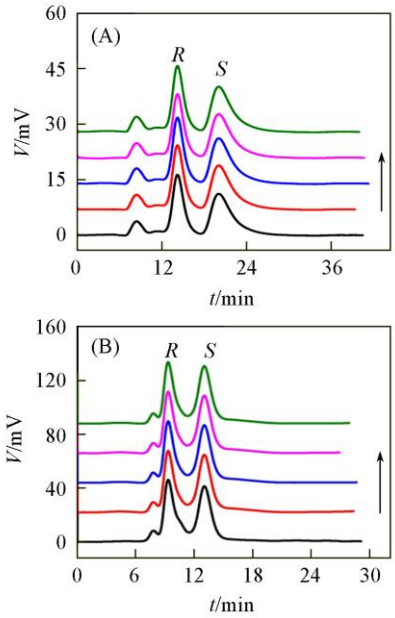

Fig.4 HPLC chromatograms of analytes on the HOCMS-packed column for five replicate separations(bottom to up)

Hydrobenzoin(A) and ketoprofen(B) at 25 °C with *n*-hexane/isopropanol(9:1, *v*/*v*) used as the mobile phase at a flow rate of 0.1 mL/min and UV detection at 254 nm.

3.4 Comparison with a Chiral Nematic Mesoporous Silica Column and a Commercial (*S*,*S*)-Whelk-O1 Column

Chiral nematic mesoporous silica(CNMS) is an inorganic mesoporous silica with a chiral nematic structure derived from nanocrystalline cellulose, and it has been used as a stationary phase for HPLC enantioseparation[30]. Although this study demonstrated the enantioselectivity of CNMS, only three racemates were separated on the CNMS column: 1-phenylethylamine, ibuprofen, and furoin. In contrast, 18 racemates, including alcohols, ketones, amines, aldehydes and organic acids, were resolved on the HOCMS column. These findings illustrate the promise of HOCMS as a candidate packing material for HPLC. We have also used a well-known commercial (*S*,*S*)-Whelk-O1 column to separate chiral compounds[31]. However, some racemates that were able to be separated on the HOCMS column were not resolved on the (*S*,*S*)-Whelk-O1 column. Therefore, there is some complementarity of these chiral columns for enantioseparations.

4 Conclusions

This research demonstrates the HPLC resolution of racemates by HOCMS as a chiral stationary phase. The packed column demonstrated excellent enantioselectivity towards a variety of chiral compounds, including alcohols, ketones, amines, aldehydes and organic acids. The study illustrates the great value of research on chromatographic enantioseparation using HOCMS. We believe that this work should enable a new approach to preparing stationary phases based on inorganic mesoporous materials for HPLC separations.

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