



Sub-1-micron mesoporous silica particles functionalized with cyclodextrin derivative for rapid enantioseparations on ultra-high pressure liquid chromatography

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ABSTRACT

Mesoporous silica particles of relatively uniform sub-1-micron size (0.6–0.9 μm) were successfully prepared by a modified synthesis strategy and applied in chiral separation in an ultra-high pressure liquid chromatography system. These particles were prepared *via* a ternary surfactant system (Pluronic P123, F127 and hexadecyltrimethyl-ammonium bromide) and subsequently derivatized with perphenylcarbamoylated- β -cyclodextrin moieties. The mesoporous silica particles, despite their submicron size, enabled low back-pressure operation on an ultra-high pressure liquid chromatography system at a maximum flow rate of 2 ml/min. In addition, the particles possessed high surface area (480 m^2/g) and thus afforded high cyclodextrin derivative loading (32 $\mu\text{mol}/\text{g}$), demonstrating rapid enantioseparation and good resolution of 6 basic and neutral racemates.

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1. Introduction

High performance liquid chromatography (HPLC) has been the most common analytical separation tool in the past 30 over years. Recently, there is an evolution towards using smaller particles as packing materials in high pressure liquid chromatography systems to improve separation efficiency, sensitivity and resolution [1]. However, the use of smaller particles could impose significant challenge to instrumentation requirement due to much higher pressure as a result of higher resistance of liquid flow through the column. Some groups had reported the beneficial effects of using small (sub-2-micron and even nano-sized) nonporous particles in enhancing chromatographic performance [2–5]. Small nonporous packing materials have limitations such as low surface functional groups loading and high back-pressure generation. Porous particles make good column packing materials due to their enhanced permeability, which could compensate the high back-pressure caused by smaller particles. Current commercial ultra-high pressure liquid chromatography systems utilize sub-2-micron porous particles as column materials and have demonstrated significant improvement in speed, sensitivity and resolution compared to conventional HPLC. This has spurred intensive research interests in column

particle fabrication to further advance chromatographic performance.

Mesoporous silica particles have garnered increasing interest in separation technologies due to their high surface area, high porosity, controllable macromorphologies and tailorable nanostructures [6,7]. Up to date, a variety of mesostructured silica spheres have been proposed as stationary phases for capillary GC [8], size-exclusion chromatography (SEC) [5,9–14] and HPLC [12,15,16]. Investigations about chiral separation using mesoporous silica have also been reported [17–19]. While most work involved in the application of 3–5 μm particles and recently some groups investigated in the performance of 1–2 μm particles [13,20], the utilization of submicron porous silica in liquid chromatography is limited. Yang and co-workers [21] fabricated C18-SBA-15 particles of size range 0.5–1 μm and demonstrated their application in an ultra-high pressure liquid chromatography system using a series of achiral analytes. However, such particles have broad size distribution, which could lower column packing quality leading to reduced column efficiency. In the aspect of chiral separation, there are no commercial sub-2 μm chiral columns, and reports on sub-2 μm particles columns are uncommon. Some works reported chiral separations using commercial sub-2 μm achiral columns by adding chiral additives [22,23]. Cancelliere et al. [24] fabricated several different chiral columns based on 4.3, 2.6 and 1.9 μm porous silica particles and performed detailed investigations on their kinetic performance and enantioselectivity. The results showed consid-

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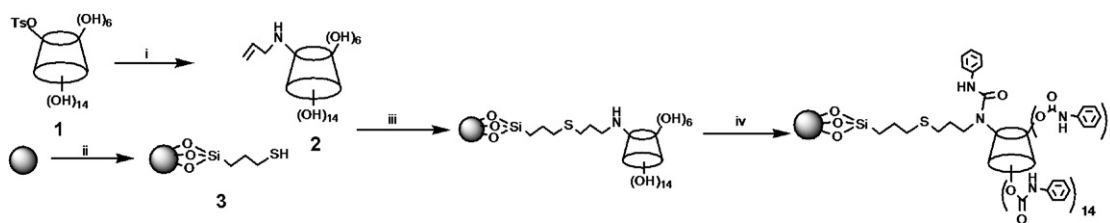


Fig. 1. Synthetic route of phenylcarbamoylated- β -CD modified mesoporous silica particles CSP. (i) Allylamine/DMF/reflux, (ii) (3-Mercaptopropyl) trimethoxysilane/anhydrous toluene/reflux, (iii) AIBN/dry toluene/80 °C, and (iv) phenyl isocyanate/anhydrous pyridine/90 °C.

erably shorter analysis time and lower solvent consumption but similar resolutions when reduced particle size and shorter columns were used. Thoelen et al. [18] reported the use of self-synthesized submicron mesoporous silica MCM-41 materials of 2.4 nm pore diameter in chiral HPLC. In the current work, we adopted a blend of templates to prepare relatively uniform sub-1-micron mesoporous silica particles with 7 nm pores, which were further modified with perphenylcarbamoylated- β -cyclodextrin (CD), and demonstrated its application in a reverse-phase chiral ultra-high pressure liquid chromatography system. The prepared column exhibited good permeability, and displayed good enantioselectivity and efficiency for 6 neutral and basic enantiomers.

2. Experimental

2.1. Chemicals and materials

Pluronic P123 (EO₂₀PO₇₀EO₂₀, M_{av} = 5800) and Pluronic F127 (EO₁₀₆PO₇₀EO₁₀₆, M_{av} = 11,200), hexadecyltrimethylammonium bromide (CTAB, ~99%), tetraethyl orthosilicated (TEOS), β -cyclodextrin (β -CD), phenyl isocyanate (\geq 98%), 2,2'-azobis(2-methylpropionitrile) (AIBN, 98%), pyridine (anhydrous, 99.8%), triethylamine (\geq 99%), acetic acid (>99.7%), hydrochloric acid (37%) and all chiral analytes were purchased from Sigma–Aldrich and used directly without further purification unless otherwise stated. Solvents for UPLC and column packing were supplied by Fisher (Fair Lawn, NJ, USA) and Merck (Darmstadt, Germany). Ultra-pure water was obtained from an Arium 611VF water system (Germany).

2.2. Instrumentation

The prepared mesoporous silica particles were observed by JEOL Field Emission Scanning Electron Microscope (JSM-6700F-FESEM). Particle size distribution was performed using a 90 plus Particle Size Analyzer (Brookhaven, USA). Nitrogen adsorption/desorption isotherms were measured with Autosorb-6b (Quanta Chrome, FL, USA). Elemental analysis was determined on a Vario EL universal CHNOS elemental analyzer (Elementar Analyse system, Hanau, Germany). The modified mesoporous silica was characterized by Fourier-transform infrared (FT-IR) spectra on a Digilab FTS 3100 FT-IR spectrometer. X-ray powder diffraction (XRD) analysis was carried out on a Bruker D8 Advance X-ray diffractometer equipped with graphite monochromatized Cu K α radiation (λ = 1.54056 Å). The fabricated materials were packed into a short stainless steel column (4.6 mm i.d. \times 50 mm) by a conventional slurry method under constant pressure (9000 psi) using a LabAlliance–Scientific System (State College, PA, USA). Chromatographic experiments were performed using a Waters Acquity UPLC system with an upper limit pressure of 15,000 psi and flow rate of 2 ml/min. All data were collected by Empower software (Waters).

2.3. Preparation of mesoporous silica

Mesoporous sub-1-micron silica particles were synthesized using a blend of triblock copolymer Pluronic P123 and F127 as the

structure directing agents and CTAB as a co-surfactant. In a typical synthesis, a mixture consisting of 3 g of P123, 1 g of F127, 0.8 g of CTAB dissolved in 27 ml of ethanol, 35 ml of deionized water and 12 ml of concentrated hydrochloric acid was stirred for more than 2 h to get a transparent mixture. Subsequently, 9 ml of TEOS was added dropwise and the solution was stirred at 313 K for 12 h. The solution was then transferred to an autoclave and aged at 368 K for 6 h, and finally kept at 393 K for 1 day. The product was collected by filtration, dried under ambient conditions, and finally calcined at 823 K in air for 6 h. A further 30 min ultrasonic treatment in ethanol was undertaken to ensure good particle dispersion before surface derivatization.

2.4. Perphenylcarbamoylated β -cyclodextrin surface derivatization of mesoporous silica

The derivatization procedure of the current mesoporous silica was similar to our previous work [25]. The synthetic scheme is depicted in Fig. 1. Typically, 3-Mercaptopropyl-bonded silica 3 and mono-(6'-N-allylamino-6'-deoxy)- β -CD 2 (synthesized from TsO-CD 1 [26]) were polymerized under the initiation of AIBN to afford thiol-functionalized silica, which was subsequently mixed with excess phenyl isocyanate in anhydrous pyridine at 90 °C for 12 h. The solid was collected and washed thoroughly, further purified by Soxhlet extraction with methanol, and then dried in vacuum.

2.5. UPLC operation

To evaluate the chiral discrimination of our column, enantioseparations of 6 neutral and basic racemates were conducted. They were aminoglutechamide (R1), diltiazem (R2), propranolol (R3), tolerisone (R4), 1-(4-iodophenyl)ethanol (R5) and 1-(4-phenylphenyl)but-3-en-1-ol (R6). Samples were prepared in 2 ml vials using a MeOH/H₂O (50/50, v/v) mixture. The mobile phase consisted of a mixture of ACN or MeOH with TEAA (1% TEA aqueous solution was adjusted to pH 4.4 by acetic acid). All samples and mobile phase were filtered before use. Sample injection volume was fixed at 1.0 μ l. All experiments were carried out at room temperature.

3. Results and discussion

3.1. Synthesis and characterization of submicron mesoporous silica particles

In the current work, we have used a blend of surfactants to obtain relatively uniform mesoporous silica particles with desired particle and pore size. P123 and F127 are two common triblock copolymers consisting of PEO–PPO–PEO segments. Different PEO/PPO ratios lead to dissimilar behavior in self-assembly process. P123 favors the formation of elongated cylindrical silicate-surfactant micelles, while F127, with longer hydrophilic chains, is more likely to induce the formation of high curvature micelles and

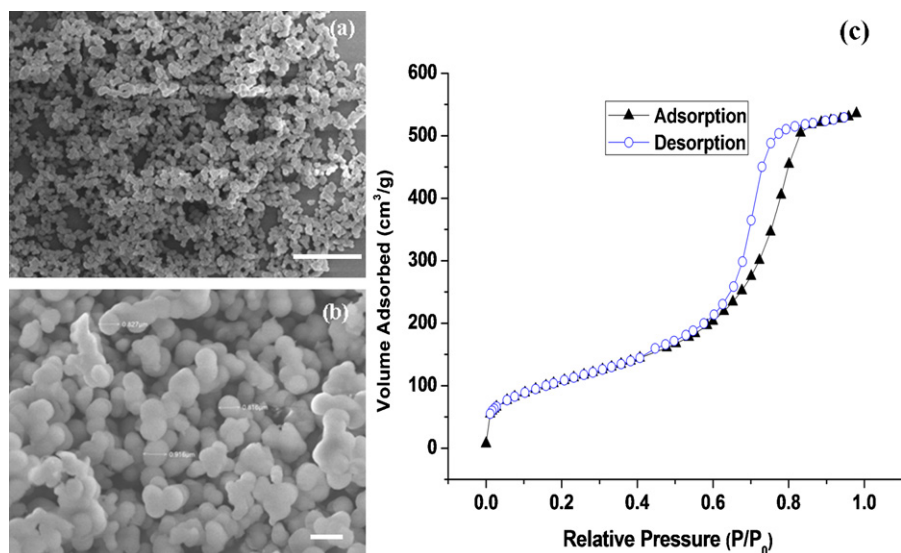


Fig. 2. Representative SEM images of obtained mesoporous silica particles with different magnifications: (a) scale bar: 10 μm and (b) scale bar: 1 μm . (c) Nitrogen physisorption isotherm and the corresponding pore size distribution of calcined mesoporous silica.

hence smaller particles with better size uniformity. Their combination is a feasible approach to tailor the hydrophilic/hydrophobic character of mixed surfactants to attain desired product. Some works on the use of these blends to adjust particle pore structure and size have been reported [27]. Since our work is focused on tailoring for chromatographic application, we are primarily concerned with the morphology, size and the porosity of the material.

We have successfully fabricated mesoporous silica particles of reasonable size distribution ranging from 0.6 to 0.9 μm . SEM images (Fig. 2a and b) showed single particles and also some agglomerated spherical particles. The aggregates can be separated without causing major damage to the single spherical particles by ultrasonic treatment [28]. The particle size distribution was determined after an ultrasonic process and the result is presented in Fig. S1 in the supplementary information. A vast majority of the particles were found to be in the range of 0.6–0.9 μm , while some larger particles were found in the 3.6–4 μm range, which were attributed to residual aggregates. The nitrogen physisorption isotherms and BJH pore size distribution curve (Fig. 2c) demonstrate that these particles have surface area of 480 m^2/g , pore volume of 0.95 cm^3/g and homogenous pores of 7 nm. The adsorption–desorption curve represents a type IV isotherm with a steep step at around $P/P_0 = 0.4$ –0.7, which reflects a highly uniform mesoporous structure. XRD pattern presents a wide diffraction peaks at 1.06° (2θ) assigned to (100), indicating a partial ordered porous structure [29].

The relationship of operation pressure *versus* flow rate (Fig. 3) illustrates that even at a maximum flow rate of 2 ml/min, a back-pressure of only 8000 psi was encountered. The specific permeability (K_f) of the column was determined to be around $2.5 \times 10^{-11} \text{ cm}^2$. The K_f value of our 5-cm packed column is similar to that of a reported highly permeable column (estimated $K_f = 9.6 \times 10^{-10} \text{ cm}^2$ for a 15-cm column packed with 5 μm particles) [31]. The high K_f value may be attributed to the high porosity and penetrating pore structure of the particles, as well as homogeneous column packing due to the relatively uniform particle size. This shows that the current sub-1-micron particle endows good permeability to the column, which significantly reduces the back-pressure and therefore enables a wider range of operability for the UPLC system.

3.2. β -CD derivatization on the mesoporous silica particles

β -CD derivatives are of the most widely used chiral selectors. Several methods of bonding of β -CD moieties onto the silica support have been developed and successfully applied in chiral separation. Our group have previously reported a series of structurally well-defined CSPs by several bonding strategies such as Staudinger reaction [26,32], Click chemistry [33,34] and polymerization [25]. Herein, we prepared a chemically bonded perphenylcarbamoylated- β -CD based CSP by a polymerization step between thiol-functionalized silica and mono-6^A-allylamino- β -CD.

FT-IR spectra (not shown here) depicted obvious C–H stretching vibration at 2961 and 2918 cm^{-1} , which evidenced organic moieties on the surface. Typical absorption of carbonyl group (C=O), assigned to 1730 cm^{-1} , was observed. The characteristic absorption bands of benzene rings were identified at the peaks 1632, 1535 and 1442 cm^{-1} . Elemental analysis (EA) indicated that the CD coverage was about $18 \times 10^{-8} \text{ mol/m}^2$ (C: 9.05%, H: 1.86%, N: 1.63% and S: 2.18%), which is slightly higher than those of the CSPs with similar selectors [34,35]. Both IR spectra and EA results indicate the successful functionalization of cyclodextrin onto the surface of the

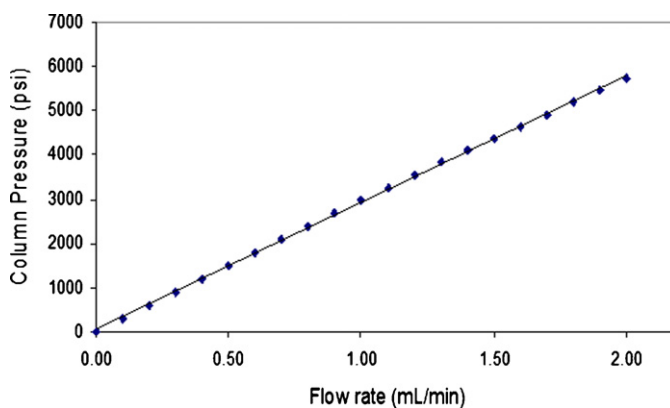


Fig. 3. Plot of column pressure *versus* flow rate with submicron mesoporous silica packed column. Column: 4.6 mm i.d. \times 50 mm, mobile phase: ACN/TEAA buffer 50/50 (v/v), pH 4.4.

Table 1
Enantioseparation results on the CSP.

No.	Selective conditions (v/v)	t_1 (min)	t_2 (min)	k_1	k_2	α	R_s	N (m)
R1 ^a	1:1ACN:MeOH/TEAA 15/85	4.977	5.571	4.01	4.61	1.15	1.19	49,500
R2 ^a	ACN/TEAA 35/65	2.207	2.686	1.20	1.68	1.40	2.90	69,300
R3 ^a	1:1ACN:MeOH/TEAA 40/60	1.002	6.233	5.22	5.94	1.14	1.11	49,700
R4 ^b	ACN/TEAA 40/60	2.897	3.254	1.10	1.36	1.23	1.79	67,100
R5 ^a	1:1ACN:MeOH/TEAA 45/55	1.83	2.134	0.86	1.17	1.36	2.43	68,600
R6 ^a	MeOH/TEAA 50/50	3.794	4.228	2.68	3.10	1.16	1.45	73,700

Conditions: column: 4.6 mm i.d. × 50 mm, composition of mobile phase are shown and flow rate.

^a 0.7 ml/min.

^b 0.5 ml/min.

mesoporous silica particles, although it is possible that the hydroxyl groups are not fully functionalized.

3.3. Chromatographic evaluation

Phenylcarbamoylated- β -CD based CSPs showed high chiral recognition towards various enantiomers in chiral HPLC [36,37]. In this work, we have chosen 6 different neutral and basic racemates to evaluate the performance of sub-1-micron CSP column. The structures of the analytes are shown as Fig. 4a and the results of enantioseparation are listed in Table 1.

It can be observed that the column demonstrated good chiral resolution and high efficiency. Baseline enantioseparations were all completed within 10 min. For instance, R2 were completely resolved within 3 min and R5 were resolved within 2.5 min. The α and R_s values were comparable to the literature results of that of a

25-cm CD-based CSP column, but the analysis time was reduced five times [26,36]. An advantage of utilizing mesoporous materials as column support is their high surface area, which affords enhanced surface functional group loading. Considering the high surface area and special pore structure of mesoporous materials, it can be expected to bear advantages on high sample loading without serious peak tailing. To examine the loading capacity of the CSP, a series of R5 solutions with concentrations ranging from 0.125 to 2.00 mg/ml were prepared and a calibration curves was constructed. The plot of peak area versus concentration (Fig. S2 in the supplementary information) shows a linear relationship ($R^2 = 0.99$), demonstrating that the current column has excellent enantioselectivity in a broad range of concentrations. The chromatograms also show symmetric peaks with a little peak broadening with increasing concentration (Fig. 4c), which suggests quick mass transfer kinetics during the separations. The stability and reproducibility of the column were also examined. By undertaking triplicate tests of day-to-day reproducibility, the standard derivation of retention time was 1.20%, 0.97% and 0.96%, respectively. This demonstrates that the column has stable performance under operation conditions in an ultra-high pressure LC system.

4. Conclusion

Sub-1-micron (0.6–0.9 μ m) mesoporous silica particles were developed with an aim to overcome the problem of small particle induced back-pressure in ultra-high pressure liquid chromatography systems. Our results demonstrated that the mesoporous particles, when used as CSP, could be operated at a relative low back-pressure (<8000 psi) at a high flow rate of 2.0 ml/min. The CSP also exhibited rapid chiral separation (within 10 min) and good resolution of 6 different neutral and basic drugs. The good permeability and high enantioselectivities of the prepared column show its promising application as a chromatographic support in ultra-high pressure liquid chromatography systems.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2010.09.061.

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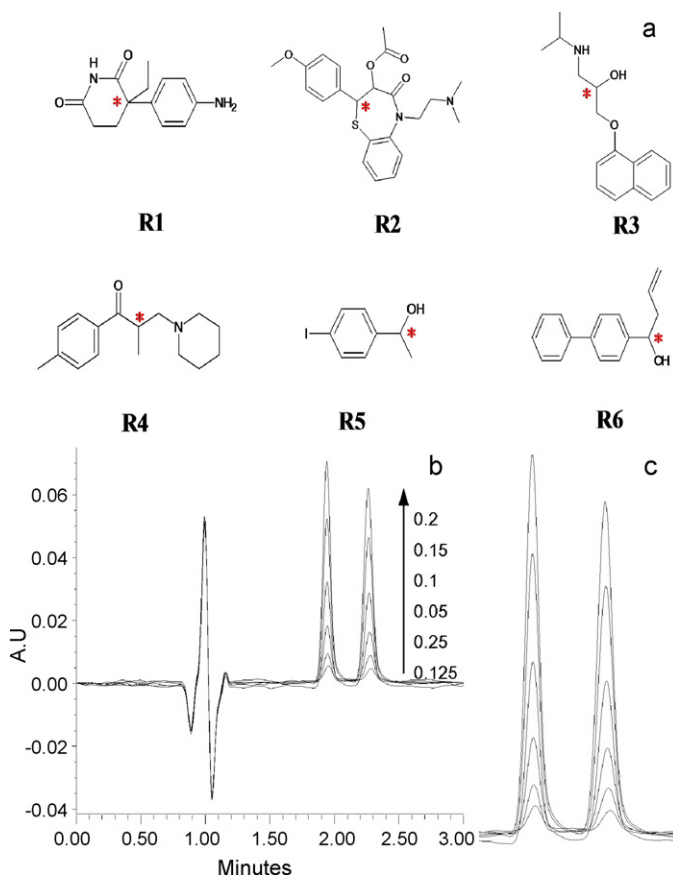


Fig. 4. (a) Structures of the racemic compounds used in the present study. (b) Chromatograms of R5 racemates with different concentrations (0.125, 0.25, 0.5, 1.0, 1.5, and 2.0 mg/ml). Mobile phases: ACN/0.1% TEAA 60/40; flow rate: 0.7 ml/min; detection wavelength: 254 nm. (c) The magnified peaks from chromatograms.

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