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Teicoplanin bonded sub-2 μm superficially porous particles for enantioseparation of native amino acids

Yi Min[a,b], Zhigang Sui[a], Zhen Liang[a], Lihua Zhang[a,*], Yukui Zhang[a]

[a] Key Laboratory of Separation Science for Analytical Chemistry, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, National Chromatographic R. & A. Center, Dalian 116023, China
[b] University of Chinese Academy of Sciences, Beijing 100049, China

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ABSTRACT

Superficially porous particles (SPPs) demonstrate superior efficiency than totally porous particles in chiral separations. In order to obtain high efficiency and fast separation, sub-2 μm SPPs with high surface area are synthesized, and with teicoplanin bonded, such materials are successfully applied into the rapid enantioseparation of native amino acids. In brief, 1.27 ± 0.06 μm nonporous silica particles are prepared by a modified seeded growth method, followed by mesoporous shell fabrication via one-pot templated dissolution and redeposition strategy, and pore size expansion via acid-refluxing. The diameter of the formed SPPs is 1.49 ± 0.04 μm, with the shell thickness as 206 nm. Nitrogen physisorption experiments show that the Brunauer–Emmett–Teller (BET) specific surface area is 213.6 m^2/g and pore size is 9 nm. After teicoplanin derivatization with bonding capacity as 83.3 μmol/g, the prepared chiral stationary phase is packed into a stainless steel tube with the geometry of 50 mm × 2.1 mm i.d.. In less than 6.4 min, six native amino acids (norleucine, alanine, valine, methionine, leucine, norvaline) are enantioseparated with resolution factors ranging from 1.9 to 5.0. Besides, the resolution for chiral separation is improved with ethanol-water instead of methanol-water as the mobile phase. Moreover, the low temperature gives higher resolution, but longer retention time and higher backpressure. Finally, the effect of flow rate on enantiomeric separation is studied and fast chiral separation within 1 min is obtained with flow rate of 0.4 mL/min. All these results show that the synthesized teicoplanin bonded sub-2 μm SPPs have great potential to achieve the enantioseparation of native amino acids with high resolution and rapid speed.

1. Introduction

HPLC is a powerful tool to separate L- and D-Enantiomers [1–3]. The properties of packing materials [2], including particle diameter, surface area and pore size, have great effects on the chiral separation. In the past two decades, 5 μm spherical silica particles dominated the matrix for chiral stationary phases (CSPs) [4]. However, according to recent studies, both the enantiomeric resolution and column efficiency increase with the decrease of particle diameter from 5 μm to 3 μm [5,6]. The performance of CSP based on chicken α_1-acid glycoprotein was in the order of 2.1 μm > 3 μm > 5 μm silica particles for chiral separations of benzoin, ibuprofen and propranolol, with the analysis time between 20 and 80 min [7].

To improve the performance of traditional CSPs, sub-2 μm totally porous particles (TPPs) and nonporous silica (NPS) particles were applied in chiral separation in recent years [8–10]. Tan's group modified the sub-2 μm TPPs with α-, β-cyclodextrins and their derivatives via click chemistry, and the particles were either self-made (0.6–0.9 μm) or from commercial source (1.4–2.0 μm), by which the enantioseparation time of diltiazem was shorted to 1/5, but the resolution was comparable with that of 5 μm TPPs [11,12]. However, if the particle aggregation and broad particle size distribution of these particles could be improved, the separations would be better. Gasparrini's team investigated the performance of sub-2 μm brush-type CSPs, i.e., 1.7 μm TPPs covalently bonded with (R,R)-Whelk-O1, 1.9 μm TPPs grafting with the bis-(3,5-dinitrobenzoyl)-derivative of *trans*-1,2-diaminocyclohexane. Although the analysis speed was improved by ~15 times, the resolution was loss ca. 33% compared to 5 μm particles [13,14]. Rocchi et al. immobilized vancomycin separately onto the 1.8 μm diol hydride-based TPPs and 5 μm TPPs, and the former demonstrated better performance for acidic samples but worse enantioresolution for basic compounds than the latter [15].

Superficially porous particles (SPPs), composed of solid core and mesoporous shell, ensured faster mass transfer [16] and

* Corresponding author. Tel.: +86 411 84379720; fax: +86 411 84379720.
E-mail address: lihuazhang@dicp.ac.cn (L. Zhang).

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higher efficiency [17] compared to TPPs for achiral separations. For chiral separation, with reduced diffusion path, the 2.3 μm core–shell silica microspheres with *trans*-(1R,2R)-diaminocyclohexane (DACH) moiety bridged in the mesoporous shell demonstrated comparable resolution, improved separation efficiency (narrower peak shape) and speed (7 min vs. 14 min) compared to DACH functionalized periodic mesoporous organosilicas [18]. It was reported that 2.6 μm SPPs coated with cellulose tris(4-chloro-3-methylphenylcarbamate) demonstrated higher separation selectivity than 3 μm TPPs coated with comparable content of chiral selector, because the SPPs were more accessible to chiral analytes and had better intra-particle mass transfer [19]. Spudeit et al. also observed higher resolution of 2.7 μm SPPs bonded with cyclofructan-6 compared to 3 μm TPPs when tested under constant retention conditions [20]. Patel et al. modified the 2.7 μm SPPs with a variety of brush-type chiral selectors, and achieved ultrafast chiral separations in the 4–40 s rage [21]. The surface area (typically 98–124 m^2/g) of most current SPPs is low [22], which may affect the binding capacity of chiral selector.

Amino acids are important constituents for life, and their enantioseparation by HPLC represents a key step to unravel their biological activities in living systems [23,24]. For the enantioresolution of native amino acids, the antibiotics are one of the best chiral selectors for CSPs [25], such as teicoplanin [26], eremomycin and ristocetin A [27]. However, to the best of our knowledge, the employment of sub-2 μm SPPs bonded with antibiotic selectors for enantioseparation of native amino acids is not explored.

To solve the problems of aggregation and broad size distribution of sub-2 μm TPPs, as well as increasing the surface area of SPPs for chiral separation, the goal of this study is to prepare monodisperse sub-2 μm SPPs with uniform particle size distribution and high surface area. With teicoplanin bonded, the prepared CSPs are successfully applied for the enantioseparation of native amino acids with high resolution and rapid speed.

2. Experimental

2.1. Material and reagents

L-Amino acids (LAA21-1KT), DL-Norleucine, DL-Norvaline, DL-Methionine (≥99%), DL-Valine (≥99%), DL-Leucine, 1,6-diisocyanatohexane (≥99.0%), hexadecyltrimethylammonium chloride (≥98%, CTAC), ammonium hydroxide solution (28–30%), tridecane (≥99%), ammonium fluoride (≥98%, NH_4F), and 3-aminopropyltriethoxysilane (99%, APS) were all purchased from Sigma–Aldrich (St. Louis, MO, USA). DL-Alanine (99%) was obtained from ACROS (Geel, Belgium). Teicoplanin was purchased from Melone (potency > 900 μg/mg, Dalian, China). Dry pyridine was provided by Aladdin (99.8%, Shanghai, China). Toluene and chloroform (≥99.0%) were purchased from Kaixin (Tianjin, China), and toluene was purified by refluxing (110 °C) over sodium and distilling (136 °C). Ethanol (≥99.8%) was purchased from Kermel (Tianjin, China). Acetone (≥99.5%) was obtained from Zhiao (Anshan, China). Methanol (≥99.9%) and acetonitrile (≥99.9%, ACN) were purchased from Merck (Darmstadt, Germany). Deionized water was used throughout the experiments (Millipore, Milford, MA, USA). Other reagents were all analytical grade.

2.2. Instrumentation

For transmission electron microscopy imaging (120 kV TEM, JEM-2000EX, Peabody, MA, USA), samples were dispersed in absolute ethanol and sonicated before added on the carbon grid (T11023, Xinxing Braim, Beijing, China). The shell thickness and ρ (ratio of core to particle) values were determined from TEM images by counting particles by software Image J. TEM images were combined in CorelDRAW 12. For physisorption experiments (ASAP 2020, Micromeritics, Norcross, GA, USA), typically 100 mg dry powder was used, and surface area was determined from the isotherms by Brunauer–Emmett–Teller (BET) method. The mesopore size was obtained from Barret–Joyner–Halenda (BJH) method. The elemental analysis was carried out in CHN mode (Elementar varioEL III, Hanau, Hesse, Germany). The particles were centrifuged with a Heraeus Multifuge 1S-R centrifuge (Thermo Scientific, Waltham, MA, USA). The calcination was carried out with a Ceramic Fibre Muffle Furnace (Michem, Beijing, China), with the maximum temperature as 1200 °C. Column packer was purchased from Teledyne Isco (65D pump, Lincoln, NE, USA). All separation was performed using 1290 infinity HPLC system (Agilent Technologies, Waldbroen, Germany) with 1 μL detection cell and 20 μL sample loop, controlled by the Chemstation software with the signal sampling rate at 80 Hz, and the extra-column volume and extra-column variance were 17.8 μL and 8–9 μL^2, respectively [28].

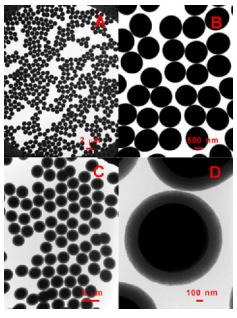

Fig. 1. TEM images of prepared NPS particles (A: 4000×, B: 20,000×) and SPPs (C: 10,000×, D: 80,000×).

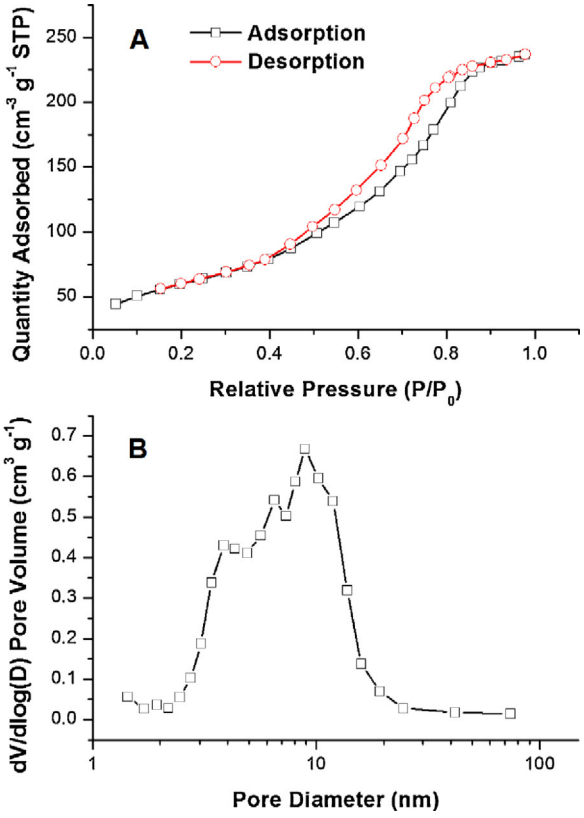

Fig. 2. Nitrogen physisorption analysis of 1.5 μm SPPs. Adsorption–desorption isotherms (A) and BJH adsorption pore size distribution (B).