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Light-assisted preparation of vancomycin chiral stationary phase based on diazotized silica and its enantioseparation evaluation by high-performance liquid chromatography

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ABSTRACT

Owing to enantiomers' identical physical and chemical properties, separation work in the chiral environments is still a great challenge.and chemical properties. Chromatographic techniques employing chiral stationary phases (CSPs) have been developed as powerful tools for the chiral analysis and preparation of pure enantiomers. Here we report a facile synthesis of vancomycin stationary phase based on diazotized silica. Monodisperse silica particles were synthesized by a modified Stöber method. The obtained silica particles were modified by self-assembly photosensitive diazoresin (DR) and vancomycin on the surface. After treatment with UV light, the ionic bonding was converted into covalent bonding through a unique photochemistry reaction of DR. Baseline separation of chiral drugs was achieved by using the vancomycin@SiO_2 particles as packing materials in high performance liquid chromatography (HPLC). The effects of separation parameters including elution mode, flow rate and analyte mass on the enantioselectivity of the CSP were investigated in detail. Due to the replacement of highly toxic and moisture sensitive silane agent by water soluble non-toxic DR in the modification of silica microspheres, this method provides a green and easy way to manufacture packing materials for chromatography applications.

1. Introduction

Enantiomers of chiral molecules show different physiological activities. The importance of enantioseparations has been increasing gradually with their extensive applications to industries such as pharmaceuticals [1–4], pesticides [5], food additives [6], and many others [7,8]. In particular, more than half of all pharmaceutical products are chiral molecules with a defined absolute stereochemistry [9] and many enantiomer may result in undesirable effects. Therefore, there is no doubt that precise analytical techniques take a vital part for chiral drugs analysis and purification.

Chromatographic techniques, especially high performance liquid chromatography (HPLC) with chiral stationary phases (CSPs), have grown into one of the most important techniques for both the determination of enantiomeric purity and the quick preparation of pure enantiomers [10,11]. Currently, silica particles bonded or coated with chiral selectors are considered state of art materials for separating enantiomers in HPLC [12,13].

A lot of chiral stationary phases(CSPs), which are based on ligand-exchange, protein and glycoprotein, cyclodextrin, polysaccharide, macrocyclic glycopeptide and crown ether, are available for separations of enantiomers in HPLC [14,15]. Since the introduction of macrocyclic antibiotics as chiral selectors by Armstrong et al. [16], macrocyclic glycopeptides, particularly vancomycin, have proved to be the most popular selectors resulting from their ability to separate enantiomers of chiral substances with different structures. In fact, the unique structure of vancomycin featuring a semi-rigid polypeptidic basket surrounded by sugars, which exactly ensures manifold sites and types of interaction (e.g., hydrogen bonding, ionic forces, dipole stacking, aromatic stacking, van der Waals interactions, hydrophobic and steric effects) [17–19].

Nowadays, there are a growing number of researchers getting effective chiral stationary phases through silica surface bonds and vancomycin. For instance, Guillarme et al. [20] reported a column based on vancomycin immobilized by reductive amination to aldehyde functionalised silica was prepared and used. They used vancomycin as chiral stationary phase to separate of some 2-arylpropionic acids in reversed-phase HPLC. Assem Abdollahpour et al. [21] reported two chiral

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stationary phases (CSPs) based on crystalline degradation products (CDPs) of vancomycin by using different synthetic methods were prepared and compared. The one synthetic method is that CDPs of vancomycin immobilized to amine functionalised silica. The another synthetic method is that CDPs of vancomycin immobilized by one or two stable ureidic functions to amine functionalised silica. Recently, Barhate et al. [22] reported that vancomycin was covalently attached to 1.9 μm narrow particle size distribution silica and 1.7 μm silica for chiral chromatography with broad selectivity and all reactions were carried out in anhydrous solvents. These CSPs have high selectivity for various classes of enantiomers, but the preparation process need too much organic solvents and toxic silylation reagents [23,24]. Moreover, highly toxic and moisture sensitive silane agents would cause serious environmental problems during the manufacture and application [25]. Thus, a much easier and greener way for modifying silica particles is essential.

Diazoresin (DR) is a positive charged water soluble non-toxic photosensitive polyelectrolyte which often used in cell culture supports [26]. According to the previously proposed preparation mechanism using the layer-by-layer self-assembly technique combined with photochemistry reactions [27], the modification of silica particles using photosensitive diazoresin is expected to be superior to traditional modified methods using moisture sensitive silane coupling agents. So the unique photocross linking reaction of DR is expected to be applied for modifying silica particles instead of silane coupling agents.

In order to improve the preparation efficiency of CSP and reduce environmental pollution, we chose the non-toxic photosensitive polyelectrolyte—DR instead of silylation reagent as a bridge between chiral selector vancomycin and silica. In this paper, we synthesized monodisperse sub-2 μm silica particles by a modified Stöber method. Furthermore we modified silica particles by self-assembly positive charged photosensitive DR modified the silica surface. With the treatment of UV light, the ionic bonding between silica particles and DR, DR and vancomycin were turned into covalent bonding through a unique photochemistry reaction of DR. Thus, vancomycin could be attached on the surface of silica particles steadily, then a novel CSP was obtained. The obtained CSP can be used in both normal phase and reversed-phase modes without any performance degradation while interchangeably using one mode or another. The brand-new CSP performs wonderful HPLC separation effect, especially in chlortrimeton, and benzoin. We introduced the CSP modification process and application process in detail, and further discussed the separation mechanism systematically.

2. Experimental

2.1. Reagents and solutions

Vancomycin hydrochloride, Tetraethyl orthosilicate (TEOS, 98%), chlortrimeton and benzoin (99%) were purchased from Aladdin Reagent Company (Shanghai, China). Methanol (99.9%), acetonitrile (99.9%), isopropanol (99.9%) and hexane (99.9%) were obtained from Tianjin Kemiou Chemical Reagent Co. (All solvents for chromatographic purposes were HPLC grade.) KCl (99.5%), NaOH (98%), ethanol (99.5%), hydrochloric acid (HCl), triethylamine (TEA), and acetic acid (AA) were obtained from Hengxing Chemical Reagent Company (Tianjin, China). Ammonia (28%) were purchased from Sanhe Chemical Reagent Company (Yantai, China). All the reagents were used as received. DR (Mn = 2500) was synthesized by polycondensation of diphenylamine-4-diazonium salt and paraformaldehyde in concentrated H_2SO_4 according to a method described elsewhere [28].

2.2. Preparation of chiral stationary phases

2.2.1. Preparation of the sub 2-μm silica particles

Monodisperse SiO_2 particles were prepared by a modified Stöber method in a semibatch chemical reactor. Firstly, solution I, which contains 0.0238 g of KCl, 9.45 g of water, 75.39 g of ethanol, 1.648 g TEOS and 4.0 mL of ammonia, were added into a 250 mL three-necked flask with mechanical stirring at 240 rpm. Secondly, solution II, which contains 26.12 g of ethanol and 4.12 g of TEOS, were continuously supplied with a syringe pump to the solution I. Furthermore, the reaction temperature was 45 °C and the supply rate of solution I was 0.1 mL/min. After further reaction for 1 h, the obtained particles were purified by centrifugation and washed with 60 mL ethanol at least three times. Finally, the 1.456 g monodisperse SiO_2 particles were obtained and dried under vacuum at ambient temperature.

2.2.2. Preparation of the vancomycin chiral stationary phases

The vancomycin chiral stationary phases were synthesized by following the routes in Fig. 1. Firstly, the SiO_2 particles were treated in 0.1 M HCl for 30 min. Then the particles were washed to neutral with water. Then the particles were added into the aqueous solution of DR (10 mg/mL) for 1 h, and washed with deionized water by centrifugation. Then diazotized coated silica particles were added into the aqueous solution of vancomycin (10 mg/mL) for 1 h, and washed with deionized water by centrifugation. The process was proceed in the dark condition and low temperature. Finally, the obtained coated silica particles were dried under vacuum at room temperature for 12 h. Afterwards, the coated silica particles were exposed to 365 nm UV light with an intensity of 350 mW/cm^2 115 for 20 min in order to form the covalently linked vancomycin coatings.

2.3. Instrumentation

The morphology and structure of the silica particles was observed by scanning electron microscopy (SEM, JEOL JSM-6309LV). The photocrosslinking of the vancomycin and SiO_2 by DR was carried out using a 365 nm UV curing system (EXFO Omnicure S1000) with a lamp power of 100 W. Thermogravimetric analysis (TGA) data were recorded using a Mettler Toledo TGA/DSC1/1600LF simultaneous thermal analyzer at a heating rate of 10 °C min^{-1} under nitrogen flow. Fourier transform infrared spectroscopy (FT-IR) was obtained using Nicolet 5700 produced by Thermo Fisher Scientific. Chromatographic study was carried out using a HPLC (SEV P500) equipped with a UV detector.

2.4. Chromatographic conditions

The CSP were packed into stainless columns (15 × 4.6 mm, I.D.) using a typical slurry-packing technique with isopropyl alcohol as the solvent by the chromatographic column packing machine (GLK 2000, GALAK). The packing pressure was maintained for at least 30 min. The column was then connected to the HPLC system with methanol passing through at a flow rate of 0.1 mL/min for about 3 h to equilibrate the column until a constant UV baseline was obtained. Samples for chromatography were dissolved in MeOH, isopropanol or ACN at a concentration of 10 mg/mL until noted elsewhere. The injection volume was 2.5 μL until noted elsewhere. Each solution was injected in triplicate and the average values are reported. Triethylammonium acetate buffer (TEAA) was prepared by dissolving 0.3% (v/v) triethylamin in ultrapure water and adjusting to the desired pH with acetate acid. All the buffers and samples were filtered through 0.45 μm membranes before use. UV detection was performed at 254 nm.

3. Results and discussion

3.1. Characterization of the vancomycin chiral stationary phases

Monodisperse nonporous silica particles were successfully prepared by a modified Stöber method in a semibatch chemical reactor. Vancomycin could be attached on the surface of silica particles steadily by the charged photosensitive diazoresin. Scanning electron