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A simple method for the synthesis of a polar-embedded and polar-endcapped reversed-phase chromatographic packing with low activity of residue silanols

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ABSTRACT

Octadecyl bonded silica (ODS) is the most popular packing for reversed-phase chromatography. However, it generally demonstrates bad resolution for polar analytes because of the residue silanols and its poor stability in aqueous mobile phase. To address the problem, a new reversed-phase packing containing both polar-embedded and polar-endcapped moieties was proposed. It was prepared by a very simple method, in which the epoxide addition reaction of 3-glycidoxypropyltrimethoxysilane with 1-octadecanethiol proceeded simultaneously with the reaction of silane coupling onto silica particles. By controlling the molecular ratio of 3-glycidoxypropyltrimethoxysilane to 1-octadecanethiol higher than 1.0 (1.56 for the present study), both polar-embedded and polar-endcapped moieties were achieved onto the packing. The performance of the packing was evaluated in detail. The results demonstrated that neutral, acidic and basic analytes were well separated on the packing. The column efficiency for phenanthrene was 34,200 theoretical plates per meter. In addition, four nucleotides can be separated in 100% phosphate buffered saline solution with good reproducibility, which indicates the packing has good stability in aqueous mobile phase. Amitriptyline, a typical basic analytes, was eluted out with relatively symmetric peak shape (asymmetry factor of 1.36), which implies that the packing has not suffered from the negative effect of residue silanols significantly. Good stability in buffer solution of pH ranging from 2.0 to 10.0 was also documented for the packing.

1. Introduction

Octadecyl bonded silica (ODS), as the most popular packing in chromatography, has been widely applied in research and industries [1,2]. The traditional synthetic method of it is based on coupling octadecyl silane onto silica particles, followed by being endcapped with trimethylchloride silane. The as-prepared ODS shows numerous advantages in separating nonpolar compounds, but it cannot deal with polar analytes very well. To achieve their separation, reducing or eliminating organic content in the mobile phase is a practical way. However, in that condition, collapse of $C_{18}H_{37}$ chains of the ODS commonly occurs, leading to serious damage of the packing.

To avoid this dilemma, some polar-embedded or polar-endcapped ODS materials were invented [3–8]. O'Gara et al. [3] prepared an octyl packing with embedded polar moieties by derivatizing silica with 3-(chlorodimethylsilyl)propyl *N*-octylcarbamate. Qiu et al. [4] prepared an imidazolium-embedded ODS packing by bonding 1-allyl-3-octadecylimidazolium bromide ionic liquid onto 3-mercapto- propyltrimethoxysilane modified silica. Liu et al. prepared several packings embedded with ether and sulfonamide [5] or amide [6]. Moreover, Guo et al. [9] prepared polar-included packing by a polar-copolymerized approach. Chromatographic evaluation has testified the success of these new packings for polar analytes. However, the synthesis methods involved are a little complicated.

In this study, we proposed a very simple method for synthesis of a new packing suitable for polar analytes. In the method, two kinds of chemical reaction, the epoxide addition of 3-glycidoxypropyltrimethoxysilane with 1-octadecanethiol and the bonding of silane onto the silica particles, proceeded

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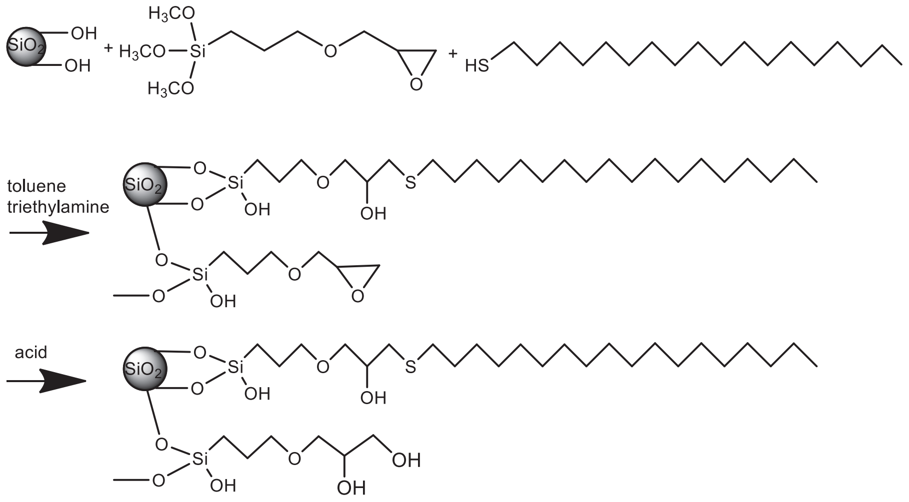

Fig. 1. Synthetic procedures of the new packing.

simultaneously, leading to the formation of a reversed-phase packing containing both polar-embedded and polar-endcapped moieties. The resultant material is not only useful for separating polar analytes in aqueous mobile phase but also suitable for analyzing basic analytes with symmetric peak shape.

2. Experimental

2.1. Materials and reagents

The silica microspheres were prepared according to a literature [10]. Biphenyl, toluene, naphthalene, phenylamine, methanol, *p*-hydroxybenzoic acid, potassium dihydrogen phosphate, dipotassium hydrogen phosphate, uracil, trichloroacetic acid (TCA) and 1-naphthylamine (98%) were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). 1-Octadecanethiol (96%) was bought from Alfa Aesar (Tianjin, China). Triethylamine was from Shanghai Lingfeng Chemical Co. Ltd. (Shanghai, China). 3-Glycidoxypropyltrimethoxysilane was purchased from Hubei Wuhan University Silicone New Material Co. Ltd. (Wuhan, China). Benzidine was from Shanghai Yuanhang Chemical Corporation (Shanghai, China). Phenanthrene, *m*-hydroxybenzoic acid, *p*-aminosalicylic acid, adenine, thymine, cytosine and guanine were bought from Aladdin (Shanghai, China). Ultrapure water was produced by a Heal Fore NW system (Shanghai, China).

The stock solutions of probe analytes were prepared in methanol (1 mg/mL for each analyte) and stored at 4 °C. When being injected into a chromatographic system, they were diluted with the mobile phase first.

2.2. Instrument and analysis

The chromatographic analysis was performed on a Shimadzu (Tokyo, Japan) system, which consists of a Prominence CBM-20A communication device, two LC-20 AT pumps, a SPD-20A UV–vis detector and an injector with a 5 μL sample loop. All chromatographic data were recorded and processed on the LC Solution (Shimadzu, Japan) software. The column was maintained at 25 °C throughout the experiment except stated elsewhere. The pH values of mobile phases were measured with a Leici PHS-3B pH meter (Shanghai, China).

Stability experiments were performed as follows. The column was flushed with methanol/pH 2.0 TCA solution (50/50, v/v) at a flow rate of 1.5 mL/min under 80 °C for 500 min to evaluate the acid stability; it was flushed with methanol/pH 10.0 phosphate buffered saline (PBS) (50/50, v/v) at a flow rate of 1.5 mL/min under 50 °C for 500 min to evaluate the base stability.

2.3. Preparation of the polar-embedded and polar-endcapped reversed phase packing

The general procedures for preparing the packing are depicted in Fig. 1. Briefly, the silica (3.0 g) was heated at 120 °C under vacuum for 5 h. Then it was mixed with 1-octadecanethiol (2.5 g), 3-glycidoxypropyltrimethoxysilane (3.0 mL), triethylamine (1.0 mL) and anhydrous toluene (50.0 mL) in a three-necked round bottom flask. The flask was heated to 110 °C and was kept at that temperature for 4 h. The resultant product was washed with toluene and methanol in sequence. Afterwards, it was refluxed in acidic solution (pH = 3.0) at 60 °C for 10 h. The as-obtained material was slurry-packed into a stainless steel column (4.6 × 100 mm) under the pressure of 6000 psi.

For comparison, an ODS material was prepared by classical method of being derivatized with octadecyltrichlorosilane and being endcapped with trimethyl chlorosilane. Moreover, a silica column was also compared with our packing.

2.4. Characterization

A JW-BK (Beijing, China) nitrogen sorption instrument was used to determine the porous structure of silica. The specific surface area was calculated according to the Brunauer-Emmett-Teller (BET) method [11]. A scanning electron microscope (SEM) of JSM-35CF (JEOL, Tokyo, Japan) was used to observe the morphology of the packing. A Fourier transform infrared spectroscope (FT-IR) of AVATAR 360 (Thermo, USA) was used to evaluate the functional moieties of the packing. A Vario EL cube instrument (Germany) was adopted for element analysis.

3. Results and discussion

3.1. Characterization of the packing

The packing has spherical morphology and relatively narrow size distribution, which can be observed from the SEM image (Fig. 2). The surface area and mean pore size of it are 146 m^2/g and 8.4 nm, respectively. Elemental analysis demonstrates that the element contents of the packing are C 12.56%, H 2.18%, and