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Hybrid organic–inorganic octyl monolithic column for in-tube solid-phase microextraction coupled to capillary high-performance liquid chromatography

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Abstract

An octyl-functionalized hybrid silica monolithic column was developed for in-tube solid-phase microextraction (SPME) to perform on-line preconcentration coupled to capillary high-performance liquid chromatography (μHPLC) analysis. A hybrid silica monolithic column functionalized with octyl groups was conveniently synthesized by a two-step acid/base-catalyzed hydrolysis/co-condensation of tetraethoxysilane (TEOS) and *n*-octyltriethoxysilane (C8-TEOS). The size of through-pores as well as the carbon content can be adjusted by changing the ratio of TEOS to C8-TEOS in the polymerization mixture. The extraction characteristics of the monolithic column prepared under optimized fabrication conditions were studied by using polycyclic aromatic hydrocarbons (PAHs) as the analytes. The sample volume that could be injected into the system was increased up to 1 mL with simultaneous increase of column efficiency, when hybrid silica monolithic column was used as a precolumn. Good linear calibration curves ($R > 0.999$) were obtained, and the limits of detection (signal-to-noise ratio, S/N = 3) for the analytes were found to be between 2.4 and 8.1 ng/mL with a UV absorbance detector, which are 299–456 times lower than those obtained without preconcentration. The column-to-column RSD values were 1.3–8.0% for recoveries of PAHs investigated.

Keywords: In-tube solid-phase microextraction; Organic–inorganic hybrid materials; Capillary high-performance liquid chromatography; Polycyclic aromatic hydrocarbon

1. Introduction

An entire analytical method involves processes such as sampling, sample preparation, separation, detection and data analysis. Surveys show that more than 80% of analysis time is spent on sample collection and sample preparation [1]. In order to achieve a practical and reliable method for the analysis of complex matrices, solid-phase microextraction (SPME) was introduced as an excellent sample preparation technique, since it possesses several attractive features including high sensitivity, solventless extraction, small sample volume, simplicity and easy automation.

In-tube SPME, which was introduced by Eisert and Pawliszyn, allows for convenient automation of the extraction process in coupling with HPLC [2]. Polymeric coatings are predominantly used in in-tube SPME [1–4]. Besides polymeric coatings, in-tube SPME devices have also been prepared by using several nonpolymeric materials [5–9]. Up till now, both organic polymer [10–12] and silica-based [13–15] monoliths have also been used as SPME medium. The synthesis of organic polymer monolithic materials requires only one-step polymerization and a simple post-treatment procedure, and the porous structure and surface properties of the polymer are usually tunable [16]. It generally provides biocompatibility and pH-stability, which makes these columns suitable for use with biological samples such as urine or serum, but they suffer from shrinking or swelling when exposed to different organic mobile phases, leading to lack of mechanical stability. Silica-based monolithic columns are typically prepared directly inside the capillary by sol–gel process and the surface is subsequently modified through silanization reaction to anchor the stationary phases in place by means of a siloxane bond. Shintani et al. [13] were

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the first to use C18 bonded monolithic silica microcolumn for preconcentration in front of a capillary LC, satisfying extraction results were obtained.

However, the conventional silica monolithic columns mentioned above must be prepared and on-column chemically modified by reaction of silica monolith under rigorously anhydrous conditions with functional silanes individually, which can lead to problems in reproducibility [17]. Another drawback of this approach is that the bonded stationary phases are subject to an insufficient hydrolytic stability of the Si–O–Si–C linkage, especially under moderately acidic or slightly alkaline conditions, and the organic coverage is relatively limited [18]. Poor hydrolytic stability and incomplete surface coverage both result in the exposure of a substantial number of surface silanols, which are thought to be primarily responsible for the residual adsorption phenomena that plague silica-based stationary phase [19].

As an alternative, the preparation and functionization of the silica matrix can typically be combined in a single step by means of adding the functional monomer into the monomer mixture for generation of the desirable chromatographic surface. It is a more straightforward and broadly applicable synthetic route for the preparation of organic–inorganic hybrid silica monolithic columns. In this way, a more hydrolytically stable bonded phase can be obtained by silica–carbon (Si–C) or silica–nitrogen (Si–NH–C) bond attachment, rather than by surface Si–O–Si–C linkage [20]. The decomposition of Si–C bonds was not observed after exposing the material to pH 10.5 for 5 days [21]. Hayes and Malik [22] have prepared silica monolith with surface-bonded C18 ligands in a single step by sol–gel process for CEC. Co-condensation of siloxane and organosiloxane precursors by the sol–gel technique in the presence of different surfactant templates to produce functionalized amorphous xerogels silica has also been extensively investigated [23–26]. Previously, a series of hybrid silica monolithic columns involving different ligands for CEC separation [27–30] have been reported.

To our knowledge, this type of organic–inorganic hybrid monolithic column has not been employed as preconcentration column in μHPLC system. The present paper described the preparation of octyl-functionalized hybrid silica monolithic column and investigated the possibility of on-line preconcentration with such hybrid materials before μHPLC separation. The extraction characteristics of the monolithic column were studied by using PAHs as the probes. Good enrichment and separation of six PAHs were achieved.

2. Experimental

2.1. Chemicals and materials

Tetraethoxysilane (98%, TEOS) and *n*-octyltriethoxysilane (97%, C8-TEOS) were purchased from the Chemical Plant of Wuhan University (Wuhan, China), which were used directly without further purification. *N*-Dodecylamine (98%) and a set of six PAHs were purchased from Sinopharm Chemical Reagent Co. (Shanghai, China). Methanol (HPLC-grade) was obtained from Haiyin Guoda Chemical Reagent Co. (Jiangxi, China). Other chemicals used in the experiment were of analytical grade, and were purchased from Shanghai Chemical Co. (Shanghai, China). Double distilled water was used for all experiments. All running buffers were freshly prepared, filtered, and sonicated for 10 min before use.

2.2. Preparation of octyl-functionalized hybrid silica monolithic column

Fused-silica capillaries (O.D. 375 μm, I.D. 250 μm), purchased from Yongnian Optic Fiber Plant (Hebei, China), were activated by 1 M NaOH and then 1 M HCl. After rinsing with double distilled water, they were dried at 160 °C under N_2 flow for 10 h. The hybrid monolith was synthesized by hydrolysis and polycondensation of precursors via a two-step catalytic sol–gel process as described in our previous work [30]. The optimal preparation conditions were as follows: 360 μL of methanol, 20 μL of H_2O, 20 μL of 0.5 M HCl. Various amounts of TEOS and 100 μL C8-TEOS were mixed together in a 1.5 mL Eppendorf vial. After thorough vortexing, the mixture was left for hydrolysis at 60 °C for 5 h. After cooling to room temperature, 10 mg of dodecylamine was added to the solution. Then the pretreated capillary was filled to a certain length with the sol by a syringe. With both ends of the capillary sealed with silicon rubber, the column was allowed to further react at 40 °C for 12 h. Subsequently, the column was rinsed with ethanol to remove the surfactant and soluble hydrolysis products, and dried at 70 °C for 48 h. The total and effective lengths of the hybrid silica monolithic column were 20 and 15 cm, respectively.

2.3. Instrument and analytical conditions

FT-IR spectra were determined by using a Thermo Nicolet 670 FT-IR (Boston, MA, USA). Elemental analysis was performed by an Elementar VarioEL β elemental analyzer (Hanau, Germany). The microscopic morphology of the monolithic column was examined by a Model X-650 scanning electron microscopy (SEM) instrument (Hitachi, Tokyo, Japan).

All SPME–μHPLC experiments were carried out on a simple laboratory-made system (Fig. 1) [31], consisting of a TrisepTM2010GV CEC system (Unimicro Technologies, Shanghai, China), a Shimadzu LC-4A six-port valve (valve A) and a stainless steel sample loop (1 mL total volume). The TrisepTM2010GV CEC system which could be applied to both μHPLC and pressure-assisted capillary electrochromatography (CEC) comprised a UV–vis detector (190–800 nm), two microflow pumps (pump A and B), a microfluid manipulation module (including a splitter and a six-port nanoinjection valve, valve B), and a data acquisition module. The splitter was the injection part of the μHPLC separation unit. The μHPLC column was inserted into the splitter.

Pump A was combined with valve A and the stainless steel sample loop (1 mL) to act as the pre-extraction segment, and all the analytical experiments were carried out on the μHPLC system. Two segments were connected by a polyether ether ketone (PEEK) tube (270 mm × 254 μm I.D.) between valves A and B.