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Preparation of attapulgite nanoparticles-based hybrid monolithic column with covalent bond for hydrophilic interaction liquid chromatography

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ABSTRACT

In current study, an attapulgite nanoparticles-based hybrid monolith was prepared by crosslinking 3-trimethoxysilylpropyl methacrylate modified attapulgite nanoparticles with acrylamide and N, N′-methylenebisacrylamide. The crosslinking of attapulgite into the hybrid monolithic matrix has notable increased the column efficiency of adenosine comparing with the neat one without attapulgite. The resulting hybrid monoliths showed good permeability and good mechanical stability. It was further applied for separation of polar compounds by hydrophilic interaction chromatography (HILIC). The key factors affecting the separation efficiency of the developed hybrid monoliths (i.e. acetonitrile content, salt concentration and pH in the mobile phase) have been completely investigated. The column efficiency was up to 147,613 plates/m for the HILIC separation of aspirin. Good repeatability of retention time was achieved, with relative standard deviations for run-to-run (n = 3), column-to-column (n = 3) and batch-to-batch (n = 3) in the range of 1.08–1.45%, 2.44–3.41% and 2.15–4.96%, respectively. We propose that the attapulgite nanoparticles-based hybrid monolith would provide a promising stationary phase for hydrophilic interaction chromatography.

1. Introduction

Since Alpert developed hydrophilic interaction chromatography (HILIC) in 1990 [1], this technique has been successfully applied for separation (e.g. pharmaceuticals [2,3], carbohydrates [4,5], amino acids [6,7], peptides [6,8], proteins [6,9], etc.), and for sample preparation [10,11]. As a complementary technique to reversed phase chromatography, HILIC could retain and separate polar and hydrophilic analytes because polar stationary phase and high organic mobile phase were used. In addition, the separation can be carried out under lower pressure with the organic solvent-rich mobile phase. There are several kinds of traditional particle stationary phase for HILIC, such as silica [12], amino [13], diol [14], and zwitterionic material [15], etc. However, it takes tremendous skill to pack particles in a capillary for microscale analysis (with advantages of low consumption of sample and solvent) while keeping good repeatability. Although particle-packed capillary HILIC columns are commercially available, the prices are very high.

In the past few decades, monolithic columns have attracted increasing attention and are widely used in capillary HPLC separation due to their distinct merits, such as easy preparation, good permeability and fast mass transfer [16,17]. Basically, monolithic columns can be categorized as polymer-based, silica-based and organic-inorganic hybrid monolithic columns by different types of monomers. Hybrid monolithic column has the advantages of both polymer-based monolith and silica-based monolith but avoids the drawbacks. Specifically, the hybrid monolithic column has the advantages of high pH tolerance, good mechanical stability and good permeability. Although there has been a lot of work on fabrication of hybrid monoliths for capillary HPLC separation [18,19], the study of such columns in HILIC separation of polar compounds is still limited [20–27].

Recently, nanoparticles contained monolithic columns have aroused widespread interest in chromatographic separations because of their good selectivity, good enrichment effect and high separation efficiency

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[28]. The incorporation of nanoparticles into monolithic columns were generally fabricated with three approaches, such as nanoparticles embedded in the monoliths [29], nanoparticles modified on the surface of monoliths [30], as well as functionalized nanoparticles as monomers to construct skeletal microstructure of monoliths [31]. The third approach is an effective way to improve the column efficiency. Zhou et al. prepared a hybrid monolith matrix by polymerization of octaglycidyldimethylsilyl polyhedral oligomeric silsesquioxane (POSS-epoxy) with poly(ethylenimine) (PEI) [27]. The high column efficiency of ca.100,000 plates/m can be achieved for separation of polar compounds in HILIC on the γ-gluconolactone modified POSS-PEI hybrid monolith. A hybrid monolith by a two-step polymerization of 1-thioglycerol-modified polyhedral oligomeric vinylsilsesquioxane and dithiothreitol was synthesized [23]. It showed HILIC separation mechanism with column efficiency of 65,000 plates/m for formamide. Wu et al. fabricated a hydrophobic hybrid monolith by crosslinking (3-aminopropyl) trimethoxysilane modified mesoporous carbon nanoparticles (MCN) with tetramethoxysilane and n-butyltrimethoxysilane, and the column efficiency of such monolith was up to ca. 116,600 plates/m for butylbenzene [32]. Although high column efficiencies were achieved, very few types of nanoparticles (e.g. POSS and MCN) were introduced into hybrid monolithic columns as skeletal microstructure for separation of small molecules, especially for the separation of polar compounds.

Attapulgite is a typical nano-structural fibrillar silicate clay material with a formula of $[(OH_2)_4(Mg, Al, Fe)_5(OH)\cdot 2Si_8O_{20}]\cdot 4H_2O$ [33]. Its structure contains ribbons of a 2:1 phyllosilicate, each ribbon being linked to the next by inversion of SiO_4 tetrahedra along a set of Si-O-Si bonds [34]. Due to its low cost, large specific surface area, reactive −OH groups and cation exchange capacity, attapulgite and organic modification of attapulgite have been applied in pollutants removal (e.g. Ni (II) [35], Pb (II) [36], Hg (II) [37], polybrominated diphenyl ethers [38], etc.) and sample preparation (e.g. benzoylurea insecticides [39], fungicide [40], melamine [41], etc.). In our previous work, attapulgite was embedded in the poly (1-vinyl-3- (butyl-4-sulfonate) imidazolium-*co*-acrylamide-*co*-N,N′-methylenebis (acrylamide)) monolithic column and exploited as a stationary phase in hydrophilic in-tube solid phase microextraction of cyromazine and melamine [33]. It had been demonstrated that the introduction of attapulgite nanoparticles into monolith matrix was a good way to improve the adsorption capacity of polar molecules. However, the monolith prepared by such technology showed poor separation efficiencies of polar compounds.

In this study, we developed an attapulgite nanoparticles-based hybrid monolithic column by crosslinking 3-trimethoxysilylpropyl methacrylate (γ-MAPS) modified attapulgite with acrylamide and N, N′-methylenebisacrylamide. To the best of our knowledge, this is the first report on using attapulgite nanoparticles-based hybrid monolithic column as the stationary phase for HILIC separation of polar compounds. The effect of composition of prepolymerization solution on the column efficiencies and permeability of the attapulgite nanoparticles-based hybrid monolith was investigated in detail. The resulting hybrid monolithic columns were characterized using scanning electron microscopy/energy dispersive analysis of X-ray (SEM/EDAX) and fourier transform infrared spectroscopy (FTIR). We also evaluated the main factors affecting the separation efficiency of the newly developed hybrid monoliths in HILIC separation of polar molecules, such as acetonitrile content, salt concentration and pH.

2. Materials and methods

2.1. Reagents and materials

Acrylamide, *N, N′*-methylenebisacrylamide and standard compounds (i.e. aspirin, thiourea, adenosine, inosine and cytidine) were all supplied by J&K Scientific Ltd (Shanghai, China). Azobisisobutyronitrile (AIBN) and toluene were bought from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Polyethylene glycol (PEG, Mn = 8,000, Mn = 10,000) and formamide were bought from Aladdin (Shanghai, China). γ-MAPS (98%) was obtained from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile (HPLC grade) was bought from Tedia (Fairfield, OH, USA). Ultra-pure water was supplied by a Milli-Q system (Millipore, Molsheim, France). Other chemical reagents were all of analytical grade.

The natural attapulgite was bought from Jiangsu Xuyi Anhalt Nonmetallic Mining Ltd. (Jiangsu, China). The polyimide-coated fused-silica capillaries with a dimension of 150 μm i.d. and 375 μm o.d. were obtained from Yongnian Optical Fiber Factory (Hebei, China).

2.2. Instrumentation

SEM/EDAX was carried out on a Hitachi S-4800 scanning electron microscope (Tokyo, Japan). FTIR spectra were acquired using a Bruker MPA FTIR spectrometer (Bremen, Germany). A calmflow-S100 capillary LC system (Lumtech, Germany) consisting of UV detector equipped with a 35 nL micro flow cell, a vacuum degasser, a binary solvent pump and a data acquisition module was used for the separation. A nano valve with electric actuater (Valco Instruments Co., Inc, USA) was used for sample injections. The permeability evaluation of the hybrid monolithic columns was performed using an HPLC pump under constant pressure (Dalian Elite Analytical Instruments Co., Ltd, China). A T-union connector was used as splitter with one end connected to the attapulgite-based hybrid monolithic column and the other end of an empty capillary (50 μm i.d. and 375 μm o.d.) with a split ratio of 1:200. The retention factor (k) is evaluated by using the following equation: $k = (t_R - t_0)/t_0$, where t_0 and t_R stand for the retention time of unretained compound (i.e. toluene) and analytes, respectively.

2.3. Preparation of the attapulgite-based hybrid monolithic column

γ-MAPS-modified attapulgite was prepared with a minor modification according to the previous work (Fig. 1A) [42]. Natural attapulgite was ground with a mortar and pestle, and then passed a 200-mesh sieve. After that, it was acidified by 4 mol/L HCl at 75 °C for 4 h, washed to neutrality with water and dried at 110 °C for 8 h. Subsequently, 6.0 g acidified attapulgite was dispersed completely in 100 mL dried toluene with ultrasound for 15 min γ-MAPS was added to the mixture with stirring in volume of 6.0 mL, and then refluxed at 110 °C for 10 h. The resulting γ-MAPS-modified attapulgite was washed with ethanol and dried for 7 h at the temperature of 85 °C.

The attapulgite nanoparticles-based hybrid monolithic column was synthesized via a one-step polymerization (Fig. 1B). 41.2 mg of γ-MAPS-modified attapulgite, 20.0 mg of acrylamide, 40.0 mg of *N, N′*-methylenebisacrylamide, 1082.6 mg of formamide, 111.5 mg of PEG 10,000 and 1.0 mg of AIBN were mixed to a completely homogeneous state with ultrasound, and then purged with nitrogen for 2 min. The homogeneous prepolymerization mixture was introduced into vinylized capillaries with a syringe [43]. After sealing both ends with silicon rubber, the capillary was immersed in a water bath at 75 °C for 20 h. The obtained attapulgite nanoparticles-based hybrid monolithic column was rinsed with methanol to remove residuals.

2.4. Calculation of permeability

Permeability (K) values of monoliths are evaluated according to Darcy's law [22], $K = F\eta L/(\pi r^2 \Delta P)$, where F and η represent the linear flow velocity and dynamic viscosity of the mobile phase, respectively, L and r represent the effective length and internal diameter of the monolithic column, respectively, ΔP is the pressure drop across the monolithic column.