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Preparation of polymer monolithic column functionalized by arsonic acid groups for mixed-mode capillary liquid chromatography

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ABSTRACT

A mixed-mode polymer monolithic column functionalized by arsonic acid groups was prepared by single-step in situ copolymerization of monomers *p*-methacryloylaminophenylarsonic acid (*p*-MAPHA) and pentaerythritol triacrylate (PETA). The prepared poly(*p*-MAPHA-*co*-PETA) monolithic column has a homogeneous monolithic structure with good permeability and mechanical stability. Zeta potential measurements reveal that the monolithic stationary phase holds a negative surface charge when the mobile phase resides in the pH range of 3.0–8.0. The retention mechanisms of prepared monolithic column are explored by the separation of selected polycyclic aromatic hydrocarbons (PAHs), nucleosides, and three basic compounds. The results indicate that the column functions in three different separation modes associated with reversed-phase chromatography based on hydrophobic interaction, hydrophilic interaction chromatography, and cation-exchange chromatography. The column efficiency of prepared monolithic column is estimated to be 70,000 and 76,000 theoretical plates/m for thiourea and naphthalene, respectively, at a linear flow velocity of 0.85 mm/s using acetonitrile/H_2O (85/15, v/v) as the mobile phase. Furthermore, an analysis of the retention factors obtained for the PAHs indicates that the prepared monolithic column exhibits good reproducibility with relative standard deviations of 2.9%, 4.0%, and 4.7% based on run-to-run injections, column-to-column preparation, and batch-to-batch preparation, respectively. Finally, we investigate the separation performance of the proposed monolithic column for select phenols, sulfonamides, nucleobases and nucleosides.

1. Introduction

The analytical separation of various compounds in complex samples has become increasingly important in recent years owing to developments in proteomics, metabolomics, environmental science, and medicinal chemistry. Presently, reversed-phase liquid chromatography (RPLC), which adopts a separation mode based on hydrophobic interaction, is the most widely employed type of high performance liquid chromatography (HPLC) applied for conducting analytical separations [1,2]. However, a number of polar and ionic compounds are too hydrophilic to be properly retained in an RPLC column, and therefore can be more efficiently separated according to hydrophilic interaction by hydrophilic interaction liquid chromatography (HILIC) [3,4]. In addition, ion exchange chromatography (IEC) has been successfully employed for the separation of ionic compounds such as inorganic ions and proteins [5–7]. Nonetheless, the separation of more complex sample matrices involving combinations of hydrophobic, hydrophilic, and/or ionic compounds remains extremely challenging for conventional single-mode HPLC techniques.

Recently, mixed-mode chromatography (MMC), where the retention behavior of analytes is determined by at least two types of interactions, has been developed to enhance the separation performance of HPLC for complex sample matrices [8]. This approach allows the chromatographic selectivity of HPLC to be fine-tuned, which results in improved resolution and increased loading capacity [9]. Specifically, a variety of anionic polymeric monoliths with sulfonic [10], phosphonic [11], carboxylic [12], and boronic acid groups [13,14] have been prepared to generate mixed-mode functionalities for capillary liquid chromatography (cLC) applications. These mixed-mode monolithic columns have demonstrated high chromatographic separation performance for a variety of compounds such as polycyclic aromatic hydrocarbons (PAHs), nucleobases, peptides, and proteins. Nonetheless, a

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number of polar compounds or mixtures containing polar compounds are still not well separated based on the developed RPLC/IEC dual-mode stationary phases [10,11]. In contrast, RPLC/HILIC/IEC mixed-mode stationary phases have been developed [12–14], and have demonstrated the efficient separation of not only nonpolar and ionic compounds, but also polar compounds. Therefore, the single-step preparation of polymeric monoliths with multifunctional monomers has attracted significant interest for conducting mixed-mode chromatographic separation.

It is well known that the selectivity of anionic stationary phases is dominated by the dissociation constants of their acid groups. In this respect, arsonic acid, which is a diprotic acid with the general formula $RAs(O)(OH)_2$, where R is an alkyl or aryl group, has significantly different acid dissociation constants ($pKa_1 \approx 4.0$ and $pKa_2 \approx 9.0$) from those of sulfonic, phosphonic, carboxylic, and boronic acid groups currently employed for generating mixed-mode functionalities [15]. Thus, an arsonic acid functionalized monolith would be a valuable alternative or complement to currently available stationary phases for improving chromatographic separation performance. However, while mixed-mode monolithic columns with sulfonic, phosphonic, carboxylic, and boronic acid groups have been prepared successfully by single-step processes, the synthesis of monolithic columns with arsonic acid groups has not yet been reported. A few attempts have been made to develop arsonic acid group functionalized materials. Zayas et al. developed poly(*o*- and *p*-methacryloylaminophenylarsonic acids) [poly(*o*- and *p*-MAPHA)] to remove trace metal ions from hard-water and sea-water because the arsonic acid ($-AsO(OH)_2$) substituent is a highly selective ligand [16]. In addition, arsenate has also been selected as chelating linkers for phosphopeptides enrichment in immobilized metal ion affinity chromatography (IMAC) techniques [17,18]. Furthermore, the ion-exchange capacity and good thermal stability of polymers containing $-AsO(OH)_2$ groups has enabled their applications as proton-exchange membrane in fuel cells [19]. The ion-exchange capacity and selectivity of $-AsO(OH)_2$ functional groups is of particular interest for developing monolithic columns that could be applied for the separation of complex samples.

In this work, we report a facile approach for the preparation of arsonic acid functionalized monolithic column for cLC via a single-step in situ copolymerization of monomers *p*-methacryloylaminophenylarsonic acid (*p*-MAPHA) and pentaerythritol triacrylate (PETA). To obtain satisfactory column permeability and chromatographic separation performance of the prepared poly(*p*-MAPHA-*co*-PETA) monolithic column, the composition of the copolymerization mixture was carefully optimized. In addition, the retention properties of the poly(*p*-MAPHA-*co*-PETA) monolithic column was investigated using a variety of test compounds such as PAHs, nucleosides, and three basic compounds. Finally, poly(*p*-MAPHA-*co*-PETA) monolithic column was applied for the separation of some small molecules with different polarities and p*K*a values.

2. Experimental

2.1. Reagents and materials

Fused-silica capillaries (100 μm i.d.) were obtained from Yongnian Optic Fiber Plant (Hebei, China). For fabricating poly(*p*-MAPHA-co-PETA) monolithic column, 3-(triethoxysilyl) propyl methacrylate was purchased from Wuhan University Silicone New Material Co. (Wuhan, China), *p*- aminophenylarsonic acid and methacryloyl chloride were purchased from Aladdin Chemical Reagent Co. (Shanghai, China), PETA was obtained from Acros (NJ, USA), azobisisobutyronitrile (AIBN), polyethylene glycol (PEG) 6000, and dimethylsulfoxide (DMSO) were supplied by Shanghai Chemical Reagent Corp. (Shanghai, China). For cLC testing, HPLC grade acetonitrile (ACN) was obtained from Fisher Scientific (Pittsburgh, PA, USA), and various nucleosides, including adenosine, uridine, and cytidine, and various nucleobases, including thymine, adenine, and cytosine, were purchased from Shanghai Kayon Biological Technology Co. (Shanghai, China), thiourea, toluene, trifluoroacetic acid, ammonium formate ($HCOONH_4$), sodium dihydrogen phosphate (NaH_2PO_4) were obtained from Sinopharm Chemical Reagent Co. (Shanghai, China), and various PAHs, including naphthalene, fluorene, and fluoranthene, and various phenols, including phenol, catechol, resorcinol, hydroquinone, and phloroglucinol, were purchased from Aladdin Chemical Reagent Co. (Shanghai, China), and various sulfonamides, including sulfamerazine, sulfamethizol, sulfadoxine, sulfisoxazole, sulfachloropyridine, and sulfadimethoxine were purchased from Laboratories of Dr. Ehrenstorfer (Augsburg, Germany). Purified water was prepared with a Milli-Q apparatus (Millipore, Bedford, MA, USA).

2.2. Instruments

The cLC experiments were performed on a Shimadzu nano-LC system (Tokyo, Japan), which was equipped with two LC-20AD nano pumps, an FCV nano valve, and a GL Sciences MU 701 ultraviolet-visible (UV–vis) detector with a 6-nL detection cell (Tokyo, Japan). The morphology of polymer monolith was examined by scanning electron microscopy (SEM) on a Quanta 200 microscope (FEI, Hillsboro, USA). The elemental compositions of the prepared monolith were analyzed by energy-dispersive X-ray spectroscopy (EDX) on a Shimadzu EDX-720 instrument (Kyoto, Japan) using Mg Kα radiation as the excitation source. Fourier transform infrared (FT-IR) spectroscopy was conducted on a Thermo Scientific Nicolet 670 FT-IR spectrometer (Boston, MA, USA) to obtain polymer monolith spectra over a wavenumber range from 4000 to 400 cm^{-1}. Mass spectrometry (MS) was conducted on an ultra-HPLC (UHPLC) –electrospray ionization (ESI) –MS/MS (UHPLC-ESI–MS/MS) system, which was equipped with a Shimadzu LC-30A system (Tokyo, Japan) and a Shimadzu MS-8040 triple-quadrupole mass spectrometer (Tokyo, Japan). The zeta potential of the monolith was measured by a Nano ZS ZEN3600 nanoparticle size and surface potential analyzer (Malvern, England).

2.3. Calculations

Column permeability (K; m^2) reflects an average through-pore size of a monolithic column [20], which can be used for the optimization of the column preparation procedure, and was calculated according to Darcy's Law [21,22]:

$$K = u\eta L/\Delta P \quad (1)$$

where u (m/s) is the linear velocity, η (Pa·s) is the dynamic viscosity of the mobile phase, L (m) is the column length, and ΔP (Pa) is the column back pressure. In this study, ACN was used as the mobile phase, for which $\eta = 0.38 \times 10^{-3}$ Pa s at 20 °C.

2.4. Synthesis of *p*-MAPHA monomer

We synthesized *p*-MAPHA monomer according to previously published methods [23]. Briefly, *p*-aminophenylarsonic acid (3.4 g, 16.0 mmol) was dissolved in 24 mL methanol, and the mixture was cooled to 4 °C. Subsequently, 2.0 g (19.2 mmol) of methacryloyl chloride was added dropwise. The reaction was performed at 4 °C under stirring for 6 h. Finally, water (4 °C) was added to precipitate *p*-MAPHA monomer, which was collected by filtration, washed with water, and dried at 60 °C in vacuum. ESI–MS m/z: 284.1 $[M-H]^-$. ^1H NMR (400 MHz, DMSO) δ 10.15 (s, 1H), 7.94 (s,

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