Journal of Chromatography A, xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Polyoxometalate incorporated porous polymer monoliths, a versatile separation media for nano liquid chromatography

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ARTICLE INFO

Article history:
Received 23 February 2016
Received in revised form 10 May 2016
Accepted 12 May 2016
Available online xxx

Keywords:
Polyoxometalates (POM)
Porous polymer monoliths
Stationary phase
Nano liquid chromatography

ABSTRACT

Here in, we present a strategy to incorporate $NBu_4SiW_{11}O_{39}(SiCH{=}CH_2)_2$, an organic-modified polyoxometalates (POM) monomer, into the monolithic poly(butyl methacrylate-*co*-ethylene glycol dimethacrylate) capillary columns. SEM analysis and permeability test indicated that the addition of POM lead to larger skeleton size and better permeability. BET and pore size distribution test confirmed the uniform porosity of the resulting POM incorporated monoliths. Hydrophobic, strong cation-exchange and H-bond interactions of the prepared monolith were evaluated by testing a series of chromatographic probes. The performance of monolith was further elaborated by separating 5 nucleobases, and 6 neurotransmitters. Chromatographic separation results showed that POM incorporated monolith exhibited much better resolution for the analytes as compared to the monolith without POM. This type of monolithic material has been reported for the first time and the work provided a promising way for preparation and application of various POM-incorporated monolithic materials in separation science.

1. Introduction

Polyoxometalates (POMs) are anionic, early transition metal–oxygen clusters comprising of large and diverse nanostructures, which exhibit specific electronic and chemical properties [1]. In particular, they can store electrons without altering their structure, and their active sites can be designed and manipulated at molecular and atomic levels [2]. Till now, POMs have been explored in several fields including catalysis [3], electrochemistry [4], photochemistry [5], semiconductors [6], and anti-viral, anti-bacterial and anti-tumor agents [7,8]. It is assumed that POMs might prove to be good sorbents for analytical separations due to their unique properties. Although, few reports have been published in this field, the potential of these materials have not been evaluated properly [9–11].

Benefiting from their high-density negative charges, POMs have been reported as ion exchangers. Bandosz et al. modified graphene oxide with two different POMs ($H_3PW_{12}O_{40}$ and $H_3PMo_{12}O_{40}$) for adsorption of ammonia [9]. Naggar et al. synthesized two novel POMs, tin potassium vanadate and zirconium potassium vanadate to adsorb metal ions [10]. Recently, Wang et al. prepared an organic–inorganic hybrid POM, ($[C_{33}H_{24}O_4]H_3PMo_{12}O_{40}$), and applied the POM to the selective isolation of β-lactoglobulin from complex samples [11]. All these successful attempts on different type of analytes demonstrated large ion-exchange capacity and excellent selectivity of POMs. However, the analytical applications of POMs are still limited. One major defect is that they can hardly be utilized directly in flow through applications, due to their compact nanostructure which would leads to high back pressure and leachability.

To solve this problem and diversify the potential applications of POMs, the development of a hybrid material based on a macroporous polymer monolith incorporated with POMs could be desirable. Polymer monoliths, the common flow through supports, have been demonstrated as one of the most efficient supports for immobilization of nanoparticles [12]. In recent years, nanoparticle-incorporated monoliths including polymer (latex) [13], carbonaceous [14], hydroxyapatite [15], metals [16], metal oxides [17], and metal-organic frameworks [18] have been developed with unique characteristic structure and selectively tailored surface chemistry for separation of targeted analytes. On the other hand, polymer monoliths usually suffer from low column efficiency, due to their smaller surface area, irregular morphology, lack of mesopores and sufficient interaction sites [14]. The incorporation of nanoparticles into polymer monoliths has been demonstrated as an effective strategy to enhance the column efficiency [19]. The main

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http://dx.doi.org/10.1016/j.chroma.2016.05.049
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Please cite this article in press as: Z. Zhang, et al., Polyoxometalate incorporated porous polymer monoliths, a versatile separation media for nano liquid chromatography, J. Chromatogr. A (2016), http://dx.doi.org/10.1016/j.chroma.2016.05.049

reason for that is improved surface area and availability of sufficient interaction sites on nanoparticles. So the simultaneous utilization of the unique properties of POMs and versatile features of polymer monoliths to develop POM–incorporated polymer monoliths could be a promising choice. This will not only introduce POMs in LC separation, but also improve the LC performance of polymer monoliths.

Here in, we present the first attempt to incorporate $NBu_4SiW_{11}O_{39}(SiCH{=}CH_2)_2$, one kind of vinyl-modified Keggin type POM (referred as $VSiW_{11}$), into poly(butyl methacrylate-*co*-ethylene glycol dimethacrylate) (BuMA-*co*-EDMA) capillary columns through free-radical-initiated polymerization, to fabricate a polymer monolith for nano liquid chromatography (nano-LC). The resulting POM incorporated monolith with more uniform mesopore size distribution showed better column efficiency and permeability. In addition, hydrophobic, strong cation-exchange and H-bonding interactions of the POM-incorporated monolith were evaluated on different analytes. Our results demonstrated that POM-incorporated monolithhas good potential separation as media for nano-LC separation of small molecules.

2. Materials and methods

2.1. Chemicals and reagents

Butyl methacrylate (BuMA, 98 wt% pure, containing 250 ppm monomethyl ether hydroquinone (MEHQ) as an inhibitor) and ethylene glycol dimethacrylate (EDMA, 98 wt% pure, containing 90–110 ppm MEHQ as an inhibitor) were purchased from Acros (New Jersey, USA). To remove inhibitors, all monomers were extracted with 10% aqueous sodium hydroxide and water; after drying over $MgSO_4$, they were filtered and distilled under reduced pressure. $NaWO_4{\cdot}2H_2O$, $Na_2SiO_3{\cdot}9H_2O$, tetrabutylammonium bromide, azobisisobutyronitrile (AIBN), PEG-6000, dimethylformamide (DMF) and dimethylsulfoxide (DMSO) were all purchased from Shanghai Chemical Reagent Corporation (Shanghai, China). Vinyl trimethoxysilane (VTMS) and 3-(triethoxysilyl) propyl methacrylate were purchased from Wuhan University Silicone New Material (Wuhan, China). HPLC grade acetonitrile (ACN) were obtained from TEDIA Company (Ohio, USA). The water used throughout all experiments was purified using a Milli-Q water purification system (Millipore, Bradford, USA). All the reagents were of analytical grade. The fused-silica capillaries were purchased from Yongnian Optic Fiber Plant (Handan, China).

The lacunary Keggin polyoxometalate, $K_8SiW_{11}O_{39}$, and its derivative $NBu_4SiW_{11}O_{39}(SiCH{=}CH_2)_2$ ($VSiW_{11}$) was synthesized according to previous report [20].

2.2. Preparation of monolithic capillary column

The fused-silica capillaries were washed with 1 mol/L NaOH (2 h), H_2O (30 min), 1 mol/L HCl (1 h), H_2O (30 min), and methanol (30 min) in sequence to activate the silanol groups, followed by drying under nitrogen flow at 160 °C for 5 h. The inner surface of the fused silica capillary was then modified with 3-(triethoxysilyl) propyl methacrylate in DMF (0.5 g/mL) at 60 °C for 12 h, and washed with methanol (30 min). Then the capillaries were allowed to dry under nitrogen stream at 120 °C for 5 h.

Monolithic poly($VSiW_{11}$-*co*-BuMA-*co*-EDMA) capillary column (30 cm-long, 100 μm i.d., 360 μm o.d.) was prepared by a one-step thermally initiated *in-situ* polymerization method. Firstly, porogen PEG-6000 and $VSiW_{11}$ were dissolved in DMSO and thoroughly mixed to ensure that the solution was completely homogeneous. Subsequently, BuMA and EDMA were added into the polymerization mixture and briefly shaken before adding the initiator AIBN (1 wt% of monomers). The mixture was completely homogenized by vortexing and ultrasonication. The resulting solution was loaded into the capillary and both ends of the capillary were sealed by silicon rubber for polymerization at 60 °C for 12 h. Finally, the prepared monolithic column was washed with DMSO and methanol successively, to remove residual reagents.

For comparison, a monolithic poly(BuMA-*co*-EDMA) capillary column (30 cm-long, 100 μm i.d., 360 μm o.d.) was also prepared by the same procedure but without the addition of $VSiW_{11}$.

2.3. Characterization and LC conditions of the monolithic columns

Surface area and pore size distribution were measured by a specific surface area and pore size distribution analyzer (Beijing JWGB Sci.&Tech., Beijing, China). Before measurement, the monolithic cubic pieces were evacuated in vacuum and heated to 120 °C for 3 h to remove the physically adsorbed substances. Specific surface area values were determined by the Brunauer–Emmett–Teller (BET) equation at P/P0 between 0.05 and 0.35 [21]. Mesopore size distributions were determined by the desorption of isotherms based on the Barrett–Joyner–Halenda (BJH) model [22]. The morphology of the monolith was examined by scanning electron microscope (SEM), using a Quanta 200 scanning electron microscope (FEI, Hillsboro, USA). The elemental contents of the prepared monoliths were determined on Shimadzu EDX-720 energy-dispersive X-ray analysis (Kyoto, Japan) by using Mg–Ka radiation as the excitation source. Fourier transform infrared (FT-IR) spectrum for the monoliths was obtained by using a Thermo Nicolet 670 FT-IR (Boston, MA, USA). Thermal gravity analysis (TGA) was performed with a TG-DTA6300 thermal analyzer (PerkinElmer, USA). Permeability measurements were performed by using a Shimadzu nano-LC-20AD pump (Kyoto, Japan) under the constant flow mode. ACN was pumped through the prepared monolithic column (30-cm long, 100 μm i.d., 360 μm o.d.) at a flow rate of 500 nL min^{-1}. The back pressure was recorded when the pressure stabilized. Permeability (K) was calculated according to Darcy's Law by using Eq. (a), where u (ms^{-1}) is the linear velocity of the mobile phase; η is the viscosity of the mobile phase (0.38×10^{-3} Pas for ACN at 20 °C in the current study); L is the length of the monolithic column (m), and ΔP is the pressure drop across the monolithic column (Pa).

$$K = u\eta L/\Delta P \tag{a}$$

The nano-LC evaluation experiments were performed on a Shimadzu nano-LC system (Tokyo, Japan), containing two LC-20AD nano pumps, two vacuum degassers, a SIL-20AC HT autosampler, a FCV nano valve, and one GL Sciences MU 701 UV–vis detector with a 6 nL detection cell (Tokyo, Japan). After connecting to the nano-LC system, the monolithic column was conditioned with the mobile phase at a flow rate of 500 nL/min for 30 min. The chromatographic data were acquired by Shimadzu LC Solution (Tokyo, Japan). Prior to use, the mobile phases were filtered through a G-4 fritted glass funnel and degassed in an ultrasonic bath for about 5 min. The pH of mobile phase referred to the pH value of aqueous solution before mixing with organic modifier.

The loading capacity was measured using frontal elution [23]. Monolith (20 cm) was equilibrated with 20% ACN, then ethylbenzene solution dissolved in 20% ACN (100 μg mL^{-1}) was pumped through the monolithic column at a constant flow rate of 1 μL min^{-1} and absorbance was measured at 214 nm. The loading capacity was calculated from the following Eq. (b) [23]:

$$Q = \frac{ct\nu}{V} \tag{b}$$

where Q is the loading capacity (μg mL^{-1}); c, the analyte concentration (μg mL^{-1}); t, saturation time (min); ν, the flow rate (μL min^{-1}); V, the volume of monolith (mL).

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