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A new anion-exchange/hydrophobic monolith as stationary phase for nano liquid chromatography of small organic molecules and inorganic anions

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ABSTRACT

In this study, an anion-exchange/hydrophobic polymethacrylate-based stationary phase was prepared for nano-liquid chromatography of small organic molecules and inorganic anions. The stationary phase was synthesized by in situ polymerization of 3-chloro-2-hydroxypropylmethacrylate and ethylene dimethacrylate inside silanized 100 μm i.d. fused silica capillary. The porogen mixture consisted of toluene and dodecanol. The pore size distrubution profiles of the resulting monolith were determined by mercury intrusion porosimetry and the morphology of the prepared monolith was investigated by scanning electron microscope. Good permeability, stability and column efficiency were observed on the monolithic column with nano flow. The produced monolithic column, which contains reactive chloro groups, was then modified by reaction with N,N-dimethyl-N-dodecylamine to obtain an anion-exchange/hydrophobic monolithic stationary phase. The functionalized monolith contained ionizable amine groups and hydrophobic groups that are useful of anion-exchange/hydrophobic mixed-mode chromatography. The final monolithic column performance with respect to anion-exchange and hydrophobic interactions was assesed by the separation of alkylbenzene derivatives, phenolic compounds and inorganic anions, respectively. Theoretical plate numbers up to 23,000 plates/m were successfully achieved in the separation of inorganic anions.

1. Introduction

The use of miniaturized techniques such as capillary liquid chromatography (cLC), capillary electrochromatography (CEC) and nano liquid chromatography (Nano-LC) has gained much attention and become the popular separation techniques in separation science and technology [1–5]. Among these chromatographic systems, Nano-LC has emerged as an alternative way to conventional LC to analyze compounds of different nature. This system refers to chromatographic separations performed in capillary columns with internal diameter (id) of 10–100 μm. Nano-LC offers to some advantages over conventinonal LC such as low sample requirement, use of small amount of reagent, good efficiency, short analysis time especially flow rate adjustment with flow splitter [3]. Nano-LC has been applied to the separation of a wide number of different organic molecules and inorganic anions. These applications are performed in packed, open tubular and monolithic capillary columns [3]. The most common of them is monolithic columns. Monolithic columns have received much attention and become the popular separation media for chromatographic analyses in all areas of separation science [5]. The interest in monolithic stationary phases is attributed to their improved flow-through properties compared to traditional packed columns and their easy of preparation in a fused silica capillary. Capillary monolithic columns can be classified according to the type of the monomers: (i) polymer-based monoliths, (ii) silica-based monoliths, (iii) hybrid (organic silica or zirconia)-based monoliths [5–9].

Polymer based monoliths are based on polystrene, polyacrylamide and polymethacrylate [10]. Among these types of macroporous polymers, the polymethacrylate-based monoliths represent the most popular and successfully explored class. These monoliths have advantages such as simple polymerization procedure and wide application range of pH values.

The polymethacrylate-based monoliths have higher separation efficiency with specific interactions. To achieve more specific interaction between the analyte and the surface of the stationary phase, chemical functionalization is often required. The application of

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these materials with surface functionality as a chromatographic stationary phase depends directly on their surface chemistry. For example, ionic groups located at the surface are required for ion-exchange chromatography and hydrophobic surfaces are necessary to achieve separations in reverse-phase mode. The preparation of functional polymethacrylate-based monoliths from a new set of monomers requires optimization of polymerization conditions [11]. Another approach that allows the introduction of desirable functionality while preserving the original structure of these types of monoliths is chemical modification of reactive groups. One of the most widely used functional polymethacrylate-based monomers is glycidyl methacrylate (GMA). Its reactive epoxy group allows a wide range of chemical conversion [12]. GMA based columns were widely used for post-modification to generate mixed mode functionalities or affinity-based monolithic stationary phases [13–15].

Mixed-mode stationary phase, which involves multiple separation mechanisms (e.g., reverse phase, ion exchange and hydrophobic interaction) simultaneously at the same cross section of capillary, has attracted wide interest in the separation of biomolecules [16].

Capillary monolithic columns prepared with mixed-mode stationary phases will have great potential in a wider application area. The development of mixed-mode stationary phase to achieve multiple separation capabilities in one column seems an important strategy for Nano-LC separations. In our recent studies, we reported the use of HPMA-Cl for the preparation of different types of capillary electrochromatography-based stationary phases [17–19].

In this study, as an alternative approach for liquid chromatography based-stationary phases, the preparation and functionalization of HPMA-Cl based polymethacrylate stationary phase were demonstrated. Due to the easy chemical functionalization of the free chloro groups, monoliths based HPMA-Cl can offer ready access to various functionalities. The HPMA-Cl-co-EDMA monolith was prepared and followed by N,N-methyl-N-dodecylamine (DMDA) functionalization. The functionalized capillary monolith showed anion-exchange/hydrophobic interactions in the separation of small organic molecules and inorganic anions in nano-LC.

2. Experimental

2.1. Chemicals and materials

3-Chloro-2-hydroxypropylmethacrylate (HPMA-Cl, 99%), ethylene dimethacrylate (EDMA, 99%) and the functionalization agent, N,N-methyl-N-dodecylamine (DMDA) were purchased from Sigma-Aldrich Chemical U.S.A. The derivatization reagent, 3-trimethoxysilylpropyl methacrylate (TMSPM, 98%) and the components of porogen mixture, toluene and dodecanol were supplied from Aldrich. Acetonitrile (ACN) (99.9%, Sigma–Aldrich), ethanol (99.8% Riedel de Haen, Germany) were used as supplied. 2,2′-Azobisizobutyronitrile (AIBN), (Merck A.G. Darmstad, Germany) was recystallized from methanol before use. All inorganic compounds used in chromatographic tests were analytical reagents. Alkylbenzene derivatives and phenolic compounds were also supplied from Aldrich.

2.2. Instrumentation

The polyimide-coated fused-silica capillaries (id: 100 μm, od: 360 μm) were obtained from Polymicro Technologies (Phoenix AZ, USA). An Ultimate 3000 Chromatography System with Ultimate 3000 pump was used for functionalization steps. Nano-LC experiments were performed on Ultimate 3000 Chromatography System with nano flow (Dionex Technologies, Munich, Germany). The system includes four parts with the following components (i) Ultimate 3000 pump, (ii) Ultimate 3000 flow manager with flow splitter, (iii) Ultimate 3000 Autosampler, (iv) Ultimate 3000 RS variable wavelength detector.

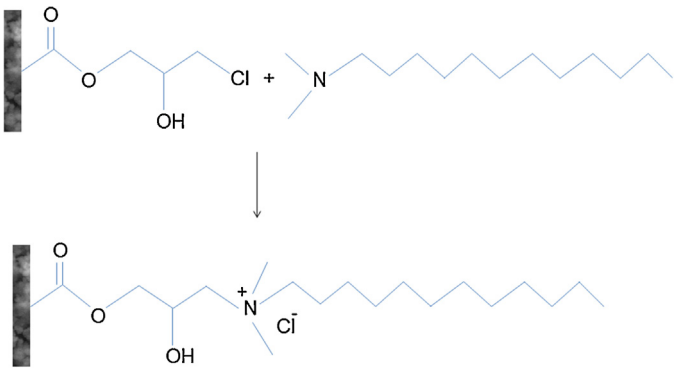

Fig. 1. The schematic reaction of a new poly(HPMA-Cl-co-EDMA) monolith with DMDA.

2.3. Monolithic stationary phase preparation

The preparation of monolithic stationary phase included in situ polymerization with the following steps: a fused silica capillary (2 m × 100 μm id) was rinsed with 0.1 M NaOH for 3 h and water for 15 min, respectively. To obtain anchoring sites for the chemical grafting of the polymer at the inner wall of the capillary, a mixture containing 50% v/v of 3-trimethoxysilylpropyl methacrylate (TMSPM) in MeOH was filled into the capillary.

After sealing both ends of the capillary, the reaction was carried out at 35 °C for 20 h. The silanized capillary column was rinsed with MeOH to remove unreacted material. A solution containing 13.6% v/v HPMA-Cl, 27.4% v/v EDMA, 48.2% v/v toluene 9.8% v/v dodecanol and 1% (w/v) AIBN (with respect to the total volume) as initiator was filled into a silanized 20 cm × 100 μm id capillary. Thereafter, both ends of the capillary were sealed and it was placed in a water bath for 8 h at 60 °C. The resulting monolith was carefully washed with ethanol for 2 h and water for 2 h, respectively.

2.4. DMDA functionalization

The modification procedure of HPMA-Cl based capillary monoliths was similar to those reported previously [20] but with a few modification. Typical procedure is as following: the prepared column was first washed extensively with the mixture including ethanol and water (50:50%, v/v) for 30 min. After that, DMDA solution was pumped through the monolithic column for 30 min using a μLC pump, and then the functionalization of the monolithic columns was carried out at 70 °C for 6 h in a water bath.

The schematic reaction is depicted in Fig. 1. Therefore, hydrophobicity and quaternary ammonium functionality, which is necessary for anion-exchange/hydrophobic chromatograpy, could be obtained. Finally, the DMDA functionalized monolithic stationary phase was rinsed with ethanol for 2 h and water for 1 h at a flow rate of 2 μL/min, respectively.

2.5. Chromatography procedures

The running buffer was prepared by mixing the phosphate buffer with an appropriate volume ACN. Phosphate buffer with different concentrations was used. Isocratic elution of the samples was performed to evaluate the extent of anion-exchange/hydrophobic interactions on the monolithic stationary phase. Alkylbenzene derivatives, phenolic compounds and inorganic anions were dissolved with ACN or MeOH and then diluted with water.