Prepared Polymethacrylate-Based Monoliths for the Separation of Cations by Non-Suppressed Capillary Ion Chromatography

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Received 10 January 2013; revised 25 February 2013

This paper describes a novel analytical system for non-suppressed capillary ion chromatography. Methacrylate monolithic columns were prepared from silanized fused-silica capillaries of 320 μm i.d. by in situ polymerization of glycidyl methacrylate and ethylene dimethacrylate in the presence of 1,4-butanediol, 1-propanol and water as the porogen solvents. The introduction of cation-exchange sites was achieved by sulfonating the matrix with sodium sulfite to produce total cation-exchange capacities in the range of 45–105 μeq/mL for a 25 cm column. The conditions (concentrations of sodium sulfite solution, reacting time and modified flow rate) of sulfonation were optimized. The hydrodynamic and chromatographic performances were estimated. Coupled with a conductivity detector, a capillary ion chromatography system was set up with the prepared column. Finally, the resultant column was used for the separations of five common univalent cations (Li+, Na+, NH4+, K+, and Cs+) using methanesulfonic acid as the eluent and four divalent cations (Mg2+, Ca2+, Sr2+ and Ba2+) by non-suppressed capillary ion chromatography; the chromatographic parameters were further researched.

Introduction

Ion chromatography (IC) was introduced by Small in 1975 (1). Until now, it has been the dominant analytical method for determining inorganic anions, some organic anions and some special analytes. Recently, related micro or capillary-sized techniques (2–5) [capillary electrochromatography (CEC) or micro/capillary IC] have attracted more attention and interest, not only because they represent low cost, portable, environmentally friendly systems with low consumption of reagents, but also because they provide easier coupling with other detection methods such as mass spectrometry (MS) for further study (life science and pharmaceutics) (6–7). Closely following the development of new types of material (monolith) (8–11) and instrumentation (contactless conductivity detector) (12–16), and the evolution of capillary liquid chromatography (LC), the micro-sized IC system has become the key point in this field (17–18).

Compared with other analytical techniques, the stationary phase is one of the most important factors for efficiency in IC. Traditional IC columns packed with ion-exchange particles are prepared by many steps, including particle-size classification, packing and frit making. The simplicity of column preparation without loss of separation performance is an important economical approach to control the procedure and the spending in the area of column preparation techniques. Because of this need to obtain simple preparation methods, the novel monolithic materials were developed. The recently developed monolithic column possesses the advantages of good hydrodynamic characteristics and low flow resistance, and provides easy and low cost preparation and the elimination of frits compared to packed-bed stationary phases. Because the fourth generation of stationary phases was developed in the 1990s (19–20), monoliths (including both silica-based and polymer-based) have been used for fast IC (21–22) and capillary IC (23–26), exhibiting obvious advantages (27–29) of easy fabrication, no fritting, high permeability, low pressure and high throughput.

Because silica-based monolithic columns for IC cannot be applied with alkaline eluents due to the chemical instability, polymer-based monoliths are more suitable for IC. In this work, a glycidyl methacrylate (GMA)-based monolithic matrix containing epoxy functional groups was prepared and sulfonated to gain cation-exchange sites for suitable cation-exchange capacity. Using a contactless conductivity detector for non-suppressed detection, nine common univalent and divalent cations were separated and determined on the prepared column with proper eluents.

Experimental

Instrument

The empty polyimide-coated fused silica capillary of 320 μm i.d. and 560 μm o.d. was purchased from Yongnian Optical Fiber Factory (Hebei, China). A thermostatic water bath (Jiangnan Instrument Factory, Jiangsu, China) was used for thermostating the columns during polymerization. The modification for columns was performed in a column oven (Abel Industries, Chengdu, China) and the modifier solution was pumped through the column by an injection pump (Gelan, China).

The capillary IC system was constructed with a pump (K120, KNAUER, Germany) with a low flow rate of 1 μL/min, a 7520 injection valve with a 500 nL injection loop (Upchurch Scientific, Oak Harbor, WA), a prepared cation-exchange capillary monolithic column and a capillary conductivity detector with two electrodes contacting with the detecting solution (with a very small flow cell of ~40 nL) supported by Professor Yang from East China Normal University.

Reagents

GMA and ethylene dimethacrylate (EDMA) were purchased from Kogyo (Tokyo, Japan). Before use, GMA was distilled under
vacuum and EDMA was washed with 5% aqueous sodium hydroxide solution and dried over anhydrous magnesium sulfate. Organic solvents [propan-1-ol, butane-1,4-diol, ethanol, tetrahydrofuran (THF), and trimethylamine] and 2,2'-azoisobutyronitrile (AIBN) were obtained from the Chemical Factory of Shanghai (Shanghai, China). AIBN was re-crystallized in ethanol to remove impurities. (γ-Methacryloxypropyl)trimethoxysilane (γ-MAPS) was purchased from the Chemical Factory of Nanjing (Nanjing, China). Methanesulfonic acid (MSA) and tetrabutylammonium hydroxide (TBAOH) were purchased from Guoyao Chemical Factory (Beijing, China). The eluent additives and analyte chemicals were of analytical reagent grade and used as obtained from Changzheng Co. (Hangzhou, China). All eluents were prepared daily. A water system (Millipore, Molsheim, France) was used to further deionize distilled water for all eluents and sample mixtures.

**Preparation of methacrylate-based monolithic column**

*Pretreatment of the capillary column tube*

For stable chromatographic performances and to make it easier to fasten the polymerized rod to the tube, pretreatment of the capillary tube was needed. The empty fused-silica capillaries (320 μm i.d.) were rinsed by ethanol and water with a syringe pump, activated with 1 mol/L of NaOH for 2 h, washed with water and with 0.1 mol/L of HCl for approximately 12 h, washed with water, flushed with ethanol and dried with a stream of nitrogen (N₂). A 50 % (v/v) solution of γ-MAPS in ethanol with pH adjusted to 5 with acetic acid was pumped through the dried capillaries at a flow rate of 5 μL/min for 20 h. The modified pretreated capillaries were washed with acetone and purged by N₂ to be completely dry for the next step.

*Preparation of methacrylate-based monolithic column*

The polymerization mixture consisted of 40% monomers (GMA–EDMA, 3:1, v/v) and 60% porogens (in the presence of 60% 1,4-butanediol, 30% 1-propanol and 10% water, v/v) with 1% solution (w/v with respect to the monomers) of AIBN as the initiator. The solution was placed in an ultrasonic bath for 10 min and purged with N₂ for 10 min. Finally, the homogenous solution was filled into the pretreated empty capillaries by using a syringe. Both ends of the capillaries were sealed with rubber septa and they were submerged into a water bath at 60°C for 20 h. To connect the columns with a pump, the unreacted porogens and monomers were removed by using ethanol for 3 h at a flow rate of 3 μL/min and washed with water for approximately 5 h.

*Preparation of cation-exchange monolithic column*

Subsequently, epoxy groups in the monolithic stationary phase were modified with a sodium sulfite solution to form strong cation-exchange groups. The GMA–co-EDMA monoliths were sulfonated by using a 1 mol/L sodium sulfite solution containing 50 mmol/L of TBAOH as the phase transfer reagent at a flow rate of 1 μL/min at 75°C for 18 h. The obtained cation-exchange monoliths were rinsed with 15 mmol/L of HNO₃ for 2 h to react with the residual epoxy sites and washed with water for 8 h for further chromatographic tests.

**Estimation of cation-exchange capacity by a breakthrough method**

The estimation was performed by passing a 10 mmol/L CuCl₂ solution through the column, and an ultraviolet (UV) detector used for the breakthrough of absorption monitoring at 210 nm.

**Chromatographic performance**

The separation of cations performed on the prepared micro-IC system consisted of a pump with the flow rate lowered to 1 μL/min, a micro-injector with injection volume of 500 μL, a conductivity detector and other tube contactors. The whole analysis procedure of capillary IC is shown in Figure 1.

The mobile phase for the separation of univalent cations was 15 mmol/L of MSA; the mobile phase for divalent cations was a mixture of 10 mmol/L diethylamine with the pH adjusted by MSA to 4.

**Result**

Methacrylate monolithic columns were prepared from silanized fused-silica capillaries of 320 μm i.d. by in situ polymerization of GMA and EDMA in the presence of 1,4-butanediol, 1-propanol and water as the porogen solvents and AIBN as the initiator at 60°C for 20 h. By sulfonating the matrix containing epoxy sites with sodium sulfite and TBAOH for 18 h, the proper cation-exchange capacities were achieved in the range of 45–105 μequiv/mL for a 25 cm column. Because of the satisfactory modification, five common univalent cations (Li⁺, Na⁺, NH₄⁺, K⁺, and Cs⁺) were smoothly separated by using 15 mmol/L MSA as the mobile phase on the prepared column by a capillary IC system. With a stronger elution (10 mmol/L of diethylamine with the pH adjusted by MSA to 4), a satisfactory result was obtained for separating four divalent cations (Mg²⁺, Ca²⁺, Sr²⁺ and Ba²⁺). More detailed discussions about the experimental results are presented in the following.

**Discussion**

*Preparation of methacrylate-based monolithic column*

GMA polymerized with EDMA is often used to prepare monolithic matrices because the epoxy-containing stationary phase can easily be modified to form a different chemical nature. The contents and constitution of the porogens in the polymerization mixture have significant influences on the hydrodynamic permeability and mechanical strength (24). Different contents of porogens (35, 40 and 45%) were tested. When the content of porogens was low (35%), the polymer globules formed slowly and the pores of the clusters formed by these globules were small, so the backpressure of the prepared column was very high (>12 MPa when flow rate was 3 μL/min); this was not suitable for further modification and chromatographic separation. If more porogens (45%) were used, the formed pores were too large to offer enough active epoxy sites for the modifier solution and the resulting exchange capacities were not desirable. Among the three kinds of porogens (propan-1-ol, butane-1,4-diol and water), the content of propan-1-ol plays the most important role in the preparation of suitable polymerized pores for reaction and separation. In this work, ternary mixtures of propan-1-ol, butane-1,4-diol and water were introduced. The
The proportion of porogens in the total reaction solution was fixed at 40% (v/v), with a mixture containing 60% propan-1-ol, 30% butane-1,4-diol and 10% water, as a good compromise among satisfactory incorporation of functional monomers, high permeability and proper mechanical strength.

**Preparation of cation-exchange monolithic column**

The epoxy site on the surface of the methacrylate-based monolithic column was very active and could be reacted with different chemicals for different modifications. Sodium sulfite was chosen to modify the stationary phase to prepare the sulfonated cation-exchange column. The cation-exchange capacity is important to the separation and has a significant effect on chromatographic performance. Thus, the degree of modification was controlled to achieve the proper capacity. The degree of modification is dependent on reaction time, concentration of the modifier and temperature. Among these parameters, reaction immersion time is the most convenient and simple parameter for forming various columns with different cation-exchange capacities. Because the modification was dynamic, the reaction times (6, 12, 18 and 24 h) and flow rates of the modifier solution (1, 3 and 5 mL/min) were further studied. Because the modifier would react with the hydrophobic polymer-based surfaces, a phase transfer reagent (TBAOH) also was needed. Figure 2 shows the influence of time and flow rate of the modifying reaction on the cation-exchange capacity. With the proper results, a 1 mol/L sodium sulfite solution containing 50 mmol/L of TBAOH was pumped slowly at 1 mL/min through the methacrylate-based monolithic stationary phase for 18 h at 75°C to react with the epoxy sites, thus forming a cation-exchange column. A column with a higher cation-exchange capability was not produced, because the stronger retention ability resulted in longer retention times of analytes, broad peaks and bad resolutions.

**Chromatographic properties**

The morphology of the polymer-based monolithic matrix was tested by scanning electron microscopy (SEM). The polymer rod was connected with the wall of the capillary tube and the polymer globules were connected with each other to form clusters, pores and channels, as shown in Figure 3.

The permeability of the monoliths was measured through the relationship between flow rate of the eluent and the backpressure of the column. With a common flow rate of 3 μL/min, a pressure of 1.2 MPa was achieved. The column showed excellent mechanical strength under a higher flow rate of 10 μL/min with only a low pressure of 0.4 MPa. Because of the excellent permeability of the monolithic column, a higher flow rate, such as 5–8 μL/min, can be used for fast separation without more changes in retention.

The prepared columns were characterized for hydrodynamic properties through the total porosity values, relative standard deviations (RSDs) of total porosity values calculated from retention times of the unretained water, column permeability values, $K$ (Equation 1), and separation impedance, $E$ (Equation 2) (30).

\[
K = \frac{\mu \eta L}{\Delta p} \quad (1)
\]

\[
E = \frac{H^2}{K} \quad (2)
\]
where $u$ is the linear velocity of the eluent, $\eta$ is the dynamic viscosity of the eluent, $L$ is the column length, $\Delta p$ is the pressure drop and the $H$ is the height equivalent to a theoretical plate.

The RSD of the total porosity values was below 1%, which proved that a unique pore size can be obtained with the optimized polymerization conditions. The $K$ values were in the range of 390–488 (10$^{12}$ m$^2$) and $E$ ranged from 105 to 164 (10$^4$).

**Capillary ion chromatography system for analysis**

At present, capillary IC systems are commercially available, but the cost is very high and most of studies concerning capillary IC do not reach beyond laboratories. The current work developed a system for cations with a conductivity detector instead of a UV detector, the applications of which are limited to few UV absorbing ions. The chromatographic performance was not efficient, but clearly demonstrated the potential of the development of low cost, low pressure and portable micro/capillary IC instrumentation.

**Separation of univalent cations with prepared capillary column**

It is difficult to prepare a cation-exchange column for IC for the separation of common univalent cations. Because of the suitable capacity and pore size of the stationary phase, a mixture of Li$^+$, Na$^+$, NH$_4^+$, K$^+$ and Cs$^+$ can be separated with 20 mmol/L of MSA as the eluent, with a back conductivity of approximately 16.2–16.6 $\mu$S at a flow rate of 6 $\mu$L/min. The obtained chromatogram is shown in Figure 4. Although the background was rather high with the non-suppressed conductivity detection, and the column efficiency was not as high as possible (<1,000), the five cations were separated smoothly and with excellent resolution.

**Separation of divalent cations with prepared capillary column**

Because of the functional sites on the stationary phase, divalent cations cannot be separated with univalent cations because of the longer analysis time. A stronger eluent was introduced into the separation by using 15 mmol/L of diethylamine with pH adjusted to 4 by MSA. Figure 5 shows the chromatographic separation of four divalent cations.

To ensure the run-to-run and day-to-day precision values in retention times, the mixture of cations was injected five times per day during a period of five days. RSD of run-to-run
reproducibility was approximately 2.8–4.3% and the RSD for day-to-day was above 6%.

Conclusion
A novel method for the preparation of strong cation-exchange monolithic columns was applied for the separation of common cations; this was performed on a settled capillary IC system consisting of a pump with a flow rate of 1 μL/min, an injector with a 500 nL injection loop, the prepared monolithic column and a capillary conductivity detector. The conditions of polymerization and modification were carefully optimized for suitable pore size and proper cation-exchange capacity. After further tests of morphology, permeability and hydrodynamic properties, nine common univalent and divalent cations were smoothly separated in this system with different eluents. Although the sensitivity was not as good as possible because of the non-suppressed conductivity detection, the potential of the low-cost system and the simplicity of the preparation of the column has been shown.

Acknowledgments
This research was financially supported by the National Natural Science Foundation of China (Nos. 20775070, J0830413, 20911140271).

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