Optimization and Investigation of Zwitterionic Monolithic Stationary Phases for Capillary Ion Chromatography

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Zwitterionic monolithic columns were synthesized by a one-pot reaction using [2-(methacryloyloxy)ethyl]dimethyl-(3-sulfopropyl)ammonium hydroxide, ethylene dimethacrylate, methanol and 2,2′-azobis(isobutyronitrile) as the monomer, cross-linker, porogen and initiator, respectively. The optimum conditions for polymerization and the efficiency of the prepared columns were examined for ion chromatography. The separation of five kinds of inorganic anions was achieved. The back pressures were monitored as increasing flow-rate, and the resulting plate heights (i.e. height equivalent of a theoretical plate, HETP) of SCN⁻ were calculated at the inspected flow-rates. It was found that the increment rates of both the back pressure and HETP were rather slight. Mobile phases containing various cations or acid increased the retention times of the anions. Divalent cations could be separated, while monovalent cations could not be resolved due to their weak retention on the stationary phases.

Keywords Inorganic anions, inorganic cations, zwitterionic monolithic columns, capillary ion chromatography

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Introduction

Monolithic columns are generally categorized into two types: organic-polymer and silica based skeleton. The advantages of monolithic columns are derived from their high porosity, because monolithic materials consisting of through-pores and mesopores, enable higher linear flow-rates and low back pressures. Another representative merit of monolithic columns are derived from their high porosity, zwitterionic structure.5,6 Jiang and coworkers reported a method to control the ratio of positively and negatively charged groups was also reported.8 Besides, the effect of the length of an alkyl chain between the positively and negatively charged groups was studied.9

Another approach is to dynamically coat the stationary phases with zwitterionic surfactants.10,11 These stationary phases are usually used for reversed-phase chromatography; the surfactants are normally absorbed onto a solid support via a hydrophobic interaction. In most of cases, zwitterionic chemicals are added to the mobile phase to keep the concentration.

Monolithic columns were introduced in the late 1990s;1–3 many scientists have made attempts to synthesize zwitterionic monolithic columns since then. Polymer-based monolithic columns were synthesized by in-situ polymerization. Monomers, cross-linkers, porogens and free-radical initiators are important for synthesizing monolithic columns. A zwitterionic monolithic stationary phase was prepared based on the thermal-initiated copolymerization of (N-(3-methacrylamidopropyl-N,N-dimethyl-N-3-sulfopropyl) ammonium betaine (zwitterionic monomer) and ethylene glycol dimethacrylate (EDMA)).14,15 Another monomer i.e. 2-methacryloyloxyethyl phosphorylcholine, was used to synthesize a phosphorylcholine-type zwitterionic monolithic column.16 Silica monolith was also modified with lysine (2,6-diaminohexanoic acid) groups.17 Moravcová and coworkers reported on the use of zwitterionic silica-based monolithic capillary columns for isocratic and gradient elution for application to hydrophilic interaction chromatography (HILIC).18

The main and ultimate aim to synthesize zwitterionic stationary phases was to separate both cations and anions simultaneously. Also, it is well-known that zwitterionic stationary phases can be applied for HILIC. By taking advantage of the easy preparation of monolithic columns, optimization for HILIC separation was fulfilled. Although zwitterionic monolithic stationary phases have been applied for the HILIC mode many times, there are few reports on the separation of anions and cations. The zwitterionic structure caused both attraction and repulsion to inorganic analyte samples. In this study, zwitterionic monolithic columns in capillaries were synthesized and applied to capillary ion chromatography.

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Experimental

Reagents and chemicals

The reagents employed were of guaranteed grade reagents and were obtained from Wako Pure Chemical Industries (Osaka, Japan), unless otherwise noted. Sodium bromide, sodium iodate, sodium iodide, sodium nitrate, sodium nitrite, sodium thiocyanate, ammonium chloride and benzyltrimethylammonium chloride were obtained from Nacalai Tesque (Kyoto, Japan). 3-(Trimethoxysilyl)propyl methacrylate was obtained from Tokyo Chemical Industry (Tokyo, Japan). Lithium chloride, magnesium chloride hexahydrate and potassium chloride were obtained from Yoneyama Yakuhin Kogyo (Osaka, Japan). 3-(Trimethoxysilyl)propyl methacrylate was obtained from Tokyo Chemical Industry (Tokyo, Japan). Lithium chloride, magnesium chloride hexahydrate and potassium chloride were obtained from Yoneyama Yakuhin Kogyo (Osaka, Japan). 3-(Trimethoxysilyl)propyl methacrylate was obtained from Sigma-Aldrich (St. Louis, USA). Ultrapure water was prepared in the laboratory by using a Simplicity UV water purification system (Millipore, MA, USA), and all solutions used in this study were prepared using this ultrapure water. All packing materials were packed in fused-silica capillary tube (100 × 0.32 mm i.d.; GL Sciences, Tokyo, Japan).

Apparatus

In this work, all experiments were conducted by using a capillary LC system constructed by a syringe pump YSP-101 (YMC, Kyoto, Japan) equipped with a gas-tight syringe (0.5 mL; Ito, Fuji, Japan) as a pump, an C4-1004-2 internal sample injector (VICI Valco Instruments, Houston, USA) with an injection volume of 0.2 µL, a microcolumn prepared from a fused-silica capillary tube (100 × 0.32 mm i.d.; GL Sciences), a UV detector (JASCO, Tokyo, Japan) with the detection wavelength set at 210 nm, and a data processor (CDS-Lite Ver. 5.0; LA soft, Chiba, Japan). The inlet pressure was monitored by an LTEX-8150 pressure sensor (LTEX Corporation, Tokyo, Japan). Separation columns were operated under room temperature (controlled at 25°C) throughout the study.

Preparation of zwitterionic monolithic columns

After 0.2 M NaOH, 0.2 M HCl and 20% (v/v) 3-(trimethoxysilyl)-propyl methacrylate in ethanol were passed through fused-silica capillary tube in a sequential order for 30 min each to attach methacrylate groups on the inner wall of the capillary tube, the tube was washed by ethanol and dried by passing nitrogen. A mixture of zwitterionic monomer, EDMA, methanol and initiator was then filled in a pretreated tube and sealed at both sides with a laboratory-made PTFE-tube-made-seal. At all times, 3 mg 2,2′-azobisisobutyronitrile (AIBN) was chosen as the initiator. The capillary was dipped in a water bath at 60°C for 18 h. After that, methanol was flushed through the tube to wash out the residuals.

Results and Discussion

Optimization of mixture composition for preparing the zwitterionic monolithic columns

Monolithic columns were prepared by a one-pot reaction, as shown in Fig. 1. The adjustable parameters were the weights/ratio of each chemical, zwitterionic monomer, EDMA and methanol. Table 1 gives all specific values of the chemicals employed in this study. The reaction temperature and the duration were kept constant at 60°C and 18 h, respectively, under all situations. The differences among columns A, B, C and D involved the concentration of methanol, i.e. the only porogen used in this study. The ratio between the zwitterionic monomer to EDMA was one to one. Column A could not pass the mobile phase due to the fact that it had the lowest concentration of methanol, which means there was not a sufficiently large space of the through-pore. Although columns B and C could be used for chromatographic separations, three peaks (IO₃⁻, NO₂⁻, NO₃⁻) were not well-separated. Column D had the highest concentration of methanol, which caused a low density of the polymer skeleton. As a result, it did not show a clear chromatogram. Columns E, F, G and H were distinguished from columns A, B, C and D based on the different ratio between the zwitterionic monomer and the cross-linker. The ratio of the zwitterionic monomer is three-time that of EDMA. The amounts of methanol were varied from 130 to 200 mg. Column E showed good separation of the anions, but the pressure was 3.3 MPa, which was still high when taking into account the property of the monolithic column. Columns F, G and H showed better results, and Column F was the best. The optimized amounts of each reagent were 60.6 mg of zwitterionic monomer, 20.4 mg of EDMA and 140.4 mg of methanol, 27.3, 9.1 and 63.3 wt% respectively. Column F was chosen for further investigation. In addition, the elution profiles using columns B-H are summarized in Fig. S1 as Supporting Information.

Table 1: Compositions of various polymerization conditions

| Column | Monomer/ Cross-linker/ Poregen/ Monomer/ Pressure/ | mg | mg | mg | wt% | MPa |
|--------|---------------------------------|-----|-----|-----|------|-----|
| A      | 40/20                           | 160 | 40/40| 67  | N/A  |
| B      | 40/20                           | 200 | 40/40| 71  | 1.6  |
| C      | 40/20                           | 220 | 40/40| 73  | 0.5  |
| D      | 40/20                           | 250 | 40/40| 76  | 0.4  |
| E      | 60/20 130                      | 60/20| 62  | 3.3  |
| F      | 60/20                           | 140 | 60/20| 64  | 0.5  |
| G      | 60/20                           | 150 | 60/20| 65  | 0.3  |
| H      | 60/20                           | 200 | 60/20| 71  | 0.4  |

a. Zwitterionic monomer, [2-(methacryloyloxy)ethyl]dimethyl-(3-sulfopropyl)ammonium hydroxide.

EDMA, ethylene glycol dimethacrylate.

Porogen, methanol.

d. Eluent, 100 mM NaCl; flow-rate, 3 µL min⁻¹.

Fig. 1 Expected reaction in the preparation of a zwitterionic sulfobetaine monolithic column.
Effect of the flow-rate

The effects of the flow-rate on the HETP (i.e., van Deemter plot) as well as the resulting back pressures are summarized in Fig. 2. As can be seen, both HETP and the back pressure increased slightly when the flow-rate was increased from 2.0 to 5.0 μL min⁻¹. The back pressure was lower than 1.0 MPa on the zwitterionic monolithic column, even when it was operated at 5.0 μL min⁻¹. This is the major advantage of monolithic columns. In addition, the increment of HETP was not badly influenced when increasing the flow-rate, which means that the column could be operated at high flow-rates without compromising the separation efficiency.

Separation of inorganic anions

The retention behavior of inorganic anions was measured by using the prepared columns. IO₃⁻, NO₂⁻, NO₃⁻, I⁻ and SCN⁻ were selected as analytes samples. Although the zwitterionic structure can repulse samples that have negative charges, it can be expected that these samples could be retained. Figure 3 showed the results of analyte anions separation. All samples were well-separated using 100 mM NaCl as the eluent; the elution order of the samples was the same as those obtained by ion-exchange chromatography. However, when lower concentrations of NaCl were used, a decrease in the retention time in all model ions was observed (the elution profiles are shown as Fig. S2 in Supporting Information). These results are opposite to those normally observed in ion-exchange chromatography; further experiments are being carried out in order to clarify the retention mechanism involved. The back pressure was 0.6 MPa at a flow-rate of 3.0 μL/min.

Effect of cations in the mobile phases

Zwitterionic stationary phases have both negative and positive charges. It can thus be presumed that cations present in the mobile phases could influence the retention behavior of anionic samples. Figure 4 shows the retention of analyte anions separated with various eluents that contained different cations. The retention times of all samples became longer as the cations varied from Li⁺, Na⁺, K⁺ to Mg²⁺. This could be explained by the retention strength of the cations. The order of retention strength is Li⁺ < Na⁺ < K⁺ < Mg²⁺ on the cation exchange site. Any cation that has strong retention, such as Mg²⁺, screens the negative charges on the external cation exchange groups, and thus analyte anions were more retained by the quaternary ammonium groups.

Effect of acid in the mobile phase

The retention behavior of inorganic anions was also investigated under an acidic condition. In this study, analyte NO₂⁻ was replaced by Br⁻. NO₂⁻ (pK_a = 3.38) is not suitable for this measurement because HNO₂ cannot be deprotonated under an acidic condition, and does not behave as an anion analyte. Figure 5 shows a comparison of the separation of anions between neutral and acidic conditions. Under the neutral condition, the retention times of IO₃⁻, Br⁻, NO₃⁻, I⁻ and SCN⁻ were 2.20, 3.61, 4.12, 11.85 and 20.27 min, respectively, while those obtained under the acidic eluent condition were 2.34, 3.67, 4.23, 12.67 and 21.44 min, respectively. The retention times given based on the acidic condition are longer than those by the neutral condition. This is because protons could screen any negative charges on the external cation exchange groups, and the anion exchange groups would attract analyte samples more strongly.
Separation of inorganic cation

Zwitterionic monolithic columns can be applied for cation separation. Figure 6 shows the result of cation separation. The salt used as the eluent was 20 mM benzyltrimethylammonium chloride, which is a neutral reagent. The divalent cations Mg\(^{2+}\) and Ca\(^{2+}\) could be retained and separated. On the other hand, the monovalent cations Na\(^+\), NH\(_4\)\(^+\) and K\(^+\) were coeluted. This is because the retention of monovalent cations was intrinsically weak, and thus repulsion from quaternary ammonium groups could prevent any electrostatic interaction prominently. As a result, it was too weak to attract monovalent cations.

Conclusions

A zwitterionic monolithic stationary phase prepared under the optimum reaction conditions could be used for anion separation. The column showed slight increment rates of the back pressure and HETP with increasing flow-rate, which is the major advantage of monolithic structures. The retention of anions could be affected by the composition of the mobile phases. Although divalent cations could be successfully separated, monovalent cations could not be separated due to their weak retention compared to repulsion of the quaternary ammonium groups. Nevertheless, the developed stationary phase is expected to also be used for applications in the HILIC mode.

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Supporting Information

This material is available free of charge on the Web at http://www.jsac.or.jp/analsci/.

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