



Separation of small inorganic anions using methacrylate-based anion-exchange monolithic column prepared by low temperature UV photo-polymerization

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ABSTRACT

A methacrylate-based anion-exchange monolithic column was prepared by a single-step UV photo-copolymerization of [2-(methacryloyloxy)ethyl]-trimethyl ammonium chloride, butylmethacrylate, and ethylene dimethacrylate at a low temperature of -15°C . The anion-exchange column exhibited good separation efficiency for the small inorganic anions of NO_2^- , Br^- , NO_3^- , and I^- in the isocratic mode. Under the conditions optimized using a mobile phase of 50% ACN containing 100 mM of NaCl, the theoretical plate heights of the anions were within the range of $12.2\text{--}15.6\ \mu\text{m}$ (N , $64,000\text{--}82,000\ \text{m}^{-1}$; k , $0.2\text{--}1.6$); and a value of $9.4\ \mu\text{m}$ (N , $110,000\ \text{m}^{-1}$) was achieved for t_0 . The flow resistance of the column was acceptably low with a permeability of $2.7 \times 10^{-13}\ \text{m}^2$. Fast gradient elution at a flow rate of 32 mm/s resulted in rapid and precise separation of the inorganic anions of IO_3^- , NO_2^- , Br^- , NO_3^- , and I^- within 20 s.

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1. Introduction

Recently, porous monolithic columns have attracted much attention as new HPLC columns. The materials used for a monolithic bed are classified into two general types: silica monoliths [1–3] and polymer monoliths [4–11]. One of the most attractive properties of a monolithic column is the low flow resistance, which allows the column length to be extended significantly [2,3]. Miyamoto et al. prepared a reversed-phase (RP) silica monolithic column with a length of over 10 m and used the column for the separation of deuterium-substituted benzenes [2]. Rapid separations using a mobile phase stream at a high flow rate has been reported to be another unique application of monolithic columns. Ultra-high speed separations at flow velocities ranging from several tens to one hundred millimeters per second have been demonstrated using RP polymer monolithic columns [6,9].

The ion-exchange mode is one of the most general separation modes in HPLC. Thus, the development of both cation- and anion-exchange monolithic columns has been frequently reported [12–18]. In the case of anion-exchange silica monolithic columns, a high separation efficiency (theoretical plate height, H , of $9\text{--}14\ \mu\text{m}$) has been achieved for small inorganic anions [15]. However, the separation efficiency of small inorganic anions on polymer

monolithic columns is still not high as that on silica monolithic columns (theoretical plate number per meter, N , of $<40,000\ \text{m}^{-1}$ or H of $>25\ \mu\text{m}$) [13,16,18]. Therefore, further advances in the separation efficiency of anion-exchange polymer monolithic columns are desired.

The preparative procedures for anion-exchange polymer monolithic columns can be divided into two categories: multi-step methods and single-step methods. In the multi-step method, a porous structure without anion-exchange ability is initially polymerized and the anion-exchange functionality is subsequently introduced. In the single-step method, the reaction solution containing anion-exchange functional materials is polymerized and a porous structure with anion-exchange functionality is prepared as long as there are available monomers with the suitable functionalities. The advantages of the single-step method are that it is a relatively simple and time-efficient preparation procedure. In this study, therefore, the latter method was used to develop a rapid and simple method preparing an anion-exchange polymer monolithic column with a high separation efficiency and high permeability that achieves the fast separation of inorganic anions.

Recently, we found that photo-initiated polymerization at low temperatures is effective for the preparation of highly efficient and highly permeable methacrylate-based RP monolithic columns [9]. Concurrently, Szumski and Buszewski also reported similar results [8]. In the present study, we developed a single-step-preparation method for the construction of an anion-exchange polymer monolithic column by low-temperature UV photo-polymerization. The column efficiency was evaluated for small UV absorbable inorganic

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Table 1

Mixing ratios by weight of reaction solutions for anion-exchange polymer monolithic column^a. Values in parentheses are ratios in wt% unit.

	BMA	EDMA	META	1-Propanol	1,4-Butanediol
AX5	16(15.8)	14(13.8)	1.5(1.5)	50(49.3)	20(19.7)
AX10	16(15.5)	14(13.6)	3.0(2.9)	50(48.5)	20(19.4)
AX20	16(15.1)	14(13.2)	6.0(5.7)	50(47.2)	20(18.9)

* 1 wt% DMPA to total weight of monomers was added as an initiator.

anions, and their rapid separation in both isocratic and gradient modes was demonstrated.

2. Experimental

2.1. Apparatus

The arrangement of the apparatus used for chromatography in this study was almost the same as that reported in our previous research [19]. The apparatus was composed of two pumps (LC-20AD, Shimadzu), a T-connector for mixing mobile phases, a splitter, a resistance tube (i.d. 0.05 mm × 50 mm), an injector (7520, Rheodyne), a capillary monolithic column, and a UV detector (CE-970, Jasco). The splitter was fitted at the outlet end of the injector and was used for split injection. The pumps were operated at a constant flow rate (0.036–20.9 mL/min), and a large fraction of the mobile phase was removed as waste from the splitter.

2.2. Chemicals

Butyl methacrylate (BMA), ethylene dimethacrylate (EDMA), [2-(methacryloyloxy)ethyl]-trimethyl ammonium chloride (META), 1-decanol, cyclohexanol, 2,2-dimethoxyphenyl-2-acetophenone (DMPA), methanol, acetonitrile (ACN), uracil, sodium nitrate, sodium nitrite, potassium bromide, sodium iodide, and sodium iodate were purchased from Wako Pure Chemicals. 3-Methacryloxypropyltrimethoxysilane was obtained from Shin-Etsu Chemicals.

2.3. Preparation of anion-exchange monolithic column

A UV-transparent fused-silica capillary (i.d. 0.1 mm, o.d. 0.375 mm, GL Science) was silanized with 3-methacryloxypropyltrimethoxysilane as described in our previous paper [9]. The capillary was cut to a length of 15 cm and filled with the reaction solution. The compositions of the reaction solutions are summarized in Table 1. Photo-polymerization was then carried out using a UV illuminator (3UV Bench top Trance Illuminator, Upland) as a UV light source (254 nm, 2 mW/cm²) in an incubator (MIR-153, Sanyo) at –15 °C for 8 min [8,9]. Following polymerization, the capillary was immediately connected to an LC pump and then washed with methanol for at least 6 h at a flow rate of approximately 2 μL/min. Finally, the column was cut to a length of 10 cm.

3. Results and discussion

3.1. Effect of amount of META in reaction solution

The method for preparing the anion-exchange monolithic column used in this study was referenced to the method used for capillary electrochromatography (CEC) columns. For the preparation of a monolithic column for CEC, a small amount of ionic monomer is often added to the reaction solution to generate a stable electroosmotic flow. In this study, a solution containing a moderate amount of anion-exchangeable monomer was polymerized. A

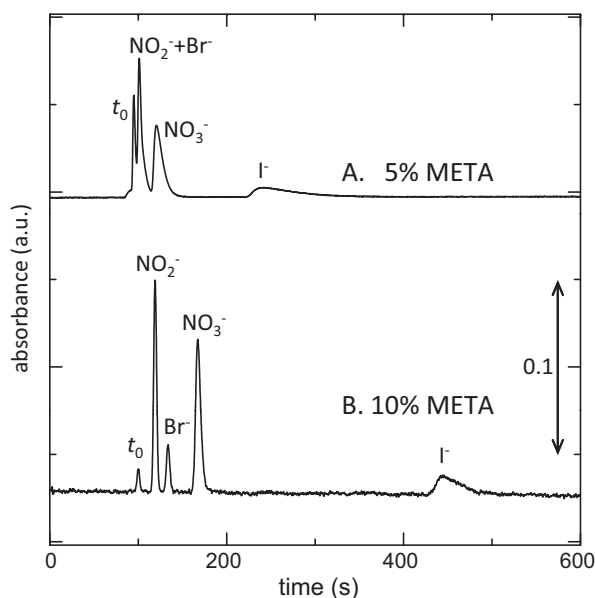


Fig. 1. Effect of META concentration in reaction solution on separation of small inorganic anions. Column: BMA-co-EDMA-co-META monolithic column ((A) AX5 and (B) AX10, i.d. 0.1 mm, length 100 mm); mobile phase: 100 mM NaCl aqueous solution; apparent flow velocity: 1 mm/s; detection: UV 190 nm; t_0 marker: uracil.

mixture composed of BMA (16 wt%), EDMA (14 wt%), 1-propanol (50 wt%), and 1,4-butanediol (20 wt%) was used as the base reaction solution [7]. META, an anion-exchange functional monomer, was added to the base solution in varying amounts of 5, 10, and 20 wt% relative to the total weight of BMA and EDMA. The compositions of the reaction solutions used in this study are listed in Table 1.

The permeability of the monolith column prepared using 20% META (AX20 in Table 1) was significantly lower than that of the monolithic column prepared using 5% and 10% META (AX5 and AX10). Because a column having a high flow resistance is not suitable for fast separation, the performance of the AX20 column was not assessed in this study. The separation efficiencies of the AX5 and AX10 columns were evaluated using small UV-absorbable inorganic anions. As shown in Fig. 1, the retention times of the anions on the AX5 column were lower than those on the AX10 column, i.e., the retention factor of NO_3^- on the AX10 column was about three times larger than that on the AX5 column. Increasing the amount of META in the reaction solution reasonably enhanced the retention ability of the columns for the analyte anions. Furthermore, the separation efficiency of the AX10 column was clearly superior to that of the AX5 column. The theoretical plate number per meter (N) of NO_3^- on the AX5 and AX10 columns was 7200 and 39,000 m⁻¹, respectively. Therefore, the anion-exchange monolithic column prepared using 10% META was further investigated in this study.

3.2. Effect of organic modifier in mobile phase

In this study, BMA, META, and EDMA were used in the copolymerization process for the preparation of the anion-exchange monolithic column. The BMA in the monolith has hydrophobic retention ability. Therefore, the content of an organic modifier, or acetonitrile (ACN), in the mobile phase was increased from 0% to 75% to assess the hydrophobic interaction. The separations of the inorganic anions using various mobile phases were shown in Fig. 2. In the case of the mobile phase without ACN (Fig. 2A), all analytes were completely separated. However, the elution time of the iodide anion, which is known as a relatively polarizable inorganic anion, was significantly longer and an unsymmetrical peak

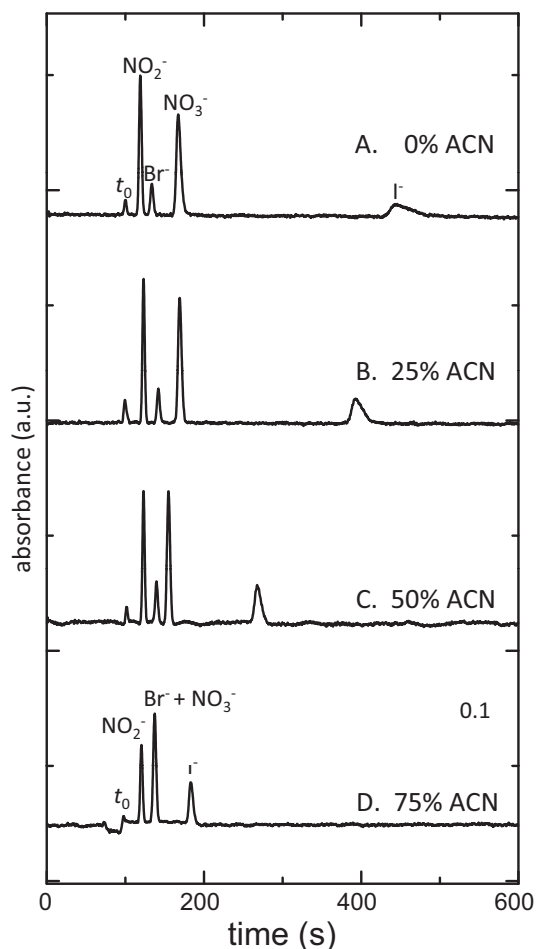


Fig. 2. Effect of acetonitrile concentration on separation of small inorganic anions. Column: BMA-co-EDMA-co-META monolithic column (AX10, i.d. 0.1 mm, length 100 mm); mobile phases: mixtures of acetonitrile ((A) 0%, (B) 25%, (C) 50%, and (D) 75%) and water containing 100 mM NaCl. The other conditions were the same as those for Fig. 1.

was obtained. As the concentration of ACN in the mobile phase was increased, the elution of the iodide anion was accelerated and the peak assumed a symmetrical shape as shown in Fig. 2. The suppression of the hydrophobic interaction between the iodide anion and the stationary phase provided the desired separation.

The addition of ACN to the mobile phase also influenced the separation behavior of other anions. The elution of all anions was slightly accelerated with the increase in the ACN content, similar to the iodide anion. The use of a mobile phase containing 75% ACN (Fig. 2D) resulted in overlap of Br^- and NO_3^- peaks. The separation efficiency, or the theoretical plate number per meter, of NO_2^- with the mobile phases containing 0, 25, 50, and 75% ACN were 54,000, 68,000, 82,000, and 66,000 m^{-1} , respectively. The column permeability (K) under these mobile phases was calculated by using the following equation: $K = \eta Lu/P$ where η , L , u , and P were viscosity, column length, t_0 -based flow velocity ($u = L/t_0$), and backpressure, respectively [20]. The permeability was decreased with the increase in ACN concentration, i.e., 3.5×10^{-13} , 2.9×10^{-13} , 2.7×10^{-13} , and $2.4 \times 10^{-13} \text{ m}^2$ were calculated for the mobile phases containing 0, 25, 50, and 75% ACN, respectively. The results suggested that the monolith was swelled according to the increase in the ACN concentration. The variation in the separation efficiency of NO_2^- would be provided by the change in the monolithic structure. In light of both the elution time and separation efficiency, the mobile phase containing 50% ACN was used for the following assays. The column had

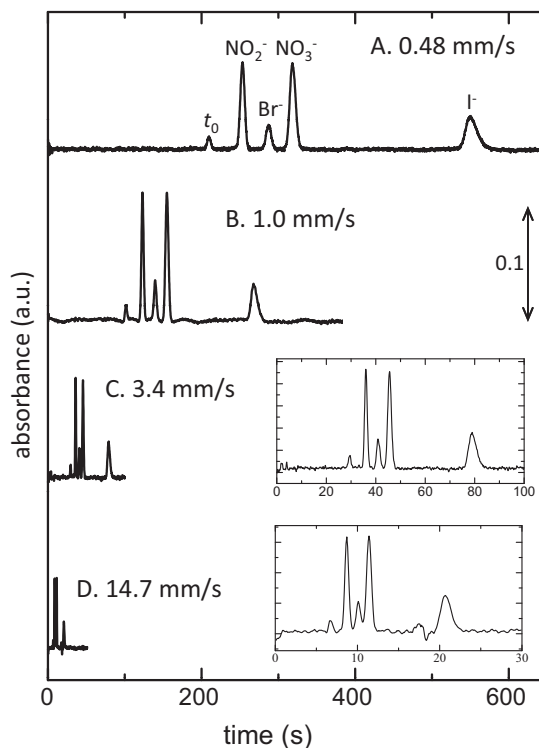


Fig. 3. Separation of small inorganic anions with various flow velocities of (A) 0.48 mm/s, (B) 1.0 mm/s, and (C) 3.4 mm/s, and (D) 14.7 mm/s. Mobile phase: acetonitrile/water (50/50) containing 100 mM NaCl; the other conditions were the same those as for Fig. 1.

been used without any deterioration in the column efficiency for at least four months with the mobile phase containing 50% ACN. The organic solvent in the mobile phase did not aggravate the column stability or column life significantly.

3.3. Separation in isocratic elution mode

The inorganic UV-absorbable anions were separated using various flow rates as shown in Fig. 3. Moreover, the relationship between the flow rate and column efficiencies, or H - u plot, is shown in Fig. 4. The best column performance was achieved at a

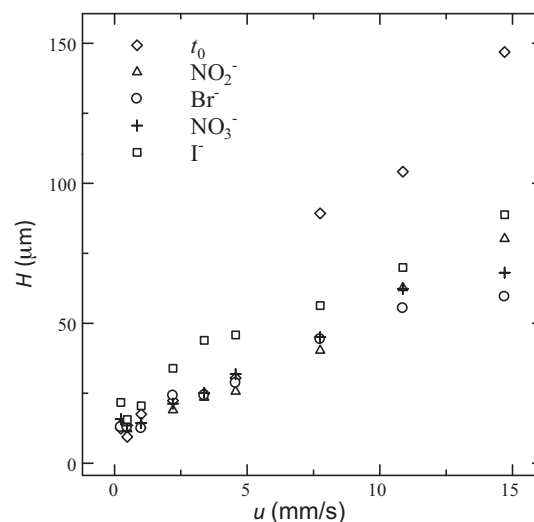


Fig. 4. Van Deemter plots of inorganic anions. Chromatographic conditions were the same as those for Fig. 3.

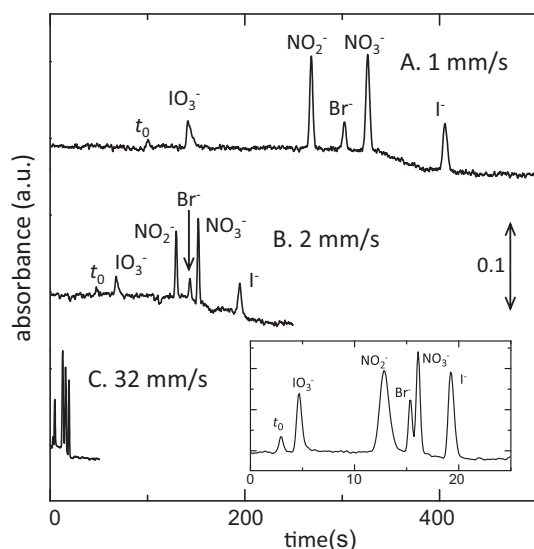


Fig. 5. Separation of small inorganic anions in gradient elution mode with various flow rates of (A) 1.0, (B) 2.0, and (C) 32 mm/s. Mobile phases: mixture of acetonitrile and water (50:50) containing (I) 10 mM NaCl and (II) 200 mM NaCl. Gradient programs: (A) 100% (I) in 120 s, 100–0% (I) in 240 s; (B) 100% (I) in 60 s, 100–0% (I) in 120 s; (C) 100% (I) in 3 s, 100–0% (I) in 6 s; other chromatographic conditions were the same as those for Fig. 3.

flow rate of 0.48 mm/s (Fig. 3A), i.e., the theoretical plate heights of the anions were 12.2–15.6 μm (N , 64,000–82,000 m^{-1} ; k , 0.2–1.6) and 9.4 μm (N , 110,000 m^{-1}) for t_0 . The separation efficiency of the anion-exchange polymer monolithic column prepared in this study was almost comparable to those of silica based monolithic columns (N , 69,000–106,000 m^{-1} ; k , 0.1–2.5) [15]. When the polymerization period was increased from 8 to 12 min, both the separation efficiency and permeability of the column were decreased. The similar behavior was also reported previously [11,21–23]. Therefore, low conversion polymerization would be a key to the high separation efficiency exhibited in this study.

Baseline separation of all analytes was obtained up to a flow rate of 3.38 mm/s as shown in Fig. 3C. The five analytes were separated within 25 s at a flow rate of 14.7 mm/s (Fig. 3D), whereas the peaks of Br^- and NO_3^- were partially overlapped. The theoretical plate heights at 14.7 mm/s ranged from 60 to 89 μm (N , 12,000–17,000 m^{-1}) except for the t_0 marker.

In the flow rate range of 0.24–14.7 mm/s, the backpressure was proportional to the flow rate ($r^2 = 0.998$). This fact indicated that the monolithic structure was not deformed in this flow rate range, or under a pressure of less than 5 MPa (backpressure at 14.7 mm/s). In the linear relationship between the flow rate and backpressure, the slope (S_{p-u}) was 0.33 MPa/(mm/s), which corresponds to the pressure necessary to produce a flow rate of 1 mm/s using 50% ACN containing 100 mM of NaCl ($\eta = 0.91$ mPa s) as the mobile phase. The column permeability in this condition was $2.7 \times 10^{-13} \text{ m}^2$ as described previous section. The flow resistance and permeability of the anion-exchange monolithic column was roughly comparable with those of a RP monolithic column previously prepared by the current authors using photo-polymerization under low-temperature [9]. The preparation of the methacrylate-based monolithic column using UV photo-polymerization under low temperature is an effective method for producing a highly permeable column with high separation efficiency.

3.4. Separation in gradient elution mode

The separation of the anions was also performed using gradient elution. Fig. 5 shows the separation of the five UV absorbable

Table 2

Repeatability for various analytes in fast gradient separation at 32 mm/s* (mean \pm SD (%RSD), $n = 5$).

Analyte	Elution time (s)	Peak height (a.u.)	Relative peak height
Uracil	3.1 \pm 0.25 (8.1)	18.7 \pm 1.9 (10.0)	(I.S.)
IO_3^-	4.8 \pm 0.25 (5.2)	70.9 \pm 6.7 (9.5)	3.79 \pm 0.08 (2.2)
NO_2^-	13.0 \pm 0.20 (1.5)	97.0 \pm 9.0 (9.3)	5.18 \pm 0.10 (2.0)
Br^-	15.4 \pm 0.12 (0.8)	62.1 \pm 4.8 (7.7)	3.32 \pm 0.16 (4.8)
NO_3^-	16.1 \pm 0.14 (0.9)	122.1 \pm 11.8 (9.7)	6.52 \pm 0.14 (2.2)
I^-	19.3 \pm 0.19 (1.0)	102.3 \pm 9.7 (9.5)	5.47 \pm 0.10 (1.8)

* The chromatographic conditions were the same as those for Fig. 5C.

inorganic anions, as well as the t_0 marker, using various flow rates with gradient elution. The concentration of NaCl in the mobile phase was increased from 10 to 200 mM over the course of the gradient to enhance the elution. The details of each gradient program are described in the figure caption. At flow rates of both 1.0 and 2.0 mm/s (Fig. 5A and B), the analytes were separated completely. Based on the increase in the flow rate, the separation period in Fig. 5B was reduced to half the value of that in Fig. 5A. A flow rate of 32 mm/s resulted in separation of the analytes within 20 s as shown in Fig. 5C. The ultra-fast separation of small inorganic anions was, therefore, successfully achieved.

The repeatability of the fast separation was statistically evaluated. The values of the mean, SD, and %RSD for elution time, peak height, and relative peak height (uracil was regarded as an internal standard) under the separation conditions employed in Fig. 5C ($n = 5$) are listed in Table 2. The SD values for elution time were less than 0.25 s for all analytes and better repeatability in the elution time was exhibited for the analytes that were eluted at longer times. The %RSD values for the peak height were approximately 10% for all analytes. In the case of relative peak height, the %RSDs were around 2% for all of the analytes except Br^- . The high precision of the fast separation using rapid gradient elution was thus confirmed.

4. Conclusions

A single-step UV photo-polymerization method was developed for the preparation of a methacrylate-based anion-exchange monolithic column at low temperature. The column exhibited good separation efficiency for some small inorganic anions. Under optimal conditions, a theoretical plate height of 9.4–15.6 μm (N , 64,000–110,000 m^{-1}) was achieved. This column efficiency was relatively high for a polymer monolithic column and was almost comparable with the efficiency of silica-based anion-exchange monolithic columns. The flow resistance of the column was acceptably low, and fast separation was successfully demonstrated with an apparent flow velocity up to 32 mm/s. In the gradient method, five inorganic anions were separated rapidly with a high precision within 20 s. In this study, the anion-exchange monolith containing hydrophobic components was prepared in the capillary and the short optical pass length of the capillary column is disadvantageous to concentration sensitivity for analyses of real environmental aqueous samples. In a future work, a combination of a suitable pre-concentration method and a high performance anion-exchange column prepared with hydrophilic monomers will be essential to analyze real samples with the organic modifier free condition.

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