Separation of Small Inorganic Anions using Methacrylate-Based Anion-Exchange Monolithic Column Prepared by Low Temperature UV Photo-Polymerization

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15   Abstract

16   A methacrylate-based anion-exchange monolithic column was prepared by a single-step UV

17   photo-copolymerization of [2-(methacryloyloxy)ethyl]-trimethyl ammonium chloride,

18   butylmethacrylate, and ethylene dimethacrylate at a low temperature of -15 ℃. The anion-exchange

19   column exhibited good separation efficiency for the small inorganic anions of NO_2^-, Br^-, NO_3^-, and

20   I^- in the isocratic mode. Under the conditions optimized using a mobile phase of 50% ACN

21   containing 100 mM of NaCl, the theoretical plate heights of the anions were within the range of

22   12.2–15.6 μm (N, 64000–82000 m^{-1}; k, 0.2-1.6); and a value of 9.4 μm (N, 110000 m^{-1}) was

23   achieved for t_0. The flow resistance of the column was acceptably low with a permeability of 2.7 ×

24   10^{-13} m^2. Fast gradient elution at a flow rate of 32 mm/s resulted in rapid and precise separation of

25   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 9–14 μm) 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 <40000 m^{-1} or H of >25 μm) [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

55   preparation procedure. In this study, therefore, the latter method was used to develop a rapid and

56   simple method preparing an anion-exchange polymer monolithic column with a high separation

57   efficiency and high permeability that achieves the fast separation of inorganic anions.

58   Recently, we found that photo-initiated polymerization at low temperatures is effective for

59   the preparation of highly efficient and highly permeable methacrylate-based RP monolithic columns

60   [9]. Concurrently, Szumski and Buszewski also reported similar results [8]. In the present study, we

61   developed a single-step-preparation method for the construction of an anion-exchange polymer

62   monolithic column by low-temperature UV photo-polymerization. The column efficiency was

63   evaluated for small UV absorbable inorganic anions, and their rapid separation in both isocratic and

64   gradient modes was demonstrated.

65

66   ## 2. Experimental

67   ### 2.1 Apparatus

68   The arrangement of the apparatus used for chromatography in this study was almost the same as

69   that reported in our previous research [19]. The apparatus was composed of two pumps (LC-20AD,

70   Shimadzu), a T-connector for mixing mobile phases, a splitter, a resistance tube (id. 0.05 mm × 50

71   mm), an injector (7520, Rheodyne), a capillary monolithic column, and a UV detector (CE-970,

72   Jasco). The splitter was fitted at the outlet end of the injector and was used for split injection. The

73   pumps were operated at a constant flow rate (0.036–20.9 mL/min), and a large fraction of the

74   mobile phase was removed as waste from the splitter.

75

76   ### 2.2 Chemicals

77   Butyl   methacrylate   (BMA),   ethylene   dimethacrylate   (EDMA),

78   [2-(methacryloyloxy)ethyl]-trimethyl ammonium chloride (META), 1-decanol, cyclohexanol,

79   2,2-dimethoxyphenyl-2-acetophenone (DMPA), methanol, acetonitrile (ACN), uracil, sodium

80   nitrate, sodium nitrite, potassium bromide, sodium iodide, and sodium iodate were purchased from

81   Wako Pure Chemicals. 3-methacryloxypropyltrimethoxysilane was obtained from Shin-Etsu

82   Chemicals (Tokyo).

83

84   2.3 Preparation of anion-exchange monolithic column

85   A UV-transparent fused-silica capillary (i.d. 0.1 mm, o.d. 0.375 mm, GL Science) was silanized

86   with 3-methacryloxypropyltrimethoxysilane as described in our previous paper [9]. The capillary

87   was cut to a length of 15 cm and filled with the reaction solution. The compositions of the reaction

88   solutions are summarized in Table 1. Photo-polymerization was then carried out using a UV

89   illuminator (3UV Bench top Trance Illuminator, Upland) as a UV light source (254 nm, 2 mW/cm^2)

90   in an incubator (MIR-153, Sanyo) at -15 ℃ for 8 min [8, 9]. Following polymerization, the

91   capillary was immediately connected to an LC pump and then washed with methanol for at least 6 h

92   at a flow rate of approximately 2 μL/min. Finally, the column was cut to a length of 10 cm.

93

94   3. Results and Discussion

95   3.1 Effect of amount of META in reaction solution

96   The method for preparing the anion-exchange monolithic column used in this study was referenced

97   to the method used for capillary electrochromatography (CEC) columns. For the preparation of a

98   monolithic column for CEC, a small amount of ionic monomer is often added to the reaction

99   solution to generate a stable electroosmotic flow. In this study, a solution containing a moderate

100   amount of anion-exchangeable monomer was polymerized. A mixture composed of BMA (16 wt%),

101   EDMA (14 wt%), 1-propanol (50 wt%), and 1,4-butanediol (20 wt%) was used as the base reaction

102   solution [7]. META, an anion-exchange functional monomer, was added to the base solution in

103   varying amounts of 5, 10, and 20 wt% relative to the total weight of BMA and EDMA. The

104   compositions of the reaction solutions used in this study are listed in Table 1.

105   The permeability of the monolith column prepared using 20% META (AX20 in Table 1)

106   was significantly lower than that of the monolithic column prepared using 5% and 10% META

107   (AX5 and AX10). Because a column having a high flow resistance is not suitable for fast separation,

108   the performance of the AX20 column was not assessed in this study. The separation efficiencies of

109   the AX5 and AX10 columns were evaluated using small UV-absorbable inorganic anions. As shown

110   in Fig. 1, the retention times of the anions on the AX5 column were lower than those on the AX10

111   column, i.e., the retention factor of NO_3^- on the AX10 column was about three times larger than that

112   on the AX5 column. Increasing the amount of META in the reaction solution reasonably enhanced

113   the retention ability of the columns for the analyte anions. Furthermore, the separation efficiency of

114   the AX10 column was clearly superior to that of the AX5 column. The theoretical plate number per

115   meter (N) of NO_3^- on the AX5 and AX10 columns was 7200 and 39000 m^{-1}, respectively. Therefore,

116   the anion-exchange monolithic column prepared using 10% META was further investigated in this

117   study.

118

119   3.2 Effect of organic modifier in mobile phase

120   In this study, BMA, META, and EDMA were used in the copolymerization process for the

121   preparation of the anion-exchange monolithic column. The BMA in the monolith has hydrophobic

122   retention ability. Therefore, the content of an organic modifier, or acetonitrile (ACN), in the mobile

123   phase was increased from 0% to 75% to assess the hydrophobic interaction. The separations of the

124   inorganic anions using various mobile phases were shown in Fig. 2. In the case of the mobile phase

125   without ACN (Fig. 2A), all analytes were completely separated. However, the elution time of the

126   iodide anion, which is known as a relatively polarizable inorganic anion, was significantly longer

127   and an unsymmetrical peak was obtained. As the concentration of ACN in the mobile phase was

128   increased, the elution of the iodide anion was accelerated and the peak assumed a symmetrical

129   shape as shown in Fig. 2. The suppression of the hydrophobic interaction between the iodide anion

130 and the stationary phase provided the desired separation.

131   The addition of ACN to the mobile phase also influenced the separation behavior of other

132 anions. The elution of all anions was slightly accelerated with the increase in the ACN content,

133 similar to the iodide anion. The use of a mobile phase containing 75% ACN (Fig. **2**D) resulted in

134 overlap of Br^- and NO_3^- peaks. The separation efficiency, or the theoretical plate number per meter,

135 of NO_2^- with the mobile phases containing 0, 25, 50, and 75% ACN were 54000, 68000, 82000, and

136 66000 m^{-1}, respectively. The column permeability (K) under these mobile phases was calculated by

137 using the following equation: $K = \eta Lu/ P$ where η, L, u, and P were viscosity, column length,

138 t_0-based flow velocity ($u = L/t_0$),, and backpressure, respectively [20]. The permeability was

139 decreased with the increase in ACN concentration, i.e., 3.5×10^{-13}, 2.9×10^{-13}, 2.7×10^{-13}, and $2.4 \times$

140 10^{-13} m^2 were calculated for the mobile phases containing 0, 25, 50, and 75% ACN, respectively.

141 The results suggested that the monolith was swelled according to the increase in the ACN

142 concentration. The variation in the separation efficiency of NO_2^- would be provided by the change

143 in the monolithic structure. In light of both the elution time and separation efficiency, the mobile

144 phase containing 50% ACN was used for the following assays. The column had been used without

145 any deterioration in the column efficiency for at least four months with the mobile phase containing

146 50% ACN. The organic solvent in the mobile phase did not aggravate the column stability or

147 column life significantly.

148

149 3.3 Separation in isocratic elution mode

150 The inorganic UV-absorbable anions were separated using various flow rates as shown in Fig. 3.

151 Moreover, the relationship between the flow rate and column efficiencies, or H-u plot, is shown in

152 Fig. 4. The best column performance was achieved at a flow rate of 0.48 mm/s (Fig. 3A), i.e., the

153 theoretical plate heights of the anions were 12.2 – 15.6 μm (N, 64000 – 82000 m^{-1}; k, 0.2-1.6) and

154 9.4 μm (N, 110000 m^{-1}) for t_0. The separation efficiency of the anion-exchange polymer monolithic

155   column prepared in this study was almost comparable to those of silica based monolithic columns

156   (N, 69000 – 106000 m^{-1}; k, 0.1-2.5) [15]. When the polymerization period was increased from 8 to

157   12 min, both the separation efficiency and permeability of the column were decreased. The similar

158   behavior was also reported previously [11, 21-23]. Therefore, incomplete polymerization would be

159   a key to the high separation efficiency exhibited in this study.

160   Baseline separation of all analytes was obtained up to a flow rate of 3.38 mm/s as shown in

161   Fig. 3C. The five analytes were separated within 25 s at a flow rate of 14.7 mm/s (Fig. 3D), whereas

162   the peaks of Br$^-$ and NO$_3^-$ were partially overlapped. The theoretical plate heights at 14.7 mm/s

163   ranged from 60 to 89 μm (N, 12000 – 17000 m^{-1}) except for the t_0 marker.

164   In the flow rate range of 0.24–14.7 mm/s, the backpressure was proportional to the flow

165   rate (r^2=0.998). This fact indicated that the monolithic structure was not deformed in this flow rate

166   range, or under a pressure of less than 5 MPa (backpressure at 14.7 mm/s). In the liner relationship

167   between the flow rate and backpressure, the slope (S_{P-u}) was 0.33 MPa/(mm/s), which corresponds

168   to the pressure necessary to produce a flow rate of 1 mm/s using 50% ACN containing 100 mM of

169   NaCl (η = 0.91 mPa·s) as the mobile phase. The column permeability in this condition was 2.7 ×

170   10^{-13} m^2 as described previous section. The flow resistance and permeability of the anion-exchange

171   monolithic column was roughly comparable with those of a RP monolithic column previously

172   prepared by the current authors using photo-polymerization under low-temperature [9]. The

173   preparation of the methacrylate-based monolithic column using UV photo-polymerization under

174   low temperature is an effective method for producing a highly permeable column with high

175   separation efficiency.

176

177   3.4 Separation in gradient elution mode

178   The separation of the anions was also performed using gradient elution. Fig. 5 shows the separation

179   of the five UV absorbable inorganic anions, as well as the t_0 marker, using various flow rates with

180 gradient elution. The concentration of NaCl in the mobile phase was increased from 10 to 200 mM

181 over the course of the gradient to enhance the elution. The details of each gradient program are

182 described in the figure caption. At flow rates of both 1.0 and 2.0 mm/s (Fig. 5A and B), the analytes

183 were separated completely. Based on the increase in the flow rate, the separation period in Fig. 5B

184 was redused to half the value of that in Fig. 5A. A flow rate of 32 mm/s resulted in separation of the

185 analytes within 20 s as shown in Fig. 5C. The ultra-fast separation of small inorganic anions was,

186 therefore, successfully achieved.

187 The repeatability of the fast separation was statistically evaluated. The values of the mean,

188 SD, and %RSD for elution time, peak height, and relative peak height (uracil was regarded as an

189 internal standard) under the separation conditions employed in Fig. 5C ($n = 5$) are listed in Table 2.

190 The SD values for elution time were less than 0.25 s for all analytes and better repeatability in the

191 elution time was exhibited for the analytes that were eluted at longer times. The %RSD values for

192 the peak height were approximately 10% for all analytes. In the case of relative peak height,

193 the %RSDs were around 2% for all of the analytes except Br^-. The high precision of the fast

194 separation using rapid gradient elution was thus confirmed.

195

196 4. Conclusions

197 A single-step UV photo-polymerization method was developed for the preparation of a

198 methacrylate-based anion-exchange monolithic column at low temperature. The column exhibited

199 good separation efficiency for some small inorganic anions. Under optimal conditions, a theoretical

200 plate height of 9.4–15.6 μm (N, 64000–110000 m^{-1}) was achieved. This column efficiency was

201 relatively high for a polymer monolithic column and was almost comparable with the efficiency of

202 silica-based anion-exchange monolithic columns. The flow resistance of the column was acceptably

203 low, and fast separation was successfully demonstrated with an apparent flow velocity up to 32

204 mm/s. In the gradient method, five inorganic anions were separated rapidly with a high precision

205 within 20 s. In this study, the anion-exchange monolith containing hydrophobic components was

206 prepared in the capillary. The short optical pass length of the capillary column is disadvantageous to

207 concentration sensitivity for analyses of real environmental aqueous samples. In a future work, a

208 combination of a suitable pre-concentration method and a high performance anion-exchange

209 column prepared with hydrophilic monomers will be essential to analyze real samples with the

210 organic modifier free condition.

211

212 References

213 [1] H. Minakuchi, K. Nakanishi, N. Soga, N. Ishizuka, N. Tanaka, Anal. Chem. 68 (1996) 3498.

214 [2] K. Miyamoto, T. Hara, H. Kobayashi, H. Morisaka, D. Tokuda, K. Horie, K. Koduki, S. Makino,

215 O. Núñez, C. Yang, T. Kawabe, T. Ikegami, H. Takubo, Y. Ishihama, N. Tanaka, Anal. Chem. 80

216 (2008) 8741.

217 [3] M. Iwasaki, S. Miwa, T. Ikegami, M. Tomita, N. Tanaka, Y. Ishihama, Anal. Chem. 82 (2010)

218 2616

219 [4] F. Svec, J. M. J. Fréchet, Anal. Chem. 64 (1992) 820.

220 [5] T. Rohr, E. F. Hilder, J. J. Donovan, F. Svec, J. M. J. Fréchet, J. M. J., Macromolecules 36

221 (2003) 1677.

222 [6] Y. Ueki, T. Umemura, Y. Iwashita, T. Odake, H. Haraguchi, K. Tsunoda, J. Chromatogr. A 1106

223 (2006) 106.

224 [7] Y. Huo, P. J. Schoenmakers, W. Th. Kok, J. Chromatogr. A 1075 (2007) 81.

225 [8] M. Szumski, B. Buszewski, J. Sep. Sci. 32 (2009) 32 2574.

226 [9] T. Hirano, S. Kitagawa, H. Ohtani, Anal. Sci. 25 (2009) 1107.

227 [10] F. Svec, J. Chromatogr. A 1217 (2010) 902.

228 [11] F. Svec, J. Chromatogr. A in press, corrected proof, available online 20 July 2011.

229 [12] P. Hatsis, C. A. Lucy, Anal. Chem. 75 (2003) 75, 995.

230   [13] P. Zakaria, J. P. Hutchinson, N. Avdalovic, Y. Liu, P. R. Haddad, Anal. Chem. 77 (2005) 417.

231   [14] S. D. Chambe, K. M. Glenn, C. A. Lucy, J. Sep. Sci. 30 (2007) 30 1628.

232   [15] J. Jaafar, Y. Watanabe, T. Ikegami, K. Miyamoto, N. Tanaka, Anal. Bioanal, Chem. 319 (2008)

233   2551.

234   [16] C. J. Evenhuis, W. Buchberger, E. F. Hilder, K. J. Flook, C. A. Pohl, P. N. Nesterenko1, P. R.

235   Haddad, J. Sep. Sci. 31 (2008) 2598.

236   [17] A. Nordborg, E. F. Hilder, Anal. Bioanal. Chem. 394 (2009) 71.

237   [18] D. Connolly, B. Paull, J. Sep. Sci. 32 (2009) 2653.

238   [19] R. Nakashima, S. Kitagawa, T. Yoshida. T. Tsuda, J. Chromatogr. A 1044 (2004) 305.

239   [20] S. H. Lubbad, M. R. Buchmeiser, J. Sep. Sci. 32 (2009) 2521.

240   [21] A. Greiderer, L. Trojer, C. W. Huck, G. K. Bonn, J. Chromatogr. A 1216 (2009) 7747.

241   [22] I. Nischang, O. Brüggemann, J. Chromatogr. A 1217 (2010) 5389.

242   [23] I. Nischang, I. Teasdale, O. Brüggemann, J. Chromatogr. A 1217 (2010) 7514.

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244

245   Figure Captions

246   Fig. 1 Effect of META concentration in reaction solution on separation of small inorganic anions.

247   Column: BMA-*co*-EDMA-*co*-META monolithic column (A. AX5 and B. AX10, i.d. 0.1 mm, length

248   100 mm); mobile phase: 100 mM NaCl aqueous solution; apparent flow velocity: 1 mm/s;

249   detection: UV 210 nm; t_0 marker: uracil.

250

251   Fig. 2 Effect of acetonitrile concentration on separation of small inorganic anions. Column:

252   BMA-*co*-EDMA-*co*-META monolithic column (AX10, i.d. 0.1 mm, length 100 mm); mobile

253   phases: mixtures of acetonitrile (A. 0%, B. 25%, C. 50%, and D. 75%) and water containing 100

254   mM NaCl. The other conditions were the same as those for Fig. 1.

255

256   Fig. 3 Separation of small inorganic anions with various flow velocities of (A) 0.48 mm/s, (B) 1.0

257   mm/s, and (C) 3.4/mm, and (D) 14.7 /mm. Mobile phase: acetonitrile/water (50/50) containing 100

258   mM NaCl; the other conditions were the same those for as Fig. 1.

259

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

261   for Fig. 3.

262

263   Fig. 5 Separation of small inorganic anions in gradient elution mode with various flow rates of (A)

264   1.0, (B) 2.0, and (C) 32 mm/s. Mobile phases: mixture of acetonitrile and water (50:50) containing

265   (I) 10 mM NaCl and (II) 200 mM NaCl. Gradient programs: (A) 100% (I) in 120 s, 100%-0% (I) in

266   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

267   chromatographic conditions were the same as those for Fig. 3.
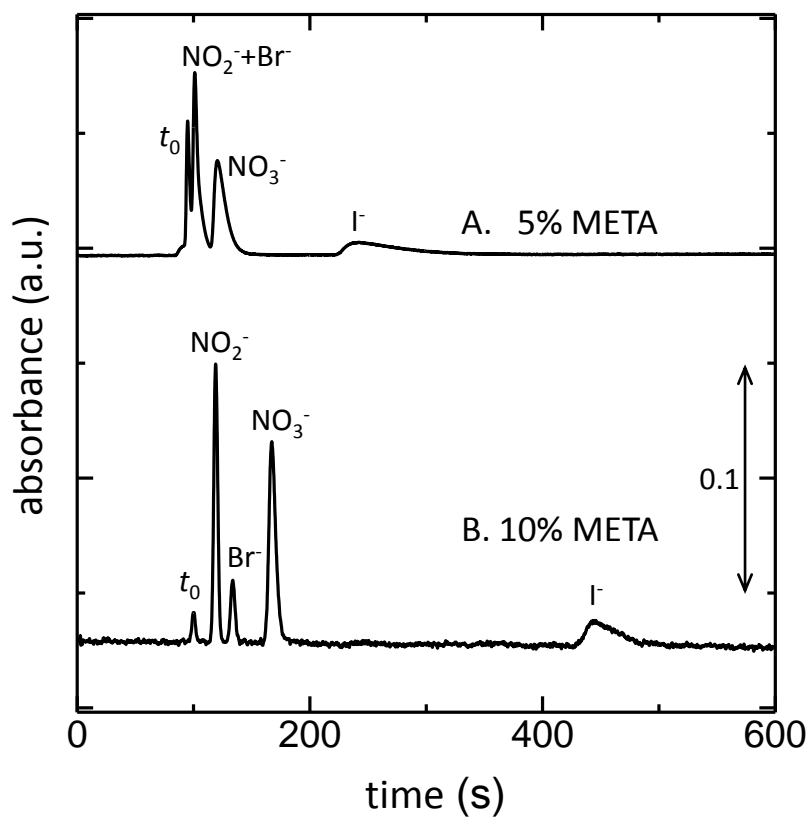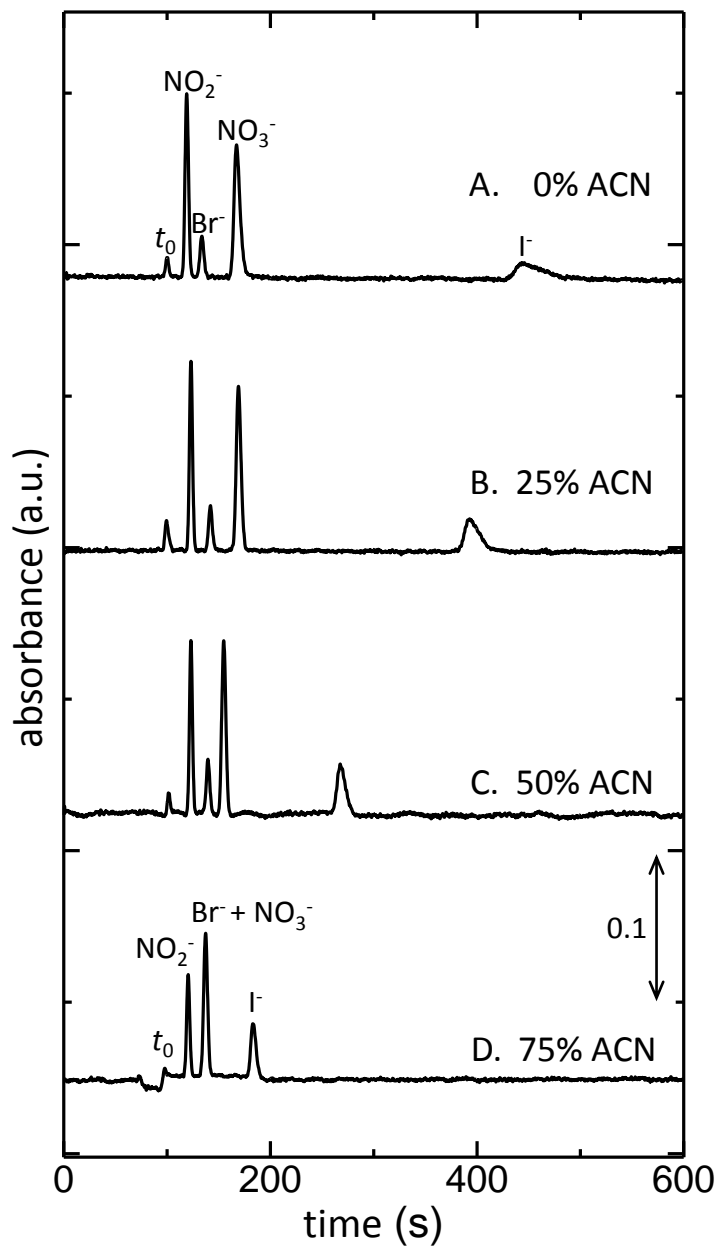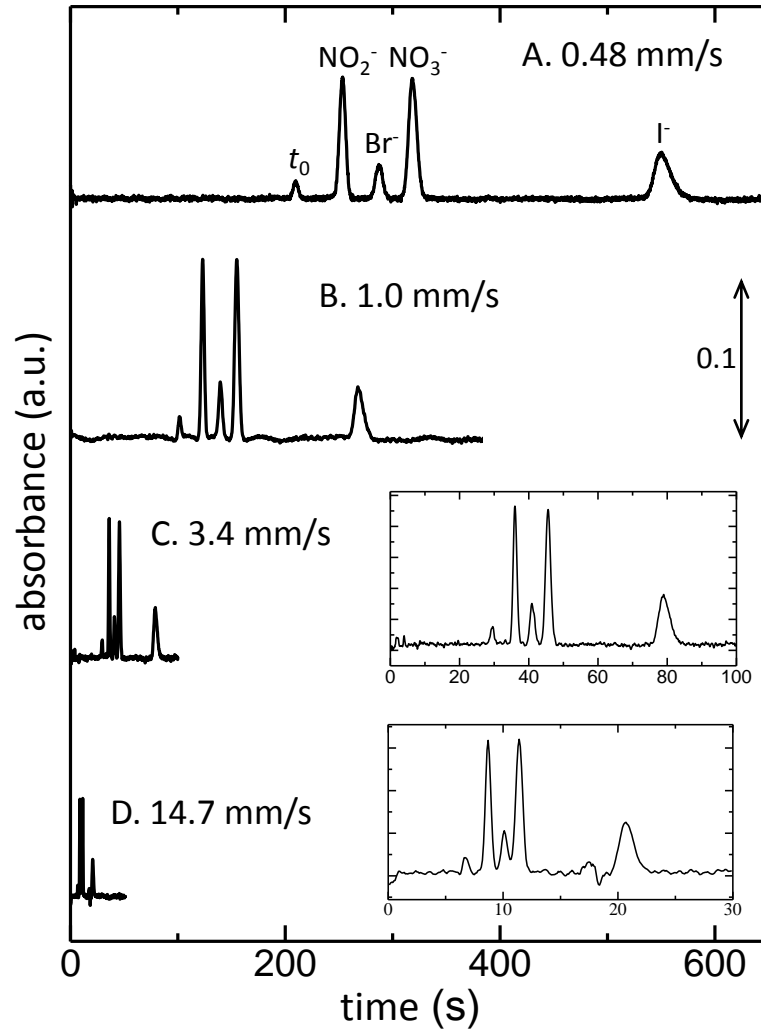
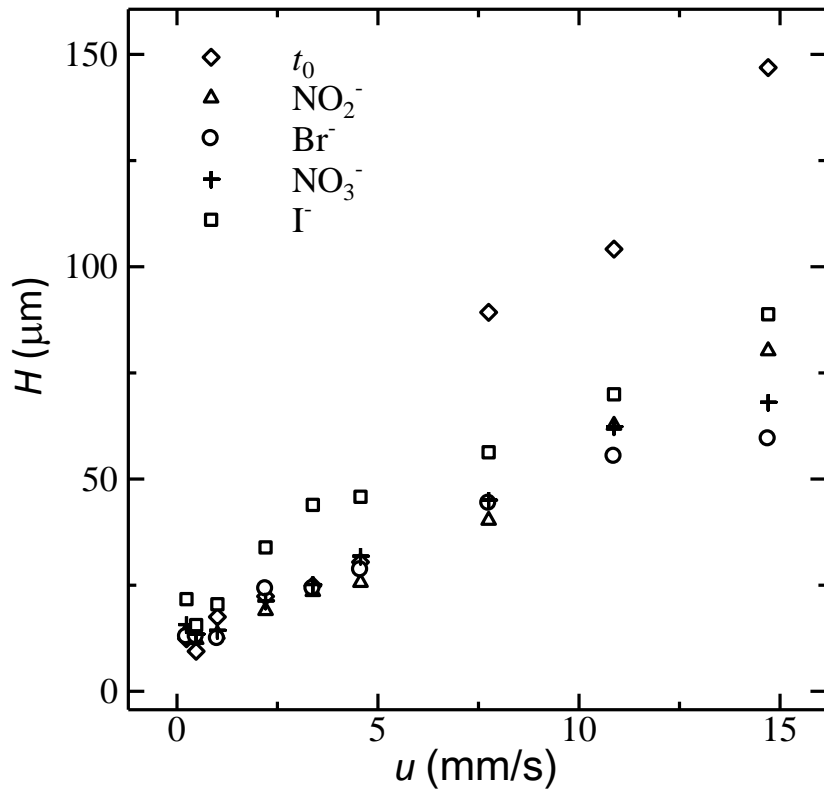**Figure 1**
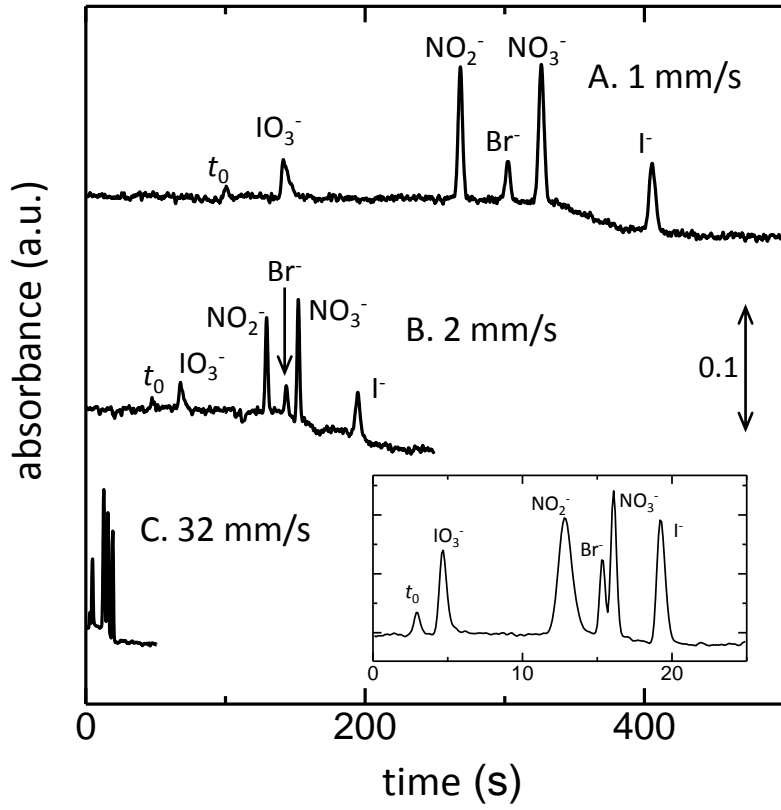
Figure 2

Figure 3

Figure 4

Figure 5

Table 1 Mixing ratios by weight of reaction solutions for anion-exchange polymer monolithic column. 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.

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 ± 0.25 (8.1)	18.7 ± 1.9 (10.0)	(I.S.)
IO_3^-	4.8 ± 0.25 (5.2)	70.9 ± 6.7 (9.5)	3.79 ± 0.08 (2.2)
NO_2^-	13.0 ± 0.20 (1.5)	97.0 ± 9.0 (9.3)	5.18 ± 0.10 (2.0)
Br^-	15.4 ± 0.12 (0.8)	62.1 ± 4.8 (7.7)	3.32 ± 0.16 (4.8)
NO_3^-	16.1 ± 0.14 (0.9)	122.1 ± 11.8 (9.7)	6.52 ± 0.14 (2.2)
I^-	19.3 ± 0.19 (1.0)	102.3 ± 9.7 (9.5)	5.47 ± 0.10 (1.8)

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