

Original paper

**Characterization and comparison of methacrylic acid with
2-acrylamido-2-methyl-1-propanesulfonic acid in the preparation of monolithic
column for capillary electrochromatography**

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Abbreviations: ACN, acetonitrile; AIBN, α,α' -azobisisobutyronitrile; AMPS, 2-acrylamido-2-methyl-1-propanesulfonic acid; BMA, butyl methacrylate; CEC, capillary electrochromatography; EDMA, ethylene dimethacrylate; EOF, electroosmotic flow; MAA, methacrylic acid

Abstract

A butyl methacrylate (BMA)-ethylene dimethacrylate (EDMA)-methacrylic acid (MAA) and BMA-EDMA-2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS) monolithic columns were prepared by varying the percentage of ionic monomers for capillary electrochromatography (CEC). Monolithic columns with higher content of ionic monomers provided better column efficiency and the performance of BMA-EDMA-MAA monoliths was better than BMA-EDMA-AMPS. In order to characterize and optimize BMA-EDMA-MAA monoliths, the effects of the content of cross-linker and the total monomer in the polymerization mixture on column performance were also studied. It was noted that plate height of 8.2 μm for unretained solute (thiourea) and 12.6 μm for retained solute (naphthalene) were achieved with monolithic column using 2.5% MAA (Column I).

1 Introduction

In recent years, monolithic columns have attracted considerable attention and are regarded as a new generation chromatographic media due to their efficiency and permeability. So far, there have been three different types of organic polymer monoliths, namely acrylate ester-based, acrylamide-based and styrene-based monoliths. Most literatures on polymer monolithic columns in CEC have focused on methacrylate-based monoliths due to their unique properties, such as high-chemical stability and excellent mechanical property in a wide pH range.

In contrast to the monolithic columns in high performance liquid chromatography, the introduction of charged moiety into the monolithic network generates electroosmotic flow (EOF) which is essential in CEC. Consequently, strongly ionic monomers, *i.e.* 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS), [2-(methacryloyloxy)ethyl]trimethyl-ammonium chloride, are extensively utilized in the preparation of polymer monoliths for CEC (1-8). An excellent separation efficiency on these columns was observed in previous works (1,4,6), while some literatures reported much lower efficiency than expected (7,8).

A weakly ionic monomer, methacrylic acid (MAA), can be an alternative to strongly acidic monomers. MAA has been used as a functional monomer for molecular imprinted polymer and solid phase extraction support (9-11); however the use of CEC monolithic columns containing MAA are limited (12-14).

In our previous study, butyl methacrylate (BMA)-ethylene dimethacrylate

(EDMA)-MAA monolith was prepared for CEC in place of AMPS which led to a high efficiency and reproducibility (15). Therefore, we think that the comparison between MAA and AMPS should be needed to clear the usefulness of MAA. At the same time, the characterization of BMA-EDMA-MAA column by systematically changing the preparation conditions is also needed.

In this study, we compared the electrochromatographic performance of BMA-EDMA-MAA and BMA-EDMA-AMPS monolithic columns with varying the percentage of ionic monomers. In addition, the effects of EDMA content as well as the ratio of monomer to porogenic solvents in the polymerization mixture on the column performance of BMA-EDMA-MAA monoliths were also investigated.

2 Materials and methods

2.1 Chemicals

BMA, EDMA, α,α' -azobisisobutyronitrile (AIBN), 1,4-butanediol and 3-(trimethoxysilyl)propyl methacrylate were obtained from Nacalai Tesque (Kyoto, Japan). MAA and AMPS were from Tokyo Chemical Industry (Tokyo, Japan). 1-propanol, acetic acid, potassium dihydrogenphosphate, dipotassium hydrogenphosphate, aniline, *N*-methylaniline, benzene, toluene and naphthalene were from Wako (Osaka, Japan). Thiourea was obtained from Kishida Chemical (Osaka).

HPLC grade of acetonitrile (ACN) was purchased from Kanto Chemical (Tokyo).

2.2 *Instrumentation*

All the CEC experiments were performed on a CAPI-3200 system equipped with an UV photodiode array detector (Otsuka Electronics, Osaka). Fused-silica capillaries (375 μm o.d. x 100 μm i.d.) were obtained from Polymicro Technologies (Phoenix, AZ, USA). The mobile phase, prepared by mixing ACN and the acetate buffer, was degassed thoroughly prior to use. At the beginning of each day's work, the capillary column was conditioned with a mobile phase for 1 h and equilibrated by applying voltage 1, 5, 10 kV for 30 min each. The separation voltage was set at 10 kV and the injections were made by applying a voltage of 3 kV for 3 sec. The detection wavelength was set at 200 nm.

2.3 *Column preparation*

All the monoliths were prepared as previously reported (15). The inner wall of capillary was treated with 3-(trimethoxysilyl)propyl methacrylate. Monoliths were prepared from polymerization mixtures containing of BMA, EDMA, MAA or AMPS and ternary porogenic solvents composed of 10% water and 90% of 1-propanol and 1,4-butanediol combined in various ratios. A mixture of water, 1-propanol and 1,4-butanediol was used as a pore-forming solvent following a recipe developed for

BMA-based monolith (1,4). AIBN (1% with respect to the monomers) was added as an initiator. The polymerization mixture was sonicated for 5 min and then purged with nitrogen gas for 10 min. From one end, 50-cm long surface-modified capillaries were filled with the polymerization mixture up to a length of 30 cm. Both ends of the capillary were plugged with a piece of septum and the reaction was initiated at 60 °C for 20 h. The monolithic column dimension was as follows: total length, 42.0 cm; packed length, 30.0 cm; length from inlet to detection window, 30.5 cm. The polyimido coating was burned away to make a detection window. The resulting columns were rinsed first with water-ACN mixture (30/70, v/v %) to remove porogenic solvents and unreacted monomers and then with mobile phase by an HPLC pump. The capillary columns were equilibrated by successively applying 1, 5, 10 kV for 10 min each.

3 Results and discussion

3.1 Comparison of BMA-EDMA-MAA and BMA-EDMA-AMPS monolithic columns

In our previous work, it was reported that BMA-EDMA-MAA monoliths with higher content of MAA provided a higher column efficiency (15). In the current study, we compared column efficiency between BMA-EDMA-MAA and BMA-EDMA-AMPS monoliths by varying the ionic monomers content (Table I). Here, 0.05% MAA vs 0.12% AMPS, 0.5% MAA vs 1.2% AMPS, and 2.5% MAA vs 6% AMPS were compared because the amount (mole) of ionic monomer introduced in the each monolith

were the same. In general, the composition of porogenic solvents has a great influence on the porous properties of monolithic materials (16-19), the composition of porogenic solvents was also varied to fairly compare the monoliths. When increasing the MAA or AMPS content, BMA content was decreased in order to maintain the ratio of total monomer and porogenic solvents, while, according to decrease in 1-propanol content, 1,4-butanediol increased and water content kept constant. Table I shows plate height and EOF mobility in the CEC separation of test solutes (thiourea, benzene, naphthalene). The permeability on the BMA-EDMA-AMPS monolithic column containing 6% AMPS was too low due to excessive swelling of monolith, this made it difficult to rinse with a mobile phase using an HPLC pump; this monolith column could not be evaluated when a low amount of 1-propanol (<27%) for making large pores was used (1). Similar to BMA-EDMA-MAA, high AMPS content provided high column efficiency (Column N and U). The monolithic columns, which showed highest column efficiency for each content of ionic monomer were compared; thus comparing Column B vs N and Column E vs U, it was realized that BMA-EDMA-MAA monoliths showed better efficiency than BMA-EDMA-AMPS monoliths. As shown in Table I, it was noted that plate height of 8.2 μm for unretained solute (thiourea) and 12.6 μm for retained solute (naphthalene) were achieved with monolithic column using 2.5% MAA (Column I). The column reproducibility obtained here (EOF mobility, retention times and retention factors) are equal to that found in our previous study (15), which meets the requirement for

widespread use of this monolithic column.

3.2 Characterization of BMA-EDMA-MAA monolithic column

3.2.1. Effect of EDMA content on column performance

It is not only the composition of porogenic solvent that affects the porosity of the monolith, monomer composition (*i.e.*, BMA/EDMA) also greatly affects on the porous properties of the monolithic materials (20, 21). In order to study the effect of EDMA content on column performance, several monoliths were prepared without changing polymerization mixture composition (monomers/porogenic solvents=40/60, v/v %). BMA content decreased in accordance with an increase in EDMA.

As seen in Fig. 1, a tendency of decrease in EOF mobility with increasing EDMA content was observed. Viklund *et al.* reported that a higher content of divinyl monomer directly translates into the formation of more highly cross-linked polymer in the early stage of the polymerization process and eventually that lead to a shift in the pore size distribution toward smaller pore size (21). The observed trend in our result may be attributed to a decrease in pore size as the content of EDMA increases. The best efficiency was obtained with monolithic column using 16% EDMA, with plate height increasing for columns with EDMA content of more than 16% (Fig. 1).

3.2.2. Effect of the composition of polymerization mixture on column performance

Methacrylate-based monolithic columns have been prepared with different ratios of monomer and porogenic solvents in the polymerization mixture (4, 22, 23); high density (40% monomers and 60% porogenic solvents), medium density (30% monomers and 70% porogenic solvents) and low density (20% monomers and 80% porogenic solvents). High density monoliths show a relatively low efficiency and permeability but high retention. On the other hand, the low density monoliths showed a higher flow permeability and column efficiency, but less retention and repeatability (24). Therefore, in order to compromise between column efficiency and retention, medium density monoliths were investigated (22, 23). In this study, BMA-EDMA-MAA monolith with low density and medium density were prepared with BMA-EDMA ratio similar to that of column I.

In contrast to all the other polymer monoliths, the permeability of low density monolithic columns was too low to rinse with a mobile phase by an HPLC pump. This might be due to higher ratio of MAA to total monomer as compared with high or medium density. In order to optimize the medium density monolith 1-propanol content was optimized. As shown in Fig 2, the best efficiency (9 μm for thiourea, 10 μm for benzene, 10 μm for naphthalene), comparable to high density monoliths (Column I), was obtained using 38.2% 1-propanol in medium density monoliths. As a result, high and medium density BMA-EDMA-MAA with high MAA content provided high column efficiency. Typical electrochromatograms of a test mixture (thiourea, aniline,

N-methylaniline, benzene, toluene, naphthalene), barbiturates (barbital, phenobarbital, amobarbital, secobarbital, thiopental) and antibiotics (moxifloxacin and ofloxacin) on BMA-EDMA-MAA were shown in Fig. 3.

4 Conclusion

It was demonstrated that BMA-EDMA-MAA monolithic column exhibits better efficiency compared to BMA-EDMA-AMPS monolithic column. Both medium density and high density monoliths of BMA-EDMA-MAA with high MAA content could provide a good column performance. Several conditions for preparation of polymer monolith *i.e.*, percentage of EDMA, composition of polymerization mixture and percentage of 1-propanol, have a great impact on the resulting column performance. Higher EOF mobility was observed by using higher content of MAA.

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The authors have declared no conflict of interest.

6 References

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via photografting. *Journal of Separation Science* (2007); 30: 407-413.

Figure captions

Fig. 1 Effect of EDMA content on column efficiency and EOF mobility on BMA-EDMA-MAA monolithic columns. Polymerization mixture: 2.5% MAA, 37.5% of combined BMA and EDMA in various ratios, 60% of ternary porogenic solvents (consisting of 10% water, 49.5%, 1-propanol and 40.5% 1,4-butanediol). For other conditions, see Table I.

Fig. 2 Effect of 1-propanol content in the porogenic solvents on column efficiency of medium density monoliths. Polymerization mixture: 11.8% EDMA, 15.7% BMA, 2.5% MAA, 70% of ternary porogenic solvents (consisting of 8.6% water and 91.4% of mixtures of 1-propanol and 1,4-butanediol in various ratios). For other condition, see Table I.

Fig. 3 Separation of test solutes and barbiturates on BMA-EDMA-MAA monolithic columns. CEC conditions in a): mobile phase, 5 mM acetate buffer (pH 5.2) / ACN = 30/70; applied voltage at 10 kV. CEC conditions in b): 5 mM acetate buffer (pH 5.2) / ACN = 40/60; applied voltage at 30 kV. Polymerization mixture: 11.8% EDMA, 15.7% BMA, 2.5% MAA, 6.0% water, 38.2% 1-propanol and 25.8% 1,4-butanediol.

Peaks: 1, thiourea; 2, aniline; 3, *N*-methylaniline; 4, benzene; 5, toluene; 6, naphthalene; 7, barbital; 8, phenobarbital; 9, secobarbital; 10, thiopental; 11, moxifloxacin; 12, ofloxacin.

Fig. 1

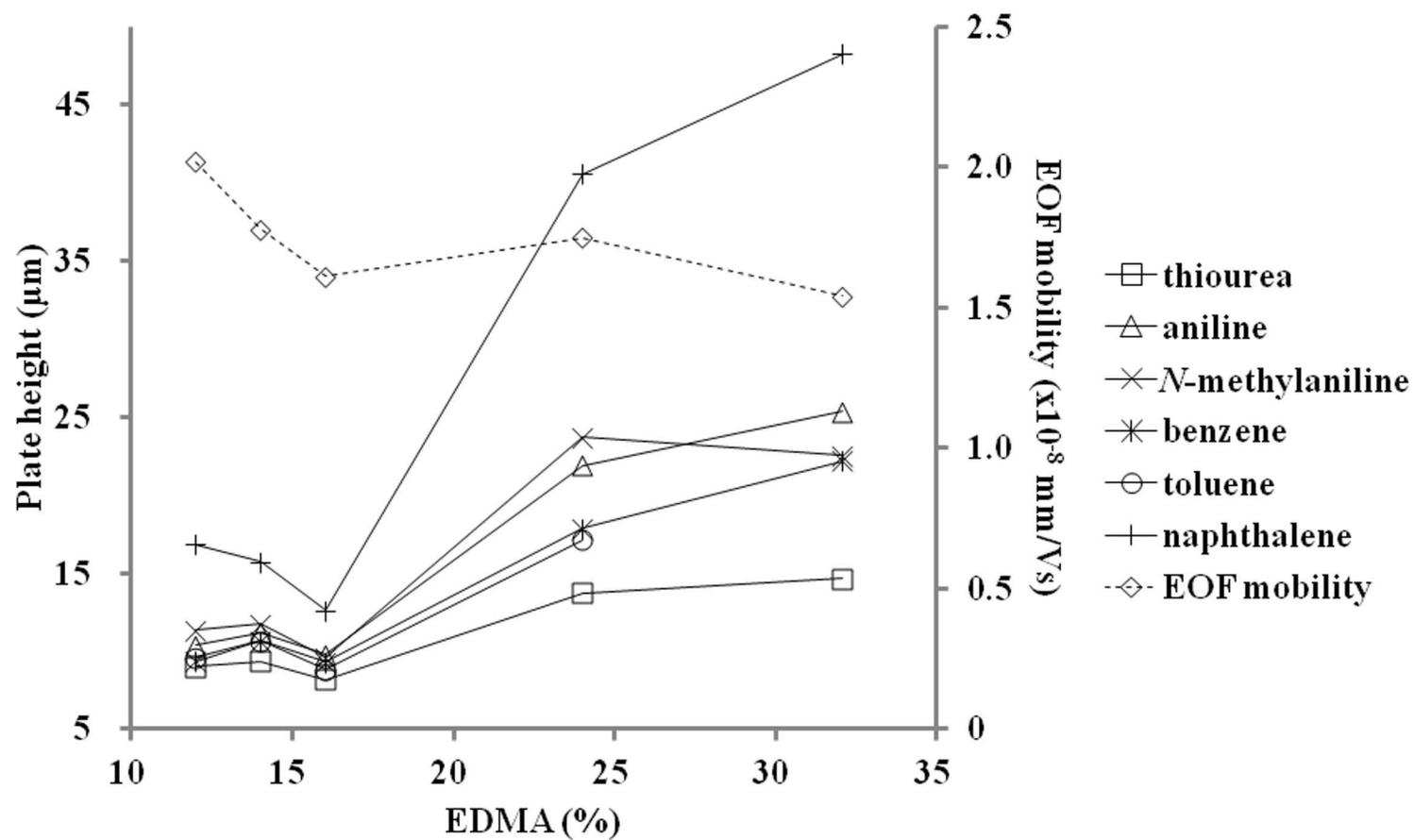


Fig. 2

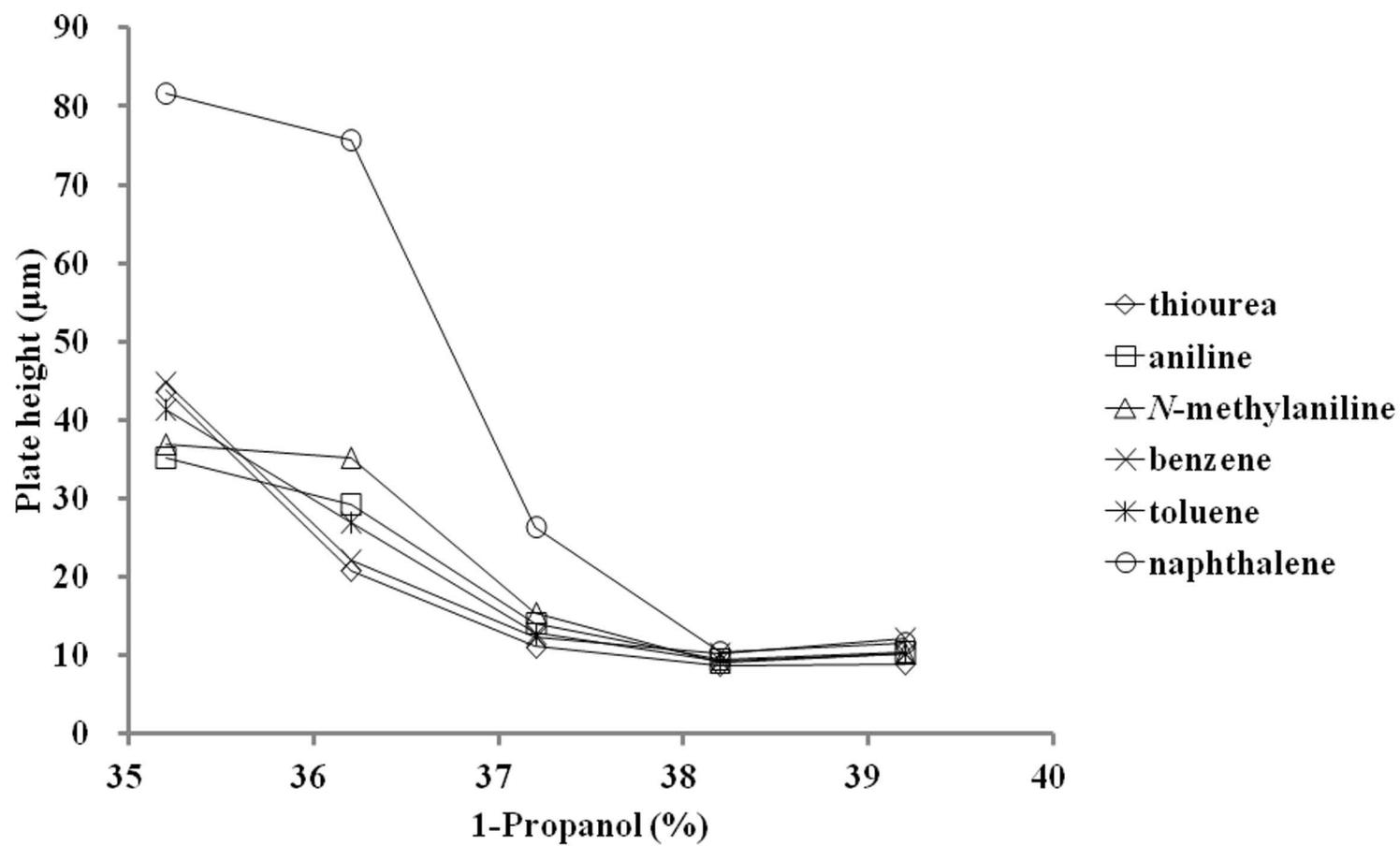


Fig. 3

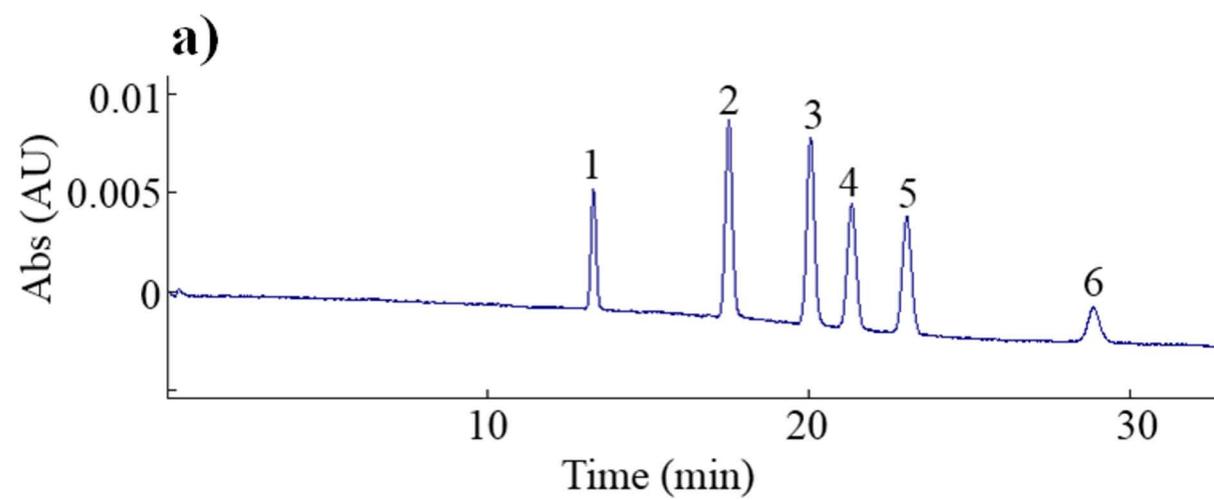


Fig. 3

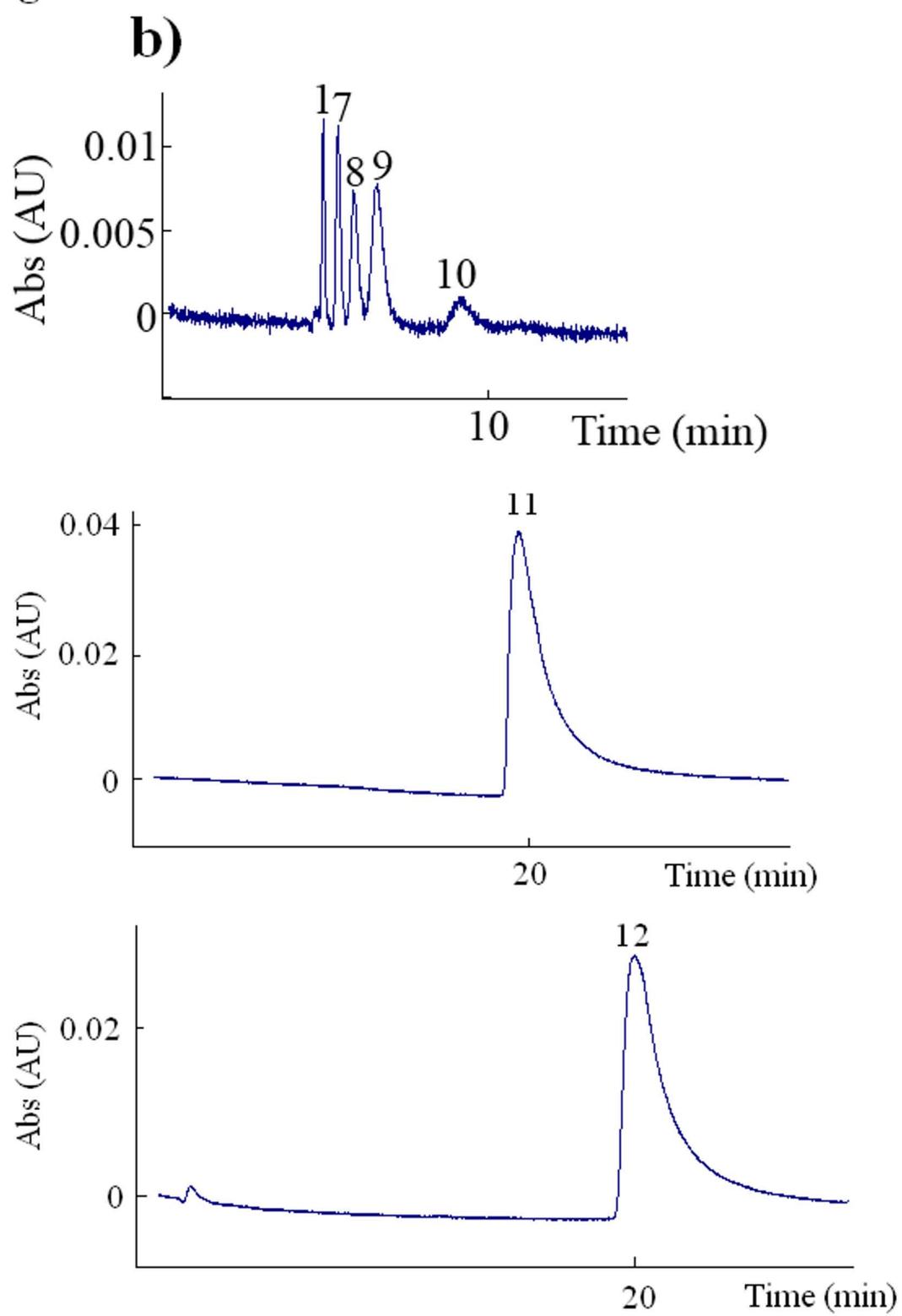


Table I Comparison of the chromatographic behavior of BMA-EDMA-MAA and BMA-EDMA-AMPS monolithic columns.

ionic monomer (wt%)	column code	1-propanol (wt%)	plate height (μm)			EOF mobility ($\times 10^{-8} \text{mm/Vs}$)	
			H_{thio}	H_{benz}	H_{naph}		
MAA ^{a)}	0.05 ^{b)}	Column A	36.2	76.9	55.6	56.2	0.69
		Column B	35.5	20.4	23.4	67.6	0.97
		Column C	29.7	25.4	54.3	303	1.30
	0.5 ^{c)}	Column D	36.2	22.6	23.5	24.4	0.98
		Column E	35.5	15.9	18.9	26.5	1.56
		Column F	34.1	41.7	36.2	141	2.02
		Column G	29.7	37.0	122	833	3.23
	2.5	Column H	30.8	12.8	13.3	15.0	1.22
		Column I	29.7	8.2	9.3	12.6	1.76
		Column J	28.7	25.8	24.0	96.2	2.04
		Column K	27.7	84.7	77.5	476	2.61
AMPS	0.12 ^{b)}	Column L	38.8	67.3	69.2	74.4	0.52
		Column M	37.8	61.9	55.4	57.5	1.16
		Column N	36.2	19.7	33.5	161	3.30
		Column O	35.5	69.4	49.8	181	3.02
		Column P	34.5	29.5	220	2100	4.33
	1.2 ^{c)}	Column Q	30.8	17.6	64.7	428	3.01
		Column R	29.7	19.1	57.8	368	3.01
		Column S	28.8	16.6	44.4	243	3.06
		Column T	27.8	13.1	23.3	98.1	2.33
		Column U	26.8	12.5	20.2	70.4	1.45
		Column V	25.8	13.9	29.5	149	3.08

^{a)} Reproduced from Ohyama, K., Horiguchi, D., Kishikawa, N., Kuroda, N.: Monolithic poly(butyl methacrylate-ethylene dimethacrylate-methacrylic acid) column for capillary electrochromatography. *J Sep Sci.* (2011); 34: 2279-228., with permission from John Wiley and Sons.

^{b)} the mol of 0.05% MAA and 0.12% AMPS are same

^{c)} the mol of 0.5% MAA and 1.2% AMPS are same

Polymerization mixture: 16% EDMA, 24% of combined BMA and MAA or AMPS in various ratios, 60% of ternary porogenic solvents (consisting of 10% water and 90% of mixtures of 1-propanol and 1,4-butanediol), AIBN 1% (with respect to monomers).
CEC Condition: mobile phase, 5 mM acetate buffer (pH 5.2) / ACN = 30/70, v/v %; injection 3 kV for 3 s; UV detection, 200 nm; column temperature at 18 °C. Test solutes: thiourea, benzene and naphthalene.