

Original

Design for Valid “Surface” Property of Polymer-based Separation Media for Capillary Electrochromatography (CEC) : Correlation between Separation Selectivity, Retentivity and Generation of Electroosmotic Flow

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Abstract

Uniformly sized, polymer-based packing materials (particles) for high performance liquid chromatography (HPLC) and CEC were prepared through two different surface modification methods using methacrylic acid (MAA) as a functional monomer. The particles prepared using “mix method” afforded higher content of MAA, while “addition method” showed lower content of MAA, probably due to high solubility of MAA monomer into the dispersion continuous phase, water.

The particle prepared using addition method showed larger retention factor for hydrophobic solutes in HPLC mode, while the polymeric external surface layer derived from MAA resulted in slightly different separation selectivity especially at pH 10 with lower column efficiency.

Although the content of MAA functionality was lower onto the particles prepared using addition method, it afforded higher electroosmotic mobility compared with that by mix method in CEC mode. On the other hand, alkylbenzenes were separated with much larger retention factor on the particle prepared by addition method and column efficiency was found to be higher on the particle prepared by addition method, while in HPLC mode, the retention factor of alkylbenzenes was found to be smaller on the particle prepared by addition method probably due to higher surface hydrophilicity.

Keywords: electroosmotic flow, capillary electrochromatography, surface modification, separation selectivity

Introduciton

Capillary electrochromatography (CEC) has potential advantage in terms of combination of “plug” type flow profile based on electroosmotic flow and chromatographic separation selectivity based on various stationary phases^{1,2,3,4,5}. These advantages result in chromatographic separations with much higher column performance compared with that in HPLC mode.

Silanol groups on fused silica capillary wall are known to contribute generation of electroosmotic flow, but separation media therein such as packing materials⁶ as well as monolithic separation media^{7,8,9} should have appropriate functional groups contributing the generation of electroosmotic flow.

In the case of silica-based -packing materials or -monolithic

separation media, silanol groups are usually utilized and consumed for surface modification by stationary phases' functionality. Therefore, further introduction of the surface functional groups as well as introduction of trimethylsilane group should decrease number of remaining silanol groups, which might reduce the generation of electroosmotic flow.

Of course, some stationary phases including ionic functional group can be prepared to overcome this problem, but it should change separation selectivity as well as retentivity of the stationary phase¹⁰ in HPLC and/or CEC mode. The introduction of ion exchange groups is believed to result in decrement of hydrophobicity of the stationary phase such as C₁₈ type stationary phase to lead smaller retentivity especially in reversed phase mode¹¹.

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On the other hand, polymer-based separation media have potential "bi-modal" separation mechanism, which is attributed to macro pore and micro or meso pores¹². We have already reported site selective modification methods for macroporous polymer-based separation media with various functional monomers, such as chiral amide type monomers¹³, temperature responsive polymer^{14,15}, or hydrophilic monomers^{16,17}.

Especially, polymer-based separation media selectively modified with hydrophilic external layers were reported to show almost comparable retentivity and separation selectivity with that on unmodified polymer-based separation media, while reasonable restriction of polypeptides such as BSA due to the enough surface hydrophilicity¹⁶.

These examples suggest that polymer-based separation media potentially have an easy and effective surface modification without loose of separation selectivity, or retentivity based on the original separation media towards small molecules.

In this paper, we wish to demonstrate preparation method of polymer-based separation media modified with methacrylic acid functionality by two different modification methods, and report that correlation between separation selectivity or retentivity and generation of electroosmotic flow in CEC mode.

EXPERIMENTAL

Materials

Water utilized through this work was purified using a E-pure (Thermolyne Co.). Methanol was just distilled to remove unexpected impurities. Tetrahydrofuran (THF) was distilled over potassium hydride, while acetonitrile was purified using distillation over phosphorus pentoxide followed by it from potassium carbonate¹⁸.

Toluene as of the highest commercial grade (Wako Pure Chemical) was distilled over potassium hydride. Styrene monomer purchased from Nacalai Tesque Co. was washed by 10% aqueous solution of sodium hydroxide as well as saturated sodium chloride aqueous solution and distilled under reduced pressure. (bp = 59 at 33 mmHg)¹⁸.

Ethylene dimethacrylate (EDMA) and methacrylic acid monomer (MAA) were purchased from Tokyo Kasei and purified using standard distillation techniques to remove polymerization inhibitors. (EDMA : bp = 64 at 0.2 mmHg, MAA : bp = 41 at 1.0 mmHg) Dibutylphthalate utilized as an activating solvent was purified using distillation technique under reduced pressure. (bp = 151 at 25 mmHg) Benzoyl peroxide (BPO) as a polymerization initiator was purified re-precipitation method¹⁸. Another chemicals utilized through this work were used as received.

Preparation of Polymer Particles

We have been working on preparation and application of uni-

formly sized, polymer particles through multi-step swelling and polymerization method and utilizing those as packing materials for HPLC as well as CEC¹⁹. First of all, seed polymer particles were prepared for this work.

Preparation of Seed Polymer Particles :²⁰ To completely deoxygenized water through helium bubbling with water boiling followed by cooling of heated water, 0.395 g of sodium chloride was added. After argon bubbling into the solution for 30 minutes, 6.0 ml of styrene monomer was added. The mixture was heated up to 75 and stirred at 350 rpm. After 30 minutes, the initiator solution of potassium persulfate (0.296 g/50 ml of the purified water) was added into the monomer mixture. 6.0 ml of styrene monomer was added every 30 minutes, where total volume of added styrene was 69.0 ml, and the polymerization was carried out for 24 hours. After the polymerization, polymer dispersion was purified using centrifugation technique and re-dispersed into water. The chemical yield of the seed polymer was of 62.3% and the dispersion concentration was about 8.0×10^{-2} ml/ml (water). The prepared dispersion of the polystyrene seed polymer was utilized for following swelling procedure.

Multi-step swelling and polymerization method²¹⁻²³

1) Mix method

Into 2.10 ml of the dispersion of seed polymer prepared, an emulsion prepared using 0.62 ml of dibutyl phthalate as activating solvent²¹⁻²³ and 0.04 g of sodium dodecylsulfate (SDS) with 10 ml of water by sonification was added. The mixture was stirred at 125 rpm for 3 hours. The complete adsorption of the added activating solvent onto the seed polymer particle was just confirmed by optical microscope.

Into the suspension, an emulsion prepared using 10.0 ml of toluene (porogenic solvent), 0.10 g of BPO (radical initiator), 20 ml of poly (vinyl alcohol) aqueous solution (4.8 wt%), and 0.16 g of SDS with 40 ml of water by sonification was added at room temperature. This swelling process was finished in 4 hours at room temperature with 125 rpm of stirring. The finish of swelling was confirmed by optical microscope.

After this swelling process, an additional emulsion prepared using 5.0 ml of EDMA (cross-linking agent), 0.56 ml of MAA (functional monomer), and 10 ml of poly (vinyl alcohol) aqueous solution (4.8 wt%), and 0.08 g of SDS with 20 ml of water by sonification were added into the suspension system. This additional swelling process with monomers was completed in 4 hours at room temperature with 125 rpm stirring. The finish of swelling was also confirmed by optical microscope.

The swollen system was heated up to 70 after 30 minutes argon bubbling to start polymerization step. After 24 hours polym-

erization, the prepared polymer particles were washed with water, methanol, and tetrahydrofuran (THF). The chemical yield of polymer particles was calculated after filtration and dry processes in vacuo.

2) Addition method¹⁶

The swelling processes were carried out using the similar processes of mix method without addition of MAA in the additional swelling process. In this case, MAA (0.56 ml) was added in the polymerization step (after 4 hours from initiation of the usual polymerization) and additional polymerization took place for 20 hours. The purification methods were the same as those in mix method.

As the reference particle, we have also prepared only EDMA particle without the ion exchange group, MAA using the similar swelling method without MAA monomer.

Packing methods

The prepared polymer packing materials were packed into stainless steel column (4.6 mm ID×100 mm) or fused silica capillary (100 μm ID) by slurry method using mixture of water, acetonitrile, 2-propanol, and/or methanol as dispersion media¹⁹.

HPLC and CEC systems

HPLC system was composed of a Jasco 880-PU HPLC pump, a Jasco UV-970 detector, and a Rheodyne 7125 valve loop injector. The measurement was carried out at 30 °C and the data was recorded with a Shimadzu C-R 4 A integrator.

CEC measurement was performed with a Hewlett Packard 3 D CE system.

RESULTS AND DISCUSSIONS

Physical Properties of the Prepared Polymer Particles

The prepared packing materials are listed in Table 1 and further information is also mentioned in Table 2. Ion exchange capacity listed in Table 2 was measured as follows: 0.200 g of each packing materials was dispersed in 10 ml of the purified water and a titration indicator, phenolphthalein solution was added in the dispersion. The titration was carried out using 0.1 N sodium hydroxide standard solution.

The particle size of the prepared particles was around 7 μm and the size uniformity (CV value) was smaller than 10%. In addition method, additional monomer (MAA) was polymerized on the surface of the EDMA particles, therefore the size uniformity became slightly worse. In addition, since MAA is an water soluble monomer, the introduction of ion exchange group (MAA) was found to be lower than that of the particle prepared by mix method, in spite of the same feed ratio, but this is the reported phenomenon.

Table 1. Composition of The Prepared Polymer Particles.

Preparation Method	Polymer Particles	Composition ^{a)} (EDMA:MAA:TOL)	Initiator (%)	Time ^{b)} (h)
—	EDMA	9 : 0 : 18	2	24
mix	10%MAA-mix	9 : 1 : 18	2	24
addition	10%MAA-add	9 : 1 : 18	2	24

a) Volume ratio

b) Total polymerization time

Table 2. Properties of The Prepared Polymer Particles.

Polymer Particles	Particle Size (μm)	CV ^{a)} (%)	Yield (%)	Ion Exchange Capacity ^{b)} (meq / g)
EDMA	6.52	4.64	100	-----
10%MAA-mix	7.30	2.94	75	0.41
10%MAA-add	6.35	6.92	100	0.31

a) CV (%) = $\{ \sum (d - d_{av})^2 / N \}^{1/2} / d_{av} \times 100$

(d : Particle size, d_{av} : Mean particle size, N : Number of particles)

b) The method was mentioned in the text.

Table 3. Physical Properties of The Prepared Particles^{a)}.

Polymer Particles	Pore Volume (cm ³ / g)	Surface Area (m ² / g)	Mean Pore Size (Å)
EDMA	0.411	471	22.9
10%MAA-mix	0.302	339	23.1
10%MAA-add	0.459	450	40.8

a) There values were measured using nitrogen adsorption method.

Table 4. Characteristics in Pore Volume And Pore Size Distribution of Each Particles^{a)}.

Polymer Particles	Eva ^{b)} (ml)	Evb ^{c)} (ml)	Evc ^{d)} (ml)	Evtot ^{e)} (ml)
EDMA	0.158	0.067	0.400	0.621
10%MAA-mix	0.156	0.057	0.345	0.558
10%MAA-add	0.118	0.078	0.414	0.609

a)Chromatography Conditions

Mobile phase :100 % THF, Column size :4.6 mm(I.D.) × 100 mm

Sample :Benzene, Hexylbenzene, Standard polystyrene(PS)

Flow rate :0.5 ml / min, Temperature :30 °C

b)Eva :Elution volume(benzene) — Elution volume(hexylbenzene)

c)Evb :Elution volume(hexylbenzene) — Elution volume(Mw = 760 PS)

d)Evc :Elution volume(Mw=760 PS) — Elution volume(Mw = 20000000 PS)

e)Evtot :Elution volume(benzene) — Elution volume(Mw = 20000000 PS)

The mix method tends to afford smaller specific surface area compared with EDMA particle prepared without addition of MAA monomer, while the addition method afforded almost comparable specific surface area with that on EDMA particle as shown in Table 3. This is also the reported phenomenon¹⁴. If the pore properties were compared in different way in size exclusion mode, we also found that mix method also gave smaller pore volume compared with those on EDMA as well as the particle prepared by addition method as shown in Table 4 (size exclusion mode). The obtained data in Table 3 and 4 are compatible each other in spite of different measurement methods. There facts suggest that a traditional

method for surface design (mix method) affects physical properties such as pore volume, pore size, and pore size distribution, while addition method can introduce some functionality without loose of the original physical properties of separation media.

Chromatographic Properties in HPLC

Chromatographic properties were measured in HPLC mode at pH 4, 7 as well as 10. Separation selectivity in terms of α values based on benzene was depicted in Figure 1. At both pH 4 and 7, similar separation selectivity was observed towards most of the solutes, while, at pH 10, the packing material prepared by addition method afforded different separation selectivity compared with those on EDMA particle towards amine compound such as p-nitroaniline. This would be explained based on charged external surface layer on the particle prepared by addition method, while

details have not been understood.

If we take a look at k' values (retention factor) of the prepared particles, EDMA, which does not include ion exchange group afforded the largest k' values towards the solutes utilized in this study at pH 4, 7, and 10 (Figure 2) except benzene and toluene at pH 10. In addition, it was also found out that the particle prepared by addition method tended to afford larger k' values compared with those on the particle prepared by mix method. This tendency was clearer especially at pH 10.

On the particle prepared by addition method, micropore regions, which mainly contribute retentivity for small molecules, are remaining without modification of MAA group¹⁶. Therefore, k' values on the particle prepared by addition method are presumably larger compared with those on the particle prepared by mix method, where hydrophilic ion exchange groups are de-localized

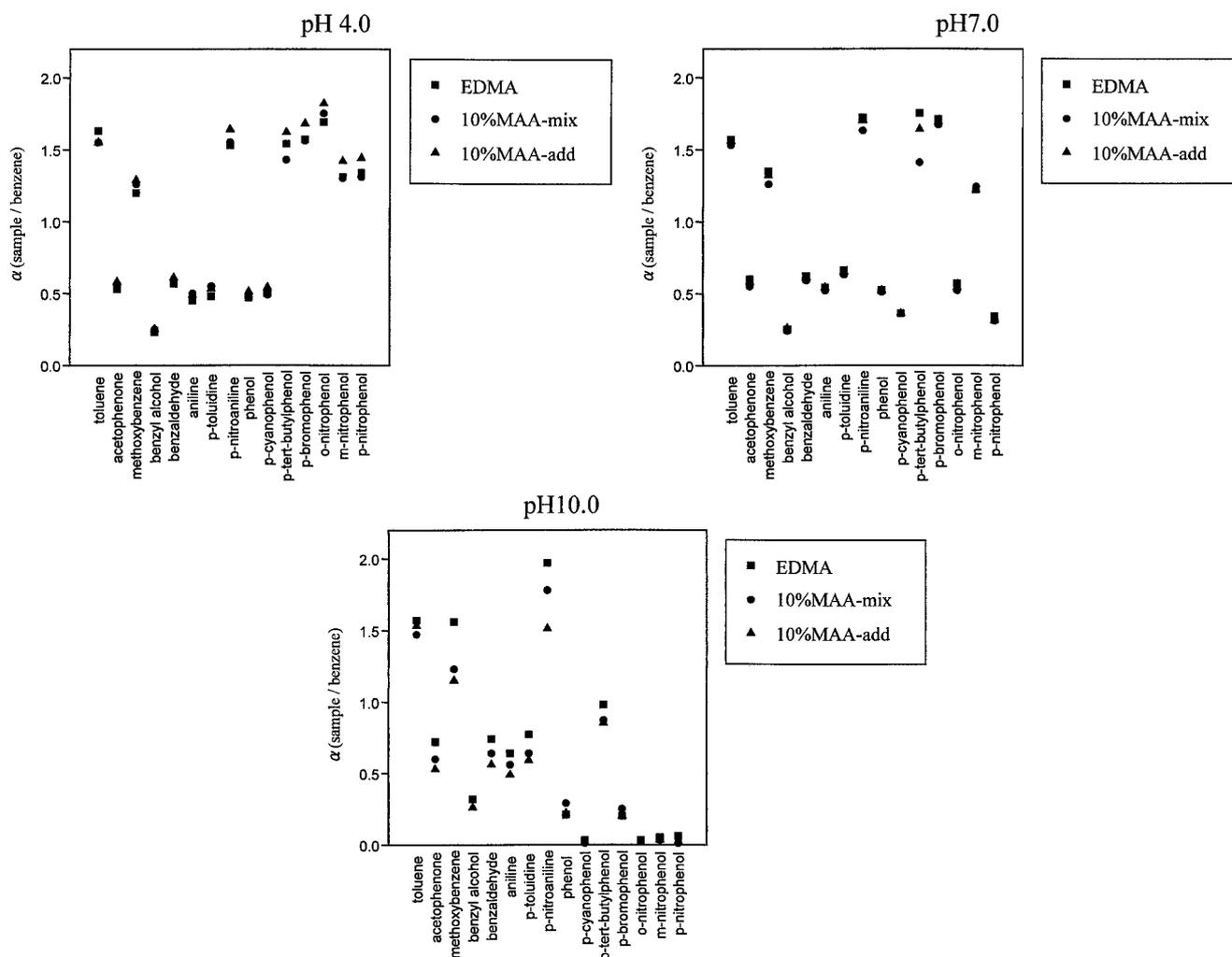


Figure 1. Separation Selectivity on The Prepared Particles in HPLC.

Column size : 4.6 mm ID×100 mm, Flow rate : 1.0 ml/min., Temperature : 30 °C, Detection : UV 254 nm

Mobile phases : 60% aqueous Methanol (citric acid buffer) pH 4. 60% aqueous Methanol (phosphoric acid buffer) pH 7.

60% aqueous Methanol (carbonate buffer) pH 10.

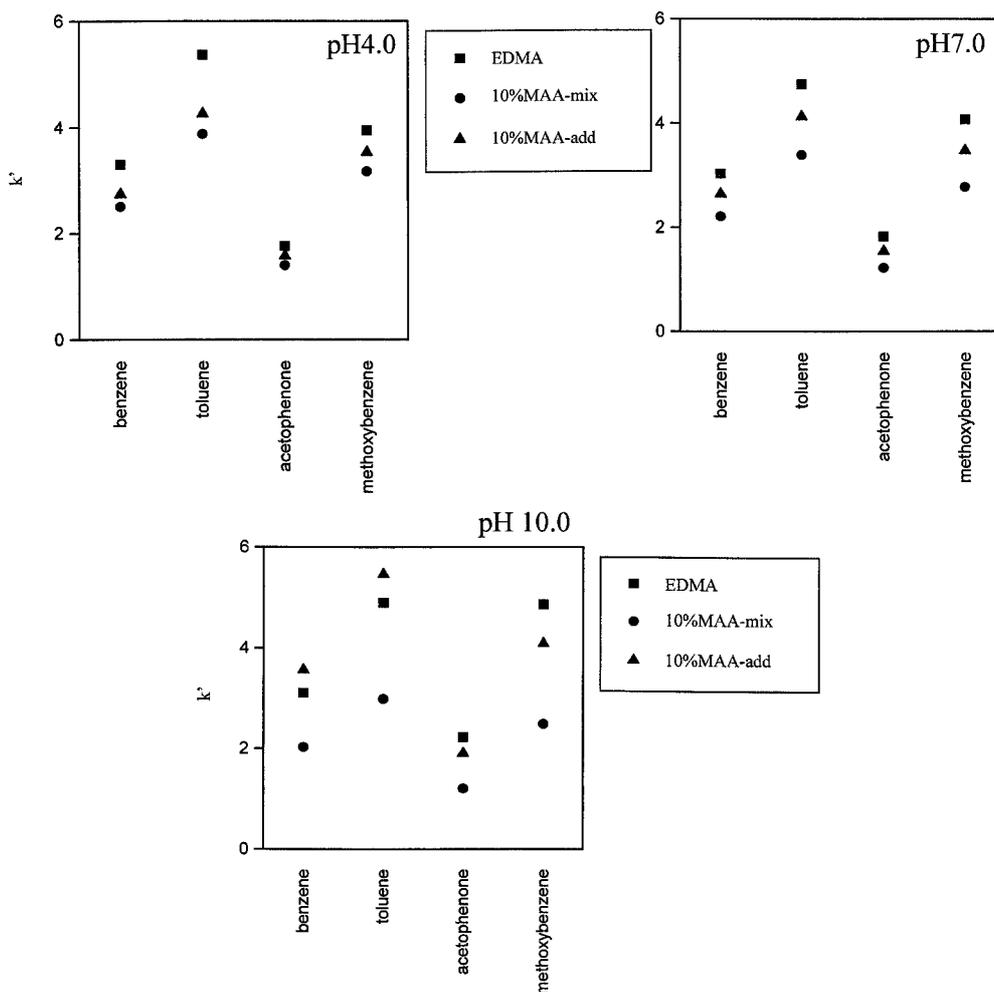


Figure 2. Retentivity on The Prepared Particles in HPLC. Chromatographic conditions are the same as those in Figure 1

on the whole surface of the particle¹⁶ to reduce retentivity of solutes such as benzene and toluene.

In fact, column performance of both particle prepared by mix and addition methods shows big difference (not mentioned in the figures). On the particle prepared by addition method, un-cross-linked surface layer has great opportunity of swelling in aqueous mobile phase. Once this swelling is occurred, mass transfer within the separation sites is greatly reduced to result in poor column efficiency. This phenomena were also found in this case.

Properties in CEC mode

Table 5 shows electroosmotic mobility measured on the particles prepared by mix method and addition method. Although ion exchange capacity was found to be lower with the particle prepared by addition method, larger electroosmotic mobility was found on it. This is clearly an advantage of external ion exchange layer on the particle in CEC mode. Chromatograms of thiourea are demon-

Table 5. Correlation Between Electroosmotic Mobility and Ion Exchange Capacity.

Polymer Particles	Electroosmotic Mobility ($\times 10^{-8} \text{ m}^2 / \text{Vs}$)	Ion Exchange Capacity (meq / g)
EDMA	—	—
10%MAA-mix	1.60	0.41
10%MAA-add	1.71	0.31

Electrochromatography was performed at 20 °C with an applied voltage of 20 kV on a 25 cm, 100. μm I.d., Polymer particles packed fused silica capillary (33.5 cm total length). Mobile phase: ACN-10 mM aqueous phosphate, pH 9.0 (6:4 v/v). Detection at 214 nm. Solute: thiourea.

strated in Figure 3.

Interestingly, this larger electroosmotic mobility on the particles prepared by addition method does not contribute faster elution of alkylbenzenes in CEC mode separation. As shown in Figure 4, the particle prepared by addition method afforded much longer retention towards alkylbenzenes compared with that prepared by mix method. In addition, column performance was better on the particle prepared by addition method than that on the particle prepared by

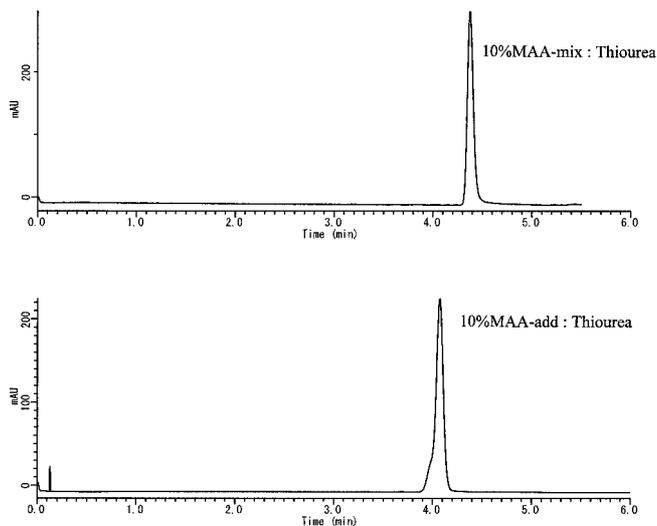


Figure 3. Peak of Thiourea in CEC mode.

Electrochromatography was performed at 20 with an applied voltage of 20 kV on a 25 cm, 100 μ m I.d., Polymer particles packed to fused silica capillary (33.5 cm total length). Mobile phase : were Acetonitrile-10 mM aqueous phosphate, pH 9.0 (6 : 4 v/v). Detection at 200 nm. Solute : thiourea

mix method. This is opposite to the result found in HPLC mode. Through the measurements, EOF was very stable on the particle prepared using addition method, while some unstable base line was observed on the particle prepared by mix method. In fact, some bubble formation was observed during measurements with the particle prepared by mix method. Although further evidence was not available now, these facts might be due to localization of ion exchange groups on the external surface with the particle prepared using addition method, which is one great advantage of this surface design.

Conclusion

A surface modification method, addition method can archive introduction of ion exchange group on the external surface of macroporous polymer-based separation media. The modified particle maintained physical properties of the original separation media and chromatographic selectivity and retention factor were also comparable to those on the original separation media.

In addition, the introduced external surface functionality contributed generation of electroosmotic flow, while separation was carried out with enough retention factor and better column efficiency.

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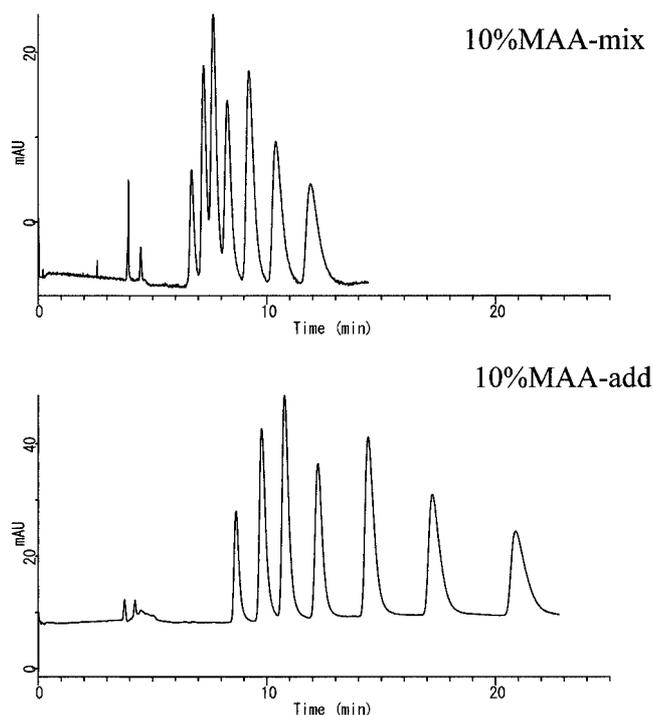


Figure 4. Separation of Alkylbenzenes in CEC mode.

Electrochromatography was performed at 20 with an applied voltage of 20 kV on a 25 cm, 100 μ m I.d., Polymer particles were packed fused silica capillary (33.5 cm total length). Mobile phase : into Acetonitrile-10 mM aqueous phosphate, pH 9.0 (6 : 4 v/v). Detection at 200 nm. Solute : benzene, toluene, ethylbenzene, propylbenzene, butylbenzene, and pentylbenzene (in elution order)

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