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# Novel surface modification of polymer-based separation media controlling separation selectivity, retentivity and generation of electroosmotic flow

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## Abstract

Uniformly sized packing materials based on synthetic polymer particles for high-performance liquid chromatography (HPLC) and capillary electrochromatography (CEC) have been prepared from polymerization mixtures containing methacrylic acid (MAA) as a functional monomer and by using a novel surface modification method. This “dispersion method” affords effectively modified separation media. Both the amount of MAA utilized in the preparation and reaction time affect the selectivity of chromatographic separation in both the HPLC and the CEC mode and electroosmotic flow. This detailed study revealed that the dispersion method effectively modified internal surface of macroporous separation media and, based on the amount of MAA introduced, exclusion mechanism for the separation of certain solutes could be observed. © 2002 Elsevier Science B.V. All rights reserved.

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## 1. Introduction

Capillary electrochromatography (CEC) has a potential advantage in terms of combination of “plug” type flow profile produced by the electroosmotic flow and chromatographic separation selectivity controlled by the chemistry of the stationary phases [1–5]. These result in chromatographic separations with a rather high column performance.

Silanol groups located on fused-silica capillary wall are known to generate electroosmotic flow. In addition, separation media therein such as packed beads [6] and monolithic separation media [7–9] should also contain functional groups contributing to

the generation of electroosmotic flow. In the case of silica-based beads and silica-based monolithic separation media, silanol functionalities are usually utilized and consumed by surface modification of the stationary phases. This decreases the number of remaining silanol groups, which might reduce the generation of electroosmotic flow.

Obviously, some stationary phases that include ionic functional group can be prepared to overcome this problem. However, these functionalities may change separation selectivity as well as retentivity of the stationary phase in the HPLC [10] and/or the CEC mode. The introduction of ion-exchange groups is believed to result in a decrease in hydrophobicity of the stationary phases such as C<sub>18</sub> silica thus leading to smaller retentivity in the reversed-phase mode [11]. On the other hand, polymer-based separation media have a potential for “bi-modal” sepa-

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ration mechanism, which is attributed to macro, micro, and meso pores [12]. We have already reported site selective modification of macroporous polymer-based separation media with various functional monomers, such as chiral amide monomers [13], temperature responsive polymer [14,15], and hydrophilic monomers [16,17]. Specifically, polymer-based separation media selectively modified with hydrophilic external layers were reported to exhibit almost comparable retentivity and separation selectivity to that on unmodified polymer-based separation media, while achieving a reasonable restriction of access for polypeptides such as bovine serum albumin due to the desired surface hydrophilicity [16].

These examples suggest that polymer-based separation media have the potential to achieve relatively easy and effective surface modification without losing separation selectivity and retentivity of the original separation medium. In this report, we wish to demonstrate the preparation method leading to polymer-based separation media modified with methacrylic acid functionality, our novel surface modification method, and report correlation between separation selectivity or retentivity and generation of electroosmotic flow in the CEC mode.

## 2. Experimental

### 2.1. Materials

Water utilized through this study was purified using an E-Pure system (Thermolyne). Methanol was distilled to remove the impurities. Tetrahydrofuran (THF) was distilled over potassium hydride, while acetonitrile was purified using distillation over phosphorus pentoxide followed by distillation in the presence of potassium carbonate [18]. Toluene of the highest commercial grade (Wako) was distilled over potassium hydride. Styrene monomer purchased from Nacalai Tesque was washed by 10% aqueous solution of sodium hydroxide followed by saturated sodium chloride aqueous solution and distilled under reduced pressure (b.p.=59 °C at 33 mmHg; 1 mmHg=133.322 Pa) [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: b.p.=64 °C at 0.2 mmHg, MAA: b.p.=41 °C at 1.0 mmHg). Dibutylphthalate utilized as an activating solvent was purified using distillation under reduced pressure (b.p.=151 °C at 25 mmHg). Benzoyl peroxide (BPO), the polymerization initiator, was purified by re-precipitation method [18]. Other chemicals were used as received.

### 2.2. Preparation of polymer particles

Multi-step swelling and polymerization method was utilized to obtain the uniformly sized polymer packing materials for both HPLC and CEC [19].

#### 2.2.1. Preparation of polymer seed particles [20]

0.395-g of sodium chloride was added to water completely de-oxygenized through helium purging of boiling water followed by cooling. After argon bubbling through the solution for 30 min, 6.0 ml of styrene monomer was added. The mixture was heated to 75 °C and stirred at 350 rpm. After 30 min, the solution of potassium persulfate (0.296 g/50 ml) was added into the monomer mixture. Another 6.0 ml of styrene monomer was added every 30 min until the total volume of added styrene was 69.0 ml. Polymerization was carried out for 24 h. After polymerization was completed, polymer dispersion was purified using centrifugation and re-dispersion into water. The yield of the seed polymer particles was 62.3% and the dispersion concentration was  $\sim 8.0 \cdot 10^{-2}$  ml/ml. This dispersion of the polystyrene seed polymer was utilized in the following swelling.

#### 2.2.2. Multi-step swelling and polymerization method [21–23]

We have previously reported two surface modification methods namely the “mix” and the “add” methods.

##### 2.2.2.1. Mix method

An emulsion prepared by sonification of 0.62 ml of dibutyl phthalate as activating solvent [21–23] and 0.04 g of sodium dodecylsulfate (SDS) in 10 ml of water was added to 2.10 ml of the seed polymer dispersion prepared above. The mixture was stirred at 125 rpm for 3 h. The complete adsorption of the

added activating solvent in the seeds was confirmed by optical microscopy.

An emulsion prepared by sonication of 10.0 ml of toluene (porogenic solvent), 0.10 g of BPO (free-radical initiator), 20 ml of 4.8% (w/w) aqueous solution of poly(vinyl alcohol), and 0.16 g of SDS in 40 ml water was added to the above suspension at room temperature. The following swelling process was completed in 4 h at room temperature upon 125 rpm stirring. The completeness of the swelling was confirmed by optical microscopy.

After this swelling, an additional emulsion prepared by sonicating 5.0 ml of EDMA (cross-linking agent), 0.56 ml of MAA (functional monomer), 10 ml of 4.8% (w/w) poly(vinyl alcohol) aqueous solution, and 0.08 g of SDS in 20 ml of water was added to the system. The additional swelling process with the new set of monomers was completed in 4 h at room temperature upon 125 rpm stirring and controlled by optical microscope.

The swollen system was first purged with argon for 30 min and then heated to 70 °C to initiate polymerization. After 24 h of polymerization, the polymer particles were washed with water, methanol and tetrahydrofuran (THF). The yield of the polymer particles was calculated after filtration and drying in vacuo.

#### 2.2.2.2. Add method [16]

The swellings were carried using processes similar to that of the mix method without the addition of MAA in the additional swelling step. In this case, MAA (0.56 ml) was added in the polymerization mixture after 4 h had elapsed from initiation of the usual polymerization, and the subsequent polymerization continued for 20 h. The purification was the same as in the mix method.

#### 2.2.2.3. Dispersion method

As a reference, we prepared particles from pure EDMA particles without adding the ionizable monomer MAA using the same swelling method. After the isolation and purification of these polymer particles, we dispersed them in acetone (100 ml) and 0.56 or 1.4 ml of MAA was added at 70 °C. This modification reaction was carried out for 4 or 24 h. The purification methods were the same as those used in the mix method.

### 2.3. Column packing

The polymer beads were packed into stainless steel column (100 mm×4.6 mm I.D.) or fused-silica capillary (100 μm I.D.) as slurry in a mixture of water, acetonitrile, 2-propanol, and/or methanol as dispersion media [19].

### 2.4. HPLC and CEC systems

Our HPLC system consisted of a Jasco 880-PU HPLC pump, a Jasco UV-970 detector, and a Rheodyne 7125 valve loop injector. The chromatography was carried out at 30 °C and the data recorded with a Shimadzu C-R4A integrator. CEC was performed using a Hewlett-Packard 3D CE system.

## 3. Results and discussion

### 3.1. Physical properties of the polymer particles

The compositions of the polymerization mixtures used for the preparation of separation media in this study are listed in Table 1 and their properties in Table 2. The ion-exchange capacity was determined by titration of 0.200 g of each packing material dispersed in 10 ml of the purified water with 0.1 mol/l sodium hydroxide standard solution using phenolphthalein as an indicator.

The size of the prepared particles was ~7 μm and the size uniformity (RSD value) was better than 10%. Using the dispersion method, additional monomer (MAA) was polymerized on the surface of the EDMA base particles. Therefore, the size uniformity became slightly worse than that obtained using the mix method. In addition, since MAA is a water soluble monomer, the incorporation of ion-exchange groups (MAA) was lower than that for particles prepared by the mix method, in spite of the same feed ratio. The polymer particles prepared by dispersion method using a longer reaction time of 24 h resulted in the highest ion-exchange capacity that played an important role in chromatographic properties described below.

The dispersion method tended to produce particles with smaller specific surface area compared to that of EDMA particles prepared without the addition of

Table 1  
Composition of the prepared polymer particles

Preparation method	Polymer particles	Composition <sup>a</sup> (EDMA:MAA:TOL)	Initiator (%)	Time <sup>b</sup> (h)
–	EDMA <sup>c</sup>	1:0:2		
Mix	10%MAA-mix	9:1:18	2	24
Addition	10%MAA-add	9:1:18	2	24
Dispersion	10%MAA-disp(6%-4 h)	9:1:18	6	4
Dispersion	25%MAA-disp(6%-4 h)	3:1:6	6	4
Dispersion	25%MAA-disp(3%-24 h)	3:1:6	3	24

<sup>a</sup> Volume ratio.

<sup>b</sup> Total polymerization time for each surface modification.

<sup>c</sup> EDMA is just same as the base polymer particle in dispersion method.

Table 2  
Properties of the prepared polymer particles

Polymer particles	Particle size (μm)	RSD (%)	Yield (%)	Ion-exchange capacity (mequiv./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
10%MAA-disp(6%-4 h)	6.49	4.00	70	0.19
25%MAA-disp(6%-4 h)	7.00	3.55	75	0.40
25%MAA-disp(3%-24 h)	6.99	3.71	78	2.08

Table 3  
Physical properties of the prepared polymer particles

Polymer particles	Pore volume (ml/g)	Surface area (m <sup>2</sup> /g)	Average pore size (Å)
EDMA	0.411	470.94	22.86
10%MAA-mix	0.302	338.87	23.13
10%MAA-add	0.459	450.00	40.76
10%MAA-disp(6%-4 h)	0.395	444.72	23.26
25%MAA-disp(6%-4 h)	0.337	387.35	22.83
25%MAA-disp(3%-24 h)	0.029	30.88	37.24

Table 4  
Size-exclusion characteristics of modified porous beads<sup>a</sup>

Polymer particles	Eva <sup>b</sup> (ml)	Evb <sup>c</sup> (ml)	Evc <sup>d</sup> (ml)	Evtot <sup>e</sup> (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
10%MAA-disp(6%-4 h)	0.105	0.060	0.364	0.528
25%MAA-disp(6%-4 h)	0.167	0.064	0.376	0.607
25%MAA-disp(3%-24 h)	0.045	0.090	0.197	0.332

<sup>a</sup> Chromatography conditions: mobile phase: 100% THF; column size: 100 mm×4.6 mm I.D. Sample: benzene, hexylbenzene, standard polystyrene (PS). Flow rate: 0.5 ml/min, temperature: 30 °C.

<sup>b</sup> Eva: elution volume(benzene)–elution volume(hexylbenzene).

<sup>c</sup> Evb: elution volume(hexylbenzene)–elution volume( $M_r$  = 760 PS).

<sup>d</sup> Evc: elution volume( $M_r$  = 760 PS)–elution volume( $M_r$  = 20 000 000 PS).

<sup>e</sup> Evtot: elution volume(benzene)–elution volume( $M_r$  = 20 000 000 PS).

MAA (Table 3). This is especially true for the particles modified with higher MAA percentage and 24 h polymerization. For example, 25%MAA-

disp(3%-24 h) beads had both a quite small pore volume and surface area as determined by BET method. Table 4 shows that the modified particles

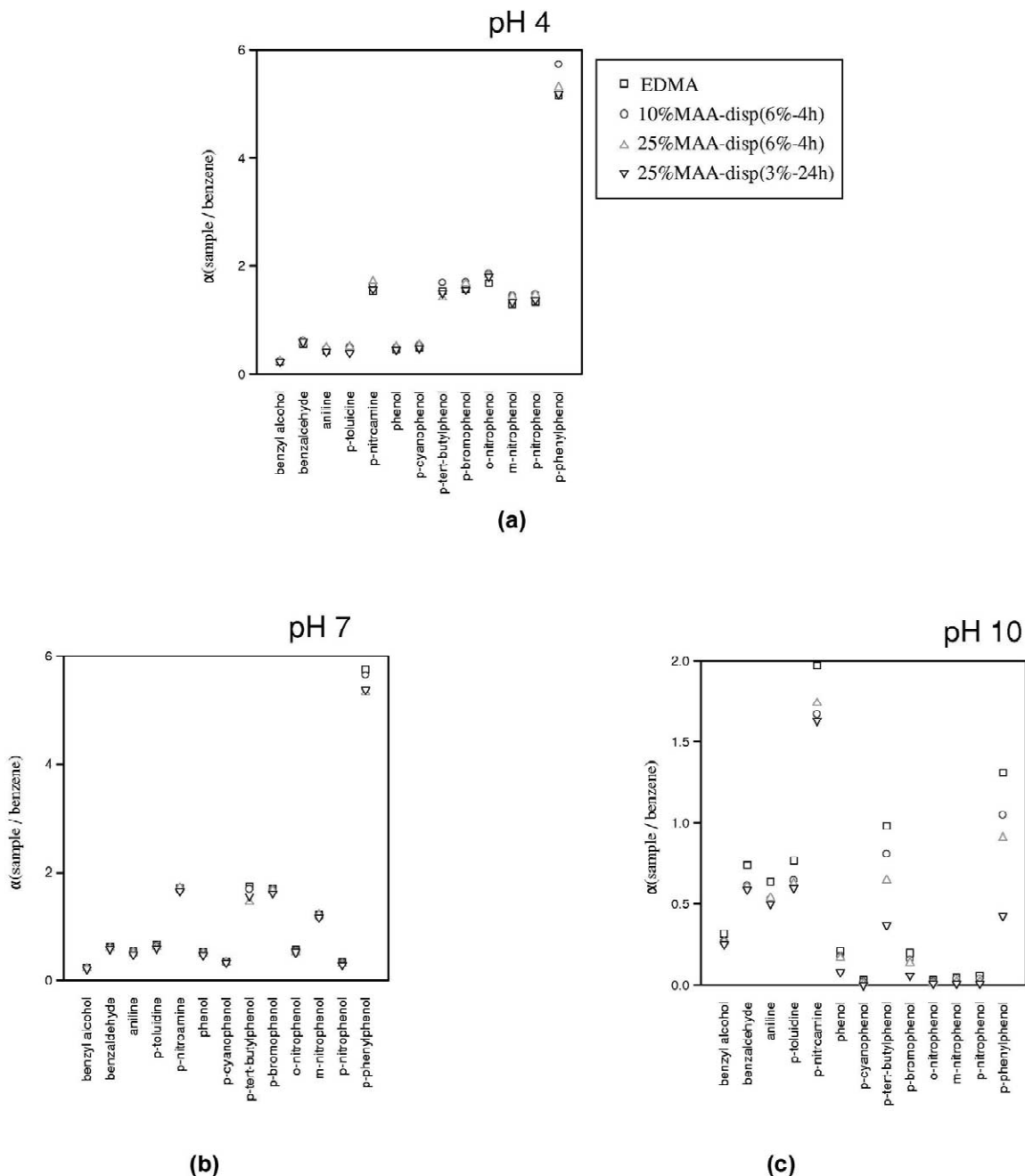


Fig. 1. Separation selectivity on the prepared particles in HPLC. Column size: 100 mm  $\times$  4.6 mm I.D., flow rate: 1.0 ml/min, temperature: 30  $^{\circ}$ C, detection: UV 254 nm. Mobile phases: 60% aqueous methanol (citric acid buffer) pH 4, 60% aqueous methanol (phosphoric acid buffer) pH 7, 60% aqueous methanol (carbonate buffer) pH 10.

25%MAA-disp(3%-24 h) also have smaller pore volume tested using size-exclusion chromatography, which is probably due to pores blocked with added MAA.

### 3.2. Chromatographic properties in HPLC

In our previous paper [24], we reported on chromatographic properties of the polymers modified using two surface modification methods, namely the addition and mix methods. The particles prepared using the mix method enabled better incorporation of MAA, while the addition method led to lower incorporation of MAA probably due to the hydrophilicity of this monomer. The particles prepared using the addition method showed larger retentivity for relatively hydrophobic solutes in the HPLC mode, while polymeric external surface layer derived from MAA resulted in slightly different separation selectivity, especially at pH 10, and much lower column efficiency.

Chromatographic properties of the modified polymer particles prepared using the dispersion method were determined in the HPLC mode at pH values of 4, 7, and 10. Separation selectivity in terms of  $\alpha$  values for benzene is shown in Fig. 1. At both pH 4 and 7, similar selectivity was observed towards most of the solutes, while at pH 10, the polymer particles prepared using the dispersion method afforded different selectivity compared to EDMA particles. Moreover, an increase in the ion-exchange capacity (Table 2) leads to an decrease in retention times for bulky

phenol derivatives such as *p-tert.*-butylphenol and *p*-phenylphenol. This can only be explained by exclusion effects introduced into internal surface (pores) by MAA.

### 3.3. Properties in CEC mode

As reported previously [24], although the percentage of MAA was lower in the particles prepared using the addition method, these beads afforded higher electroosmotic flow in CEC mode compared to those prepared by the mix method. In addition, alkylbenzenes exhibited much larger retention on the particles prepared by the addition method and column efficiency was higher.

Table 5 shows electroosmotic mobility determined for the particles prepared by both mix and addition methods. Although the polymer particles 25%MAA-disp(3%-24 h) had much higher ion-exchange capacity, electroosmotic mobility was found to be comparable to those of the mix and addition methods despite having much lower ion-exchange capacity.

The separation of alkylbenzenes in CEC mode is shown in Fig. 2. Although the ion-exchange capacity of 25%MAA-disp(6%-4 h) was higher than that of 10%MAA-disp(6%-4 h), retention times of alkylbenzenes, especially pentyl- and hexylbenzene, were longer on the 25%MAA-disp(6%-4 h). In contrast, 25%MAA-disp(3%-24 h) beads afforded much shorter retention times. The reason for this is not clear at the moment, but exclusion phenomenon seem to play an important role in retentivity in the CEC mode. In

Table 5  
Correlation between electroosmotic mobility and ion-exchange capacity

Polymer particles	Electroosmotic mobility <sup>a</sup> ( $\times 10^{-8} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$ )	Ion-exchange capacity <sup>b</sup> (mequiv./g)	<i>a/b</i>
EDMA	–	–	–
10%MAA-mix	1.60	0.41	3.90
10%MAA-add	1.71	0.31	5.52
10%MAA-disp(6%-4 h)	1.06	0.19	5.57
25%MAA-disp(6%-4 h)	1.27	0.40	3.18
25%MAA-disp(3%-24 h)	1.90	2.08	0.91

CEC was performed at 20 °C at 20 kV with a 25 cm  $\times$  100  $\mu\text{m}$  I.D., fused-silica capillary (33.5 cm total length) packed with polymer particles. Mobile phase: acetonitrile–10 mM phosphate buffer, pH 9.0 (60:40, v/v). Detection at 200 nm. Solute: thiourea.

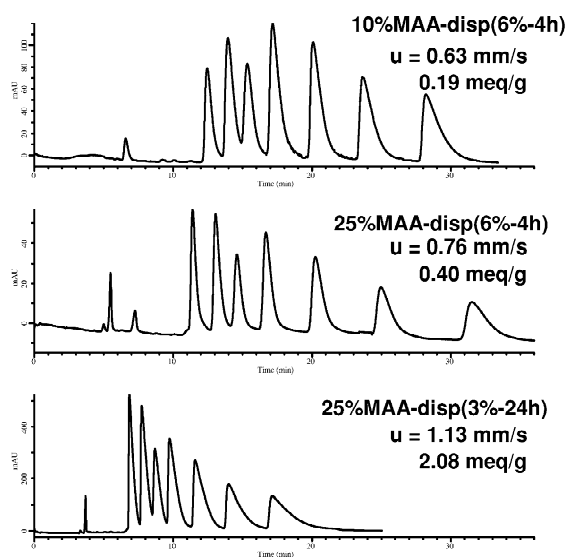


Fig. 2. Separation of alkylbenzenes in the CEC mode. Electrochromatography was performed at 20 °C with an applied voltage of 20 kV on a 25 cm  $\times$  100  $\mu$ m I.D., polymer particles packed fused-silica capillary (33.5 cm total length). Mobile phase: acetonitrile–10 mM aqueous phosphate, pH 9.0 (6:4, v/v). Detection at 200 nm. Solutes: benzene, toluene, ethylbenzene, propylbenzene, butylbenzene, and pentylbenzene (in elution order).

these cases, the packing methods as well as flit preparation have not been optimized. Therefore, the peaks were found to be eluted with large tailings.

But there may not be potential problems with the beads. Improvements will be done in other applications.

#### 4. Conclusion

Novel surface modification dispersion method affords various polymer particles having MAA groups located mainly inside the pores. These beads exhibit rather different chromatographic selectivity as well as retentivity in both the HPLC and CEC separation modes. Although the detailed mechanism is currently not completely clear we believe that it follows the schematic shown in Fig. 3. Using the combination of other surface modification methods, we can obtain various polymer-based separation media which can be utilized in both HPLC and CEC. Further studies are now in progress.

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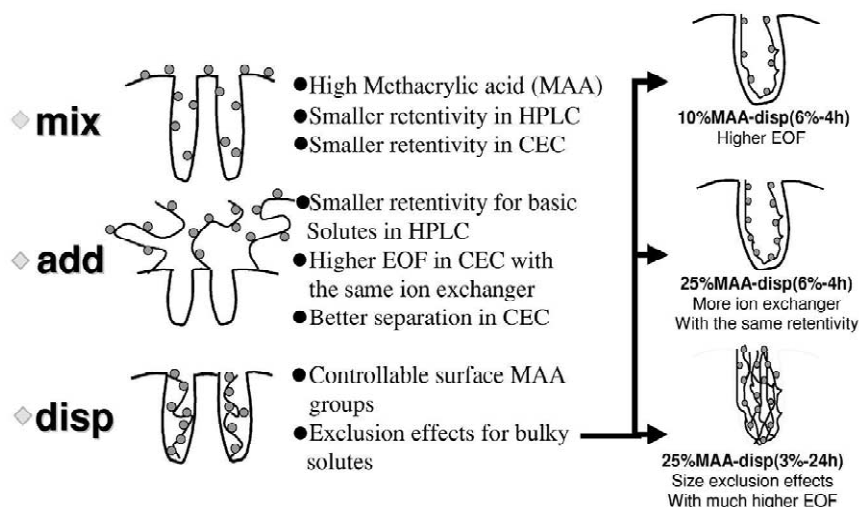


Fig. 3. Schematic image of the surface modified polymers.

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