

Separation of copolymers using high-performance liquid chromatography with polymeric stationary phase and liquefied carbon dioxide as adsorption promoting solvent

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Abstract

Chromatographic separation of copolymers depending on the chemical composition was studied by a solvent gradient method using liquefied carbon dioxide (CO₂) as an adsorption promoting solvent. As the high polar stationary phase, non-bonded silica gel, crosslinked acrylamide (AA) gel and crosslinked acrylonitrile (AN) gel were utilized. All columns showed the typical normal phase type of adsorption. Polymeric stationary phases showed the higher sample recovery for styrene–methyl methacrylate (St–MMAs) copolymers, indicating suitability for quantitative analyses. The separations of butyl methacrylate (BMA)–methyl methacrylate, and 2,2,3,3,4,4,4-heptafluorobutyl methacrylate (FBMA)–methyl methacrylate copolymers were also carried out, and the latter copolymers were separated based on the CO₂-philicity with acrylonitrile column.

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1. Introduction

Synthetic polymers usually have intermolecular heterogeneity such as molecular weight distribution, and chemical composition distribution (CCD) of copolymers. Characterization of these heterogeneities is important because the relationships between heterogeneity and polymer properties must be elucidated in order to produce the high quality of materials. Indeed, it is reported that the CCD of copolymers relates to the mechanical and hydrodynamic properties [1,2]. To characterize intermolecular heterogeneity, polymer sample must be fractionated based on some specific property. Therefore, chromatographic approaches are especially promising methods. Indeed, molecular mass distribution is commonly analyzed by a gel permeation chromatography, and oligomers have been separated by adsorption

high-performance liquid chromatography (HPLC) including supercritical fluid chromatography according to the specific degrees of oligomerization.

As for the characterization of chemical composition distribution of copolymers, gradient HPLC has been successfully utilized [3]. Teramachi et al. [4] originally separated copolymers by chemical composition with gradient HPLC in 1979. Polymer samples are adsorbed on the stationary phase strongly if the interaction between the sample and stationary phase exist [3]. In order to desorb the polymer solvent and/or temperature gradient must be conducted in the direction to make the interaction weaker. Chemical compositional separations are based on the fact that the strength of interaction was dependent on the copolymer chemical composition, We also separated various types of copolymers by normal and reversed-phases of HPLC [5–9]. Previous studies indicated the combination of polar stationary phase and non-polar mobile phase (normal phase conditions) or non-polar stationary phase and polar eluent

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(reversed-phase conditions) provided the highly resolved separation governed by the adsorption mechanism [7].

Carbon dioxide (CO₂) is inexpensive, non-flammable, and easily liquefied. Liquefied or supercritical CO₂ exhibits non-polar nature [10], and it has been utilized as a mobile phase for separation of oligomers based on their molecular mass [11–16]. Non-viscous nature of CO₂ or high diffusion coefficient of the solute affords higher resolution compared with conventional HPLC systems. However, molecular mass of samples investigated using HPLC or SFC has been limited below ca. 10,000, since common polymers are generally not soluble in CO₂. Olesik and co-workers [17–19] have reported the separation of polymeric samples with enhanced-fluidity liquid mixtures using CO₂. This fascinating solvent has the possibility to play a role of non-polar organic solvent in normal phase HPLC for the compositional separation of copolymers. It is expected that high resolution is achieved based on the non-viscous nature of CO₂ [17–19]. At the same time, the amount of environmentally harmful organic solvent can be reduced. In addition, since the solvent properties such as density, solvating power can be tuned by the change of temperature and/or pressure, various types of gradient elution can be used for the compositional separation of copolymers as well as the solvent gradient.

We recently reported the compositional separation of styrene–methyl methacrylate copolymers (St–MMAs) with the solvent gradient method using liquefied CO₂ as an adsorption promoting solvent and non-bonded silica gel as a stationary phase [20]. This is the first report concerned with the compositional separation of high molecular mass copolymers using CO₂ as a component of mobile phase. Hydrogen bonding played an important role in the adsorption–desorption process. In the case of HPLC utilizing solvent gradient method, solubility of a sample in CO₂ is not a serious problem when a desorption promoting solvent has the ability to dissolve the sample and CO₂ play a role of an adsorption promoting solvent. It was also found that the elution behavior is practically independent of the molecular mass in the examined range of 2.5×10^4 to 1.6×10^6 .

In this paper, two kind of polar polymer beads were applied as a stationary phase and their advantage was indicated compared with conventional non bonded silica gel in the separation of St–MMA. Sato et al. [5,6] also have demonstrated that the polymeric stationary phases are superior to those based on unmodified or modified silica gels for separating polymers by the conventional adsorption HPLC, because of the good reproducibility and proportionality between the peak area and sample amount due to the smaller amount of irreversible adsorption. The same advantage is expected for the compositional separation with CO₂ as the one of the components of eluents. The separation of other methacrylate copolymers containing fluorinated alkyl chain was carried out. The effect of CO₂-philicity of fluorinated component on the elution behavior was investigated.

2. Experimental

2.1. Samples

Statistical St–MMA, butyl methacrylate (BMA)–MMA, and 2,2,3,3,4,4,4-heptafluorobutyl methacrylate (FBMA)–MMA copolymers were synthesized by a radical polymerization in bulk under nitrogen atmosphere. The conversion of a copolymer was kept below 10% to obtain a sample with a narrow chemical composition distribution. The average chemical composition in each sample was determined by ¹H NMR spectroscopy (JEOL α-500 spectrometer, Tokyo Japan). Effective average molecular masses M_n , and M_w , of obtained copolymers were determined by gel-permeation chromatography (GPC) using two columns (30 cm × 7.6 mm i.d.) packed with styrene–divinylbenzene copolymer beads (the median pore sizes in a swollen state are 180 and 50 Å, respectively) [21] and THF (Wako Chemical, Osaka, Japan) as an eluent at a flow rate of 0.5 ml/min. GPC was carried out at an ambient temperature using HPLC pump (JASCO, 880-PU, Tokyo, Japan), RI detector (JASCO, RI-2031 Plus, Tokyo Japan). A 10 μl portion of the sample (10 mg/ml each in THF solution) was injected through a Rheodyne 7125 injector (Cotati, CA, USA). The calibration curve for polystyrene standards (Shodex, Tokyo, Japan) was used to estimate the molecular mass. Table 1 shows the characteristics of copolymers used in this study.

2.2. HPLC

HPLC measurements using CO₂ as a component of an eluent were carried out JASCO Super 201 System (Tokyo, Japan) equipped with a 880-81 type back pressure regulator (JASCO, Tokyo, Japan) using 4.6 mm i.d. × 25 cm stainless-steel column packed with non-bonded silica gel (SFC pak SIL-5, pore size; 60 Å, JASCO, Tokyo Japan), crosslinked acrylonitrile

Table 1
Characteristics of synthesized copolymers

Code	M_n^a (10 ⁵)	M_w^a (10 ⁵)	M_w/M_n^a	MMA content ^b (mol%)
St–MMA 1	1.2	2.4	2.0	33
St–MMA 2	1.6	3.3	2.1	40
St–MMA 3	0.95	2.6	2.7	57
St–MMA 4	0.90	3.1	3.4	84
BMA–MMA 1	3.2	8.0	2.5	10
BMA–MMA 2	5.0	9.2	1.8	34
BMA–MMA 3	5.8	9.6	1.7	59
BMA–MMA 4	3.7	7.7	2.1	74
BMA–MMA 5	3.8	7.0	1.8	94
FBMA–MMA 1	1.2	3.1	2.5	28
FBMA–MMA 2	0.73	2.2	3.0	44
FBMA–MMA 3	0.96	1.7	1.8	67
FBMA–MMA 4	0.73	1.8	2.5	87

Abbreviations: St—styrene; MMA—methyl methacrylate; BMA—butyl methacrylate; FBMA—2,2,3,3,4,4,4-heptafluorobutyl methacrylate.

^a Determined with GPC calibrated by polystyrene standards.

^b Determined by ¹H NMR.

(AN) or acrylamide (AA) gel [9]. The molecular masses of the exclusion limit were 3×10^4 , 5×10^5 , and $> 10^6$, respectively. Outlet pressure was regulated at 15 or 20 MPa. The column effluent was monitored with JASCO UV-970 detector (Tokyo, Japan) equipped with a high pressure proof cell at the wavelength of 254 nm or an evaporative light scattering detector (Alltech ELSD 800, Deerfield, IL, USA) operated at 40 °C using N₂ as a nebulizer gas (2 atm). The outlet stream from the back pressure regulator was directly introduced to ELSD. The column temperature was maintained from 50 to 70 °C by a JASCO CO965 column oven (Tokyo, Japan). The system consists of two pumps for mobile phase, one is for the delivery of CO₂ and the other for the delivery of CHCl₃/EtOH or THF. The flow rate of CO₂ was kept at 0.5 ml/min and that of organic solvent was linearly changed from 0.25 to 2.5 ml/min in 30 min. A 10 μl portion of the sample (10 mg/ml each in CHCl₃ solution) was injected through a Rheodyne 7125 injector (Cotati, CA, USA).

3. Results and discussion

3.1. Effect of stationary phase on separation of St–MMA copolymers

In the chromatography using CO₂ as the component of eluents including supercritical fluid chromatography, non-bonded or modified silica gels have been exclusively utilized. Here it is demonstrated that polymeric stationary phases are applied for the chemical compositional separation of copolymers.

Fig. 1 shows the separation of St–MMA copolymers using silica (a), AA gel (b) and AN gel (c). In all cases, the sample with higher styrene content eluted earlier, indicating a normal phase type elution. Silica column provided the highest resolution but we observed the lower recovery ratio especially for the sample with higher MMA content. Table 2 shows the recovery ratio obtained with three type of gels. To obtain the precise data, the samples were injected individually, and three experiments were conducted for each sample. We assumed that the recovery of polystyrene homopolymer is 100% and only styrene unit shows the absorption at 254 nm. The recovery ratios were calculated from the peak area and styrene content. In the case of silica,

Table 2
Comparison of recovery ratio obtained with silica, AA and AN column

MMA content (mol%)	Recovery ratio (%) ^a		
	Silica	AA	AN
33	93	86	86
40	85	89	92
57	51	85	85
84	30	91	92

Abbreviations: AA—acrylamide column; AN—acrylonitrile column; MMA—methyl methacrylate.

^a It is assumed that the recovery of polystyrene is 100% and only the styrene unit shows the absorption at 254 nm.

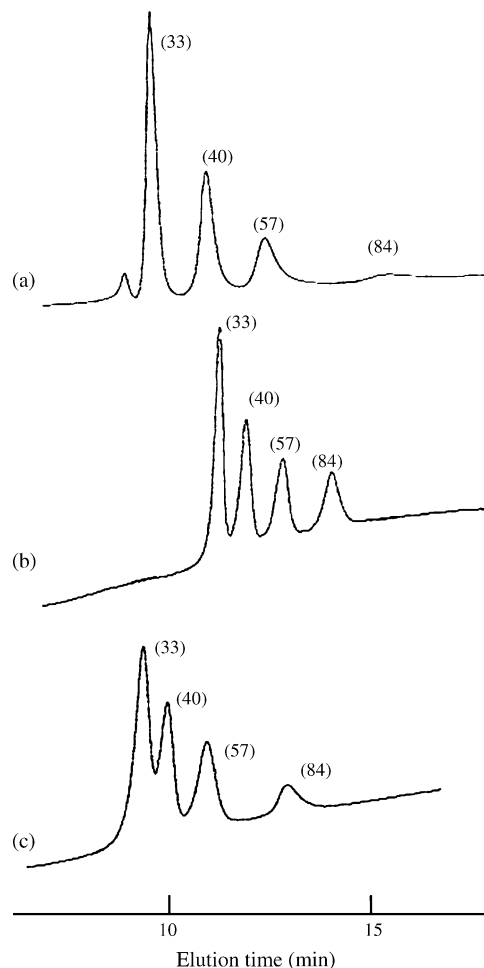


Fig. 1. Separation of poly(styrene-co-methyl methacrylate)s with solvent gradient method using (a) silica, (b) acrylamide, and (c) acrylonitrile gels as a stationary phase. Values in the parentheses indicate MMA content of the copolymer. Flow rate: CO₂ (0.5 ml/min); CHCl₃ (for silica and acrylamide 3.5 vol% of ethanol was contained) (0.25–2.5 ml/min in 30 min); column temperature: 60 °C; back pressure: 20 MPa; detector: UV (254 nm).

the recovery ratio decreased as the MMA content of copolymers increased. This is probably due to the irreversible adsorption. Both polymeric gels showed higher recovery ratio. It is found that polymeric AA and AN gels are more suitable for quantitative analyses than silica gel, for example the determination of CCD. Sato et al. [5] also reported that polymeric packing material was superior to silica gel concerned with the recovery and reproducibility for the compositional separation of styrene–butadiene copolymers utilizing a conventional normal phase type HPLC. In chromatogram (a) an additional small peak was observed at around 9 min. This peak always appeared in the case of the sample injection. The intensity and elution time was almost independent of the type of the sample injected. This unknown peak was never observed in HPLC with both polymeric gels.

Fig. 2 shows the temperature dependencies of sample elution (silica (a), AA and AN (b)). The chloroform contents at the sample elution were plotted against the styrene content

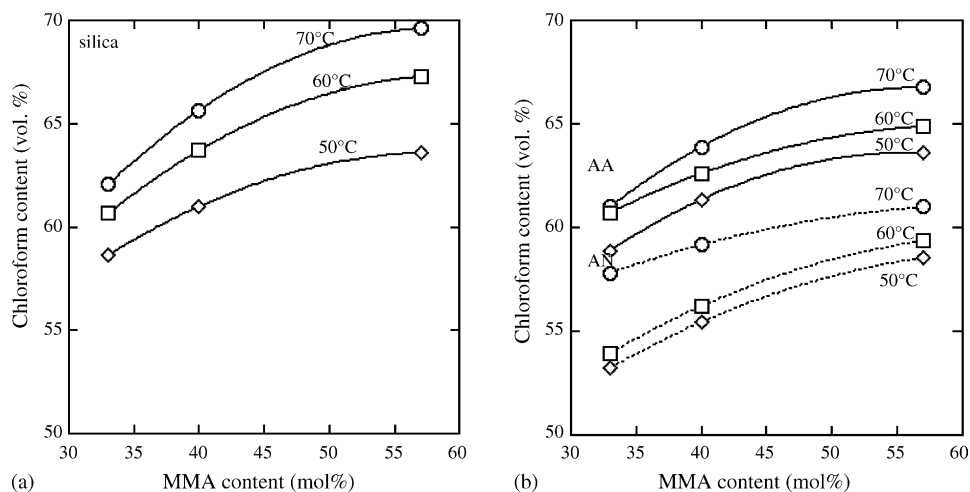


Fig. 2. Temperature effect on chloroform content of the eluent. (a) For silica column, and (b) acrylamide (solid line) and acrylonitrile (dotted line) columns, column temperature: 50°C (diamond), 60°C (square), 70°C (circle). The other chromatographic conditions are the same as those in Fig. 1.

of the sample. In all cases, the higher the column temperature, the stronger the adsorption, i.e., the sample elution was retarded as the temperature increased. In the case of silica (Fig. 2a), the strongest dependence was observed. Mori and co-workers reported the similar temperature dependence to ours in the separation of St–MMA copolymers by a chemical composition in normal phase conditions [22]. In their system, adsorption of samples to the stationary phase (silica gel) was mainly due to the hydrogen bonding, and a small amount of EtOH was used as a desorption promoting solvent. In the chromatographic conditions where the component of eluent (EtOH) strongly interacts with the stationary phase, not only the changes of enthalpy and entropy of the solute involved in the adsorption or desorption process but those of EtOH should be taken into consideration. It can be assumed that when the polymeric sample desorbs, solvent molecules (such as EtOH) adsorb at the sites. Solvent molecules in the mobile phase have more freedom compared with polymer molecules in the mobile phase. This consideration suggests that the polymer desorption process (together with the adsorption of solvents) is at least entropically unfavored. Compared with silica, AA gels shows weaker dependence. It is probably due to the fact that the hydrogen bonding interaction between gels and ethanol is not so strong as that in silica gel system. AN gel is considered to have poorer hydrogen bonding ability, and in fact, no EtOH (strongly interacts with high polar stationary phase) was added to the desorption promoting solvent, which explains the weakest temperature dependence.

3.2. Separation of methacrylate copolymers

Judging from the results for St–MMA copolymers, the sample with higher MMA content tends to adsorb irreversibly on silica gel. Therefore, polymeric stationary phases are more favorable for the analyses of methacrylate type copolymers, BMA–MMAs and FBMA–MMAs, and all the following experiments were carried out using AA and AN columns.

Fig. 3 shows the chromatograms for FBMA–MMA (a) BMA–MMA (b) using AA column. In both cases, the sample with lower MMA content eluted earlier still indicating the normal phase type of separation. For BMA–MMAs, acceptable separation was observed. On the other hand, significant peak broadening was observed for FBMA–MMA. The peak width increased, as the FBMA content increased. There are two possibilities to explain the peak broadening. One is due to the CCDs of samples. Since the elution times are similar between the samples, CCD is not the main factor for peak broadening. Another possibility is due to the molecular mass distribution. As previously reported, molecular mass effect is negligible for the samples having more than 2×10^4 for St–MMA copolymers. Recently Brun and Alden [23] reported the gradient separation of polymers at critical

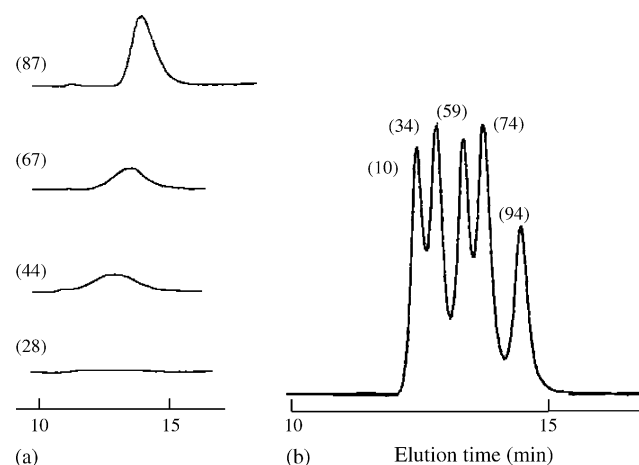


Fig. 3. Separation of poly(2,2,3,3,4,4,4-heptafluorobutyl methacrylate-co-methyl methacrylate)s (a) and poly(butyl methacrylate-co-methyl methacrylate)s (b) with solvent gradient method using acrylamide gel as a stationary phase. Values in the parentheses indicate MMA content of the copolymer. Flow rate: CO₂ (0.5 ml/min); CHCl₃ containing 3.5 vol% of ethanol (0.25–2.5 ml/min in 30 min); column temperature: 60°C; back pressure: 15 MPa; detector: ELSD (40°C, 2 atm).

point of adsorption. Novel parameter, Q , is introduced in their theory, which is proportional to the mean square radius and therefore increases with molecular mass. It was demonstrated theoretically and experimentally that when the polymer homologous series is subjected to gradient elution, lower molecular mass fractions can be effectively separated according to the size ($Q < 1$), whereas the macromolecules with higher molecular masses ($Q > 1$) will have practically the same retention. It can be said that our experimental conditions for St–MMA are sufficient for such a situation, but not for FBMA–MMA. Moreover, peak area decreased as the FBMA content increased. For the quantitative discussion, the calibration of ELSD should be conducted.

It is well-known that the polymers of fluorinated alkyl methacrylates or acrylates are “CO₂-philic”, and soluble in supercritical CO₂ [24,25]. However, the retention time of BMA–MMA sample was similar to that of FBMA–MMA with the corresponding MMA content, indicating that CO₂ philicity does not play an important role in this system. It is considered that the strong interaction between the samples and high polar AA gels hinders the difference of the CO₂-philicity. It is probably possible that above-mentioned molecular mass effect comes from CO₂ philicity of FBMA unit.

Fig. 4 shows the chromatograms for FBMA–MMA (a) BMA–MMA (b) using AN column. For both copolymers, the elution of sample with higher MMA content was retarded. Compared with AA column, the peak width increased for BMA–MMA, but decreased for FBMA–MMA. As the results of peak sharpening and higher selectivity between samples, it was possible to separate four copolymers as shown in Fig. 4 (a). FBMA–MMA copolymers eluted earlier compared with

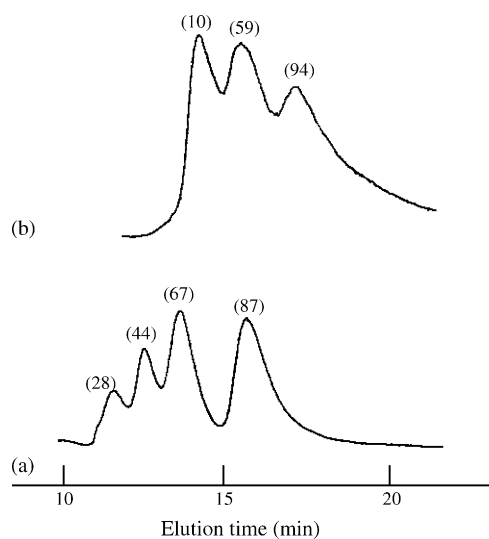


Fig. 4. Separation of poly(2,2,3,3,4,4,4-heptafluorobutyl methacrylate-co-methyl methacrylate)s (a) and poly(butyl methacrylate-co-methyl methacrylate)s (b) with solvent gradient method using acrylonitrile gel as a stationary phase. Values in the parentheses indicate MMA content of the copolymer. Flow rate: CO₂ (0.5 ml/min); CHCl₃ (0.25–2.5 ml/min in 30 min); column temperature: 60 °C; back pressure: 15 MPa, detector: ELSD (40 °C, 2 atm).

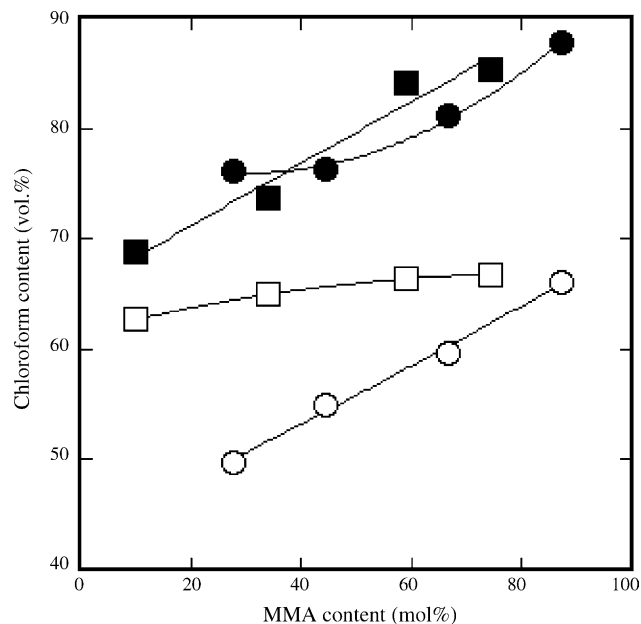


Fig. 5. Chloroform content of the eluent at peak maximum utilizing CO₂ (open symbols) and hexane (filled symbols) as an adsorption promoting solvent for poly(butyl methacrylate-co-methyl methacrylate) (square) and poly(2,2,3,3,4,4,4-heptafluorobutyl methacrylate-co-methyl methacrylate) (circle). Experimental conditions in utilizing CO₂, flow rate: CO₂ (0.5 ml/min); CHCl₃ (0.25–2.5 ml/min in 30 min); column temperature: 60 °C; back pressure: 15 MPa, in utilizing hexane: flow rate: hexane/CHCl₃; 0.45/0.05 → 0/0.5 ml/min in 25 min; column temperature: 30 °C, and in both case ELSD (40 °C, 2 atm) was used as a detector.

BMA–MMA copolymers with the corresponding MMA content, indicating the CO₂-philic nature of fluorinated alkyl moiety.

Fig. 5 shows the comparison of CO₂ with hexane in the case of AN column. Chloroform contents at the sample elution was plotted against the MMA content of the sample. Open symbols represent the results with utilizing CO₂ as an adsorption promoting solvent, and filled symbols with utilizing hexane. In the case of hexane, more chloroform was necessary to elute the sample and no selectivity was observed between BMA–MMA and FBMA–MMA. On the other hand, in the case of CO₂, FBMA–MMA eluted earlier, and higher selectivity was observed. This is probably due to the specific interaction between fluorinated group and CO₂. It is considered that this selectivity is attributed to the difference of CO₂-philicity among copolymers and moderate strength of the interaction between the samples and AN gels.

4. Conclusions

Separations of copolymers (St–MMA, BMA–MMA, FBMA–MMA) based on the chemical composition were successfully conducted by solvent gradient method utilizing CO₂ as the one of the components of mobile phase (adsorption promoting solvent). For all cases normal phase type of elution was observed. For the quantitative analyses of methacrylate

containing polymers, polymeric stationary phases, i.e., AN and AA gel were more suitable than silica gel. At the higher temperature, the stronger adsorption was observed, and silica showed strongest temperature dependence. The mixture of FBMA–MMAs was separated only utilizing AN–CHCl₃ system with the specific interaction between fluorinated group and CO₂. The separation is based on the difference of CO₂-philicity among copolymers and moderate strength of the interaction between the samples and AN gels.

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