Chemical compositional separation of styrene–methyl methacrylate copolymers using high-performance liquid chromatography with liquefied carbon dioxide as eluent

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Abstract

Chromatographic separation of styrene–methyl methacrylate (MMA) copolymers depending on the chemical composition was studied using liquefied carbon dioxide as an adsorption promoting solvent, and tetrahydrofuran, chloroform containing ethanol as a desorption promoting solvent in the mobile phase and the column packed with non-bonded silica gel by a solvent gradient method. With the increase of MMA content, the elution was retarded indicating that the typical normal-phase type of adsorption occurred. The effects of type of desorption solvents, molecular mass of sample, and column temperature on the elution were investigated.
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1. Introduction

Copolymers prepared by radical polymerization usually have the chemical heterogeneitic distributions among molecules such as molecular mass distribution and chemical composition distribution (CCD). Even in the case of low-conversion polymerization, the copolymers show a statistical CCD [1]. These distributions relate to the mechanical and hydrodynamic properties of polymers [2,3]. Therefore, it is important to analyze them in terms of some special characterization systems. Currently, molecular mass distribution is commonly analyzed by gel permeation chromatography (GPC), and oligomers have been separated by adsorption high-performance liquid chromatography (HPLC) according to the specific degrees of polymerization. Polymer samples are adsorbed on the stationary phase irreversibly if the interaction between the sample and stationary phase exists [4]. In order to desorb the polymer solvent and/or temperature gradient must be conducted in the direction to make the interaction weaker. Based on the fact that the strength of interaction was dependent on the copolymer chemical composition, Teramachi et al. originally separated copolymers by chemical composition with gradient HPLC in 1979 [5]. We also separated various types of copolymers by normal- and reversed-phase HPLC [6–9]. Previous studies indi-

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cated the combination of polar stationary phase and non-polar mobile phase (normal-phase conditions) or non-polar stationary phase and polar eluent (reversed-phase conditions) provided the highly resolved separation governed by the adsorption mechanism [7].

Carbon dioxide (CO_2) is inexpensive, non-flammable, and easily liquefied. Liquefied or supercritical CO_2 exhibits non-polar nature [10]. 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 CO_2 [11–13]. 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.

Liquefied or supercritical CO_2 has been utilized as a mobile phase for separation of oligomers based on their molecular mass [14,15]. Non-viscous nature of CO_2 or high diffusion coefficient of the solute affords higher resolution compared with conventional HPLC systems. Ute et al. obtained 100mer isotactic methyl methacrylate (MMA) [16] and 25mer syndiotactic styrene [17] with preparative supercritical fluid chromatography (SFC) using silica gel as stationary phase. Kinugasa et al. separated oligo(ethylene glycol) by molecular mass with SFC in order to obtain the uniform size of standard samples [18]. Pasch and co-workers separated block co-oligomers of ethylene oxide and propylene oxide depending on length of each monomer unit [19,20]. Molecular mass of samples investigated using HPLC or SFC has been limited below ca. 10 000, since common polymers are generally not soluble in CO_2. Olesik and co-workers have reported the separation of polymeric samples with enhanced-fluidity liquid mixtures using CO_2 [11–13]. In the case of HPLC utilizing solvent gradient method, solubility of a sample in CO_2 is not a serious problem when a desorption promoting solvent has the ability to dissolve the sample and CO_2 play a role of an adsorption promoting solvent.

In this paper, the separation of styrene–methyl methacrylate (St–MMA) copolymers was investigated with HPLC utilizing CO_2 as an adsorption promoting solvent. To our best knowledge, this is the first paper concerned with the compositional separation of high-molecular-mass copolymers using CO_2 as a component of mobile phase. The effects of type of desorption solvent, molecular mass of sample, and column temperature on the elution behavior were investigated.

2. Experimental

2.1. Samples

Statistical St–MMA copolymers were synthesized by a radical polymerization in bulk under a nitrogen atmosphere. Characteristics of these polymers are shown in Table 1. 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 ^{1}H nuclear magnetic resonance (NMR) spectroscopy. Effective number average (M_n) and weight-average (M_w) molecular masses of obtained copolymers were determined by GPC using two columns (30 cm×7.6 mm I.D.) packed with styrene–divinylbenzene copolymer beads [21] and tetrahydrofuran (THF) as an eluent at a flow-rate of 0.5 ml/min. GPC was carried out at an ambient temperature using a HPLC pump (Jasco, 880-PU), and a refractive index (RI) detector (Jasco, RI-2031 Plus). A 10-μl portion of the sample (10 mg/ml each in THF solution) was injected through a Rheodyne 7125 injector. The calibration curve for polystyrene standards (Shodex) was used to estimate the molecular mass. St–MMA 2 (Table 1) was fractionated by molecular mass using the same GPC apparatus. The

Table 1
Characteristics of styrene–methyl methacrylate copolymers

Code	$M_n^a/10^5$	$M_w^a/10^5$	M_w/M_n^a	St content[b] (mol%)
1	1.2	2.4	2.0	67
2	1.6	3.3	2.1	60
3	0.95	2.6	2.7	43
4	0.90	3.1	3.4	16

[a] Determined with GPC calibrated by polystyrene standards.
[b] Determined by ^{1}H-NMR.

column effluent for 10 20-μl portions of the sample (20 mg/ml) was collected and seven fractions were recovered.

2.2. HPLC

HPLC measurements using CO_2 as an component of an eluent were carried out using 25 cm×4.6 mm I.D. stainless steel column packed with non-bonded silica gel (SFC pak SIL-5, Jasco) and a Jasco Super 201 system equipped with a 880-81 type back-pressure regulator. Outlet pressure was regulated at 20 MPa in all experiments. The column effluent was monitored with a Jasco UV-970 detector equipped with a high-pressure proof cell at a wavelength of 254 nm. The column temperature was maintained at 40 to 70 °C by a column oven (Jasco, CO965). The system consists of two pumps for mobile phase, one is for the delivery of CO_2 and the other for the delivery of $CHCl_3$–EtOH or THF. The flow-rate of CO_2 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 $CHCl_3$ solution) was injected through a Rheodyne 7125 injector.

3. Results and discussion

3.1. Effect of desorption promoting solvent

Fig. 1 shows the chromatograms for the separation of St–MMA copolymers using THF (a) and $CHCl_3$–EtOH (96.5:3.5, v/v) (b). The small unknown peaks at around 3–4 min resulted from solvent dissolving polymers, which was confirmed by the solvent injection. In Fig. 1b 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. Until now, the origin of this peak is unclear. In the both cases, the polymers with the higher styrene content eluted earlier indicating that normal-phase type of elution was observed even in using CO_2 [7]. In the case of THF, elution time for each copolymer was shorter than that in the case of $CHCl_3$ (b) and the peaks in chromatogram severely overlapped each other. On
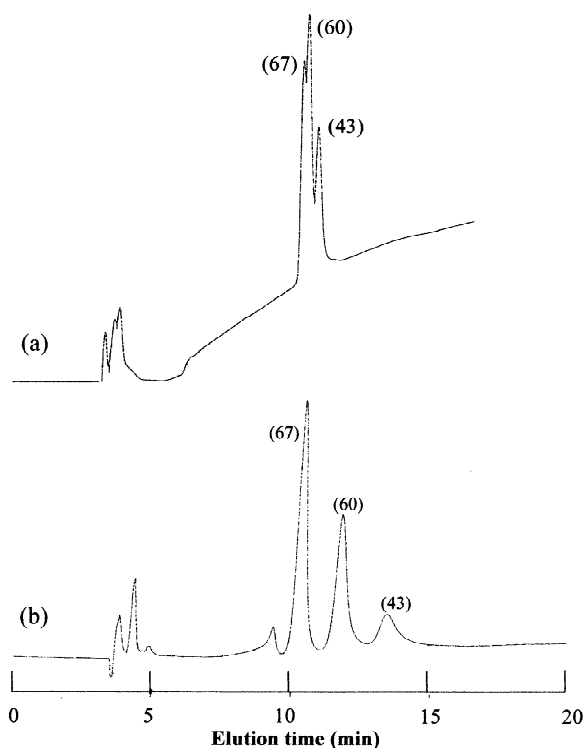

Fig. 1. Separation of poly(styrene–co-methyl methacrylate)s with solvent gradient method using (a) THF and (b) $CHCl_3$ (EtOH 3.5%) as desorption promoting solvent. Values in the parentheses indicate styrene content of the copolymer. Flow-rate; CO_2 (0.5 ml/min), THF or $CHCl_3$ (0.25 to 2.5 ml/min in 30 min), column temperature; 60 °C, back pressure; 20 MPa.

the other hand, in Fig. 1b elution was retarded and almost a baseline separation was achieved. THF molecules can form hydrogen bonding with silanol group in the stationary phase, and destroy the hydrogen bonding between the sample (especially the carbonyl group in MMA unit) and silica. On the other hand, the $CHCl_3$ molecule has a poorer ability to form hydrogen bonds. Therefore the hydrogen bonds between the sample and the stationary phase played an important role in the adsorption–desorption process.

Fig. 2 shows the relationships between the styrene content of copolymer and the content of desorption promoting solvent in the mobile phase. In the case of THF, the elution curve is shifted downside and the slope is moderate, indicating that THF easily desorbs
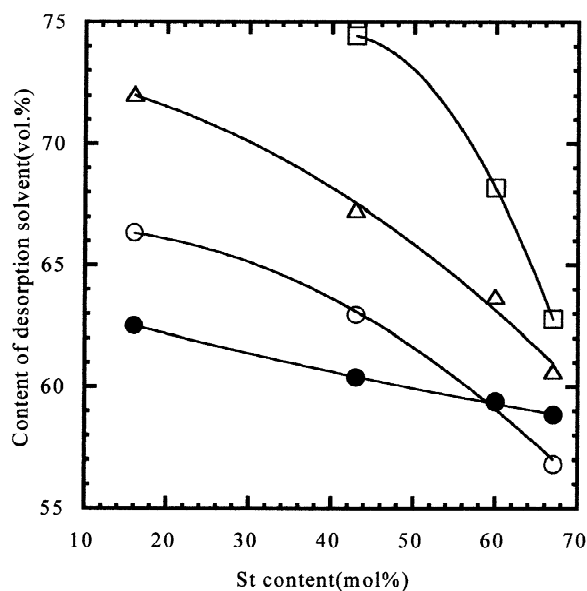
Fig. 2. Content of desorption promoting solvent of the eluent at peak maximum for THF (●), and CHCl$_3$ [EtOH content 2% (□), 3.5% (△), 5% (○)]. Chromatographic conditions as in Fig. 1.

Fig. 3. Chloroform content of the eluent at peak maximum (○) and cloud point (●). CHCl$_3$ contains 3.5% (v/v) of EtOH.

the adsorbed polymer, and lowers selectivity among samples. In the case of CHCl$_3$, the elution behavior was strongly dependent on EtOH content. As EtOH content increases, the desorption ability becomes stronger. As shown in Fig. 2, the elution time was shorter with the increase of EtOH. EtOH molecules also interact with the stationary phase, and break hydrogen bonds between polymer molecule and silica gel. These results also indicate the crucial role of hydrogen bonds in the compositional separation of copolymers. Although the line for 2% EtOH has the steepest slope, the samples with high MMA content adsorbed irreversibly. Therefore, the following experiments were carried out using CHCl$_3$ containing 3.5% EtOH as a standard desorption solvent.

In Fig. 3 CHCl$_3$ contents at the peak top are plotted against the styrene content in the copolymer together with each cloud point. Cloud points of samples in CO$_2$–CHCl$_3$ systems were determined as follows. A 10-μl portion of polymer solution was coated on the inner wall of an empty stainless steel column (20 mm×4.6 mm I.D.). This column was set in place of analytical column in the system described
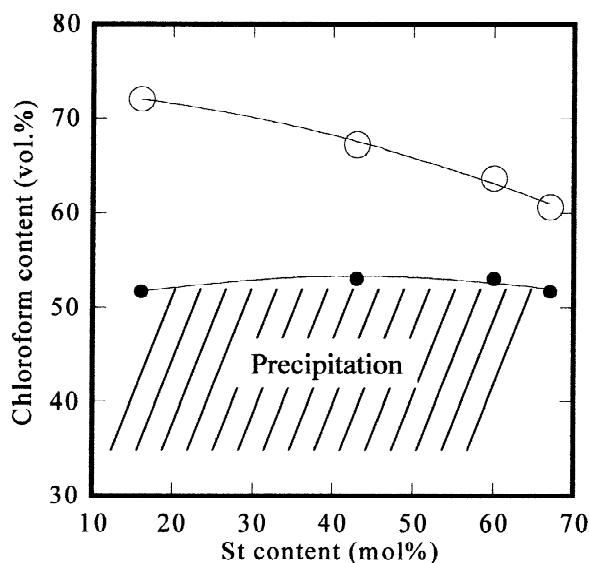
in the Experimental section. Solvent gradient was conducted in the same manner as in HPLC measurements and column effluent was monitored to obtain an elution profile. Under the initial conditions, examined copolymers were not soluble in the mobile phase. With the increase of CHCl$_3$ content, which is a good solvent for copolymers, the sample was dissolved and eluted out. The cloud point was defined as the onset point of the elution profile. As shown in Fig. 3, the sample did not elute from the HPLC column until CHCl$_3$ content increased about 10–20% higher than the cloud point for each sample. It is confirmed that the sample adsorbed to silica gel and the separation was governed by the adsorption–desorption mechanism.

In order to compare the property of CO$_2$ with hexane, which is non-polar and typical adsorption promoting solvent in conventional normal-phase HPLC, CHCl$_3$ content with 3.5% (v/v) EtOH at peak top was plotted against the styrene content together with that obtained utilizing hexane in Fig. 4. In the case of CO$_2$, the higher CHCl$_3$ content was necessary for sample to elute out, indicating that the sample was more strongly adsorbed on the stationary phase. The resolutions for neighboring pairs of
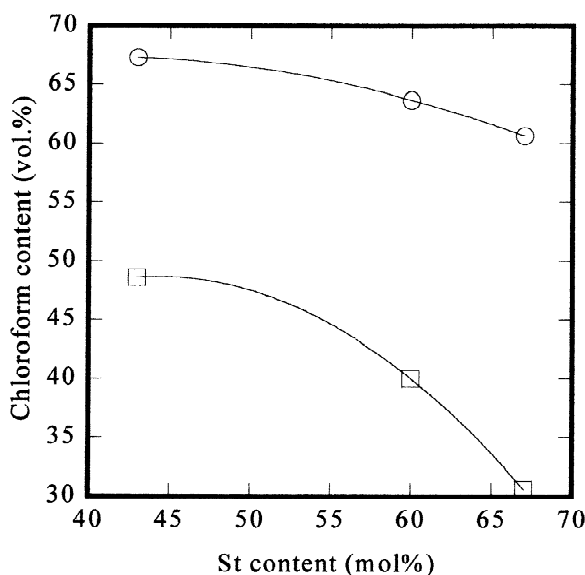
Fig. 4. Chloroform content of the eluent at peak maximum utilizing CO$_2$ (○) and hexane (□) as an adsorption promoting solvent. CHCl$_3$ contains 3.% (v/v) of EtOH. Experimental conditions using hexane are as follows: hexane–CHCl$_3$; 0.45/0.05→0/0.5 ml/min in 25 min.
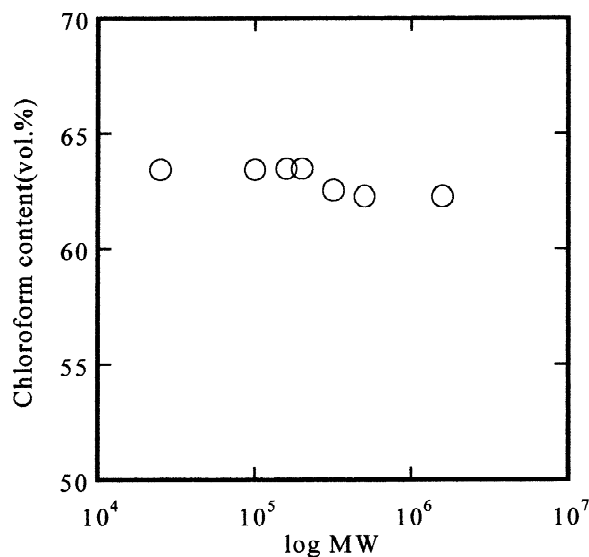

Fig. 5. Molecular mass effect on chloroform content of the eluent. Poly(styrene–co-methyl methacrylate) 2 (Table 1) was fractionated into seven fractions using preparative GPC. Chromatographic conditions as in Fig. 1.

samples were in the range of 2.1–2.4 and almost comparable to those obtained using hexane.

3.2. Molecular mass effect

It is well-known that the molecular mass as well as the chemical composition affects the separation of copolymers [7,22]. In Fig. 5 the CHCl$_3$ content in an eluent at the peak top is plotted against the molecular mass of the sample. The CHCl$_3$ content for each fraction is almost the same. This indicates that the elution behavior is practically independent of the molecular mass in the examined range of $2.5 \cdot 10^4$ to $1.6 \cdot 10^6$. In general if the attractive interaction exists between the sample and the stationary phase, the sample with higher molecular mass shows the higher adsorption energy consisting of additive contributions of the individual segments of repeating unit according to Martin's rule [23]. However it has been reported that the relationship between the enthalpy of adsorption, ΔH and molecular mass, M of sample is not completely linear and the slope ($=d\Delta H/dM$)

decreases with the increase of molecular mass [24–27]. In the case of the compositional separation by a conventional HPLC, it has been also reported that molecular mass effect is negligible for the samples having more than 10^5 of molecular mass [7].

More recently Brun and Alden reported the gradient separation of polymers at critical point of adsorption [28]. The 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 are sufficient for such a situation.

3.3. Temperature effect

It is reported that column temperature also influenced the elution of polymeric samples

[22,27,29,30]. To investigate the temperature effect in this system, HPLC measurements were carried out at various temperatures using $CHCl_3$ containing 3.5% EtOH. As shown in Fig. 6, elution curves are shifted upside with increasing column temperature indicating that adsorption is more favorable at higher temperature. The inverse temperature dependence has been reported by Chang and co-workers [27,29,30]. They have successfully applied the temperature gradient interaction chromatography to the molecular mass distribution analyses of various polymers. In their experiments under normal- [27] and reversed-phase [29,30] conditions, higher temperature has been necessary to elute out the sample with higher molecular mass. This indicates that desorption ability increases with the increase of temperature assuming that the sample with higher molecular mass shows the higher adsorption energy as mentioned above.

On the other hand, Mori and Uno reported the similar temperature dependence to ours in the separation of St–MMA copolymers by a chemical composition under normal-phase conditions [22]. In their system, adsorption of samples to the stationary phase (silica gel) was also 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 desorb, EtOH molecules adsorb at the site. EtOH 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 EtOH) is at least entropically unfavored. Therefore, it does not seem strange that temperature dependence observed here is different from that of Chang and co-worker's.

Fig. 7 indicates the temperature effect in the case of THF together with selected data in Fig. 6. When THF was used as a desorption promoting solvent, the same temperature dependence was also observed. However, the effect was not so remarkable compared with the case of $CHCl_3$. As mentioned above, THF molecules can form hydrogen bonding with silanol
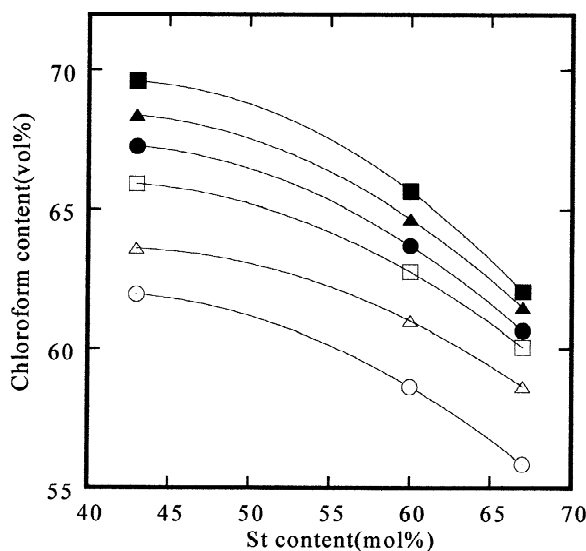

Fig. 6. Temperature effect on chloroform content of the eluent. Column temperature: 40 °C (○), 50 °C (△), 55 °C (□), 60 °C (●), 65 °C (▲), 70 °C (■). Other chromatographic conditions as in Fig. 1.
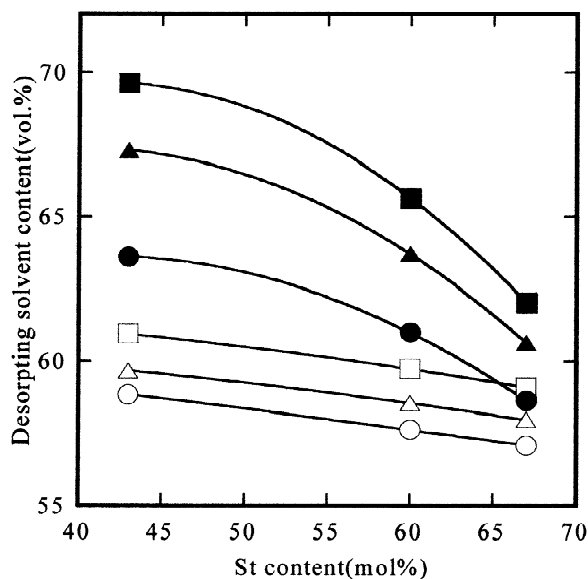

Fig. 7. Dependence of desorption promoting solvent on temperature effect. ■ □: 70 °C, ▲ △: 60 °C, ● ○: 50 °C, □ ▲ ●: $CHCl_3$ (3.5%, v/v, EtOH), □ △ ○: THF. Other chromatographic conditions as in Fig. 1.

group in the stationary phase. Therefore, the interaction of sample with the stationary phase via hydrogen bonds is not expected. These results indicate that hydrogen bonding interaction dominates the column temperature effect.

4. Conclusions

St–MMA copolymers were successfully separated depending on the chemical composition by solvent gradient method using CO_2 as the one of the components of mobile phase. Normal-phase type separation was observed. The utilization of THF or the increase of EtOH content in $CHCl_3$ decrease the retention times indicating that the hydrogen bonding plays an important role in the adsorption–desorption process of copolymers. Molecular mass dependence was negligible in the molecular range we examined. As column temperature increased, the retention time increased.

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