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Temperature Gradient Interaction Chromatography and MALDI-TOF Mass Spectrometry Analysis of Stereoregular Poly(ethyl methacrylate)s

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Temperature gradient interaction chromatography (TGIC) was applied for the separation of stereoregular poly(ethyl methacrylate) (PEMA) according to the tacticity. The three PEMA samples with differing tacticity (rr triad content: 0, 53, and 91%) prepared by anionic polymerization were used. C18 bonded silica and a mixture of CH_2Cl_2 and CH_3CN (30/70, v/v) were used as stationary and mobile phase, respectively. TGIC was able to separate the PEMA samples, showing the increasing retention in the order of decreasing rr triad contents; however TGIC elution peaks of the three PEMAs were not fully resolved but, rather, were partially overlapped. To isolate the tacticity effect from the molecular weight effect on the TGIC retention, the PEMA samples were fractionated by TGIC, and the accurate molecular weight of the fractions was determined by MALDI-TOF mass spectrometry. The fractions showed a much narrower molecular weight distribution than the mother PEMAs. The TGIC fractions of similar molecular weight but with different tacticity were fully resolved by TGIC, but mother PEMAs were not. These results indicate that the retention in TGIC is affected by both tacticity and molecular weight.

Synthetic polymers, even a homopolymer, can have various heterogeneities in molecular weight, chain architecture, and microstructures, such as tacticity and isomeric structure, which influence their physical properties a great deal. These heterogeneities usually occur together, and it is difficult to isolate the effect of single origin, for example, tacticity, which is the major concern of this study. Therefore, it is often assumed that the microstructure is developed randomly, and the average ads composition is used as the parameter to represent the tacticity. However, there are many examples of polymerization in which microstructures are developed by non-Bernoullian or non-Markovian process.[1] One way to probe the tacticity heterogeneity is to fractionate the polymer in question according to the tacticity distribution.

In recent years, several polymerization techniques have been developed to produce highly stereoregular polymers, and their chromatographic separation method has drawn attention. Polymers of different tacticity have slightly different hydrodynamic volumes, but the difference is not large enough for size exclusion chromatography (SEC) to distinguish them effectively. There have been a number of reports on the separation of stereoregular polymers by various interaction chromatography (IC) techniques. Inagaki and co-workers were the first to report the separation of poly(methyl methacrylate) (PMMA) according to tacticity by thin-layer chromatography.[2,3] When one considers small molecules, the polymers with different tacticity are diastereomers. Therefore, it is not surprising that the chromatography technique can separate them. For example, Lewis et al. and Mourey et al. reported that the separation of individual stereoisomers was possible, in addition to the separation according to the number of repeating units by the solvent gradient HPLC.[4,5] However, such diastereomeric separation becomes more and more difficult as the molecular weight increases, since the chromatographic retention is determined by both tacticity and molecular weight, and all of the synthetic polymers have finite distributions in both. Therefore, the chromatographic separation of polymers is restricted to the polymers with well-defined stereoregular structures.[6,7] The relative contribution of the tacticity and molecular weight to the IC retention has not yet been well-established. Recently, liquid chromatography at the critical condition (LCCC) was applied to separate stereoregular PMMA[8,9] and poly(ethyl

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(1) Cheng, H. N.; Kasehagen, L. J. *Macromolecules* **1993**, *26*, 4774−4782.
(2) Inagaki, H.; Miyamoto, T.; Kamiyama, F. *J. Polym. Sci., Part B: Polym, Phys.* **1969**, *7*, 329−333.
(3) Miyamoto, T.; Inagaki, H. *Polym. J.* **1970**, *1*, 46−54.
(4) Lewis, J. J.; Rogers, L. B.; Pauls, R. E. *J. Chromatogr.* **1983**, *264*, 339−356.
(5) Mourey, T. H.; Smith, G. A.; Snyder, L. R. *Anal. Chem.* **1984**, *56*, 1773−1777.
(6) Sato, H.; Sasaki, M.; Ogino, K. *Polym. J.* **1989**, *21*, 965−969.
(7) Hatada, K.; Nishiura, T.; Kitayama, T.; Ute, K. *Macromol. Symp.* **1997**, *118*, 135−141.
(8) Berek, D.; Janco, M.; Kitayama, T.; Hatada, K. *Polym. Bull.* **1994**, *32*, 629−635.
(9) Berek, D.; Janco, M.; Hatada, K.; Kitayama, T.; Fujimoto, N. *Polym. J.* **1997**, *29*, 1029−1033.

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methacrylate) (PEMA).[10,11] In particular, Janco et al. demonstrated by coupling SEC and LCCC that it was possible to discriminate the highly stereoregular PEMAs according to their molecular weight and tacticity.[10] SEC can separate the polymers in terms of molecular weight first, and subsequently, LCCC separates them in terms of tacticity.

In this study, we employed temperature gradient interaction chromatography (TGIC) on the separation of stereoregular PEMAs. Unlike SEC, IC utilizes mainly the interaction between the solutes and the stationary phase, and in TGIC, the interaction strength between the solutes and the stationary phase is controlled by varying the column temperature.[12] TGIC has shown much higher resolution than SEC in the polymer separation according to the molecular weight.[13,14] If an interaction free energy difference exists among the different tactic polymers, TGIC is expected to show a high sensitivity in tacticity separation, also due to its band broadening effect that is smaller than SEC. In addition, another powerful tool for the molecular weight characterization technique, matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOFMS), was employed to measure the molecular weight distribution apart from the tacticity effect. The coupling of various liquid chromatography techniques and MALDI-TOFMS is also well-established for the analysis of complex polymers.[15−20]

EXPERIMENTAL SECTION

Materials. The PEMAs with different tacticity and narrow molar mass distribution were prepared by stereospecific living polymerizations at low temperatures.[21,22] The highly syndiotactic PEMAs (s-PEMA) were prepared by polymerizing ethyl methacrylate (EMA) in toluene at −78 °C with *t*-BuLi/*n*-Bu_3Al. The heterotactic PEMA (h-PEMA) was obtained by polymerization of EMA in toluene at −55 °C with *t*-BuLi/methylaluminum bis(2,6-di-*tert*-butylphenoxide) as the initiator. The highly isotactic PEMA (i-PEMA) sample was obtained by the polymerization of EMA in chloroform at −60 °C with *t*-BuMgBr/Me_3Al. The tacticity was determined from carbonyl signals in 125 MHz ^{13}C NMR spectra measured in $CDCl_3$ at 55 °C. The molecular weights of the PEMAs were characterized by SEC with two mixed-bed columns (Polymer Lab, Mixed C, 300 × 8 mm i.d.) as well as MALDI-TOFMS. The SEC chromatograms were recorded by a multiangle light scattering detector (MALLS, Wyatt, mini-DAWN) and a refractive index detector (Wyatt, Opti-Lab) using tetrahydrofuran (THF, Duksan, HPLC grade) as a mobile phase. Chromatograms were collected and processed by Astra software. The characteristics of the PEMA samples are listed in Table 1.

Table 1. Characteristics of Stereoregular Poly(ethyl methacrylates)

sample code	M_w (g/mol)/ M_w/M_n[a]	M_w (g/mol)[b]	tacticity (%)		
			mm	mr	rr
s	12 600/1.02	13 300	0	9	91
s1	6300/1.05		0	9	91
s2	51 200/1.09		0	7	93
h	13 800/1.01	13 400	2	45	53
i	9800/1.10	10 600	96	4	0

[a] Determined by SEC-MALLS. [b] Determined by MALDI-TOFMS.

Chromatography. The TGIC experiments were carried out on a typical isocratic HPLC system equipped with a C18 bonded silica column (LUNA, 100 Å pore, 250 × 4.6 mm, 5-μm particle size). The mobile phase was a mixture of CH_2Cl_2 and CH_3CN (Duksan, HPLC grade, 30/70 in volume), and the flow rate was 0.45 mL/min. Each PEMA sample was dissolved in the mobile phase (2.0 mg/mL), and injected through a Rheodyne 7125 injector equipped with a 100-μL sample loop. The column temperature was varied in a preprogrammed manner by circulating a fluid through a homemade column jacket from a bath/circulator (Neslab, RTE-111). The chromatograms of PEMA were recorded using a UV absorption detector (TSP, UV100) operating at a wavelength of 235 nm.

MALDI-TOFMS. In the MALDI-TOFMS experiments, a Bruker REFLEX III mass spectrometer was used. The spectrometer was equipped with a nitrogen laser (λ = 337 nm), a pulsed ion extraction, and a reflector. This instrument operated at an accelerating potential of 20 kV in reflector mode. Polymer solutions were prepared in HPLC-grade THF. The matrix, *trans*-3-indoleacrylic acid (IAA, 99%, Aldrich) was dissolved in THF at a concentration of 10 mg/mL. A 5-μL portion of the polymer solution was mixed with 40-μL of the matrix solution and 1 μL of sodium iodide solution (1 mg/mL in THF), respectively. A 0.5-μL portion of the final solution was deposited onto a sample target plate and allowed to dry in air at room temperature.

RESULTS AND DISCUSSION

In the TGIC experiment, the right choice of an eluent system is important for successful separation. In the case of PEMA samples, we found that the mixture of CH_2Cl_2 and CH_3CN is adequate as an eluent. Figure 1 shows the TGIC chromatograms of the three PEMA samples with differing tacticity recorded by a UV detector. The temperature of the column was raised in three steps from 30 to 50 °C, as shown in the plot. The TGIC chromatograms show that i-PEMA is most strongly retained and s-PEMA is least retained, but the three different tactic polymers are not fully resolved. The finite distributions of the synthetic polymers in molecular weight and in tacticity lead to the finite peak width, which would result in the partial peak overlap. The small peaks appearing at low t_R (5−10 min) seem to be due to the impurity in the polymers, which is present in the largest

(10) Janco, M.; Hirano, T.; Kitayama, T.; Hatada, K.; Berek, D. *Macromolecules* **2000**, *33*, 1710−1715.
(11) Kitayama, T.; Janco, M.; Ute, K.; Niimi, R.; Hatada, K.; Berek, D. *Anal. Chem*. **2000**, *72*, 1518−1522.
(12) Chang, T.; Lee, H. C.; Lee, W.; Park, S.; Ko, C. H. *Macromol. Chem. Phys.* **1999**, *200*, 2188−2204.
(13) Lee, W.; Lee, H. C.; Chang, T.; Kim, S. B. *Macromolecules* **1998**, *31*, 344−348.
(14) Lee, W.; Lee, H.; Cha, J.; Chang, T.; Hanley, K. J.; Lodge, T. P. *Macromolecules* **2000**, *33*, 5111−5115.
(15) Montaudo, M. S.; Puglisi, C.; Samperil, F.; Montaudo, G. *Rapid Commun. Mass Spectrom*. **1998**, *12*, 519−528.
(16) Montaudo, M. S.; Montaudo, G. *Macromolecules* **1999**, *32*, 7015−7022.
(17) Pasch, H. *Adv. Polym. Sci*. **2000**, *150*, 1−66.
(18) Pasch, H.; Ghahary, R. *Macromol. Symp.* **2000**, *152*, 267−278.
(19) Lee, H.; Lee, W.; Chang, T.; Choi, S.; Lee, D.; Ji, H.; Nonidez, W. K.; Mays, J. W. *Macromolecules* **1999**, *32*, 4143−4146.
(20) Lee, H.; Chang, T.; Lee, D.; Shim, M. S.; Ji, H.; Nonidez, W. K.; Mays, J. W. *Anal. Chem*. **2001**, *73*, 1726−1732.
(21) Kitayama, T.; Shinozaki, T.; Masuda, E.; Yamamoto, M.; Hatada, K. *Polym. Bull.* **1988**, *20*, 505−510.
(22) Kitayama, T.; Zhang, Y.; Hatada, K. *Polym. J.* **1994**, *26*, 868−872.

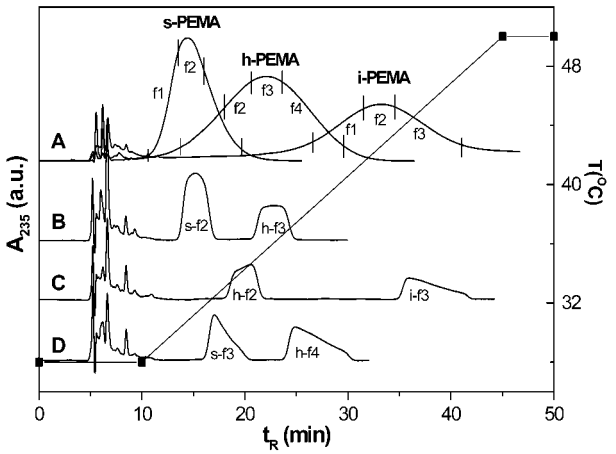

Figure 1. TGIC chromatograms of three PEMA samples with differing tacticities (A) (s-, h-, and i-PEMA: rr triad content = 0, 53, and 91%), and TGIC chromatograms of fractions of similar molecular weight but of different tacticity (B−D). Column, one reversed-phase column (LUNA C18, 100 Å, 250 × 4.6 mm); eluent, CH_2Cl_2/CH_3CN (30/70, v/v). Temperature program is also shown in the plot.

amount in i-PEMA. The strongly UV-adsorbing impurities in the i-PEMA may come from the bromine derivatives of the initiator, *t*-BuMgBr.

To isolate the effects on the retention from the molecular weight and tacticity, the three PEMA samples were fractionated by TGIC, as shown in Figure 1(A). s-PEMA was fractionated into 3 fractions (f1, 10.50−13.5; f2, 13.5−16; f3, 16−19.5 min); h-PEMA, into 4 fractions (f1, 13.5−18; f2, 18−20.5; f3, 20.5−23.5; f4, 23.5−29.5 min); and i-PEMA, into 3 fractions (f1, 26.5−31.5; f2, 31.5−34.5; f3, 34.5−41 min). The collected fractions were subjected to TGIC analysis again, as displayed in the three chromatograms at the bottom (B−D). The peak shapes of the fractions shown in the three chromatograms are very much like the portion of the mother chromatogram in which each fraction was taken, which clearly shows that each of the different tactic polymers was fractionated sharply. This is indicative of a very small band-broadening of TGIC, which is not possibe to realize with SEC.[14] We also notice that there exist even more intense multiple peaks at low t_R. These peaks look alike for all three chromatograms of the fractions but look different from the one observed in the top chromatogram of the mother polymers. We confirmed from the blank experiments that they come from the impurity (or additive) in the eluent that was concentrated during the fraction collection, drying, and redissolution.

For the PEMAs and their fractions, MALDI-TOFMS spectra were taken to determine the accurate molecular weight, as shown in Figures 2, 3 and 4. From the mass spectra of the tactic PEMAs, weight-average molecular weights were determined and are listed in Table 1. They are compared favorably with the molecular weight determined by SEC-MALLS measurements. The s-PEMA shows the narrowest distribution, and h- and i-PEMA show relatively long tails toward the low molecular weight. Furthermore, i-PEMA shows an envelope of the subsidiary peaks, of which structure will be discussed later. The average molecular weights of s- and h-PEMA are similar, although the TGIC retention time was significantly different, as shown in Figure 1. In addition, i-PEMA has the lowest average molecular weight, but it is retained most

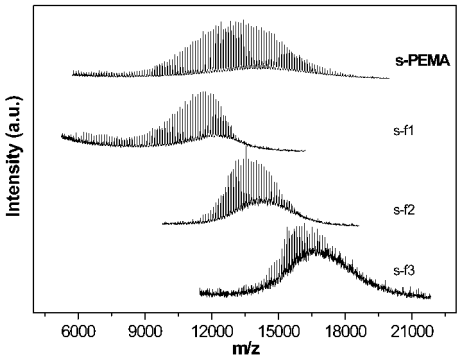

Figure 2. MALDI-TOFMS spectra of the mother s-PEMA and the three TGIC fractions taken, as shown in Figure 1A.

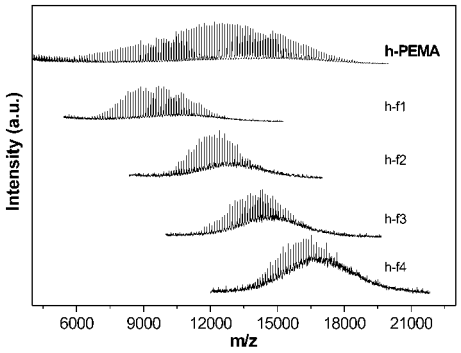

Figure 3. MALDI-TOFMS spectra of the mother h-PEMA and the four TGIC fractions taken, as shown in the Figure 1A.

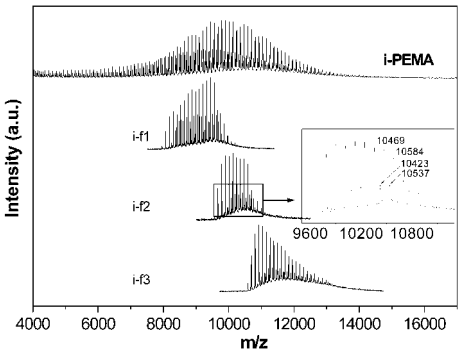

Figure 4. MALDI-TOFMS spectra of the mother i-PEMA and the three TGIC fractions taken, as shown in the Figure 1A. In the insert, a magnified spectrum of the i-f2 fraction is shown.

strongly. These observations clearly indicate that the IC retention is strong in the sequence of i-, h-, and s-PEMA.

To compare the retention due to tacticity more unambiguously, we chose three pairs of the fractions having similar molecular weight but different tacticity: s-f2 and h-f3, s-f3 and h-f4, and h-f2 and i-f3. Comparing the chromatograms obtained from these pairs, as shown in Figure 1 (B−D), we can clearly see that TGIC can separate the polymers completely in terms of tacticity only if the molecular weight distribution is narrow enough. Another interesting feature is the shape of the MALDI-TOFMS spectra of the PEMA fractions. Although the chromatographic peak shape of the fractions in Figure 1 indicates very sharp fractionations, MALDI-TOFMS spectra of the fractions of s- and h-PEMA in Figure 2 lack the feature. The overlap of the envelope is more serious in h-PEMA than in s-PEMA. On the other hand, the mass spectra of the i-PEMA fractions show shapes quite similar to the

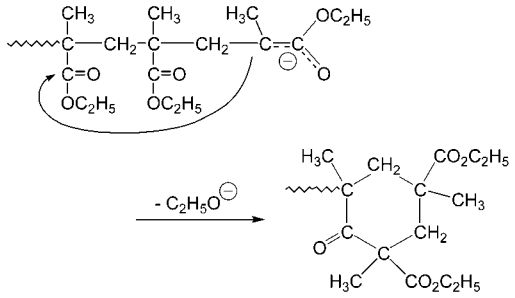

Figure 5. Self-termination reaction scheme of i-PEMA anions by back-biting cyclization.

chromatographic peak shape. These observations are another indication of the chromatographic band dispersion due to both molecular weight and tacticity. The i-PEMA is highly pure in the stereoregularity, and the TGIC retention was controlled almost exclusively by the molecular weight, as determined by the mass spectra. On the other hand, for s- and h-PEMA, the molecular weight distribution found in the mass spectra is relatively broad, since the dispersion is due to the combined effect of molecular weight and tacticity.

Another feature to notice from the MALDI-TOFMS analysis is the envelope of subsidiary peaks found in the i-PEMA. The expanded MALDI-TOF mass spectrum is shown as an inset in Figure 4. The molecular weight of the major envelope is consistent with the *tert*-butyl-initiated PEMA of degree of polymerization, *n*, terminated with a hydrogen atom. The major envelope is represented by $114.14\times n$ (*n* EMA units) + 58.12 (ends groups: *tert*-butyl initiator and one hydrogen) + 22.99 (Na^+ ion). For example, with $n = 90$, the molar mass of the PEMA terminated by hydrogen was found to be 10 469, as compared to the calculated mass of 10 467.9. The subsidiary peaks show molecular weights of 46 that are smaller than the major peaks. We think that the peaks result from a different chain end, a cyclic β-ketoester structure, as shown in Figure 5. The minor envelope is represented by $114.14\times(n - 3)$ + 57.12 (*tert*-butyl initiator) + 297.36 (cyclic end group) + 22.99 (Na^+ ion). For example, with $n = 90$, the molar mass of the cyclic structure was found to be 10 423, as compared to the calculated mass of 10 421.8.

The i-PEMA living anions are considered to be self-terminated by cyclization reaction much more easily than other tactic PEMA living anions. In the isotactic-specific living polymerization of methyl methacrylate by *t*-BuMgBr, the self-termination by cyclization occurs mostly after complete consumption of monomer in the polymerization system. Although the i-PMMA anion prepared at −78 °C in toluene is perfectly living, even after the complete consumption of monomer, ∼90% of the polymer chain underwent cyclization when the living i-PMMA anions were kept at 0 °C for 24 h.[23] It may be related to the fact that the i-PMMA has a tendency to have a helical conformation,[24] which would facilitate the back-biting reaction. The present i-PEMA was prepared at −60 °C in chloroform. The cyclization reaction would take place mainly after the near complete consumption of the monomers, but some might also take place earlier, which made the molecular weight distribution somewhat broader. This would explain the intensity inversion of the major and minor peaks at the low-molecular-weight region of the mother i-PEMA observed in Figure 4. It indicates that a minor part (∼10−20%) of the polymer chains are terminated earlier by the cyclization reactions, but the major surviving polymer anions keep on adding a few more monomers to complete the polymerization.

This example shows a distinct advantage of MALDI-TOFMS in the analysis of synthetic polymers. Such a high-precision analysis of the molecular weight enables good structural identification, which was not possible with any other analytical technique. One more thing to mention is that the TGIC retentions of the major and minor peaks are slightly different. If we look at the edge of the MALDI-TOF mass spectrum of the fractions of i-PEMA, we can find the dispersion at the cutting edges mainly occurred by the uneven distribution of two peak envelopes. For example, if we look at the magnified spectrum of i-f2 in Figure 4, the minor peaks extend further toward the higher-molecular-weight side but depleted at the lower-molecular-weight end. This indicates that the polymer chains with cyclic ends are retained less and a higher-molecular-weight polymer with a cyclic end elutes together with a lower-molecular-weight i-PEMA terminated normally.

In summary, TGIC showed high sensitivity on both molecular weight and tacticity distributions of PEMA. Because of the combined effect of molecular weight and tacticity on the TGIC retention, TGIC alone cannot characterize both distributions. The combination of TGIC fractionation and MALDI-TOFMS analysis was found useful to elucidate the relative contribution of tacticity and molecular weight on the IC retention of the stereoregular PEMAs. In addition, the coupling with SEC, which is less susceptible to the change in tacticity, can be considered for the characterization of both molecular weight and tacticity distribution.

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(23) Hatada, K.; Ute, K.; Tanaka, K.; Okamoto, Y.; Kitayama, T. *Polym. J.* **1986**, *18*, 1037−1047.

(24) Sawatari, N.; Konishi, T.; Yoshizaki, T.; Yamakawa, H. *Macromolecules* **1995**, *28*, 1089−1094.