Analysis of Poly(ethyl methacrylate)s by On-Line Hyphenation of Liquid Chromatography at the Critical Adsorption Point and Nuclear Magnetic Resonance Spectroscopy

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Liquid chromatography at the critical adsorption point (LC CAP) with on-line NMR detection (on-line LC CAP NMR) was utilized for analysis of tacticity distribution of stereoregular poly(ethyl methacrylate)s (PEMAs). The separation of a model PEMA sample composed of four constituents with similar molar masses ($M_w = (14 - 16) \times 10^2$ g mol$^{-1}$) differing in their tacticity (rr triad content = 0, 33, 68, and 89%) was achieved by LC CAP with a mixed eluent composed of acetone, acetone-$d_6$, and cyclohexane. The tacticity composition within each peak eluted from the LC CAP column was directly determined by a 750-MHz $^1$H NMR spectrometer that was used as a real-time detector in the continuous-flow mode. Tacticity distribution in a particular PEMA sample with $mm/rr/rr = 2/45/53$ and narrow molar mass distribution of $M_w/M_n = 1.05$ has been revealed by the LC CAP NMR technique.

Recently, we showed that liquid chromatography at the critical adsorption point (LC CAP) is a powerful technique for separation of stereoregular polymers;¹² mixtures of isotactic and syndiotactic poly(methyl methacrylate)¹⁰ and isotactic and syndiotactic poly(ethyl methacrylate)¹¹ (PEMAs) were separated into single constituents. More recently, a coupling of size exclusion chromatography (SEC) and LC CAP was applied to simultaneous discrimination of stereoregular PEMAs according to their molar mass and tacticity in its entire range using a tetrahydrofuran (THF)/cyclohexane mixture as an eluent.² By an on-line SEC-LC CAP technique, the macromolecules were first discriminated solely according to their molar masses in the SEC column. Narrow SEC fractions were successively forwarded into the LC CAP column where their separation according to tacticity was achieved. In previous work,¹³ we employed an evaporative light-scattering detector for the LC CAP analyses. To give conclusive confirmation of tacticity separation by LC CAP as well as to directly determine the tacticity composition of macromolecules within each peak eluted from LC CAP column, the hyphenation of LC CAP NMR has to be performed.

Recent progress in the sensitivity and resolution of NMR spectrometers together with introduction of commercially available LC NMR probes and development of methods for effective suppression of eluent signals⁵ opens the possibilities of use of NMR spectrometers as real-time detectors in different modes of liquid chromatography. Thus, the NMR spectrometer has been used as a detector in SEC for direct determination of accurate molar mass and molar mass distribution of macromolecules⁶⁻⁸ as well as for the study of molar mass dependence of tacticity⁹ and copolymer composition¹⁰¹¹ in polymer samples.

In the present work, a 750-MHz $^1$H NMR spectrometer was employed as a detector in LC CAP for direct and continuous determination of tacticity to prove the tacticity separation by this technique. Acetone/acetone-$d_6$/cyclohexane mixtures were used as eluents, which are proper solvents for $^1$H NMR measurements because the mixtures show only two singlet peaks at the spectral regions apart from the analyte (PEMA) resonances. Both eluent resonances were able to be eliminated simultaneously by applying

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the WET (water suppression enhanced through T1 effects) technique. Acetone-d$_6$ was added to the eluent for internal lock and shimming of the magnetic field. The reliability of LC CAP NMR measurements was demonstrated by the tacticity determination of a model PEMA mixture composed of four constituents with different tacticities. LC CAP NMR analysis of a particular PEMA sample has revealed the tacticity distribution in the sample, which may be difficult to detect by other conventional analytical methods.

**EXPERIMENTAL SECTION**

**Conditions for LC CAP.** LC CAP measurements were carried out on a chromatograph composed of an eluent container protected against moisture, a Jasco model PU-980 pump (Tokyo, Japan), a Rheodyne injector provided with a 100 µL sample loop, and an analytical column placed into a Jasco model CO-965 column oven operated at 35 °C. The column effluent was monitored by using a model EMD-960 evaporative light-scattering detector (Polymer Laboratories, Church Stretton, U.K.) operated by using compressed air as carrier gas from a Hitachi model 0.2LP-7S compressor (Tokyo, Japan). The carrier gas outlet pressure and flow rate were set at 0.2 MPa and 5 L min$^{-1}$, respectively. The temperature of the detector evaporative chamber was set at 65 °C. The detector signal was collected on-line by using a Shimadzu model C-R7A data acquisition system (Kyoto, Japan).

The LC CAP separations were performed using an analytical column (8 mm i.d. × 250 mm) packed with silica gel bonded with aminopropyl groups (Develosil SG-NH$_2$, pore size 12 nm, particle size 5 µm) purchased from Nomura Chemical (Seto, Japan).

Analytical grade acetone and cyclohexane were obtained from Nacalai Tesque (Kyoto, Japan) and distilled just before use. Acetone-d$_6$ was purchased from Wako Ltd. (Osaka, Japan) and used as received. Acetone/acetone-d$_6$/cyclohexane mixtures, used as eluents and sample solvents, were prepared by weighing single components and therefore the mixture compositions are expressed in w/w ratio in all cases.

**Conditions for SEC.** SEC measurements were carried out on a 7.5 mm i.d. × 300 mm column packed with nonpolar poly(styrene-co-divinylbenzene) gel (PLgel, M ixed-D, particle size 5 µm), maximum porosity 4 × 10$^4$ purchased from Polymer Laboratories. The other conditions follow those of LC CAP measurements.

**LC CAP NMR and SEC NMR Measurements.** A Varian Unity-INOVA 750 spectrometer was employed as the real-time LC CAP and SEC detector. An $^1$H($^{15}$N–$^{31}$P) inverse-geometry LC NMR probe with pulsed field gradient coils was used for observation. The flow cell in the probe had a detection volume of ~60 µL. The column outlet was connected to the probe by using a PEEK capillary (0.25 mm i.d. × 2.7 m). The eluent flow rate was set at 0.2 mL min$^{-1}$ to achieve sufficient nuclear polarization of analyte during its residence in the NMR flow cell. A total of 120 spectra were collected every 18 s in an elution period from 25 to 61 min. Each spectrum was obtained by the accumulation of eight scans using 90° pulses (8.9 µs) with a pulse repetition time of 2.25 s and stored into 65 536 data points covering a spectral width of 16 000 Hz. Digital signal processing with an oversampling factor of 25 was applied. The peaks at 1.43 and 2.02 ppm from cyclohexane and acetone, respectively, were suppressed by WET with $^{13}$C decoupling. Figure 1 shows an example of an $^1$H NMR spectrum of a model PEMA sample observed in continuous-flow NMR with the WET method. Prior to Fourier transformation, an exponential apodization function was applied to the free induction decays corresponding to a line broadening of 2.0 Hz.

The tacticity composition of PEMA was determined from the relative intensity of α-CH$_3$ resonances due to mm, mr, and rr triads. The intensities of mm, mr, and rr triad resonances in every spectrum of the LC CAP NMR data were measured by integrating the spectral regions of 1.18–1.28, 0.98–1.07, and 0.82–0.92 ppm, respectively. The ester CH$_3$ resonance at 1.23 ppm overlapped with the α-CH$_3$ resonance due to mm triad, and thus the intensity of mm triad resonance was evaluated by subtracting 1.47 times the intensity of the ester OCH$_2$ resonance at 3.92–4.06 ppm from the intensity of the spectral region from 1.18 to 1.28 ppm (see Figure 1). Although the theoretical relative intensity of ester CH$_3$ and OCH$_2$ resonances is 1.5:1.0, it was found to be 1.47:1.00 under the acquisition conditions due to the difference in their relaxation times employed in the above-mentioned NMR measurements.

**Materials.** The PEMA samples with different tacticity and narrow molar mass distribution (Table 1) were prepared by stereospecific living polymerizations at low temperatures. Four samples of highly syndiotactic PEMA (rr triad content 89–93%) with average molar mass $M_w$ ranging from $6 \times 10^3$ to $45 \times 10^3$ g mol$^{-1}$ were prepared by polymerizing EMA in toluene at −78 °C with t-BuLi/n-Bu$_4$A$^{13}$ and used for identification of the critical adsorption point. One predominantly heterotactic PEMA sample (P1, mr = 63%) and two predominantly syndiotactic PEMA samples (P2 and P3 with rr = 53 and 68%) were obtained by the polymerization of EMA in toluene at −60, −55, and −50 °C, respectively, with

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RESULTS AND DISCUSSION

We have shown that on-line SEC NMR hyphenation^9 is a powerful technique that gives information on both molar mass and tacticity distributions of analyzed polymer when its tacticity varies together with molar mass. When macromolecules have similar or the same molar mass and differ in their tacticity, however, the method will provide only average tacticity values. Figure 2 shows the results of the SEC NMR analysis of a model PEMA mixture composed of four constituents (S3, P1, P3, and I in Table 1) with similar molar mass (MW = (14.3–16.3) × 10^5 g mol^-1) and different tacticity (rr triad content = 0, 33, 68, and 89%) by using a PLgel column in an acetone/acetone-d_6/cyclohexane mixture (34/5/61 w/w) monitoring the ester OCH_2 resonance from 3.92 to 4.06 ppm. Tacticity compositions, determined from α-CH_2 proton resonance in the range of 0.82–0.92, 0.98–1.07, and 1.18–1.28 ppm due to rr (●), mr (+), and mm (○) triads, respectively, are also shown.

We have already reported^3 that elution behavior of stereoregular PEMAs is strongly influenced by tacticity when polar column packings such as silica gel modified with aminopropyl groups were used with eluent mixtures of THF and cyclohexane.

In this study, we have observed similar elution behavior of stereoregular PEMAs on silica gel bonded with aminopropyl groups by using acetone/acetone-d_6/cyclohexane mixtures as an eluent. Figure 3 illustrates calibration curves of syndiotactic PEMAs at four different eluent compositions. With an increase of cyclohexane content, the calibration curves shifted to higher retention volumes. The critical adsorption point for highly syndiotactic PEMAs was identified in an acetone/acetone-d_6/cyclohexane mixture (34/5/61 w/w) at 35 °C, where molar mass-independent elution of highly syndiotactic PEMA was achieved as shown in Figure 3. Under the same conditions, PEMA samples

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### Table 1. Characteristics of Stereoregular Poly(ethyl methacrylate)

<table>
<thead>
<tr>
<th>sample</th>
<th>initiator</th>
<th>temp/°C</th>
<th>M_w × 10^{-3}</th>
<th>M_w/M_n</th>
<th>tacticity/%</th>
<th>mm</th>
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<td>−78</td>
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<td>1.05</td>
<td>0</td>
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</tr>
<tr>
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<td>1.06</td>
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<tr>
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<td>1.06</td>
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<td>33</td>
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<td>1</td>
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<td>68</td>
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</table>

^a^ A, prepared with t-BuLi/ n-Bu_3Al (1/3) in toluene; B, prepared with t-BuMgBr/M eAl (1/1) in chloroform; C, prepared with t-BuLi/bis(2,6-di-tert-butylphenoxy)methylaluminum (1/5) in toluene. ^b^ Determined from carbonyl signals in 13C NMR spectra measured in CDCl_3 at 55 °C at 125 MHz.

dBuLi/ bis(2,6-di-tert-butylphenoxy)methylaluminum as the initiator. The highly isotactic PEMA sample (I) with MW = 16.3 × 10^5 g mol^-1 was obtained by the polymerization of EMA in chloroform at −60 °C with t-BuMgBr/M eAl. Both a single P2 sample (rr = 53%) and a model PEMA mixture prepared by mixing four constituents (S3, P1, P3, and I in Table 1) differing in tacticity (rr triad content: 0, 33, 68, and 89%) were used for LC CAP NMR investigations. A 100μL aliquot of sample solution with a concentration of ~8 mg mL^-1 for each constituent was injected into the LC CAP column.

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**Figure 2.** 1H NMR-detected SEC trace of a model PEMA sample composed of four constituents (S3, P1, P3, and I in Table 1) with similar molar mass (MW = (14.3–16.3) × 10^5 g mol^-1) and different tacticity (rr triad content = 0, 33, 68, 89%) by using a PLgel column in an acetone/acetone-d_6/cyclohexane mixture (34/5/61 w/w) monitoring the ester OCH_2 resonance from 3.92 to 4.06 ppm. Tacticity compositions, determined from α-CH_2 proton resonance in the range of 0.82–0.92, 0.98–1.07, and 1.18–1.28 ppm due to rr (●), mr (+), and mm (○) triads, respectively, are also shown.

**Figure 3.** Calibration curves of highly syndiotactic PEMAs on a Develosil-NH_2 column (pore size 12 nm, particle size 5 μm, 8 × 250 mm) in acetone and in acetone/acetone-d_6/cyclohexane mixtures at 35 °C. Weight percent of cyclohexane in eluent: (□) 0; (○) 55; (●) 61, and (○) 65.
with lower rr content (rr = 68, 33, and 0%) were eluted in SEC mode and their retention volumes decreased with decreasing rr triad content. Figure 4 illustrates an LC CAP chromatogram of the model PEMA mixture, which clearly indicates the separation of PEMA by tacticity. Elution volume increased linearly with increasing rr content.

The separation according to tacticity (rr triad content in a strict sense) in LC CAP mode was confirmed by on-line NMR detection. Figure 5 illustrates a stacked trace plot of continuous-flow LC CAP NMR data for the model PEMA mixture. A series of 110 spectra with spectral regions of 0.75–1.35 and 3.90–4.10 ppm was plotted against elution time from 27.7 to 60.7 min. A cross section of the stacked plot at 3.99 ppm where the ester OCH₂ resonates gives an ¹H NMR-detected chromatogram.

Figure 4. Chromatogram of LC CAP separation of the model PEMA sample (see Figure 2) in an acetone/acetone- d₆/cyclohexane (34/5/61 w/w) mixture at 35 °C. The plot of rr triad of sample against its retention volume is also plotted. An evaporative light-scattering detector was employed to monitor LC CAP column effluent.

Figure 5. Stacked trace plot of the 750-MHz continuous-flow LC CAP NMR spectra of the model PEMA sample (see Figure 2) in an acetone/acetone- d₆/cyclohexane (34/5/61 w/w) mixture at 35 °C. A series of 110 spectra with spectral regions of 0.75–1.35 and 3.90–4.10 ppm was plotted against elution time from 27.7 to 60.7 min. A cross section of the stacked plot at 3.99 ppm where the ester OCH₂ resonates gives an ¹H NMR-detected chromatogram.

Number tacticity values are depicted at the top of each peak.

Figure 6. ¹H NMR-detected LC CAP trace of the model PEMA sample (see Figure 2) monitoring the CH₂ in ethoxy ester group resonance. Tacticity compositions, determined from α-CH₃ proton resonance in the range of 0.82–0.92, 0.98–1.07, and 1.18–1.28 ppm due to rr (●), mr (+), and mm (○) triads, respectively, are also shown. Number tacticity values are depicted at the top of each peak.

Figure 7. ¹H NMR-detected LC CAP trace of a single PEMA sample (P2) with molar mass, Mₑ = 14.6 × 10³ g mol⁻¹, and average tacticity, mm/mr/rr = 2/45/53, by using a Develosil-NH₂ column in an acetone/acetone- d₆/cyclohexane mixture (34/5/61 w/w) monitoring the ester OCH₂ resonance. Tacticity compositions determined from α-CH₃ proton resonance due to rr (●), mr (+), and mm (○) triads are also shown.
The tacticity determined from the sum of whole LC CAP NMR spectra \((\text{mm}/\text{mr}/\text{rr})_{27/24/49}\) agreed well with the average tacticity of the four constituents \((\text{mm}/\text{mr}/\text{rr})_{25/27/48}\). These results demonstrate the validity of tacticity determination by LC CAP NMR in the continuous-flow mode.

The tacticity distribution in single PEMA sample P2 with molar mass, \(M_w = 14.6 \times 10^3 \text{ g mol}^{-1}\), and average tacticity, \((\text{mm}/\text{mr}/\text{rr})_{2/45/53}\) was inspected by LC CAP NMR in the continuous-flow mode (Figure 7). Despite narrow molar mass distribution, the polymer showed the tacticity distribution as clearly seen in Figure 7 in which \(\text{rr}\) triad content increases from 40 to 70\% and \(\text{mm}\) triad content decreases from 20 to 0\%. To confirm this tacticity distribution, the PEMA sample was fractionated by LC CAP into three fractions as indicated in Figure 8 and the tacticity composition of each fraction was determined by 188-MHz \(^{13}\text{C}\) NMR used in a conventional way. The values of tacticity indicated in the parentheses in Figure 8 agreed fairly well with those determined by summing up the LC CAP NMR spectra at corresponding elution time (Figure 7).

**CONCLUSIONS**

Liquid chromatography at the critical adsorption point with on-line nuclear magnetic resonance spectroscopic detection was used for separation of stereoregular poly(ethyl methacrylate)s according to their tacticity and for direct determination of tacticity composition of LC CAP effluent. The separation of PEMAs according to their tacticity (\(\text{rr}\) triad content in the strict sense) has been achieved by using silica gel bonded with aminopropyl groups and acetone/acetone-\(d_6\)/cyclohexane mixture (34/5/61 w/w) at 35 °C. The stereoregular PEMAs eluted from column packing in order highly isotactic, predominantly heterotactic, predominantly syndiotactic, and highly syndiotactic. The tacticity composition within each peak eluted from the LC CAP column was determined with a 750-MHz \(^{1}\text{H}\) NMR spectrometer operated in the continuous-flow mode. LC CAP NMR hyphenation is the only technique to obtain information on tacticity distribution when stereoregular polymers have the same or similar molar mass and differ in tacticity. The information obtained by the LC CAP NMR technique can be useful for better understanding of stereoregulation processes in stereospecific polymerizations. Undoubtedly, the use of NMR detection greatly enhances the applicability of LC CAP method in the characterization of a variety of complicated polymer systems.

**ACKNOWLEDGMENT**

This work was partially supported by the Ministry of Education, Science, Sports and Culture (Monbusho), project No 14401. M.J. gratefully acknowledges the financial support obtained from the Japan Society for the Promotion of Science (JSPS) for his research stay at Osaka University (JSPS fellowship P97 107). Thanks are due to the Venture Business Laboratory, Osaka University, for assistance in performing the LC CAP NMR experiments on a 750-MHz NMR spectrometer.

Received for review September 14, 1999. Accepted January 18, 2000.

AC991065R