Direct Determination of Molecular Weight Distribution by Size Exclusion Chromatography with 750 MHz ¹H NMR Detection (On-Line SEC-NMR)

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ABSTRACT: A size exclusion chromatograph coupled to a 750 MHz ¹H NMR spectrometer (on-line SEC-NMR) was used to analyze molecular weight distributions of isotactic PMMAs with \overline{M}_n ranging from 3.27×10^3 to 2.22×10^4 . The molecular weight of the polymer in flowing eluate was determined directly (without a conventional calibration procedure) from the relative intensity of NMR signals due to the end-group and repeating units. The molecular weight distribution determined in this manner was proved to be accurate by SEC-NMR experiments on uniform isotactic PMMAs consisting of exactly 23, 40, and 80 repeating units. Investigation was also undertaken to optimize flow rate and NMR parameters for quantitative SEC-NMR measurements. The signal-to-noise ratio of 750 MHz SEC-NMR spectra was 9.2 times that obtained previously with 500 MHz SEC-NMR operated under similar conditions.

KEY WORDS Size Exclusion Chromatography / Gel Permeation Chromatography / Liquid Chromatography-Nuclear Magnetic Resonance / Poly(methyl methacrylate) / Molecular Weight / End Group / Tacticity / Uniform Polymer /

Great progress has been made in the last decade in the direct coupling of NMR spectroscopy to liquid chromatography (on-line LC-NMR). This is partly due to the introduction of commercially available, dedicated LC-NMR probes which ensure high sensitivity and resolution in flow NMR measurements together with efficient suppression of solvent (eluent) signals. Recent review articles describe the development and applications of LC-NMR.¹⁻³

From the point of view of polymer analysis and characterization, size exclusion chromatography (SEC) should be the most useful mode of separation for online detection by NMR. As shown in previous experiments using a 500 MHz ¹H NMR spectrometer as a detector of SEC,⁴⁻⁹ on-line SEC-NMR is quite useful for studying the molecular weight dependence of chemical structures such as tacticity⁷ and comonomer composition⁵ in a polymer sample.

In the present work, directly coupled SEC-750 MHz ¹H NMR operating in the continuous-flow mode was used to analyze the molecular weight distribution of isotactic poly(methyl methacrylate) (PMMA) whose chemical structure is expressed as follows.

$$CH_{3}-CH_{3} \xrightarrow{CH_{3}}_{CH_{2}-C} H_{2} \xrightarrow{CH_{3}}_{n} H$$

$$CH_{3} \xrightarrow{CH_{3}}_{C=0} \xrightarrow{CH_{3}}_{n} H$$

$$CH_{3} \xrightarrow{CH_{3}}_{OCH_{3}} H$$

This polymer has a t-C₄H₉ group at the chain end, and thus the number average degree of polymerization can be determined from the relative intensity of NMR signals due to the t-C₄H₉ group and repeating units (α -CH₃ or OCH₃ group). This method requires accurate determination of the end group which is small in quantity and thus the benefit of using high field NMR is obvious. The high sensitivity of 750 and 800 MHz NMR has recently been shown in the LC-NMR analysis of equilibrium mixtures of ester glucuronides¹⁰ and metabolites of tolfenamic acid.¹¹

The present study investigates the validity of molecular weight determination by 750 MHz SEC-NMR and demonstrates the usefulness of this technique in polymer characterization.

EXPERIMENTAL

Materials

Four samples of isotactic PMMA, denoted as A, B, C, D, were prepared by the stereospecific living polymerization of MMA with t-C₄H₉MgBr in toluene at -78° C.¹² The isotacticity of these PMMAs ranged from 96.3 to 97.9 % in triads. Uniform PMMAs consisting of 23, 40, and 80 repeating units, respectively, were obtained by the fractionation of similar isotactic PMMAs (see formula I) with supercritical fluid chromatography.^{13,14}

SEC-NMR Measurements

SEC-NMR measurements were performed on a Varian Unity-INOVA 750 spectrometer using an ¹H{¹⁵N-³¹P} inverse-geometry LC-NMR probe with pulsed field gradient coils. A detection volume of the flow cell (2 mm i.d.) is approximately $60 \,\mu$ l. The sample inlet of the probe was connected to a Jasco PU-980 chromatograph equipped with a Shodex "linear" SEC column K-805L (8 mm i.d. × 300 mm, maximum porosity 4×10^6) *via* a PEEK tubing (0.25 mm i.d. × 2.4 m). CDCl₃ (99.8 + atom%, stabilized with silver foil, ISOTEC Inc.) was used as the eluent.

The conditions for 750 MHz ¹H NMR measurements in the continuous-flow mode were investigated in the present work, and the relevant parameters are described in the next section. The following are the SEC-NMR

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conditions for molecular weight determination of PMMA at a flow rate of 0.2 ml min^{-1} (see Figure 2). A total of 74 spectra were collected every 18 s in an elution period from 34.8 to 57.0 min. Each spectrum was obtained by the accumulation of 8 scans using 90° pulses (7.7 μ s) with a pulse repetition time (t_{pr}) of 2.25 s, and stored into 32 K data points covering a spectral width of 7490.6 Hz. Digital signal processing (DSP) was applied, and the oversampling factor was 40. The peak at 1.51 ppm from a small amount of water in the eluent was suppressed by digital filtering. Prior to Fourier transformation, an exponential apodization function was applied to the free induction decays (FIDs) corresponding to a line broadening of 2.2 Hz. Chemical shift values were determined with reference to the resonance at 7.245 ppm due to residual $CHCl_3$ in the eluent.

Molecular Weight Determination by the Conventional Methods

 \overline{M}_n of samples A to D were determined from the relative intensities of t-C₄H₉ and OCH₃ signals in the ¹H NMR spectra recorded by the use of 5 mm o.d. probe in nitrobenzene- d_5 at 110°C (see ref 15) and 750 MHz.

Measurements of molecular weight distribution by the conventional SEC method were carried out on the above-mentioned chromatograph connected to a Shodex SE-61 refractive index (RI) detector instead of the NMR spectrometer. $CDCl_3$ was used as the eluent and the flow rate was 0.2 ml min^{-1} . The molecular weight was calibrated against uniform PMMAs (23-, 40-, and 80mers). A linear calibration function was assumed.

RESULTS AND DISCUSSION

Investigation of Flow-NMR Conditions

The sensitivity and accuracy of on-line NMR detection are major issues in molecular weight determination by SEC-NMR. For optimization of the experiments, the first parameter to be considered is flow rate.

Table I shows the line shape and sensitivity of CHCl₃ resonance recorded at various flow rates. A mixture of CHCl₃ and CDCl₃ (5:95, v/v) was used as the sample solution, and measurements were carried out without an SEC column. At each flow rate, 8 transients accumulated with a t_{pr} specified in Table I. t_{pr} was chosen so that a single pulse excitation would be applied to the analyte during a residence time in the flow cell ($\tau = [detection]$ volume]/[flow rate]). The line widths at half height $(W_{1/2})$ and at the height of the ¹³C satellites (W_s) were within a range of 0.76-0.81 and 12.7-14.7 Hz, respectively, when flow rate was 0.50 ml min^{-1} or below. Slight broadening of the resonance was observed at larger flow rates, which is mostly attributable to a shortening of the apparent spin-spin relaxation time $(T_{2 \text{ flow}})$ relative to static measurements $(1/T_{2 \text{ flow}} = 1/T_{2 \text{ static}} + 1/\tau)$.¹⁶

The signal-to-noise ratio (S/N) for CHCl₃ resonance decreased gradually with increasing flow rate. At flow rates above 0.5 ml min⁻¹, S/N dropped to about one-fifth that in the non-flow state. The decrease in S/N is mainly because the sample transfer from the outside of magnetic field to the inside is too fast at larger flow rates to achieve pre-equilibration of nuclear polarization. The spin-lattice relaxation time (T_1) of CHCl₃ was 15.4 s under the

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Table I. Effects of flow rate on ¹H NMR signal due to CHCl₃-CDCl₃ (5:95, v/v) measured at 750 MHz using an LC-NMR probe with a 60 μl flow cell^a

Flow rate	τ ^b	t _{pr} ^c	$W_{1/2}^{d}$	W _s ^e	S/N^{f}
ml min ⁻¹	s	s	Hz	Hz	104
0.0	x	60	0.81	14.3	9.59
0.1	36	50	0.81	14.7	9.15
0.2	18	25	0.77	14.0	8.42
0.5	7.2	10	0.76	12.7	5.88
1.0	3.6	10	1.18	15.5	1.98
2.0	1.8	10	1.55	17.0	1.76

^a Pulse width, 90°; acquisition time, 9.7 s; number of transients, 64; spectral width, 6740 Hz; data points, 131072; digital resolution, 0.103 Hz; line broadening factor, 0.03 Hz; oversampling factor, 59; temperature, 28° C. ^b Residence time (=detection volume/flow rate). ^c Pulse repetition time. ^d Line width at half height. ^e Line width at the height of the ¹³C satellites. ^f Signal-to-noise ratio.

Table II. Effects of flow rate on the intensity of ¹H NMR signals due to the α -CH₃ and OCH₃ resonances relative to that of the *t*-C₄H₉ resonance in the 750 MHz flow-NMR measurements for isotactic PMMA sample A in CDCl₃ $(2 \text{ mg ml}^{-1})^{a}$

Flow rate	τ ^b	t _{pr} ^c	Relative intensity ^d	
ml min ⁻¹	S	s	α-CH ₃	OCH ₃
0.0	00	75	10.38	10.29
0.1	36.0	50	10.56	10.46
0.2	18.0	25	10.54	10.37
0.5	7.2	10	10.50	10.28
1.0	3.6	10	10.36	10.12
2.0	1.8	10	9.79	9.50

^a Pulse width, 90°; acquisition time, 9.7 s; number of transients, 4; spectral width, 6740 Hz; data points, 131072; line broadening factor, 2.2 Hz; oversampling factor, 40; temperature, 28°C. ^b Residence time (= detection volume/flow rate). ^c Pulse repetition time. ^d Intensity of *t*-C₄H₉ resonance as 1.00.

present conditions and thus approximately 80s is required for full premagnetization. Such an effect of flow rate on S/N may be less significant in the flow NMR detection of PMMA having much smaller T_1 's. T_1 for the α -CH₃, CH₂, OCH₃, and t-C₄H₉ groups in sample A were 0.38, 0.55–0.60, 1.73, and 0.49 s, respectively.

To examine the effects of flow rate on the relative intensity of NMR signals due to the end-group and the repeating units in the PMMAs, continuous-flow NMR measurements were made at various flow rates on a 2 mg ml^{-1} solution of sample A. Table II shows the intensity of α -CH₃ and OCH₃ resonance relative to that of t-C₄H₉ resonance. The values were almost independent of flow rate, except for those measured at 2.0 ml min⁻¹. When the flow-NMR experiments were carried out at 0.2 ml min⁻¹ varying t_{pr} from 9.0 to 1.8 s, the relative intensity of OCH₃ resonance decreased considerably due to saturation (Figure 1). Accordingly, the OCH₃ resonance was unsuitable for the quantitative flow-NMR measurements in the present work. The relative intensity of α -CH₃ resonance was unaffected by t_{pr} above 2.0 s.

SEC-NMR Measurements of Isotactic PMMA Samples Based on the above, on-line SEC-NMR measurements



Figure 1. Intensity of α -CH₃ (\bigcirc) and OCH₃ (\bigcirc) resonance relative to *t*-C₄H₉ resonance as a function of pulse repetition time (t_{pr}) in the 750 MHz flow NMR of isotactic PMMA (sample A). [PMMA]/[CDCl₃] = 10 mg ml⁻¹; flow rate, 0.2 ml min⁻¹; pulse width, 90°; number of transients, 4; acquisition time, 1.09 s; spectral width, 7491 Hz; data points, 16384; line broadening factor, 1.0 Hz.



Figure 2. 750 MHz on-line SEC-NMR data of isotactic PMMA (sample A).

for the PMMAs were made at a flow rate of 0.2 ml min^{-1} . Time resolution of the measurements, which corresponds to the time interval between consecutive spectra, was adjusted to the τ at this flow rate (18 s). Figure 2 shows a stacked trace plot of the serial spectra for sample A. A cross section of the plot at 1.18 ppm where the α -CH₃ group resonates gives an ¹H NMR-detected SEC curve. The solid lines in Figure 3 represent SEC curves obtained for the four PMMA samples. Those recorded by an RI detector are displayed together in dotted lines. The peak profiles of ¹H NMR- and RI-detected chromatograms agree well with each other, except that a time lag (ca. 0.2 min) exists between them. The amounts of samples A, B, C, and D injected to the chromatograph were 1.0, 0.8, 0.4, and 0.4 mg, respectively. Increasing the amount caused appreciable peak-broadening.

Figure 4a illustrates the spectrum acquired at the elution maximum of sample A (50.1–50.4 min). For comparison, Figure 4b shows the spectrum obtained previously by the 500 MHz SEC-NMR operated under similar conditions.⁶ A striking difference between the two spectra can be seen in their S/N. S/N of the α -CH₃ resonance in Figure 4a was 4450, which was 9.2 times that in Figure 4b. This marked improvement in sensitivity appears due to magnetic field strength, probe design, and spectrometer performance. According to our investi-



Figure 3. ¹H NMR- (solid lines) and RI-detected (dotted lines) SEC curves of isotactic PMMAs. Amounts of samples A, B, C, and D introduced to the chromatograph were 1.0, 0.8, 0.4, and 0.4 mg, respectively, and the volume of sample solution was $50 \,\mu$ l.



Figure 4. ¹H NMR spectra of isotactic PMMA recorded at the elution maximum in the 750 MHz (a) (see Figure 3) and 500 MHz (b) (see ref 6) SEC-NMR operated under similar conditions.

gation using 500 and 750 MHz spectrometers supplied as the same model (Varian Unity-INOVA), sensitivity increases by a factor of about 2.3 as the field strength increases from 500 to 750 MHz. Approximately 20—30% gain of sensitivity may be due to the recent progress in spectrometers such as the introduction of low-noise preamplifiers and DSP. It follows that additional increase in sensitivity (by a factor of \sim 3) resulted from the improvements in LC-probes.

The high sensitivity of 750 MHz SEC-NMR is advantageous for the quantitative determination of endgroup content in an analyte polymer. The intensity of the α -CH₃ and t-C₄H₉ resonances in a spectrum recorded at a certain elution time $[I_i(\alpha$ -CH₃) and $I_i(t$ -C₄H₉)] was measured by integrating the spectral regions of 0.95—1.35 and 0.75—0.95 ppm, respectively. The ratio of these intensities yields the molecular weight

Table III. \overline{M}_n and $\overline{M}_w/\overline{M}_n$ of isotactic PMMAs determined by 750 MHz SEC-NMR and conventional methods

Sample	¹ H NMR ^a	SEC-NMR		SEC-RI ^b	
	$\bar{M}_{n}/10^{3}$	$\bar{M}_{n}/10^{3}$	$ar{M}_w/ar{M}_n$	$\bar{M}_n/10^3$	${ar M}_w/{ar M}_n$
Α	3.27	3.35	1.143	2.98	1.231
В	5.75	6.00	1.107	5.40	1.215
С	10.16	10.22	1.088	10.80	1.170
D	24.22	20.54	1.080	28.35	1.135

^a Determined from relative intensity of α -CH₃ resonance to t-C₄H₉ resonance in the 750 MHz NMR spectrum measured by the use of a 5 mm o.d. probe. ^b Molecular weight was calibrated against uniform isotactic PMMAs (23-, 40-, and 80mers).



Figure 5. Plots of $\log(M_i)$ determined by 750 MHz SEC-NMR against elution time for isotactic PMMA samples A (\triangle), B (\blacktriangle), C (\bigcirc), and D (\bigcirc). Calibration curve made by SEC-RI measurements is shown in a straight line together with the log(M) against elution time plots for uniform isotactic 23-, 40-, and 80mers (\times).

of polymer detected in the spectrum (M_i) by the following equation:

$$M_i = 100.1 \times 3I_i (\alpha - \text{CH}_3) / I_i (t - \text{C}_4 \text{H}_9) + 58.1$$
 (1)

where 100.1 and 58.1 are the formula weights of a repeating unit $(C_5H_8O_2)$ and the end-groups $(C_4H_9 + H)$. When M_i is determined for every spectrum in a SEC-NMR data, the \overline{M}_n and \overline{M}_w of a whole polymer are given by

$$\bar{M}_n = \sum_i I_i (t - C_4 H_9) M_i / \sum_i I_i (t - C_4 H_9)$$
 (2)

$$\bar{M}_{w} = \sum_{i} I_{i}(t - C_{4}H_{9})M_{i}^{2} / \sum_{i} I_{i}(t - C_{4}H_{9})M_{i}$$
(3)

or,

$$\bar{M}_n = \sum_i I_i (\alpha - CH_3) / \sum_i [I_i (\alpha - CH_3) / M_i]$$
(4)

$$\bar{M}_{w} = \sum_{i} I_{i}(\alpha - CH_{3})M_{i} / \sum_{i} I_{i}(\alpha - CH_{3})$$
(5)

It should be noted that $I_i(t-C_4H_9)$ is sensitive to the number of polymer chains while $I_i(\alpha$ -CH₃) is sensitive to polymer weight. In practice the evaluation of M_i is difficult for several spectra acquired at the trailing edges

Table IV. Apparent \overline{M}_n and $\overline{M}_w/\overline{M}_n$ of uniform isotactic PMMAs found by 750 MHz SEC-NMR and SEC-RI

Sample	Expected M	SEC-NMR		SEC-RI	
		\bar{M}_n	${ar M}_w/{ar M}_n$	\bar{M}_n	$ar{M}_w/ar{M}_n$
23mer 40mer 80mer	2360.8 4062.8 8067.5	2384 4042 8100	1.0003 1.0007 1.0001	2286 3845 7772	1.042 1.043 1.033



Figure 6. ¹H NMR- (solid lines) and RI-detected (dotted lines) SEC curves of the uniform isotactic PMMAs consisting of 23, 40, and 80 repeating units. The amount of sample injected to the chromatograph was 0.2 mg dissolved in 50 μ l CDCl₃.

of an elution peak because of low S/N of the end-group resonance (especially at higher molecular weight regions), so those consecutive spectra were co-added to enhance the S/N (>10) and the elution time was averaged. Table III summarizes the \overline{M}_n and $\overline{M}_w/\overline{M}_n$ calculated for the four PMMA samples by eq 2 and 3. \overline{M}_n agreed well with those determined from the end-group analysis of the total polymers by ¹H NMR. $\overline{M}_w/\overline{M}_n$ were 5—9% smaller than those by the conventional SEC-RI method.

As shown in Figure 5, plots of $\log(M_i)$ against elution time were linear over the elution period of each PMMA. These linear relationships, regarded as absolute calibration curves for the PMMAs, had slopes less steep than the calibration curve made by SEC-RI. This would be responsible for the discrepancy between $\overline{M}_w/\overline{M}_n$ by SEC-NMR and SEC-RI. The decrease in the slopes of absolute calibration curves indicates that instrumental spreading (axial dispersion)¹⁷ of elution bands cannot be disregarded in the SEC measurements under the present conditions.

To elucidate the dispersion problem, SEC-NMR and SEC-RI measurements were carried out for isotactic uniform 23-, 40-, and 80mers of MMA. Each uniform polymer has a definite molecular weight (*M*) and no molecular weight distribution ($\bar{M}_w/\bar{M}_n=1$), and is therefore useful for such experiments.^{13,14} When uniform PMMAs were subjected to SEC-RI, apparent \bar{M}_w/\bar{M}_n ranging from 1.033 to 1.042 were observed (Table IV). Theoretical plates estimated from the chromatograms were 8100 to 8300. The deviation of the apparent \bar{M}_w/\bar{M}_n from unity clearly shows the instrumental spreading in the SEC as well as the imperfection of calibration procedure in SEC-RI. This problem arises from the fact that the absolute calibration curve for uniform polymers has a slope equal to zero. In contrast, quite naturally, SEC-NMR gave $\overline{M}_w/\overline{M}_n$ values very close to unity. The \overline{M}_n by SEC-NMR agreed with M within an error of 1.0%. The determination of average molecular weights by SEC-NMR is thus unaffected by the slope of absolute calibration curve and gives accurate \overline{M}_n and $\overline{M}_w/\overline{M}_n$.

SEC-NMR analysis of uniform PMMAs showed dispersion due to nonideal flow characteristics in the LC-NMR probe. The NMR-detected SEC curves of uniform PMMAs had half height widths broader by 22—35% than RI-detected SEC curves (Figure 6). Such broadening of an elution band can hardly be found in the NMR-detected SEC curves of non-uniform PMMAs (*cf.* Figure 3).

The present results demonstrate the applicability of high-field SEC-NMR in the analysis of molecular weight distribution through quantitative determination of endgroup content in a polymer sample. When measurements are carried out under appropriate conditions, reliable data on \overline{M}_n and $\overline{M}_w/\overline{M}_n$ can be obtained in a molecular weight region exceeding 2×10^4 .

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