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Off-line size-exclusion chromatographic fractionation–matrix-assisted laser desorption ionization time-of-flight mass spectrometry for polymer characterization
Theoretical and experimental study

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Abstract

The parameters affecting the fractionation performance in size-exclusion chromatography (SEC) of broad polymer samples were investigated. Some equations were derived which enable the prediction of polydispersity (PD) in an SEC fraction. Good agreements were obtained between the calculated data and the experimental values. Based on these equations, SEC fractionation conditions were optimized. In the off-line SEC–matrix-assisted laser desorption ionization (MALDI) time-of-flight (TOF) mass spectrometry (MS), two different modes can be employed, i.e., using MALDI-MS to provide an absolute calibration curve for SEC, or using SEC as a sample preparation step for MALDI-MS measurements. It was demonstrated that it is more reliable to use the latter combination, because most problems inherent in SEC can be circumvented. Some guidelines for the optimization of off-line SEC fractionation–MALDI-TOF-MS were given. It was found that under optimized conditions normally only a few SEC fractions are already sufficient to separate a highly polydisperse sample into portions of low PD that can accurately be measured by MALDI-TOF-MS.

Keywords: Matrix-assisted laser desorption/ionization mass spectrometry; Size-exclusion chromatography; Molecular mass; Polydispersity; Polymers

1. Introduction

Techniques for polymer synthesis are becoming more and more sophisticated and various macromolecules have been developed for specific applications, which has exerted increasing challenge on polymer analysis. Benefited from recent advances in ionization methods, mass spectrometry (MS) is a viable technique for polymer characterization. This is especially the case with the development of matrix-assisted laser desorption ionization (MALDI). MALDI is a new soft ionization mode that allows desorption and ionization of macromolecules with molecular mass up to hundreds of kilodaltons with very little or no fragmentation [1,2]. Although introduced recently, MALDI time-of-flight mass spectrometry (TOF-MS) has already been considered to be a very powerful method for the characterization of polymer samples. Structural information on the repeating unit mass, end group mass, copolymer

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sequence and mechanism of polymerization were acquired by using MALDI-TOF-MS [3–9]. Many efforts have also been made to obtain molecular mass average values, M_n (number-average molecular mass $\Sigma n_i M_i / \Sigma n_i$), M_w (weight-average molecular mass $\Sigma n_i M_i^2 / \Sigma n_i M_i$) and PD (polydispersity M_w / M_n). Unfortunately, it has been reported that for highly polydisperse polymers the values of M_n, M_w and PD could not be reliably determined by using MALDI-MS alone, because of the possible differences in desorption rate, ionization probability, transmission efficiency in the flight tube and detection sensitivity for ions with different masses [10–12].

Size-exclusion chromatography (SEC) is the method routinely employed for the polymer molecular mass determination [13]. In SEC the polymer molecules are separated according to their size, with the largest eluting first. Due to the band broadening of the chromatographic system, the chromatogram of even a monomeric compound appears not as a straight line but as a bell-shaped Gaussian peak. Therefore, instead of separating into the individual compounds, a distribution of molecular sizes is normally present in the detector. Thus correction of band broadening might frequently be required, which needs complicated mathematical calculations [14]. More importantly, SEC is not an absolute method, and requires secondary calibration, which very possibly to introduces errors [15].

Coupling of SEC and MALDI-TOF-MS can overcome most of the limitations that would occur if the analytical techniques were used separately. On-line coupling of MALDI with SEC has been reported [16–18]. So far, however, the off-line coupling is still more attractive than the on-line combination because it allows both MALDI and SEC to be operated at their respective optimal conditions. Basically, two arrangements can be envisaged in off-line SEC–MALDI-TOF-MS. One is to use MALDI-MS to obtain an absolute calibration curve for SEC, and the other to use the SEC as one of the sample preparation steps for MALDI-MS. In the first combination, after the establishment of the calibration curve, the molecular mass values can be acquired through an SEC operation, while in the second combination, the values can be calculated by summing the MALDI-MS data of all the SEC fractions.

When MALDI-MS is employed to obtain an absolute SEC calibration curve, some small fractions that have much lower PD are collected after SEC separation. The MALDI-MS measurements of these fractions can provide accurate M_n and M_w values, and thus, the SEC calibration curve against absolute molecular mass. The curve can then be used to calculate the average molecular mass and molecular-mass distribution of the original sample via SEC software. In this way, a wide variety of synthetic polymers with high PD have been characterized. Among the successfully characterized polymers are poly(dimethylsiloxane) [19], poly(butylene adipate) and poly(butylene adipate–co-butylene succinate) [20], caprolactone oligomers [17], polydextrans [21], poly(methyl methacrylate) [22], polystyrene, polybutylacrylate, poly(bisphenol A carbonate), aromatic polyester resin and methyl methacrylate–methacrylic acid copolymer [23]. Another important aspect of this method is that the MALDI-MS spectra of the fractions containing the lowest-molecular-mass species allow the identification of the polymer structure and of the end-groups present in the polymer chain [20,23]. More recently, a micro-scale SEC–MALDI-TOF-MS system with a robotic interface was developed in which the MALDI matrix was coaxially added to the column effluent and directly spotted onto the MALDI target [24]. The system is claimed to be less laborious and less time-consuming. Despite all the impressive progress, the band-broadening of the SEC system is generally not corrected due to its complexity, which could very well possible make the analytical results inaccurate. In addition, the exact retention volume in each fraction corresponding to the M_n or M_w is generally not in the middle of the fraction and difficult to predict. This will also introduce errors, especially for early-eluting fractions and when large fraction volumes are taken. Moreover, a very important fundamental aspect concerning the parameters affecting the polydispersity in an SEC fraction that is critical for a successful SEC–MALDI-MS analysis, has not been fully investigated yet. So far, the selection of SEC fractionation conditions is still highly empirical. It is one aim of this work to study how the experimental parameters, such as the origin of the polymer, the performance of the SEC column and the width of the fraction window, control the PD in an SEC fraction. Based on the study, some guide-

lines for optimizing the fractionation conditions are given.

Using SEC as one of the sample preparation steps for MALDI-MS is also a very attractive combination for polymer analysis. As will be demonstrated later in this article, it can circumvent most, if not all, of the problems encountered in SEC. By analyzing the molecular masses of all the fractions with MS, the molecular mass distribution of the polymer sample can be calculated without considering the band broadening and calibration of the chromatographic system. Unfortunately, this combination has not received enough attention yet. In this contribution, some equations were derived which enable the prediction of PD in an SEC fraction. Based on these equations the SEC fractionation conditions were optimized and a highly polydisperse poly(methyl methacrylate) sample was analyzed by summing the data of all the SEC fractions measured by MALDI-MS. The results were compared with those obtained in SEC with a calibration standard or with an absolute calibration using MALDI-TOF-MS.

2. Theory

MALDI-TOF-MS has been exhibited to be a very useful tool for polymer analysis. When combined with SEC, it can provide more accurate information about molecular mass distribution for polymers even with high polydispersity. As discussed in the Introduction, there can be two arrangements for off-line SEC–MALDI-TOF-MS. In order to get right results, it is very important that the PD in the fractions measured by MALDI-MS should be low enough, no matter which arrangement is employed. The average molecular mass values and PD are defined by:

$$M_n = \sum n_i M_i / \sum n_i \tag{1}$$

$$M_w = \sum n_i M_i^2 / \sum n_i M_i \tag{2}$$

$$\mathrm{PD} = M_w / M_n \tag{3}$$

where n_i represents the number of oligomer molecules having a mass of M_i, M_n the number-average molecular mass, and M_w the weight-average molecular mass.

The polydispersity in an SEC fraction is controlled by many parameters. Due to the band broadening of the SEC chromatographic system, the elution profile can be described by the Tung axial dispersion equation [25] if a concentration-sensitive detection method like UV–Vis is used:

$$F(v) = \int_{-\infty}^{\infty} W(y)G(v,y)\mathrm{d}y \tag{4}$$

where $F(v)$ is the function representing the chromatogram height at retention volume v, $W(y)$ the height of the chromatogram that would be obtained if resolution were perfect, and $G(v,y)$ the shape of the unseen chromatograms originated from each molecular size present in the sample. We shall use v and y interchangeably to denote the eluent volume, y is mainly used to denote the eluent volume as the variable under the definite integral sign.

The molecular-mass averages in an SEC fraction can be expressed by:

$$M_n = \frac{\int_{V_1}^{V_2} F(v)\mathrm{d}v}{\int_{V_1}^{V_2}\left[\int_{-\infty}^{\infty}\frac{W(y)}{M}G(v,y)\mathrm{d}y\right]\mathrm{d}v} \tag{5}$$

$$M_w = \frac{\int_{V_1}^{V_2}\left[\int_{-\infty}^{\infty} MW(y)G(v,y)\mathrm{d}y\right]\mathrm{d}v}{\int_{V_1}^{V_2} F(v)\mathrm{d}v} \tag{6}$$

where M is the molecular mass, and V_1 and V_2 are the start and the end volume of the fraction window, respectively. Obviously, V_1 and V_2 must be inside the range between the initial elution volume (V_a) and the final elution volume (V_b) of the chromatogram. In principle, the polydispersity in any SEC fraction (PD_1) can be calculated by combining Eqs. (3), (4), (5) and (6). The calculation can be carried out by using the mathematical technique convolution. It is very well possible that no general solutions for PD_1 are available. Fortunately, for a familiar molecular

mass distribution function, the log–normal distribution, some very useful equations can be derived. The log–normal distribution [25] can be written as:

$$w(M) = \frac{1}{\beta\sqrt{\pi}M} \cdot e^{-\frac{\ln^2(M/M_0)}{\beta^2}} \quad (7)$$

where $w(M)$ is the molecular mass distribution function, and β a parameter denoting the width of the distribution. By combining Eqs. (4), (5), (6) and (7) into Eq. (3), the PD of the polymer with a log–normal distribution can be calculated by:

$$\mathrm{PD} = e^{\frac{\beta^2}{2}} \quad (8)$$

Assuming that the eluent volume in SEC is proportional to the logarithm of the molecular mass and, the chromatogram of a monomeric compound is a Gaussian-shaped peak [25], we have:

$$v = C_1 - C_2 \ln M \quad (9)$$

$$G(v,y) = \frac{C}{\sqrt{2\pi}\sigma_c} \cdot e^{-\frac{(v-y)^2}{2\sigma_c^2}} \quad (10)$$

where C_1 and C_2 are constants, C is a constant related to the concentration of the compound, σ_c the band variance in SEC.

Transformation of Eq. (7) to the y coordinate gives:

$$W(y) = \frac{1}{C_2 N\beta\sqrt{\pi}} \cdot e^{-\frac{(y-y_0)^2}{C_2^2\beta^2}} \quad (11)$$

where $y_0 = C_1 - C_2 \ln M_0$, N the normalization factor for $W(y)$:

$$N = \frac{1}{\int_{-\infty}^{\infty} W(y)\mathrm{d}y} \quad (12)$$

According to Tung [25], under this condition the chromatogram retains the Gaussian form with the peak maximum at y_0:

$$F(v) = \frac{1}{C_2 N\sqrt{\pi(\sigma_c^2 + \sigma_p^2)}} \cdot e^{-\frac{(v-y_0)^2}{\sigma_c^2+\sigma_p^2}} \quad (13)$$

where:

$$2\sigma_p^2 = C_2^2\beta^2 \quad (14)$$

By combining Eqs. (3), (4), (5), (6), (10) and (11), we have:

$$\mathrm{PD}_1 = e^{\frac{\sigma_c^2\sigma_p^2}{C_2^2(\sigma_c^2+\sigma_p^2)}} \cdot \frac{\int_{V_1}^{V_2} e^{-\frac{C_2(v-y_0)^2+2\sigma_p^2 v}{2C_2(\sigma_c^2+\sigma_p^2)}}\mathrm{d}v \cdot \int_{V_1}^{V_2} e^{-\frac{C_2(v-y_0)^2-2\sigma_p^2 v}{2C_2(\sigma_c^2+\sigma_p^2)}}\mathrm{d}v}{\left[\int_{V_1}^{V_2} e^{-\frac{(v-y_0)^2}{2(\sigma_c^2+\sigma_p^2)}}\mathrm{d}v\right]^2} \quad (15)$$

The PD value becomes lower when the fraction window $(V_2 - V_1)$ gets smaller. In a very small fraction, Δv, Eq. (15) can be simplified as:

$$\mathrm{PD}_0 = e^{\frac{\sigma_c^2\sigma_p^2}{C_2^2(\sigma_c^2+\sigma_p^2)}} \quad (16)$$

Combined with Eq. (14) and taking into account the fact that σ_c^2 and C_2 are both proportional to the SEC column length (L), Eq. (16) can be rewritten as:

$$\mathrm{PD}_0 = e^{\frac{\beta^2}{2(1+a\beta^2 L)}} \quad (17)$$

where ''a'' is a constant controlled by the nature of the stationary phase, the type of polymer and the performance of the SEC column.

From Eqs. (15), (16) and (17) the following conclusions can easily be drawn.

(1) For a polymer sample with a known log–normal distribution, the polydispersity in any SEC fraction can be calculated, since all the parameters in Eq. (15) can be determined.

(2) Many parameters can affect the polydispersity in an SEC fraction. Among these are the width of the fraction window, the polymer identity and its distribution, the type of the SEC column and its efficiency.

(3) In a very small fraction the polydispersity will be lower if a longer column is used.

(4) Due to the band broadening of the SEC system, a polydispersity of 1 cannot be obtained even in a very small fraction ($V_2 - V_1 = \Delta V$), except for the case when a monomeric sample ($\sigma_p = 0$) is to be analyzed, or when a SEC column with infinite efficiency ($\sigma_c = 0$) is used.

Although the calculations described above are based on polymers with a log–normal distribution, some of these conclusions might also be applicable to polymers with other types of distribution. This will be studied in our future investigations.

After SEC separation, polymers are divided into small fractions with much lower PD. The overall values of molecular mass distribution of the original polymer can be acquired either by using the absolute calibration curve obtained by MALDI-MS and via the SEC software, or more reliably by adding up the values of all the fractions determined by MALDI-MS. The summation can be carried out using the following equations:

$$M_n = \frac{\sum n_i M_i}{\sum n_i} = \frac{AB}{\sum_{j=1}^{n}\left(\sum_{i=1}^{m} n_i\right)_j} \tag{18}$$

$$M_w = \frac{\sum n_i M_i^2}{\sum n_i M_i} = \frac{\sum_{j=1}^{n}\left(\sum_{i=1}^{m} n_i M_i^2\right)_j}{AB} \tag{19}$$

where j is the serial number of fraction, A the SEC peak area which corresponds to the overall polymer mass concentration, and B a constant related with the sensitivity of the detector.

In any fraction, the M_{nj} and M_{wj} values can be accurately determined by MALDI-MS:

$$M_{nj} = \frac{A_j B}{\sum_{i=1}^{m} n_i} \tag{20}$$

$$M_{wj} = \frac{\sum_{i=1}^{n} n_i M_i^2}{A_j B} \tag{21}$$

where A_j is the SEC peak area corresponding to the jth fraction. By combining Eqs. (18), (19), (20) and (21) we have:

$$M_n = \frac{A}{\sum_{j=1}^{n} \frac{A_j}{M_{nj}}} \tag{22}$$

$$M_w = \frac{\sum_{j=1}^{n} A_j M_{wj}}{A} \tag{23}$$

Dividing Eq. (23) by Eq. (22) yields:

$$PD = \frac{\left(\sum_{j=1}^{n} A_j M_{wj}\right) \cdot \left(\sum_{j=1}^{n} \frac{A_j}{M_{nj}}\right)}{A^2} \tag{24}$$

3. Experimental

Two broad polymer samples, polystyrene (PS) and poly(methyl methacrylate) (PMMA) were purchased from American Polymer Standards (Mentor, OH, USA). Dithranol, sliver trifuoroacetate and 2,5-dihydroxybenzoic acid (DHB) were obtained from Aldrich (Milwaukee, WI, USA). The narrow PS and PMMA standards were from Polymer Labs. (Amherst, MA, USA). All the solvents used were of HPLC grade and used without further purification.

SEC separations were carried out in a PLgel 500 Å column (30 cm×7.5 mm I.D., 5 μm particles) from Polymer Labs. The chromatographic system consisted of an LC-10AT pump (Shimadzu, Kyoto, Japan) operating at 1 ml/min. Chloroform was used as the mobile phase. The highly polydisperse synthetic polymers were dissolved in chloroform (typically 20 mg/ml). Samples of 20 μl were injected with a Midas autosampler (Spark Holland, Emmen, The Netherlands). The UV–Vis detector (UVIS-205, Reno, NV, USA) was operated at 254 nm and 240 nm for the detection of PS and PMMA, respectively. The chromatographic data were collected and calculated using DAX software (PP van Mierlo, Eindhoven, The Netherlands).

The MALDI-TOF-MS measurements were performed with a Voyager-DE Pro (PerSeptive Biosystems, Framingham, MA, USA) instrument equipped with a 337-nm nitrogen laser, capable of executing both linear and reflectron modes. Spectra were acquired by summing spectra from 256 selected laser shots. DHB was selected as the matrix for

PMMA, and dithranol together with the silver trifluroracetic acetate as the cationization reagent (dithranol–silver trifluoroacetate, 10:1, w/w) for PS. All the MALDI matrices were dissolved in tetrahydrofuran (THF) at a concentration of 20 mg/ml.

When SEC was used as a sample preparation step for MALDI-MS, the chromatographic peak of a broad PMMA sample could be included in eight fractions of 0.4 ml. If, on the other hand, MALDI-MS was used to obtain an absolute calibration curve for SEC, 20 fractions of 10 s were taken. The fractions were concentrated appropriately, mixed with the matrix solution, and finally 0.5 μl of the mixed solution was pipetted onto the MALDI target plate and allowed to dry at room temperature. All MALDI-MS measurements were performed in the linear mode with delayed extraction.

4. Results and discussion

4.1. Polydispersity in an SEC fractionation

SEC can separate synthetic polymers into small fractions with low PD that is a key prerequisite for a successful SEC–MALDI-TOF-MS analysis. As can be seen in the Theory section, many parameters can affect the polydispersity in an SEC fraction (PD_1). For samples with log–normal distribution, the polydispersity in a very small fraction (PD_0) can easily be calculated using Eq. (16). The results are listed in Table 1. In order to make the theoretical calculations more practical, we used a PLgel 500 Å column (30 cm×7.5 mm I.D., 5 μm particles), which was used in our experiments, as the reference. With this column the C_2 value for the polystyrene standard was found to be 0.853 (ml).

It can be seen from Eq. (16) that the PD_0 value depends on σ_c, β and C_2. In order to see the dependence more clearly, the data shown in Table 1 are presented according to the effects of these parameters. PD_0 reaches 1 if the plate number of the column is infinite ($\sigma_c = 0$). In a real case with a finite plate number, however, there will be a mixture of polymer molecules with different molecular masses in the fraction due to the band broadening of the chromatographic system. As expected, the lower the column efficiency, the higher the PD_0 value. This effect becomes less significant for samples of lower β values. If a single pure compound is injected, PD_0 is obviously one despite the band broadening. Actually, for polymers with low PD, no SEC fractionation is required prior to MALDI-MS measurements. For synthetic polymers with high PD values, on the other

Table 1
Calculated results of polydispersity in a hypothetically very small SEC fraction[a]

Effects of σ_c ($C_2 = 0.853$ ml, $\beta = 1$)							
σ_c^2 ($\cdot 10^3$ ml^2)	0	3	6.14[b]	12	24	48	
PD_0	1	1.004	1.008	1.016	1.031	1.060	
Effects of β ($C_2 = 0.853$ ml, $\sigma_c^2 = 6.14 \cdot 10^{-3}$ ml^2)							
β	0	0.1	0.5	1	1.5	2	
PD[c]	1	1.005	1.133	1.649	3.080	7.389	
PD_0	1	1.003	1.008	1.008	1.008	1.008	
Effects of C_2 ($\sigma_c^2 = 6.14 \cdot 10^{-3}$ ml^2, $\beta = 1$)							
C_2	0.2	0.4	0.853[b]	1.6	3.2	6.4	
PD_0	1.125	1.036	1.008	1.002	1.001	1.0002	
Effects of column length ($\beta = 1$)[d]							
Length (cm)	30/8	30/4	30/2	30[b]	2×30	4×30	8×30
PD_0	1.061	1.032	1.016	1.008	1.004	1.002	1.001

[a] Values were calculated according to Eq. (16).
[b] Values for polystyrene obtained with a PLgel column (500 Å, 5 μm particles, 30 cm×7.5 mm I.D.).
[c] Calculated according to Eq. (8).
[d] σ_c^2 and C_2 are both proportional to the column length.

hand, the profit of SEC fractionation becomes immediately evident. The PD_0 value calculated is 1.008 for an extremely highly polydisperse sample (PD= 7.39) at the conditions used in Table 1. From Table 1 it can also be found that the PD_0 value first increases with increasing β and then remains constant. At high β values ($\sigma_p^2 >> \sigma_c^2$), Eq. (16) can be reduced to:

$$PD_0 = e^{\frac{\sigma_c^2}{C_2^2}} \quad (25)$$

C_2 is the slope of the calibration curve (v vs. ln M) that represents the difference in elution volume of two compounds with their variance in logarithm molecular mass of one. A high C_2 value means a better separation. Clearly from Eq. (16), it will yield a low PD_0 value as well. Another parameter which should also be considered in estimating PD_0 is the column length. In a chromatographic separation, longer columns will normally give better resolutions. This is also true in SEC, as it is apparent from Eq. (17) that longer columns will yield lower PD_0 values.

The prediction of PD_1 in a normal SEC fraction requires the integration of several complex functions (Eq. (15)). In addition to the parameters that affect PD_0, PD_1 is also influenced by the width of the fraction window ($v_f = V_2 - V_1$). If the chromatogram of a polymer with log–normal distribution retains Gaussian shape (Eq. (13)), the initial and final elution volumes can generally be assumed as $y_0 - 3\sigma$ and $y_0 + 3\sigma$, respectively ($\sigma^2 = \sigma_c^2 + \sigma_p^2$ and y_0 is the elution volume at the peak maximum). Thus, the effects of v_f on PD_1 can be studied in this region. In our calculations, similar effects of the various parameters on PD_1 were observed at different y_0 values. Therefore, in the following estimations we artificially let $y_0 = 8$ (ml).

The PD_1 value in an SEC fraction depends on how many compounds are present in the fraction. Some calculated data are listed in Table 2. If the fraction volume (v_f) was reasonably small, no considerable variations of PD_1 in different fractions were observed. For a given polymer, PD_1 is only determined by v_f and C_2 if the band broadening of the chromatographic system can be neglected. Evidently, PD_1 is lower with smaller v_f. At a given fraction volume, a larger C_2 will result in a lower PD_1 value because of the higher selectivity of the SEC column. The effects of σ_c^2 on PD_1 are relatively straightforward. Higher σ_c^2, i.e., lower column efficiency, will yield higher PD_1 values. However, it can be seen from Table 3 that at different σ_c^2 values the ratio of PD_1/PD_0 remains constant that is mainly determined by v_f and C_2. As discussed in a previous paragraph, a high σ_c^2 value will result in a high PD_0. Thus, the effects of

Table 2
Effects of SEC fractionation volume on polydispersity (PD_1) at various conditions[a]

	v_f (ml)						
	0.01	0.05	0.1	0.2	0.4	0.8	1.6
Different C_2 (ml) ($\beta=2$, $\sigma_c^2=0$)							
$C_2=0.4$	1	1.001	1.005	1.020	1.084	1.33	1.95
$C_2=0.853$[b]	1	1	1	1.004	1.018	1.070	1.28
$C_2=1.6$	1	1	1	1.001	1.005	1.020	1.084
Different σ_c^2 (10^{-3} ml^2) ($\beta=2$, $C_2=0.853$ ml)							
$\sigma_c^2=0$	1	1	1	1.004	1.018	1.070	1.28
$\sigma_c^2=6.14$[b]	1.008	1.008	1.008	1.012	1.026	1.080	1.30
$\sigma_c^2=12.28$	1.018	1.018	1.018	1.021	1.035	1.090	1.43
Different β ($\sigma_c^2=6.14\cdot10^{-3}$ ml^2, $C_2=0.853$ ml)[b]							
$\beta=1$	1.008	1.008	1.008	1.012	1.025	1.077	1.22
$\beta=2$	1.008	1.008	1.008	1.012	1.026	1.080	1.30

[a] Values calculated according to Eq. (15).
[b] Values for polystyrene obtained with a PLgel column (500 Å, 5 μm particles, 30 cm×76 mm I.D.).

Table 3
Values of PD_1/PD_0 at various conditions and fraction volumes[a]

	v_f (ml)						
	0.01	0.05	0.1	0.2	0.4	0.8	1.6
Different C_2 (ml) ($\beta=2$, $\sigma_c^2=0$)							
$C_2=0.4$	1	1.001	1.005	1.020	1.084	1.33	1.95
$C_2=0.853$[b]	1	1	1	1.004	1.018	1.070	1.28
$C_2=1.6$	1	1	1	1.001	1.005	1.020	1.084
Different σ_c^2 (10^{-3} ml^2) ($\beta=2$, $C_2=0.853$ ml)							
$\sigma_c^2=0$	1	1	1	1.004	1.018	1.070	1.28
$\sigma_c^2=6.14$[b]	1	1	1	1.004	1.018	1.070	1.28
$\sigma_c^2=12.28$	1.001	1.001	1.001	1.004	1.018	1.072	1.28
Different β ($\sigma_c^2=6.14\cdot10^{-3}$ ml^2, $C_2=0.853$ ml)[b]							
$\beta=1$	1	1	1	1.004	1.017	1.068	1.21
$\beta=2$	1	1	1	1.004	1.018	1.070	1.28

[a] PD_1 and PD_0 values were calculated with Eqs. (15) and (16), respectively.
[b] Values for polystyrene obtained with a PLgel column (500 Å, 10 μm particles, 30 cm×7.5 mm I.D.).

column efficiency on PD_1 are essentially exerted through its influence on the PD_0 value. Compared with the effects of σ_c^2, the effects of β on PD_1 are a little more complicated. In SEC fractionation of high polydisperse samples, β together with C_2 are the most important parameters determining the initial and final elution volumes. High β values will result in broader SEC peaks and require more fractions if other experimental conditions remain unchanged. When the v_f value is reasonably small (e.g., <0.1 ml in Table 2) and is kept constant, only marginal effects of β on PD_1 are observed. If an unrealistically large v_f is collected, however, smaller β values will give lower PD_1. For example, in an extreme case of collecting all the compounds in one large fraction, i.e., no fractionation has been performed, PD_1 is obviously the PD of the original polymer which is a function of β. Nevertheless, it can be seen in Table 2 that even for an extremely high disperse polystyrene sample (PD=7.39), a PD_1 of 1.03 can be achieved by simply taking a few SEC fractions of 0.4 ml using a PLgel 500 Å column (30 cm×7.5 mm I.D.). From this it can be concluded that SEC is a very powerful technique for the fractionation of highly polydisperse samples.

From the discussions above, it is clear that high column efficiency, large slope of the v vs. ln M curve and small fraction volume together will result in a low PD_1. In order to obtain an expected PD_1 value, the polydispersity in a very small fraction (PD_0) should first be estimated. This is because PD_0 is the lowest value that could be reached. Under our experimental conditions for polystyrene, if v_f is below 0.1 ml (see Table 2) PD_1 is very close to PD_0 which can easily be calculated by using Eq. (16). For separations of different polymers, PD_0 can be adjusted by using columns with different length or different stationary phase.

To evaluate the accuracy of the theoretical calculations, a series of experiments were carried out with different fractionation volumes. In these experiments, SEC separation was performed with a PLgel 500 Å column using a broad polystyrene sample ($M_w=$ 14 900 and $M_n=6000$, according to the manufacturer) as the model polymer. Polystyrene was employed because our SEC system was well calibrated using a series of polystyrene calibration standards and, thus can provide an accurate C_2 value for the calculation. In addition, polystyrene is amenable to MALDI-TOF-MS characterization. Some calculated results and experimental data are listed in Table 4. Fig. 1 shows some representative MALDI-MS spectra obtained with different amount of fractionation volume. As can be seen in Table 4, very good agreements were observed between the calculated values and the experimental data.

Table 4
Comparison of calculated and experimental values of polydispersity in an SEC fraction

	Fraction volume (ml)		
	0.4	0.8	1.6
Calculated[a]	1.026	1.080	1.299
Experimental	1.03±0.004[b]	1.09±0.006[c]	1.31±0.02[d]

[a] Calculated according to Eq. (15).
[b] Average values of 16 fractions (four SEC runs with four selected fractions).
[c] Average values of eight fractions (four SEC runs with two selected fractions).
[d] Average values of four fractions (four SEC runs with one selected fraction).

4.2. SEC fractionation–MALDI-TOF-MS

It is widely recognized that MALDI-MS alone is not suitable for the analysis of highly polydisperse polymers. For these samples, SEC might be able to provide nice results if calibration standards with the same properties of the analytes would be available. However, only very few types of calibration standards are obtainable. Moreover, the molecular masses of the standards are generally obtained by indirect measurements, such as viscosity and light scattering. Recently, the direct determination of molecular mass and molecular mass distribution by, for example, using SEC–MALDI-TOF-MS has attracted great interest among polymer scientists. In off-line SEC–MALDI-TOF-MS so far, MALDI-MS is mostly used to obtain an absolute calibration curve for SEC, and MS analysis of a few selected fractions might already be sufficient. Despite the absolute calibration curve, however, SEC might still not be able to obtain accurate results. This is because of the difficulties to assign the exact values of elution volume in the SEC fractions corresponding to the M_n and M_w obtained by MALDI-MS, and to correct the band broadening of the chromatographic system. In contrast, by using SEC as a sample preparation step and measuring all the SEC fractions by MALDI-MS, most problems occurred in SEC can be circumvented, and the molecular mass data of a broad polymer can be calculated according to Eqs. (22–24). In order to distinguish these two arrangements, we name one combination SEC–MALDI-TOF-MS calibration, and the other SEC fractionation–MALDI-TOF-MS. In this section, the usefulness of the SEC fractionation–MALDI-TOF-MS was tested with a broad PMMA sample. For the SEC–MALDI-MS calibration, interested readers are referred to recent literature [3,26,27].

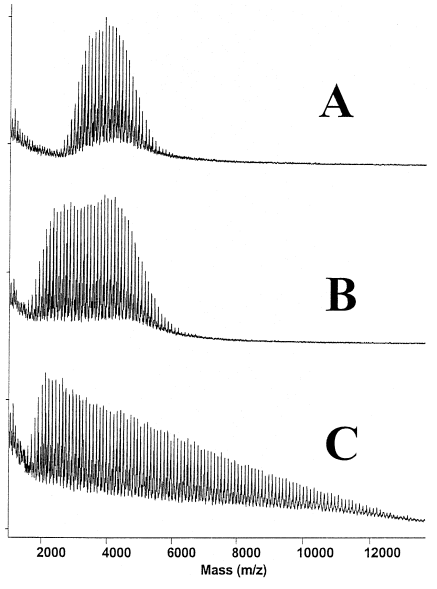

Fig. 1. Representative MALDI-TOF-MS spectra of a highly polydisperse polystyrene sample with different SEC fractionation volumes. SEC conditions: column PLgel 500 Å (30 cm×7.5 mm I.D., 5 μm particles), mobile phase $CHCl_3$ at 1 ml/min. MALDI-MS conditions: Voyager-DE with a 337-nm nitrogen laser, linear mode, dithranol (silver trifluoroacetate as cationization reagent) as the matrix. (A) 0.4 ml; (B) 0.8 ml; (C) 1.6 ml.

In SEC fractionation–MALDI-TOF-MS, one might expect that many SEC fractions and MALDI-MS measurements might be required. Opposite to this expectation, only a few fractions are normally needed, which makes the method quite applicable. For example, as can be calculated with the equations derived in the Theory section, only 18 fractions of 0.4 ml are needed to divide a highly polydisperse polystyrene sample (PD=7.39) into fractions with PD of 1.03 using a PLgel 500 Å column (30 cm×76 mm I.D.). In our experiments with this column, only eight fractions of 0.4 ml were found to be sufficient for the PMMA (PD=1.82, according to the supplier) sample. The results are listed in Table 5. Also in this table, the data obtained using MALDI-MS and SEC alone, and with SEC–MALDI-MS calibration are

Table 5
Molecular mass distribution data of poly(methyl methacrylate)

	Manufacturer	SEC[a]	MALDI-MS	SEC–MALDI-MS calibration		SEC fractionation–MALDI-MS[d]
				Start[b]	End[c]	
M_n	9650	5700	3900	4700	6200	5400
M_w	17 600	10 600	6600	8800	11 200	9500
PD	1.82	1.86	1.69	1.87	1.81	1.76

[a] Values obtained based on the calibration curve with narrow standards.
[b] Calibration using the data of starting elution volume of SEC fractions as the elution volume for M_n obtained by MALDI-MS.
[c] Calibration using the data of ending elution volume of SEC fractions as the elution volume for M_n obtained by MALDI-MS.
[d] Summation of eight fractions of 0.4 ml according to Eqs. (22–24).

listed for comparison. Some typical MALDI-MS spectra are shown in Fig. 2. As expected, MALDI-MS alone gives incorrect results for the broad polymer. The discrimination against oligomers of larger molecular masses in a broad polymer leads to the M_n and M_w determined by MALDI-MS considerably lower than its actual values. From Table 5, it can be seen that reasonably good agreements were observed between the data obtained in SEC fractionation–MALDI-TOF-MS and in SEC with narrow PMMA calibration standards. Nevertheless, it should be remarked here that conventional SEC method using calibration standard might not be accurate, and SEC fractionation–MALDI-TOF-MS is more reliable. It should also be noted that our data was considerably different from those supplied by the manufacturer. The reason for this is still not quite clear yet.

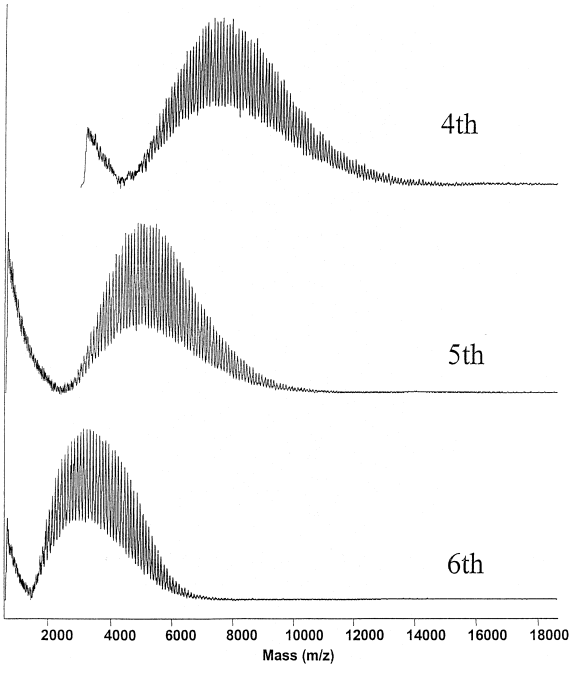

Fig. 2. MALDI-TOF-MS spectra of a highly polydisperse poly(methyl methacrylate) sample from the fourth to the sixth SEC fractions. SEC conditions: column PLgel 500 Å (30 cm×7.5 mm I.D., 5 μm particles), mobile phase $CHCl_3$ at 1 ml/min. MALDI-MS conditions: Voyager-DE with a 337-nm nitrogen laser, linear mode, 2,5-dihydroxybenzoic acid (DHB) as the matrix.

Using MALDI-MS to obtain an absolute calibration curve for SEC has been applied for the characterization of polymers, especially when suitable calibration standards are not available. However, since the exact values of elution volume corresponding to the respective M_n or M_w (determined by MALDI-MS) in each fraction are generally not in the middle of the fraction and difficult to predict in SEC–MALDI-MS calibration, some considerable deviation by the method itself might occur. This can clearly be seen in Table 5 where the elution volumes were artificially assigned at the start or the end of the fraction, with the fractionation window of only 10 s. Further minimizing the fraction window can partly solve the problem. Unfortunately, extremely small fractions are difficult to collect and handle. In contrast, using SEC as a sample preparation step for MALDI-MS, no calibration is required. The overall values of molecular mass and molecular mass distribution can simply be calculated by the summation of the data of all SEC fractions obtained by MALDI-MS.

Although SEC fractionation–MALDI-TOF-MS can provide accurate molecular mass and molecular mass distribution data, it is not a method for routine

analysis. This is because in every run, a polymer must be separated into several SEC fractions and then all the fractions be measured by MALDI-MS for which the instrumentation is quite expensive. For everyday analysis, therefore, one should first consider using SEC alone (calibrated with narrow standards, or with MALDI-MS when suitable calibration standards are not available) if a lot of samples are to be analyzed and only reasonable accuracy is required. Though the making of a calibration curve is sometimes tedious, once the curve is established, the polymer analysis can be carried out simply by using the chromatographic technique with SEC software. Nevertheless, when a small number of sample is to be analyzed and more reliable data are required, SEC fractionation–MALDI-TOF-MS is the method of choice.

4.3. Optimization of SEC fractionation for MALDI-TOF-MS

As demonstrated above, SEC fractionation–MALDI-TOF-MS is a very attractive method for the characterization of highly polydisperse polymer samples. By summing up the data in all the fractions measured with MALDI-MS, most problems occur in SEC, such as band broadening and calibration can be circumvented. Since in SEC fractionation–MALDI-TOF-MS all the SEC fractions should be measured, the optimization of the fraction volume (v_f) is very important. On the one hand, if the volume is too large, the PD_1 value in an SEC fraction might be too high to obtain accurate MS results. On the other hand, however, if the volume is too small, excessive MS measurements are required. The following is a guideline of how to optimize an SEC fractionation–MALDI-TOF-MS experiment. Due to the fact that the method is still in its early stage of development, the discussion here can only be general.

(1) For the separation of a given polymer, the first step is to select a right column. This can be carried out by estimating the PD_0 value that can be achieved with different columns, because PD_0 is the lowest possible value. It can be seen from Eq. (25) that for a highly polydisperse sample, PD_0 is determined by the ratio of σ_c^2/C_2^2. Regarding σ_c^2, it is advantageous to use highly efficient SEC columns (packed with small particles). As for C_2, it depends on the identity of the sample and the type of the column, and can change greatly with different compounds or columns. Longer columns will result in lower σ_c^2/C_2^2 ratios and, thus lower PD_0 values. However, excessively large C_2 values and long columns are not always applicable in SEC fractionation–MALDI-TOF-MS. This is because large C_2 values and long columns will result in very broad SEC peaks which require large amounts of solvent to completely elute the sample. Therefore, a column is normally selected which would yield a PD_0 value slightly below the PD_1 value demanded by MALDI-MS.

(2) After the selection of column, the number of fractions required and fraction volume (v_f) should be assessed according to the maximum allowable PD_1 value. PD_1 in an SEC fraction can be calculated with Eq. (15).

(3) In MALDI-TOF-MS, a sample solution is normally mixed with a suitable matrix solution before they are added onto the target plate. Because of the possible large difference of analyte molecular number in different fractions, it might be necessary to concentrate SEC fractions differently. For example, it might be required to remove more solvent in the early fractions (with oligomers of large molecules) than in the fractions near the peak top.

(4) Finally, the MALDI-MS parameters, e.g., the ion mode, laser intensity, accelerating voltage, delay time, should all be carefully optimized in order to get good MALDI-MS spectra.

After the selection of experimental conditions, the polymer sample is fractionated by SEC, and then all the fractions are measured by MALDI-TOF-MS. The M_n, M_w and PD values of the polymer are calculated according to Eqs. (22–24).

5. Conclusions

MALDI-TOF-MS using SEC as a sample preparation step is a very attractive technique for the characterization of highly polydisperse samples. Some equations were derived which enable the prediction of polydispersity in an SEC fraction, and the predicted results were in very good agreements with experimental data. Under optimized conditions, only a few fractions are sufficient to separate a broad

synthetic polymer into portions of much lower polydispersity. By summing up the molecular mass data of all these SEC fractions obtained using MALDI-MS, the broad polymer can reliably be analyzed.

6. Nomenclature

A	SEC peak area corresponding to the overall polymer mass concentration
A_j	SEC peak area corresponding to the jth fraction
a	Constant (Eq. (16))
B	Constant related to the detection sensitivity
C_1	Coefficient of linear SEC calibration curve (Eq. (8))
C_2	Coefficient of linear SEC calibration curve (Eq. (8))
$F(v)$	Measured SEC chromatogram
$G(v,y)$	The shape of unseen chromatograms originated from each molecular size
M	Molecular mass
M_0	The most probable molecular mass (Eq. (7))
M_n	Number-average molecular mass
M_{nj}	Number-average molecular mass in the jth fraction
M_w	Weight-average molecular mass
M_{wj}	Weight-average molecular mass in the jth fraction
N	Nomalization factor (Eq. (11))
n_i	Number of oligomer molecules having a mass of M_i
PD	Polydispersity
PD_1	Polydispersity in an SEC fraction
PD_0	Polydispersity in a very small SEC fraction
v	Variable (Eq. (4))
V_1	Start volume of an SEC fraction
V_2	End volume of an SEC fraction
V_a	Initial elution volume
V_b	Final elution volume
v_f	SEC fraction volume
$w(M)$	Molecular mass distribution function (Eq. (7))
$W(y)$	The height of chromatogram that would be obtained if resolution were perfect
y	Variable (Eq. (4))
β	Parameter denoting the breadth of distribution (Eq. (7))
σ_c	SEC band variance
σ_p	Parameter denoting the breadth of distribution (Eq. (13))

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