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Use of Pulsed Gradient Spin−Echo NMR as a Tool in MALDI Method Development for Polymer Molecular Weight Determination

Michaël Mazarin,[†] Stéphane Viel,[†] Béatrice Allard-Breton,[‡] André Thévand,[†] and Laurence Charles*,[†]

JE 2421 TRACES (Théorie, Recherche et Applications Concertées en Spectrométries), Universités Aix Marseille I & III, Faculté de Saint-Jérôme, Service 511, 13397 Marseille Cedex 20, France, and ARKEMA Centre de Recherche Rhône Alpes, Service Analyse, Rue Henri Moissan BP63, 69493 Pierre-Bênite, France

This study shows how mass spectrometry and pulsed gradient spin−echo (PGSE) nuclear magnetic resonance can be advantageously combined to achieve more reliable molecular weight information for polymers. Specifically, PGSE was shown to be a convenient tool for a rapid evaluation of M_w values to be further used as guidelines in matrix-assisted laser desorption/ionization (MALDI) sample preparation. PGSE calibration curves, established under given experimental conditions, were shown to be particularly robust, as they could be applied satisfactorily on different commonly available NMR instruments and different time frames. PGSE results were shown to compare well with size exclusion chromatography data used as a reference to validate this alternative technique. Moreover, because PGSE is relatively fast, it can be used interactively with MALDI analysis to check and understand mass spectrum profiles. This approach was first tested on poly(methyl methacrylate) (PMMA) standards and then successfully applied to determine the molecular weight of two unknown samples, a PMMA and a poly(ethylene glycol) monomethacrylate polymer.

Knowledge of a polymer molecular weight distribution is vital to correlating and understanding properties of a particular polymer system. Matrix-assisted laser desorption/ionization (MALDI) time-of-flight (TOF) mass spectrometry (MS) has been shown to be applicable to more types of synthetic polymers than any other MS technique and generally leads to spectra displaying singly charged oligomers with little or no fragmentation.[1,2] Molecular weight data can then be obtained for polymers of narrow polydispersity with precision and speed.[3−5]

A key point in the success of a MALDI-MS analysis is sample preparation, that is, proper matrix and cation selection. Because of the diversity of polymeric materials and the ambiguous role the matrix plays in desorption and ionization of the analyte, no standard protocol is available. Sample preparation methods are usually developed from published protocols that were shown to work for a given polymeric system. However, the choice of the experimental conditions for sample preparation should be based not only on the polymer chemical nature but also on its size. Indeed, different sample preparations are often needed for different molecular weight ranges within a polymer type.[6,7] For example, dithranol was shown to be a useful matrix for low-mass polydienes whereas *all-trans*-retinoic acid significantly improved the detection limit of greater molecular weight polymers.[8] As the polymer molecular weight increases, there is also a definite need to increase the molar ratio of the matrix to analyte in order to generate a strong polymer signal.[8−10] It has been shown by a number of groups that the distribution of the oligomers in MALDI mass spectra can depend on the nature of the cation added as part of the MALDI sample preparation.[11−13] The cation may thus have to be selected according to the size of the polymer. In addition, the instrumental conditions used to analyze oligomer ions by MS are to be considered as a function of the molecular weight. Typically, samples under 15−20 ku are analyzed in the reflectron mode whereas the linear mode is expected to provide higher transmission for higher weight species due to its shorter flight path and the absence of the refocusing region.[10] Moreover, the higher molecular weight ions are more massive and require a higher extraction, postacceleration, or both to maintain detector

* To whom correspondence should be addressed. Phone: (33) 491 28 8678. Fax: (33) 491 28 2897. E-mail: laurence.charles@univ.u-3mrs.fr.

† JE TRACES.

‡ ARKEMA.

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Table 1. Self-Diffusion Coefficients *D* and M_w Estimates of the Three PMMA Test Standards A, B, and C

std	M_w^{SEC}	D^a ($\times 10^{-10}$ m^2 s^{-1})	M_w^{PGSE}	PGSE accuracy[b] (%)	M_w^{MALDI}
A	4540	2.88 ± 0.06[c]	4400 ± 200[c]	3.1	3690; 3750[d]
B	40300	0.89 ± 0.02	38000 ± 2 000	5.7	40000[e]
C	106000	0.50 ± 0.01	107000 ± 4 000	1.0	102000[e]

[a] Determined by PGSE at 300 K in dilute $CDCl_3$ solution. [b]With respect to the M_w^{SEC} values supplied by the manufacturer. [c] Calculated from measurements performed on three distinct solutions at identical concentration. [d]Calculated from the $[M + Na]^+$ and $[M + K]^+$ distributions, respectively. [e] Values actually measured as M_p.

sensitivity.[14] Although not a prerequisite to MALDI-MS analysis, estimation of the polymer size range would help in rationalizing the development of MALDI sample preparation.

Such estimation is usually obtained by size exclusion chromatography (SEC), a well-established method for the routine molecular weight determination of polymers. At least for narrow-distribution polymer standards, many studies have shown similar molecular weight values were obtained from SEC and MALDI.[3,10,15,16] However, SEC analysis requires calibration with standards of identical or closely related chemical composition.[17] In case of inhomogeneous polymer samples, such as polymer chains with different end groups, considerable systematic errors may affect SEC determination of molecular weight distribution due to uncontrolled polymer/support interactions.[18] In addition, as a time- and solvent-consuming technique, SEC is not desirable when rapid analysis is required.

As an alternative, pulsed gradient spin−echo (PGSE)[19−21] nuclear magnetic resonance (NMR) can be used to estimate the molecular weight of a polymer from the measurement of its molecular self-diffusion coefficient. Self-diffusion is defined in the liquid state as the random translational motion of molecules due to Brownian movement and is described by the self-diffusion coefficient *D*, which depends on both the solvent viscosity and the molecular size.[22] The diffusion of polymers is well understood and has been extensively studied.[23] In addition, PGSE has been applied to investigate fundamental aspects of polymer diffusion[24,25] as well as to study polymer mixtures[26] and molecular weight distribution.[27−29] The weight-average molecular weight M_w of a polymer can be related to its self-diffusion coefficient *D*, as measured in very dilute solutions, according to[23]

$$D = KM_w^{-a} \quad (1)$$

where *K* and *a* are scaling parameters that depend on the polymer structure as well as experimental conditions (solvent viscosity and temperature). Typically, these scaling parameters are obtained by analyzing a series of monodisperse polymer standards under a given set of experimental conditions. In fact, similarly to SEC, the determination of M_w by PGSE is based on the hydrodynamic volume of the molecule and thus requires a calibration. However, the absence of a stationary phase is a clear advantage of PGSE over SEC as no adverse adsorption effect would be deplored. Moreover, as long as the contribution of the end groups to the hydrodynamic volume of a polymer chain is low, the use of standards having the same end groups as the analyte is not a requirement. Unlike other techniques such as photon correlation spectroscopy,[29] PGSE can be applied to study samples spanning a wide range of molecular weights. Finally, PGSE does not require any specific sample preparation and is a rather fast technique that can easily be performed on generally available NMR instrumentation.

To date, the use of PGSE in combination with MALDI for molecular weight determination has not been reported. In this study, commercial polymer standards were first analyzed to test the accuracy of M_w determination by PGSE. Then, PGSE was used as a preliminary technique to evaluate the M_w value of unknown polymers prior to their MALDI mass analysis. Specifically, this approach was applied to the analysis of poly(methyl methacrylate) and poly(ethylene glycol) monomethacrylate polymers.

EXPERIMENTAL SECTION

Samples and Reagents. Poly(methyl methacrylate) (PMMA) standards **A**, **B**, and **C** were supplied by Polymer Standards Service (Mainz, Germany) with molecular weight information as measured by SEC (Table 1). Seven poly(ethylene glycol) (PEG) standards (M_w = 200, 400, 1000, 1500, 26 000, 85 000, and 885 000) were from Sigma Aldrich (St. Louis, MO). Two unknown samples, PMMA and poly(ethylene glycol) monomethacrylate (PEGMMA) polymers, were provided by ARKEMA (Centre de Recherche Rhône-Alpes, Lyon, France). For MALDI sample preparation, dithranol was purchased from Bruker Daltonik (Leipzig, Germany) whereas 2,5-dihydroxybenzoic acid (DHB) was from Sigma-Aldrich. Potassium chloride, cesium fluoride, and sodium iodide

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were from Sigma-Aldrich. All materials were used as received. The solvent used in MALDI PMMA sample preparation was acetone (SDS, Peypin, France). Deuterated solvents used in NMR experiments (D_2O and $CDCl_3$) were from Euriso-Top (Saint-Aubin, France).

MALDI Sample Preparation. PMMA **A** standard solution (5.0 mg mL^{-1}) was mixed in a 10:10:5 volume ratio with dithranol (10.4 mg mL^{-1}) and KCl (5.1 mg mL^{-1}) solutions. PMMA **B** (4.3 mg mL^{-1}) or PMMA **C** (1.9 mg mL^{-1}) standard solutions were mixed in a 10:50:5 volume ratio with DHB (30.8 mg mL^{-1}) and CsF (14.0 mg mL^{-1}) solutions. All solutions of PMMA samples were prepared in acetone. A 1-μL aliquot of the different mixtures was deposited onto the sample plate and allowed to air-dry. The protocol applied to the PEGMMA polymer was a solvent-free sample preparation method, where 29.8 mg of dithranol was ground together with 2.3 mg of sample and 7.0 mg of NaI with a mortar and pestle for ~5 min. A few grains of the solid mixture were applied to the MALDI target then pressed with a small spatula to form a thin film.

Mass Spectrometry. MALDI-TOF MS experiments were carried out using a Bruker Autoflex (Bruker Daltonics, Leipzig, Germany). The instrument is equipped with a nitrogen laser emitting at 337 nm, a single-stage pulsed ion extraction source, and dual microchannel plate detectors for linear and reflectron modes. Positive-ion mode was used for all analyses with an accelerating voltage of 20 kV for linear mode and 19 kV for reflectron mode. The delay time used in delayed extraction mode was optimized based on the mass range of the individual polymer distributions.

PGSE Experiments. PGSE experiments were performed on three distinct Bruker Avance spectrometers operating at 400, 500, and 600 MHz for the ^1H Larmor frequency. The 400- and 600-MHz spectrometers were equipped with a 5-mm multinuclear inverse probe whereas the 500-MHz spectrometer was equipped with a 5-mm triple resonance inverse cryoprobe optimized for ^1H detection. All probes were from Bruker and were equipped with an actively shielded *z*-gradient coil. The gradient coils were calibrated by measuring the diffusion coefficient of the residual proton in D_2O[30] and were found to be 53, 55, and 56 G cm^{-1} for the 400-, 500-, and 600-MHz instruments, respectively. For all instruments, the temperature was set and controlled to 300 K with an air flow of 545 L h^{-1} to avoid temperature fluctuations due to sample heating during the gradient pulses. D_2O and $CDCl_3$ solutions were prepared by weighing an amount of sample directly into the NMR sample tube and adding 0.6 mL of deuterated solvent. For each sample, PGSE measurements were performed at different decreasing concentrations until reaching a constant D value indicating the dilution level was appropriate to apply eq 1. These repeated experiments increase the whole analysis time but are required for D measurement, hence M_w value, validation. Optimal concentration level was found to be 1.0 mg mL^{-1} for all samples except for the PEGMMA polymer, which was 0.1 mg mL^{-1} (see Results and Discussion section). In these conditions, no significant difference in solution viscosity was noted.

The pulse sequences used for the measurements in D_2O and $CDCl_3$ were based on a stimulated echo and a double-stimulated echo, respectively, and both sequences incorporated bipolar gradients and a longitudinal eddy current delay,[31] used for minimizing spectral artifacts resulting from eddy currents. Note that the double-stimulated echo sequence was used to avoid artifacts caused by thermal convection.[32] In these pulse sequences, the amplitude of a NMR resonance observed at the echo is given by[20,21]

$$I = I_0 \exp(-D(\gamma g \delta)^2(\Delta - \epsilon(\delta))) \quad (2)$$

where I_0 is the resonance amplitude at zero gradient strength, γ is the magnetogyric ratio of the observed nucleus, g and δ are the strength and the duration of the gradient pulses, respectively, and Δ is the diffusion time, i.e., the time during which the diffusion is monitored. $\epsilon(\delta)$ is a correction factor that depends on both δ and the pulse sequence. Usually, all delays are kept constant to avoid any complication arising from magnetic relaxation, and only the gradient strength is varied. Specifically, the gradient strength was quadratically incremented in 16 steps from 2 to 95% of its maximum value. Diffusion times and gradient pulse durations were optimized for each experiment to achieve at the largest gradient amplitude a decrease in the resonance intensity higher than 95%; typically, diffusion times between 150 and 1000 ms and bipolar sine gradient pulses between 1.0 and 2.3 ms were employed. The gradient pulse recovery time and the longitudinal eddy current delay were set to 0.1 and 25 ms, respectively. On average, a PGSE experiment lasted ~25 min, but recent development of this technique allows further time-saving to be envisioned in the next future.[33] After Fourier transformation and phase correction, the baseline of the spectra was carefully adjusted. The data were analyzed by plotting the signal intensities (areas) as a function of the gradient strength and fitting the resulting decays to eq 2 with a nonlinear least-squares fit.

RESULTS AND DISCUSSION

Similarly to SEC, PGSE determines relative M_w values and thus requires a calibration curve, which is established for a given polymer class under specific solvent and temperature conditions. In principle, however, the diffusion coefficient obtained by PGSE should not depend on the instrument used to measure it. In other words, provided that the temperature and the gradient coil of the probe are properly calibrated, a calibration curve obtained with one instrument should be applicable to any other, especially if standard NMR equipment is used.

To address this issue, three PMMA standards **A**, **B**, and **C**, used as "test standards", were analyzed by PGSE on a 500-MHz instrument. The so-obtained D values are reported in Table 1. To estimate their respective corresponding M_w values, a calibration curve established from a set of six PMMA standards analyzed on a 600-MHz instrument was used. These data had been obtained ~1 year before in the frame of another study (unpublished results). Figure 1 shows the calibration curve obtained by fitting the data to eq 1. The following relationship was found: $D = 2.92 \times 10^{-8} M_w^{-0.55}$. By using this calibration curve, the M_w values of

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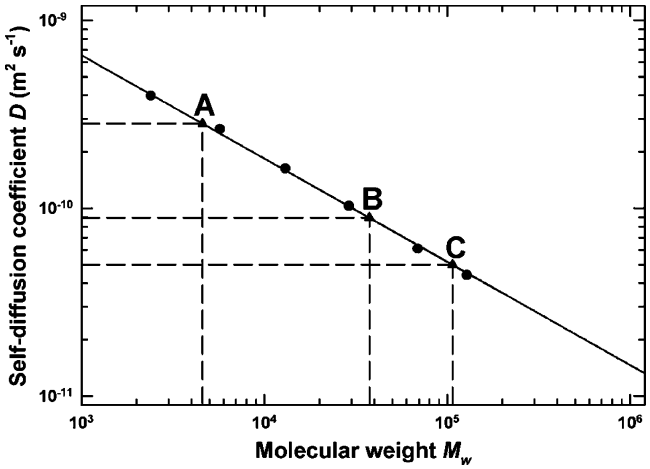

Figure 1. Double logarithmic plot of D versus M_w for a series of six PMMA standards (●) as measured by PGSE experiments at 300 K in dilute $CDCl_3$ solutions. By fitting these data to eq 1, a calibration plot is obtained (—), which allows the M_w of the PMMA test standards **A**, **B**, and **C** (▲) to be estimated from their respective D values.

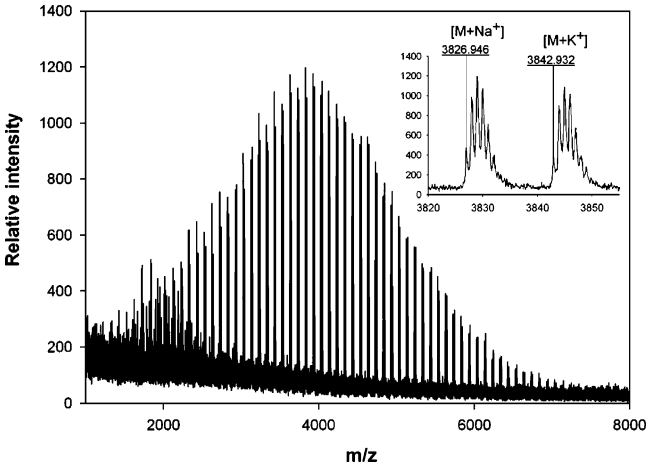

Figure 2. MALDI-TOF mass spectrum of PMMA **A** recorded in reflectron mode TOF, with 10-Hz laser frequency and 50% laser fluence. The mass spectrum is the sum of 128 shots. Inset: details of the mass spectrum showing PMMA **A** is detected as sodium and potassium adducts.

the test standards **A**, **B**, and **C** could be estimated (Table 1). Clearly, the SEC data and PGSE results are consistent, which confirms that PGSE is a valuable technique for determining M_w values. Data in Table 1 also show that a calibration curve obtained on one instrument can effectively provide pretty good accuracy when applied on another NMR spectrometer and in a different time frame. Therefore, for the studied standards, PGSE is shown to be particularly robust. In contrast to SEC, the calibration curve used in PGSE may thus be established only once, provided no major instrumental change occurs, allowing for considerable time-saving.

As indicated by both SEC and PGSE, M_w values estimated for PMMA **A** are ~4500. Sample preparation for MALDI analysis was then adapted from a published protocol developed for low molecular weight PMMA.[34] A MALDI mass spectrum was readily obtained in reflectron mode TOF, which showed two oligomer distributions (Figure 2). The appearance of many distributions is a common occurrence in the high-resolution analysis of polymers by MALDI and can be due to cation adduction with different ionic species. The two distributions, which could be attributed to sodium and potassium adducts, are of the same abundance in the mass spectrum although only a potassium salt was used in the sample preparation. The matrix is suspected to be the source of sodium, as described in a recent study that showed dithranol can be highly polluted with sodium, depending on the supplier.[35] We took advantage of these two distributions to calculate two M_w values, 3690 and 3750 from the $[M + Na]^+$ and $[M + K]^+$ distributions, respectively. MALDI results are in the mass range predicted by both PGSE and SEC but appear to be slightly underestimated, when taking these alternative techniques as references.

Applying the same sample preparation to PMMA **B** and **C**, no signal could be obtained, either in the reflectron or in the linear mode TOF. This result confirms sample preparation should be

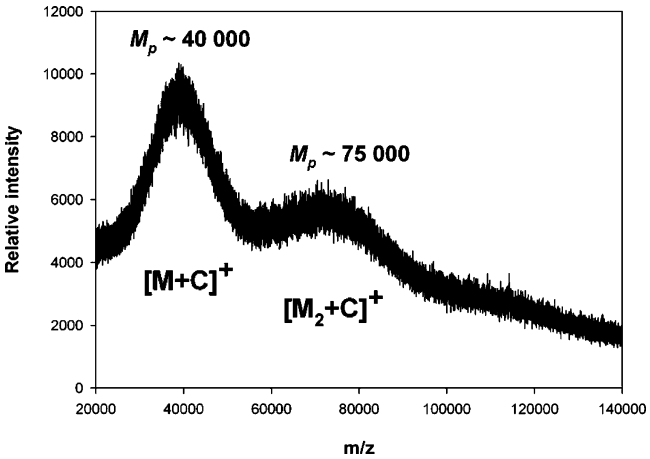

Figure 3. MALDI-TOF mass spectrum of PMMA **B** recorded in linear mode TOF, with 10-Hz laser frequency and 70% laser fluence. The mass spectrum is the sum of 500 shots.

adapted according to the analyte molecular weight within the same polymer class. Matrix-to-analyte molar ratio was then varied, as larger amounts of matrix sometimes help to better solvate increasing size polymer chains.[7] However, this approach was unsuccessful, and so was the use of alkali metals of increasing size as the cationizing agent. A new sample preparation was then developed by trial and error, starting from different protocols used for PMMA.[34,36] MALDI mass spectra were finally obtained for standards **B** and **C** using a combination of DHB and cesium fluoride. The associative preference of PMMA with cesium has been qualitatively demonstrated for small polymers,[36] and higher molecular weight oligomer ions are further expected to be more stable when associated with bigger cation.[34] Figure 3 shows the mass spectrum obtained from MALDI analysis of PMMA **B** in linear mode TOF. Two unresolved distributions are observed, from which only M_p values (peak average molecular weight) could

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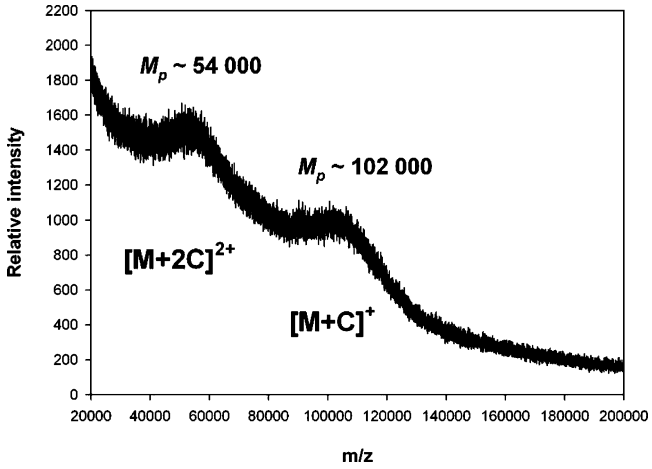

Figure 4. MALDI-TOF mass spectrum of PMMA **C** recorded in linear mode TOF, with 10-Hz laser frequency and 70% laser fluence. The mass spectrum is the sum of 500 shots.

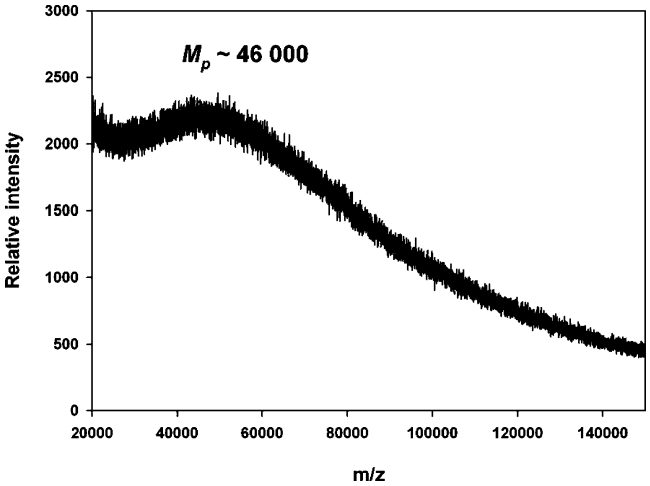

Figure 5. MALDI-TOF mass spectrum obtained for the unknown PMMA sample. The mass spectrum was recorded in linear TOF mode, with 10-Hz laser frequency, 70% laser fluence, and is the sum of 500 shots.

graphically be estimated to be about 40 000 and 75 000, respectively. As both SEC and PGSE data indicated M_w values for this sample around 40 000, the distribution detected at lower m/z in Figure 3 could thus be attributed to singly charged oligomers whereas sample clustering into dimeric ions would account for the higher m/z distribution. This clustering effect, already described in the case of PMMA, usually indicates favorable polymer chain associations.[5] However, experiments where matrix-to-analyte ratio was progressively increased gave rise to a general sensitivity loss and did not reduce aggregation phenomena. The best result obtained for PMMA **C** is presented in Figure 4. Based on SEC and PGSE indications, the two distributions observed in this MALDI mass spectrum could be attributed to a singly charged ($M_p \approx 102\,000$) and a doubly charged ($M_p \approx 54\,000$) oligomer distribution. During the MALDI process, multiple charging of increasing molecular weight polymers had already been reported, particularly in the case of PMMA.[5] Interestingly, a similar behavior was shown for polystyrene for which MALDI mass spectra of molecular weight below 100 000 exhibit singly charged dimers and trimers whereas multiple charged ions are formed from polymer of molecular weight above 100 000.[7]

Available M_w estimations prior to MALDI analysis are shown here to be extremely valuable as a guide for optimizing MALDI sample preparation as well as to understand signals observed in MALDI mass spectra. Data obtained for these two high molecular weight PMMA standards are summarized in Table 1. Although MALDI analyses systematically lead to lower molecular weight values as compared to SEC and PGSE, a pretty good agreement is shown between the three techniques. Note, however, that data reported as M_p are systematically lower than M_w values.[37] This allows PGSE to be applied to estimate the molecular weight of an unknown PMMA, for which no SEC data were available, prior to MALDI analysis. Unlike the PMMA standards analyzed before, the PGSE decay curve obtained for the unkown PMMA sample showed a slight deviation from the usual monoexponential decay, indicating a slight polydispersity of the polymer.[27] Studies reported in the literature[26−28] have shown that sample polydispersity may complicate the determination of diffusion coefficient from PGSE data. However, in the case of slight polydispersity, the monoexponential approximation can be used,[38] and accordingly, a value of 53 000 ± 3000 was found. The unknown sample was thus submitted to the sample preparation protocol that was previously applied successfully to PMMA **B** ($M_p \approx 40\,000$). Under these conditions, a MALDI mass spectrum was obtained in linear mode TOF that showed a single unresolved distribution centered around m/z 46 000 (Figure 5). In contrast to PMMA **B** experiments, the absence of cluster distribution may indicate polymer interactions are not favored in the case of this unknown sample, maybe because of chemically different end groups. Note that, even though a monoexponential fit was used to process PGSE data, the molecular weight estimates obtained by PGSE and MALDI were in good agreement.

The PGSE approach to predetermine sample M_w prior to MALDI analysis was then applied to a PEG-based polymer. This sample arose from the reaction of a PEGMMA polymer ($M_w \approx 2000$) with different species to produce macromolecules with functionalized end groups. Although polymers with large and chemically distinct end groups were expected, the PGSE calibration was built using H- and OH-terminated PEG standards, and the following relationship was established: $D = 6.51 \times 10^{-9} M_w^{-0.49}$. The PEGMMA polymer M_w was first estimated at 9000 from the D value (7.5×10^{-11} m^2 s^{-1}) measured on a 1.0 mg mL^{-1} solution. As done for all samples, the validity of this PGSE measurement was checked by analyzing solutions at decreasing concentrations. Indeed, intermolecular interactions may, for instance, result in "microaveraging effects" that were shown to lead to systematically biased D values.[39] A first dilution to 0.1 mg mL^{-1} yielded a much higher D value (1.12×10^{-10} m^2 s^{-1}), indicating the PGSE measurement from the 1.0 mg mL^{-1} solution was biased. A further dilution to 0.01 mg mL^{-1} did not significantly change the obtained D value (1.08×10^{-10} m^2 s^{-1}, i.e., within the

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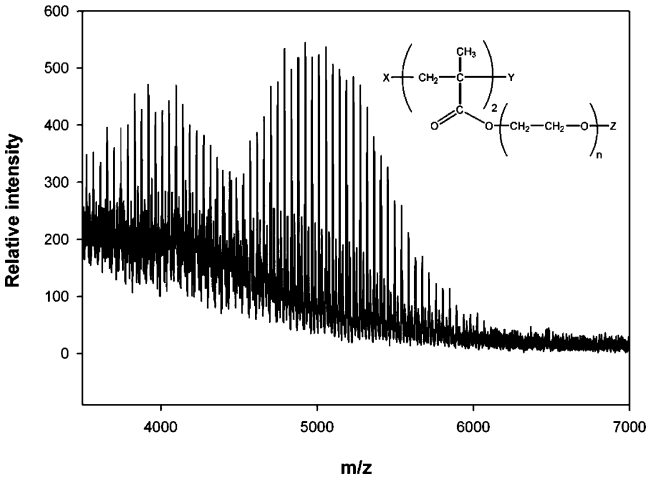

Figure 6. MALDI-TOF mass spectrum produced for the unknown PEGMMA sample using the solvent-free preparation method. The mass spectrum was recorded in linear TOF mode, with 10-Hz laser frequency, 45% laser fluence, and is the sum of 372 shots.

±2% experimental errors). This indicated that the last two solutions were sufficiently diluted and allowed an average D value (1.10×10^{-10} m^2 s^{-1}) to be used for estimating M_w at 4100 ± 200. It should be noted that, in the case of PMMA samples, the dilution did not modify the obtained D values, suggesting the extent of intermolecular interactions was lower. Finally, similarly to the unknown PMMA sample, a single-exponential fit was used to approximate the slightly non-monoexponential PGSE decay of the PEG-based polymer.

As for PGSE experiments, where it was assumed the PEG-based polymer could behave as a linear PEG polymer, MALDI sample preparation was first developed from a published protocol used for PEG.[40] Many experiments using different matrixes and cationizing agents were unsuccessfully performed. Solvent-free MALDI-MS has been shown to often yield better spectra than solvent-based MALDI-MS, particularly for synthetic polymers.[41,42] Therefore, this new sample preparation method was alternatively tested and gave rise to the mass spectrum shown in Figure 6. The mass spectrum was recorded from m/z 3 500 as a much more intense signal from unreacted PEGMMA dominated the mass spectrum in the low m/z range. This huge signal lead to detector saturation after a few laser shots and thus prevented less concentrated species to be detected. From Figure 6, four distributions could be observed, all corresponding to singly charged PEG-based polymers as the distance between two consecutive oligomer peaks within each distribution was found to be $\Delta m/z = 44$. Simple calculation allowed us to conclude each distribution corresponded to a distinct PEG-based polymer and did not arise from different cation adduction of the same polymer with different ionic species. In the low-mass range of the MALDI spectrum, M_w 4030 and M_w 4020 could be calculated from the main and the secondary distributions, respectively. In the high-mass range, data from the main and the secondary distributions were used to reach M_w 4990 and M_w 4840, respectively. These M_w values indicate the formation of polymeric species consisting of two PEGMMA units ($M_w \approx$ 2000) and different end groups (see inset in Figure 6). Calculation of the end group masses from the MALDI data should further be considered together with the chemistry involved in this polymer synthesis to fully characterize the polymer. Signal from the MALDI mass spectrum can fairly be utilized to propose structural hypotheses. Indeed, as PGSE did not indicate the presence of larger compounds in the sample, the observed ions would not arise from fragmentation, during the ionization process or the flight time, of much bigger ionic species, but would reflect the composition of the polymer mixture, at least qualitatively.

Noteworthy, MALDI and PGSE values are in good agreement although the PGSE calibration curve was established with PEG standards with very different end groups as compared to the PEG-based sample. This result confirms that, in contrast to SEC, the use of standards having end groups similar to those of the analyte is not a requirement in PGSE. Moreover, in contrast to MALDI, a single M_w value was reached by PGSE. Some M_w values indicated by MALDI could clearly not be distinguished in PGSE (such as M_w 4030 and 4020 or M_w 4990 and 4840). However, the two main polymer distributions observed in the mass spectrum should have been resolved in PGSE since it allows for a ±5% precision for M_w values, as established in this work. As mentioned before, a slight deviation from a monoexponential behavior was observed. This could be attributed to the presence of either a polydisperse sample or multiple components with similar molecular weights. Nevertheless, a monoexponential function was used to fit the signal decay as it would have been hazardous to predict the number of components in the mixture and thus the appropriate multiexponential fit to be used. However, in this study, PGSE was not aimed at a detailed description of the sample composition but was mainly used to give a first indication of the M_w range of polymer samples. In the case of the PEG-based polymer, PGSE provided good M_w indications to be used as a guide in the MALDI sample preparation. In addition, it allowed MALDI data to be interpreted.

CONCLUSION

The combination of PGSE and MALDI-TOF-MS was shown to be very helpful to rationalize MALDI sample preparation and also to confidently interpret MALDI mass spectra. The PGSE approach to polymer M_w evaluation can be chosen as an alternative to SEC because it is a rather fast, robust, and reliable method. PGSE does not suffer from typical SEC drawbacks, such as uncontrolled molecule adsorption on the stationary phase and the need for the polymer standards to have chemically similar end groups with the analyte. A great advantage of PGSE is that old calibration curves, obtained from a different instrument, can be used. Although repeated experiments at decreasing sample concentrations are required to validate M_w results, the duration of a PGSE analysis still compares well with that of SEC. Finally, this technique can be implemented satisfactorily on different commonly available NMR instruments.

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to the 600-MHz NMR spectrometer. All the experiments were performed using the MALDI-TOF-MS and the NMR spectrometers of Spectropole, the analytical center of Aix-Marseille Universities.

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