

Experimental investigation on the reliability of routine SEC–MALLS for the determination of absolute molecular weights in the oligomeric range

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Abstract

Absolute number-average molecular weights were carefully measured for very-low to low molecular-weight poly(diisopropyl trimethylene-1,1-dicarboxylate) polymers that had been obtained from diisopropyl cyclopropane-1,1-dicarboxylate using a living anionic ring-opening polymerization technique (degree of polymerization in the range of 11–45 and polydispersity indices <1.13). Results obtained from four different analytical techniques, including end-group analysis (¹H NMR), vapor pressure osmometry (VPO), size-exclusion chromatography coupled to a multi-angle laser light-scattering detector (SEC–MALLS), and matrix-assisted laser desorption ionization–time-of-flight mass spectrometry (MALDI–ToF), were compared and discussed. Although only crude estimates could be obtained by end-group analysis using ¹H NMR (experimental errors of up to 20%), \bar{M}_n values estimated by SEC–MALLS were in perfect agreement with results obtained by VPO and MALDI–ToF. As the overall experimental protocol had been designed to prevent bias arising from some initial knowledge upon the exact molecular weights by the operator during the SEC–MALLS experiments, these results confirm a previous claim that SEC–MALLS is effective in measuring molecular weights in the oligomeric range. © 2002 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Recent advances in cell design and electronics coupled to the availability of more powerful laser sources and very sensitive light detectors have enabled the commercial production of multi-angle laser light scattering (MALLS) chromatography detectors that, coupled to size-exclusion chromatography (SEC–MALLS), are theoretically able to cover a very large range of molecular weights [1]. Of particular interest is the claim by the manufacturer of a MALLS instrument that a monodisperse polystyrene sample of very-low molecular weight—down to 580—can be measured accurately by SEC–MALLS [1,2]. This claim, although never challenged in the literature, has in the author's experience met with considerable skepticism in the polymer community. In practice, it does not appear that examples of low molecular weight characterization using SEC–MALLS have ever been reported except of course for the claim mentioned earlier.

This paper summarizes the results obtained during a recent critical examination of the reliability obtained by SEC–MALLS when characterizing absolute molecular weights and molecular weight distributions in the low molecular weight regime (2500–10 000). Absolute number-average molecular weights were carefully measured for low-to-medium molecular weight poly(diisopropyl trimethylene-1,1-dicarboxylate) samples (PDiPTD) that had been obtained by living anionic ring-opening polymerization of diisopropyl cyclopropane-1,1-dicarboxylate (\bar{M}_n values in the range of 2–10 × 10³ and polydispersity indices <1.13). Results obtained from four different analytical techniques, including end-group analysis (¹H NMR), vapor pressure osmometry (VPO), size-exclusion chromatography coupled to multi-angle laser light-scattering detector (SEC–MALLS), and matrix-assisted laser desorption ionization–time-of-flight mass spectrometry (MALDI–ToF), are compared and discussed.

2. Experimental

Polymer samples were obtained using a procedure recently reported in the literature [3]. ¹H NMR spectra

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Table 1
Molecular weight and molecular weight distribution measured by NMR, SEC–MALLS, VPO, and MALDI–ToF

Sample numbers	\bar{M}_n ($\times 10^{-3}$)				\bar{M}_w/\bar{M}_n	
	NMR	SEC–MALLS	VPO	MALDI	SEC–MALLS	MALDI
1	3.04	2.49	2.42	–	1.13	–
2	6.04	5.96	5.99	5.6092	1.08	1.029
3	7.91	6.56	6.70	6.6249	1.08	1.027
4	9.2	8.31	8.49	8.2898	1.05	1.021
5	> 10.0	9.10	9.70	9.4172	1.06	1.018

were recorded on a 200 or a 300 MHz Varian spectrometer using CD_2Cl_2 as the solvent. SEC–MALLS analyses were obtained at 40 °C based on a setup including a Waters 510 pump, two SHODEX K80M and one K802.5 columns. Carefully filtered chloroform was used as the eluent at a flow rate of 1 ml min^{-1} . Appropriate standard precautions to avoid the presence of dust and insoluble residues in the eluent and polymer solution were implemented in all SEC–MALLS experiments. A Wyatt DAWN DSP multi-angle light scattering photometer and a Wyatt OPTILAB DSP interferometric refractometer were used as detectors. The refractive index increment (dn/dc) value of PDiPTD in chloroform was measured at 40 °C, using the Wyatt OPTILAB DSP refractometer in its stand-alone configuration ($\lambda = 632.8$ nm). The ASTRA 4.0 software (Wyatt Technology) was used to analyze the data. VPO measurements were conducted in toluene at 51 °C using a Jupiter VPO 833 instrument calibrated with sucrose octaacetate (MW = 678.6). All MALDI–ToF analyses were performed on a Bruker Reflex III MALDI mass spectrometer with a 2 GHz ADC. The instrument was externally calibrated using protein standards: bombesin (MW = 1620.8) and cytochrome c (MW = 12 361.1). Matrix solutions of 10 mg ml^{-1} dithranol in THF, 5 mg ml^{-1} polymer in THF were mixed in 1:1 ratio. One microliter of this mixture was spotted on top of KCl crystals sitting on the MALDI target. The spectra were collected using the reflectron detector in positive ion mode with laser power optimized to obtain the best signal/noise ratios.

3. Results and discussion

3.1. Polymer synthesis and analytical protocol

The synthetic procedure used in obtaining the poly(diisopropyl trimethylene-1,1-dicarboxylate) samples (PDiPTD) requested in this project has been described in a previous publication [3]. The living character of the polymerization leads to monodisperse polymers whose molecular weights can be easily adjusted by either varying the polymerization time (conversion) or the initial monomer/initiator ratio. For this study, five PDiPTD samples were synthesized under the same conditions, except for the polymerization time. The living nature of the polymerization also allows for the struc-

ture of the end-groups to be varied and controlled by the experimental conditions (nature of the initiator and end-capping reaction). In the present experiments, initiator and end-capping techniques were selected in order to introduce a phenylthio end-group on one side and a hydrogen atom on the other side. The structure of the polymers obtained under these conditions can be summarized by the following structural formula:



This structure, and more generally the associated family of polymers with two esters on every third atom on a carbon-only polymer backbone, had never been obtained before, and no information is available on the relationships between molecular weights and physical properties such as intrinsic viscosity.

All obtained polymers were characterized by end-group analysis (^1H NMR), SEC–MALLS, VPO, and MALDI–ToF in that particular order. Results for the molecular weight and molecular weight distribution measurements using these techniques are summarized in Table 1 and will be discussed in more detail later.

3.2. Molecular weight measurements by end-group analysis, VPO and MALDI–ToF

Number-average molecular weights (\bar{M}_n) for the five PDiPTD samples were determined by ^1H NMR based on the ratio between the signals at 5.0 and 7.1–7.4 ppm, which correspond to the CH on the isopropyl group and the five aromatic protons on the phenylthio end-group, respectively. The exact values are listed in Table 1. Number-average molecular weights were also measured by VPO, a reliable colligative method for polymers in the low molecular weight range (<25 000). A traditional plot of $\Delta V/c$ against concentration c for PDiPTD sample **5** is shown in Fig. 1. The absence of a slope in the obtained straight line indicates that the value of the second virial coefficient A_2 is very close to zero (Θ conditions), meaning that additional data treatments suggested in the literature is not necessary [4]. All other polymer samples (**1–4**) exhibited the same behavior as **5**. \bar{M}_n values were obtained from the corresponding $\Delta V/c$ versus c curves and are listed in Table 1. \bar{M}_n and \bar{M}_w/\bar{M}_n values were also measured by MALDI–ToF and are included in Table 1 as well. Further details on the

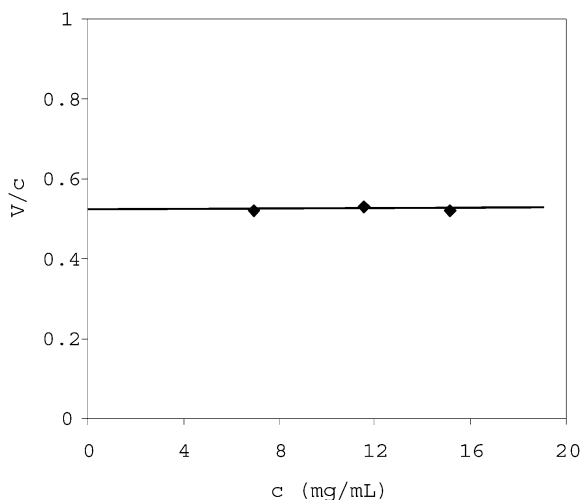


Fig. 1. $\Delta V/c$ versus c plot for PDiPTD sample 4.

MALDI–ToF experiments not directly relevant to this study will appear in a separate paper.

3.3. Molecular weight measurements by SEC–MALLS

The use of light scattering as a method for molecular weight measurements relies on the Rayleigh–Gans–Debye approximation (Eq. (1)):

$$\frac{Kc}{R(\theta)} = \frac{1}{MP(\theta)} + 2A_2c \quad (1)$$

with

$$K = \frac{4\pi^2(dn/dc)^2 n_0}{N_a \lambda_0^4}, \quad (2)$$

$$\frac{1}{P(\theta)} = 1 + \left(\frac{16\pi^2 n_0^2 \sin^2(\theta/2)}{3\lambda^2} \right) \bar{R}_g^2$$

where K is an optical constant, c the concentration of the polymer solution, $R(\theta)$ the excess Rayleigh ratio at angle θ , M the weight-average molecular weight, $P(\theta)$ a form factor describing the angular and size dependence of the scattered light intensity, \bar{R}_g^2 the mean-square radius of gyration, A_2 the second virial coefficient, n_0 the refractive index of the solvent, dn/dc the differential refractive index increment, N_a the Avogadro number, and λ_0 the wavelength of the incident light under vacuum [5,6].

When $c \rightarrow 0$ and $\theta \rightarrow 0$, Eq. (1) becomes:

$$\frac{Kc}{R(\theta)}_{c \rightarrow 0, \theta \rightarrow 0} = \frac{1}{M} \quad (3)$$

The value for $R(\theta)_{c \rightarrow 0, \theta \rightarrow 0}$ in Eq. (3) is not directly measurable, so a traditional light scattering experiment measures the light scattering intensity $R(\theta)$ at different scattering angles θ for a series of dilute polymer solutions. A Zimm plot in which $R(\theta)$ is plotted against $\sin^2(\theta) + kc$ enables the determination of the $R(\theta)_{c \rightarrow 0, \theta \rightarrow 0}$ value by extrapolating to

$c \rightarrow 0$ and $\theta \rightarrow 0$. Weight-average molecular weights M can then be calculated from Eq. (3).

In SEC–MALLS, a size-exclusion chromatography (SEC) system is combined with multi-angle laser light scattering (MALLS) and differential refractive index (DRI) chromatography detectors [1]. Light scattering intensities at different angles (with the MALLS detector) and concentrations (with the DRI detector) are obtained as a function of elution time. The chromatogram is divided into small slices that polymers eluting in the range of elution intervals defined by the slice limits have the same molecular weights.

In order to determine the sample concentration for each data slice in the chromatogram, two of the three requirements have to be met: (1) known dn/dc ; (2) known calibration constant α for the refractive index detector (Eq. (4)); or (3) 100% mass recovery. It is strongly recommended by the manufacturer to determine the value of dn/dc and calibration constant α since the assumption of quantitative mass recovery is questionable in most experimental setups [7]. Some preliminary experiments in our group to determine the molecular weight of a polystyrene standard based on the 100% mass recovery assumption indicated experimental errors of up to 20% for our system. As a consequence, dn/dc values for the polymer and calibration constant α for the refractive index detector were determined experimentally prior to the actual molecular weight measurements by SEC–MALLS. Based on the obtained numbers, the sample concentration of each fraction (for example the i th slice on the chromatogram), Δc_i can be calculated as follows:

$$\Delta c_i = \frac{\Delta n_i}{dn/dc} = \frac{\alpha(V_i - V_{i,\text{baseline}})}{dn/dc} \quad (4)$$

where Δn_i is the change in the refractive index compared to pure solvent, V_i and $V_{i,\text{baseline}}$ are the AUX signal and baseline voltages, respectively.

As indicated in Eqs. (2) and (4), dn/dc is a key experimental parameter that has to be known or determined off-line in a separate experiment. For homopolymers in the low molecular weight regime, dn/dc increases with molecular weight until a plateau is reached, generally around a molecular weight of 10 000 [1]. This means that, in theory, measurement of individual dn/dc values for each monodisperse polymer sample whose molecular weight is below 10 000 is required. In practice, however, experimentally measuring dn/dc is a very time-consuming process and an additional assumption was made in this study that the molecular-weight dependence would not notably affect the accuracy of the measurement. That this assumption is reasonable can be rationalized by analyzing Eqs. (5) and (6). If for a monodisperse sample, dn/dc for each fraction is assumed to be the same, by substituting Eqs. (2) and (4) in Eq. (3), one obtains:

$$\frac{4\pi^2(dn/dc)^2 n_0}{N_a \lambda_0} \frac{\Delta n_i}{(dn/dc)} = \frac{1}{M_i} \quad (5)$$

which in turn gives:

$$M_i = \frac{N_a \lambda_0 R(\theta)_{c_i \rightarrow 0, \theta \rightarrow 0}}{4\pi^2 (dn/dc) n_0 \Delta n_i} \quad (6)$$

It is known that dn/dc increases by about 4% from molecular weights 3000 to 10 000 for monodisperse polystyrene standards [8]. Eq. (6) indicates that assuming no variation for dn/dc value introduces a systematic error of 4% on the molecular weight during a SEC–MALLS measurement. Although the relationship between dn/dc and molecular weights is somewhat dependent upon the nature of the polymer, this example clearly indicates that only minor systematic errors will be introduced by the earlier assumption. In this study, the molecular weights of the PDiPTD samples are in the range extending from about 3000 to 10 000 (as previously estimated by end-group analysis), and a single dn/dc value of 0.0254 as measured off-line for sample **5** was used for all PDiPTD samples.

Another approximation that is traditionally used in SEC–MALLS is to neglect the $2A_2C$ term in Eq. (1) by maintaining the concentration of polymer very small [1]. Under these conditions, the intensity of a light scattering signal at 0° (magnitude of excess Rayleigh ratio) is proportional to the product of the solution concentration and the molecular weight. For a low molecular weight polymer sample, this requirement to work at low polymer concentration has to be balanced against the need to maintain a concentration large enough to generate a significant signal-to-noise ratio. In order to check the earlier hypothesis, the effect of the polymer concentration on the SEC–MALLS results was investigated. A series of solutions of PDiPTD sample **5** ($\bar{M}_n > 10\,000$ (NMR)) ranging from 5.0 to 75.0 mg ml⁻¹ were subjected to SEC–MALLS analysis under otherwise identical conditions. The results are summarized in Fig. 2, and demonstrate that the measured value of \bar{M}_n does not depend on the polymer concentration in the investigated range. A small variation that can be observed in the low

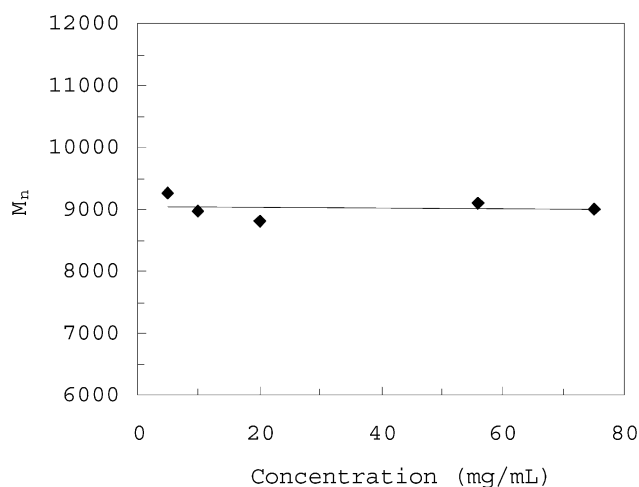


Fig. 2. Dependence of \bar{M}_n as measured by SEC–MALLS on the concentration of polymer in the injected solution.

concentration range is due to the difficulty to accurately measure the intensity of the weak signals obtained during the measurement.

As a result, molecular weights for the other PDiPTD samples were measured at relatively high concentrations (60–80 mg ml⁻¹) to obtain stronger signals and more reproducible results. The absolute values of \bar{M}_n and the distributions of all five PDiPTD samples measured using SEC–MALLS are listed in Table 1.

3.4. Comparison between molecular-weight measurement techniques

Soluble non-associative polymers can have their absolute molecular weights determined by several techniques—including colligative methods, end-group analysis, light scattering, and mass spectrometry, but most synthetic research groups do not appear to use absolute methods routinely, and rather focus on relative ones. A quick survey of the recent polymer synthesis literature indicates that most molecular weights reported for newly synthesized polymers of previously unknown structures were measured by two techniques only: capillary viscometry and size exclusion chromatography (also known as gel permeation chromatography (GPC)) calibrated with some polymer standards of unrelated structures (polystyrene, PMMA, etc.). Unfortunately, neither of these two techniques can provide more than a crude estimate of the absolute molecular weights when applied to new polymers. In most cases, viscometry only indicates whether the molecular weights are in the oligomeric regime or not, while examples are known where molecular weights measured by SEC will over- or underestimate very significantly the real values [9,10]. This apparent lack of interest in measuring absolute molecular weights in a systematic fashion for new polymers is surprising since accurate measurement of molecular weights and molecular weight distributions is of fundamental importance in polymer science and engineering: these molecular attributes directly impact materials properties, and knowledge of the molecular weights also helps in clarifying polymerization mechanisms and optimizing synthetic procedures. The focus on the two techniques mentioned earlier results from several factors, including inaccessibility to the relevant analytical instruments and total cost of the instrumentation needed (several techniques are generally required in order to cover a realistic range of molecular weights), time commitment and/or lack of local expertise.

Among absolute methods, light scattering is the only one (so far) that has the potential to make the determination of absolute molecular weight a more routine task. In theory, it can cover the entire range of molecular weights relevant to synthetic polymer chemists, from a few hundreds to hundreds of millions Daltons. Historically, however, light scattering methods have mostly been confined to high molecular-weight determination. This has generated a common misconception that light scattering is ineffective

at determining low molecular weights, while in fact, the range of molecular weights that can be covered is only limited by technical factors such as the power of the light source and the sensitivity of the photo-detector(s).

The polymer samples considered in this study are of low molecular weights (2500–10 000). As mentioned in Section 1, light scattering techniques can theoretically be used to measure molecular weight in that range [1,2]. Although it has been claimed by the manufacturer of the equipment used in this study that SEC–MALLS could be used to measure molecular weights down to several hundreds, there had been no other report on low molecular weight determination (<10 000) using this technique. Our experiments summarized in Table 1 indicate that the values of \bar{M}_n obtained using SEC–MALLS are in very good agreement with those determined by VPO. This finding strongly supports the manufacturer's claim and suggests that SEC–MALLS is a reliable technique in measuring low molecular weights for monodisperse polymers in the range 2500–10 000. A comparison between the molecular weights estimated by ^1H NMR and those measured by either VPO or SEC–MALLS shows differences of up to 20%. Such differences are not surprising due to the relative inaccuracy of end-group analysis by ^1H NMR. Among the analytical techniques used in this study, this method provides a very rapid and convenient way to estimate molecular weights, yet its accuracy decreases with increasing polymer molecular weights due to the increasing difficulty in measuring an accurate ratio between a strong signal and an always weaker end-group signal. When the molecular weight was relatively high, as in the case of sample 5, no value of sufficient accuracy could be obtained. The \bar{M}_n values determined by SEC–MALLS agree well not only with those determined by VPO but also with the MALDI–ToF values, which further confirms the earlier conclusions on the reliability of SEC–MALLS.

It must be emphasized that the experimental design used during this series of experiments prevented the operator in charge of the SEC–MALLS experiments to have more than a rough estimate on the molecular weight of the samples he was analyzing. This was purposefully designed so as to prevent any bias during the data treatment of the SEC–MALLS experiments (choice of a baseline and other parameters). Experience acquired during training periods in our group, using polystyrene standards of known molecular weights, had previously indicated the importance of a fair data treatment (baseline, normalization, etc.) on the quality of the final results and how possible bias from novice experimentalists played a significant role on the conclusions.

4. Conclusions

The results presented in this contribution include the first

reported example of molecular weight measurement down to 2500 using SEC–MALLS. The results were obtained for a new synthetic polymer with no a priori knowledge of the molecular weights. The \bar{M}_n values obtained by SEC–MALLS are in perfect agreement with the values from VPO and MALDI–ToF, corroborating previous claims from the commercial literature that SEC–MALLS can be used to measure molecular weight in the low molecular weight range (from 2000 to 10 000). The assumption made during the SEC–MALLS analysis that dn/dc is identical for all five PDiPTD samples does not cause any major error in the SEC–MALLS results. A comparison of the \bar{M}_n values determined using SEC–MALLS and MALDI–ToF with the values predicted by Poisson distribution illustrates that MALDI–ToF can provide very accurate values of the molecular weight distribution while SEC–MALLS overestimates the molecular weight distribution most probably due to the band broadening effect. Approximate values of \bar{M}_n with an error up to 20% can be estimated by end-group analysis using ^1H NMR.

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