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Fast and Accurate Determination of Absolute Individual Molecular Weight Distributions from Mixtures of Polymers via Size Exclusion Chromatography−Electrospray Ionization Mass Spectrometry

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ABSTRACT: We present a method to determine accurate and absolute molecular weight distributions (MWDs) as well as absolute concentrations of the individual macromolecular components in mixtures of polymers of the same monomer class yet differing in their end groups. Data gained from size exclusion chromatography coupled online to refractive index (RI) detection and electrospray ionization mass spectrometry (ESI-MS) are processed by a sophisticated computer algorithm based on the maximum entropy principle. The procedure yields, for the first time, absolute molecular weight distributions of each component corrected for chromatographic band broadening. Molecular weights of up to 10 kDa are accessible with a conventional quadrupole ion-trap mass analyzer. The method is applicable to variable polymer systems regardless of the monomer class and without the need for an external calibration or additional model assumptions. It was validated using binary and ternary mixtures of poly(methyl methacrylate) carrying labile functional groups. For the ternary mixture, maximum deviations of the reconstructed molecular weight averages and mass concentrations from the original values were 8% and 5%, respectively.

Introduction

Mass spectrometry has become a powerful and ubiquitous analytical technique in modern macromolecular science. In the past two decades, electrospray ionization (ESI)[1] and matrix-assisted laser desorption ionization (MALDI)[2] mass spectrometry have proven to be especially useful for the analysis of functional polymers. These soft ionization techniques allow the unfragmented polymer molecule to be introduced into the mass analyzer. Determination of the molecular weight of the individual polymer molecules with up to ppm accuracy, depending only on the performance of the employed instrumentation, is thus possible.

These exact mass measurements allow the determination of the chemical constitution of the polymer including the identity of the monomer repeat units and, more importantly, of the functional end groups. As every identified end group species involves a specific reaction pathway during the polymerization, essential mechanistic information can be obtained from the product range of polymerization reactions. A number of scientific publications including those from our own group show that electrospray ionization MS is an extremely powerful tool for the elucidation of reaction mechanisms in free radical polymerization.[3−8] The method yields data that are complementary to information obtainable by NMR and IR experiments.[9]

Accurate determination of the molecular weight distribution of polymers generated by controlled (including laser controlled) polymerization experiments stands at the basis of many modern approaches aimed at finding kinetic rate coefficients in free radical polymerizations. For example, pulsed laser polymerization−size exclusion chromatography (PLP-SEC), introduced by Olaj in 1985,[10] has significantly improved the determination of the rate coefficient of propagation, k_p. The experiment involves a nonstationary photopolymerization process initiated by the pulsing action of a laser. k_p can be easily derived from the inflection points in the obtained molecular weight distribution. The rate coefficient of termination, k_t, and its dependence on the chain length of the propagating radical can be determined by low-frequency pulsed laser experiments followed by an analysis of the molecular weight distribution.[11,12] These and other kinetic methods depend on an accurate determination of the full molecular weight distribution as well as the degree of polymerization, DP, as any error in the distribution or the DP is directly reflected in the accuracy of the measured rate coefficients.

Today, size exclusion chromatography (SEC) is almost exclusively used for the determination of molecular weight distributions. The method suffers a number of significant drawbacks. First, it requires calibration with polymer standards whose molecular weights need to be determined by independent techniques.[13] For many polymer classes well-characterized standards are not available. In these cases universal calibration, heavily relying on the accuracy of Mark−Houwink parameters and the validity of the Flory−Fox equation[14−18] or—alternatively—online calibration by light scattering and viscosimetric detection have to be employed, which can lead to errors in the molecular weight distribution of up to 30%.[3] Chromatographic band broadening further deteriorates the SEC results, with an especially strong impact on the apparent MWD of polymers exhibiting sharp peaks or shoulders as in the case of distributions derived from pulsed laser kinetic experiments.[19] Buback[20] and Schnöll-Bitai[19] have shown that k_p values obtained in the presence of typical chromatographic band broadening can be too low by as much as 20%.

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The mass-to-charge (m/z) axis in mass spectrometry is extremely accurate and does not suffer from the aforementioned impediments. MS therefore has the potential to circumvent the problems encountered in SEC with a significant impact on the quality of measured kinetic rate constants. A number of attempts at using MS with soft ionization to derive molecular weight distributions have therefore been made.[21−24] Application of MALDI-MS in pulsed laser experiments has also been investigated to some extent.[25,26] Despite the accuracy of the m/z axis, the values obtained by direct introduction of the polymer sample into the mass spectrometer are inevitably biased. This is because the complex physical and chemical processes involved in ion formation, mass spectrometric separation, ion transfer, and ion detection all show a functional dependence on the molecular weight/structure of the polymer.[27,28] Compared to MALDI, the abundance response in electrospray ionization exhibits a somewhat greater mass bias, and elucidation of the MWD from the spectra is further complicated by the occurrence of multiple charging.

We have recently shown that the individual strengths of both techniques—SEC with concentration detection and ESI-MS—can be successfully combined to derive very accurate molecular weight distributions of synthetic polymers.[29] In the employed chromatographic setup[30,31] (Figure 1), a concentration sensitive refractive index (RI) detector and the electrospray mass spectrometer are coupled to the chromatographic effluent of a size exclusion column. The method accounts for the individual strengths and limitations of both detectors by deriving the absolute polymer concentration solely from the RI-detector trace. The electrospray mass spectrometer is used only in its ability to accurately measure the concentration profiles of the individual oligomers eluting from the chromatographic system for further processing. No use is made of mass spectrometry to derive absolute concentration data. The elution profiles of the individual oligomers derived by MS contain accurate retention time information. This allows for a precise calibration of the SEC retention time dependence on chain length. A calibration can be derived without additional knowledge of the polymer class or any other physical assumptions as long as the polymer molecule is compatible with electrospray ionization. In addition to the position in time, the exact shape of the elution profile can be derived from online ESI-MS, which allows characterization of the chromatographic band-broadening function as well as corrections to be made for band-broadening effects in the derived MWDs.

Although MALDI could in theory be used as an alternative ionization technique, it must be coupled to liquid chromatography off-line. Hyphenation involves many individual work steps, such as sample fractionation and collection, matrix addition, drying, and measurement, making it a time-consuming and costly process. ESI-MS can be coupled easily online to liquid chromatography and is therefore ideally suited as a high-throughput, relatively low-cost liquid chromatographic detector.

Combining both data sets to gain absolute molecular weight distributions free from band broadening requires a sophisticated data processing algorithm. Use was made of the so-called maximum entropy (MaxEnt) principle for this purpose,[32−35] as data from the present kind of experiment contain a great amount of redundancy, usually leading to amplification of noise in the restored MWDs. With the employed system, MWDs of low and high polydispersity standards with a molecular weight of up to 15 kg mol^{-1} corrected for chromatographic band broadening could successfully be determined.[29]

Other detectors that are usually coupled online to SEC include light scattering photometers or viscosimetric detectors which yield quantitative information on the physical solution properties of the polymer as well as the absolute molecular weight and structural factors after some model assumptions.[13,36]

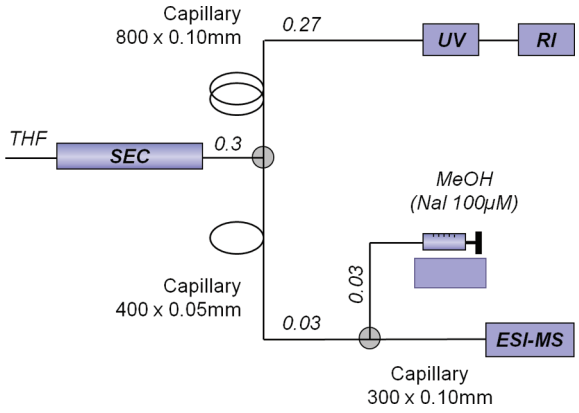

Figure 1. Chromatographic setup employed for coupling the concentration sensitive RI and UV detectors and the electrospray ionization mass spectrometer to the column effluent in parallel. Numbers indicate flow rates in mL min^{-1}.

No chemical information can be derived from these devices. Spectroscopic detectors that can be coupled online or offline to SEC, such as UV/vis, NMR,[37] and FT-IR detectors,[38] yield valuable chemical information about all species eluting from the chromatographic system at one time, but they usually lack the ability of molar mass or individual species discrimination.

The great potential of mass spectrometry in polymer analysis lies in the ability to measure the polymer molecular weight with an accuracy that allows both the unambiguous identification of the chemical identity of the polymer and its end groups as well as an accurate molecular mass calibration of the SEC system. In the current article we exploit this strength of mass spectrometry to successfully extend our previously described method[29] to elucidate the individual molecular weight distributions and absolute concentrations of components in mixtures of polymers. The method is based on the assumption that—although mass bias inhibits the direct measurement of the individual molecular weight distributions—ESI-MS can be used to successfully derive the relative concentrations of polymers eluting from the SEC column, as long as they feature the same chain length. To the best of our knowledge, this is the first time such an approach has been taken.

Knowing the identity of the individual polymer species together with their concentrations provides both the synthetic chemist and the polymer kineticist with new routes toward the characterization and optimization of polymerization processes.

Method

Chromatographic Setup. A parallel setup was employed for coupling the concentration-sensitive RI and UV detectors and the mass spectrometer to the chromatographic system (Figure 1).[30,39] In this way, all detectors could operate under optimum flow conditions and pressure strain on the RI detector was minimized. The column effluent was split in a ratio of 9:1 by introducing narrow silica capillaries of differing dimensions into the flow paths. In order to improve the ionization efficiency, a 100 μM solution of sodium iodide in methanol was added to the eluent stream, prior to introduction into the electrospray source. Flow rates, instrument settings, and salt concentrations were optimized to yield maximum ionization efficiency while keeping salt cluster formation to a minimum.[40] Capillary dimensions were chosen carefully in order to avoid a possible influence of viscosity gradients on the split ratio (see Supporting Information).

Data Processing. The current approach features a direct extension of the method of absolute molecular weight

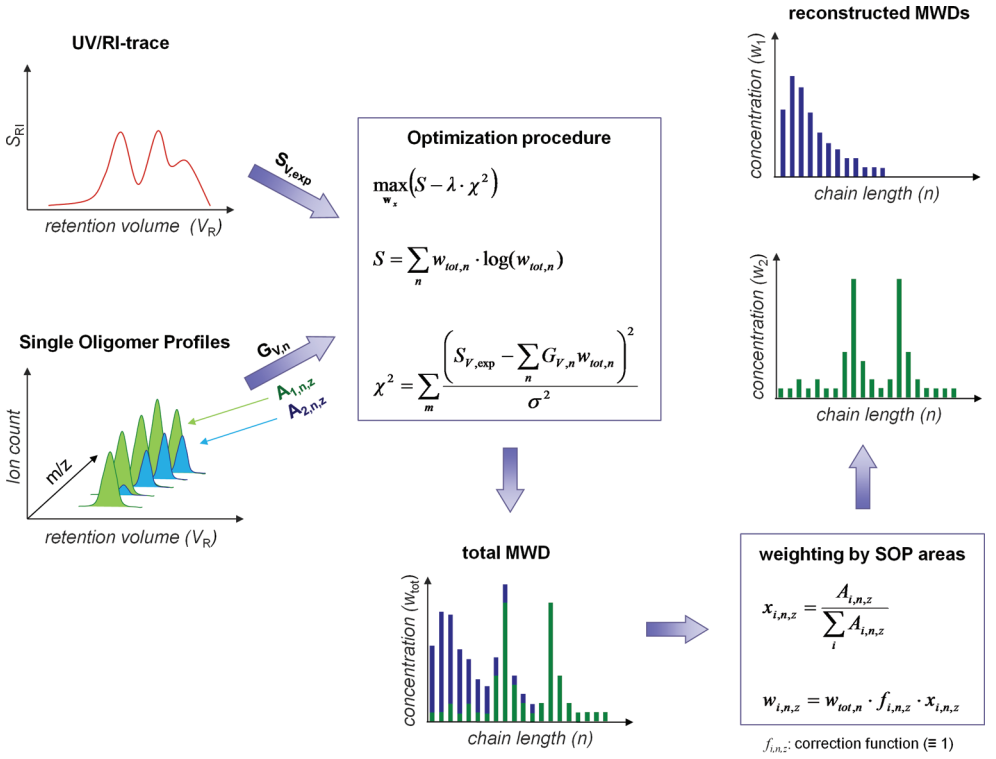

Figure 2. Flow diagram of the principal data processing approach. The instrumental calibration and band-broadening matrix $G_{V,n}$ derived by online mass spectrometry as well as the RI detector trace $S_{V,exp}$ are processed to arrive at the deconvoluted total molecular weight distribution w_{tot}. Weighting of w_{tot} by the areas of the SOPs of the functional oligomers $A_{i,n,z}$ yields the reconstructed individual molecular weight distributions w_i.

derivation for pure polymers (previously described by our group)[29] to mixtures of individual polymer species. The total molecular weight distribution w_{tot} of all polymer species is calculated from the absolute calibration and broadening data derived by online ESI-MS. Absolute mass concentrations are derived solely from the RI detector trace.

An assumption is made that ESI mass spectrometry can be used to successfully derive the relative concentrations of polymers eluting from the SEC column, as long as they have the same chain length. In other words, it is assumed that there is no significant end group bias on ionization efficiency. Furthermore, it is assumed that influences of the polymer end groups and the chain length of the polymer on the refractive index increment, dn/dc, are negligible. As demonstrated by a number of authors, this assumption is generally valid except for low-molecular-weight oligomers with less than about 20 repeat units.[41−43] We address this issue in the last section of the current study. A simple weighting procedure based on the area of the single oligomer profiles of the functional oligomers is then employed to derive the individual molecular weight distributions w_i (see Figure 2).

As can be seen in Figure 2, two data streams are generated from a chromatographic run. The trace recorded by the RI detector $S(V_R)$ corresponds to the total mass concentration of polymer eluting from the SEC column at a certain retention volume V_R. The mass spectrometer provides a two-dimensional array of data, which consists of the ion count recorded over a certain m/z range against the retention volume.

The so-called single oligomer profile (SOP) can be extracted from this array. It is the ion count recorded at the mass-to-charge ratio (m/z) corresponding to an oligomer molecule of chain length n and charge state z with monomer molar mass m_M, end group molar mass m_E carrying z cations with molar mass m_{Me}. This mass-to-charge ratio can be calculated from the chemical constitution of the functional polymer if known beforehand, or it can be derived from the recorded mass spectrum itself.

$$m/z = \frac{nm_M + zm_{Me} + m_E}{z} \quad (1)$$

The single oligomer profiles contain the retention time of every polymer species present as well as information on the band-broadening behavior of the chromatographic system, necessary to construct the calibration and broadening function $G(V_R,n)$.

In size exclusion chromatography, the functional relationship between the RI detector trace $S(V_R)$ and the total mass weighted molecular weight distribution $w_{tot}(n)$ of a mixture of polymers is given by Tung's equation.[44] For a mass concentration detector with linear response, the detector trace $S(V_R)$ is the convolution of $w_{tot}(n)$ with the instrumental calibration and broadening function $G(V_R,n)$.

$$S(V_R) = \int_1^\infty G(V_R,n) w_{tot}(n)\, \mathrm{d}n \quad (2)$$

Analogously to a pristine polymer, the total molecular weight distribution of all polymers in a mixture regardless of their end group can be calculated by inversion of the convolution equation (2). Unfortunately, the solution of (2) is a so-called "ill-conditioned" problem. Analytical inversion leads to an amplification of instrumental noise and a highly oscillatory behavior of $w_{tot}(n)$ with possibly negative values, lacking physical meaning.[32,45] Sophisticated numerical approaches therefore have to be used for the calculation of $w_{tot}(n)$. Maximum entropy methods[46−49] have successfully been applied in a number of related scientific problems in image reconstruction[33,34] spectroscopy[35] and chromatography.[32] We have therefore chosen this approach to calculate w_{tot} by the procedure outlined in Figure 2 (note the change in variable nomenclature, due to the fact that the procedure works with discrete data).

The employed algorithm starts by calculating the theoretical total mass−concentration trace from a trial molecular weight distribution w_{tot}. This concentration trace is then compared to the measured RI detector trace. The software iteratively manipulates w_{tot} to obtain the closest possible fit to the measured trace. The total squared sum of error (χ^2) is used to assess the agreement between the measured and the theoretical mass concentration trace. In a typical least-squares approach, the single objective would be to minimize χ^2, yielding w_{tot} as the maximum likelihood estimator of the molecular weight distribution. However, in the current case, such an approach would lead to an excessive amplification of noise from the RI detector trace and the aforementioned oscillatory behavior of w_{tot}. This is due to the fact that in SEC the individual oligomers elute very closely to each other in time, so that many individual elution profiles overlap. This feature of SEC complicates accurate calculation of the individual contribution of each oligomer to the RI detector trace and leads to great variance in the obtained distributions. The problem can be alleviated if a regularization filter—in our case maximum entropy (MaxEnt) regularization—is imposed on the estimated molecular weight distribution.[32] As can be seen in the inset in Figure 2 ("optimization procedure"), the optimization routine still aims at achieving a close fit to the data by minimizing χ^2. An additional entropy term, S,[47−49] introduced in the objective function by means of a Lagrangian multiplier λ, ascertains that the molecular weight distribution w_{tot} is as smooth as permitted by the instrumental variance σ^2 of the RI detector trace.[50] A more detailed description of this approach can be found in a previous publication.[29]

The molecular weight distribution of a single species w_i is subsequently calculated by simply weighting w_{tot} with the ratio $x_{i,n,z}$ of the SOP area $A_{i,n,z}$ of the species to the total area of all single oligomer profiles at the fixed charge-state z and repeat unit number n (see Figure 2, "weighting by SOP areas").

$$w_{i,n,z} = w_{tot,n,z} f_{i,n,z} x_{i,n,z} \quad \text{with } x_{i,n,z} = \frac{A_{i,n,z}}{\sum_i A_{i,n,z}} \qquad (3)$$

This approach is possible, even in the presence of strong molecular weight influences on the ionization efficiency, as only a comparison is made between the abundance of the different end-group-carrying polymers of the same repeat unit. Such a methodology is feasible as long as there is only a negligible effect of the end group on ionization efficiency in the electrospray source. Furthermore, all species with the same repeat unit need to arrive at the mass spectrometer at the same retention time, so that ionization occurs in the same chemical background. The latter assumption is valid in most cases, as the influence of the end group on hydrodynamic volume is typically negligible when compared to the polymer backbone. However, special care should be taken to avoid secondary interactions of the polymer with the stationary phase as well as adsorption on capillaries. To account for possible end group bias, a correction factor $f_{i,n,z}$ may be introduced. Because of a lack of a proper functional description of the ionization process, negligible end group influences were assumed in the current approach ($f \equiv 1$). A validation of this assumption as well as of the general applicability of the proposed method will be given in the following section.

Experimental Section

Materials. Methyl methacrylate (99%, Sigma-Aldrich) was freed from inhibitor by passing through a column of basic alumina prior to use. The RAFT agent cyanopropyl dithiobenzoate (CPDB) was synthesized according to literature,[51] and its purity was confirmed by ^{1}H NMR spectroscopy. 2,2′-Azobis(isobutyronitrile) (98%, Sigma-Aldrich) was recrystallized twice from ethanol prior to use. Copper(I) bromide (98%, Sigma-Aldrich) was purified by stirring in glacial acetic acid for 24 h, followed by washing with ethanol and diethyl ether and drying under high vacuum for 72 h. *N*,*N*,*N*′,*N*″,*N*″-Pentamethyldiethylenetriamine (Merck), methyl α-bromoisobutyrate (99%, Sigma-Aldrich), anisole (99%, Fluka), sodium iodide (puriss. p.a., Fluka), tetrahydrofuran (multisolvent, 250 ppm BHT, Scharlau), and methanol (chromasolv, Sigma-Aldrich) were used as received. Poly(methyl methacrylate) standards were received from Polymer Laboratories/Varian (Church Stretton, UK).

Cyanopropyl Dithiobenzoate-Mediated Polymerization of Methyl Methacrylate. A solution of cyanopropyl dithiobenzoate (431 mg, 48.7 mmol L^{-1}) and 2,2′-azobis(isobutyronitrile) (33.5 mg, 5.11 mmol L^{-1}) in methyl methacrylate (40 mL) was degassed by three freeze−pump−thaw cycles. The solution was heated to 60 °C for 150 min, after which the reaction was stopped by cooling in liquid nitrogen. The residual monomer was removed under vacuum and the polymer precipitated in cold methanol. Molecular weights determined by our previously described method[29] were the mass weighted average molecular weight M_w = 2550 Da, the number-average molecular weight M_n = 1560 Da, and the polydispersity index PDI = 1.64. The observed, rather broad PDI is consistent with previous observations in RAFT systems[52,53] at this low conversion; the end group identity was not affected.

ATRP of Methyl Methacrylate. In a typical procedure, methyl methacrylate (6.7 mL, 62.6 mmol) was diluted with anisole to 20 mL in a Schlenk tube, and the solution was degassed by three consecutive freeze−pump−thaw cycles. A second Schlenk tube containing copper(I) bromide (62.1 mg, 0.417 mmol) was evacuated and subsequently flushed with nitrogen two times. The degassed monomer solution was transferred into the Schlenk tube containing CuBr using a canula, and *N*,*N*,*N*′,*N*″,*N*″-pentamethyldiethylenetriamine (135 μL, 0.417 mmol) was added via a degassed syringe. After dissolution of the CuBr, the pale green solution was heated to 80 °C under constant stirring. The initiator methyl α-bromoisobutyrate (404 μL, 3.12 mmol) was rapidly added via a degassed syringe after which the solution turned dark green. The polymerization was terminated after 100 min by cooling in liquid nitrogen and opening to ambient air. The polymerization mixture was diluted with 40 mL of tetrahydrofuran, and the copper complex was removed by passing over a column of neutral alumina. Molecular weights of the standard used for preparation of the binary mixtures as determined by our previously published method[29] were M_w = 2410 Da, M_n = 1280 Da, and PDI = 1.89. The high PDI of the standard was most likely due to the low molecular weight of the polymer, requiring a high concentration of initiator at a reduced concentration of copper(I) catalyst. However, the end group fidelity of this standard was not significantly affected, and corrections were made for the small amount of saturated side product[54] formed during polymerization. Molecular weights of the standard used for preparation of the ternary mixture were M_w = 5260 Da, M_n = 4730 Da, and PDI = 1.11.

SEC/RI/ESI-MS. Spectra were recorded on a LXQ mass spectrometer (ThermoFisher Scientific, San Jose, CA) equipped with an atmospheric pressure ionization source operating in the nebulizer-assisted electrospray mode. The instrument was calibrated in the m/z range 195−1822 using a standard containing caffeine, Met-Arg-Phe-Ala acetate (MRFA), and a mixture of fluorinated phosphazenes (Ultramark 1621) (all from Aldrich). A constant spray voltage of 4.5 kV, a dimensionless sweep gas flow rate of 2, and a dimensionless sheath gas flow rate of 12 were applied. The capillary voltage, the tube lens offset voltage, and the capillary temperature were set to 60 V, 110 V, and 275 °C, respectively. The LXQ was coupled to a Series

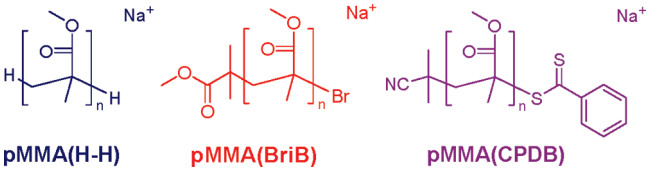

Figure 3. Structural formulas of the polymer standards used to prepare the binary and ternary mixtures for validation of the proposed method.

1200 HPLC-system (Agilent, Santa Clara, CA) consisting of a solvent degasser (G1322A), a binary pump (G1312A), and a high-performance autosampler (G1367B), followed by a thermostated column compartment (G1316A). Separation was performed on two mixed bed size exclusion chromatography columns (Polymer Laboratories/Varian, Church Stretton, UK, Mesopore 250 × 4.6 mm, particle diameter 3 μm) with precolumn (Mesopore 50 × 4.6 mm) operating at 30 °C. THF at a flow rate of 0.30 mL min^{-1} was used as eluent. The mass spectrometer was coupled to the column in parallel to an RI detector (G1362A with SS420x A/D). 0.27 mL min^{-1} of the eluent was directed through the RI detector, and 30 μL min^{-1} infused into the electrospray source after postcolumn addition of a 100 μM solution of sodium iodide in methanol at 20 μL min^{-1} by a microflow HPLC syringe pump (Teledyne ISCO, Model 100DM). 20 μL of a polymer solution with a concentration of 3−6 mg mL^{-1} was injected onto the HPLC system.

Results and Discussion

Three polymer species were used to validate the developed method (see Figure 3). Commercial standards of poly(methyl methacrylate) synthesized by anionic group transfer polymerization and carrying only hydrogen as end group are denoted by pMMA(H-H). These standards were mixed in different weight ratios with poly(methyl methacrylate) synthesized by atom transfer radical polymerization (ATRP) carrying a bromine end group denoted by pMMA(BriB) as well as pMMA(CPDB) synthesized by cyanoisopropyl dithiobenzoate-mediated reversible addition−fragmentation chain transfer (RAFT) polymerization.

Binary Mixture and Validation. The linearity of the mass spectral response is of primary concern when using the relative peak area ratios derived by mass spectrometry for concentration reconstruction. The dynamic range was therefore tested by varying the absolute concentration of a binary polymer mixture of pMMA(H-H) and pMMA(BriB) by a factor of 3 (see Figure 4) as well as by varying the concentration ratio of the two constituents over 1 order of magnitude (see Figure 5).

In the current method it is assumed that the end groups have only negligible influence on the ionization of the macromolecules. However, Gidden et al.[55] have shown for singly charged oligomers (n < 11) that this may not be the case. These authors used molecular modeling techniques in conjunction with ion mobility spectrometry (IMS) to determine the prevalent conformation of singly charged pMMA oligomer ions. It was found that a U-shaped conformation is assumed, with the metal ion bound to both carbonyl end groups of the oligomer, suggesting that end group functionality may play an important role in the ionization process. A decrease in end group bias is expected with increasing charge, as sites on the polymer backbone become more important for metal coordination. To our knowledge, no information on the conformation of multiply charged pMMA macroions is available. IMS of the more flexible poly(ethylene glycol) oligomers has shown that there is a trend from the more compact, globular structures toward open linear conformations with increasing charge due to Coulombic repulsion.[56,57]

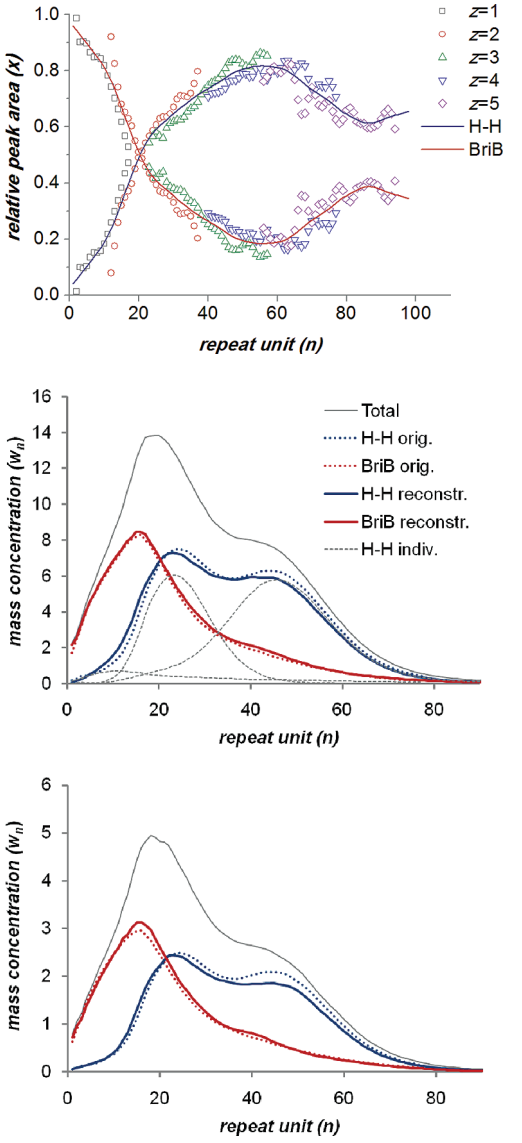

Figure 4. Deconvolution of a binary mixture of poly(methyl methacrylate) carrying hydrogen end groups pMMA(H-H) and bromine end groups pMMA(BriB). (top) Relative peak areas from the single oligomer profiles at different charge states and smoothing spline used for reconstruction of the molecular weight distributions. (middle) Original and reconstructed mass weighted molecular weight distributions of a 1:1$_w$ mixture of pMMA(H-H) and pMMA(BriB) with a total concentration of 6 mg mL^{-1}. (bottom) Reconstruction for the same 1:1$_w$ mixture but at a reduced total concentration of 2 mg mL^{-1}. To span a wider molecular weight range, the polymer pMMA(H-H) consisted of a 2:3$_w$ mixture of two polymer standards with manufacturer stated M_w of 2700 and 4930 g mol^{-1}. Some H-capped polymer was present as impurity in the standard pMMA(BriB) (see "H−H indiv.").

These conformational changes will have an additional influence on the end group bias in the different charge states.

The bromine as well as the dithiobenzoate end groups of pMMA(BriB) and pMMA(CPDB) are only weakly bound to the polymer backbone and can be easily lost if ionization conditions are too harsh. Electrospray ionization is arguably the softest method available to date for the ionization of synthetic polymers. Species that fragment during ionization or mass analysis will be underrepresented in the mass spectrum. Thus, successful restoration of the molecular weight distributions will serve as a proof of both, i.e., the absence of end group bias as well as the softness of the ionization process.

Figure 4 (top) shows the SOP peak area fractions derived by online ESI-MS detection. As can be taken from the figure, ions of the same repeat unit length may appear in two or more charge states. There was usually good agreement between all charge states except between $z = 1$ and $z = 2$, where the apparent abundance of the bromine functional polymer is overestimated for $z = 2$. This is possibly due to the low ion abundances of the doubly charged species at the low molecular weight end, leading to some bias due to baseline and noise influences. The effects of variations and discontinuities in the obtained area ratios on the individual molecular weight distribution were minimized by interpolation with a smoothing spline (continuous curves in the figure). Although smoothing was deemed necessary at this stage, some broadening may be reintroduced in the final molecular weight distributions by this procedure.

In Figure 4 (middle), the reconstruction of the individual molecular weight distributions from a binary $1{:}1_w$ mixture of pMMA(H-H) and pMMA(BriB) with a total concentration of 6 mg mL^{-1} is shown. In the case of the hydrogen-terminated polymer pMMA(H-H), which was free from impurities detectable by ESI-MS, the original molecular weight distributions were determined by our previously described SEC/RI/ESI-MS method.[29] The original distribution of hydrogen-functional polymer is given by the dotted blue curve in the figure. To span a wider molecular weight range, the polymer pMMA(H-H) consisted of a 2:3 mixture of two polymer standards with manufacturer stated M_w of 2700 and 4930 g mol^{-1}. Side reactions in the synthesis of the bromine functional poly(methyl methacrylate) by ATRP employing the aliphatic amine-containing ligand pentamethyldiethylenetriamine (PMDETA) lead to formation of a saturated side product, with the same structural formula as the polymer pMMA(H-H).[54] An estimated 10% of this impurity were present in the standard pMMA(BriB) used in the current validation. Therefore, it was necessary to calculate the original distribution of the pMMA(BriB) (red dotted line) already by a reconstruction using the current method. The three individual pMMA(H-H) distributions are given by the dotted gray curves in Figure 4.

The two restored molecular weight distributions are shown as continuous red and blue curves in the figure. An excellent overlap between the original and the reconstructed molecular weight distributions can be seen. The concentration of pMMA(H-H) is slightly overestimated at repeat unit numbers below 20, possibly due to some minor end group bias, whereas a slight underestimation occurs for the higher repeat unit numbers. Deviation of the reconstructed from the original weight-average (DP_w) and number-average degree of polymerization (DP_n) was lower than or equal to 4%. The reconstructed total mass concentration was around 3% lower than the original for the pMMA(H-H) and 4% higher for pMMA(BriB). The successful restoration can serve as an indication of the absence of significant end group bias on ionization in this case, as especially at the low molecular weights there is good overlap of the distributions. As can be seen in Figure 4 (bottom), a reduction of the total concentration of polymer by a factor of 3 to 2 mg mL^{-1} did not significantly alter the restored molecular weight distributions, except for a slightly enhanced underestimation of the concentration of pMMA(H-H) at around 50 repeat units.

No significant fluctuations in any of the reconstructed molecular weight distributions of pMMA(BriB) are witnessed. It is therefore concluded that the presence of a narrow molecular weight species in the spectrum does not seem to significantly influence the accurate estimation of the concentration of a second, broader distribution via the current method.

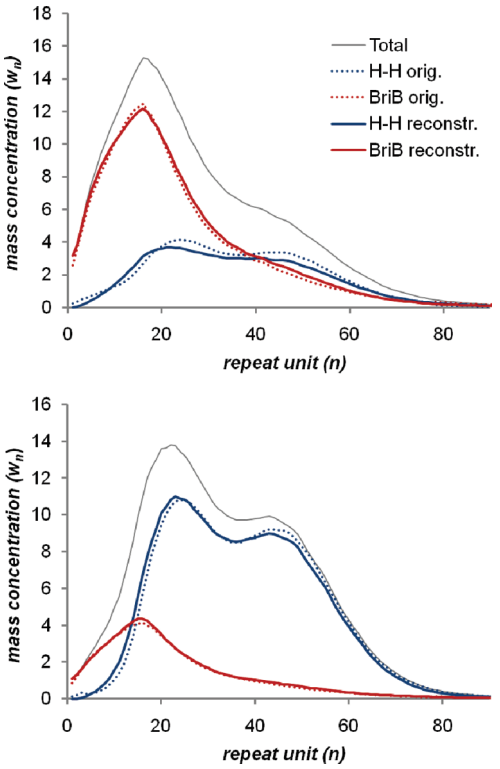

Figure 5. Variation of the component ratios of the binary mixture of poly(methyl methacrylate) carrying hydrogen end groups (H−H) and bromine end groups (BriB) with a total concentration of 6 mg mL^{-1}. (top) Reconstructed mass weighted molecular weight distributions of a $1{:}3_w$ mixture of pMMA(H-H) and pMMA(BriB). (bottom) $3{:}1_w$ mixture of pMMA(H−H) and pMMA(BriB). To span a wider molecular weight range, the polymer pMMA(H-H) consisted of a $2{:}3_w$ mixture of two polymer standards with manufacturer stated M_w of 2700 and 4930 g mol^{-1}.

To further assess the dynamic range of the method, the ratio of the components was varied by about 1 order of magnitude, while keeping the total concentration constant at 6 mg mL^{-1}. The results are shown in Figure 5. Restoration was virtually unhampered, when the relative concentration of the pMMA(H-H) was greater by a factor of 3 (Figure 5, bottom), except for a somewhat overestimated concentration of pMMA(H-H) at the lower molecular weights. Some influence could be seen upon increasing the relative concentration of pMMA(BriB) by a factor of 3, as the reconstructed total area of the hydrogen functional polymer was underestimated by around 8% (Figure 5, top). This effect may have been caused by additional impurities present in the polymer pMMA(BriB) that were not accounted for in the current data treatment, but which may have influenced the response of the ESI source and hampered the calculation of correct area fractions.

Ternary Mixture and Validation. With the current setup and procedure, the reconstruction of a mixture of three functional poly(methyl methacrylates) was attempted. The original molecular weight distributions of each species together with the reconstructions from a $1{:}1{:}1_w$ mixture of pMMA(H-H), pMMA(BriB), and pMMA(CPDB) are given in Figure 6. Generally a good agreement was attained between the individual original molecular weight distributions and those that were reconstructed. Table 1 lists the original and the restored molecular weight averages of each component together with the total area under the mass

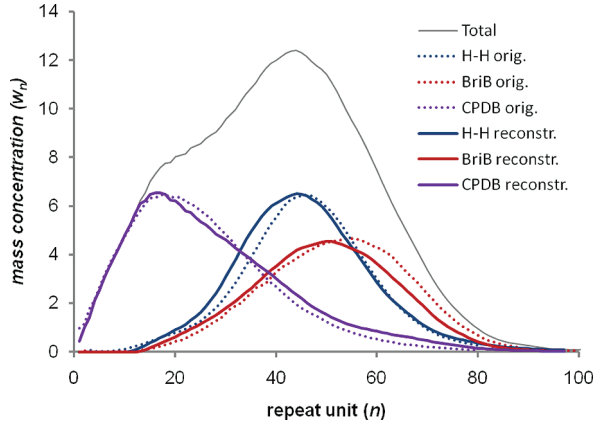

Figure 6. Reconstructed and original mass weighted molecular weight distributions for a ternary 1:1:1_w mixture of pMMA(H-H), pMMA(BriB), and pMMA(CPDB) with total concentration of 6 mg/mL.

concentration distribution. As can be taken from Table 1, the average degrees of polymerization of pMMA(CPDB) are overestimated by around 8%, whereas the degrees of polymerization of pMMA(H-H) and pMMA(BriB) are underestimated by around 2% and 5%, respectively. Agreement between the original and reconstructed area under the distribution (total mass concentration) is better, featuring a maximum deviation of 5%.

The roughly 5% lower molecular weight averages of the original distribution of pMMA(H-H) compared to the manufacturer stated values are in agreement with earlier findings. We are certain that the differences reveal systematic errors in the techniques applied by the manufacturer rather than in our method; this was proven previously by quantitative NMR measurements.[29] We cannot at this point give a conclusive explanation for the slight systematic deviations of the reconstructed molecular weight distributions of pMMA(BriB) and pMMA(H-H) to the lower molecular weights. End group effects may be at the basis of this. The cumyldithiobenzoate end group of pMMA(CPDB) has the potential to act as coordination site for the attached sodium ion but would compete with the harder Lewis base groups of the acrylate backbone. Since the absolute areas were accurately reconstructed, a degradation of the polymer species is also most likely not the cause of the current (very minor) bias.

The systematic error in the reconstructed molecular weights is in our view likely to be due to resolution limitations of the mass analyzer toward higher molecular weights as well as to possible unrecognized side products in the polymer standards. Baseline subtraction in the single oligomer profiles proved to be difficult in some cases due to background suppression and unresolved peaks, thereby influencing correct calculation of the SOP area ratios. Even more accurate reconstruction may be possible by the introduction of suitable baseline estimation and denoising techniques based on wavelet transforms.[58−60]

Limits of the Current Method. A number of authors have pointed out that—although RI detection generally gives a chain length independent response for higher molecular weight polymers—deviations of the refractive index increment dn/dc may be expected when very low molecular weight oligomers of only a few repeat units are analyzed.[41−43] We have recently quantitatively evaluated such a potential RI response dependence using a number of low-molecular-weight poly(methyl methacrylate) standards of low PDI,

Table 1. Comparison of the Reconstructed Molecular Weight Averages of the Ternary Mixture with Those Determined for the Pure Polymer Standards

	original				reconstructed			
	DP_w	DP_n	PDI	area	DP_w	DP_n	PDI	area
pMMA(H-H)	46.6	41.5	1.12	195	45.8	41.6	1.10	200
manufacturer	50.0	45.4	1.09		(−2%)	(0%)	(+1%)	(+3%)
pMMA(BriB)	52.6	47.4	1.11	195	49.8	45.2	1.10	185
					(−5%)	(−5%)	(−1%)	(−5%)
pMMA(CPDB)	25.5	15.6	1.64	215	27.4	17.0	1.61	220
					(+7%)	(+8%)	(−2%)	(+2%)

carrying only hydrogen as end groups, using tetrahydrofuran as solvent.[29] It was found that for the investigated polymer−solvent system RI detector response approached a constant limit value above 20 repeat units (see the Supporting Information for additional details). However, beneath this value a chain length dependence was observed, where the RI detector response decreased to about 40% of the limit value at low molecular weights. Because of additional potential contributions of the functional end group to the refractive index increment of the low-molecular-weight polymers, it was concluded that in the current case correction for molecular weight bias of the RI detector signal in the fashion of ref 29 would not be universal. Data recorded with the current method should therefore be regarded with some care in the molecular weight range beneath 20 repeat units, where increased dependence of the ESI response on the functional end groups may also be expected. Although with the current data no estimations of the molecular weight dependence of the RI detector response can be made, the successful reconstruction of the molecular weight distributions clearly demonstrates that influences of the functional bromine end groups are rather minor.

The mass range of the current method is restricted mainly by the fact that both mass spectrometry and size exclusion chromatography show a decline in resolution with increasing molecular weight. Coelution of the oligomer fractions, mass spectral peak broadening due to isotope effects, and mass overlap between oligomer ions in different charge states limit the number as well as the molecular weight of the components that can be successfully restored.

In order to obtain an approximation of the total number of end group species that can be distinguished by mass spectrometry, we introduce the so-called peak capacity, C. This is the maximum number of species with different end groups that can be fitted in the m/z space between two repeat units of the same polymer with monomer molecular mass m_M, without overlapping, assuming an equal spacing between the peaks.

To calculate C, a Gaussian instrumental spreading function of the MS with $\sigma_{MS} = 0.25$ Th was assumed, and the minimum distance between two resolved peaks was required to be 4σ. The ^{13}C isotope envelope of a single polymer peak was assumed to be normally distributed, with an estimated width σ_{iso}. The peak capacity, which is a function of the repeat unit number n, the number of carbon atoms per monomer unit n_C, the charge state z, and the natural abundance of the heavier ^{13}C isotope $p = 0.0107$, is thus approximated by the following equation:

$$C = \frac{m_M/z}{4\sqrt{\sigma_{MS}^2+\sigma_{iso}^2/z^2}} = \frac{m_M/z}{4\sqrt{\sigma_{MS}^2+nn_Cp(1-n_Cp)/z^2}} \quad (4)$$

For large repeat unit numbers, the impact of instrumental spreading on the peak capacity is outweighed by the

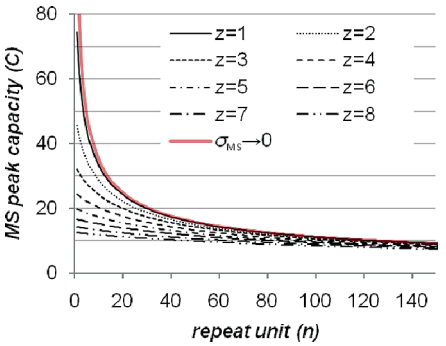

Figure 7. Mass spectral peak capacity C for the analysis of poly(methyl methacrylate) (m_M = 100 u) with a typical quadrupole ion trap MS featuring a spectral peak width of σ = 0.25 Th.

broadness of the isotope envelope, and eq 4 can be reduced to

$$C = \frac{m_M}{4\sigma_{iso}} = \frac{m_M}{4\sqrt{nn_C p(1-n_C p)}} \quad (5)$$

The dependence of the peak capacity on the charge state is eliminated in eq 5. This behavior can be seen in Figure 7, for the analysis of poly(methyl methacrylate), where the peak capacities of all charge states reach a common asymptote ("$\sigma_{MS} \rightarrow 0$") around a repeat unit number of n = 140 that is inversely proportional to the square root of n. At 80 repeat units, a theoretical number of 10 evenly spaced peaks can be resolved by the mass spectrometer in the charge state z = 5. As can be taken from eq 5, this number can only be improved by increasing the monomer molecular mass or by working with isotopically pure monomers. We suggest that in a real mixture around half of the number of peaks estimated by C can be successfully restored with the current SEC/ESI-MS method. We base this assertion first on the fact that in reality peaks are not evenly distributed over the spectrum. Second, limitations of the chromatographic separation leading to overlap between species of different charge states in the mass spectrum were not included in the calculation of C.

It should be mentioned that the current estimate of the peak capacity is valid only for instruments whose resolution is insufficient to yield isotope resolved spectra at higher charge states, which is the case for the quadrupole ion trap MS employed in the current analysis. It is expected, however, that high-resolution time-of-flight, Fourier transform ion cyclotron resonance, or Orbitrap mass spectrometry can significantly enhance the number of resolvable species by up to an order of magnitude. This great potential improvement is due to the fact that for these mass analyzers the broadness of the isotope distribution is not the limiting factor anymore, since isobaric species can often be resolved. The instrumental resolution itself and not the isotope envelope will therefore limit peak capacity at the high molecular weight end for high-resolution MS.

Conclusions

For the first time a method has been introduced to gain quantitative molecular weight data of the individual components in mixtures of polymers differing in their end groups. The method is calibration free with regard to both the construction of a correct molecular weight axis as well as the calculation of the relative species concentrations. It is therefore applicable to a broad range of polymers with molecular weights of up to around 10 kg mol^{-1}, as long as these are amendable to electrospray ionization. It is expected that future improvements in data processing as well as the use of higher resolving instruments will broaden the accessible molecular weight range to up to 30 kg mol^{-1} as well as further improve the already high accuracy of the attainable data.

A validation of the method was given by the successful restoration of molecular weight data from binary and ternary mixtures of polymers. Slight variations could be seen upon variation of the concentration ratio over 1 order of magnitude, but generally an excellent agreement between the restored and original MWDs was achieved. Systematic errors in the reconstructed molecular weight averages and total mass concentrations of the binary and ternary mixtures were less than 8%.

Although a final proof of the absence of end group influences on the MWDs is impossible, the successful restoration serves as an indicator of the excellent ability of online mass spectrometry to function as a quantitative end group selective detector. End group bias will certainly depend on the chemical nature of the end group, such as the presence of surface active or coordinating groups as well as its size. We are certain however that for the typical polymer systems obtained via free radical polymerization SEC/RI/ESI-MS may be used to gain accurate molecular weight and concentration data.

Because of the extreme softness of the ionization process, SEC coupled to electrospray ionization is ideally suited for the fast and accurate analysis of polymers produced by controlled/living radical polymerization and other processes furnishing the polymer with very labile functional groups. The method has great potential use as an analytical tool in the kinetic investigation of polymerization processes, in high throughput experimentation, and in synthetic polymer chemistry.

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Supporting Information Available: Additional SOP peak area ratios for the analyzed polymers, data on the analysis of the variation of the RI detector response with molecular weight, and information on the proper selection of the split capillary dimensions. This material is available free of charge via the Internet at http://pubs.acs.org.

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