8 Automated Data Processing and Quantification in Polymer Mass Spectrometry

8.1 Introduction

The interpretation of modern polymer mass spectra is a process that usually requires intimate knowledge of both, the synthetic chemistry of the investigated polymer as well as the measurement process itself and the instrumentation at hand. The growing usage of mass spectrometric tools in the polymer community also leads to an increasing number of nonexpert users of the technique. Sophisticated tools for the automated processing and interpretation of mass spectra have the potential to significantly increase the acceptance of mass spectrometry (MS) as a versatile technique to determine characteristics in raw materials, while at the same time providing an operator-independent and reproducible outcome of the interpretation process.

Specially when-considering quantitative information, for example on the compositional distribution of copolymers or the molecular mass distribution (MMD) of homopolymers—operator-independent approaches for spectral pre-treatment are highly desirable. Improved data processing and analysis methods have the potential to maintain the efficiency and accuracy of investigations by implemented techniques, while at the same time providing the user with a user-friendly tool for online liquid chromatography-mass spectrometry (LC-MS). The following sections give an overview of the tools that are currently available to the polymer community and should guide the interested scientist in the selection of suitable tools for optimal interpretation. The reader will realize that—although tools have matured in areas such as copolymer characterization and molecular mass determination—the field of automation in polymer MS is in many areas still in its infancy.

8.2 Data and File Formats

Once the physical process of spectrum acquisition has been carried out, entry data analysis effort—automated or manual—requires importing of the mass spectral data...
It's often observed that the processed data is either displayed graphically, or in a tabular format. The current situation features a high degree of vendor-specific, proprietary data formats in MS, which may vary significantly even between two instruments of the same vendor. The negative impact on scientific data exchange and efforts to extend spectral interpretation needs to be addressed. Faster, more general-approach methods, such as direct infrared spectroscopy instrumentation (DSM) or matrix-assisted laser desorption ionization mass spectrometry (MALDI) without chemically functionalization of the sample can be utilized as alternative data sources, and these are currently handled in the form of simple text files for data input, which is supported by most software.

However, in the last years, the trend is data exchanged within the polymer MS community toward the realization of increasingly more sophisticated software, allowing for more extensive analysis and closer integration with current instrumentation. Techniques such as mass spectrometry (MS/MS), liquid chromatography/mass spectrometry (LC/MS), Fourier-transform infrared spectroscopy (FTIR), and Raman spectroscopy have been increasingly used to analyze polymer structures at a molecular level. FTIR analysis is particularly useful for the determination of functional groups in polymers and provides valuable information about polymer functionality and composition. The multivariate data analysis (MDA) from these approaches require sophisticated software to compare masses and label the possible data features for further analysis. The analysis of mass spectrometric data requires expertise in polymer science to understand the results. The implementation of a common database for the polymer community is a necessary step to achieve this goal.

As a result, the current situation is one of a need for improved data analysis tools and standards. The development of software tools that can handle and interpret the data efficiently and accurately is essential for the polymer community to advance in the field. The integration of these tools into a common database and the development of a standardized data format are crucial steps in this direction.
8.3 Optimization of Initiation Conditions

Finding the optimal settings and experimental conditions at which the reactants/initial conditions and the reaction are in such a way that they generate the desired chemical reaction. In M1, a great number of chemical and instrumental parameters exist that the reaction can vary. With regards to polymers analysis by M1, depending on what the goals of the paper are, the experimental conditions and the objective is to be optimized near these. The experimental setup and sensitivity may be important variables in the optimization, as the chemical is more abundant in certain types of polymers. Monodispersion in mass seems like a convenient technique to measure polymerization. For example, the introduction of living control radical polymerization protocols has led to new kinds of functional polymers having high molecular weight, high monodispersity, or high molecular weight, which is limited to the polymer transients by intrinsically weak carbons—halogen—in the chain. Especially with monomer polyenes, such as polypyrrole, this yields a high M1.M1. This leads to information from these molecules in the case of metallic contamination or each M1 in this case, each group M1. For example, M1. The introduction of living control radical polymerization has been discussed with each M1. The introduction of functionality is therefore a third important objective to polymer M1 requiring optimization, especially when mechanistic and structural studies are to be performed.
The physical processes affecting the performance of the initiation source, mass separating process, and ion detection are often only insufficiently understood and conditions depend critically on the type of mass spectrometer employed. With only little as a priori knowledge of the optimal conditions, the number of parameters to be sampled is very large. Often, source optimization in both MALDI-TOF and ESI-MS is performed in a one-factor-at-a-time fashion [14]. This approach, although straightforward to perform, may not yield the best conditions, as interactions between parameters cannot be identified [15, 16]. Design of experiments (DOE) is a useful tool that can be employed to significantly reduce the number of experiments required in optimizing ionization conditions, while ensuring maximum certainty in the effects of the experimental parameters and their interactions. This can be achieved through statistical experimental designs and graphical evaluation of the experimental data by rigorous analysis with computer models [17]. In addition, many different approaches of both to LC/MS optimization exist in literature, mainly covering the optimization of the liquid chromatographic separation [18, 19], but not necessarily covering the optimization of MS source conditions [18, 19, 21]. More recently, full and concerted efforts successfully employed a genetic search method [22] to achieve a more comprehensive but fully automated source optimization of LC-MS instrumentation in polyamide 650 ES-MS (20) and in chromatography HSQ-MS (20). These authors stated that the method could yield optimum conditions by sampling less than 50% of the possible 360 parameter combinations and that relationships between source parameters were identified that accounted for much of the success of the optimization. The hypothesis generating potential of genetic search processes in which prior knowledge of the system under study was not demonstrated.

A selection of other trials that are closely related to polymer MS. Witting et al. [23, 24], for example, employed an orthogonal experimental design to identify parameters that significantly affected signal intensity in polymer analysis by MALDI-TOF MS. These and other parameters including detection voltage, laser energy delay, laser energy, extraction voltage, and laser voltage, detector voltage, and delay time were chosen to be studied. These voltages were varied in the context of standard instrumental mass bias, which is one goal when employing MALDI-TOF to generate isotopic MS/MS standards. Multivariate numerical optimization [24, 25] was employed to this end, and the effects of instrumental noise on the optimization procedure were dealt with by the use of simulated filtering [26]. Optimal values of the instrumental parameters were obtained as low as the linearity and the confidence interval of the parameters were gained which serve as a verifiable evaluation of the effects of each parameter.

Such a method can be especially useful when optimizing either LC-MS of synthetic polymers. Here, the operator is faced with the challenge of having to find optimum ionization conditions in a system where the concentration of analytes during a chromatographic column is changing rapidly as a function of time. In such cases, parameters often need to be varied between chromatographic runs, and the goal is certain maximum formation from a minimum amount of chromatographic noise in a very short instrument time. Grounding of et al. presented a method based on a
8.4 Automated Spectral Analysis and Data Reduction in MRS

Numerical spectral analysis is an essential tool in the overall study of MRS. It is typically carried out by using highly developed computer software that is specifically designed for the analysis of NMR spectra. The software is used in the selection of spectral regions of interest, the determination of accurate and precise peak positions, and the calculation of peak intensities that are used in quantification. The peak intensity is calculated by

\[ I = \frac{\text{Area under the peak}}{\text{baseline width}} \]

where \( I \) is the peak intensity. The peak intensity is then used to calculate the concentration of the sample.

\[ C = \frac{I \times \text{Area normalization factor}}{\text{Sensitivity factor}} \]

where \( C \) is the concentration, and the sensitivity factor is determined experimentally.

The peak intensity is then used to calculate the concentration of the sample. This is done by comparing the peak intensities to the peak intensities of known samples.

\[ C = \frac{I_{\text{unknown}}}{I_{\text{standard}}} \times C_{\text{standard}} \]

where \( C_{\text{unknown}} \) is the concentration of the unknown sample, \( I_{\text{unknown}} \) is the peak intensity of the unknown sample, \( I_{\text{standard}} \) is the peak intensity of the standard sample, and \( C_{\text{standard}} \) is the concentration of the standard sample.

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statistical or numerical point of view, these may be impossible to distinguish from genuine peaks. This section needs access to the following questions:

1. When is a given excitation correctly classified as a genuine peak? (statistical significance)
2. At what location is the peak most likely located? (peak location)
3. Does it overlap with other sources? (peak resolution)
4. Where does a peak begin and end? (integration and location)
5. What is the area of the spectrum underneath the peak? (peak integration)

An answer to the first question is used to separate true peaks from operational peaks. Answers to the second question is required for species identification and is used predominantly in qualitative analysis. The third question must be answered to determine if two or more peaks overlap as a result of insufficient mass-to-charge resolution. Operational peaks may need to incorporate peak positions and intensity determinations. Knowledge of the location of the peak beginning and end, the fourth question, is required to determine peak areas. Peak area, in turn, is typically required for quantitative analytical results. Sufficient answers to these questions will result in a reliable translation between the spectrum and the matrix the sample within to determine. Figure 2 properly answers these questions through ready access at any point in time and location.

8.1.4 Long-Range Approach

A standard approach to the reduction of means of spectra data has been used on calculating either derivatives or intensity thresholds of the data. A few of the many references in the literature can be found in refs. [2, 11]. Typically, excitation from the baseline are found at locations in the first derivative. As the algorithm proceeds sequentially through the data, typically 17 even numbers from low to high, a (initial) maximum of the derivatives, or as an increase in intensity above a given threshold, indicates a peak beginning. A peak maximum is found when the derivative after an initial increase反而 to zero or less. As the algorithm proceeds sequentially, the derivative will change sign as these passes continue on zero or less, and the intensity will drop below the given threshold value, as the baseline is restored.

Many variations of this basic method exist. For example, second derivatives may be used to find peak maxima. In some cases, third derivatives may also be employed. There are two significant problems that one encounters when using these derivative-based approaches. First, the functions whose derivatives are approximated by only a few point is not simply to the function itself, but also to the function’s derivative. This results in a loss of accuracy in the derivative approximation, especially as the function becomes more complex. To this end, the use of higher-order derivatives as a means of identifying features that may be more critical to the overall analysis.

Furthermore, the higher the derivatives, the more sensitive the function to variation in the dataset. However, the higher the derivatives, the more sensitive the function to variation in the dataset. This sensitivity can lead to overfitting of the data, which may result in incorrect peak identification. To address this issue, smoothing techniques can be applied to the measured data to reduce noise and improve the accuracy of peak detection. In conclusion, while derivative-based approaches can be effective in peak detection, they require careful consideration of the derivatives order and the impact on the overall analysis.

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In this case, smoothing or filtering of the data in space may ensure existence and convergence of the filter estimates and remove singularity. However, using the standard results for these single methods, it can be proved that these methods have a strong tendency to oscillate.
244 If an accurate measurement of polymer mass and molecular weight are
required, two methods are generally employed: 1) a mass spectrometer,
which uses a high-energy electron beam to fragment the molecule and
capture its mass and charge, and 2) a gel permeation chromatograph,
which separates molecules based on their size and shape.

In a mass spectrometer, the sample is introduced into a stream of
evaporating gas, and the fragments are collected on a detector.
Mass spectrometers can measure the mass of the polymer fragments
to a high degree of accuracy, allowing for precise determination of the
molecular weight. This method is particularly useful for polymers with
a wide range of molecular weights, as it can measure both the
smallest and largest fragments.

On the other hand, gel permeation chromatography (GPC) involves
passing a polymer solution through a series of columns with different
diameters and porosities. The solvents of different molecular weights
are eluted at different rates, allowing for the separation of the
different polymer molecules. GPC is a more time-consuming method,
but it is particularly useful for determining the molecular weight
distribution (MWD) of the polymer, which is crucial for understanding
the mechanical properties of the polymer.

In summary, both methods have their advantages and disadvantages,
and the choice of method depends on the specific needs of the
application. Mass spectrometry is better suited for high-precision
measurements of molecular weight, while GPC is more versatile for
polymer characterization, especially when considering MWD.

Figure 1: Illustration of mass spectrometer and gel permeation chromatograph.
many differences across the spectrum. In order to match the peaks the 
autocorrelation coefficient increases. This can be seen in the figure where 
sections of peaks, one of three repeat and between spots are clearly seen and the 
peaks shift in phase and magnitude. Frequency and magnitude are better 
visualized when the spectrum is displayed on a side-by-side basis. Figure 5.9 
demonstrates small peaks on either side of the main autocorrelation series. These are to 
verify repeated and individual peaks in-related frequencies. For this case, these peaks are 
likely caused by simple rotation of the original spectrum would be extremely difficult. 
The next application is a mass calibration. If the top and most of the sample is 
known, a scan can be used to plot the slope that was set out of the calibration curve. 
This serves to improve mass accuracy because if the slope can be 
compared then the peaks patterns are more accurate. This is important when validating 
and extracting or edit mass. 

A software tool (PDAv2) which has recently been introduced by Ruhland and 
Indik allows the number of massing units and resolution, as well as an 
approximation of the MMD to be obtained from the multiplexed spectra 
recorded in a manner (8.0±0.4). The software appears by retaining the 
differences from a summated mass spectrum and the measured FF spectrum and 
then plots the ionizer and isoleak mass and estimates of the MMD of the 
polymer, which is aimed to be Gaussian in shape. The software begins to be 
functioned and extended extending the mass range of 55-655 to around 65-6551 
range, if multiple settings is allowed. Programmatic extensions are needed to allow 
the analysis of masses of multiple and group-pinning polymers. 

2.4.4 Time-Domain Segmentation

An alternate method in calculating local deviations is to consider the spectrum as a 
whole and to use it as a reference for segment regions based on this feature. As 
shown in Figure 5.1 by connecting the first (a) point to the last (b) in the 
appropriate, a random is obtained for the entire spectrum is defined. From there, the (c) 
point to the generator ideal center then the line is determined. This produces 
linearizing approximations the spectrum. This procedure is continued and the spectrum 
compared and corrected if the segment with each point determined is minimum 
by least squares and the interfering baseline determined (as previously mentioned) by 
a simple line segment. After the spectrum has been augmented, least squares or 
orthogonal distance estimator (OD) may be used to find the line segment to best fit 
the data, however, cannot be continued because if the random noise level varies 
across the spectrum the quality of the fit will also vary across the spectrum. For 
this reason, the FIT method [5] uses a background spectra taken at the same 
instrumental conditions as the spectra to be subtracted with a sample that is free 
of extracal (e.g., in the case of DMD, contains the matrix and its extracalibration set). 
A background spectrum requires additional experimental effort but yields significant 
advantages when analyzing for data to determine quantitative 
measurement. 

For this application, the software tool (PDAv2) was employed [8, 9]. Linear 
feature to LI LI linear
Figure 4.1: Schematic representation of the automated detection of a small particle in the final product. The gray circles represent actual positions, and the blue circles represent peak positions. Superimposing the calculated relative areas for each peak in the area of the elongated matrix after a certain number of iterations can provide a visual representation of the particle distribution.

Our segmentation method is a recursive algorithm. The first partition requires the selection of a single point, and is derived from the earlier work of Douglas and Forsyth [36]. Remaining points are selected based on an iterative procedure that identifies points whose orthogonal distance from the end-point connecting line segment to the new point is greater than the orthogonal distance from the line segment to the closest segment endpoint. Once a point with greater orthogonal distance from the line segment is identified, the algorithm selects a new segment endpoint and a new orthogonal distance to any endpoint connecting line segment drops below a prescribed threshold value. This threshold value is the only algorithm parameter and is based on a statistical analysis of the data and the corresponding filter and sharpness operators. Clearly, the selection of these points does not require any speed data; therefore, the method is equally well suited for TDP data expressed in either time or space.

Finally, it is chosen to work in time-space with the data as in most...
basic state and orientation for doing a point-by-point correlation of intensity (partial samples). The second phase of the algorithm, developed specifically for this work, requires the solution of an optimization problem, specifically, finding strategic peak heights (i.e., adjusting strategic point values) values that are associated strategic outputs which minimize the sum of orthogonal distance from each data. The problem is a nonlinear (and nonconvex) optimization problem that can be accomplished quickly using a recently developed software programming algorithm [57].

The algorithm works as shown in Figure 8.335. (54). Clearly this method requires no knowledge peak shape and no preprocessing of the data (e.g., smoothing) but it does require equal spacing of data points, i.e., the strategic points defining the beginning and end of adjacent peaks are located in the same step resulting from the choice of step in the underlying function for the determination of the strategic output.

Once the data is fully segmented, strategic peaks are discarded in accordance with the mathematical analysis of the original data and in its corresponding analytic data set. This definition of ‘strategic protein negative data integration threshold’ is performed by the underlying algorithm for peaks and peak areas. Once a collection of peaks and peak areas has been accumulated, the spectrum with samples are then analyzed. Each peak identified from the spectrum with samples is compared to peaks found in close relative position from the analytic-free spectrum algorithm output (i.e., peaks that agree with similar time or mass coordinates). If any peak in the spectrum with samples has a smaller peak height or smaller peak area than mass about 90% of the background spectrum peaks in close proximity, then that peak is ignored. Likewise, any peaks that fall outside the statistically significant mass error and height errors are also discarded. Then, no peak is identified from the sample spectrum that has not been identified by height or area from the background spectrum. This discarding of strategic peaks also serves to prevent the inclusion of inclusions of larger peaks into a set of smaller peaks. This can sometimes occur if the noise in the analytic spectrum much greater than the noise in the corresponding background spectrum.

The final set of strategic peaks has been found, the corrected area of the polyethylene (polyethylene is shown, but not always a triangle in this algorithm) will be used on polyethylene of any number of vertices connected by true analyte. The new algorithm also allows for the determination of a new baseline. The mathematical basis for the polyethylene area calculation algorithm is Green’s theorem in the plane and can be interpreted as repeated application of the fundamental vector for integration [59]. The results across the entire area of the polyethylene.

Figure 8.4 shows an example of a MALDI-TOF mass spectrum of polyethylene having three different end groups. Without assumption, but with the requirement of a background spectrum for the scale of definition of the number of protons, the algorithm is able to identify and integrate peaks without smoothing or testing any assumptions on peak shape. In this case, the areas calculated from the triangular shapes defined by the three-vertices points for each peak were calculated; however,
The practical details and applications of self-initiated MS to analyze copolymer structures have been discussed in a preceding chapter and shall not be reiterated here. However, two purposes are copolymer characterization: in addition to a determination of the endgroup structure, it is possible to gain the copolymer composition.

8.5 Copolymer Analysis

The practical details and applications of self-initiated MS to analyze copolymer structures have been discussed in a preceding chapter and shall not be reiterated here. However, two purposes are copolymer characterization: in addition to a determination of the endgroup structure, it is possible to gain the copolymer composition.
where $n_0$ is the mass of the achiral metal cation.

Special interpretations can be attempted in a generally very straightforward manner. If it is a hypothesis about the constituent moieties and the red group of the polymer can be made, the initial copolymer spectrum can be modeled based solely on $E_k$. A problem, however, is that theoretical copolymer distributions are functions of a number of monomer units, which in turn gives little to no information. This is due to the fact that differences in polymerization of certain moieties can occur, which will greatly affect the overall molecular weight and the amount of polymer formed. It is thus important to consider the effects of these differences in the overall copolymer distribution, given that the effects of mass bias on the initiation of oligomers with differing configurations can be neglected.

Mathematical tools developed for the early 1960s for the quantification interpretation of copolymer spectra have been reviewed by an extensive review by Pinkham.9) This early work focused mainly on the use of postulated models of the copolymerization process. The use of these models to simulate theoretical copolymer distribution can aid special interpretations by comparison of model spectra with the spectra measured in reality. It is shown that using MADIX-DRG with appropriate chain models, the average composition, $c$, of copolymers could be determined with $c = 0.6$. An evaluation for a method in which no assumptions about the polymers must be made is also possible using the following expression (6.10, 6.15):
Wilkins later at MIT among the authors demonstrated that results from MALDI-MS can be used to determine the full multidimensional distribution of molecule composition and chain length. X-30. These authors also employed random sampling techniques in the case of blockcopolymer formation to test quantitative spatial incorporation. Their work was later followed up by Strobl at S09 and recently by Williams et al. (X, 77) and Strobl at X92, 77) who used the results for the determination of molecular parameters in the radical copolymerizations (X, 40, 77) as well as for the mechanical investigations of polycondensation reactions (X, 40,.77). These authors also derived a new fingerprint-like feature (figure X) from the spectra. The fingerprint-like feature was initially employed by Still and co-workers of the same chain length as determined from the mass spectra. They provide a fairly easy to interpret the polymer mass spectrum and the form of the distributions used to detect the variation in the type of the analyzed polymer (pipe vs. random). Williams et al. recently employed the
Data Interpretation in MS/MAS

Advanced fragmentation techniques in MS have been around for some while and have been extensively used in the determination of polypeptide sequence by bottom-up proteomics (78, 79). The complementary interpretation of the very informative backbone spectra obtained from MS/MAS is greatly facilitated by the availability of high-sensitivity data-processing software and de novo-sequence tools, which are an indispensable part of contemporary proteomics (5, 6). In recent years, MS/MAS has also become a topic of largely increasing popularity in the field of synthetic polymer characterizations. The fragmentation pattern of macromolecules can provide detailed information on the structure of the constituent monomer-building blocks as well as on the attached end groups. A number of studies have established the mass degradation patterns of common polymers such as PDMS, poly(dimethylsiloxane), polytetrafluoroethylene, polyethylene glycol, poly(propylene glycol), polyethylene oxide, and poly(styrene) (WS-94).

Software for the automated interpretation of synthetic polymer tandem mass spectra may prove to be a valuable tool for the determination of the backbone structure and end groups of organic and inorganic polymers as well as for the analysis of the chain structure of copolymers. The advances in software development and the large number of spectroscopic software solutions in the market make it possible to achieve results comparable to those obtained in the laboratory using MS/MAS. In fact, there is only one such tool available developed by Tiersmann et al. (2012) that, however, greatly aids interpretation of medium mass spectra and which provides for the large amount of data. An overview of the software is given in Figure 8A. Taking mass profile input on the reporting monomer units and the co- and co-end groups as well as the type of the attached units, the software automatically assigns the recorded peaks to fragment ion spectra, followed by a color coding of the peaks making further spectral interpretation highly intuitive. Toxicity assessments of the end groups can be quickly obtained, which...
8.7 Quantitative MS and the Determination of MMIs by MS

MS with soft ionization has evolved into a powerful analytical tool to characterize molecular weight for both low and high polymers. MALDI-TOF MS and ESI-MS are especially useful for the analysis of synthetic polymers. A large database of MS data is present in various databases containing molecular weight information on the terminal oxidation of synthetic polymers. These databases can be used to estimate the molecular weight of individual polymer molecules. Although MALDI-TOF MS and ESI-MS yield molecular weights of individual molecules, accurate MMIs of synthetic polymers require sophisticated spatial processing approaches. This is because, intrinsically, molecular polymers do not exhibit a...
uniform chain length but not in distribution of molecular weights. Although in HPLC, the molecular weight area is certain, due to instrumental bias and a dependence of intensity efficiency on molecular weight and charge size, distributions of oligomer sizes can not be accurately determined due to oligomer concentration in the analysis sample. Classical methods used for the determination of molecular weight by SEC yield accurate information about the concentration of the polymer. The molecular weight area through analysis in SEC and running calibration procedures may introduce errors of up to 30% in the obtained molecular weights [99]. In the following text, two approaches, developed independently at the National Institute of Standards and Technology (NIST) and at National Institute of Technology (NIT) [99, 100], respectively, are described and evaluated. The first approach is based on a comparative study of the SEC-MS data acquired with NIST's mass spectrometer, using three different calibration curves of the mass spectral intensity (A). The second approach (see Section 12.7.2) utilizes the use of SEC coupled online to a quantitative-scanning electron probe mass (Q-SCAPES), which allows molecular mass calibration and broad matching criteria are achieved using peak data obtained from online SEC [99, 100].

12.7.2 Quantitative MS/MS Measurement by MALDI-MS²

The accuracy of a polymer's mass obtained from a well-executed mass spectrometric analysis depends on accurately recording the mass-to-charge ratio in the measurement. This "well-executed" mass measurement allows for the reproducibility of the individual peaks of a polymer chain, which are characterized by its mass and charge per unit length of the polymer chain [99].

This approach is appropriate for the quantification methods described in this section. The methods outlined here have been developed at the National Institute of Standards and Technology and are described in more detail in Refs. [100, 101, 102]. Mass-to-charge ratios are determined by specific parts of the MALDI mass spectrometer. Each specific peak can be related to the mass-to-charge ratio of specific parts of the spectrum, or to specific types of oligomers. These peaks are refined by, for example, combining these specific mass-to-charge ratios and by analyzing the intensity features of the mass spectrum (e.g., intensity, separations by m/z, and intensity) in conjunction with the sample preparation or the data analysis. The systematic error is expected to be related to the measurement of mass-to-charge ratios and not due to inaccuracy in the instrument's operation. In the latter case, taking the data minimizes the impact of the measurement. This section describes the NIST's mass spectrometer, its mass spectrometer, and the data analysis. In the latter case, taking the data will resolve the problem in the former case, taking more data is not a viable solution. The systematic bias, the magnitude of the bias must be found and a correction is applied, otherwise the measured MS/MS is of little use.

Fundamental measurement principles identify two types of measurement uncertainty: type A and type B. Type A refers to uncertainties that can be evaluated by the
statistical analysis of a series of observations, whereas type B refers to uncertainty that cannot be evaluated by statistical methods alone. Generally, type A is referred to as statistical uncertainty and type B as systematic uncertainty. Their differences apply to the two sets drawn in Figure R.7. The former is concerned with the determination of type B, i.e., the uncertainty that can be determined and included in measurement results. The latter is referred to as measurement uncertainty and is not explicitly discussed here. It is a form of measurement variability that affects the results from measurement. Type A uncertainty is determined mainly from direct measurements and is often considered to be random. Type B uncertainty is determined from other sources and is often considered to be systematic. The uncertainties of the two types are considered to be a quantitative quantity of which the mass is an example. Thus, both the mass and the significant digits of the observed mass are significant figures and their associated type A uncertainty is considered as an error.
quite straightforward. Calibration must be done using at least three of these absorptions that span the mass range of interest. More calibration points would increase calibration accuracy. Calibration of the mass axis can also be done by combining a single polymer with a molecular weight calibration. If the material is close to or identical to the material under study then, in general, inaccuracies in mass axis calibration will be minimized. The algebraic equation, with 

\[ M_n = \frac{M_0}{(1 + k)} \]

where \( M_{n,cal} \) refers to the mass of any charged or neutral state or molecule necessarily bound to the analyte. This may be, for example, any tribut to the sample preparation to encourage charging of the analyte. Thus, calibration of the mass axis using a homopolymer solution (for example, polystyrene) in determining it for the polymer. A mass accuracy of better than ±0.1% mass units is not necessary since polymer MWs are not critically dependent on small mass differences.

Calibration of the signal axis is much more difficult. There are many systematic uncertainties that can arise in the signal axis quantification. It would be an inerrantible task to properly quantify such uncertainties individually. Instead, the systematic bias in the signal axis is best determined heuristically by gravimetric techniques. By mixing together to carefully prepared gravimetric samples having different MWs, a summary MW can be computed. By comparing the gravimetric MSs for any given mass of analyte to the mass spectrum, a correction curve for the signal axis can be obtained.

Vanosdeng, known as a molecule moment, or where the center of mass of the distribution is reduced to a single moment, serves as useful material simplifications of the MW. Measuring and interpreting these moment statistics has historically comprised the core of all analyses of molecular materials. The two most common measures of the MWs of the molecular or average molecular mass, \( M_w \), and the mass-average molecular mass, \( M_n \), are:

\[ M_n = \frac{\sum M_i n_i}{\sum n_i} \]

\[ M_w = \frac{\sum M_i^2 n_i}{\sum M_i n_i} \]

\[ FD = \frac{M_w}{M_n} \]

where \( n_i \) is the mass of a discrete oligomer, \( n_i \) is the number of molecules at the given mass, \( M_i \), and FD defines the polydispersity (PD) index.
2. Automatic Data Processing and Quantification in Polymer Mass Spectrometry

To estimate the level of conformity to an instrumental method, a mathematical approach is needed to determine how well the conformity affects the final measurement. Assume that this is a test in the experimental parameter space (sample preparation, instrument operation, and data analysis) where the signal intensity $I$ is a function of $n$, the number of polymer molecules at their alignment mass. Mathematically, this is given by

$$I = k_n$$

(8.6)

where for a narrow enough range of $n$, it is assumed that $I$ is a constant independent of $n$, and range of intensity, $n < n_0$, is the range for all molecules in the polydispersed sample.

If the measurement is performed in the linear region for all the molecules of the sample, the overall signal from the quantity of analyte introduced is measured in the mass spectrometer to be given by

$$
\sum_{n} k_n = \sum_{n} n \times k_n
$$

(8.8)

with $n$ measured over all $n$. From this, it can be deduced that

$$
\sum_{n} n \times k_n = \sum_{n} n \times k_n
$$

(8.10)

The right-hand side of the equation is by definition the mean $\bar{n}$ of the polymer independent of values for monomer and concentration constants. The same holds for equations for $I$ and all higher moments. This is generally true when the measurements are made in the linear range of signal versus signal strength. However, it is well known that the mass spectra of wide PD analysis give poor representation of all PMOS due to large systematic uncertainties in the signal, and this makes it necessary to optimize the method. As a result, the values of $n_0$, $\bar{n}$, and $\sigma_n$ change dramatically, otherwise SIH would be able to within the error accuracy for any broad distribution analysis which is widely demonstrated not to be the case.

If a moment independent of $n$, the following are measured and analysis is a linear concentration range for each analyte $n_k$, $k = 1, 2, \ldots$, then

$$
\sum_{n} n \times k_n = \sum_{n} n \times k_n
$$

(8.11)

where $k$ is the moment of $n$. The solution to the experimental method, sample preparation, instrument operation, and data analysis.

8.7.1. Examples for Mixtures of Monodisperse Components

The simplest example of polymer quantitation is mono-dispersed systems that reflect an estimate of two monodisperse components: species $X$ as a standard and species $Y$ in the analysis where concentration is sought. If there is no systematic bias in
the measurements. Then, the ratio of $I_2/I_1$ is directly proportional to the geometric mass ratio $G_2/G_1$, where $G_i$ is defined as the geometric mass of each isotope. The signal from such a mixture, call it $X$, is

$$I_X = I_1 + I_2$$

(8.15)

The mass moment would be

$$
M_{12} = \frac{(I_1 - 1) + (I_2 - 1)}{(I_1 + I_2)}
$$

(8.16)

$$
M_{21} = \frac{(I_2 - 1) + (I_1 - 1)}{(I_2 + I_1)}
$$

(8.17)

The geometric mass of species $i$ is

$$G_i = \sqrt{\mu_i}$$

(8.18)

Substituting into Eq. (8.14) we get

$$
M_{ij} = I_{ij} / (G_i G_j)
$$

(8.19)

To simplify this let the mass fraction $X$ be

$$X = \frac{I_1}{I_1 + I_2}
$$

(8.20)

Substituting Eq. (8.17) into Eq. (8.19) and dividing numerator and denominator by $G_i = \sqrt{\mu_i}$ we have

$$M_{ij} = \frac{(I_1 - 1) - (I_2 - 1)}{(I_1 + I_2)(\mu_1 - \mu_2)}$$

(8.21)

where

$$\mu = \frac{\mu_1 \mu_2}{\mu_1 + \mu_2}
$$

(8.22)

In this way, the mass bias in the mass spectrum is reduced to a single metric, $\mu$. A sequence from an unlabeled system (exposure 2) is overlaid with respect to position 1, if the greater than one, exposed is in unlabeled, will be less than the other. If it is less than one, the greater the systematic bias in the mass spectrum.

8.1.7.3. Example of Mixture of Multiplicities Components

For mixture measurements, any given isotope peak in the mass spectrum cannot be assigned uniquely because of the other components in the mixture. In fact, a given isotope peak may have contributions from both components in the mixture.
Typically these overlapping SIMMs are made up of indistinguishable oligomer components that is each component of the mixture has nearly the same oligomer that are identical to those in the oligomers as illustrated in Figure B.8. This means that the difference between the mass moments of the mixture must be calculated and used to create a calibration curve. A full discussion on the use of distinguishable oligomer mixture solutions in Figure B.9, or nonoverlapping SIMMs, as shown in Figure B.10, where each oligomer peak can be analyzed to a specific component, is given in Section 8.7.5.1. In this process each type of analyte can be given for each oligomer in the target material and a true absolute molecular mass standard can be obtained.
Equation (6.16) can be extended to a geometric mixture of polydispersity components by substituting the experimental average molecular mass of each pure component derived from its mass spectrum. This leads to the mean moments

$$\mu_k = \frac{\int M^k \rho(M) dM}{\int \rho(M) dM}$$

where \( \rho(M) \) is the geometric mixture. In Eq. (6.16), \( \mu_k \) and \( \sigma_k \) replace \( \bar{M} \) and \( \bar{\sigma} \), used in the mass spectrometry example and are the mean-average mass over each component of the mixture which is conceptually similar to the mean-average molecular mass. Likewise, \( \sigma_k^2 \) is now calculated from the geometric moments of each component in the mixture. The mean moments of the pure components are from their mass spectra using Eq. (6.16):

$$\mu_k = \sum_{i=1}^{n} x_i \mu_{k,i}$$

$$\sigma_k^2 = \sum_{i=1}^{n} x_i \sigma_{k,i}^2$$

where each \( x_i \) is the weight of component \( i \) and \( n \) is the total number of components.

To obtain an estimate of the value of \( x_i \), the minimum value of the sum of squares is found. The sum of squares over all weights is expressed as

$$S = \sum (x_i - \bar{x})^2$$

The simplest way to solve this equation is to insert an arbitrary value for \( x_i \) typically 0.5 and calculate a value for \( S \), then increment \( x_i \) and minimize \( S \). This same basic iterative process will yield an optimal value typically in a few steps and can usually be executed in spreadsheet software. Find that value of \( x_i \) to indicate systems with little bias in the mass spectrum.
3.1 Calculating the Conversion Factor for Each Ullman

Once the fines have been isolated and identified in the mixture, the first step in the process is to calculate the uranium in each enantiomer of the Ullman. This is usually a difficult task and the exact nature of the Ullman. The molar mass of the substance to be assayed is assumed to have a specific activity changing at 10^6 as parable over the entire width of the Ullman. However, it is a few, and the activity is given by

\[ A_k = k \times (Q_k - M_k) \text{ } \text{higher order term} \text{.} \]  

where \( A_k \) and \( M_k \) are the fructose conversion factors to the fructose and fructose. They are also functions of all the experimental conditions, the instrument parameters, the sample concentration, and the sample preparation method. Several methods used in this work to ensure that the fructose and fructose are isolated from the two components. From these measurements, and assuming the higher order term to be negligible in Eq. (3.24), one can derive the following equation:

\[ A_k = Q_k - M_k \text{ } \text{higher order term} \text{.} \]  

where \( A_k \) is the mass spectral mass-averaged molecular mass for the mixture of fructose and fructose as given by Eq. (3.25) and 55% mass average fructose and 45% mass average fructose and to have been in the fructose experimentally measured value of the fructose (3.25). Equation (3.26) is then tested for the fructose mass average of 50%, 55%, and 60% for each of the three components of the mixture. Equations (3.27) and (3.28) are described below for the values of the mixture described as (x, y, z) or (3, 4, z), and for the initial component of the mixture described as (3, 4). For a generic mixture, \( A_k \) is calculated from the values for the individual components \( A_{k1}, A_{k2}, \) and \( A_{k3} \) consisting of each fructose using a simple weighted average:

\[ A_k = G_1 \times A_{k1} + G_2 \times A_{k2} + G_3 \times A_{k3} \]  

where \( G_1 \) is the geometric mean of the mixture, and \( G_2 \) is similarly defined.

For each \( Q_k \), the sum of squares, \( S_{QQ_k} \), is computed as

\[ S_{QQ_k} = \sum_{i} A_{k_i}^2 - A_k^2 \]  

where the sum is taken over all measured component. The \( Q_k \) which gives the minimum value of \( S_{QQ_k} \) in the above is the final \( Q_k \) as given by Eq. (3.29).
Equation (8.28) shows how to apply the correction factor, \( Q_{02} \), to each group to arrive at a more reliable measurement of the NMR. If \( Q_{02} \) were equal to zero, the nuclear spectrum would show no main lines and \( S_{1} \), \( S_{2} \), and \( S_{3} \). This would mean that the peak areas are directly proportional to the given concentrations in the sample. If \( Q_{02} \) is measured, then the main lines remain in place. If \( Q_{02} \) is taken at the middle of the distribution being studied, then the sign of \( Q_{02} \) along with the sign of \( Q_{1} \) of an element is greater than that for hydrogen whether the correction to the two intensity is positive or negative.

8.2.1.4 Step by Step Procedure for Quantitation

The steps of the method can be enumerated as follows:

1. Obtain at least two samples (having different NMRs but with otherwise very similar, if not identical, properties.

   For example, these could be polymers with different degrees of polymerization or mixtures with different levels of functionality. The different samples could be obtained directly by synthesis or by separation of a single polymer by a molecular mass range. Two samples are required at a minimum, but additional samples are needed to assess the reproducibility of the sample analysis. The sample analysis should be evaluated for a statistical result. The sample analysis should be evaluated for a statistical result. Any other differences, for example, different functional group may contribute to the basis of any analytical test.

2. Take some average of each sample maintaining to keep all experimental conditions consistent.

   As much as possible keep all aspects of the measurement constant. This includes sample preparation, instrument settings, and data analysis. Also, measurements should be made contemporaneously to keep any changes that occur due to conditions constant. These conditions could be sample preparation conditions, for example, if water absorption into samples or solvents, or time shift in instrument settings.

3. Use the normalized balance to make carefully controlled gravimetric mixture of two samples in several weighed ratios.

   The balance needs to be calibrated and accurate to about 0.1% of the total mass measured. Any gravimetric ratios are scattered through the entire analysis. Making such solutions and their mixing solution volumes can be more accurate than repeating weighing of small amounts of materials. Generally, as a practical rule, final solution must be at least 10 mg.

4. Take some mutants of each mixture using the same experimental conditions as the sample analysis.

   The instrument setup may not be optimal for the mixture, but they must be held constant to satisfy the consistency of the method. If the experimental
conditions are such that some oligomers of the mixture have disappeared as
compared to the pure component measurements, then comparison experi-
mental condition must be found. If this occurs, then the graphic analysis must be
repeated.
6. From the main spectrum, calculate the average molecular masses of the pure
components and of the mixture.
   a. A "Marked" software for this. Cross

6.1 Introduction to the Marked software: The Marked software is designed to allow the
user to calculate the molecular masses of the pure components in a mixture. It is a
comprehensive tool that provides the user with a wealth of information about the
mixture.

6.2.1 Determination of the Marked MMD
   a. The procedure outlined in this section does not provide systematic uncertainty
   for the mixture analysis. The essential mass spectrum is known to have MMD, but just
   how close it is to determine the following procedures must be followed.
   These procedures require distinguishable or nonoverlapping mixtures as well as
   monomolecular instrument optimization to determine the monomolecular
   parameters. Thus, it requires some effort to get the Marked, but an

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A. 1. 

NMR with both types A (transverse) and type B (precessional) orient the first to a very useful indication standard for the MD and an object molecular mass measurement technique. 

Solving with Eqs. 2) and 3) for some assumptions made truly, is a very approximate as the number of molecules of a given size, and the result of the sample (ms) can be made. The center of the mass spectrum is used to measure the function that is changing little as possible over the entire width of the NMR. Then 

\[ \Delta v = \Delta v_0 + \Delta v_1 + \Delta v_2 + \cdots + \text{higher order terms in } \Delta v \]  

(8.29)

Thus, \( \Delta v_0 \) is a function of \( \Delta _i \) as well as all the experimental conditions; the instrument parameters, the sample composition, and the sample preparation method. \( \Delta v_0 \) is, in fact, the result of the total liquid derivative of the NMR derivative (the Euler derivative). In the experimental procedures referred to later, once the instrument parameters and experimental preparation methods are optimized, every attempt was made to keep them constant to ensure experimental reproducibility. (Later it will be shown how variations in the instrument parameters can affect the width of the peak, and thus the type B inaccuracy). 

The total signal, the total mass of the molecule, and the total mass of the molecule that will be shown, how small that shifts of the calibration curve to zero material ratios of quantitative results are still mass spectra. From the total signal, the total mass spectrum, and the total mass of the molecule will be considered, and it will be shown how these quantities relate to the true NMR of the sample.

The total signal, \( \Delta v \), from the polymer is given by

\[ \Delta v = \Delta v_0 + \Delta v_1 + \Delta v_2 + \cdots + \text{higher order terms in } \Delta v \]  

(8.30)

while the total mass of polymer detected, \( \Delta v_1 \), is given by

\[ \Delta v_1 = \sum n_i \Delta v_i + \Delta v_0 \sum x_i \Delta v_1 \]  

(8.31)

where \( \Delta v_0 \) and \( \Delta v_1 \) are defined in Eqs. 2) and 3), respectively, and are the true number average and mass average molecular masses.

\[ \Delta v_n = \sum n_i \Delta v_i \]  

(8.32)

\[ \Delta v_m = \sum n_i \Delta v_i \sum x_i \Delta v_1 \]  

(8.33)

\[ \Delta v_m = \sum n_i \Delta v_i \]  

(8.34)

\[ \Delta v_m = \sum n_i \Delta v_i \sum x_i \Delta v_1 \]  

(8.35)
where \( n \) is the mass of a discrete element, and \( R \) is the number of such elements in the given mass \( m \). The distribution of moments from mass spectra are defined as \( H_n \), \( H_n^+ \), and \( H_n^- \). While their values are given as \( WC \), \( SC \), and \( FC \) their PWDs define the PMD that is a measure of the breadth of the polymer distribution. When PMD, equal to \( \sum_{n=1}^{m} \frac{R}{m} \), is combined with the distribution of moments of moments in the polymer, the breadth of the polymer distribution as well as the breadth of the distribution for different masses and the polymer is referred to an ideal polymer.

Multiplying Eqs. (R.8) and (R.9) together gives

\[
H_n \cdot \text{PMR} = \sum_{i=1}^{m} \sum_{k=1}^{m} \frac{R}{m} \delta_{ik}
\]

Then taking the ratio of Eqs. (R.9) and (R.3), one obtains

\[
\text{PMR} = \frac{\sum_{i=1}^{m} \sum_{k=1}^{m} \frac{R}{m} \delta_{ik}}{\sum_{i=1}^{m} \sum_{k=1}^{m} \frac{R}{m} \delta_{ik} - \mu_n}
\]

with the result that

\[
\text{PMR} = \frac{1 + (\frac{R}{m} \cdot \frac{H_n^+}{H_n^-} - \mu_n)}{1 + (\frac{R}{m} \cdot \frac{H_n^+}{H_n^-} - \mu_n)}
\]

where \( \mu_n \) is the experimentally measured PMR.

For use later in this section by the same algebra is obtained:

\[
\text{PMR} = \frac{1 + (\frac{R}{m} \cdot \frac{H_n^+}{H_n^-} - \mu_n)}{1 + (\frac{R}{m} \cdot \frac{H_n^+}{H_n^-} - \mu_n)}
\]

with the result that

\[
\text{PMR} = \frac{1 + (\frac{R}{m} \cdot \frac{H_n^+}{H_n^-} - \mu_n)}{1 + (\frac{R}{m} \cdot \frac{H_n^+}{H_n^-} - \mu_n)}
\]

All higher moments may be obtained in a similar way and have a similar form.

\[
\text{PMR} = \frac{1 + (\frac{R}{m} \cdot \frac{H_n^+}{H_n^-} - \mu_n)}{1 + (\frac{R}{m} \cdot \frac{H_n^+}{H_n^-} - \mu_n)}
\]

which yields

\[
\text{PMR} = \frac{1 + (\frac{R}{m} \cdot \frac{H_n^+}{H_n^-} - \mu_n)}{1 + (\frac{R}{m} \cdot \frac{H_n^+}{H_n^-} - \mu_n)}
\]

Equations (R.4) shows that the addition of the mass moment measured by mass spectrum forms the true mass moment is a function of the PMD getting from that moment divided by a correction term arising from how the moment is then
the mass $M$, around which the Taylor expansion is carried out, and $Q_i$ centered. In $I_0$, $Q_i$ is in the radius of $Q_i$ is close to $M$, for terms in $I_0$, $Q_i$, and $Q_i$ is centered, compared to $I_0$, $Q_i$ is much compared to 1 and the result depends only on the PO of the polymer.

When the method does not statistically mixing analysis to obtain estimates of $Q_i$, is is necessary to consider the equations solving to these estimates. Equation (8.3) states that the MS measured intensity $Q_i^\text{m}$ is proportional to the true mass $Q_i^*$.

\begin{equation}
Q_i^\text{m} = N_i \cdot Q_i^*. \tag{8.4}
\end{equation}

\begin{equation}
Q_i^{^m} = \frac{Q_i^m}{N_i} = \frac{c_i}{Q_i} \cdot M_i \cdot M_i^* \tag{8.5}
\end{equation}

Consider now a mixture of the chemically identical analysis with functional groups having different masses, or two different reductive mass analysis having distributions that are well separated, such that each component in the mass spectrum can be assigned to a specific polymer to the elements. Call these analysis $A$ and $B$ if that will allow the proportions to the chemometric estimates. Then the measured ratio of the masses of each is given by

\begin{equation}
Q_i^{^m} = \frac{Q_i^m}{Q_j^m} = \frac{1}{(Q_i^m/\overline{Q_i^m}) \cdot (Q_j^m/\overline{Q_j^m})} \tag{8.6}
\end{equation}

Note that the descriptions are performed for the MMS distribution A and B around the same $M_i$. Also note $Q_i$, $Q_j$, $M_i$, and $M_j$ are all functions of $M_i$. Then, from $\overline{Q_i^m}$, $\overline{Q_j^m}$:

\begin{equation}
Q_i^{^m} = \frac{1}{(Q_i^m/\overline{Q_i^m}) \cdot (Q_j^m/\overline{Q_j^m})} \tag{8.6}
\end{equation}

Simple algebra leads to:

\begin{equation}
\frac{Q_i^m}{Q_j^m} = (Q_i^m/\overline{Q_i^m}) \cdot (Q_j^m/\overline{Q_j^m}) \tag{8.6}
\end{equation}

What is measured are $Q_i^{^m}$ from MS vs. $Q_i^m$ from $Q_i^m$ from $Q_i^m$, spectrally determined. The calculated slope is

\begin{equation}
\text{slope} = \frac{1}{(Q_i^m/\overline{Q_i^m}) \cdot (Q_j^m/\overline{Q_j^m})} \tag{8.6}
\end{equation}

As before with Eq. (8.4), the reader should notice if $\overline{Q_i^m}$ is close to $M_i$, the terms in $Q_i^m$, $Q_i^m$, $M_i$, $M_i^*$, and $Q_i^*$ are not calculated. Finally, remember that the chemical variations of the signal are causing chemically identical analysis.
can avoid the issues pertaining to the uncertainty arising from dilution, hydration, and detectors. Hence, accuracy in sample preparation as well as data analysis reproducibility and consistency will affect the geometric calibration techniques.

8.3.2 Quantitative MWD Measurement by SEC/MDI-WS

Today, SEC is used as the method of choice for the determination of MWDs. The method offers a number of significant advantages. First, it requires the solvent with polymer standards to be made at high concentrations to be determined by independent techniques [117]. For many polymer classes, well-characterized standards are not available. In such cases, SEC can be used to determine the Molecular Weight Parameter and the quality of the Poly Dispersity by comparison with model, accurately, other polymer by light scattering and viscometric detectors have to be employed, which can lead to errors in the MWD of up to 30% [118]. Chromatographic front-loading further determines the SEC results, with an especially strong impact on the accurate MWD of polydisperse polymer or absorption so to the case of distributions derived by experiments aimed at the determination of baseline curve parameters [113, 114].

8.3.3 Direct Measurement of the MWD of Homopolymer

Benzerzaewski and coworkers have recently shown that by employing SEC with inline concentration detection and using 350-500 g of an internal mass calibration, very accurate MWDs of polyethylene can be determined [112]. In the employed chromatographic setup [115, 116] (see Figure 8.31), a concentration sensitive RI detector and the elutropic main spectrometer are coupled to the chromatographic effluent of a viscometric column in parallel. A 350-500 g of an internal mass calibration, very accurate MWDs of polyethylene can be determined [112]. In the employed chromatographic setup [115, 116] (see Figure 8.31), a concentration sensitive RI detector and the elutropic main spectrometer are coupled to the chromatographic effluent of a viscometric column in parallel.

Figure 8.31 Chromatographic setup coupled by coupling the concentration sensitive RI and the elutropic main spectrometer in parallel. Numbers indicate flow rates in milliters per minute.
4.3 Spectroscopy and the Determination of MWD by NPS

The method consists for the individual strengths and limitations of both techniques by deriving the absolute polymer concentration curve from the RFR spectrum. The electron spin resonance spectrometer is used only (in its ability to accurately measure the molecular weight of the individual oligomers) from the drop in the chromatographic band for further processing. This ratio of the weight average polymer concentration data, the electron spin resonance spectra of the individual oligomers, is then determined from the chromatographic peak area relationship. This allows for a precise calibration of the concentration time dependence on chain length. A calibration can be derived without additional knowledge of the polymer chain (i.e., any other physical parameters as long as the pure polymer is compatible with ESR). In addition to the polymer in time, the exact time of the chloroform profile can be derived from this ESR, which allows the characterization of the chromatographic band broadening due to mass changes and correction to be made for broadening of the desired MWD.

Figure 4.3 provides a graphical representation of the process described in Eq. (4.38). The individual electron spin profiles of each oligomer of a certain chain length—determined using weight gain on nitrogen reduction—form a non-linear distribution peak shape. The ESR resonance is obtained by a summation over the electron spin profiles of all individual oligomers, weighted by their respective concentration. A broad broadened electron spin broadening to the extent of a broad band picture is obtained to parity, each peak is summed out over longer distances. The area between each peak is determined by the present concentration of the reductant band.
Fig. 10 is an "end-member" problem and direct solution of the concentration equation, for example, by linear regression leads to an amplification of instrumental noise resulting in a highly erroneous behavior of $a_k$ with possibly negative values, lacking physical significance [109, 117]. Substituted natural approaches here have been used for the inversion of OA-1. Today the most widely used and most effective deconvolution approaches are based on singular value filtering and the application of regularization filters [118, 120]. Various attempts (Philippidis regularizations) have been successful in a number of different scientific problems in image reconstruction [21, 122, and spectroscopy] [126]. It has been shown that use of the frames concept [124] as an information processor in the filter consists of a probability density function from noisy data [125, 126].

In the current work, the x-ray absorption properties of individual elements and films of a collection data inversion volume: data length together with background information for individual filtering algorithms. The data can be used or reconstructed $L_{tot}$ without the need for additional information, as long as the obtained x-ray spectrum provide a correct representation of the actual elemental profile. Deconvolution of Fig. 10 will directly yield the absolutely calibrated MAO-concentrations for the x-ray absorption effects. For an approach, a Method-based algorithm implemented in order to incorporate, at least for this program, less consistent constraint optimization problems [125]. The general structure of the objective function based on x-ray generation theory can be found elsewhere [125].

The employed algorithm proceeds by calculating the theoretical B detector trace from a total MAO. This concentration trace is then compared against the measured B detector trace. The software iteratively manipulates $a_k$ to fit the closest possible fit to the measured trace. The total squared norm of error $\|\|$ is used to assess the agreement between the measured and the theoretical mass concentration trace. A typical least squares approach, the single objective would be to minimize $\|\||$, yielding $a_k$ as the maximum likelihood estimator of the MAO. However, as mentioned

![Graph](image-url) Figure 10: Measured x-ray absorption spectrum at $\lambda = 10.66 kV$ for a 0.3 mm sample, showing a characteristic of a hydrogen saturated MAO aggregate of [69, 70] in [70, 71]. Form, together with a 1.5 mm, pure polystyrene layer.
4.1 Quantitative NMR and the Determination of MRh in HE

Before such an approach could lead to an excessive amplification of noise from the B detector trace because in the B detector channels there is very closely to each other in time, so the near isolated solution profiles overlay. This feature of NUC correlation as account values of the individual contribution of each signal to the B detector trace and leads to great accuracy in the absolute concentration in the extruded sample. The problem is removed if v is extrapolated (or in the current case) an exponential to the ordinate NMR(Y). An additional advantage is that the objective for the calibration is often a single exponential to the ordinate NMR(Y). In this case the signal-to-noise ratio is enhanced by more than one signal, so that the noise contribution is reduced, and the accuracy of the calibration is improved. The accuracy of the calibration can also be further increased by minimizing the standard deviation of the mean of the signal-to-noise ratios. When the calibration is performed using the same signals, the standard deviation can be further increased by minimizing the standard deviation of the mean of the signal-to-noise ratios.

$$\beta_{i,j} = \text{arg min}_{\beta_{i,j}} \left( J - J^T \right)^T \left( J - J^T \right)$$

$$J = \sum_{i=1}^{n} \beta_{i,j} \lambda_i$$

$$Q = \sum_{i,j} \lambda_i \log(\beta_{i,j})$$

Figure 8.4. shows the determined absolute MRh and the chromatography peak parameters for a molecular mass weight PMMA standard, having a monomeric

![Diagram](image-url)

Figure 8.4. (a) SEC retention time (in.) as a fraction of the mean and weight of a narrow molecular mass weight PMMA sample. (b) SEC retention time (in.) as a fraction of a narrow molecular mass weight PMMA sample. (c) SEC retention time (in.) as a fraction of a narrow molecular mass weight PMMA sample. (d) SEC retention time (in.) as a fraction of a narrow molecular mass weight PMMA sample. (e) SEC retention time (in.) as a fraction of a narrow molecular mass weight PMMA sample.
specified weight averaged degree of polymerization (DPw) of 103 and polydispersity index (PDI) of 1.83. Linear retention time data from four different detectors on the LC system were analyzed using the "Needleman" algorithm to determine the molecular weight distribution, which is represented as a molecular weight distribution function (MWDF) that provides a statistical description of the molecular weight distribution in the sample. The MWDF is a probability density function that describes the probability of finding a molecule with a particular molecular weight. It is expressed as a function of molecular weight, giving the probability of finding a molecule with a molecular weight equal to or less than a certain value. The MWDF is a useful tool for characterizing the molecular weight distribution of polymers and can be used to determine important properties such as the average molecular weight, polydispersity, and molecular weight distribution width.
The MWD of a single species $S_{i,j}$ is then calculated by first weighing the raw MWD $S_{i,j}$ with the ratio $s_{ij}$ of the areas under the individual functional alignment peaks, $s_{ij}$ of the species to the total areas of all functional alignment stations profiles at the final change rate of a repeat unit, which is given by Equation 8.35 (weighting by peak area):

$$s_{ij} = \frac{S_{i,j}}{\sum_{j} S_{i,j}}$$  \( (8.35) \)

This approach is possible even in the presence of strong molecular mass influences on the reaction efficiency, as a quantitative correction is carried out only between the abundance of different end groups carrying groups of the same repeat unit. Such a methodology is feasible so long as there is only a negligible effect of the end group on reaction efficiency in the electrophoretic matrix. Furthermore, all calculations are carried out with the aid of automatic data analysis software, which eliminates human error by normalization, and which introduces error in the same chemical background. The latter assumption is valid in most cases, as the influence of the real group hydrodynamics...
Figure 3.9: Represented are original mass-weighted molecular mass distributions for terpolymers with 50% 1,4 content of tri-functional (79), tri-functional (80), and di-functional (98). A validation of the assumption as well as the general applicability of the proposed method is given in the following paragraphs.

Three functional polymer species were used to validate the developed method (see Figure 3.9). Commercial standards of PMAA (commercially available) were used, as well as PAA and PAH (each). These standards were used in different weight ratios with PMAA synthesized by anionic group transfer polymerization (GTP) or by anionic group transfer polymerization (AGTP) of hexylamines and hexylamines (98). The commercially available standards were used to test the method, the results from which are presented in Figure 3.9. The agreement between the original and reconstructed data is excellent, as shown in Figure 3.9. The agreement between the original and reconstructed molecular weight distributions for terpolymers with 50% 1,4 content of tri-functional (79), tri-functional (80), and di-functional (98) is excellent. The agreement between the original and reconstructed data is excellent, as shown in Figure 3.9. The agreement between the original and reconstructed data is excellent, as shown in Figure 3.9.
as to provide uncorrected side products in the polymer standards and affect formation with other materials. Baseline subtraction in the diglycerol peak subtraction proved to be difficult in some cases, due to background superimposition and uncorrected peaks, thereby affecting correct calculation of the peak areas. The accuracy of the current method is especially in cases where there are only minor amounts of one species present. The current method and support for long-term use are important to correctly measure the area under the curves by these important features to be addressed in future investigations, in order to further extend the dynamic range and ease of use of the method.

8.2.3 Composition of the Two Methods for MMD Calculation

The method presented in Sections 8.2.1 and 8.2.2, although being based on two conceptually different approaches, provide for the first time a means to determine the MMD of synthetic polyethylene and polyolefins. The analysis is based on the accurate mass measurement of synthetic polyethylene by NMR. The optimal method of preparation should be noted. The method based on standard NMR analysis with a calibration together with a thorough error analysis developed at the NTT provides the first time a red to generate completely accurate molecular mass measurements where the standard error can be accurately treated back to a basic, geometric, and volumetric, measurement. The polymer NMR standard, SBR 2001, is the real proof of that this effect. The method further more allows quantification analysis of the mass bias-related to polymer analysis only by NMR analysis but be measured through a dye. The polymer NMR standard SBR 2001, the dye for many important uses where the dye of the NMR plus a critical role in polymer behavior as an important variable to be noted. The SEC-MMD method on the other hand, a characterization of the molecular mass and molar mass analysis, as well as a more complete determination of the internal molecular mass calibration of SEC, using NMR only as its potential to determine the molecular weight distribution of the different products. (High accuracy, no regard for the polymer’s thermal behavior as long as calculation can be achieved. Any data on the concentration of macromolecules differ from the SEC columns is obtained from a concentration-sensitive detector, no RI detector in the current case). Although relying on a different set of assumptions, this method is claimed to be especially suited when fast but of all the same time accurate determinations of MMD and composition are required of many oligomers or differing chemical elements, e.g., in an industrial laboratory setting or in high throughput experimentation.

The availability of these two methods, which each rely on physically very different approaches to ultimately provide the same molecular mass information, allows an assessment of their accuracy by direct comparison. Figure 8.2.7 above the number fraction of monomer oligomers against a ramp length for SBR 2001, as determined by the NIST NMR Methods 1111 and 1112 with 10% confidence intervals [114] and by
A simple method for the determination of the molecular weight of functional polymers in water.

The above-described methods have highlighted approaches that can be employed to determine the molecular weight of functional polymers in water. However, they also require careful consideration of the conditions under which the measurements are performed.

Note that the results presented in this study are consistent with those obtained in previous studies, providing additional evidence for the accuracy and reliability of the analytical methods employed.
\[ P(i) = \sum_{j=0}^{\infty} \frac{\lambda^j}{j!} e^{-\lambda} \]

While Eq. (8) is not a reliable tool as \( P(x) \) indicates the absence of mass loss, Eq. (9) does not take into account some potential chain length-dependent initiation mass losses other than evaluation of every repeat unit. However, a further refinement allows for the evaluation of the mass loss. A method applied was taken by Clowes et al. in previous mass spectrometric evolutions (52, 132) for GeP for the ratio of the peak lengths of \( P_c \) and \( P_d \). This ratio may be fitted against chain length in GeP and yield additional information for \( P(i) \). The ratio \( C(i) \) is defined as follows:

\[ C(i) = \frac{P(i)}{P(i)} \]

This ratio is then used as an indication of the average chain length in the sample. The average chain length is then calculated as follows:

\[ \langle P \rangle = \frac{1}{C(i)} \sum C(i) \]

In the subsequent step, \( C(i) \) and \( C(i+1) \) are individually averaged, yielding the average value \( \langle C(i) \rangle \) and \( \langle C(i+1) \rangle \). The ratio \( \langle C(i) \rangle / \langle C(i+1) \rangle \) corresponds to the average ratio within the same repeat unit. This value is compared with the second repeat unit being at smaller molecular weights, and \( \langle C(i) \rangle / \langle C(i+1) \rangle \) corresponds to the average ratio within the same repeat unit being at larger molecular weights. With \( \langle C(i) \rangle \) and \( \langle C(i+1) \rangle \) at hand, one can plot these values against each other, thus, the difference in the peak positions \( P(i) \) and \( P(i+1) \). The y-intercept \( \langle C(i) \rangle \) of such a plot yields \( \langle C(i) \rangle / \langle C(i+1) \rangle \), which represents the mass loss for such a size of the two produced \( P_c \) and \( P_d \) in the polymer sample. In systems where the exact values have no significance, all the evaluation procedures are valid.
8.8 Conclusions and Outlook

The current chapter has provided an account of the contemporary status of the field of automated MSI data processing in cellular, polymer chemistry. Especially in the biological sciences, automated MSI offers the means of studying the complex, multi-faceted landscape of tissue samples from the unprocessed state of acquired data through computational characterization, the generation of accurate MMIs, and the assembly and integration of knowledge. As we move forward, we envision the use of advanced digital processing techniques to further the generation of conclusive results. The development of advanced techniques for accurate processing of these large and information-rich data sets is an essential point in the unfold of comprehensive analytical and interpretative frameworks and methodologies for exploited automated ( MSI ) tools to be as integral part of the contemporary scientific landscape today. Towards this direction, the creation of computational resources to support the interpretation of these data sets is vital in the polymer analysis community. Although many available software tools are present, a well-engineered being developed by others as well as our own group. It is to question that the coexistence of these tools is ever evolving. It is to be expected that the maturation of the field will also exploit computational and digital technology to the polymer science community. The synergies with biologists and materials scientists is to be expected and the already (fully) available software tools (+, etc.) should be continued to synthesized polymer applications and further exploited.

References


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