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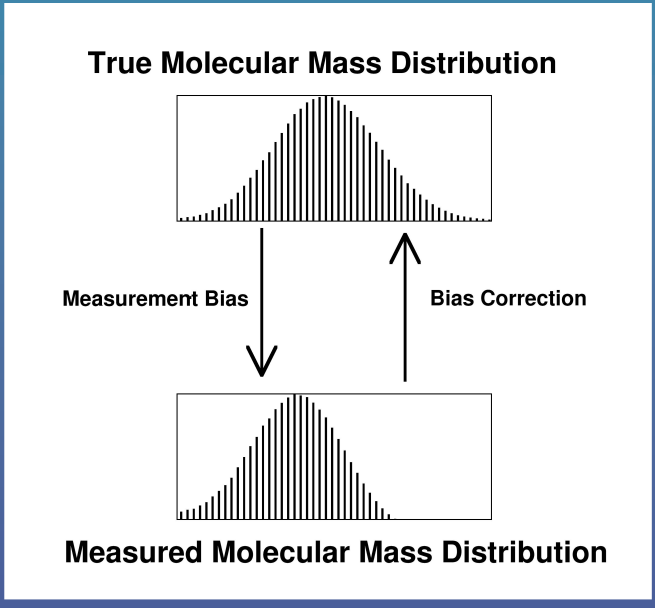
Molecular Mass Distribution Measurement by Mass Spectrometry



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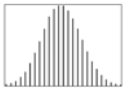

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Molecular Mass Distribution Measurement by Mass Spectrometry

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U.S. Department of Commerce
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Preface: Purpose of the Guide

This NIST Recommended Practice Guide presents a method that uses mass spectrometry to make a quantitative determination of the molecular mass distribution of narrow polydispersity synthetic molecular materials. The main obstacle for converting an oligomer-resolved mass spectrum (MS) into a valid molecular mass distribution (MMD) is the systematic uncertainty in the ion intensity axis of the mass spectrum. This guide demonstrates how to make gravimetric mixtures of analytes having the same general chemistry but differing in molecular mass distribution to 1) ascertain if mass bias is present for a given set of measurement conditions, 2) create a calibration curve to properly convert the MS to a valid MMD, and 3) determine systematic uncertainties for the calibration curve when certain specific conditions are met.

Within the framework of this Recommended Practice Guide two broad classes of mixtures exist: those where oligomer peaks in the mass spectrum cannot be attributed to a particular component of the mixture and those where they can. For example, mixtures of two polymers having the same repeat units and the same end groups fall into the former category (see Figure 4), while if the components have the same repeat units but different mass end groups they fall into the latter category (see Figure 5). However, if the components have the same repeat unit and the same end group but the mass distributions are far enough apart such that they do not overlap, their mixture falls into the second category: each oligomer can be assigned to a specific component of the mixture (see Figure 6). The first type is referred to as “indistinguishable” and “overlapping” and the second type as “distinguishable” or “non-overlapping”.

The first type is most often encountered in practice. Two molecular materials may be made that have different average molecular masses, or a broad molecular mass material may be separated, often chromatographically. In such situations when the mixtures are indistinguishable and overlapping, moments of the molecular mass distribution of the pure components and of gravimetric mixtures are used to create a calibration curve. It is this case that is covered in Section 4 of this Recommended Practice Guide.

The second type of mixture, distinguishable or non-overlapping, is encountered less often. It often requires a separate synthesis to create a different chemical end group, or perhaps chemical modification after synthesis, to systematically change the mass of one component. However, the advantage of such a situation is that an absolute molecular mass distribution standard can be created. Appendix A and references therein describe this situation. NIST Standard Reference Material 2881, “A Polystyrene Absolute Molecular Mass

Distribution Standard," was created this way. This second method is useful in creating standards, especially of proprietary materials, that may be used to calibrate other types of instruments, for example, chromatography equipment.

Section 1 introduces the broad classes of molecular materials to which this method applies and the basic concepts of polydispersity, molecular mass distribution, and mass moments. Section 2 gives a conceptual overview of mass spectrometry and introduces the concept of mass bias. Section 3 describes basic concepts in metrology and delves into the mathematical rationale behind the method for indistinguishable, overlapping mixtures. Section 4 gives step-by-step instructions of how to implement the approach described in Section 3.

Appendix A presents the full theory used to determine systematic uncertainties for the calibration curve when an absolute molecular mass distribution standard is desired and requires distinguishable or non-overlapping mixtures. Appendix B gives a computational approach to implement the method found in Section 4 that can easily be implemented in software. Appendix C lists documentary standards in force when this document was prepared for the measurement of molecular mass distribution by mass spectrometry.

This document is available online at:
http://www.nist.gov/public_affairs/practiceguides/practiceguides.htm

More information of the NIST Quantitative Polymer Mass Spectrometry Project can be found at: http://www.nist.gov/maldi

1 The Molecular Mass Distribution

1.1 Importance to molecular materials

As an unavoidable consequence in their synthesis, man made molecular materials (polymers, dendrimers, macromers, nanoparticles, nanotubes, etc.) are rarely obtained as pure, single molecular mass entities. Rather they are formed as mixtures of molecules having multiple molecular masses with regularly repeating mass differences indicative of the molecular building blocks from which they were created. This common feature of molecular materials is called polydispersity and is described fully and quantitatively by the material's molecular mass distribution (MMD). Research chemists compare the measured MMD to predictions from mathematical models of reaction kinetics in order to a gain fundamental understanding of the synthesis mechanism. Chemical engineers use the measured MMD for process control in large scale industrial operations. Materials scientists find that the MMD is important in determining material properties (mechanical, rheological, thermodynamic, etc.) and use it in materials processing and in consumer product design. For this last reason the MMD is used as a common specification between buyers and sellers in domestic and international commerce. To this end the National Institute of Standards and Technology (NIST) has been a provider of molecular mass standards and advances in molecular mass distribution measurement for over 50 years.

1.2 Definition of mass moments

Various averages, known as molecular moments (MM), where the entire shape of the distribution is reduced to a single number, serve as useful numerical simplifications of the MMD. Measuring and computing these summary statistics has historically comprised the core of the analysis of molecular materials. The two most common measures of the MMD are the number average molecular mass, M_n, and the mass average molecular mass, M_w. [1]

$$M_n = \sum_i m_i n_i \Big/ \sum_i n_i \tag{1}$$

$$M_w = \sum_i m_i^2 n_i \Big/ \sum_i m_i n_i \tag{2}$$

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

where m_i is the mass of a discrete oligomer i, n_i is the number of molecules at the given mass m_i, and PD defines the polydispersity index. When the polydispersity index is equal to one (i.e., in statistical terms the variance

of MMD is zero), all of the polymer molecules in a sample are of the same molecular mass and the polymer is referred to as monodisperse. Generally the mass moments of polydisperse molecular materials give an incomplete description of the overall MMD because all the information in a complex curve is reduced to a single value, or a small set of values. Properties like melt viscosity, tensile strength, and impact strength often depend on the tails of the MMD rather than its central portion that is defined by M_n and M_w. Consequently, it is critically important to measure the entire MMD. Furthermore, it is not uncommon, due to purposeful blending of materials, or due to the details of the synthetic chemistry (for example from two competing mechanisms going on simultaneously), that the polymer MMD will be multimodal. If so the central moments, M_n and M_w, will be exceedingly poor representations of the MMD and must be used as descriptors of a material's molecular mass distribution only with great caution.

1.3 Measurement methods

Chromatographic separations are typically used to measure the MMD with size-exclusion chromatography and high-performance liquid chromatography being the most useful for organic molecular materials. However, each suffers from the fact that it is not absolute, that is, each requires calibrants of known molecular mass, that are chemically and architecturally identical to the material being studied for proper mass calibration. On the other hand, methods to determine the absolute molecular mass of polydisperse samples return only one moment of the MMD. M_n is obtained by measurement of colligative properties like osmotic pressure, or alternatively by end group analysis via spectroscopic or titration techniques. M_w is obtained typically by light scattering or by ultracentrafugation. These measurements may yield absolute moments but, because they return only a single moment, are incomplete descriptors of the MMD. Recently it has been demonstrated by NIST that mass spectrometry (MS) can be used to determine the absolute MMD. [2] MS combines the mass dispersive aspects of chromatography with the absolute aspects of the moment measuring methods. This practice guide describes how mass spectrometry can be used to find a material's absolute MMD conceptually illustrated in Figure 1.

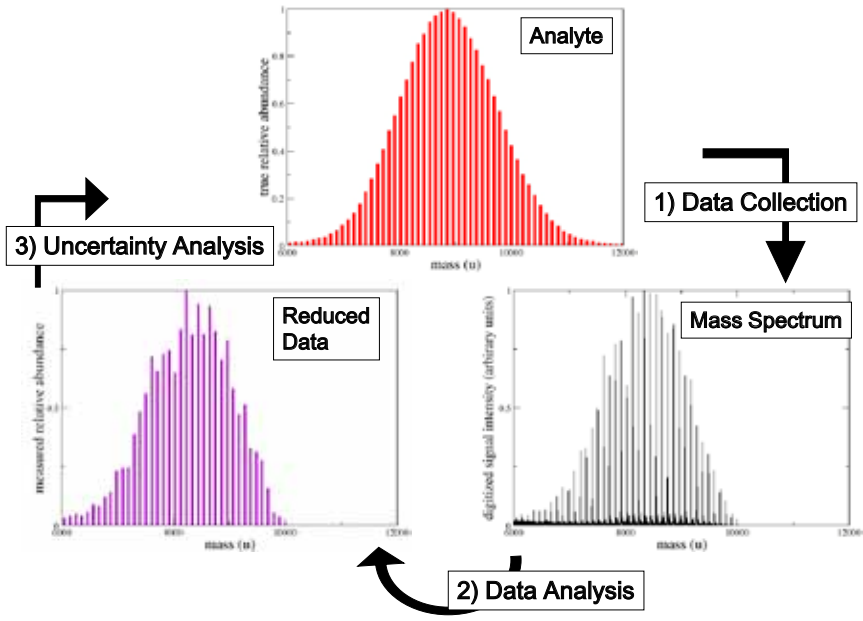

Figure 1— Steps of a complete measurement: data collection, data analysis, and uncertainty analysis.

2. Mass Spectrometry Overview

2.1 Ion creation, separation, and detection

Mass spectrometry (MS) is an analytical technique for the determination of the mass of individual molecules. As such it is well suited to the study of the chemical composition of molecular materials. In principle MS entails the ionization of chemical compounds in vacuum to generate isolated charged molecules and subsequently separating them by their mass-to-charge ratio. Thus, the mass spectrometer must perform three basic functions: create gas-phase ions, separate these ions by their mass to charge ratio, and detect the ions once separated. A mass spectrometry experiment, schematically shown in Figure 2, adds to these three basic functions two additional steps: sample preparation and data analysis. Samples often need some sort of chemical or physical manipulation before they can be introduced into the mass spectrometer. An example of sample preparation could be dissolution of the analyte into a solvent with perhaps the addition of an agent to assist in gas-phase ionization. Energy input is required to drive the molecules into the vacuum and to ionize them. This energy can come from many sources, for example, lasers in the case of matrix-assisted laser desorption ionization

(MALDI) or high electric field strengths in the case of electrospray ionization (ESI). The mass spectrometer itself produces a mass spectrum which in its most simple form is a histogram of the number of ions detected versus ion mass to charge ratio. This mass spectrum is then converted into a molecular mass distribution, typically using a computer code. Some computational steps for this may include baseline subtraction and peak integration.

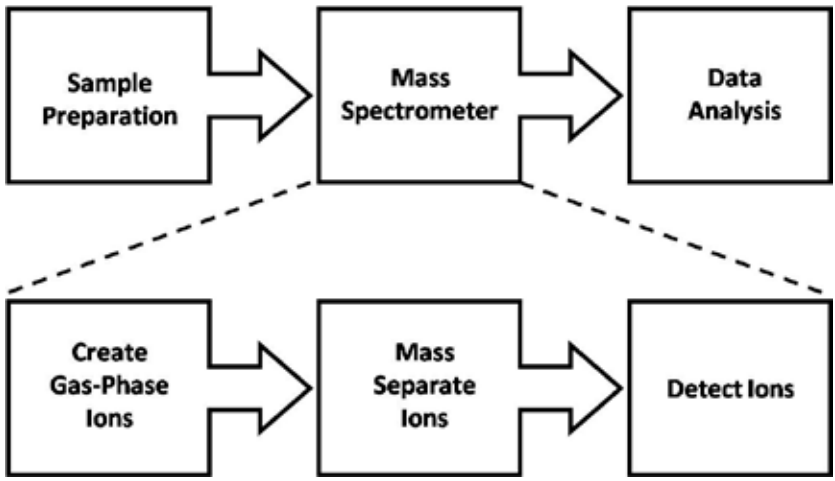

Figure 2— Steps in a mass spectrometry experiment including the three core functions of the mass spectrometer: ion creation, separation by mass to charge ratio, and ion detection.

2.2 Mass resolution requirements

Mass spectrometers can be thought of as modular instruments where an ion source is coupled to ion separation optics and from there to an ion detector to create a complete instrument. There are about a dozen fundamental ionization methods embodied in a bewildering array of ion sources. These ion sources may be coupled to about a half-dozen prominent ion separation methods that are embodied in a wide array of ion optic configurations. Lastly, while the types of basic ion detection technologies is fewer than a half-dozen their number multiplies the possible configurations for any given mass spectrometer. However, in the end no matter the configuration of the mass spectrometer only one factor matters for measurement of the molecular mass distribution of a molecular material by mass spectrometry: can the instrument resolve the individual oligomers in the sample? Here to “resolve” means having the ability to separate the mass spectrometric peaks to baseline of two oligomers

whose mass differs by one unit of their (typically) periodic mass spacing. This ability is required for the quantitation methods described in the next section. An instrument's mass resolution is defined as the smallest mass difference Δm between two equal magnitude peaks such that the valley between them is a specified fraction of the peak height. [3] Similarly, mass resolving power is defined as the observed mass divided by the difference between two masses that can be separated: $m/\Delta m$ where the method by which Δm was obtained and the mass at which the measurement was made must also be reported. In the method described in this practice guide oligomer mass resolution is a key requirement.

2.3 Mass bias

The determination of the MMD from a well-resolved mass spectrum depends on accounting for the mass bias in the measurement. Mass bias in the systematic over or under counting of specific parts of the molecular mass distribution by the mass spectrometer. Here "specific parts" can refer to the high mass or low mass parts of a mass spectrum, or to specific types of oligomers as defined by, for example, end group or molecular architecture. Mass bias can occur in any of the three basic functions of the mass spectrometer, as well as in the sample preparation or data analysis. By systematic it is meant that the bias is an inherent aspect of the measurement method and not simply due to imperfect counting statistics. In the latter case taking more data will resolve the problem, in the former case taking more data is not a solution. For systematic bias the magnitude of the bias must be found and a correction applied, otherwise the measured MMD is of little use. How to perform this correction is what this Recommended Practice Guide demonstrates.

To achieve the best results in measuring the absolute molecular mass distribution with mass spectrometry, it is best to minimize as far as possible the systematic mass bias. It is generally not possible to eliminate this bias completely; therefore, control experiments must be undertaken that elucidate the source of mass bias and determine ways to minimize it. This will help insure that the systematic bias is not so great as to defy correction, for example, in the case were certain oligomers are not detected at all which represents a degree of bias that is impossible to correct. Due to the enormous number of mass spectrometry approaches, this guide does not describe how to best tune an instrument and analyze any given sample to reduce bias. Previous work in instrument tuning [4], and data analysis [5] suggests some ways to approach this task. Sample preparation is an often overlooked source of mass

bias through such effects as mass-dependent solubilities. The analyst should carefully consider all preparation steps and be cognizant of possible sources of mass bias. In Section 4 methods are given to determine how much mass bias is inherent in your chosen method. And how to correct for it if it is within defined limits. Reducing mass bias will ultimately give a more accurate measured molecular mass distribution by keeping the correction factors as small as possible.

3. The Metrology behind Molecular Mass Distribution Measurement by Mass Spectrometry

3.1 Random and systematic measurement uncertainties

Fundamental metrological principles identify two types of measurement uncertainty type A and type B. Type A refers to uncertainty that can be evaluated by the statistical analysis of a series of observations, whereas type B refers to uncertainty that cannot be evaluated by the statistical analysis of a series of observations. [6] Generally, type A is spoken of as *statistical* or *random* uncertainty and type B as *systematic* uncertainty. Their differences applied to the mass spectrometry of organic molecular materials are shown in Figure 3. This guide is concerned with the determination of type B. Type A uncertainty that can be determined (and reduced) by repeat measurements is not explicitly discussed here; however, interlaboratory comparisons [2,7] have been used to address the statistical uncertainty in the MMD determination of organic molecular materials measurement by mass spectrometry. It will be noted that measurement repeatability is critical. If the operator cannot repeat the measurement from run to run and from day to day the chances of measuring the correct molecular mass distribution decrease dramatically. The measurement method must be repeatable and reliable before it can be considered for quantitative, much less standards, work.

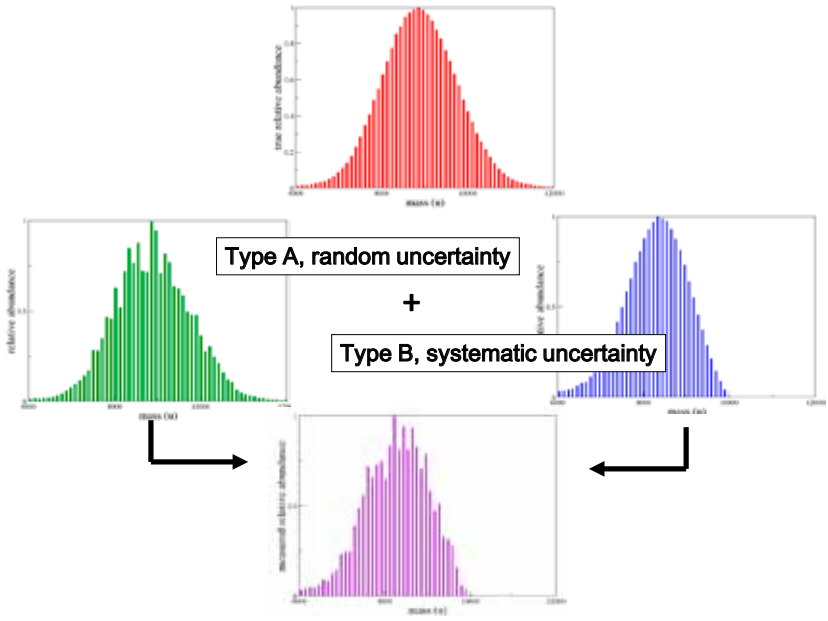

Figure 3— Schematic illustration of type A ("random") and type B ("systematic") uncertainties in molecular mass distribution measurement.

3.2 Systematic uncertainties in mass spectrometry

Many of the physical quantities measured for which uncertainties are determined, for example, the mass of an object, the wavelength of light, or the viscosity of a solution, are single point measurands; therefore, the instrument calibration and estimates of uncertainty need only be in a single dimension. Specifically, this applies to the absolute molecular moment determination methods discussed in Section 1.3. The molecular mass distribution is a two dimensional quantity of which the mass spectrum is its (imperfect) representation. Thus, both the mass axis and the signal axis (i.e., the intensity of the ion signal at a given mass) have to be calibrated separately, and their associated type B uncertainties considered separately.

Mass axis quantification is the most easily performed of the two and is not a significant source of uncertainty in determining the MMD from the mass spectrum. Calibration of most mass spectrometers is usually done with biopolymers of known molecular masses. These biopolymers are selected because they typically provide a single major peak whose mass is known accurately; thus, mass axis quantification is quite straightforward. Calibration

must be done using at least two or three of these biopolymers 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 biopolymer with a molecular material calibrant. If this material is close to, or identical to, the material under study then, in general, inaccuracies in mass axis calibration will be minimized. The oligomeric masses, m_i, with *n* repeat units of mass *r* and masses of the end group, m_{end}, of the polymer calibrant are given by:

$$m_i = nr + m_{end} + m_{adduct} \quad (4)$$

where m_{adduct} refers to the mass of any charged or neutral atoms or molecules non-covalently bound to the analyte. This may be, for example, any salts added to the sample preparation to encourage charging of the analyte.

Thus, calibration of the mass axis using a homopolymer calibrant (for example) reduces to determining *n* for one of the peaks. This is accomplished through use of the biopolymer mass as follows. The main peak from the biopolymer is assigned to its mass. The biopolymer peak will either lie between the masses of two n-mers of the calibrant, or exactly correspond to the mass of an n-mer. If it is at exactly the same mass as one of the *n*-mers of the calibrant, use equation 4 to find the degree of polymerization, *n*, for the *n*-mer. If the peak of the biopolymer lies between the masses of two *n*-mers of the calibrant, use equation 4 to find n_1, the mass of the *n*-mer whose mass is less than that of the repeat unit lower than the mass of biopolymer. Find additional calibration points by selecting calibrant peaks at intervals between five to ten repeat units less than and greater than n_1 and compute masses from equation 4. Generally, a total of four or five calibration points are selected. For obtaining a MMD of a typical organic material, mass accuracy of better than a few mass units may not be necessary since polymer MMD are often not critically dependent on such accurate masses.

Calibration of the signal axis is much more difficult and will occupy most of the rest of this practice guide. There are many systematic uncertainties that can arise in the signal axis quantitation as discussed in the previous section. It would be an insurmountable task to try to quantify each of these uncertainties individually. Instead, the systematic bias in the signal axis is determined heuristically by a gravimetric calibration technique. By mixing together samples having different molecular mass distributions in carefully prepared

gravimetric ratios, a mixture's molecular mass distribution can be controlled. By comparing the gravimetric ratios to the total signal intensity in the mass spectrum, a calibration curve for the signal axis can be obtained.

3.3 Linearity in signal response versus analyte concentration

To estimate the level of uncertainty in an instrumental method, a theoretical construct is needed to determine how type B uncertainties affect the final measurand. Assume that there is a point in the experimental parameter space (sample preparation, instrument operation, and data analysis) where the signal intensity, Si , for an oligomer of mass mi is linearly proportional to ni , the number of polymer molecules at that oligomer mass. Mathematically this is given by:

$$S_i = kn_i \tag{5}$$

where for a narrow enough range of m_i it is assumed that k is a constant independent of m_i and the range of linearity, $n_i < n_0$, is about the same for all molecules in the sample.

If the measurement is performed in the linear region for all the oligomers of the sample, the overall signal from the quantity of analyte introduced into the mass spectrometer is given by:

$$\sum_i S_i m_i = k \sum_i n_i m_i \tag{6}$$

with n_i m_i summed over all i. From this it can be derived that:

$$\sum_i S_i m_i \Big/ \sum_i S_i = k \sum_i n_i m_i \Big/ k \sum_i n_i \tag{7}$$

The right hand side of the equation is the exact M_n of the polymer (see equation 1) independent of k since k in numerator and denominator cancels out. The same holds for equations for M_w and all higher moments. This is generally true when the measurements are made in the linear range of analyte versus signal strength. However, it is well known that the mass spectra of wide polydispersity analytes give poor representations of the molecular mass distribution due to large systematic uncertainties in the signal axis. That is, if the values of the m_i span too great a mass range then the values for k and/or the n_0 saturation limits must change dramatically, otherwise mass spectrometry would be able to obtain the MMD correctly for very broad distribution analytes.

If *k* is not a constant independent of *i* then, and if the measurements are made in a linear concentration range for each oligomer *i* (that is $n_i < n_0$), then:

$$S_i = k_i n_i \tag{8}$$

where k_i is now a function of the oligomer *i* for a fixed experimental method: sample preparation, instrument operation, and data analysis.

3.4 Example for mixtures of monodisperse components

The simplest example of gravimetric quantitation to test mass spectrometer response is to create a mixture of two monodisperse compounds: species 1 as a standard and species 2 as the analyte whose concentration is sought. If there is no systematic bias in the measurement then the ratio of S_2/S_1 is directly proportional to the gravimetric mass ratio G_2/G_1 where G_i is defined as the gravimetric mass of each species.

The signal from such a mixture, call it A, is:

$$S_A = k_1 n_1 + k_2 n_2 \tag{9}$$

The mass moments would be:

$$M_{wA}^{grav\_\exp} = \frac{\left(k_1 m_1 n_1 + k_2 m_2 n_2\right)}{\left(k_1 m_1 n_1 + k_2 m_2 n_2\right)} \tag{10}$$

$$M_{wA}^{grav\_\exp} = \frac{\left(k_1 m_1^2 n_1 + k_2 m_2^2 n_2\right)}{\left(k_1 m_1 n_1 + k_2 m_2 n_2\right)} \tag{11}$$

The gravimetric mass of species *i* is

$$G_i = m_i n_i \tag{12}$$

Substituting into equation (11)

$$M_{wA}^{grav\_\exp} = \frac{\left(k_1 m_1 G_1 + k_2 m_2 G_2\right)}{\left(k_1 G_1 + k_2 G_2\right)} \tag{13}$$

To simplify this let the mass fraction X be

$$X = \frac{G_1}{G_1 + G_2} \tag{14}$$

Substituting equation (14) into equation (13) and dividing numerator and denominator by ($G_1 + G_2$) yields:

$$M_{wA} = \frac{\left(k_1 m_1 X + k_2 m_2 (1 - X)\right)}{\left(k_1 X + k_2 (1 - X)\right)} = \frac{\left(m_1 X + \theta\, m_2 (1 - X)\right)}{\left(X + \theta (1 - X)\right)} \tag{15}$$

where

$$\theta = k_2 / k_1 \tag{16}$$

In this way the mass bias in the mass spectrum is reduced to a single metric, θ. θ equals one for an unbiased system. If species 2 is overcounted with respect to species 1 θ will be greater than one, if species 2 is undercounted θ will be less than one. The further θ is from one the greater the systematic bias in the mass spectrum.

3.5 Example for mixtures of polydisperse components

For most mixtures encountered, any given oligomer peak in the mass spectrum cannot be assigned exclusively to one or the other component of the mixture. In fact, a given oligomer peak may have contributions from both components in the mixture. Typically these overlapping molecular mass distributions are made up of indistinguishable-oligomer components, that is, each component of the mixture has some (but not all) oligomers that are identical to those in the other component as illustrated in Figure 4. This means that in this case the mass moments of the mixtures must be calculated and used to create a calibration curve. A full theory for the atypical case of distinguishable-oligomer mixtures (shown in Figure 5), or non-overlapping molecular mass distributions (shown in Figure 6), where each oligomer peak can be assigned to a specific component is given in Appendix A. In this special case, true type B uncertainties can be given for each oligomer in the target material and a true absolute molecular mass standard can be created. Refer to Appendix A for full details.

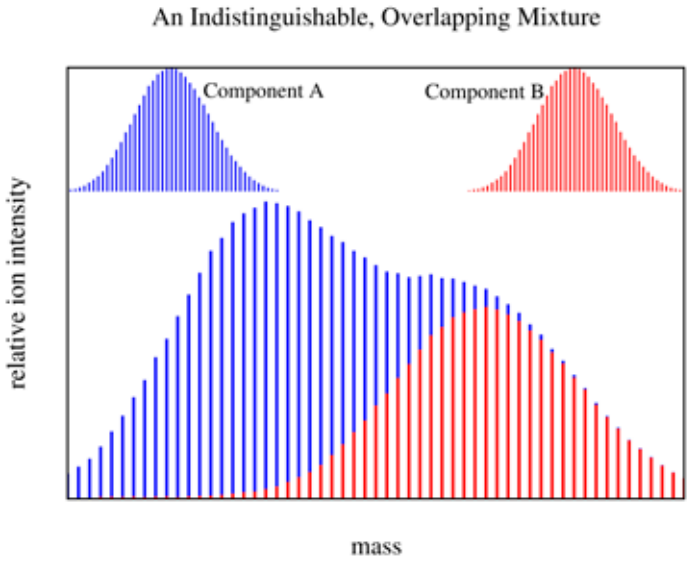

Figure 4— Schematic illustration of an indistinguishable and overlapping mixture of two components, where the peak intensities in the mixture are simply sums of component oligomer intensities.

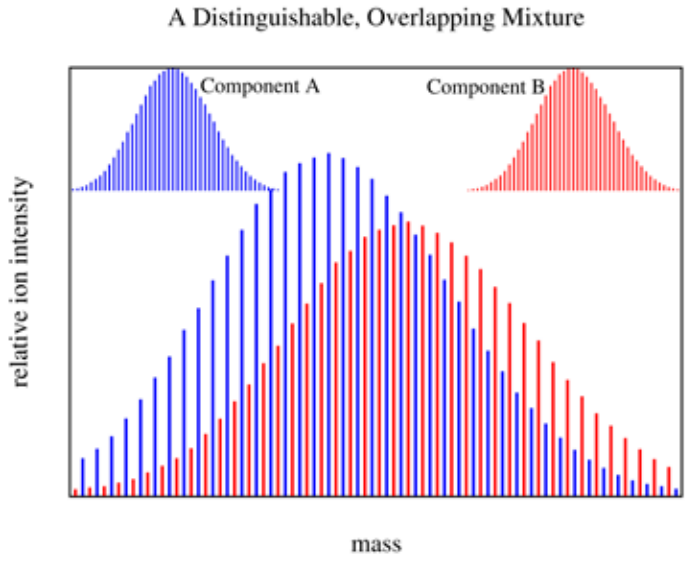

Figure 5— Schematic illustration of a distinguishable but overlapping mixture of two components.

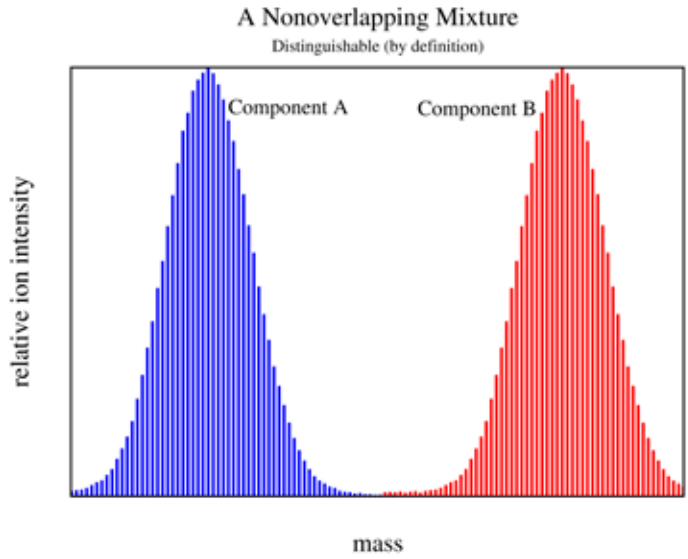

Figure 6— Schematic illustration of a non-overlapping mixture of two components which is distinguishable by definition.

Equation (15) can be extended to a gravimetric mixture of polydisperse components by substituting the experimental average molecular mass of each pure component derived from its mass spectrum. This leads to the mass moments:

$$M_{wq}^{grav} = \frac{\left(\hat{k}_1 M_{w1}^{\exp} X + \hat{k}_2 M_{w2}^{\exp}(1-X)\right)}{\left(\hat{k}_1 X + \hat{k}_2(1-X)\right)} = \frac{\left(M_{w1}^{\exp} X + \theta\, M_{w2}^{\exp}(1-X)\right)}{\left(X + \theta(1-X)\right)} \qquad (17)$$

Where q represents a given gravimetric mixture. In equation 17 $\hat{k}_1$ and $\hat{k}_2$ replace k_1 and k_2 used in the monodisperse example and are the mass-average means over each component of the mixture which is conceptually similar to the mass-average molecular mass. Likewise, X is now calculated from the gravimetric amounts of each component in the mixture. The mass moments of the pure components are from their mass spectra using equation (18):

$$M_{wq}^{\exp} = \sum_i S_{iq} m_i^2 \Big/ \sum_i S_{iq} m_i \qquad (18)$$

To obtain an estimate of the value of θ, the minimum value of the sum of squares is found. The sum of squares over all mixtures q is expressed as:

$$SS_{\vartheta} = \sum_{q} (M_{wq}^{grav} - M_{wq}^{\exp})^2 \tag{19}$$

The simplest way to solve this equation is to insert an arbitrary value for θ (typically θ=1) and calculate a value for SS_{θ} then increment θ and recalculate SS_{θ}. This most basic iterative process will yield an optimal value typically in a few steps and can easily be encoded in spreadsheet software. Recall that values of θ near one indicate systems with little bias in the mass spectrum.

3.6 Calculating the Correction Factor for Each Oligomer

Once θ has been calculated and found to be near one, the next step in the process is to calculate the various k_i in order to correct the molecular mass distribution. If the k_i are a smoothly and slowly varying function of i (or m_i), a Taylor expansion on k_i may be made around a mass peak near the center of the MMD, termed M_0. The center is used to assure that the function is changing as little as possible over the entire width of the MMD; however, mathematically the choice is arbitrary. Thus:

$$k_i = k_0 + Q(m_i - M_0) + \text{ higher order terms in } m_i \tag{20}$$

$$S_i = k_0 n_i + Q(m_i - M_0) n_i + \text{ higher order terms in } m_i \tag{21}$$

where k_0 and Q are the first two coefficients in the Taylor expansion. They are also functions of all the experimental conditions: the instrument parameters, the sample concentrations, and the sample preparation method. (By k_0 it is not meant the k of the zeroth index oligomer but rather the zeroth derivative of the Taylor expansion). In this way the entire physics of the experiment is folded into these two coefficients. From these assumptions, and dropping the higher order terms in equation (21) one can derive the following important relationship:

$$M_{wq}^{\exp} = M_{wq}^{0} \left\{ \frac{1 + (Q/k_0)(PD_{wq} M_{wq}^{0} - M_0)}{1 + (Q/k_0)(M_{wq}^{0} - M_0)} \right\} \tag{22}$$

where $M_{wq}^{\exp}$ is the mass spectral mass-average molecular mass for the mixture of analytes given in equation (18). PD_w is mass average polydispersity (M_z/M_w) and is taken here to be the experimentally measured value (M_z^{exp}/M_w^{exp}).

Equation (22) is then solved for M_{wq}^{0} for various values of Q/k_o at a fixed M_o chosen as described below for the values of the mixtures described by q = A, B, C, etc. and for the initial components of the mixtures described as j = 1 and j = 2.

For a gravimetric mixture *A*, $M_{wA}^{grav\_0}$ is calculated from the values for the individual components M_{w1}^{0} and M_{w2}^{0} computed for each Q/k_o using a simple weighted average:

$$M_{wA}^{grav\_0} = \frac{G_1}{G_1 + G_2} M_{w1}^{0} + \frac{G_2}{G_1 + G_2} M_{w2}^{0} \tag{23}$$

where G_1 is the gravimetric mass of component 1 in the mix, and G_2 is similarly defined.

For each Q/k_o the sum of squares, $SS_{(Q/k_0)}$, is computed as:

$$SS_{(Q/k_0)} = \sum_{q} (M_{wq}^{grav\_0} - M_{wq}^{o})^2 \tag{24}$$

where the sum is taken over all measured mixtures. The Q/k_o which gives the minimum value of the $SS_{(Q/k_0)}$ is then taken as the best fit. As with equation (19), solution of equation (24) required iteration over incremented values of Q/k_o.

Dropping the higher order terms and rearranging equation (21) yields:

$$\frac{S_i}{k_0 n_i} = 1 + \frac{Q}{k_0}(m_i - M_0) \tag{25}$$

Equation (25) shows us how to apply the correction factor Q/k_0 to each oligomer *i* to arrive at a more reliable measure of the molecular mass distribution. If Q/k_0 were equal to zero, then the mass spectrum would show no mass bias and $S_i = k_0 n_i$. This would mean that the peak areas are directly proportional to the oligomer concentrations in the sample. If Q/k_0 is non-zero, then mass bias is present. If M_0 is taken at the middle of the distribution being calibrated, then the sign of Q/k_0 along with where the mass m_i of an oligomer *i* is greater than or less than M_0 determines if the correction to the ion intensity is positive or negative.

4. Step by Step Procedure for Quantitation

The steps of the method can be summarized as follows:

1. Obtain at least two samples having different molecular mass distributions but with otherwise very similar, if not identical, properties.

For example, these could be polymers with different degrees of polymerization or nanoparticles with different levels of functionalization. The different samples could be obtained directly by synthesis or by separation of a single broader molecular mass sample. Two samples are required at a minimum, but additional samples will allow for more calibration points. If possible the only difference between the two should be molecular mass. Any other differences, for example, different functional groups may contribute to mass bias in an uncontrolled way.

2. Take mass spectra of each sample endeavoring to keep all experimental conditions constant.

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 constant any variables that may change over time. These variables could be sample preparation conditions, for example water absorption into samples or solvents, or time drift in instrument settings.

3. Use a laboratory balance to make carefully controlled gravimetric mixtures of two samples in several well-spaced ratios.

The balance needs to be calibrated and accurate to about least 0.1% of the total mass measured. Any gravimetric errors are carried through the entire analysis. Making stock solutions and then mixing solution volumes can be more accurate than repeated weighing of small amounts of material. Generally, as a practical matter, final weights must be at least 25 mg.

4. Take mass spectra of each mixture using the same experimental conditions as used for the pure components.

The instrument settings may not be optimal for the mixtures, but they must be held constant to satisfy the self 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 compromise experimental conditions must be found. If this occurs, then it suggests strong mass bias in the measurement.

5. From the mass spectra calculate the mass-average molecular masses of the pure components and of the mixtures.

Be careful in the application of "black box" software for this step. Unseen algorithms for data processing can lead to substantial errors in converting the mass spectrum to a molecular mass distribution. [7] Smoothing can introduce mass bias into a spectrum that is not a product of the measurement itself but of the data analysis method applied. [5]

6. Use equation (19) to iteratively calculate the minimum value of SS_0 at a given θ.

The most direct way to do this is to set up a simple spread sheet. Start with $\theta=1$ and change it systematically by small steps until a minimum in SS_0 is found. If θ is between 0.5 and 2 then the possibility exists that the molecular mass distribution can be corrected. If not, the results should be treated with caution, and the error is too great to be corrected using only the linear term in the Taylor expansion. See Appendix B for an example of computer code to make this calculation.

7. Choose M_0, a mass near the center of the average molecular masses of the two components.

The exact choice of M_0 is not critical; however, the correction to the distribution will be more accurate near M_0 and less accurate the farther any given oligomer mass is from M_0. If a certain mass range is more critical, then choose M_0 at the center of that range.

8. Use equation (24) to iteratively calculate the minimum value of $SS(Q/k_0)$ at a given Q/k_0.

See Appendix B for computational assistance.

9. Use equation (25) and the value for Q/k_0 to correct the ion intensities S_i in the mass spectrum to arrive at a new molecular mass distribution.

Individual oligomer intensities may increase or decrease depending on whether they were under counted or over counted in the mass spectrum.

At this stage the analyst should have a good feel for the degree of mass bias in the mass spectra. Furthermore, if this bias is not too large it can be corrected using the methods outlined in this section. If the bias is large, higher order terms in equations (20) and (21) need to invoked; however, methods to determine the values of the higher order coefficients have not been created. This is a fruitful topic for future research.

The procedures outlined in this section do not provide systematic uncertainties for the corrected values. The corrected mass spectrum is closer to the true molecular mass distribution, but just how close is it? In order to determine this, the procedures of Appendix A must be invoked. These procedures require distinguishable or non-overlapping mixtures as well as numerical instrument optimization [4] to determine the systematic uncertainties inherent in the instrument. This requires extra effort on the part of the analyst, but a molecular mass distribution with both type A (random) and type B (systematic) uncertainties is a very useful calibration standard for mass spectrometry and any other molecular mass measurement technique.

5. Appendix A — Full mathematical theory for distinguishable/ non-overlapping mixtures

If the desired outcome of a quantitation effort is the certification of an absolute molecular mass distribution reference material, including both type A and type B uncertainties, then calibration of the signal intensity axis must be performed using mixtures of analytes where each oligomer peak in the mass spectrum can be identified with a specific component of the gravimetric mixture. These are termed distinguishable-oligomer mixtures. The non-overlapping aspect of the mixture can be created in two ways. The first is by having molecular mass distributions that are well separated so that the highest mass oligomers of one are lower than the lowest mass oligomers of the other. The difficulty here is the requirement of a linear (not quadratic or higher) correction term. Over wide mass ranges higher order correction terms are more likely to be required. The second is by using components that are chemically similar with one distinguishing mass feature. For example, in the case of NIST Standard Reference Material 2881 [2] polystyrene homopolymers with different alpha end groups (butyl and octyl) but the same omega end group were used to positively identify which oligomers belong to which component in the gravimetric mixture. Identification was possible even though the mass ranges of the components overlapped. The inert nature of the alkyl end groups was shown not to introduce mass bias into the mass spectrometry experiments.

Starting with equation 8 from Section 3.3, $S_i=k_in_i$, if an assumption is made that k_i is a slowly varying function of *i* (hence also of m_i), then a Taylor expansion around a mass peak near the center of the MMD, termed M_0, can be made. The center of the mass spectrum is used to assure that the function is changing as little as possible over the entire width of the MMD. Then:

$$S_i = k_o n_i + Q(m_i - M_0)n_i + \text{ higher order terms in } m_i \qquad \text{(A.1)}$$

Here Q and k_o are functions of M_0 as well as of all the experimental conditions: the instrument parameters, the sample concentrations, and the sample preparation method. (By k_o it is not meant the k of the zeroth index oligomer but rather the zeroth derivative of the Taylor expansion). In the experimental procedures, once the instrument parameters and experimental preparation methods are optimized, every attempt should be made to keep them constant to insure experimental reproducibility. Variation in the machine parameters can affect the variation of Q/k_o and thus the type B uncertainty.

The implications of the model embodied in equation (A.1) will now be explored and it will be shown how small linear shifts of the calibration constant Q over limited mass ranges effects quantities derivable from mass spectral data. First the total signal, the total detected mass, and the mass ratios of mixtures will be considered, and it will be shown how these quantities relate to the true MMD of the analyte.

The total signal, S_T, from the polymer is given by:

$$S_T = \sum_i S_i = k_o \sum n_i + Q(M_n^0 - M_0)\sum_i n_i \tag{A.2}$$

while the total mass of polymer detected, $G_T^{\exp}$, is given by:

$$G_T^{\exp} = \sum_i m_i S_i = k_o M_n^0 \sum_i n_i + Q M_n^0 (M_w^0 - M_o)\sum_i n_i \tag{A.3}$$

where M_n^0 and M_w^0 are defined in equations (A.4) and (A.5), and are the true number average and mass average molecular masses.

$$M_n^0 = \sum_i m_i n_i / \sum_i n_i \tag{A.4}$$

$$M_w^0 = \sum_i m_i^2 n_i / \sum_i m_i n_i \tag{A.5}$$

$$PD_n^0 = M_w / M_n \tag{A.6}$$

$$M_z^0 = \sum_i m_i^3 n_i / \sum_i m_i^2 n_i \tag{A.7}$$

$$PD_w^0 = M_z / M_w \tag{A.8}$$

where m_i is the mass of a discrete oligomer, n_i is the number of molecules at the given mass m_i. The experimental moments from mass spectrum are defined as M_n, M_w, and M_z, while the true values are given as M_n^0, $M_w^0$and M_z^0. PD_n defines the polydispersity index which is a measure of the breadth of the polymer distribution. When PD_n is equal to one (i.e., in statistical terms the variance

of MMD is zero), all of the polymer molecules in a sample are of the same molecular mass and the polymer is referred to as monodisperse.

Multiplying equations (A.4) and (A.5) together gives:

$$M_w^0 M_n^0 = \sum_i m_i^2 n_i / \sum_i n_i \tag{A.9}$$

Then taking the ratio of equations (A.2) and (A.3), one obtains:

$$M_n^{\exp} = \sum m_i S_i / \sum S_i \tag{A.10}$$

with the result that:

$$M_n^{\exp} = M_n^o \left\{ \frac{(1+(Q/k_o)(M_w^o - M_o)}{(1+(Q/k_o)(M_n^o - M_o)} \right\} \tag{A.11}$$

where $M_n^{\exp}$ is the experimentally-measured M_n^0 .

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

$$M_w^{\exp} = \sum m_i^2 S_i / \sum m_i S_i \tag{A.12}$$

with the result that:

$$M_w^{\exp} = M_w^o \left\{ \frac{(1+(Q/k_o)(M_z^o - M_o)}{(1+(Q/k_o)(M_w^o - M_o)} \right\} \tag{A.13}$$

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

Equation (A.13) gives by simple division:

$$M_w^0 = M_w^{\exp} \left\{ \frac{1+(Q/k_o)(M_w^o - M_o)}{1+(Q/k_o)(M_z^o - M_o)} \right\} \tag{A.14}$$

which yields:

$$M_w^0 = M_w^{\exp} \left\{ 1 - \frac{(Q/k_o)M_w^o(PD_w - 1)}{1+(Q/k_o)(M_z^o - M_o)} \right\} \tag{A.15}$$

Equation (A.15) states that the deviation of the mass moment measured by mass spectrum from the true mass moment is a function of the polydispersity (PD) (arising from that moment) divided by a correction term arising from how far that moment is from the mass M_o around which the Taylor expansion to obtain k_0 and Q is centered. In equation (A.15) the reader should notice that if

M_z^o is close to M_o the term in $(Q/k_o)(M_z^o - M_o)$ is small compared to 1 and the result depends only on the polydispersity of the polymer.

Since the method depends on gravimetrically mixing analytes to obtain estimates of Q/k_0, it is necessary to consider the equations relating to these mixtures. Equation (A.3), states that the MS measured total mass, $G_T^{\exp}$, is proportional to the true mass, G_T^0:

$$G_T^0 = M_n \sum n_i \tag{A.16}$$

$$G_T^{\exp} = k_o G_T^0 \left\{1 + (Q/k_o)(M_w^0 - M_0)\right\} \tag{A.17}$$

Consider now a mixture of the chemically identical analytes with functional groups having different masses, or two different molecular mass analytes having distributions that are well separated, such that each oligomer in the mass spectrum can be assigned to a specific polymer in the mixture. Call them analyte A and analyte B that will make up the components of the gravimetric mixtures. Then the measured ratio of the masses of each is:

$$\frac{G_{TA}^{\exp}}{G_{TB}^{\exp}} = \frac{k_{oA} G_{TA}^o}{k_{oB} G_{TB}^o} \left\{ \frac{1 + (Q_A/k_{oA})(M_{wA}^o - M_o)}{1 + (Q_B/k_{oB})(M_{wB}^o - M_o)} \right\} \tag{A.18}$$

Notice the expansions are performed for both polymer distributions A and B around the same M_o. Note Q_A, Q_B, k_{oA} and k_{oB} are all functions of M_o.

Thus from equation (A.18):

$$\frac{G_{TA}^{\exp}}{G_{TB}^{\exp}} = \frac{G_{TA}^{o}}{G_{TB}^{o}} \left\{ \frac{1+(Q/k_o)(M_{wA}^{o} - M_o)}{1+(Q/k_o)(M_{wB}^{o} - M_o)} \right\} \tag{A.19}$$

Simple algebra leads us to:

$$\frac{G_{TA}^{\exp}}{G_{TB}^{\exp}} = \frac{G_{TA}^{o}}{G_{TB}^{o}} \left\{ 1 + \frac{(Q/k_o)(M_{wA}^{o} - M_{wB}^{o})}{1+(Q/k_o)(M_{wB}^{o} - M_o)} \right\} \tag{A.20}$$

What are measured are $\frac{G_{TA}^{\exp}}{G_{TB}^{\exp}}$ from mass spectrometry versus $\frac{G_{TA}^{o}}{G_{TB}^{o}}$ gravimetrically determined. The calculated slope is:

$$slope = \left\{ 1 + \frac{(Q/k_o)(M_{wA}^{o} - M_{wB}^{o})}{1+(Q/k_o)(M_{wB}^{o} - M_o)} \right\} \tag{A.21}$$

As before with equation (A.17) the reader should notice if M_{wB}^{o} is close to M_o the term in $(Q/k_o)(M_{wB}^{o} - M_o)$ is small compared to 1 which means the slope depends only on the difference $(M_{wA}^{o} - M_{wB}^{o})$ and, thus, (Q/k_o) maybe easily calculated. This concept with these equations can be used to obtain estimates of (Q/k_o) in a self consistent approach to the data analysis. Lastly, remember that the gravimetric calibration of the signal axis using chemically identical analytes can avoid the issues pertaining to the uncertainties arising from ablation, ionization, and detection. However, uncertainties in sample preparation as well as data analysis repeatability and consistency still affect the gravimetric calibration techniques.

6. Appendix B — Computer Code to Obtain Estimate of Q/k_0 from Experimental Data

This section gives sample Visual Basic (VBA) code to solve for M_w^o by varying Q/k_o (referred to as Qset in the code) assuming $PD_0 = PD_{\exp}$. Using equation 22 from Section 4 for the $M_w^{\exp}$ of a mixture made up of components j = 1 and 2:

$$M_{wq}^{\exp} = M_{wq}^0 \left\{ \frac{1+(Q/k_0)(PD_{wq}M_{wq}^0 - M_0)}{1+(Q/k_0)(M_{wq}^0 - M_0)} \right\} \tag{B.1}$$

where the subscript q can take on values of A, B, C, etc. for various gravimetric mixtures of components 1 and 2. Assuming $PD_{wq} = PD_{wq}^{\exp}$, then M_{wq}^0 is quadratic and is solved for each j. From equation (23) of Section 4:

$$M_{wq}^{grav\_0} = \frac{G_1}{G_1+G_2} M_{w1}^0 + \frac{G_2}{G_1+G_2} M_{w2}^0 \tag{B.2}$$

Equation (24) from Section 4 is then invoked. That is, the sum of squares of the difference between $M_{wq}^{grav\_o}$ and M_{wq}^o with $PD_0 = PD_{\exp}$ is calculated for any Q/k_o for all the mixtures:

$$SS_{(Q/k_o)} = \sum_{all\_q} (M_{wq}^{grav\_0} - M_{wq}^0) \tag{B.3}$$

Equation (B.3) is solved iteratively on a simple spread sheet with Q/k_0 varied in steps over an expected range. M_o is arbitrarily chosen near the middle of the component spectrum to be quantified. Once the code calculates a range of $SS_{(Q/k_o)}$ values, the minimum is found by simple inspection. This minimum gives the slope of the correction line, Q/k_0, to be applied to each peak in the mass spectrum. If the minimum is too broad the algorithm can be repeated with a smaller step size around the minimum.

Sample VBA Code:

```
‘  All lines with single quote mark, “  ‘ “ , at beginning of line are comments
‘  All line  with Cells function or with  Irow or Icol variables  in them are for
‘  input or output onto  the spreadsheet
‘  Input Experimental MMD  moments of mixtures as well as Mn, Mw and Mz
‘  of two species being mixed
‘  Input gravimetric mixtures fractions of each of the mixtures
‘  Need to have at least 3 mixtures
‘  Vary Q/ko (as Qset) to compute Mno (or Mn_true) assuming PDo=PDexp .
‘  Using Mno for each species in mixture
‘  and fraction of each species computing M_grav computing M_grav_n
‘  moment
‘  Try to optimized quadratic difference between Mn_grav and Mn_true for
‘  mixtures of first and last species
‘  The next few lines of code take equation B.1 and solve the quadratic in Mwj0
‘  (called M_true(2,ik))for
‘  where ik=j
‘  equations   assume PDo is known and is PDexp
‘  equations   Rw=M_exp(2,ik)*Qset
‘  equations for Mw Uw=1/(2*PD(2,ik)*Qset)
‘  equation square  Vw=((1-Ro*Qset-Qset*M_exp(2,ik))^2+2*
   (1-Ro*Qset)*Qset*M_exp(2,ik)
‘    if Vw< 0 then stop 003
‘  equation  M_true(2,ik)= Uw*((1-Ro*Qset-Qset*M_exp(2,ik))+sqr(Vw))
‘  equation  M_true(2,ik)= Uw*((1-Ro*Qset-Qset*M_exp(2,ik))-sqr(Vw))
‘   check on whether + or - is correct by seeing if in limits
‘  The next few lines of code take equation B.1 and solve the quadratic in Mnj0
‘  (called M_true(1,ik))for
 ‘ where ik=j
‘  equations for Mn Un=1/(2*PD(1,ik)*Qset)
‘  equation   Vn=((1-Ro*Qset-Qset*M_exp(1,ik))^2+2*(1-Ro*Qset)*Qset*M_
‘  exp(1,ik)
‘    if Vn< 0 then stop 003
‘  equation  M_true(1,ik)= Un*((1-Ro*Qset-Qset*M_exp(1,ik))+sqr(Vn))
‘  equation  M_true(1,ik)= Un*((1-Ro*Qset-Qset*M_exp(1,ik))-sqr(Vn))
‘   check on whether + or - is correct by seeing if in limits
‘  equation for polydispersity
‘  equation  PD_exp(i,ik)=PD_true(i,ik)*(1-Ro*Qset+Qset*M_true(i+2,ik))*(1-
‘ Ro*Qset+Qset*M_true(i,ik))/((1-Ro*Qset+Qset*M_true(i+1,ik))^2)
```

```
‘Input Data for run other the raw data
LimitUp = 1147  ‘data from range of distribution--generally upper limit of
smallest peak seen
LimitLow = 720  ‘data from range of distribution--generally lower limit of
smallest peak seen
Mo = 950
Mo = Cells(Irowin - 4, Icolin + 1)
‘  The values below  are established for our specific data—reader needs to
estimate his own limits
LowQSet = -0.0043
StepQset = 0.00012
IstepMaxQset = 100
QupperLim = Abs(1 / (Mo - LimitUp))
QLowLim = Abs(1 / (Mo - LimitLow))
QLim = QupperLim
If QLim > QLowLim Then QLim = QLowLim
‘ fix up bounds on limits and outputs
‘output starts here
Cells(Irowin - 5, 1) = “MacroName=”
Cells(Irowin - 5, 3) = “Opt_Qset_Moments_Mixtures_Plus()”
Cells(Irowin - 4, 1) = “Mo=”
Cells(Irowin - 4, 2) = Mo
Cells(Irowin - 4, 3) = “QLim=”
Cells(Irowin - 4, 4) = QLim
‘  Icol set previously in software
Icol0 = Icol + 2
For IQavg = 1 To IstepMaxQset
‘  set Q/ko
Qset = LowQSet + StepQset * IQavg
‘  this section is shifting active cells for output
irowStart = Irowin + IQavg * (NumSheet + 7)
Cells(irowStart - 3, Icol0) = “Qset=”
Cells(irowStart - 3, Icol0 + 1) = Qset
icol = Icol0
Irow = irowStart
For ik = 1 To NumSheet
PDw(ik) = M_exp(3, ik) / M_exp(2, ik)
 ‘ equations to solve quadratic in  Mwo  for various values of Qset
 Uw = 1 / (PDw(ik) * Qset)
```

```
Ww = -(1 - Mo * Qset - Qset * M_exp(2, ik)) / 2
Vw = Ww ^ 2 + PDw(ik) * (1 - Mo * Qset) * Qset * M_exp(2, ik)
If Vw < 0 Then
Cells(Irow, Icol0 + 5) = " Vw negative-thus skip"
GoTo stop_Vn
End If
' M_true(2, ik) is Mw^o
M_true(2, ik) = Uw * (Ww + Sqr(Vw))
' input Cells(Irow, icol + 1) = M_true(2, ik)
'M_true(2, ik) = Uw * (Ww - Sqr(Vw))
'   check on whether + or - is correct by seeing if in limits
' output Mw^o  spread sheet
'Cells(irow + 1, icol + 1) = M_true(2, ik)
Irow = Irow + 1
Next ik
Cells(irowStart - 2, icol + 1) = "Mw_true"
icol = icol + 2
Irow = irowStart
For ik = 1 To NumSheet
' calculate Mw^o_grav using equation B2
'  FractionSeries1(ik) refers to mass fraction of series
' M_grav(2,ik) is  Mw^o_grav
M_grav(2, ik) = (FractionSeries1(ik) * M_true(2, 1) + (1 - FractionSeries1(ik))
* M_true(2, Iend))
'  Output  Mw^o_grav  to spread sheet
Cells(Irow, icol + 1) = M_grav(2, ik)
Irow = Irow + 1
Next ik
Irow = irowStart
SumVar2 = 0
For ik = 1 To NumSheet
M_Var(2, ik) = (M_true(2, ik) - M_grav(2, ik)) ^ 2
SumVar2 = SumVar2 + M_Var(2, ik)
' Output squared differences
Cells(Irow, Icol0 + 7) = M_Var(2, ik)
Irow = Irow + 1
Next ik
Irow = Irow + 1
'  output  Sum of squares are in equation B4
```

```
Cells(Irow, Icol0 + 7) = SumVar2
Irow = Irow + 1
‘ Label column
Cells(irowStart - 2, icol + 1) = “Mw_grav”
stop_Vn:
Next IQavg
End Sub
```

7. Appendix C — Documentary standards

Documentary standards for quantitative molecular mass distribution measurement by mass spectrometry in force at the time of publication of this Recommended Practice Guide.

1. ASTM International D7034-05 “Standard Test Method for Molecular Mass Averages and Molecular Mass Distribution of Atactic Polystyrene by Matrix Assisted Laser Desorption/Ionization (MALDI) Time of Flight (TOF) Mass Spectrometry (MS)”; http://www.astm.org/

2. International Organization for Standardization (ISO) DIS10927 “Determination of the molecular mass and molecular mass distribution of polymer species by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDITOF-MS)”; http://www.iso.org/

3. Deutsches Institut für Normung (DIN) 55674 “Synthetic Polymers - Determination of molecular mass and molecular mass distribution of polymers by matrix assisted laser desorption/ionization-time-of-flight-mass spectrometry”; http://www.din.de/

8. References

[1] According to International Organization for Standardization standard 31-8 (31. Quantities and units -- Part 8: Physical chemistry and molecular physics), the term "molecular mass" has been replaced by "relative molecular mass," symbol M_r. If that nomenclature and notation were followed in this publication, one should write $M_{r,w}$ instead of the historically conventional M_w for the weight average molecular mass with similar changes for M_n, and M_z. M_w would be called the "mass average relative molecular mass." The conventional notation, rather than the ISO notation, has been used in this publication.

[2] C.M. Guttman, K.M. Flynn, W.E. Wallace, A.J. Kearsley, Quantitative Mass Spectrometry and Polydisperse Materials: Creation of an Absolute Molecular Mass Distribution Polymer Standard, Macromolecules 42 (2009) 1695-1702.

[3] International Union of Pure and Applied Chemistry (IUPAC) "Standard Definitions of Terms Relating to Mass Spectrometry"; http://www.iupac.org/web/ins/2003-056-2-500

[4] W.E. Wallace, C.M. Guttman, K.M. Flynn, A.J. Kearsley, Numerical optimization of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry: Application to synthetic polymer molecular mass distribution measurement, Anal. Chim. Acta 604 (2007) 62-68.

[5] W.E. Wallace, A.J. Kearsley, C.M. Guttman, An Operator-Independent Approach to Mass Spectral Peak Identification and Integration, Anal. Chem. 76 (2004) 2446-2452.

[6] Guide to the Expression of Uncertainty in Measurement, International Organization for Standardization, Genève, Switzerland, 1993.

[7] C.M. Guttman, S.J. Wetzel, W.R. Blair, B.M. Fanconi, J.E. Girard, R.J. Goldschmidt, W.E. Wallace, D.L. VanderHart, NIST-Sponsored Interlaboratory Comparison of Polystyrene Mass Distribution Obtained by Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry, Anal. Chem. 73 (2001) 1252-1262.

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