The molecular weight distribution of low molecular weight polymers and oligomers can be measured by matrix assisted laser desorption ionization (MALDI) mass spectrometry (MS) as well as by the more conventional method of size exclusion chromatography (SEC). However, in the former case a number distribution of species is measured and the separation scale is the square root of the molecular weight, whereas in the case of SEC a weight-fraction distribution is measured and the separation is by the logarithm of molecular weight. The molecular weight distributions are shown to be in good agreement for both broad and narrow molecular weight distributions when the data are interpreted correctly. MALDI-MS has been shown to provide peak molecular weight values, $M_p$, for poly(methyl methacrylate) polymers that are low relative to manufacturers $M_p$ values measured by SEC. Comparison of theoretical $M_p$ values determined by MS or SEC is shown to be a function of how the data is displayed. For narrow molecular weight distributions, the theoretical $M_p$ value determined by MS will be 2 monomer units smaller than the $M_p$ determined by SEC. For wide, polydisperse, molecular weight distributions, the $M_p$ value determined by MS will be considerably lower than those obtained from SEC and depends on the type of polymerization. In condensation polymers, the presence of a cyclic molecular weight distribution, in addition to the linear MWD complicates interpretation of the data. The $M_p$ value reported should be reserved for weight fraction vs. log mass plots as in SEC. The modal molecular mass, $M_m$, should be used for data represented as number fraction vs. linear mass as with MS data.

Part of this study is discussed in more detail in C.Jackson, B. Larsen and C. McEwen, *Anal. Chem.*, (68) 8, 1303, 1996.
INTRODUCTION

Matrix-assisted laser desorption/ionization provides an important new method for characterizing polymers using time-of-flight mass spectrometry.\textsuperscript{1-10} The method is especially valuable for structural characterization of individual oligomers of low mass polymers.\textsuperscript{10-14} However, considerable attention has been focused on the ability of MALDI to provide accurate molecular weight distributions.\textsuperscript{6-10,14} Because polymer properties can be correlated with measurable characteristics of the molecular weight distribution such as number average ($M_n$), and weight average ($M_w$) molecular weights, and molecular weight polydispersity (D), accurate determination of these values is important for characterizing polymers.

Mass spectrometry provides absolute molecular weights and has the potential to provide molecular weight distributions that are more accurate than those determined by methods such as size exclusion chromatography (SEC), light scattering, and osmometry. However, because synthetic polymers are a mixture of oligomers that differ in molecular weight, end groups, branching, etc., accurate molecular weight analysis requires that both the mass and abundance of each oligomer species be correctly measured over a rather wide mass range. In mass spectrometry, an accurate abundance measurement for polymers requires that the ionization process, ion transmission, and ion detection be independent of mass (or at least a known function of mass) and oligomer structure (end groups, number of repeat units, etc.).

Results obtained from different laboratories using MALDI for polymer analysis have reached different conclusions concerning the applicability of MALDI for polymer molecular weight analysis.\textsuperscript{1,7,8,10} This is not unexpected because MALDI is an ionization method which itself has considerable variation,
especially in sample preparations, as well as instrumental effects. Significant variation of polymer molecular weight distributions are possible even with time-of-flight mass analyzers because of differences in ion acceleration, ion focusing, ion detection, etc. Thus, instrumentation or sample preparation may be the cause of different conclusions concerning polymer molecular weight analyses rather than the MALDI ionization process. If this is the case, it should be possible with an instrument properly designed for polymer analysis to find a matrix and sample preparation which will provide accurate molecular weight results for a given polymer type. Knowing how to evaluate when MALDI provides accurate molecular weight distributions is important.

The common method for determining the accuracy of mass spectral molecular weight distributions for synthetic polymers is by comparison with values provided by the manufacturer for polymer standards. These values are frequently obtained using size exclusion chromatography (SEC). The polymer standards are often identified by the $M_p$ values. $M_p$ is the mass value for the most probable (abundant) point on the SEC chromatogram. Because the mass of the most abundant peak is easy to determine by mass spectrometry, it is common to compare mass spectral generated $M_p$ values with the manufacturer's $M_p$ values. \cite{3,6,7} $M_p$ values thus become one measure of the accuracy of MALDI for polymer analysis. \cite{7} This paper discusses the applicability of comparing $M_p$ values generated by SEC and mass spectrometry and evaluates the accuracy of MALDI generated $M_p$ values.
EXPERIMENTAL

Poly(methyl methacrylate) (PMMA) standards were obtained from Polymer Laboratories (Church Stretton, U.K.) with the exception of the PMMA 17K standard which was obtained from American Polymer Standards Corporation (Mentor, Ohio). The poly(tetramethylene ethylene glycol) (PTMEG) used is a commercial product of Hodogaya Chemical Company, (Japan). The sample preparation for MALDI involved dissolving the polymers in tetrahydrofuran (THF) to make 10 - 100 μmolar solutions. 2,5-Dihydroxybenzoic acid (DHB) (Sigma) was dissolved in THF at ca. 10 mg/ml. Either, the two solutions were mixed 3:1 matrix to polymer and 0.5 μl spotted on the target, or 0.7 μl of matrix solution was dried on the target and 0.4 μl of polymer solution was added to the dried matrix. The polymer solution was prepared in soft glass vials as a means of increasing the sodium ion content of the solution.

The MALDI mass spectrometer used for these experiments was a Finnigan MAT Vision 2000 (Hemel Hempstead, England) and was operated in the reflectron mode using 5 kV ion acceleration and 20 kV post acceleration. Spectra obtained in the linear mode were acquired using 30 kV ion acceleration with no post acceleration. Spectra were acquired by summing spectra from ca. 100 selected laser shots. A 337 nm nitrogen laser (Laser Science, Inc., Newton, MA) was used with the beam attenuated to just above threshold for polymer ions.

The chromatograph and detectors used in these experiments consists of a Waters 150C size exclusion chromatograph (Waters Associates, Milford, Mass.), a Viscotek 150R bridge viscometer (Viscotek Corporation, Houston, TX) and a DAWN DSPF laser photometer (Wyatt Technology, Santa Barbara, CA) equipped with a 5 mW helium neon laser operating at 632.8 nm. The detectors are connected in series with the photometer first after the columns and the refractometer last.

Four 300mm x 7.5 mm i.d. columns packed with MIXED-C5 micron PLGel packing (Polymer Laboratories, Amherst, MA) were used in tandem. The mobile phase was HPLC grade THF and the operating temperature was 30°C. Polymer solutions were prepared at a concentration of 2 mg/mL and the injected volume was typically 100 μl.
RESULTS AND DISCUSSION

Narrow Distribution Case

Figure 1 shows the MALDI mass spectrum for a narrow distribution PMMA standard. The $M_p$ value for the oligomer as determined by MALDI-TOF mass spectrometry operated in the reflectron mode is 2100 while that given by the manufacturer of the standard using carefully obtained SEC values is 2400. An $M_p$ value of 2100 was also reported for the same standard using a different manufacturer’s MALDI-TOF instrument operated in the linear mode. For narrow distribution PMMA standards, MALDI-MS consistently produces $M_p$ values that are low compared to size exclusion chromatography and has been a point of discussion concerning the accuracy of MALDI results. While the discrepancy between MALDI and SEC $M_p$ values has been cited as an error in the MALDI measurement, it can be shown that even for narrow distributions, a difference between the MALDI and SEC $M_p$ values is expected. A narrow polymer distribution can be described by a Poisson Distribution. Displaying the data from a Poisson distribution using the normal SEC format (weight average intensity vs. a log mass scale) produces $M_p$ values that are approximately two repeat unit masses higher than when the same data is displayed using the mass spectral format (number average intensity vs. linear mass scale).

Theoretical Description of a Narrow Molecular Weight Distribution

Poisson Distribution

A polymer formed by monomer addition without termination, such as in anionic "living" polymerizations, has a number fraction distribution $P$ of molecules of degree of polymerization $x$ described by a Poisson distribution, $^{15}$
$$P(x) = \frac{e^{-v x^{-1}}}{(x-1)!}$$  \hspace{1cm} (1)$$

where $v$ is the number of monomers reacted per initiator. The molecular weight is given by $x(M_0)$ where $M_0$ is the monomer molecular weight and $x$ is the number of monomers. This function describes the distribution measured in the mass spectrometry experiment. An alternative description of the distribution is as a weight fraction distribution $W$ given by

$$W(x) = \frac{1}{x_n} \frac{x e^{-v x^{-1}}}{(x-1)!}$$  \hspace{1cm} (2)$$

where the factor $1/x_n$ is a normalization factor and $x_n$ is the number average degree of polymerization, $x_n = v + 1$.

In size exclusion chromatography, the separation of the molecules depends on the logarithm of the molecular weight and so the calibration curve of log molecular weight is a linear function of the elution volume. The shape of the weight fraction distribution, $W(\log x)$, from such a separation is given by\textsuperscript{17, 18}

$$W(\log x) = \frac{x^2}{x_n x_w} \frac{e^{-v x^{-1}}}{(x-1)!}$$  \hspace{1cm} (3)$$

where $x_w$ is the weight average degree of polymerization,

$$x_w = \frac{v^2 + 3v + 1}{v + 1}$$  \hspace{1cm} (4)$$

The degree of polymerization at the peaks of these three distribution, $P(x)$, $W(x)$ and $W(\log x)$, can be found by calculation and are listed in Table 1. The peak molecular weight measured by SEC is found to be always 2 monomer units higher than that measured by mass spectrometry for a polymer
with a Poisson molecular weight distribution ($v > 3$).

<table>
<thead>
<tr>
<th>Distribution</th>
<th>Peak Molecular Weight $M_p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P(x)$</td>
<td>$(v + \frac{1}{2}) M_0$</td>
</tr>
<tr>
<td>$W(x)$</td>
<td>$(v + 1 \frac{1}{2}) M_0$</td>
</tr>
<tr>
<td>$W(\log x)$</td>
<td>$(v + 2 \frac{1}{2}) M_0$</td>
</tr>
</tbody>
</table>

**Table 1.** Values of the peak molecular weight for the Poisson molecular weight distribution.

Figures 2 and 3 are simulated distributions for PMMA ($M_p = 100$), for $v = 20.5$, demonstrating the difference between MS and SEC data, respectively. In Figure 2 the peak molecular weight is 2100 whereas in Figure 3 it is 2300 g/mol. In practice the molecular weight distributions are slightly broader than theoretically predicted due to impurities and a difference of 3-4 monomer units in molecular weight would be expected. At low molecular weights this difference in peak molecular weights will be apparent. However, at higher molecular weights the difference will become insignificant compared to experimental errors.

The above discussion demonstrates that for a narrow distribution (polydispersity is approaching 1), $M_p$ measured using a number fraction abundance scale and a linear mass scale, as is the case with mass
spectrometry, will be approximately two repeat units lower in mass (for a homopolymer) than when measured using a weight fraction abundance scale and a log mass scale, as is the case with SEC. Thus, in the case of the PMMA 2400 standard shown in Figure 1, the comparable $M_p$ value measured by mass spectrometry and graphed like SEC would be 2300. The one repeat unit difference (4% mass error) is well within the accuracy of the measurements. The $M_n$ and $M_w$ determined for PMMA 2400 by MALDI-MS and SEC differs by 100 Daltons. Therefore, small differences in $M_p$ values are expected for narrow distributions which are a result of how the data is plotted.

Wide Distribution Case

For wide polydisperse polymers, large discrepancies in $M_p$ as well as polydispersity measurements are observed between MALDI and SEC measurements. Montaudo, et. al. plotted a relationship between polydispersity and the "error" in measuring $M_p$ by MALDI. The error in measuring $M_p$ increased with increasing polydispersity.

This can also be seen in results from our laboratory. Figure 5 shows the MALDI distribution measured for a commercial poly(tetra methylene ethylene glycol) (PTMEG). The measured $M_p$ by mass spectrometry is ca. 1400 but by SEC is ca. 4000 (Figure 6). However, plotting the SEC data as a number fraction vs. a linear mass scale (Figure 7) gives an $M_p$ value of 1450 (382), in close agreement with the MALDI results. Clearly, plotting the mass spectral data as a number fraction intensity vs. a linear mass scale has a considerable influence on the measured values of $M_p$. We suggest that the peak molecular weight, as measured by MALDI mass spectrometry, be referred to as $M_m$ for modal molecular weight so that it is not confused with $M_p$ as measured by SEC.
Theoretical Description of a Wide Molecular Weight Polymer Distribution

The molecular weight distribution produced by linear condensation polymerization and by many addition polymerizations can be described by the Schulz distribution,\textsuperscript{19-21}

\[ F(x) = \frac{(-\ln p)^k x^k p x}{\Gamma(k)} \]  

(5)

For linear condensation polymerization, \( p \) is the extent of the reaction and \( k \) is equal to 1. In this case eq 10 reduces to the Flory most probable distribution. For addition polymerization, \( p \) is the probability of propagation steps among the combined total of propagation and termination steps. In general, \( k = 1 \) for termination by disproportionation and \( k = 2 \) for termination by second-order combination. The corresponding weight fraction distribution is given by

\[ W(x) = \frac{(-\ln p)^{k+1} x^{k+1} p^2 x}{\Gamma(k+1)} \]  

(6)

and the weight-fraction distribution measured by SEC is given by

\[ W(\log x) = \frac{(-\ln p)^{k+2} x^{k+2} p^2 x}{\Gamma(k+2)} \]  

(7)

The number and weight-average degrees of polymerization are given by

\[ x_n = \frac{k}{\ln p} \]  

(8)

\[ x_w = \frac{k+1}{\ln p} \]  

(9)

As in the case of the Poisson distribution, the molecular weights at the
peaks of these distributions are going to be different. In addition, in the case of polydisperse molecular weight distributions, the peak in the MS spectra depends upon the value of \( k \) in equation 10. The peak molecular weights are summarized in Table 2.

<table>
<thead>
<tr>
<th>Distribution</th>
<th>Peak Molecular Weight ( M_p )</th>
<th>( M_p ) for ( k = 1 )</th>
<th>( M_p ) for ( k = 2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( P(x) )</td>
<td>( M_n (k-1)/k )</td>
<td>( M_o )</td>
<td>( \frac{1}{2} M_n )</td>
</tr>
<tr>
<td>( W(x) )</td>
<td>( M_n )</td>
<td>( M_n )</td>
<td>( M_n )</td>
</tr>
<tr>
<td>( W(\log x) )</td>
<td>( M_w )</td>
<td>( M_w )</td>
<td>( M_w )</td>
</tr>
</tbody>
</table>

Table 2. Values of the peak molecular weight for the Schulz molecular weight distribution.

The peak molecular weight measured by SEC is always the weight-average molecular weight. However, measured by MS the peak molecular weight varies from the monomer mass (\( k = 1 \)) to half the number average molecular weight depending on the type of termination. Figures 8 and 9 show calculated molecular weight distributions with \( p = 0.90 \) and \( k = 1 \) measured by MS and SEC respectively. In Figure 8 the monomer is clearly the most abundant species by number fraction and in Figure 9 the peak molecular weight is the weight-average value of 1,900 g/mol (equation 14). Figures 10 and 11 show calculated molecular weight distributions with the same value of \( p, 0.90 \), but with \( k = 2 \). The peak from the MS distribution (Figure 10) is between 900 and 1000 g/mol which is half the number-average molecular weight (equation 14) and the SEC peak (Figure 11) is at 2,850 g/mol, the weight-average molecular weight.
Molecular Weight Distributions with Cyclics

In many condensation polymerizations it is possible for cyclic molecules to form when one end of the polymer reacts with its other end. In this case the molecular weight distribution consists of a distribution of linear species and a distribution of cyclic species. The distribution of the linear species is still described by the most probable distribution. The proportion of cyclic to linear molecules at a given molecular weight depends upon the probability of the cyclic reaction of the ends to the reaction of one or other of the ends with another molecule, given by the molar cyclization equilibrium constant $K_x$. The first theoretical treatment of ring formation in polymers was given by Jacobson and Stockmayer\textsuperscript{22,23} and extended by Flor\textsuperscript{24}. This treatment predicted a number fraction distribution of cyclic polymers that rapidly decreased with increasing size. The decrease in number fraction is much more rapid than the corresponding decrease for the linear polymers formed, and the cyclic polymers are limited to very low molecular weights. When the weight-fraction distribution is considered, the cyclic polymer distribution still decreases rapidly and monotonically, in contrast to the linear polymer molecular weight distribution which has the familiar broad single peak distribution. The molecular weight distribution of the cyclic species $W_c(x)$, where $x$ is the degree of polymerization, was derived by Jacobson and Stockmayer. For the case where each monomer can react with itself, such as nylon 6,

$$W_c(x) = \frac{2BM_0}{c} \frac{p^x}{x^{3/2}}$$

(10)

where

$$B = \frac{((3/2)\pi c)^{3/2}}{2l^3N_A}$$

(11)
where \( M_0 \) is the molecular weight of the structural unit, \( c \) is the concentration, \( \varepsilon \) is the number of atoms in the repeat unit backbone, \( l \) is the effective link length of each atom, \( N_a \) is Avogadro’s number and \( p' \) is a revised extent of reaction.

Figure 12 shows the combined number fraction distribution of linear and cyclic species for a condensation polymer with \( p = 0.9 \). Figure 13 shows the SEC chromatogram for the same distribution. In the number fraction distribution there are more cyclic polymers than linear polymers at low molecular weights, but the fraction decreases rapidly and above a certain molecular weight, in this case about \( n = 10 \), the distribution is entirely linear. In the SEC chromatogram the cyclic species cannot be completely resolved from the linear species but form an additional peak, or series of peaks, at high elution volumes. The presence of cyclic species makes it difficult to calculate a true molecular weight distribution. In the case of MALDI-MS, the cyclic and linear polymers are (generally) resolved, and in principle, both the cyclic and linear molecular weight distributions can be measured. In SEC, the two distributions cannot be resolved, in addition the calibration curves for linear and cyclic polymers are very different so it is not possible to estimate the molecular weight from the elution volume calibration curve.
CONCLUSION

Comparison of $M_p$ values as measured by mass spectrometry vs. SEC is shown not to be valid. $M_p$ values are a function of how the data is displayed. In SEC, the data is displayed as weight fraction intensity vs. a log mass scale. Mass spectrometry, uses a number fraction abundance axis and a linear mass axis. Changing from a linear to a log mass scale or from weight fraction to number fraction intensity will change the mass at which the most probable peak appears.

For narrow distributions, the difference between $M_p$ values as determined by mass spectrometry and SEC will be small (ca. 2 repeat units), but increases with increasing polydispersity. For wide polydisperse samples, $M_p$, as measured by SEC represents $M_w$ but as plotted from mass spectral data is dependent on the type of polymerization and can vary from the monomer mass in condensation polymerization to $\frac{1}{2} M_n$ for addition polymerization. It is therefore recommended that $M_p$ be reserved for weight fraction vs. log mass plots. Because the most abundant peak in a number fraction plot is related to the conditions used for polymerization, it can be an important parameter and should have its own identity. We suggest the acronym Mm for modal molecular weight which happens to be easily obtained by MALDI Mass Spectrometry.
REFERENCES

14.) Our paper to JASMS
25.) After this work was published a similar study of experimental rather than theoretical distributions was brought to our attention, and we recommend it to the reader: P.M. Lloyd et al., European Mass Spec., 1, 293 (1995).
Figure 1 MALDI spectrum of Poly(methyl methacrylate) 2400.
Figure 2  Simulated narrow distribution of oligomers for v=20.5 plotted on a linear scale.
Figure 3  Simulated narrow weight-fraction distribution of oligomers for ν=20.5 plotted on a logarithmic scale.
Figure 4. The difference in degree of polymerization between peak molecular weight values measured by SEC and MALDI-MS plotted as a function of the number average degree of polymerization for theoretical narrow molecular weight distributions. For high molecular weights, the peak value measured by MALDI-MS is two monomer units lower than that measured by SEC.
Figure 5  MALDI spectrum of Poly(tetra methylene ethylene glycol).
Figure 6  SEC chromatogram of the PTMEG sample (Figure 5) plotted on a logarithmic scale.

Figure 7  SEC chromatogram of PTMEG sample plotted as a number-fraction on a linear scale against the square-root of the molecular weight.
Figure 8  Theoretical distribution for a wide distribution of oligomers ($K=1$, $p = 0.9$); simulated mass spectrum plotted on a linear scale.

Figure 9  Theoretical distribution for a wide distribution of oligomers ($K=1$, $p = 0.9$); simulated SEC chromatogram plotted on a logarithmic scale.
Figure 10  Theoretical distribution for a wide distribution of oligomers (K=2 p = 0.9); simulated mass spectrum plotted on a linear scale.

Figure 11  Theoretical distribution for a wide distribution of oligomers (K=2 p = 0.9); simulated SEC chromatogram plotted on a logarithmic scale.
Figure 12 Theoretical number fraction distribution for a condensation polymer \((K=1, p=0.9)\) with cyclics.
Figure 13  Theoretical SEC chromatogram for a condensation polymer (K=1, p=0.9) with cyclics
Analysis of Low Molecular Weight Heparin Products by SEC/MALLS
James E. Knobloch and Patrick N. Shaklee
Scientific Protein Laboratories

Abstract only to be published