Laser desorption analyses in trapped ion mass spectrometry systems
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Isotope Beating Effects in the Analysis of Polymer Distributions by Fourier Transform Mass Spectrometry

The signals of the ions cyclotron orbital motions in Fourier Transform Ion Cyclotron Resonance Mass Spectrometry periodically undergo destructive interference on the time scale of the measurement if the frequencies of these motions are closely-spaced. The result is a beat pattern in the measured time-domain signal. If transients are sampled for less than two beat periods, it is likely that mass spectra obtained by Fourier transformation of these transients will show significant errors in the measured relative abundances. The effect was found to be significant in investigations of synthetic polymer samples, leading to errors in the derived average molecular weights. Here, we present experimental data on synthetic polymer samples which demonstrate that the measured molecular weight distributions exhibit distortions if the acquired transients are short compared to the beating period. These errors decrease for increasing transient lengths and become insignificant when the transient is acquired for longer than twice the beat period. Computer simulations show a similar behavior and indicate that the distortions are induced by isotope beating. By virtue of the newly constructed AWG system (Chapter 5) it was possible to obtain experimental data on an isotope depleted polymer distribution similar to the one studied in the computer simulations. This distribution was produced by SWIFT ejection of all $^{13}$C-containing ions from the analyzer cell. Distortions that depend on the transient length are not observed in these spectra. This proves that isotope beating is responsible for distortions in measured molecular weight distributions if transients are acquired for too short durations.
6.1 Introduction

In Chapter 3 it was already discussed that MALDI-FTICR-MS has distinct advantages in the analysis of (synthetic) polymers. However, reasonable care must be taken to reduce the amount of artificial error introduced at each step of the experiment. For example, mass discrimination effects in trapping either externally or internally generated ions can skew the observed oligomer distribution, as was extensively examined in section 3.4. In Chapter 2 it was outlined that the use of SWIFT [121] excitation waveforms with a relatively flat power frequency spectrum reduces artifacts that result from the uneven power distribution found in the commonly used linear chirp excitations [141]. This was one of the driving forces behind the instrumental development described in Chapter 5. Other sources of error include ion densities that induce amplitude errors due to space-charge effects [142], energy-dependent ion trapping [93, 143, 144], and dynamic Coulomb interactions of ion clouds with different frequencies during detection [145].

In this chapter we discuss another newly discovered source of error in measurements of the proper relative abundances of oligomers in a polymer distribution with FTICR-MS. This source of error is inherent to the measurement of the image charge induced on the detection electrodes by the coherent cyclotron motion of ions trapped in the analyzer cell. The detected signal is simply the sum of the instantaneous phases and amplitudes of the individual oscillators. Depending on the relative phase of the oscillators, this may lead to constructive or to destructive interference. The result is a complex waveform that contains a manifold of frequency components, which correspond to the different oscillators.

It is often observed that interference of the signals from ions of slightly different \( m/z \) produces modulation effects in the measured transients. This is especially the case in ESI-FTICR-MS experiments, because the frequency separation between isotopes may become very small for highly charged, high mass ions. This phenomenon is known as isotope beating [146] and is illustrated by the data in Figure 6.1, which shows the time-domain signal corresponding to the ESI-FTICR-MS spectrum of cytochrome c presented in Chapter 5 (Figure 5.6 A).

The theoretical description of modulation effects between closely spaced oscillators is fairly simple, and can be derived from basic trigonometric entities [146]. In the case of isotope beating, the modulation frequencies are determined by the frequency separation between adjacent isotopic peaks. Although the number of isotopic peaks for heavy ions can be quite considerable, the variation in the frequency difference between any pair of adjacent peaks is small. This allows the estimation of the overall beat frequency by the characteristic frequency spacing for a given charge state. This characteristic beat frequency (in Hz) is in good approximation given by:

\[
f_{\text{beat}} \approx \frac{zB}{2\pi m^2} \times 10^8 \tag{6.1}
\]

Here, \( z \) is the integer number of elementary charges on the ion, \( m \) is the average ion mass in u, and \( B \) is the magnetic field strength in Tesla. For example, cytochrome c ions with
Figure 6.1: Time domain transient measured at a magnetic field strength of 7 T for the 15+ charge state of cytochrome c (MW = 12,358). The periodic regions of diminished signal with a beat frequency of 11 Hz (eqn. 6.1) clearly illustrate the effect of isotope beating. Additional beats are observed from ions of other molecular weights that are present at low intensity.

a average mass of 12,358 and 15 elementary charges attached exhibit a major beat of 11 Hz ($\tau = 90$ ms) at a magnetic field strength of 7 T.

Some degree of modulation is usually also observed for other ionization techniques in the time domain transients of FTICR-MS spectra of most materials. However, the modulation periods for heavy ions produced by ESI can approach 10-20 seconds in length, even for highly charged ions. High mass ions, with large numbers of closely spaced isotopic peaks, produce a transient signal in which short bursts of data are separated by long periods (dictated by the modulation frequency) of little or no observable signal above the broadband spectral noise (Figure 6.1). Although the mass information provided by these transients is not compromised by modulation of the time domain data, longer acquisitions are required to achieve high resolution spectra that can be used to determine the charge state from the separation of isotopic peaks [146].

Previous work on isotopic modulation effects concerned the effect on mass resolution [146], but the impact on the magnitude of spectral peaks was not examined. However, the key question for polymer analysis is to what extent the beating phenomenon alters the individual intensities of mass peaks and how these changes affect the quantitative characterization of polymer distributions. Therefore, the effect of isotope beating on the measured relative abundances of a distribution of ions is investigated in this chapter. Experimental data on synthetic polymer samples are presented, which demonstrate that the measured molecular weight distributions exhibit distortions if the acquired transients are short compared to the beating period. The behavior of transients was numerically modelled to investigate the effect of multiple modulation frequencies on the determination of polymer distributions independently of other significant physical effects in the experiments. These computer simulations showed the same behavior as the experimental data.
Finally, experimental data on an isotope depleted polymer distribution are presented. This distribution was produced by SWIFT ejection of all $^{13}$C-containing ions from the analyzer cell. The absence of transient length dependent distortions in these measurements prove unambiguously that isotope beating is responsible for distortions in measured molecular weight distributions if transients are acquired for too short durations.

Although the data and models presented here are primarily involved with the mass information found in polymer distributions, it is reasonable to assume that samples with multiple, closely spaced masses such as petrochemicals, combinatorial libraries, and natural products would exhibit similar effects.

### 6.2 Experimental

#### 6.2.1 Instrumental

MALDI FTICR-MS spectra were acquired with the heavily modified APEX 7.0e FTICR-MS (Bruker-Spectrospin, Fallanden, Switzerland) equipped with a 7-T magnet. Instrumental and experimental details can be found in Chapter 2. In short, the MALDI samples were deposited on the stainless steel tip of a direct insertion probe and introduced into the external MALDI ion source via a vacuum lock. Ions were produced by irradiating the samples with the 337.1 nm wavelength beam of a nitrogen laser and subsequently transferred by electrostatic ion optics to the analyzer cell. After trapping, the ions were excited to a large, coherent cyclotron orbit and detected at an acquisition rate of typically 500 kHz into 128k data points. This time domain signal was zero-filled to 256k, followed by discrete Fourier transformation and magnitude calculation.

Ion selection was carried out using the home-built AWG system (Chapter 5). The experiments described in this paper use an adapted SWIFT algorithm [60, 121, 125] to distribute the excitation power over the duration of the excitation pulse. The calculated waveform is downloaded from the SGI workstation into the AWG memory. A small relay switching circuit connects either the output of the programmable frequency synthesizer or the AWG output to the transmitter. A TTL trigger pulse toggles this switching circuit. A second TTL trigger pulse is generated 3 ms later, when the relays have settled. This pulse triggers the AWG to output the loaded excitation waveform.

#### 6.2.2 Sample Preparation

The poly(methyl methacrylate) (PMMA) standard with an average molecular weight of 1140 was obtained from Polymer Laboratories (Amherst, MA). DHB (2,5-dihydroxybenzoic acid) was used as the MALDI matrix and was purchased from Sigma Chemical Co. (St. Louis, MO). The MALDI samples were prepared by mixing a 1-M matrix solution in ethanol with an approximately 10-g/L analyte solution in ethanol to yield a molar ratio matrix:analyte = 1000:1. This mixture was electrosprayed onto a stainless steel probe tip.
Approximately 0.1 mL analyte/matrix was consumed during sample deposition. The total sample loading on the probe was approximately 10 ng.

6.2.3 Numerical Simulations

To separate the effects of signal interactions from other physical phenomena, simulated transients were evaluated. These transients were produced by M. Easterling with a custom program [147] that runs on a 180 MHz MIPS R5000 based Silicon Graphics workstation (Silicon Graphics, Mountain View, CA) and uses arrays of mass and abundance values describing the fictional ion population in the analyzer cell. The mass and abundance values were either coded manually or generated automatically by providing the program with key (polymer) distribution parameters such as mean, standard deviation, and repeat mass. Isotopic patterns were calculated using the IsoPro program [148]. The amplitude of the transient signal \( I \) that would be measured for the fictional ion population is calculated for different time points according to

\[
I(n) = \sum_i \sum_j A_i C_j \sin \left( \frac{qB}{m_{i,j}} n \Delta \right)
\]  

(6.2)

where \( A_i \) is the abundance of the oligomer with a monoisotopic mass \( m_{i,0} \), \( C_j \) the natural abundance of the \( j \)-th isotope at mass \( m_{i,j} \), \( qB/m_i \) its unperturbed cyclotron frequency, and \( \Delta \) is the time step (or fictional reciprocal sample rate). In physical terms, the instantaneous amplitude is calculated by summing together the amplitudes for the individual ions. It is hereby assumed that all ions are instantaneously excited to a high cyclotron radius and that all phases are initially equal to zero. Broad band noise was not included in the simulation, and isotopic abundance ratios for each oligomer were treated the same as that of the central peak, as the abundances do not vary greatly for the major peaks in distributions with low polydispersity indices. Actual experimental conditions with respect to experimental phase lag due to chirp excitation, the delay between excitation and detection or ion cloud evolution effects such as dephasing [149, 150] signal locking [151–154], or damping [155] were not considered. Data manipulation and evaluation including FFT and mass calibration and were performed using the XMGR data analysis program compiled for the SGI workstation. This program assisted in handling the large data sets produced by the simulations, which approached 30 MB in size. All simulated distributions were windowed with the Hanning function prior to the Fourier transformation. Additionally, the data sets were zero-filled to eliminate significant random errors in intensity. Data sets shorter than 256 kword were zero-filled to 256 kword, while 256 kword transients were zero-filled to 512 kword.
6.3 Effect of Isotope Beating on Measured Polymer Distributions

6.3.1 Distortions in Measured Distributions

A wide range of industrial homopolymers are extensively used for diagnostics and calibration, in particular in MALDI-FTICRMS [113, 143]. Also within the framework of this thesis, many experiments were carried out on polymeric samples to characterize instrument performances and optimize experimental conditions utilizing their characteristic symmetrical molecular weight distributions. It was discovered, however, that changes in the size of the data set of acquired spectra results in changes in the observed polymer molecular weight distribution. This effect is illustrated with MALDI-FTICR-MS measurements on a PMMA1140 sample. Figure 6.2 shows a measured time domain signal. It was acquired over 131 ms at a fixed trapping delay of 1000 ms (i.e., no correction for

![Figure 6.2: Measured time domain transient for a MALDI generated PMMA1140 distribution. The dashed lines indicate the boundaries for sets of 4 K data points extracted at various times to compare the instantaneous distribution of amplitudes (Figure 6.5).](image)

the flight-time distribution distortions was applied) at a sample rate of 1 MHz. Isotope beating is not readily recognized in the depicted signal. This is not surprising, because the various different beating frequencies associated with the different oligomers spread the beats evenly in time.

The dependence of the observed PMMA distribution on the size of the data set is examined by Fourier transformation of different portions of the transient in Figure 6.2. The results are shown in Figure 6.3. For the top spectrum (Figure 6.3 A), the complete data set was part of the calculation. The observed distribution resembles the expected profile for this sample given the mass discrimination during the ion transport. The data set was truncated to 64 kword and 32 kword for the spectra in Figure 6.3 B and 6.3 C, respectively. Although a regular distribution is generally retained, it is clearly visible that the distribution is skewed and shifted about the center mass for smaller data sets.
Figure 6.3: MALDI-FTICR-MS spectra of a PMMA1140 distribution obtained by Fourier transformation of the full 128 kword time domain data set (A), the first 64 kword (B), and the first 32 kword of the data set (C).

In Figure 6.4 it is demonstrated that modulation effects are present in the transient of Figure 6.2 by examination of small portions of the transient. It shows the contour plot of mass spectra obtained by transformation of 1 kword segments of the PMMA transient as a function of their occurrence in the transient. The plot clearly depicts that the signals of the individual components of the molecular weight distribution exhibit profound beating patterns. The frequencies of the beating patterns were determined from the contour plot and are listed in Table 6.1. Also the theoretical frequencies, which were calculated with eqn. 6.1, are included. It shows that the observed and the calculated values agree well. This indicates that isotope beating causes the staggered nature of the contour plot.

The contour plot in Figure 6.4 also indicates that the mass dependency in the beating frequencies causes “instantaneous” errors in the molecular weight distribution. This could well explain the changes in the measured distributions for different acquisition times. For example, a sampling time of less than 0.02 s would not completely sample the first beat
Figure 6.4: Contour plot of PMMA1140 mass spectra obtained by Fourier transforming 1 kwrd segments of the transient in Figure 6.2 as a function of the position of the segment in the transient (i.e., time). An intensity modulation is observed for each component of the oligomer distribution.

Table 6.1: Beating frequencies of the components of the PMMA1140 distribution. The observed values were determined from the contour plot in Figure 6.4, the theoretical values were calculated with eqn. 6.1.

<table>
<thead>
<tr>
<th>Mass (u)</th>
<th>Observed Beating Frequency (Hz)</th>
<th>Theoretical Isotope Beating Frequency (Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1425</td>
<td>54</td>
<td>55</td>
</tr>
<tr>
<td>1325</td>
<td>62</td>
<td>63</td>
</tr>
<tr>
<td>1225</td>
<td>76</td>
<td>74</td>
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<td>1125</td>
<td>87</td>
<td>88</td>
</tr>
<tr>
<td>1025</td>
<td>104</td>
<td>106</td>
</tr>
<tr>
<td>925</td>
<td>127</td>
<td>130</td>
</tr>
<tr>
<td>825</td>
<td>160</td>
<td>164</td>
</tr>
</tbody>
</table>
of the \( m/z \) 1425 oligomer and therefore would underestimate its abundance. This effect is even stronger than is suggested by the contour plot, as is illustrated with the spectra shown in Figure 6.5. The PMMA transient was split in 32 blocks of 4 kword each in order to obtain these spectra. Selected blocks, which are indicated in Figure 6.2 with dashed lines, were transformed into mass spectra. The equal length of the blocks makes that the spectral line width is similar in all spectra so that peak height, rather than area, can be used as a consistent measure of relative ion abundance. It is seen that each section of the transient produces a set of significantly different amplitudes. In addition, comparison of the spectrum obtained from the full data set (Figure 6.3 A) with those of the selected blocks reveals that it matches nowhere. It is obvious that this raises serious doubts about the correlation between the measured and the true ion population in the analyzer cell.

6.3.2 **Numerical Simulation of the Distortions**

The effects of signal interferences on the spectral amplitude distribution can not be completely determined *in situ*. Although the frequency dependent power differences resulting from chirp excitation might be eliminated by SWIFT excitation [121], and space charge amplitude effects could be reduced by using a relatively low ion population, several other uncontrollable or unknown processes might effect the phase or amplitude of the rotating
ion packets over time [150, 151, 156, 157]. To avoid these problems, numerically calculated time domain transients were used to investigate the effect of interference between closely-spaced isotopic frequencies on the measured polymer distributions. In these numerical investigations, dynamic processes that occur naturally during ion detection such as broadening mechanisms, collision induced signal dampening [155] and signal locking [149, 152, 154, 158] were not considered.

Two transients of 256 kword size were generated for a molecular weight distribution closely matching that of a PEG distribution with average molecular weight of 2030 u. One was the result of summing sine waves with frequencies corresponding to each of the peaks including isotopes, whereas the other contained only the monoisotopic masses at the polymer repeat interval. Frequencies were calculated for a 4.7 T magnetic field strength. Transformed data using Hanning apodization for the leading 32 K points of the isotope containing data set is shown in Figure 6.6 A. For comparison with expected values, theoretical peak magnitudes used to generate the waveforms are shown as circles with the highest abundance value normalized with the transformed data set. Although the

Figure 6.6: Mass spectra obtained by Fourier transform of computer generated transients corresponding to an isotope containing (A & B) and a monoisotopic PEG2000 mass distribution (C). For the top and bottom spectra, only the first 32 kword data points were part of the calculations, whereas the middle spectrum was the result of the full 256 kword transient. The circles correspond to the abundance values of the distribution used to generate the transient.
distribution is somewhat symmetric, skewing in favor of the lower half of the distribution is observed for the short transient from the isotope-containing model. These errors resemble those found for experimentally-obtained transients of similar length (data not shown). Figure 6.6 B shows that the full 256 K data set transforms into a mass distribution that closely matches the expected values for the isotope-containing transient. Figure 6.6 C shows the transformation of the monoisotopic time domain data of equal length as in Figure 6.6 B. The masses calculated in this case lie within the peak heights of the model distribution and shifts in the centroid distribution are not apparent. A qualitative correlation between these results and experimental data indicates that the centroid shifts observed for polymer distributions are mainly attributable to the interference effects of closely-spaced isotope frequencies. It should be stressed that while amplitudes are affected, the measured frequencies are not altered by this effect, as dictated by the superposition theorem [57].

The effect of “instantaneous” errors in the amplitude distribution, which was observed in the experimentally obtained PMMA distribution (Figure 6.5), was also numerically investigated. The computer generated transients corresponded to the expected distribution of singly charged PMMA ions with an average molecular weight of 1000 u. They were calculated for a 7 T magnetic field strength and were of 128 kword size (131 ms transient for 1 MHz sampling rate). Again, one was calculated for the isotope-containing and one for the monoisotopic case. The data sets were split into 32 blocks containing 4 kword of data per block, and selected blocks were transformed into mass spectra to evaluate the signal evolution in time. As in the experimental data, the equal length of the blocks allowed the use of peak heights as a measure of relative ion abundance. Figure 6.7 shows the results of Fourier transformation of the selected blocks of the isotope-containing computer-generated transient. Each section of the transient is observed to produce a set of significantly different amplitudes for the given range of masses, although the distributional shape is fairly well defined for all of the data blocks. Comparison with the measured distributions (Figure 6.5) reveals that the variations in the numerically simulated data set are similar to those observed in the measured distributions. However, the S/N ratio in the experimentally obtained mass spectra is observed to decrease towards the end of the transient, whereas in the case of the simulated transient this ratio remains roughly constant. This effect results from signal dampening, which was not included in the simulations. Computer-generated mass spectra from monoisotopic oligomer distributions, shown in Figure 6.8, indicate no observable oscillation in the magnitudes of the polymer masses, but rather exhibit identical amplitudes for each transformed block. This demonstrates that the presence of isotope beating is the origin of variations during the lifetime of the transient in the amplitude associated with each oligomer in the mass distribution. It also indicates that the actual distribution is measured if transients are acquired sufficiently long or if the naturally occurring isotopes are removed.
6.3.3 Elimination of the Beating Induced Distortions by Removal of Isotopes

To prove that oscillations in the measured relative abundances of the components of a polymer distribution can be fully attributed to isotope-induced beat frequencies, isotope depleted experimental data were obtained for the PMMA sample. In these experiments, the arbitrary waveform generator was used to isolate the monoisotopic peaks by elimination of all isotopes from the analyzer cell by resonant ejection. Figure 6.9 shows mass data obtained by transformation of 1 kword segments of the monoisotopic transient as a function of time. It shows that the beating patterns that were observed in the contour plot in Figure 6.4 are no longer present.

In Figure 6.10, mass spectra of the full transient and selected blocks of 4 kword size are presented. As observed in the simulations, the shape of the mass distribution remains unchanged over the entire transient. Comparison of the mass spectra of the entire transients in Figures 6.5 and 6.10 reveals that the molecular weight distribution changes significantly by removal of the isotopes from the analyzer cell. Closer inspection of the distribution in Figure 6.10 indicates that the abundance of the \( m/z \) 925 ion is severely underestimated. Imperfections in the isolation waveforms come to mind as a potential
Figure 6.8: Spectra obtained by Fourier transforming selected 4 kword blocks of computer generated transients corresponding to a monoisotopic PMMA1140 mass distribution.

Figure 6.9: Contour plot of PMMA1140 mass spectra obtained by Fourier transforming 1 kword segments of the transient measured from the monoisotopic distribution as a function of the position of the segment in the transient (that is, time). No intensity modulation is observed.
Figure 6.10: MALDI-FTICR-MS spectra of the isotope depleted PMMA 1140 distribution for the full transient and selected 4 K blocks. Abundances are not observed to vary significantly as in the isotope containing spectra shown in Figure 6.5.

explanation of this underestimation. Waveform imperfections could affect the trapped ion distribution through off-resonance excitation. However, it is unlikely that this would affect only one component in the distribution as all notches in the frequency spectrum are symmetrically centered around the monoisotopic peaks and of equal amplitude. Furthermore, previous isolation experiments have never revealed such effects. Another explanation could be a variation in ion cloud evolution. If we assume that the experimentally determined distribution in Figure 6.10 represents the actual ion population in the cell, it is striking to see the similarity between the spectra of block 23 and 30 in Figure 6.5 (containing all isotopes) and Figure 6.10 (containing only the monoisotopic oligomer ions). The convergence of these two series of spectra is most likely a result of ion cloud evolution that favors the most populated mass channels. This effect has recently been attributed to coulombic shearing forces that preferentially dephase sparser ion clouds causing them to dampen before the denser ion clouds [159]. Additionally, the weak presence of only a few mass channels virtually eliminates the beating effect, as observed as smoothness
in the contour plots. As a result of these effects, the “instantaneous” mass distributions for both the isotope containing and depleted transients are observed to converge over the duration of the transient. The similarity of the final mass spectra from the final blocks of data supports our contention that the initial oligomer distributions were the same.

Examining small pieces of the time domain transients would be expected to yield mass spectra with varying amplitudes if beating effects created a discriminating sampling condition. In this case, a non-uniform number of maxima, or beats, are sampled for each frequency in the spectrum. Of greater importance to the analytical study of polymers is how the beating phenomena affects amplitude information for larger data sets, those in which the sample size provides Fourier-limited resolving power for baseline isotope separation. Experimental observations analogous to the one presented in Figure 6.3 (data not shown) indicated that centroid shifts are less significant for larger data set sizes. Table 6.2 shows that the theoretically produced data sets also exhibit this behavior. The calculated weight average for the isotope-containing PEG2000 theoretical distribution with an expected average molecular weight of 2032.13 u is seen to drop from a 5% error for the 32 kword data set, to an error of about 1% for the 128 kword transient. The 32 kword transient would represent an acquisition time of about 66 ms sampling for a bandwidth of 250 kHz, slightly longer than the isotopic beat period for the central mass (58 ms). The 8 ms sampling time provided by the 4 kword transient only represents about 15% of the beat period, resulting in large errors observed for transients of that length. A significant decrease in error is observed for the 64 kword transient, which utilizes 131 ms of magnitude information and contains at least two isotopic beats for each component of the distribution. From these data, it can be inferred that sampling for a period equal to the duration of two isotope-induced beats should be a minimum requirement to obtain a reasonable quantitative description of a polymer distribution. Slightly greater precision is obtained by further averaging of the instantaneous differences for an isotope containing distribution, as mentioned above for the 128 kword transient which samples more than four isotope beats of the center mass.

As determined earlier, the errors for the monoisotopic distribution are not observed to

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Table 6.2: Average molecular weights calculated from theoretical transients of different length for a PEG 2000 distribution with an average molecular weight of 2032.13 u. Significant error is observed for the small data set of the isotope containing transient. The error is sharply reduced as more data is sampled. In contrast, the data generated for monoisotopic distributions provides an accurate average molecular weight even for small data sets.

<table>
<thead>
<tr>
<th>Data set size</th>
<th>With isotopes</th>
<th>Without isotopes</th>
</tr>
</thead>
<tbody>
<tr>
<td>32 kword</td>
<td>1922.49 u (-98.64 u)</td>
<td>2031.26 u (-0.87 u)</td>
</tr>
<tr>
<td>64 kword</td>
<td>2024.93 u (-7.20 u)</td>
<td>2030.00 u (-2.13 u)</td>
</tr>
<tr>
<td>128 kword</td>
<td>2029.45 u (-2.68 u)</td>
<td>2031.41 u (-0.72 u)</td>
</tr>
</tbody>
</table>
significantly fluctuate for changes in the size of the data set. These results quantitatively support the earlier finding that longer transients tend to provide better molecular weight values for polymers. The longer sampling times inherently provide larger windows in which the disparity in the number of amplitude maxima for represented frequencies becomes smaller.

6.4 Conclusions

To accurately measure relative abundances for large distributions of ions that contain closely-spaced masses, transient duration must be considered to avoid the errors produced by isotopic beating in the time domain transient. The appropriate duration will depend on the magnetic field strength and mass and intensity distributions. Providing an analytical expression for the required sampling time for polymers is somewhat complex because the beat period is significantly different for the two ends of the distribution. To be conservative, the minimum sample time should cover several beat periods calculated for the frequency difference between isotopic peaks at the high mass end of the oligomer distribution. Verification that the transient is sufficiently long would require the performance of Fourier transformations on multiple truncations of the data set. As described earlier, the distributions will converge to a given central value as the data set size is increased. Convergence of the centroid indicates that sufficient sampling has been performed. It should be noted however, that the data found towards the end of the experimentally obtained transient is usually weighted much lower than that found at the beginning due to collision-induced signal dampening [155]. Also other temporally-evolving effects such as non-uniform decay and magnitude dependant ion cloud destabilization [145] should be considered when seeking longer transients. These considerations aside, accurate relative intensities can be obtained by sampling sufficient data points or by measuring isotope depleted spectra.

Finally, it is worth mentioning that the strategy of removing isotopes from the ICR-cell is also promising in the determination of the charge state of highly charged, high mass ions. For example, if the beating period is larger than the dampening constant of the transient, it would not be possible to detect the second beat in the transient. This is however required for isotopic resolution in the mass spectra [146] and, consequently, the charge state can not be determined from the mass-to-charge difference between adjacent isotopes. A set of experiments in which a charge state is isolated with increasing resolution could solve this problem. After all, if the isolated mass interval becomes smaller than the isotopic separation, only a single isotope would remain inside the ICR-cell. This would be expressed by a sudden increase in transient length. The charge state can then be determined from the width of the isolated mass interval.