Electrospray ionisation FT-ICR mass spectrometry of linear and hyperbranched polymers
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Chapter 4

Accurate determination of the polymer endgroup and monomer mass of polyoxyalkylenes

Accurate monomer and endgroup masses were measured of three polyoxyalkylenes in the mass range of 400-8000 Da using electrospray ionisation (ESI) Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS). Use of all multiple charge states observed with ESI lead to a threefold increase in precision of the endgroup and monomer mass determination when compared with MALDI. The improvement is attributed to the increased number of data points used for the regression procedure. Endgroup masses are determined with a mass error better than 5 and 75 millimass units for the molecular weight range of 400-4200 Da and 6200-8000 Da, respectively. A mass error of better than 1 millimass unit was observed for all monomer mass determinations. With ESI, endgroup and monomer masses have been determined for PEG molecules with a mass higher than 8000 Da. This is almost two times larger than observed with MALDI on the same instrument.

4.1. Introduction

One of the most commonly used techniques to obtain quantitative endgroup information is titration. However, this technique fails to provide information about the structure of the endgroup. NMR can provide information on both the endgroup and monomer structure but only yields an average of these polymer characteristics. Mass spectrometry provides detailed molecular information on the composition and structure for a large variety of polymer classes using only minute amounts of sample. Matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI TOF) has proven to be a good technique for the characterisation of the molecular weight distribution and the monomer/endgroup composition up to a molecular weight of ~ 50,000 Da. However, the mass resolution is not sufficient to distinguish the different isotopic peaks at high m/z.
Isotopic resolution is not necessary for the determination of the molecular weight distribution but it is required for an accurate endgroup and monomer mass determination as will be shown in this chapter.

MALDI Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) has been explored for the characterisation of synthetic polymer endgroups. However, given the single charge state generated with MALDI, the technique is limited to relatively low-molecular weight polymer systems (typically <10,000 Da) since ions with a high mass (high m/z) are difficult to trap. A technique that overcomes this problem is electrospray ionisation (ESI). The main advantage of ESI compared to other ionisation techniques is that multiply charged polymer ions are formed that are easier to trap and enables an analysis at lower m/z values. ESI also provides a low internal energy deposition, like MALDI, resulting in minimal fragmentation of the polymer ions. ESI can be used online with SEC, spraying conventional solutions. Solubility properties and the inability to interact with cations of for example polyolefins can be a limitation for polymer analysis with ESI.

Due to the multiple charge states created by ESI, overlapping distributions of polymeric ions are observed in the mass spectra. Mass spectrometers such as quadrupoles, sector and TOF instruments do often not provide enough mass resolution to distinguish between the isotopic peaks. The combination of ESI and FT-ICR MS provides enough mass resolution to distinguish the isotopic peaks of the polymer molecules in the different charge states of a molecular weight up to 23,000. Only a few research groups have used ESI FT-ICR MS for the characterisation of synthetic polymers, while most groups use MALDI.

We have combined the advantages of ESI and FT-ICR MS for the characterisation of polyethylene glycol (PEG), polypropylene glycol (PPG) and polytetrahydrofuran (PTHF) on monomer and endgroup mass. A novel method is introduced that uses all charge states in one linear regression procedure. We will demonstrate how this method can be used to improve the endgroup characterisation substantially compared to the linear regression procedure using a single charge state, as done with MALDI.

4.2. Experimental

The electrospray ionisation Fourier transform ion cyclotron resonance mass spectrometer (ESI FT-ICR MS) experiments are performed with a modified Bruker-Spectrospin (Fällanden, Switzerland) APEX 7.0e FT-ICR MS equipped
with a 7-T super-conducting magnet and a Bruker Infinity™ Cell (see chapter 2).

The polyethylene glycol (PEG): PEG3000 and PEG6000 and polypropylene glycol (PPG): PPG1150 and PPG3250 standards are obtained from Fluka Chemical (Buchs, Switzerland). PEG1000 is obtained from Serva (Heidelberg, Germany). The polytetrahydrofuran PTHF2000 sample is supplied by DSM (Geleen, The Netherlands). The numbers in the names denote the average molecular weight of the samples. All samples contain sodium from origin and are used without further purification. The PEG samples are sprayed in an ~ 1 μM solution with a solvent composition of 70:30 MeOH:H2O. PPG and PTHF are sprayed with concentrations of ~5 and 100 μM, respectively. The average molecular weight of the polymer standards (e.g. 3000 g/mol for PEG3000) is used for the calculation of the polymer concentration. Approximately 10 μM NaI (Aldrich) was added to the polymer solutions to be sprayed via a concentrated stock solution in H2O to increase the S/N ratio.

4.3. Results and discussion

A series of polyoxyalkylenes with different average molecular weights in the range 400-8000 Da was used for endgroup determination. Charge states of 1+ to 12+ are generated depending on the average molecular weight of the polymer standards. In figure 3.1 the spectra of PEG3000 and PTHF2000 are shown. All polymer peaks are isotopically resolved (see insert of figure 3.1) with a resolution (m/Δm)50% of ~25,000 at m/z ~1000 in broadband mode.

The spectrum of PEG6000 is more complex than the spectrum of PEG3000 although the samples contain approximately an equal number of polymer molecules. This difference is caused by three factors. First, higher mass molecules are observed in more charge state distributions. Second, the spacing between the molecules and isotopic peaks becomes smaller for higher charge states. Third, the number of isotopic peaks increases with the degree of polymerisation. Figure 4.1 shows a simulation of the isotopic distribution of three polymer molecules (upper figures) with degrees of polymerisation 25, 75 and 150, which are comparable to the size of a molecule in PEG1000, PEG3000 and PEG6000, respectively. Four isotopic peaks are observed for PEG1000 while for a PEG6000 molecule, about 10 isotopic peaks show up in the spectrum. Another feature in figure 4.1 is that the intensity of the mono isotopic peak decreases with increasing molecular weight. The intensity has become almost zero for PEG6000. This is because the probability to observe a molecule containing only 12C atoms decreases with an increasing number of carbon atoms. Some of the experimentally observed isotopic peaks of
PEG6000 (lower figure) show deviations from the simulated isotopic pattern. This is probably caused by an overlap with peaks of other polymer molecules. In general, the experimentally observed isotopic patterns correspond well with the simulated pattern.

4.3.1. Theory of the endgroup determination using the linear regression and averaging methods with electrospray ionisation

Two methods have been introduced by others for the calculation of the endgroup mass, monomer mass and mass accuracy of synthetic polymers from MALDI FT-ICR mass spectra. The method has been applied successfully to linear and hyperbranched polymers. The multiple charge states observed with ESI require a linear regression procedure similar to the procedure applied to the MALDI mass spectrum in which only singly charged ions are observed. In ESI, an ion in charge state \( z \) will contain \( z \) cations. The charge state is determined from the \( m/z \) spacing between the isotopic peaks. Since \( z \) can be very large, and the mass accuracy of the FT-ICR MS is very high, the mass of \( z \) electrons is taken into account. Fenn et al. estimated more than 4200 charges on a PEG with a molecular weight of 5 million, which will result in an error of \( \sim 2.3 \) Da if a correction for the electron is not performed. The mass of an electron \( m_{\text{elec}} \) has to be subtracted from the mass of the neutral atom or molecule that is responsible for the charge, to obtain the cation mass (for example \( m_{\text{atom}} - m_{\text{electron}} \)). The measured mass over charge ratio, \( (m/z)^{z+} \) _meas_, for the monoisotopic polymer molecule is given by

\[
\left( \frac{m}{z} \right)^{z+}_{\text{meas}} = \frac{n \cdot m_{\text{mon}} + m_{\text{end}} + z \cdot m_{\text{cat}}}{z} = n \cdot \frac{m_{\text{mon}}}{z} + \frac{m_{\text{end}}}{z} + m_{\text{cat}}
\]

where \( m_{\text{mon}} \) is the mass of the monomer unit, \( n \) is the number of monomers, \( m_{\text{end}} \) is the endgroup mass and \( m_{\text{cat}} \) is the mass of the cation. Equation (4.1) has to be extended with the term \( x \cdot (\text{mass } ^{13}\text{C} - \text{mass } ^{12}\text{C})/z \) if the selected molecules contains \( x \) \(^{13}\text{C} \) atoms. We have considered the masses of the cation and electron to be known exactly. Later in this chapter we will describe a method that enables the determination of the identity of an unknown cation.
Figure 4.1  Simulated (upper) and experimentally observed isotopic pattern of PEGs with \( n=25, z=2 \) (a), \( n=75, z=5 \) (b) and \( n=150, z=9 \) (c). Peaks marked with an asterisk (*) are the mono isotopic peaks. Peaks marked with an \# originate from other polymer molecules.

A more manageable way to determine the endgroup and monomer masses is by plotting the mass \( m_{\text{meas}} \) (by multiplying \((m/z)^{\pm}_{\text{meas}}\) with the charge state) as a function of \( n \) (equation (4.2)) assuming that \( z \) is known. In this equation the monomer mass and the sum of the endgroup and \( z \) cation(s) follow immediately from the intercept and slope, respectively

\[
m_{\text{meas}} = n \cdot m_{\text{mon}} + m_{\text{end}} + z \cdot m_{\text{cat}}
\]

(4.2)

In order to determine the degree of polymerisation \( n \) of an ion, all possible combinations, \( m_{\text{end}}=m_0^\pm n_i m_{\text{mon}} \), have to be considered. Here, \( m_0 \) is the smallest possible endgroup mass for \( n_i=0,1,2,\ldots \). The calculated endgroup mass must be consistent with the information that is known about the polymer system (synthesis etc).

Note that for unknown polymers only isotopic peaks may be visible in the mass spectrum that contains an unknown number of \(^{13}\)C atoms. The resulting \( m_0 \), which is calculated with linear regression is the sum of the endgroup and this unknown number of \(^{13}\)C atoms. To determine the number of \(^{13}\)C atoms the following procedure is followed. First, the monomer mass is the slope of
equation (4.2), which is accurately determined (see further). Second, the elemental composition of the monomer is determined using the monomer mass. Third, by comparing the theoretical isotopic pattern of a given molecule with the experimentally observed pattern, as done in figure 4.1, the number of \(^{13}\text{C}\) atoms that a given ion contains can be estimated. Note that for relatively large monomer masses, e.g. \(> 100\) Da, various elemental compositions can correspond with the determined monomer mass. This requires additional information about the polymer from techniques such as NMR.

For large \(n\), the distance between the measured points and the intercept becomes large. As a result, small deviations in the slope will introduce a large error in the endgroup mass. This can easily be seen from the expression for the precision of the endgroup mass \(\sigma_{\text{end}}^2\) that results from the propagation of errors in the extrapolation procedure

\[
\sigma_{\text{end}}^2 = \sigma_b^2 + \bar{n}^2\sigma_a^2 = \frac{\sigma_{\text{data}}^2}{N} + \bar{n}^2\sum_{i=1}^{N}n_i^2
\]

(4.3)

Where \(\sigma_b^2\), \(\sigma_a^2\) and \(\sigma_{\text{data}}^2\) are respectively the uncertainties at the centre of gravity in the fit procedure, the slope and the mass measurements, \(\bar{n}\) is the transformed average degree of polymerisation as described by van Rooij and coworkers \(^{93}\), \(N\) is the number of data points, \(n_i\) is a polymer molecule with degree of polymerisation \(i\) and \(n_i' = n_i - \bar{n}\). A more detailed description of equation (4.3) can be found elsewhere.

A method which is unaffected by small fluctuations in the slope has been evaluated by van Rooij et al. for MALDI FT-ICR MS.\(^{93}\) This method, 'the averaging method', requires a known elemental composition of the monomer. The method does not involve an extrapolation procedure to \(n=0\). By subtracting \(n\) times the mass of the monomer from the mass of the singly charged ions formed by MALDI, the endgroup plus cation mass is obtained. This procedure is followed by an averaging of the results of all polymer molecules. For multiply charged ions, as formed by ESI, the same result is obtained by subtracting \(n\) times the mass of the monomer from \(m^{z+}_{\text{meas}}\). The charge state follows from the spacing between the isotopic peaks. Hence isotopic resolution is absolutely necessary.
4.3.2. Results of the endgroup determination using the linear regression and averaging methods

The results for the determination of the endgroup and monomer masses of PEG1000, PEG3000 and PEG6000 determined with linear regression ($m_{\text{end,regression}}$ and $m_{\text{mon,regression}}$) and the averaging method ($m_{\text{end,average}}$) are shown in tables 4.1 and 4.2. The endgroup masses have been calculated for all observed charge states separately. Only peaks with S/N >3 were used for the endgroup calculations.

The elemental composition of the monomers of PEG, PPG and PTHF is confirmed with linear regression as C$_2$H$_4$O ($m_{\text{mon,exact}}$=44.0262 Da, $m_{\text{mon,regression}}$=44.025 Da), C$_3$H$_6$O ($m_{\text{mon,exact}}$=58.0419 Da, $m_{\text{mon,regression}}$=58.042 Da) and C$_4$H$_8$O ($m_{\text{mon,exact}}$=72.0575 Da, $m_{\text{mon,regression}}$=72.058 Da), respectively. The mass accuracy resulting from linear regression is very high ($\Delta m_{\text{mon,regression}}$<<0.1%) as can be seen from tables 4.1 and 4.2. Note that $m_{\text{mon,regression}}$ is determined for all charge states separately.

The endgroup mass $m_{\text{end,regression}}$ calculated with equation (4.2) includes the mass of the cation(s) as well (similar to the method used by van Rooij et al.\textsuperscript{93}) and therefore increases with the charge state. When the mass of the cations and $^{13}$C atoms is subtracted from the calculated endgroup, $m_{\text{end-cation,regression}}$ in tables 4.1 and 4.2, the elemental composition of the endgroups is confirmed as –H and –OH ($m_{\text{end,exact}}$=18.0106) for all polymers studied. This corresponds to what is known about the synthesis of polyoxyalkylene polymers. A comparison of the theoretical and the experimentally observed endgroup masses is described in more detail later in this chapter.

The $\sigma_{\text{mon,regression}}$ and $\sigma_{\text{end,regression}}$ values are the uncertainties of the monomer and endgroup mass respectively as determined with the linear regression method. The $\sigma_{\text{end,average}}$ value is the uncertainty of the endgroup mass as determined with the averaging method. Note that $\sigma_{\text{mon,average}}$=0 because the average method assumes the monomer mass to be known exactly. The determination of the accuracy in the experimental data ($\sigma_{\text{data}}$) has been done for all charge states separately. We have taken the maximum observed experimental mass error in the measurements as $\sigma_{\text{data}}$. The $\sigma_{\text{data}}$ value is considered for all charge states separately since the mass error increases with decreasing charge state (see below). Large fluctuations of $\sigma_{\text{data}}$ for the highest charge states have not been observed, e.g. for 3+ to 7+ of PEG3000.
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Table 4.1 Calculated monomer and endgroup masses (Da) and accuracy for PEG1000 and PEG3000 of all observed charge states determined with the regression and averaging method. Only the monoisotopic peaks have been used for the determination of the monomer and endgroup masses. The $\Delta$ values denote the difference between the theoretical and measured masses. The $\sigma$ values are the corresponding standard deviations, which follow from linear regression or equation 4.3 (average method). $m_{\text{end-cation, regression}}$ is the endgroup mass as calculated by equation 4.2 minus the mass of the cation(s). $m_{\text{end-cation, average}}$ is similar to $m_{\text{end-cation, regression}}$ but determined by the averaging method. $\sigma_{\text{data}}$ is considered for all charge states separately and is the maximum observed experimental mass error.

<table>
<thead>
<tr>
<th>Masses (Da) and PEG1000</th>
<th>PEG3000</th>
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</thead>
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<tr>
<td>deviations</td>
<td>z 1+</td>
</tr>
<tr>
<td>$n$ ($N$)</td>
<td>10-26</td>
</tr>
<tr>
<td>(17) (13)</td>
<td>(22) (34)</td>
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<tr>
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<tr>
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</tr>
<tr>
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<tr>
<td>$\sigma_{\text{data}}$</td>
<td>0.0117</td>
</tr>
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</table>
Polymer endgroup determination

<table>
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<th>Masses (Da) and</th>
<th>PE G6000</th>
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</thead>
<tbody>
<tr>
<td>deviations</td>
<td></td>
</tr>
<tr>
<td>( n(N) )</td>
<td>143-146</td>
</tr>
<tr>
<td>( m_{\text{max. regression}} )</td>
<td>( \Delta m_{\text{max. regression}} )</td>
</tr>
<tr>
<td>17</td>
<td>(26)</td>
</tr>
<tr>
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</tr>
<tr>
<td>44.0265</td>
<td>0.0056</td>
</tr>
</tbody>
</table>

Table 4.2 Calculated monomer and endgroup masses (Da) and accuracy for PEG6000 of all observed charge states determined with the regression and averaging method. For the determination of the exact masses, only the peaks containing 4 \(^{13}\)C isotopes were used. The \( \Delta \) values denote the difference between the theoretical and measured masses. The \( \sigma \) values are the corresponding standard deviations, which follow from linear regression or equation 4.3 (average method). \( m_{\text{end-cation,regression}} \) is the endgroup mass as calculated by equation 4.2 minus the mass of the cation(s) and C atoms. \( m_{\text{end-cation,average}} \) is similar to \( m_{\text{end-cation,regression}} \) but determined by the averaging method. \( \sigma_{\text{data}} \) is considered for all charge states separately and is the maximum observed experimental mass error.

The range of polymer molecules \( n \) and the corresponding number of datapoints \( N \) observed for each charge state \( z \) and used for the calculations are given in the tables. In most cases \( N \) is determined by the range of polymer molecules \( n \) observed in the mass spectrum. However, for some polymer molecules it was difficult to locate the peak due to peak overlap with other polymer molecules. Therefore, \( N \) and the range of polymer molecules \( n \) do not correspond in all cases.

The \( m_{\text{end-cation,regression}} \) values of PEG1000 and PEG3000 are \( \approx 18.01 \) Da which is less than 0.08% difference with the theoretical endgroup mass for \(-\text{OH and }-\text{H}\). For PEG6000 this value is smaller than 0.7%. The 2+ charge state of PEG3000 and 4+ - 5+ charge states of PEG6000 are the only exceptions due to the decreasing mass accuracy at higher \( m/z \) (see further). For PEG6000 and PPG3250 only the peaks containing 4 and 1 \(^{13}\)C isotopes were used for the endgroup and
monomer calculations, respectively. This is necessary since the intensity of the monoisotopic peaks becomes too low.

![Graph showing mass error as a function of m/z](image)

**Figure 4.2** Mass error \((m_{\text{exact}} - m_{\text{meas}})\) for PEG3000 (2+, 3+ and 5+ charge states) as a function of m/z for the observed charge states.

The \(\sigma_{\text{mon,regression}}\) and \(\sigma_{\text{end,regression}}\) values are highest for the ions which are observed in the m/z range of ~1300 to 1800, such as the 2+ charge state for PEG3000, and the 4+ and 5+ charge states of PEG6000. This is illustrated in figure 4.2 where the difference between exact and measured mass \((m_{\text{exact}} - m_{\text{meas}})\) is given for PEG3000 as a function of m/z. Only the 2+, 3+ and 5+ charge states have been plotted in figure 4.2 to prevent an overlap of different data points from different charge states. The average mass error fluctuates around zero if m/z is between 500 and 1200. An increase in the mass error is observed for m/z values above 1200. This increase is attributed to the decreasing number of data points that constitutes a peak. Ions of higher m/z have a lower cyclotron frequency and are therefore reconstructed with fewer data acquisition points. This decrease in accuracy at higher m/z partially explains the higher \(\sigma_{\text{mon,regression}}\) and \(\sigma_{\text{end,regression}}\) values. Another consequence of the increasing mass error with m/z is that the slope of the fitted line (equation (4.2)) will decrease, which results in lower \(m_{\text{mon,regression}}\) and higher \(m_{\text{end,regression}}\) values. This can easily be seen from the tables where the \(\Delta m_{\text{mon,regression}}\) value becomes more negative for lower charge states while \(\Delta m_{\text{end,regression}}\) increases. In general, the best mass accuracy is achieved at the highest charge states.

The linear regression procedure requires an extrapolation from about \(n=60\) to \(n=0\) for PEG3000. Small deviations in the slope of the line will dramatically
alter the extrapolated endgroup mass. This does not hold for the average method since this method does not require an extrapolation. For PEG1000, PPG1150, PPG3250 and PTHF2000 higher $\sigma_{\text{mon,regression}}$ and $\sigma_{\text{end,regression}}$ values at lower charge states, compared to higher charge states, are not observed. PEG1000 and PPG1150 are observed below $m/z$ 1300 where the mass accuracy is relatively high. The lowest charge states (2+ and 3+) observed for PPG3250 contain at least twice as many data points as the higher charge states and increase the endgroup accuracy according to equation (4.3). For PTHF2000 a similar reasoning can be held. The $\Delta m_{\text{end-cation,average}}$ values of PEG1000 and PEG3000 have a relative error of less than 0.02% with the 2+ and 3+ charge states of PEG3000 being the only exceptions. For PEG6000 these values have a relative error of ~0.4%. The $\sigma$ values determined with the averaging method are about one order of magnitude lower than the $\sigma$ values determined with the linear regression procedure.

The magnitude of the $\sigma$ values discussed in this chapter is determined by the number of data points $N$ used for the calculations, the distance between the measured points and the intercept (not for the averaging method) and the $m/z$ range in which the polymer molecules are observed (see figure 4.2). Other effects that might influence the magnitude of the $\sigma$ values such as space charge effects have not been considered.

4.3.3. Endgroup determination combining all charge states

Taking advantage of the generation of multiple charge states in ESI can increase the mass precision in the endgroup determination. According to equation (4.2), the mass of $z$ cations can be subtracted from the $m_{\text{meas}}$ values observed in all charge states. The resulting mass values only account for the monomer and endgroup mass. Since the mass of the cation can be considered exact, all mass values can be combined in one linear regression procedure. The advantage of this method is that the number of data points $N$ for the linear regression procedure increases. This results in an endgroup determination with a higher precision (equation (4.3)). The $\sigma^2_{\text{data}}$ value introduced in equation (4.3) is different for each charge state. In order to combine all charge states, $\sigma^2_{\text{data,all}}$ is introduced which is the weighted average of the $\sigma^2_{\text{data}}$ values over the different charge states

$$\sigma^2_{\text{data,all}} = \frac{\sum_{z=1}^{\bar{z}} \sigma^2_{\text{data}}(z) \cdot u(z)}{\sum_{z=1}^{\bar{z}} u(z)}$$

(4.4)
Where $\sigma^2_{\text{data}(z)}$ is the $\sigma^2_{\text{data}}$ value in charge state $z$ and $v(z)$ is the statistical degree of freedom of the number of data points $N$ observed in charge state $z$.

The charge carrying cation is sodium in all cases. This is confirmed by the addition of different alkali salts to the solution (spectra not shown). A major disadvantage of this method for cation identification is that additional analysis with other cations have to be carried out. A faster and less laborious confirmation is obtained from figure 4.3 for PEG3000. In this figure the measured masses ($m_{\text{meas}}$) in all charge states are plotted as a function of $n$. We have used the same $m_{\text{meas}}$ values as we have used for the endgroup determination. The vertical spacing between the different lines gives the mass of the cation. This can also be seen from equation (4.2) if $z$ is increased by one charge. For PEG3000 a cation mass of $22.989 \pm 0.001$ is determined from figure 4.3.

The results of the method that combines the data of all charge states are shown in table 4.3. In general, the endgroup precision increases when all charge states are combined instead of considering them separately. This is explained as due to the increasing number of datapoints $N$ that is used in the linear regression procedure (equation (4.3)). For PEG1000 a $\sigma_{\text{end,average}}$ of 0.0020 is observed after combining the two observed charge states. When considering the charge states separately, a 1.4 times higher value of 0.0028 is found. For PEG3000 a value of 0.0026 is observed which is on average 2.2 times higher than the $\sigma_{\text{end,average}}$ values of the separate charge states. For PEG6000 a factor of approximately three is observed. Similar results are obtained for PPG1150 and PPG3250.

4.3.4. Comparison of the endgroup accuracy determined with ESI and MALDI FT-ICR MS

The mass accuracies for the endgroup determination of PEG1000 and PEG4000 found by van Rooij et al.\textsuperscript{93} with MALDI FT-ICR MS are compared with the results for the PEGs reported in this chapter (see summary of the MALDI results\textsuperscript{93} in the last two columns of table 4.3). The $\sigma$ values for PEG1000 determined with MALDI FT-ICR MS are in good agreement with the values found in this chapter with ESI FT-ICR MS when all charge states are considered separately. The $\sigma$ values of PEG4000 are of the same order of magnitude as those of PEG3000 and PEG6000. This demonstrates that the $\sigma$ values generated with ESI data are of the same order as the MALDI data. Note that the number of data points used with MALDI FT-ICR MS described by van Rooij et al.\textsuperscript{93} is lower than the number of data points used in this chapter with ESI FT-ICR MS. This influences the $\sigma$ values. Therefore, the endgroup and monomer mass calculations have been done with a similar number of data points as used by van Rooij et al.\textsuperscript{93} for several
charge states of PEG3000 and PEG6000. As expected, the resulting $\sigma$ values are lower. However, the $\sigma$ values are still of the same order of magnitude as the results found with MALDI for PEG4000.

![Graph of mass vs. number of monomers](image)

**Figure 4.3** Determination of the mass of the cation for PEG3000. Plot of an enlarged mass scale of all observed charge states. The distance between two adjacent parallel lines determines the mass of the cation.

The $\sigma$ values of PEG6000 have improved by a factor of about two compared to those of PEG4000 when all charge states are combined in one linear regression procedure (fourth column of table 4.3). This is in spite of the fact that the molecular weight of PEG6000 is larger than PEG4000. Large polymer molecules can be measured more accurately using ESI compared to MALDI because ESI yield lower $m/z$ values, which can be measured more accurately using FT-ICR MS.
Table 4.3  Calculated monomer and endgroup masses (Da) and precision for PEG1000, PEG3000, PEG6000, PPG1150, PPG3250 and PTHF2000 combining all charge states. The values of PEG1000 and PEG4000 displayed in the last two columns are obtained with MALDI FT-ICR MS from reference 93 (mass of cation and $^{13}$C subtracted).

The largest polymer molecule observed with MALDI has a degree of polymerisation of 105. For ESI a polymer molecule with 178 monomeric units (PEG6000) has been observed on the same FT-ICR MS. This demonstrates clearly that when ESI is used instead of MALDI the mass range is extended by almost a factor of 2. Note that for other polymers, like polyolefins, multiply charging is difficult and an extension of the mass range will not be obtained. Polyethylene glycol samples with molecular weights of up to 20,000 Da have also been measured successfully using ESI. Accurate endgroup determinations for polyethylene glycol samples with a molecular weight larger than 20,000 Da require a mass resolving power only provided by a high field FT-ICR MS.

4.4. Conclusions

Two methods, a linear regression and an averaging method, have been developed and evaluated for the accurate analysis of the monomer and endgroup composition of synthetic polymers using ESI FT-ICR MS. All charge states of a
polymer, observed with ESI, are combined in one linear regression or averaging procedure, in order to increase the precision of the endgroup determination. The charge states can be combined under condition that the mass of the cation is known. By plotting \((m/z)^{z+}\) multiplied by the charge state as a function of the degree of polymerisation the identity of the cation is easily determined. This procedure prevents multiple experiments in which different cations have to be added to the polymer sample.

The endgroup accuracy determined from a single charge state with ESI FT-ICR MS is of the same order of magnitude as determined by MALDI FT-ICR MS on the same instrument. However, when all charge states measured with ESI are combined in one procedure, the endgroup precision increases up to threefold. This demonstrates that more accurate monomer and endgroup masses are obtained using ESI instead of MALDI. ESI exceeds MALDI in the study of larger polymeric systems due to the multiple charging nature of ESI. Multiply charging results in a lower \(m/z\) value, which are measured with a higher accuracy in the FT-ICR MS. However, multiply charging of polymers like polyolefins is difficult, which will not result in more accurate monomer and endgroup masses and an extension of the mass range.
The largest polymer molecule observed with MALDI was a cluster of polyethylene of 105. For ESI a polymer molecule with 178,000 monomer units (PEG-600), has been observed on the same FT-ICR MS. This desorption/ionization method that is used instead of MALDI, the mass range is increased by almost a factor of 2. More true for other polymers, like polystyrene, multiply charged ions of different mass are observed in the mass range that will not be observed. Polyelectrolytes, for example, have different charge states with molecular weights of up to 20,000 Da have also been examined successfully using ESI. Accurate endgroup determinations for non-spherical objects samples with a molecular weight larger than 20,000 Da are possible using MALDI FT-ICR MS. All charge states of a