Laser desorption analyses in trapped ion mass spectrometry systems
van Rooij, G.J.

Citation for published version (APA):
van Rooij, G. J. (1999). Laser desorption analyses in trapped ion mass spectrometry systems

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.

UvA-DARE is a service provided by the library of the University of Amsterdam (http://dare.uva.nl)
Determination of the Individual Block Length Distributions of poly(oxypropylene) and poly(oxyethylene) Block Copolymers by MALDI-FTICR-MS

External ion source MALDI-FTICR-MS was used to analyze the block length distributions of triblock polymers of poly(oxypropylene) and poly(oxyethylene) (EOPO). The results presented demonstrate that flight-time induced mass discrimination (see Chapter 3), which distorts molecular weight distributions generated by MALDI in external ion source FTICR-MS, is not the only effect that must be considered before accurate information on block length distributions can be obtained. The variation of isotopic patterns over the measured mass range and overlap of peaks in the spectrum also complicate the analysis of the block length distributions. An analytical treatment of the spectral data is presented to correct measured molecular weight distributions for these effects. The corrected EOPO molecular weight distribution was used to determine the individual block length distributions. By virtue of this methodology it was possible to obtain for the first time detailed and accurate molecular weight data on such a complex sample. The results independently validate the data provided by the manufacturer. The experimentally verified random coupling hypothesis supports the validity of the flight-time correction method and the proposed treatment of the spectral data.

4.1 Introduction

In Chapter 3, the properties of polymer molecular weight distributions were utilized to characterize and optimize the performance of external ion source MALDI-FTICR-MS with respect to mass accuracy and flight-time induced mass discrimination. The results clearly demonstrated that MALDI-FTICR-MS can provide detailed information on the
individual components of polymeric samples based on the technique’s unequaled mass accuracy and mass resolving power. A superimposition methodology was proposed to compensate for mass distortions induced by mass dependent flight-times, which allowed accurate description of polymer molecular weight distributions. Together, these properties make the technique well suited for many of the questions addressed in polymer analysis [61,77,90,91,106–113]. The methodology is evaluated in this chapter by the investigation of a Pluronic block copolymer.

Pluronics are water-soluble triblock polymers of poly(oxypropylene) and poly(oxyethylene) and find widespread application as nonionic surface-active agents. The surfactant properties of polyoxyalkylene block copolymers have been utilized as lubricants, dispersants, antistatic agents, foam control agents, solubilizers, and numerous other applications in the areas of pharmaceuticals, cleaning agents, foods and personal care products [114, 115]. The diversity in applications results from the various structural possibilities of poly(oxyalkylene) block copolymers. In these materials, ethylene oxide blocks provide the hydrophilicity and propylene oxide blocks the hydrophobicity necessary for surfactancy. The chemical composition can be optimized to achieve the desired surfactant performance. A complete description of the molecular composition distribution of the individual blocks in the copolymer is required to understand the structure-function relationships of surfactants with respect to physical, rheological, and mechanical properties. Important information is the detailed knowledge about the sequence and distributions of EO and PO blocks, the degree of polymerization and block size, the identity and structure of particular end groups (initiator and terminator type), and the presence of impurities. Various analytical approaches have been followed to investigate these properties. Detailed reviews on the analysis of poly(oxyalkylene) nonionic surfactants [116–119] describe useful methods such as liquid chromatography, infrared spectroscopy, raman spectroscopy, viscosimetry, calorimetry and NMR spectroscopy. In general, these methods provide only average data on specific structural features and are not suitable for the characterization of the individual components of the polymeric samples. In recent years, it has been demonstrated that mass spectrometric methods in combination with soft ionization techniques such as MALDI [18, 32, 33, 79] can provide this information [95].

In this Chapter, a method is developed to process spectral data obtained by MALDI-FTICR-MS to yield the individual block length distributions in a Pluronic sample. The Pluronic sample is a challenging test case as its constituent monomer units are small relative to the overall size of the copolymer, which means that overlap between different components of the molecular weight distribution is likely and high resolution mass spectrometry is imperative. It is demonstrated that the Pluronic sample offers a unique possibility to test the corrections on distortions induced by the experimental technique. An equal distribution in the block length of one of the constituents for different block lengths of the other constituent would confirm the validity of the correction method if the random coupling hypothesis is assumed. The final results will be shown to independently validate the manufacturers data and demonstrate the unique potential of MALDI-FTICR-MS for the analysis of this type of polymers.
4.2 Experimental

4.2.1 Instrumental

The MALDI-FTICR-MS experiments were performed on the heavily modified Bruker-Spectrospin (Fällanden, Switzerland) APEX 7.0e FTICR-MS with the external ion source operating in the MALDI mode. The instrumental layout and experimental procedures are described in detail in Chapter 2 and 3. Briefly, the MALDI samples were deposited on the stainless steel tip of a direct insertion probe and introduced into the external ion source via a vacuum lock. Ions were produced by irradiation of 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 high, 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. The resulting frequency spectrum was transformed to a mass spectrum.

4.2.2 Sample Preparation

The copolymeric sample investigated was Pluronic L31, a triblock copolymer of poly(oxypropylene) and poly(oxyethylene) from BASF (Mount Olive, NJ). According to the specifications of the manufacturer, Pluronic L31 has the general structure HO-(C₂H₄O)ₓ-(C₃H₆O)ᵧ-(C₂H₄O)₂-H. Further, it was specified that the average molecular weight of the PO part was 950 u and that the EO parts constituted 20% of the final molecules. For the MALDI experiments, 2,5-dihydroxybenzoic acid (DHB) from Sigma Chemical Co. (St. Louis, MO) was used as the matrix.

The MALDI samples were prepared by mixing a 1 M matrix solution in ethanol with approximately 10 g/L analyte solution in ethanol in a molar ratio of approximately 1000:1. The resulting analyte matrix solution was electrosprayed on the stainless steel probe tip (Section 3.2.2) over a distance of approximately 7 mm and a voltage difference of typically 4 kV between the capillary and the probe tip. A total of 2 µg sample was deposited on the probe tip.

4.3 Experimental Determination of the Distribution of Units

The distributions in the EO and PO monomers of the Pluronic copolymer were evaluated on the basis of MALDI-FTICR-MS measurements. Figure 4.1 shows an example of a MALDI-FTICR-MS magnitude spectrum of Pluronic L31 in broadband mode. For this spectrum the trapping delay was optimized to be 900 µs for maximum signal-to-noise at the center of the molecular weight distribution, i.e., it was not compensated for the flight-time induced mass discrimination discussed in section 3.4.1. The spectrum was obtained by summing the spectra of 250 consecutive laser shots. The expansion of the mass scale shows that the resolution is sufficient to resolve the naturally occurring isotopes of a
Figure 4.1: MALDI-FTICR-MS spectrum of poly(oxyethylene)-poly(oxypropylene). For this measurement the trapping delay was optimized to be 900 μs for the center of the molecular weight distribution. The spectra from 250 laser shots were summed. The series of poly(oxypropylene) homopolymers is indicated (the first number refers to $n_{\text{EO}}$, the second number to $n_{\text{PO}}$). In the expanded mass scale the composition of all monoisotopic copolymers is indicated.
component molecule. For example, the resolution in broad band mode \( (m/\text{dn})_{50\%} \) is 15,000 with a S/N ratio of 330 at \( m/\text{z} = 969.6 \).

Each copolymer (pseudo-)molecular ion consists of several units of propylene oxide (molecular weight \( M_{\text{PO}} = 58.0419 \)), a few segments of ethylene oxide (molecular weight \( M_{\text{EO}} = 44.0262 \)), the end groups -H and -OH (total molecular weight of the end groups \( M_{\text{end}} = 18.0106 \)), and the cationizing species (molecular weight \( M_{\text{cat}} \)). Therefore, the molecular weight of the cationized molecular ions can be written as:

\[
m/\text{z}_{\text{calc}} = n_{\text{PO}} M_{\text{PO}} + n_{\text{EO}} M_{\text{EO}} + M_{\text{end}} + M_{\text{cat}}
\]  

(4.1)

where \( n_{\text{PO}} \) and \( n_{\text{EO}} \) are the number of segments of PO and EO respectively. From eqn. 4.1 follows that, if the identity of the ionizing species is known, the values for \( n_{\text{PO}} \) and \( n_{\text{EO}} \) can be obtained by comparison of the measured values for \( m/\text{z} \) in the spectrum with the calculated masses. The identity of the cation was determined by evaluation of the series of peaks which are relatively abundant in the spectrum, i.e., \( m/\text{z} = 1027, m/\text{z} = 1085, m/\text{z} = 1143, m/\text{z} = 1202 \), etc. The equidistant intervals between these peaks are nominally 58 u (which corresponds to the mass of the PO unit). Linear regression (Section 3.3) on these measured masses versus the estimated degree of polymerization, yields from eqn. 4.1 the mass of the cation plus the mass of the EO segments: \( M_{\text{cat}} + n_{\text{EO}} M_{\text{EO}} = 22.990 + i \cdot 58.0419 \) u \( (i = 0, 1, 2, ...) \) (note that the identity of the end groups was known, and that the mass of the end groups has already been subtracted here). For \( i = 0 \), this series of peaks corresponds to the sodiated poly(oxypropylene) homopolymers (i.e., \( n_{\text{EO}} = 0 \)). For \( i > 0 \), there are no plausible candidates for the cationizing species and the copolymer composition. In heterodyne mode (similar to Figure 4.4), the presence of cationizing species other than sodium was investigated for the poly(oxypropylene) homopolymers. There was no evidence for other cation adducts; therefore, it is concluded that only signals for sodiated molecules are present in the spectrum. The peaks in the spectrum in Figure 4.1 that correspond to monoisotopic (pseudo-)molecular ions and with \( S/N > 2 \) were labeled and were used for further analysis. The composition of these copolymer molecules is indicated for the series of poly(oxypropylene) homopolymers in the figure and in the expanded mass scale for all monoisotopic peaks. Here the first number refers to the number of EO units present in the copolymer \( (n_{\text{EO}}) \), and the second number refers to the number of PO units present \( (n_{\text{PO}}) \). In this way, 130 monoisotopic peaks were identified, leading to 130 data objects, which are referred to with the following notation (which is in accordance with the notation used in the paper of Wilczek-Vera et al. [80]):

<table>
<thead>
<tr>
<th>peak no.</th>
<th>( m/\text{z} )</th>
<th>( n_{\text{EO}} )</th>
<th>( n_{\text{PO}} )</th>
<th>relative intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>( (m/\text{z})_1 )</td>
<td>( n_{1}^{\text{EO}} )</td>
<td>( n_{1}^{\text{PO}} )</td>
<td>( I_{1}^{\text{exp}} )</td>
</tr>
<tr>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>( k )</td>
<td>( (m/\text{z})_k )</td>
<td>( n_{k}^{\text{EO}} )</td>
<td>( n_{k}^{\text{PO}} )</td>
<td>( I_{k}^{\text{exp}} )</td>
</tr>
</tbody>
</table>

(in this data format, \( m/\text{z} \) increases for increasing \( k \)). The distribution of both components of the copolymer is visualized by the measured intensities as a function of both \( n_{\text{EO}} \) and
Figure 4.2: Distribution of monomer units as a function of the individual monomer units in the polymer. The data plotted are extracted from the spectrum in figure 4.1.

$n^{\text{PO}}$ in the contour map in Figure 4.2. This plot indicates that a strong coupling exists between the molecular weight distributions of the individual parts because there is a significant shift of the center of the distribution in the PO part of the copolymer as a function of the number of EO units. However, for this type of block copolymer the polymerization process is expected to follow the random coupling hypothesis (i.e., no correlation between the molecular weights of the individual parts). In fact, the observed asymmetric distribution reflects again the flight-time induced mass discrimination discussed in the previous section. In order to use the superimposition method to correct for this discrimination, a series of 10 experiments were performed, in which the trapping time was varied from 600 $\mu$s up to 1000 $\mu$s, and from 1050 $\mu$s up to 1450 $\mu$s in steps of 100 $\mu$s. At each trapping time setting, multiple laser shots (in the measurements presented typically 250 laser shots) have been averaged to minimize the “shot-to-shot” variations in the MALDI process. All the spectra were recorded from the same spot, which eliminated “spot-to-spot” variations.

Finally, a possible decrease in the total ion production in the MALDI process was monitored by taking reference spectra (with the trapping delay at 900 ms) in the course of the series of measurements. No decrease was observed, and therefore it was allowed to apply the superimposition method. The corrected spectrum is shown in Figure 4.3. It can be seen that the molecular weight distribution in Figure 4.3 has become significantly broader in comparison to the spectrum that resulted from the single measurement in Figure 4.1. Especially the lower mass range was affected by the flight-time effect. This explains the shift of the center of the distributions in the PO part toward higher numbers for lower numbers of EO units in Figure 4.2. After all, discrimination of lower masses means a discrimination of the lower end of the distributions in the PO part, which becomes less apparent for larger EO lengths.

Before the spectrum in Figure 4.3 can be processed to reveal the individual block
length distributions, two additional phenomena, which affect the measured molecular weight distribution, have to be taken into account. First, the contribution of the naturally occurring isotopes in the molecular ion changes over the mass range of interest. For example, 62% of the sodium cationized homopolymer with 13 PO units at m/z 796 will be monoisotopic, whereas only 40% of the molecular ions will be monoisotopic for the homopolymer with 25 PO units at m/z 1492. Taking the monoisotopic peaks as the relative abundance of the molecular ions will evidently induce significant errors and, therefore, will distort the molecular weight distribution.

The second effect can be illustrated with the peak at m/z 971.6 in the expanded mass scale in Figure 4.1, where it is seen that no distinction can be made between the second isotopic peak of the homopolymer with \( n^{\text{EO}} = 0 \) and \( n^{\text{PO}} = 16 \) (monoisotopic peak at m/z
969.6) and the monoisotopic peak of the copolymer with $n_{\text{EO}}^\text{mono} = 4$ and $n_{\text{PO}}^\text{mono} = 13$ at $m/z = 971.6$. Because all the components of the molecular weight distribution of the Pluronic sample differ by an even number of mass units, and because contributions of isotopic peaks of polymers with a monoisotopic molecular weight less than or equal to $(m/z)_{k-4}$ may be neglected as these can be estimated to be smaller than 1% in the molecular weight range considered here, the intensities of the peaks in the spectrum at $(m/z)_k$ which satisfy

$$(m/z)_k - (m/z)_{k-1} \cong 2$$  \hspace{1cm} (4.2)$$

have to be corrected for the contribution from the molecular ions with the monoisotopic peak at $(m/z)_{k-1}$. To illustrate the importance of this correction, let us consider the two homopolymers used in the previous example. In the case of the 13-mer, 8% of the molecular ions will contain two $^{13}$C isotopes (or $^{13}$C$^{17}$O or $^{21}$O or $^{18}$O); for the 25-mer this will be 17%. On the basis of the intensities in the mass spectrum in Figure 4.3, it was determined that the contribution of the $^{13}$C isotope of the 13-mer to the peak that corresponds to the monoisotopic peak would be 50%; for the $^{13}$C isotope of the 25-mer and for the $(n_{\text{EO}}, n_{\text{PO}}) = (4, 22)$, this was determined to be 10%. This second effect is further illustrated by utilization of the high mass resolution of the instrument in the heterodyne mode. The mass range $m/z = 930 - 1015$ was recorded in this high-resolution mode and resulted in the spectrum shown in Figure 4.4. The inset reveals that the resolution is sufficient to separate the two peaks around $m/z = 971.7$.

In principle, the heterodyne mode would be a potential way to overcome the problem of peak overlap. However, it would take approximately 20 experiments for each different trapping delay (i.e., a total of 200 experiments) to cover the entire molecular weight distribution. Such large numbers of experiments can practically not be correlated due to inevitable variations in ion yields. Therefore, it is proposed to apply a correction method instead. This method is checked by comparison of the data from the high resolution spectrum in Figure 4.4 with those from the broad band spectrum in Figure 4.3. It is determined from the peaks at $m/z = 969$ and $m/z = 971.65$ in the high-resolution spectrum that the ratio between the abundance of the monoisotopic copolymer and the abundance of the monoisotopic copolymer would be 0.19. Determination of this ratio from the spectrum in Figure 4.3 yields the value 0.36. After correction of the intensities for the expected overlap (according to the method which is proposed below) this value became 0.18, which is in excellent agreement with the result from the high-resolution measurement, and thus confirms the validity of the correction method. This example also illustrates that the precision of the correction process for the individual intensities is of the order of 5% (off course, for very low S/N ratios, this value might increase significantly).

Corrections for the change of the isotopic patterns over the mass range and the overlap of peaks can easily be calculated with analytical expressions. The first step in the derivation of these expressions is the calculation of the probability that the poly(oxyethylene)-poly(oxypropylene) molecule is monoisotopic. Standard probability theory [120] yields
Figure 4.4: High resolution (heterodyne) MALDI-FTICR-MS spectrum of poly(oxy-ethylyene)- poly(oxypropylene) around m/z 970. The expansion of the mass scale shows that the second isotopic peak of the \( (n_{EO}, n_{PO}) = (0, 16) \) copolymer and the monoisotopic peak of the \( (n_{EO}, n_{PO}) = (4, 13) \) copolymer are separated, which can not be accomplished in broad band experiments.

For the probability that a copolymer which consists of \( n_{EO}^{k} \) EO units and \( n_{PO}^{k} \) PO units (i.e., \( [\text{HO-(C}_2\text{H}_4\text{O})_{n_{EO}^{k}}\text{-(C}_3\text{H}_6\text{O})_{n_{PO}^{k}}\text{-H]} \) ) has a mass of \( (m/z)_{k} \) (i.e., it is monoisotopic):

\[
P(n_{EO}^{k}, n_{PO}^{k}, (m/z)_{k}) = A^{2n_{EO}^{k}+3n_{PO}^{k}}(^{12}\text{C}) \cdot A^{n_{EO}^{k}+n_{PO}^{k}+1}(^{16}\text{O})
\]

where \( A^b(mX) \) denotes the \( b \)th power of the natural abundance of the isotope \( mX \). If there is no polymer present in the spectrum with a monoisotopic molecular weight \( (m/z)_{k-2} \), then the corrected abundance for this component of the molecular weight distribution is
simply given by:

\[ I_{k}^{\text{corr}} = \frac{I_{k}^{\text{exp}}}{P(n_{k}^{\text{EO}}, n_{k}^{\text{PO}}, (m/z)_{k})} \]  \hspace{1cm} (4.4)

where \( I_{k}^{\text{exp}} \) refers to the measured intensity of the peak at \((m/z)_{k}\). If there is a peak at \((m/z)_{k-2}\), the probability has to be calculated that also the mass of the copolymer described by \(n_{k-1}^{\text{EO}} \) and \(n_{k-1}^{\text{PO}} \) equals \((m/z)_{k}\). This probability is equal to the sum of the individual probabilities that this molecule contains two \(^{13}\text{C}\) isotopes, one \(^{13}\text{C}\) and one \(^{17}\text{O}\) isotope, or one \(^{18}\text{O}\) isotope (the chance that two \(^{17}\text{O}\) isotopes are present is negligible). The calculation of this probability is analogous to the one in eqn. 4.3.

With these probabilities, the corrected abundance becomes

\[ I_{k}^{\text{corr}} = \frac{1}{P(n_{k}^{\text{EO}}, n_{k}^{\text{PO}}, (m/z)_{k})} \left( I_{k}^{\text{exp}} - \frac{P(n_{k-1}^{\text{EO}}, n_{k-1}^{\text{PO}}, (m/z)_{k-1})}{P(n_{k-1}^{\text{EO}}, n_{k-1}^{\text{PO}}, (m/z)_{k-1})} I_{k-1}^{\text{exp}} \right) \]  \hspace{1cm} (4.5)

A small computer program was developed to aid in the interpretation of the copolymer spectra and the processing of the large data sets. This program uses the elemental composition of the two different monomeric units and the end groups of the copolymer as input in order to determine the composition of the copolymer for each peak in the spectrum with eqn. 4.1. Subsequently, it calculates the corrected intensities for all the components of the molecular weight distribution with eqns. 4.4 and 4.5. Finally, it calculates the characterizing numbers presented below.

The effect of the corrections can be quantified by calculation of the number- and the weight-average molecular weights (\(M_{n}\) and \(M_{w}\), respectively), and the polydispersity factor \((M_{w}/M_{n})\) for the copolymers with \(n^{\text{EO}} = 4\) for the uncorrected data, for the data corrected only for the isotope effect, and for the fully corrected data. The resulting values are listed in Table 4.1. It should be mentioned here that all peak intensities were determined by integration over the peaks, because otherwise the correction for the peak overlap would not hold. For example, if the overlapping peaks do not entirely coincide, then peak broadening will occur, and the use of peak heights will underestimate the actual intensities. It can be seen from the table that, for the sample under investigation, the change induced by each correction is about the same. The total percentile change in

<table>
<thead>
<tr>
<th></th>
<th>(M_{n})</th>
<th>(M_{w})</th>
<th>(M_{w}/M_{n})</th>
</tr>
</thead>
<tbody>
<tr>
<td>uncorrected</td>
<td>1047</td>
<td>1104</td>
<td>1.055</td>
</tr>
<tr>
<td>corrected for isotope effect</td>
<td>1083</td>
<td>1136</td>
<td>1.049</td>
</tr>
<tr>
<td>fully corrected</td>
<td>1125</td>
<td>1177</td>
<td>1.047</td>
</tr>
</tbody>
</table>
the values listed in the table is 6-8%. Off course, these differences in molecular weight do not lead to measurable differences in physical properties. However, the corrections appear to be valuable in the evaluation of the second step of the synthesis of the block polymer (where the propylene block is sandwiched between ethylene blocks). In order to fully characterize the sample under investigation, we calculated the usual molecular weight averages (number average), (weight average), and the polydispersity factor from the complete molecular weight distribution. In addition, some averages which describe the chemical composition of the block copolymer in terms of the individual segments are introduced:

1. the average number of units for segment X (X=EO,PO) in the block copolymer:

   \[ \bar{n}^X_n = \frac{\sum_k n^X_k I^X_k}{\sum_k I^X_k} \]  

2. the weight average number of units for segment X:

   \[ \bar{n}^X_m^w = \frac{\sum_k (n^X_k)^2 I^X_k}{\sum_k n^X_k I^X_k} \]  

3. the polydispersity factor for segment X:

   \[ \frac{n^w_X}{\bar{n}^X_n} \]  

4. the chemical composition of segment X:

   \[ \bar{x}^X = \frac{M_X \bar{n}^X_n}{M_n} \]  

The values of these quantities were calculated from the data extracted from the spectrum in Figure 4.3 before as well as after correction and are listed in Table 4.2. The characterizing numbers according to the manufacturer are \[ n^\text{PO} = 16(\approx 95)/38.04; \text{error margin } \approx 0.5 \] \[ \text{and } n^\text{EO} = 0.2 (\text{error margin } \approx 0.03). \] When these numbers are compared with the values presented in Table 4.2 \( n^\text{PO} = 15.57 \text{ and } n^\text{EO} = 0.17 \) after correction, it can be seen that our results derived from MALDI-FTICR-MS confirm the manufacturers specifications with greater accuracy.

The individual block length distributions are visualized in contour maps. Figure 4.5A shows the data after flight-time compensation and Figure 4.5B shows the fully corrected data. Comparison of the two contour maps shows that the full correction removes the discontinuity in the EO distribution around \( n^\text{EO} = 4 \) (a local maximum). This discontinuity arose from the peak overlap of the second isotopic peaks with \( n^\text{EO} = 0 \) (i.e., the poly(oxypropylene) homopolymers) and the monoisotopic peaks with \( n^\text{EO} = 4 \). The contour map in Figure 4.5B clearly reveals the random coupling hypothesis for this type of
copolymers, as the distribution in the PO units remains the same for different EO segments. Moreover, the fact that the experiment verifies the random coupling hypothesis indicates that the correction of MALDI-FTICR-MS spectra with the methods described above leads to the real molecular weight distribution. This means also that other possible mass discrimination effects in MALDI-FTICR-MS with an external ion source (for example, discriminations in the MALDI processes, mass-dependent transfer efficiencies, mass-dependent trapping, and discriminations in the excitation and detection event) are negligible over the mass range considered here. The random coupling hypothesis can be verified more accurately by calculation of the experimental marginal probabilities for the

Table 4.2: Properties of the Pluronic L31 sample calculated from the spectrum in Figure 4.3.

<table>
<thead>
<tr>
<th></th>
<th>uncorrected</th>
<th>corrected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EO block</td>
<td>PO block</td>
</tr>
<tr>
<td>$n_X$</td>
<td>4.53</td>
<td>14.78</td>
</tr>
<tr>
<td>$n_X^W$</td>
<td>8.51</td>
<td>15.83</td>
</tr>
<tr>
<td>$n_X^W/n_X^N$</td>
<td>1.88</td>
<td>1.07</td>
</tr>
<tr>
<td>$\bar{X}$</td>
<td>0.19</td>
<td>0.81</td>
</tr>
<tr>
<td>$M_n$</td>
<td>1098.4</td>
<td>1125.7</td>
</tr>
<tr>
<td>$M_w$</td>
<td>1175.9</td>
<td>1200.6</td>
</tr>
<tr>
<td>$M_w/M_n$</td>
<td>1.07</td>
<td>1.07</td>
</tr>
</tbody>
</table>

Figure 4.5: Contour maps of the overall distribution of monomer units as a function of the individual monomer units in the polymer, both before (A) and after correction (B) for the isotope effect and peak overlap. The data plotted are determined from the reconstructed molecular weight distribution in Figure 4.3.
two components of the copolymer [80, 120]. For the PO units the marginal probability
\( \Gamma_{\text{exp}}^{\text{PO}} \) is defined by:

\[
\Gamma_{\text{exp}}^{\text{PO}}(n_j^{\text{PO}}) = \sum_i \Gamma_{\text{exp}}^{\text{PO}}(n_i^{\text{EO}} , n_j^{\text{PO}}) \tag{4.10}
\]

where \( \Gamma_{\text{exp}}^{\text{PO}}(n_i^{\text{EO}}, n_j^{\text{PO}}) \) is the experimental distribution function, which is equal to the measured intensity for given \( n_i^{\text{EO}} \) and \( n_j^{\text{PO}} \). According to this formula, all the measured intensities for a given \( n_j^{\text{PO}} \) are summed. Similarly, the marginal probability \( \Gamma_{\text{exp}}^{\text{EO}} \) for the EO units is defined. If the random coupling hypothesis holds, then the experimental distribution function can be represented as the product of the two marginal distributions [120]:

\[
\Gamma_{\text{exp}}^{\text{PO}}(n_i^{\text{EO}}, n_j^{\text{PO}}) = \Gamma_{\text{exp}}^{\text{PO}}(n_i^{\text{EO}})\Gamma_{\text{exp}}^{\text{PO}}(n_j^{\text{PO}}) \tag{4.11}
\]

In order to test eqn. 4.11, it was assumed that the distributions in the PO units for each number of EO units would follow the Gaussian function. If the heights of the (fitted) Gaussian functions are considered to be representative for the distribution in the EO units, it is possible to rewrite eqn. 4.11 as:

\[
\Gamma_{\text{exp}}^{\text{PO}}(n_i^{\text{EO}}, n_j^{\text{PO}}) = A(n_i^{\text{EO}})\exp - \left( \frac{(n_j^{\text{PO}} - n_i^{\text{PO}})^2}{w} \right) \tag{4.12}
\]

If eqn. 4.12 holds, it follows that the fitted PO distributions have equal centers \( n_c^{\text{PO}} \) and equal widths \( w \). This is evaluated in Figure 4.6, were the results of the individual fits are

**Figure 4.6:** Test of the random coupling hypothesis for the Pluronic L31 copolymer. The measured distribution in the propylene oxide units is fitted with a Gaussian function for different numbers of ethylene oxide units. The center and width of the fitted Gaussian functions are plotted as a function of the number of ethylene oxide units.
plotted as a function of $n^{\text{EO}}$. It is seen that values for $w$ and $n^{\text{PO}}_c$ are randomly distributed around the average value of 4.26 and 13.67, respectively. The observed fluctuations are most probably caused by differences in S/N ratios. At lower S/N ratios ($<3$), the accuracy in the determination of the peak intensities becomes of the order of the corrections for peak overlap; consequently these corrections will induce additional errors to the sides of the overall 2D distribution. As there are no significant trends visible in Figure 4.6, it is concluded that the random coupling hypothesis is valid for this particular Pluronic copolymer system.

### 4.4 Summary and Conclusions

The molecular weight distribution of a triblock polymer of poly(oxypropylene) and poly-(oxyethylene) was measured with MALDI-FTICR-MS. The superimposition method that was proposed in Chapter 3 was applied to compensate for flight-time induced mass discriminations during the ion transport from the ion source to the ICR-cell. An additional correction algorithm was developed to process the data from the MS peaks in order to correct for the change in the isotopic patterns over the measured molecular weight distribution and for the overlap of different isotopic peaks. The magnitude of these corrections on the molecular weight data for the copolymer sample under study is as large as 10%. The results demonstrate that the experimental technique is very useful for characterization of the individual block length distributions, even in those cases where the difference in mass of the different components of the molecular weight distribution is very small (down to several milli-mass units).

The random coupling hypothesis was tested and was found to hold for the copolymer system studied. This result is also a further experimental verification of the superimposition method and proves that the actual molecular weight distribution of synthetic polymers can be obtained with MALDI performed in the external ion source of an FTICR-MS.