Laser desorption analyses in trapped ion mass spectrometry systems
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The design and performance of a newly configured external ion source Matrix Assisted Laser Desorption and Ionization quadrupole Ion Trap Mass Spectrometer (MALDI-ITMS) is described. The performance was characterized in detail with respect to mass discrimination effects and space charge induced mass shifts. Mass dependencies in the trapping efficiency were probed by comparison of measurements of poly(methyl methacrylate) standards in the mass range m/z 400 to 3400 with MALDI-TOF-MS measurements of the same samples. The results indicated that it is possible to determine ideal experimental conditions for analyzing mass ranges smaller than 1000 u with negligible mass discrimination. This was demonstrated on the basis of measurements on a tri-block copolymer of poly(ethylene oxide) poly(propylene oxide). Mass measurements were observed to be highly influenced by the magnitude of the trapped ion population in measurements of phthalocyanine blue and poly(ethylene glycol). Consequently, reproducible results can only be obtained by controlling the total space charge in the trap. In MALDI experiments, this can be achieved by tuning the laser power just above threshold. This approach allowed the mass determination of three different end groups in a complex Jeffamine D2000 sample with an accuracy of better than 0.1 u.

7.1 Introduction

It was already discussed in Chapter 4 that soft ionization techniques, which minimize fragmentation during ionization and thus produce (pseudo-) molecular ions, have proven to be most successful for lifting and converting macromolecules from the condensed phase to
gaseous ions. Within this range of techniques, especially electrospray ionization [160] and matrix-assisted laser desorption/ionization (MALDI) [9] have proven their importance in providing accurate and detailed molecular weight data on high molecular weight materials. Additional properties of MALDI that make it especially suited for many applications include the dominance of singly-charged (pseudo) molecular ions (which makes the resulting mass spectra easier to interpret) and its tolerance for salts, buffers and other common additives and impurities. Since its introduction by Tanaka and coworkers [33] and by Karas and Hillenkamp [32] the technique has successfully been applied to volatilize and ionize a wide variety of molecules, such as peptides, proteins, oligosaccharides, and synthetic polymers. Several reviews illustrate the continuously growing scope of MALDI applications [16, 17, 34].

Traditionally, MALDI is coupled to time-of-flight (providing high sensitivity and a theoretically unlimited mass range) [83,87], or Fourier transform ion cyclotron resonance mass spectrometers (providing superior mass accuracy, mass resolution and multistage tandem mass spectrometry) [108]. A relatively new and unexplored area is the use of the quadrupole ion trap mass spectrometer (ITMS) in analytical applications of MALDI. The ITMS seems particularly well-suited to combine with MALDI. For example, the pulsed nature of the ion production in a MALDI ion source matches well with the pulsed operation of the ITMS, whereas the capacity of the ITMS to store all ions created in the MALDI process, irrespective of the time scale in which these were formed, offers the possibility of panoramic registration of the ions from each laser shot. Moreover, the ITMS provides ultra-high sensitivity in the measurements and possibilities for multistage tandem mass spectrometry (MS^n). These are great potentials for the analysis of large molecules in complex mixtures.

Various publications have dealt with the coupling of the MALDI ionization technique and ITMS, which was realized either by performing the MALDI ionization event near the trap or by producing MALDI ions in an external ion source and subsequently transferring these ions to the trap. In the near-trap geometry, the MALDI samples were positioned near the internal surface of the ring electrode (radial introduction) [161–163] or at one of the end cap electrodes (axial introduction) [164]. The generation of ions in an external ion source has obvious advantages, such as flexibility in the nature and size of the samples and switching of ion sources [165–168]. Despite the attractiveness of a MALDI-ITMS instrument outlined above, these investigations encountered several problems revealing that this coupling is not trivial. Crucial among these is the efficiency in the trapping of the MALDI ions. Ions produced by MALDI have wide, mass-independent velocity distributions [96]. Therefore, difficulties in the trapping of ions over wide mass ranges without large (mass-dependent) losses can be expected. In the early experiments, no sophisticated trapping schemes were employed, and the MALDI ions are injected into a constant trapping field (static trapping). Trapping is realized by reducing the kinetic energy of the ions by means of collisions with buffer gas molecules. Several theoretical studies have discussed this process [169–171] and experiments utilizing this method of trapping have been reviewed extensively [172,173]. The main drawback of this method
is found to be a mass dependent, relatively low, trapping efficiency, where the amplitude of the rf field is found to determine the ion mass at which maximum trapping efficiency occurs [174, 175]. Recently, new methods for improved trapping of ions produced in a pulsed ionization technique have been developed. It has been demonstrated that the ion potential energy can be reduced if the rf field is switched on after the ions reached the central region of the trap, whereas it is gated during ion injection [176]. Following an alternative method, which is called dynamic trapping [177–179] the rf field is initially set to a low level to allow the ions an easy penetration of the trapping field. Subsequently, the amplitude of the trapping field is rapidly ramped to a high level to realize trapping before the ions have reached the opposite trap boundary and are lost. In the case of a near-trap geometry, dynamic trapping was demonstrated to give an improvement in the trapping efficiency of 1 order of magnitude [164]. In a more subtle version, called matched dynamic trapping [180], the trapping efficiency was found to be improved by an additional factor of 4 for an external source geometry. This improvement was accomplished by initially ramping the amplitude of the trapping field to an even higher level, followed by a down ramp to a lower level. Finally, it was demonstrated that also applying a d.c. retarding voltage to the end-cap electrodes improves the trapping efficiency [171].

The second essential problem encountered in MALDI-ITMS experiments results from the strong dependence of the performance of the ion trap as a mass spectrometer on the total number of ions that are held inside the trap. When the ion population within the ion trap becomes too dense, then the electrical fields to which the ions are being subjected are substantially modified by the electrostatic forces associated with the trapped ions, that is, the space charge. Low levels of space charge result into slight shifts in the secular frequencies of the ions [181–183] which are observed as small shifts in the mass assignment. At higher space charge levels, also peak broadening is observed, up to levels at which the analyzer performance is seriously degraded [184]. These space charge effects are especially in the case of MALDI measurements easily encountered, because the MALDI process will generate a large excess of matrix ions in addition to the ions of interest. The most straightforward method for diminishing the ion population inside the trap is optimizing the amplitude of the rf field to satisfy \( q_e > 1 \) for the matrix ions and \( q_e < 1 \) for the analyte ions, which means that only the polymer ions will be trapped. The disadvantage of this method is (next to the previously mentioned relatively low trapping efficiency) that this amplitude does not necessarily provide optimal trapping for the ions under study. Another option is ejection of the matrix ions from the trap during or after trapping [185]. In the case of dynamic trapping in an external source geometry matrix ion discrimination is also feasible on basis of differences in flight-times [168]. During the transport to the trap, the low-mass matrix ions separate in space from the higher mass analyte ions and consequently these will arrive earlier at the trap entrance. Appropriate adjustment of the timing in ramping the amplitude of the rf trapping field should accomplish the desired discrimination.

In this chapter, we describe the design and performance of a new external ion source MALDI-ITMS instrument. No sophisticated trapping schemes were employed in the pre-
presented experiments, but the MALDI ions were injected into an active trapping field. The performance of the instrument, in particular with respect to mass dependencies in trapping efficiency and mass shifts due to space charge effects, was investigated by measuring the molecular weight distributions of low-molecular-weight synthetic polymer samples. These samples are ideal for this purpose for two reasons. The first reason is that the polymer distributions cover substantial mass ranges and mass dependencies in the trapping efficiency will therefore be reflected in distortions of the measured distributions. These distortions were quantified by comparison of the MALDI-ITMS distributions with the distributions measured by MALDI-TOF and results obtained by MALDI-FTICR-MS (described in Chapter 4 and previously published publications). On basis of these results it was possible to determine the optimal experimental conditions for measuring the molecular weight distribution of a complex block copolymer sample without significant distortions. Evaluation of the distributions in the individual block lengths demonstrated that mass discrimination effects were negligible in the copolymer measurements. The second reason is that this type of samples is demanding for the total number of trapped ions. Polymer distributions generally consist of many components. This means that the total ion signal is divided over the different components and therefore it is necessary to accumulate many ions in order to obtain an adequate signal-to-noise ratio for each component. Consequently, mass shifts induced by the total associated space charge are likely. These mass shifts were monitored in detail as a function of the total ion load on basis of deviations in the mass difference between adjacent components in a single spectrum. In the final series of measurements on a complex polymer sample it is demonstrated that carefully controlling the total space charge in the trap sufficiently minimizes these shifts. This is illustrated on basis of end group mass determinations with an accuracy of $\sim 0.1 \text{ u}$ from the measured spectra.

7.2 Experimental

7.2.1 MALDI-ITMS

Figure 7.1 shows a schematic representation of the newly constructed MALDI-ITMS instrument. In this instrument, the ions are generated external to the ion trap in a home-build MALDI ion source, which is described in detail in Chapter 2. These are subsequently accelerated towards and focused onto the entrance of the ion trap using electrostatic ion optics. This ion trap (including the control electronics) was obtained from Bruker-Franzen Analytik GmbH (Bremen, Germany), and is identical to the one in the commercial Bruker Esquire instrument.

The MALDI samples were deposited on the stainless steel tip of a direct insertion probe. This probe was inserted into the external ion source via a vacuum lock. After insertion, it was in electrical contact with the source housing. The frequency-tripled output of a Quanta-Ray GCR-11 (Spectra-Physics Inc, Mt. View, California) pulsed Nd:YAG laser (producing 355 nm pulses with an energy of 60 mJ and a pulse length of 5 ns) was
focused onto the MALDI probe with an incident angle of $45^\circ$. The area at the MALDI probe illuminated by the laser beam was approximately $2 \times 1 \text{ mm}^2$ and the power density on target was measured to be maximally $100 \text{ MW/cm}^2$. The ions were created at a potential of typically $15 \text{ V}$ and extracted from the ionization region by applying a potential difference of approximately $10 \text{ V}$ between the source housing and the extraction plates. Subsequently, the ions were alternately accelerated and decelerated between $80 \text{ eV}$ and $30 \text{ eV}$ in a modified Heddle geometry, consisting of eight elements, to transport the ions over the distance of $0.2 \text{ m}$ between the MALDI source and the ion trap. At the end of the transport lenses a set of three electrodes, operated at $-40 \text{ V}$, $-150 \text{ V}$, and $-10 \text{ V}$, was installed to focus the ion beam onto the entrance of the ion trap. The ions are injected into the active trapping field of the ion trap, and He background gas was introduced into the ion trap at an estimated pressure of $10^{-3} \text{ mbar}$ to realize trapping by collisional cooling of the ion kinetic energies.

At the beginning of each experiment the laser was triggered by a TTL pulse from the ion trap control electronics. A delay of typically $70 \text{ ms}$ was installed allowing the ions to travel from the source to the cell, and to translationally cool to the center of the trap. The amplitude of the rf voltage during ion injection was determined by the value of the so-called cut-off mass $M_{\text{cut-off}}$, which corresponds to the mass of the ions that are in resonance with the dipole field at that amplitude. The relation between the amplitude and the cut-off mass in the standard operation mode is given by:

$$V_{rf} = \frac{0.2}{\varepsilon} \Omega^2 r_0^2 M_{\text{cut-off}}$$  \hspace{1cm} (7.1)

Here, $V_{rf}$, $\varepsilon$, $\Omega$, and $r_0$ are the amplitude of the rf field, the elementary charge, the fre-
quency of the rf field, and the radius of the trap (10 mm) respectively. Finally, the mass spectrum was recorded in the resonant ejection technique by scanning the amplitude of the rf voltage on the ring electrode and the phase-locked resonant ejection voltage over the two end caps. Ejected ions were accelerated over 7 kV towards a conversion dynode and the secondary electron signal was recorded by a channeltron detector operated at -1.2 kV. The entire multiplier signal as function of time was stored if only limited mass ranges were examined (see Chapter 2.3.4). Otherwise, the multiplier signal was converted in a bar graph. In that case the signal intensity is only stored for integer masses. The software packages Bruker DataAnalysis and m.a.c.s Labstar (Bruker-Franzen Analytik GmbH, Bremen, Germany) running on an IBM compatible computer under OS2 controlled the measurements and performed data acquisition and processing.

7.2.2 MALDI-TOF-MS

Reference spectra of the samples used to characterize the performance of the MALDI-ITMS instrument were recorded by MALDI-TOF-MS. In this instrument, a Bruker Biflex mass spectrometer, desorption and ionization is achieved using a nitrogen laser, which produces 337 nm pulses with a pulse length of 5 ns. Samples for the MALDI experiments were deposited on a stainless steel disk containing 26 targets. After drying, this disk was transferred into the mass spectrometer via a vacuum lock. A CCD camera, connected to a video monitor, allowed visual selection and examination of the area of laser interaction with the sample. Selection of the MALDI target and positioning of the target under the desorbing laser beam were computer controlled. Optimization of the MALDI signal was achieved by varying the laser power and the position of the desorption spot. The spot size on the sample surface was approximately 50 μm in diameter. The laser beam was attenuated using a variable neutral density filter. The laser irradiance was typically optimized to be slightly above threshold. During measurements, a source pressure of ~ 10^-6 mbar at analyzer pressure of ~ 10^-7 mbar was maintained. Ions were detected in the linear mode using an accelerating voltage of 20 kV and a detector voltage of 1000 V by a MCP detector (1 GHz A/D converter). All spectra were signal averaged over 100 laser shots. All time of flight spectra were calibrated using a mixture of PEG1000, PEG2000 and PEG3000.

7.2.3 Sample preparation

The poly(methyl methacrylate) (PMMA) standards were obtained from Polymer Laboratories (Amherst, MA). Four different standards were studied with an average molecular weight of 640, 1140, 1850, and 3100 u, respectively. With these standards, also a polymer blend with an extremely large polydispersity was obtained by mixing these in ratio to their number-averaged molecular weight. The polyethylene glycol (PEG) sample with average molecular weight of 1000 u was obtained from Serva (Heidelberg, Germany). Also the Jeffamine D2000 is commercially available (Texaco Chemical Company). According
to the manufacturers data sheet (SC-024 102-0411), the Jeffamine D2000 is an amine-
terminated polypropylene glycol with the general structure \( \text{H}_2\text{N}-(\text{O})_n\text{C}_3\text{H}_7\text{N} \). The
PLURONIC L31 is a tri-block copolymer of poly(ethylene oxide) poly(propylene oxide)
and was obtained from BASF (Mount Olive, NJ). The average molecular weight of the
propylene oxide part was specified to be 950, and the ethylene oxide fraction of the final
polymer sample was specified to be 20%. Liquitex acrylic phthalocyanine blue paint was
manufactured by Lefranc & Bourgeois (Le Mans, France). The matrix in the MALDI ex-
periments was 2,5-dihydroxybenzoic acid (DHB) from Sigma Chemical Co. (St. Louis,
MO).

The polymer samples were prepared for MALDI mass spectrometry by mixing a 1-
M matrix solution in ethanol with an approximately 10-g/L analyte solution in ethanol
yielding a molar ratio matrix:analyte \( = 1000:1 \). For the experiments on PMMA the an-
alyte solutions were first mixed yielding unity molar ratios. The resulting mixture was
electrosprayed onto a stainless steel probe tip Chapter 3. Approximately 0.1 mL ana-
lyte/matrix is consumed during sample deposition. The acrylic paint was applied to the
probe without prior sample preparation (that is, direct laser desorption instead of MALDI
was performed). The total sample load on the probe was approximately 10 ng. Each sam-
ple was used to produce ions for thousands of laser shots by rotating the sample probe
over \( 360^\circ \) and translating it over 5 mm (in this way a total area of \( 30 \text{ mm}^2 \) could be
exposed to the laser spot).

7.3 Results

7.3.1 Evaluation of Mass Dependencies in the Trapping Efficiency with
Broad Polymer Distributions

Characterization of the blend of PMMA standards was realized by measuring a MALDI-
TOF-MS reference spectrum. This spectrum was obtained by averaging 100 laser shots
and is shown in Figure 7.2. It demonstrates that the molecular weight distribution of
the PMMA blend ranges from \( m/z \) 500 (corresponding to a degree of polymerization
\( n = 5 \)) to 5500 (\( n = 55 \)). The expansion of the mass scale illustrates that predomi-
nantly sodium cationized pseudo-molecular ions are formed in the MALDI process. It
further reveals that the resolution in the MALDI-TOF experiment is insufficient to dis-
tinguish between the natural occurring \( ^{12}\text{C}/^{13}\text{C} \) isotopes. No further optimization of
the resolution was attempted because only information on the shape of the molecular
weight distribution was required. The MALDI-TOF molecular weight distribution is
characterized by calculating the molecular weight averages from the spectrum in Fig-
ure 7.2. For these calculations, the measured masses were corrected for the mass of the
sodium adducts and the intensities were determined by integration over the com-
plete isotopic patterns. The calculated averages are the number-average molecular weight
\( M_n = \frac{\sum (N_i M_i)}{\sum N_i} \), where \( N_i \) and \( M_i \) denote the signal intensity and measured
mass at peak \( i \), the weight-average molecular weight \( M_w = \frac{\sum (N_i M_i^2)}{\sum (N_i M_i)} \), the
z-average molecular weight $M_z = \frac{\sum (N_i M_i^2)}{\sum (N_i M_i^2)}$, and the polydispersity index ($M_w/M_n$). The results are listed in Table 7.1. In the following paragraphs it is assumed that these MALDI-TOF-MS results represent the true molecular weight distribution.

Next, the same sample was analyzed in the MALDI-ITMS instrument to monitor mass dependencies in the trapping efficiency of the instrument. The mass range $m/z$ 500 to 3400 was examined in the high-mass mode (as was mentioned before, $m/z$ 3400 is presently the software imposed upper mass limit of the system). In a series of experiments, the amplitude of the active rf field (that is, the value of $M_{cut-off}$) was varied because this parameter was expected to primarily determine the relationship between trapping efficiency and ion mass [174, 175]. In each experiment, spectra were recorded during 80

<table>
<thead>
<tr>
<th>TOF</th>
<th>MALDI-ITMS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100 u 150 u 200 u 250 u 300 u</td>
</tr>
<tr>
<td>$M_n$</td>
<td>2094 1363 1668 2106 2348 2466</td>
</tr>
<tr>
<td>$M_w$</td>
<td>2644 1544 1810 2265 2486 2586</td>
</tr>
<tr>
<td>$M_z$</td>
<td>3085 1720 1955 2408 2604 2688</td>
</tr>
<tr>
<td>$M_w/M_z$</td>
<td>1.26 1.13 1.09 1.08 1.06 1.05</td>
</tr>
</tbody>
</table>

Table 7.1: The characterizing weight averages of the PMMA blend, determined from the TOF measurement in Figure 7.2 (column 2) and from the IT measurements in Figure 7.3 for different cut-off settings (column 3-7).
successive laser shots. The resulting total ion current (TIC, the overall intensity in the individual scans as a function of time) for the experiment with $M_{\text{cut-off}} = 100$ u is presented in the upper plot in Figure 7.3. This plot shows that the MALDI-ITMS experiments are subjected to large shot-to-shot variations. The origin of these shot-to-shot variations is not likely to be in the MALDI ion source for two reasons. First, the ionizing laser beam has been tested to be stable in the course of the measurements, and second, MALDI experiments on our MALDI-FTICR-MS instrument utilizing an identical MALDI ion source did not reveal such large variations. Also the instabilities in the ion transport are not expected to be the cause, because previous experiments utilizing continuous ion beams did not reveal large temporal variations in the total ion yield. Therefore, we presently attribute the observed shot-to-shot variations to changes in the trapping efficiency due to random
variations in the phase of the rf trapping field at the laser firing [179]. Elimination of the shot-to-shot variations was achieved by averaging the individual scans composing the TIC-signal, yielding the top spectrum of Figure 7.3. The other spectra in Figure 7.3 were similarly obtained with $M_{\text{cut-off}} = 150, 200, 250, \text{ and } 300 \text{ u}$, respectively. Experiments performed with $M_{\text{cut-off}}$ smaller than 100 u or larger than 300 u were observed to give no significant signals. It is immediately seen from this set of spectra that the shape of the measured molecular weight distribution, and thus the mass range of efficient trapping, is strongly dependent on the setting for $M_{\text{cut-off}}$. However, experiments on the individual PMMA standards in the same mass range (data not shown) proved that the (mass dependence of the) trapping efficiency is not solely determined by the amplitude of the rf field. For example, comparison of the spectra in Figure 7.2 and Figure 7.3 suggests that the high-mass measuring mode is not suitable for masses smaller than $m/z \sim 1000$, whereas experiments on the PMMA standard with an average molecular weight of 640 u ($M_{\text{cut-off}}=50-100 \text{ u}$) yielded relatively much higher intensities in the lower mass range. Moreover, an experiment (with $M_{\text{cut-off}}=150 \text{ u}$) in which the PMMA oligomer at $m/z 625$ was isolated after trapping and prior to the ejection scan yielded significantly higher intensities for this oligomer (data not shown). A similar effect was not observed for the higher mass end of the distribution. The total space charge apparently causes lower mass ions to be inefficiently ejected during the ejection scan or to become unstable prior to the ejection scan.

The distortions in the observed molecular weight distributions induced by the mass dependent trapping efficiency are nicely reflected by the characterizing weight averages. These have been calculated for the individual spectra in Figure 7.3 and are included in Table 7.3. As expected from the measured spectra, the weight averages increase for increasing $M_{\text{cut-off}}$. In a rough approach, the number-average molecular weights are indicative for the center of the mass range in which optimal trapping occurs at a given setting of $M_{\text{cut-off}}$. Unfortunately, the exact relationship between the trapping efficiency (as a function of mass) and $M_{\text{cut-off}}$ can not be obtained. This is caused by the mass discrimination effects due to space charge, which can not be recovered from the present measurements. The observed decrease of the polydispersity index for increasing $M_{\text{cut-off}}$ is mainly the result of the upper mass limit of $m/z 3400$ in the MALDI-ITMS experiments. After all, the spectra in Figure 7.3 suggest that for $M_{\text{cut-off}} \geq 200 \text{ u}$ components with $m/z > 3400$ would be detected if the upper limit could be extended to $m/z 5000$, which would lead to higher values of the polydispersity factor.

7.3.2 Characterization of the Trapping Efficiency

It follows from the previous experiments that, in order to characterize the trapping efficiency as a function of mass for different amplitudes of the active rf field, it is necessary to minimize mass discrimination due to space charge. This was accomplished by evaluating the PMMA standard with an average molecular weight of 640 u for different values of $M_{\text{cut-off}}$ and comparing the results with a MALDI-TOF-MS reference spectrum. The
MALDI-TOF-MS reference spectrum is plotted in Figure 7.4 A and shows the entire distribution, ranging from \( m/z \) 425 to 1525 \((n = 4 - 15)\) with a maximum at \( m/z \) 725. It is seen that the PMMA640 sample has a much more moderate polydispersity index in comparison to the PMMA blend, which means that the number of trapped ions can be kept much lower. The MALDI-ITMS experiments were performed in the standard mass range for \( M_{\text{cut-off}} \) ranging from 50 u to 180 u in steps of 10 u. The individual spectra were averaged over 25 laser shots. As an example, the MALDI-ITMS spectrum recorded with \( M_{\text{cut-off}} = 60 \) u is shown in Figure 7.4 B. This reveals again a severe distortion in the mass distribution recorded by the MALDI-ITMS instrument. The trapping efficiency was determined as a function of mass and \( M_{\text{cut-off}} \) by dividing the relative intensities in the series of MALDI-ITMS spectra by those in the MALDI-TOF-MS spectrum:

\[
P(M_i, M_{\text{cut-off}}) = \frac{I_{\text{ITMS}}}{I_{\text{TOF}}} \tag{7.2}
\]

Here, \( I_{\text{ITMS}} \) and \( I_{\text{TOF}} \) are the intensity of the polymer component measured by MALDI-ITMS and the intensity of the same polymer component measured by MALDI-TOF, respectively, at mass \( M_i \), and \( P \) is the trapping efficiency. The results are visualized as a function of \( M_{\text{cut-off}} \) in the 3D plot in Figure 7.4 C. The 3D plot suggests that for \( M_{\text{cut-off}} > 90 \) u the trapping efficiency becomes fairly constant over considerable mass ranges. For example, at \( M_{\text{cut-off}} = 120 \) u the PMMA distribution measured by MALDI-ITMS was almost identical to the one measured by MALDI-TOF-MS in the mass range \( m/z \) 600 to 1200.

This “flatness” of the detection efficiency curve was examined in more detail by evaluating the distributions in the individual components of the PLURONIC L31 block copolymer, which was previously characterized with MALDI-FTICR-MS (Chapter 4). The FTICR-MS results demonstrated that the PLURONIC copolymer follows the random coupling hypothesis, i.e., no correlation exists between the lengths of the different constituents. A correlation between these lengths in the ITMS results would therefore be a strong indication for mass discrimination effects which distort the measured molecular weight distribution. The PLURONIC copolymers are fabricated by first synthesizing a poly(propylene oxide) polymer and subsequently adding ethylene oxide units to both sides of the initial poly(propylene oxide) polymers which yields a distribution of triblock polymers of the type \( \text{HO} - (\text{C}_2\text{H}_4\text{O})_x - (\text{C}_3\text{H}_6\text{O})_y - (\text{C}_2\text{H}_4\text{O})_z - \text{H} \). Figure 7.5 shows the MALDI-ITMS spectrum. In order to obtain this spectrum, two experiments were performed over the mass ranges \( m/z \) 500-1300 and \( m/z \) 1100-1900, respectively, in which 50 laser shots were averaged in the standard mass mode with \( M_{\text{cut-off}} = 70 \) u. In this way it was possible to store the complete multiplier signal as a function of time and thus to preserve peak information. The overall spectrum was reconstructed by overlaying the two individual spectra. The mass range shown covers the entire molecular weight distribution. Spiking the samples with sodium, potassium, and lithium salts verified that only sodium adduct ions are present in the spectrum. The most dominant ion series was identified as the distribution of the poly(propylene oxide) homopolymers. The composition of the
Figure 7.4: Molecular weight distribution of PMMA 640 recorded by MALDI-TOF (A), by MALDI-ITMS with $M_{\text{cut-off}} = 60$ u (B), and by MALDI-ITMS as a function of the value of $M_{\text{cut-off}}$ (C).
Figure 7.5: MALDI-ITMS spectrum of Pluronic L31. For this measurement, \( M_{\text{cut-off}} \) was optimized to minimize mass discrimination over the mass range covered by the molecular weight distribution. The series of poly(propylene glycol) 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.

copolymer molecules is indicated for the series of poly(propylene glycol) homopolymers in the spectrum, and in the expanded mass scale for all monoisotopic peaks. Here the first number refers to the number of ethylene oxide units present in the copolymer (\( n^{\text{EO}} \)) and the second number refers to the number of propylene oxide units present (\( n^{\text{PO}} \)). In this way we have identified all the components with S/N > 2, yielding 210 peak intensities with their corresponding copolymer compositions.

Before evaluating the individual block length distributions two effects have to be considered. Firstly, it can be seen from the expanded mass scale 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 \( n^{\text{EO}} = 4 \) and \( n^{\text{PO}} = 13 \) at \( m/z \) 971.6. Secondly, it is evident that taking the intensity of monoisotopic peaks to be representative for the relative abundance of the corresponding component will introduce errors as the relative abundance of the monoisotopic peak will significantly vary over the mass range under study. In Chapter 4 we have dealt with these problems and proposed a correction method to reveal the actual relative abundances.
After applying this correction method to the data obtained from Figure 7.5, the corrected peak intensities are plotted as a function of \( n_{\text{EO}} \) and \( n_{\text{PO}} \) in Figure 7.6. The contour plot reveals that the distribution in the propylene oxide block remains approximately constant for the different total lengths of the ethylene oxide blocks. This result demonstrates that our MALDI-ITMS instrument can be optimized to have a constant trapping efficiency in the mass range \( m/z \) 500 to 1500.

### 7.3.3 Mass Shifts Induced by Space Charge

The dependence of the measured mass upon the signal intensity has been investigated for a phthalocyanine blue pigment in a series of measurements on the acrylic paint sample. For these experiments the complex paint formulation was applied directly to the probe, that is, direct Laser Desorption and Ionization (LDI) was employed instead of MALDI. Spectra were recorded for single laser shots in the high-resolution mode with \( M_{\text{cut-off}} = 100 \) u over the mass range \( m/z \) 400 to 700. Variations in the intensity of the molecular ion were induced by changes in the attenuation of the laser beam at the one hand, and by the statistical shot-to-shot variations in the MALDI-ITMS experiments at the other hand. The intensity of the molecular ion cluster was determined by integration of the ion signal over the mass range \( m/z \) 574 to 580. The monoisotopic peak of the molecular ion cluster was fitted with the Gauss function

\[
I_{\text{meas}} = A \cdot \exp \left( -\frac{(m_{\text{meas}} - m_c)^2}{w^2} \right) \tag{7.3}
\]

where \( I_{\text{meas}} \) was the measured intensity at mass \( m_{\text{meas}} \). The fit result for the peak center \( m_c \) was taken as the measured mass of the pigment. The fit results for the peak height (\( A \))
and the peak width ($w$) were not evaluated. The results are plotted in Figure 7.7. The plot indicates that a linear relationship exists between the measured mass and the molecular ion intensity for intensities lower than $4 \cdot 10^6$ cps. The total mass shift over the complete set of measurements is expounded by the inset in Figure 7.7. This inset shows the measured isotopic pattern for an integrated intensity of $0.5 \cdot 10^6$ cps (upper spectrum) and $7 \cdot 10^6$ cps (lower spectrum), respectively, revealing the total shift of $\sim 1.1$ u. Note that the isotopic pattern is severely distorted in the lower spectrum. Inspection of the individual spectra revealed that this is the case for integrated intensities larger than $\sim 1.5 \cdot 10^6$ cps. The increase in the measured mass for an increasing ion load can be explained by recalling that the mass axis (y-axis) can be regarded as a time-of-ejection axis, whereas the intensity of the molecular ion (x-axis) can be regarded as the total number of ions present inside the trap. It is evident that the space charge associated to the ion cloud inside the trap slightly shields the electrical field induced by the rf voltage on the ring electrode. This shielding increases for increasing numbers of ions, inducing a decrease of the effective electrical field strength. As a result of the decreased effective electrical field, the secular motion of ions with a particular $m/z$ will get in resonance with the alternating dipole field across the end caps at higher rf voltages, and thus will be ejected and detected at later times (that is, at higher masses). The asymptotic behavior of the plot for intensities larger than $4 \cdot 10^6$ cps is presently not fully understood. Future experiments in combination with ion trajectory calculations are planned for further investigation of this effect.

After the previous results, which only concerned ions of one specific mass, it is interesting to investigate shifts in the measured mass of a particular ion induced by the
presence of ions at other masses. This was studied by measuring the distribution of the poly(ethylene glycol) sample. Mass spectra were produced by averaging 25 spectra from individual scans in the extended mass range with $M_{\text{cut-off}} = 90$ u, covering the molecular weight distribution in the mass range $m/z$ 745 to 1362 (corresponding to a degree of polymerisation of 16 and 30 respectively). First, the distribution was measured with the laser intensity just above threshold, which implies that mass shifts are minimal. The instrument was calibrated on basis of this measured distribution. Next, a spectrum was taken at a much higher laser intensity. The influence of the total ion load on the measured mass was evaluated by plotting the difference between the mass determined from the spectrum and the calculated mass as a function of the number of ions trapped at the time of ejection for each monoisotopic peak in the spectrum. The latter quantity was assumed to be proportional to the integration of the ion signal starting two mass units lower than the mass of the ejected ions up to the upper limit of the mass scan. The result is plotted in Figure 7.8. The corresponding high-intensity MALDI-ITMS spectrum is shown in the inset in Figure 7.8. It should be noted that $M_{\text{cut-off}}$ was optimized for optimal signal-to-noise and no attempt was made to produce a molecular weight distribution close to the expected one. The linear dependence between the mass shift and the integrated intensity demonstrates

**Figure 7.8:** Mass deviations as a function of the integrated intensity for a PEG1000 sample measured by MALDI-ITMS. The mass deviation is defined as the difference between the measured mass of a particular component of the molecular weight distribution and its actual mass. The corresponding integrated intensity is determined by integration of the mass spectrum over the mass range starting at (and including) this particular component up to the upper limit of the measured mass range. This quantity is assumed to be proportional to the magnitude of the trapped ion population at the time of its ejection.
that shifts in the measurement of an ions mass are entirely determined by the magnitude of the total space charge at the time of ejection. An important consequence of this result is that the number of trapped ions does not change the calibration function by a constant offset. Instead, the change depends on both the masses as well as the intensities of all trapped ions, resulting in a complex calibration function which is hard to predict.

It is obvious that mass shifts complicate the mass measurements. A challenging test for the possibilities of MALDI-ITMS with respect to accurate mass measurements is the determination of the polymer end groups in a complex polypropylene sample. According to the specifications of the manufacturer, the Jeffamine D2000 sample consists of a series of amine terminated polypropylene glycols and an average molecular weight of 2000 u. As was mentioned before, the present set-up only allows to acquire scan spectra in standard mass mode over mass ranges smaller than 800 u. In order to preserve information on single isotopic peaks, experiments were performed in the standard mass mode with $M_{\text{cut-off}}=70$ u over three different mass ranges, namely $m/z$ 500-1300, $m/z$ 1100-1900, and $m/z$ 1700-2500 respectively. In this way the complete molecular weight distribution of the Jeffamine sample could be analyzed. The laser intensity was optimized to be just above threshold. Spectra of 50 individual scans were summed for each of the three mass ranges. First, these settings were used to record spectra of the PMMA1140 standard (data not shown) for calibration of the instrument. The results of the experiments on the Jeffamine sample are combined in Figure 7.9 to reconstruct the complete molecular weight distribution. It is seen that the molecular weight distribution in the mass range $m/z$ 500-2500 is bimodal, pointing to contamination with other polymers, early termination reactions during polymerization, or oxidation. The expansion of the mass scale reveals that the resolution $(m/dm)_{50\%}$ of 2500 at $m/z$ 1722 is sufficient to resolve the naturally occurring $^{12}$C/$^{13}$C isotopes of the component molecules. Only sodium adduct ions are

![Figure 7.9: MALDI-ITMS spectrum of Jeffamine D2000. The value of $M_{\text{cut-off}}$ was optimized for optimal signal-to-noise at the lower mass end of the molecular weight distribution.](image-url)
present in the spectrum, as was again verified by spiking the samples with sodium and potassium salts. The distribution was characterized by calculating the molecular weight averages and the polydispersity index from the intensities of all monoisotopic peaks in the spectrum. The results are presented in Table 7.2, which also lists the results of previous analyses on the same sample by MALDI-FTICR-MS [112]. It is seen that the average

Table 7.2: $M_n$, $M_w$, $M_z$, $M_p$, and $M_w / M_n$ values calculated from the molecular weight distributions of Jeffamine D2000 measured by MALDI-FTICR-MS [112] and MALDI-ITMS (Figure 7.9).

<table>
<thead>
<tr>
<th></th>
<th>MALDI-FTICR-MS Complete MWD</th>
<th>MALDI-ITMS Complete MWD</th>
<th>$C_3H_8N$</th>
<th>$C_3H_5$</th>
<th>$C_3H_2O$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$M_n$</td>
<td>1559</td>
<td>1347</td>
<td>1785</td>
<td>997</td>
<td>912</td>
</tr>
<tr>
<td>$M_w$</td>
<td>1746</td>
<td>1517</td>
<td>1817</td>
<td>1062</td>
<td>958</td>
</tr>
<tr>
<td>$M_z$</td>
<td>1779</td>
<td>1653</td>
<td>1847</td>
<td>1119</td>
<td>1002</td>
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<td>1779</td>
<td>1779</td>
<td>1067</td>
<td>793</td>
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<tr>
<td>$M_w / M_n$</td>
<td>1.12</td>
<td>1.13</td>
<td>1.02</td>
<td>1.07</td>
<td>1.05</td>
</tr>
</tbody>
</table>

molecular weights obtained from the ITMS data are structurally lower than those obtained from the FTICR-MS data. This results from an optimization of the value for $M_{cut-off}$ for especially the lower mass range (there signal intensities are lower). Consequently, masses above $m/z$ 1600 were slightly discriminated which causes an underestimation of the average molecular weights.

End group analysis (Chapter 3) of the mass spectrum of the Jeffamine D2000 spectrum demonstrated that there are at least three different series of peaks at equidistant intervals of 58 u present in the molecular weight distribution of Jeffamine D2000. The masses of the end groups $M_{end, meas}$ were calculated by subtracting the mass of the cationizing species and the product of the degree of polymerization and the mass of the repeating unit from the measured masses for each component in the molecular weight distribution. Subsequently, these values were averaged for the three series of peaks. The results are presented in Table 7.3, along with a proposal for the elemental composition of the end groups, the corresponding calculated masses of the end groups $M_{end, calc}$, and the differences between the measured and calculated end group masses $\Delta M_{end}$. In addition, the characterizing molecular weights were calculated for the individual series, which are included in Table 7.2. The results in Table 7.3 show that the dominant distribution in Figure 7.9 consists of the amine terminated polypropylene glycols of the type $[H_2N-(C_3H_8O)_n-C_3H_8N + Na]^+$, as specified by the manufacturer. The molecular weight distribution of this series ranges from $m/z$ 1316 ($n=21$) to $m/z$ 2303 ($n=38$) and exhibits an $M_p$ of $m/z$ 1779. The second prominent ion series is observed in the low molecular weight part of the spectrum, ranging from $m/z$ 544 to 1473, and with an $M_p$ of $m/z$ 1067. End group analysis on this series yielded 57.26, which corresponds to the sum of the masses of $C_3H_5$.
Table 7.3: Masses and deviations in the end group analysis of Jeffamine D2000 by MALDI-ITMS.

<table>
<thead>
<tr>
<th>End Group</th>
<th>M\text{end, calc}</th>
<th>M\text{end, meas}</th>
<th>ΔM\text{end}</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₂N-(C₃H₆O)ₙ-C₃H₆N</td>
<td>74.08</td>
<td>73.96</td>
<td>-0.12</td>
</tr>
<tr>
<td>H₂N-(C₃H₆O)ₙ-C₅H₇</td>
<td>57.06</td>
<td>57.01</td>
<td>-0.05</td>
</tr>
<tr>
<td>H₂N-(C₃H₆O)ₙ-C₃H₅O</td>
<td>73.05</td>
<td>73.02</td>
<td>-0.03</td>
</tr>
</tbody>
</table>

and NH₂ (Table 7.3). Such unsaturated structures are well known for poly-ethers, and may result from dehydration of terminal hydroxyl groups during polymerization. This would point to early termination of the polymerization process, and thus explain the relatively low average molecular weight numbers of this series (Table 7.2). The last ion series is observed in the molecular weight range from m/z 560 to 1315 and exhibits a M\text{p} of m/z 793. The mass of the corresponding end group was determined to be 73.25, which is in agreement with the sum of the masses of C₃H₅O and NH₂. This would indicate that for example oxidation processes have induced ketone or aldehyde functions in the polymer.

In the MALDI-FTICR-MS experiments an additional ion series with an end group mass corresponding to the sum of the masses of H and NH₂ was observed. This ion series has not been recovered in the present investigation, as the resolution of the MALDI-ITMS experiment is not sufficient to resolve it from the naturally occurring \(^{13}\)C isotopes of the \([H₂N-(C₃H₆O)ₙ-C₅H₇O + Na]^+\) series.

It can be concluded that the results of the end group analysis are in good agreement with those obtained from the previous MALDI-FTICR-MS experiments, which demonstrates that mass accuracies better than 0.3 u can be achieved by optimizing the laser intensity just above threshold.

7.4 Discussion and Conclusions

The presented results clearly reveal that external ion source MALDI-ITMS experiments are easily complicated by mass discriminations and shifts in the mass determination. Mass discriminations are attributed to mass dependencies in the trapping efficiency because the amplitude of the rf field during injection highly determines the mass range of efficient trapping. Nevertheless, the instrument can be optimized to properly analyze samples with narrow mass distributions in a single experiment. This was demonstrated with the experimental confirmation of the random coupling hypothesis for the PLURONIC copolymer. On basis of the measurements of the PMMA blend it is expected that this generally holds for mass ranges smaller than \(\sim 1000\) u up to at least m/z 3400. Analyses of systems that are more polydisperse are highly affected by mass discriminations. Possible experimental strategies for such samples are correction of the measured spectra for the mass discrimination or recording a series of spectra in which successive mass intervals of 1000
u are optimized for minimal mass discrimination. Of course, these remarks assume that additional mass discriminations due to space charge can be avoided.

The shifts in the mass determination result from variations in the total space charge. It was demonstrated that these can effectively be minimized by minimizing the number of trapped ions. At larger ion signals, mass shifts can be kept within reasonable limits if the shape of the isotopic patterns is evaluated. It was shown that these become substantially distorted when a mass shift of approximately 0.3 u is observed (see Figure 7.7). This is however only true if the sample contains a few different components. Calibrant spectra have to be recorded to characterize the mass shifts for the actual experimental conditions if many different components are present in the ion population. This can be used to estimate the space charge induced mass shifts for the individual components on basis of shift versus ion intensity graphs (similar to Figure 7.8) as a correction of the measured masses.