Electrospray ionisation FT-ICR mass spectrometry of linear and hyperbranched polymers
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Chapter 2

Basics and experimental set-up of the ESI FT-ICR MS

The basics of electrospray ionisation and Fourier transform ion cyclotron resonance mass spectrometry are discussed in this chapter. A detailed description of the instrument and experimental set-up is given. All experiments discussed in this thesis were performed with a modified Bruker ESI FT-ICR MS.

2.1. Electrospray ionisation (ESI)

Magnetic and electrical fields, time-of-flight and ion cyclotron resonance are the most commonly used mass spectrometric techniques to separate ions by their \( m/z \) ratio. One of the most crucial steps in mass spectrometry is the generation and transfer of ions from a sample to the gas phase. Until recently, the most common ionisation technique was electron impact ionisation, which is based on the irradiation of vaporised sample molecules with electrons resulting in ionised fragments. The most important advantage of electron impact is that structural information of many classes of compounds is obtained. However, polymers are not volatile. Also, the technique is not the best choice if one desires to obtain structural information of one molecule in a mixture of molecules, for example, one polymer molecule in a MWD. A fragment that appears in the mass spectrum upon electron impact can originate from different polymer molecules making the interpretation of the mass spectra difficult.

During the last decades, new ionisation techniques have been developed to transfer the molecules \textit{intact} to the gas phase and produce \textit{intact} molecular ions (see section 1.1.1). Fragmentation during the ionisation was absent or minimised with the introduction of the soft ionisation techniques matrix-assisted laser desorption/ionisation (MALDI) and electrospray ionisation (ESI).

Electrospray ionisation is based on the dispersion of a dilute solution of (macro)molecules as a fine spray of charged droplets at atmospheric pressure. The
spray is produced by applying a potential of 2-5 kV between the spray needle and inlet of the mass spectrometer, as shown in figure 2.1. The solution to which a salt or acid is added is pumped through the spray needle where a Taylor cone is formed due to the electrical field. Charge separation takes place in the Taylor cone through the electrophoretic mechanism and a nebula of positively charged droplets is formed when operated in the positive ion mode. Since the overall number of charges must be constant, some negative ions (e.g. OH⁻) have to be oxidised at the surface of the spray tip or alternatively the metal surface of the spray tip is oxidised. For example, Zn²⁺ ions were formed and detected when a spray tip was used made of Zinc.

![Figure 2.1](image)

**Figure 2.1  Schematic representation of the ESI process.**

The charged droplets are directed to the inlet of the mass spectrometer. On their way to the mass spectrometer the solvent of the droplets is made to evaporate leading to an increase of the charge density on the surface of the droplets. The evaporation of the solvent can be enhanced by directing the droplets through a heated capillary. It is assumed that the number of charges on the droplets remains constant because the evaporation of a cation (e.g. sodium) is very endothermic. When the electrostatic repulsion between the charges becomes equal to the force resulting from the surface tension, the droplet will explode into smaller charged droplets. This can theoretically be described by the Rayleigh stability limit. This process continues several times until ionised (macro)molecules enter the gas phase.

The final stage in electrospray ionisation, the transfer of an ion from the droplets to the gas phase, is not well understood but can be described by two models. The first is the Charged Residue Model (CRM) developed by Dole and holds that gaseous ions are formed from droplets containing a single analyte.
molecule bound to a certain number of electrolyte ions. When all solvent molecules evaporate, a charged molecule remains. The second model, the Ion Evaporation Model (IEM), developed by Iribarne and Thomson states that the field on the surface of the droplet increases due to the shrinkage of the droplet by evaporation. The field can become so high that an ion ‘desorbs’ from the surface of the droplet entering the gas phase, which is a similar process as in field desorption. The main advantages of electrospray ionisation are:

- The detection of high mass compounds at relative low \( m/z \) values due to multiply charging.
- Conventional solutions can be sprayed.
- Minimal fragmentation.
- Combination with separation methods.

Multiply charging can also be a disadvantage for some applications. When samples are measured that consist of different molecules, like synthetic polymers, the peak density in the mass spectrum can become too high to resolve all polymer molecules. This disadvantage can be overcome with high resolution mass analysers like time-of-flight and Fourier transform mass spectrometry. Another disadvantage is that only molecules with polar groups, necessary for the interaction with cations, can be sprayed. A more detailed description of the electrospray process can be found elsewhere.

2.2. Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS)

The cyclotron motion of ions in a magnetic field was first used in 1932 to accelerate protons to high kinetic energies for nuclear physics experiments. It took however four decades before image charge detection of the cyclotron motion of ions in combination with Fourier transformation algorithms was introduced. This development resulted in a new mass spectrometric technique: Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS). The basics of this technique will be discussed briefly in the next paragraphs. More detailed introductions of the technique can be found elsewhere.
2.2.1. The cyclotron motion

The heart of the Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR MS) is the ICR cell, which is a Penning trap and is positioned in the centre of a (super-conducting) magnet. Several ICR cell designs have been investigated in the literature including rectangular, \(^{167}\) cubic \(^{168,169}\) and cylindrical \(^{170}\) cells. The main disadvantage of these cells is the undesirable loss of ions along the z-axis. The loss of ions was minimised with the introduction of the ‘infinity’ trap. \(^{171}\) However, the main disadvantage of this cell is that ions are introduced through a small hole in the front trap electrode making the trapping of ions difficult. With the introduction of the open-ended cell, \(^{172-174}\) ions were easier to trap because the front trap electrode has a diameter of typically 6 cm. Trapping with an open cell requires a gas pulse during the ion introduction. This reduces the kinetic energy of the ions allowing ions to be trapped.

Ions are trapped radially, in the x-y direction, by the magnetic field and axially (z-axis) by an electrostatic potential applied to the two trapping electrodes as is shown in figure 2.2. An ion with charge \(q\) and velocity \(v\) moving in magnetic and electrical fields \(\mathbf{B}\) and \(\mathbf{E}\) experiences a Lorentz force \(F_L\)

\[
F_L = q\mathbf{E} + q(\mathbf{v} \times \mathbf{B})
\]  
(2.1)

Only \(q\), \(v\) and the strength of the magnetic field influence the magnitude of the Lorentz force if the electrical field is considered negligible. The magnetic field is homogenous and uniform in the volume of the ICR cell. The Lorentz force becomes \(qv_y B\) and is compensated by a centripetal force \(F_C\) opposite to the Lorentz force as shown in figure 2.3, which is given mathematically by the following equation

\[
qv_y B = \frac{mv_y^2}{r}
\]  
(2.2)
where $r$ is the orbit radius of the ion in the ICR cell. In the absence of collisions the orbit radius is constant. By introducing the angular velocity $\omega = v_\phi / r$, equation (2.2) becomes the well known cyclotron frequency $\omega_c$ of ions in a magnetic field

$$\omega_c = \frac{qB}{m} \quad (2.3)$$

It is important to point out two important advantages that follow from equation (2.3). First, the cyclotron frequency is a function of the mass, charge and the strength of the magnetic field (which is constant in time and space). Moreover, the frequency of the ions is independent of the orbit radius. Secondly, mass resolution and frequency resolution are the same (except for a minus sign) as can be seen by taking the derivative $dldm$ of equation (2.3) ($\omega_c/\Delta \omega_c = -m/\Delta m$).
Figure 2.3  Motion of the ions with velocity $v_{xy}$ in an FT-ICR in the presence of a magnetic field $B$. The Lorentz force $F_L$ is opposed by the centripetal force $F_C$.

The electrical field of the trapping electrodes has been neglected in equation (2.3) but can have a significant contribution on the cyclotron motion and must therefore not be disregarded. Most Fourier transform mass spectrometers operate with an external ion source requiring that the ions have a kinetic energy along the z-axis to enter the ICR cell. The potential on the trapping electrodes makes sure that the ions are trapped along the z-axis. Consequently, the ions oscillate along the z-axis with a trapping frequency $\omega_T$ given by

$$\omega_T = \sqrt{\frac{qV_T}{md^2}}$$

(2.4)

where $V_T$ is the potential applied to the trapping electrodes and $d$ the diameter of the ICR cell. The trapping potential induces an axial electric field $E=V_Tr/2d^2$ that opposes the Lorentz force. Equation (2.2) has to be extended with $qE$ to correct the cyclotron frequency for the trapping potential according to equation (2.1) and can be written as $m\omega^2r=qB_0r-q(V_T/2d^2)$. It can be shown that two solutions exist for $\omega$. 

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2.2.2. Excitation and detection

It was shown in the previous paragraph that ions have a cyclotron motion in a magnetic and electrical field. The mass and charge of the ions can be determined with high accuracy by detection of the cyclotron frequency $\omega_c$. To detect the cyclotron frequency and to calculate the corresponding mass and charge of the ions, the ions must be excited into a coherent motion. An ion population that is excited without a coherent motion is not detected. Ions can be coherently excited by applying an RF electric field between two opposite excitation electrodes (see figure 2.2) of the form

$$E(t) = E_0 \cos \omega_c t \mathbf{j} = \frac{V_{pp}}{d} \cos \omega_c t \mathbf{j}$$  \hspace{1cm} (2.6)$$

where $\alpha$ is the geometry constant of the ICR cell, $V_{pp}$ is the peak-to-peak amplitude of the excitation RF signal. The geometry constant $\alpha$ is 0.897 (5% accuracy)\textsuperscript{175} for the Infinity Cell and 2.26 for the home build open cell (based on a comparison of breakdown diagrams measured with the Infinity and open cell).

The electrical field $E(t)$ consists of two counter rotating components $E_1(t) + E_2(t)$ that continuously push the ions coherently to a higher orbit in the ICR cell.

$$E_1(t) = \frac{E_0}{2} (\sin \omega_c t + \cos \omega_c t)$$ \hspace{1cm} (2.7a)$$

$$E_2(t) = \frac{E_0}{2} (\sin \omega_c t - \cos \omega_c t)$$ \hspace{1cm} (2.7b)$$
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Only the component, which rotates with the same frequency and in the same direction as the ion of interest, will increase the kinetic energy of the ions. The counter-rotating component does not influence the kinetic energy but ensures the coherent motion of the ions. If it is assumed that all power absorbed by the ions \( A(t) = E_0^2 q^2 t / 4m \) is converted into kinetic energy, the radius \( r \) of the ions becomes

\[
r = \frac{E_0 f_{\text{exc}}}{2B}
\]

(2.8)

The nice feature of this equation is that all ions are excited to the same radius in the ICR cell independent of the mass and charge of the ions. However, most excitation methods provide an excitation power that is not completely independent of the frequency. These methods include rectangular pulse or impulse excitation, single frequency excitation and a frequency chirp. A method that does excite the ions to the same radius is called stored waveform inverse Fourier transform (SWIFT) and was introduced by Marshall et al. in 1985. The frequency range for excitation is specified followed by an inverse Fourier transformation to obtain the time domain excitation waveform. Quadratic phase scrambling is used to spread the power of the time domain signal.

The coherent motion of the ions is detected with the detection electrodes after kinetic excitation of the ions to an orbit with a radius smaller than the diameter of the ICR cell. The two detection electrodes are opposite to each other and positioned next to the excitation electrodes as shown in figure 2.2. An alternating image current is induced each time the ions pass the detection electrodes. The frequency of the alternating image current matches the cyclotron frequency of the ions. After conversion of the alternating current to an alternating voltage the time domain voltage signal \( f(t) \) is obtained

\[
f(t) = \sum_{i=1}^{M} N_i e^{-t/\tau} \cos(\omega_i t + \varphi_i)
\]

(2.9)

where \( t \) is the length (in seconds) of the transient, \( N_i \) is the number of ions \( i \) and \( \varphi_i \) the phase of the ions. The intensity of the time domain signal decreases with damping constant \( \tau \) due to collisions with background gas and space charge effects. These effects result in a dephasing of the coherent motion of the ions, magnetron expansion and a decrease of the orbit radius.
An example of a time domain signal of an ion with a cyclotron frequency $\omega$ of 114.179 Hz ($m/z$ of 940.6933) is shown in figure 2.4a (see insert). The beating pattern that is observed originates from the presence of other ions with cyclotron frequencies 114.057 Hz and 113.936 Hz ($m/z$ 941.6967 and 942.7001). These ions are the isotopic peaks of the ion with a cyclotron frequency of 114.179 Hz due to the natural abundance of isotopes ($^2$H, $^{13}$C, $^{15}$N and $^{18}$O). Another feature of equation 2.9 that becomes clearly visible in figure 2.4a is that the intensity of the time domain signal decays in time, which can be described by $\tau_i$. After Fourier transformation of the time domain spectrum the frequency spectrum is obtained. The mass spectrum (figure 2.4b) is calculated by equation (2.3).

Figure 2.4  *Time domain signal of a synthetic hyperbranched oligomer made by the polycondensation of di-isopropanolamine and phthalic acid (a). Mass spectrum of the Fourier transformed time domain signal (b).*

2.2.3. **Collisional excitation for fragmentation**

A frequently used method to obtain structural information of ions is by collisionally activated dissociation (CAD). The ion of interest is kinetically excited in the ICR cell and collided with a collision gas. A part of the collision energy is converted into internal energy until the dissociation threshold is reached and fragmentation occurs. Kinetic excitation is performed by applying a well-defined RF voltage on the excitation electrodes. The two most common methods to activate the ions are on-resonance excitation and sustained-off resonance irradiation (SORI). The alternating field in on-resonance excitation has the same frequency as
the cyclotron frequency of the ion of interest. The laboratory frame kinetic energy $E_{\text{kin,lab}}$ at time $t_{\text{exc}}$ can be controlled by $t_{\text{exc}}$ and $E_0$ and is given by the following relation

$$E_{\text{kin,lab}} = \frac{q \omega_c E_0^2 t_{\text{exc}}^2}{8B} = \frac{q^2 E_0^2 t_{\text{exc}}^2}{8m}$$  \hspace{1cm} (2.10)$$

Note that the orbit radius $r$ in equation (2.8) is independent of the mass and charge of the ions. The increase of the orbit radius during the excitation is schematically shown in figure 2.5a.

(a) \hspace{5cm} (b)

Figure 2.5 \hspace{0.5cm} Increase of the orbit radius during the excitation upon on-resonance excitation (a) and increase of the orbit radius during sustained off resonance irradiation (SORI) (b). Solid line represents acceleration of the ions, dotted line deceleration. Note that the radius of the ion trajectory in the ICR cell during the SORI excitation is much smaller.

An electrical field that is off resonance with the cyclotron frequency of the ions is applied on the excitation electrodes in SORI. The radius is continuously modulated as is shown in figure 2.5b. The following relation gives the laboratory frame kinetic energy and orbit radius.
The main difference with on-resonance excitation is that the ions are continuously (sustained) accelerated and decelerated. In on-resonance excitation the ions are accelerated to a relatively high kinetic energy in approximately 100 – 300 μs. The kinetic energy of the ions relaxes in ~0.1 second after acceleration due to multiple collisions. In SORI, the kinetic energy can be modulated for seconds. The collision energy of each collision is typically lower than in on-resonance excitation, which allows the study of the lowest energetic fragmentation pathways.

2.2.4. Instrumental layout and experimental procedures for ESI FT-ICR MS analysis

The electrospray ionisation Fourier transform ion cyclotron resonance mass spectrometry (ESI FT-ICR MS) experiments are performed with a modified Bruker-Spectrospin (Fällanden, Switzerland) APEX 7.0e FT-ICR MS equipped with a 7T super-conducting magnet. The heart of the system, the ICR cell, is positioned in the centre of the super-conducting magnet and is held at $10^{-9} - 10^{-10}$ mbar.

A polymer solution to be electrosprayed is made that contains ~1 mg/ml polymer. Several types of solvents can be chosen for this purpose but the polymer must be soluble in the solvent and the solvent must be electrospray compatible. Commonly used solvents are THF, methanol and propanol. The ‘magic’ solvent hexafluoroisopropanol (HFI) can often be used for polymers that are difficult to dissolve. Depending on the type of cations that is desired, ~1 mM NaI (for sodium cationised ions) or ~ 2% acetic acid (for protonated ions) is added to the solution.

Ions are generated in an in-house constructed ESI source shown in figure 2.6. The sample is pumped with a Harvard syringe pump model 55-1111 (Kent, U.K.) at a flowrate of 0.1 ml/hr through a fused silica capillary (0.18 mm internal diameter). Positively charged electrospayed droplets are generated by applying a 3000-5000 V potential difference between a stainless steel spray needle and the stainless steel capillary, which is the inlet of the mass spectrometer. The stainless steel capillary is at a distance of ~0.5 cm from the spray needle. The capillary has
an internal diameter of 0.75 mm, length of ~20 cm (Alltech Assoc., Inc., Deerfield, IL) and is placed in a ceramic heater tube. To aid the evaporation of the solvent, the stainless steel capillary is held at approximately 170 °C by passing a current of 1.8 A (~12 W) through a metal wire woven in the ceramic heater tube.

![Image of ESI source with spray needle (A), heated capillary (B), skimmer (C), quadrupole (D), and extraction lenses (E).]

**Figure 2.6** Schematic representation of the ESI source with spray needle (A), heated capillary (B), skimmer (C), quadrupole (D), and extraction lenses (E).

Five stages of differential pumping are employed to bridge between the atmospheric pressure conditions in the ESI source to the FT-ICR cell at $10^{-9} - 10^{-10}$ mbar. In the first pumping stage, the nozzle skimmer region, the pressure is approximately 1 mbar. Ions are focused by a tube lens (not shown) through a copper skimmer with an orifice diameter of 1 mm. Excess neutrals are removed by the skimmer. Typical nozzle skimmer voltage differences are about 5 V to minimise fragmentation in the ion source. The second pumping stage contains a RF-only quadrupole at a pressure of $10^{-5}$ mbar. Electrostatic ion optics is used (at a pressure of $10^{-7}$ mbar) in the third region to guide the ions from the exit of the quadrupole in the direction of the ICR cell. Ions are accelerated to 3000 eV in the intermediate region (~$10^{-8}$ mbar) at the exit of the quadrupole to prevent radial ejection by the magnetic field. Before entering the ICR cell the ions are decelerated to approximately 1 eV to facilitate trapping in the ICR cell at $10^{-9} - 10^{-10}$ mbar.

Two ICR cells have been used in this thesis. The analysis described in chapter 3 and 4 were performed with a Bruker Infinity™ ICR Cell. All other experiments were performed with an in-house constructed capacitively coupled open cell shown in figure 2.7. The copper electrodes of the open cell are mounted in an Al$_2$O$_3$ cylinder with an internal diameter of 6 cm and an outer diameter of 8 cm. The 1 cm thick Al$_2$O$_3$ wall contains sixteen channels with a diameter of 3 mm.
In these channels 12 electrically shielded heater elements are placed. The coaxial heater elements consist of a closed rigid copper cylinder, a UHV compatible coaxial isolation material and a central heater wire. These elements were chosen as they can be electrically shielded from the ICR cell electrodes to prevent the introduction of noise from the heater current and to keep the heater current sufficiently low to have only a minimal effect on the magnetic field homogeneity. A Pt100 temperature sensor was mounted on the outside of the Al$_2$O$_3$ cylinder to measure and control the cell temperature. A Shinyo thermostat was used to readout the Pt100 sensor and controls the temperature of the open cell.

A standard ESI FT-ICR MS experimental time sequence is shown in figure 2.8a. The experiment starts with a quench on the rear trapping electrode to make sure that all ions are ejected from the ICR cell. In the next step the emptied ICR cell is filled with the ion population. Argon gas is pulsed (~$10^{-6}$ mbar) during the introduction of the ions to kinetically cool down the ions and enhance trapping. Kinetic excitation and detection of the ion population takes place after a delay of several seconds to pump down to a pressure of $\sim 10^{-8}$-$10^{-9}$ mbar. Collisional cooling is only required if the experiments are performed with the open cell. Data acquisition (128k datapoints) and control is performed using XMASS (Bruker-Daltonics, Billerica MA) running on an SGI Indigo R4000 (Silicon Graphics, Mountain View, CA) UNIX-based workstation. The total duration of one experiment is in the order of seconds.

The experimental time sequence of a mass spectrometry/mass spectrometry (MS/MS) experiment is shown in figure 2.8b. After the ions are trapped and the Argon pressure is reduced to $\sim 10^{-8}$-$10^{-9}$ mbar the precursor ion is selected. Isolation of the ions is performed with an in-house constructed arbitrary waveform generator (AWG) with a memory of 192 Mb. Tailor made isolation pulses can be generated including single frequency excitation, frequency chirp and SWIFT. These pulses have in common that the cyclotron motion of all ions, except the ion of interest, is kinetically excited to make sure that these ions collide with the electrodes of the ICR cell. The ion of interest remains in the ICR cell for further study. The AWG can also be used for detection. A factor of 1500 more data points are available by detecting with the AWG compared with the 128 Kb of the Bruker hardware. This allows detecting the coherent motion of ions for a longer period and hence an increase of the resolution can be obtained. MS/MS experiments are started with an Argon pulse in the ICR cell after isolation of the ion. A delay of $\sim 0.5$ s is implemented before the ions are excited by on-resonance or sustained off resonance collisionally activation as described in paragraph 2.2.3. Collisions of the ions with Argon take place in approximately 2 seconds for on-resonance excitation. The collision time can be longer or shorter for SORI. After the collision experiment a pumping delay of 5 seconds is introduced followed by the regular
excitation and detection event. In a similar way other experiments can be designed. For example, MS/MS/MS experiments can be performed with an additional isolation and MS/MS step. MS\textsuperscript{n} experiments were performed in chapters 6 and 7.

A typical time sequence of a H/D exchange experiment is depicted in figure 2.8c. The experiment is very similar to the previous two experiments. The only difference is that the ion excitation/activation event of figure 2.8b is replaced by a D\textsubscript{2}O gas pulse (~5·10\textsuperscript{-7} mbar) via a pulsed valve. The pulsed valve is in-between a reservoir containing liquid D\textsubscript{2}O and the ICR cell. A combination of gas phase H/D exchange experiments and MS\textsuperscript{n} was used in chapter 8 for the study of the isomeric structures of hyperbranched polyesteramides. The electron capture dissociation (ECD) experiments described in chapter 9 for the study of hyperbranched polyesteramides have been performed in a similar manner as the experiment described in figure 2.8c. The only difference is that an electron pulse for ECD is used instead of the D\textsubscript{2}O pulse. A Rhenium filament is positioned in the back of the ICR cell centred on the z-axis of the cell. The filament is heated by a current of 2.95 A, which is sufficient to generate electrons. During the ECD event the electrons are accelerated to ~ 1.5 eV and allowed to be captured by the ions.

![Figure 2.7: The two ICR cells used in this thesis. Infinity\textsuperscript{TM} Cell (a) and in house constructed capacitively coupled open cell (b).](image-url)
Basics and experimental set-up of the FT-ICR MS

Figure 2.8 Time sequence of a standard ESI FT-ICR MS experiment (a), MS/MS experiment (b) and H/D exchange experiment (c).
A typical time sequence of a H/D exchange experiment is presented in figure 2.6. The key difference is that the samples to be exchanged are placed in the D$_2$O protons. After a period of time, the exchange is complete. The structures of the hydrogenated polymers can then be determined by hyperfine NMR (1H NMR) experiments. In addition, the experiment has been performed on a sample of polymer described in figure 2.6. The only difference is that a helium atom was used instead of the D$_2$O probe. A scheme similar to that described in figure 2.6 is used to determine the H/D exchange and the polymer composition. The results are consistent with the predictions in figure 2.6.