Electrospray ionisation FT-ICR mass spectrometry of linear and hyperbranched polymers
Koster, S.

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Chapter 5

Quantitative analysis of copolymers: The influence of the monomers on the ionisation efficiency in electrospray ionisation

The influence of the ionisation efficiency on the measured copolymer sequence distribution is discussed. Large differences in ionisation efficiency were observed for mixtures of homopolymers containing dipropoxylated bisphenol-A/adipic acid and dipropoxylated bisphenol-A/isophthalic acid and the corresponding copolyester dipropoxylated bisphenol-A/isophthalic acid/adipic acid. The adipic acid structure has a higher affinity for the sodium cation, which results in more intense peaks for adipic acid containing oligomers. Relative sodium affinities of the oligomers were found to increase with an increasing number of acid endgroups in favour of adipic acid containing oligomers. The ESI response of the oligomers depends on the polymer concentration in the sprayed mixture and other parameters. This makes it impossible to correct for the ionisation efficiency necessary for copolymer analysis. If differences in ionisation efficiency are not corrected, the ion intensities in the copolymer mass spectra will show large deviations from the real composition and no conclusion can be drawn about the chemical (in)homogeneity of the MWD nor the random or block structure of the copolymer. This will also be valid for other cationisation techniques like MALDI and FAB.

5.1. Introduction

Copolymers consist of a complex mixture of molecules that differ in size, chemical composition and sequence. Complete characterisation of such complex materials demands new analytical approaches. A technique that has become increasingly popular for copolymer analysis during the last ten years is mass spectrometry. Soft ionisation techniques like matrix-assisted laser desorption/ionisation (MALDI) and electrospray ionisation (ESI) have been
successfully used to ionise intact polymer molecules, which made it possible to study the chemical composition distribution of the copolymer directly from the mass spectrum.

The sequence of copolymers can be studied using Bernoullian and Markovian chain statistics applied to the intensity profile of the polymer distribution. The Bernoullian chain statistics are used to model random copolymerisation reactions. Markovian statistics allow the modelling of both random and non-random copolymerisation reactions. Non-random polymerisation reactions in step (radical) polymerisation normally result in the formation of block copolymers.

The chain statistical models rely on a uniform mass spectrometric response i.e. an equal ionisation efficiency (IE) for the various components in the molecular weight distribution (MWD), but this uniformity is questionable because the overall efficiency of a molecule to become charged in electrospray ionisation is influenced by several processes. Ions with relatively low solvation energy or a high surface tension will be situated preferentially on the surface of the droplets and are desolvated more easily during ESI. Ions with larger solvation energies will be distributed throughout the entire droplet and will be ionised less easily.

Other factors that influence the ionisation efficiency of an analyte are the polarity of the solvent, the structure of the ion (i.e. the organic functionalities in the molecule), the flexibility of the chain (for example cyclic versus linear molecules), pH or the nature of the cation, charge state, counter ion effects, drying gas flow rate and the ESI source temperature (selective nozzle skimmer ion activation). Several studies have been reported in the literature in which ionisation efficiencies of mixtures of different cyclic compounds like crown ethers and peptides with a variety of cations were studied using FAB MS, MALDI MS and ESI MS. Most studies indicate that a good correlation exists between the selectivity of analytes towards metal ions in the liquid phase and the intensity in the mass spectrum, which allows a correction for differences in ionisation efficiency. Synthetic polymer mass spectrometric studies carried out sofar do not take the ionisation efficiency (or response factors) of the different components in copolymers into account. This is probably because synthetic polymers are not single molecules but consist of mixtures with a distribution of different molecules, which are impossible to quantify with NMR. Liquid Chromatography (LC) allows only a quantification of the low molecular weight part of the MWD. Besides, the determination of the ionisation efficiency of all individual polymer molecules would be a very laborious process.
In this chapter Bernoullian and Markovian chain statistics are applied to the ESI FT-ICR MS spectra of the copolyester poly(di-propoxylated bisphenol-A/adipic acid/isophthalic acid). Table 5.1 presents the structures of the different monomers. The copolymer composition determined with the chain statistics will be different from the real copolymer composition if differences in ionisation efficiency (IE) between the adipic and isophthalic acid structures exist. The relative IE of the adipic and isophthalic acid structures are determined with well defined mixtures of the homopolyesters poly(di-propoxylated bisphenol-A/adipic acid) and poly(di-propoxylated bisphenol-A/isophthalic acid). Gradient polymer elution chromatography (GPEC) was used to quantify the weight fractions of the oligomers in the low molecular weight part of the molecular weight distribution of the homopolyesters, a necessary requirement for the determination of the relative IE. This is the first time synthetic polymers were measured with GPEC for quantitative analysis with ESI FT-ICR MS.

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<th>Notation</th>
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Table 5.1 Notation, mass and structure of the monomers used in this chapter.

5.2. Methods and materials

5.2.1. ESI FT-ICR MS analysis

The ESI FT-ICR MS (Bruker-Spectrospin APEX 7.0e, Fällanden, Switzerland) used in this work has been described in chapter 2 and elsewhere. The cell is an in-house constructed open cell. The electrospray generated ions
were trapped for 2-4 s. Argon gas is introduced through a pulsed valve to enhance trapping \((P_{Ar} = 5 \times 10^{-6} \text{ mbar})\).

5.2.2. GPEC ESI TOF analysis

GPEC analyses were performed at Akzo Nobel Chemicals Research Arnhem as described elsewhere. The Hewlet Packard LC system model 1050 (Palo Alto, CA, USA) was operated with an autoinjector model Basic Marathon (Spark Holland, Emmen, The Netherlands) with a loop volume of 10 \(\mu\)l. Solution A contained unstabilised THF: \(\text{H}_2\text{O}\) 5:95 and solution B unstabilised THF: \(\text{H}_2\text{O}\) 95:5 (v/v). To both solutions 0.1 \% glacial acetic acid was added. The applied gradient ran from 45\% of solution A at \(t=0\) to 10\% of solution A at \(t=35\) min, at a flow of 1 ml/min. The column was a Waters Symmetry C18 (5 \(\mu\)m particle size), 150 mm x 4.6 mm ID. Approximately 30 \(\mu\)l/min of the eluent from the column was split with a 0.01 inch i.d. stainless steal T-piece (Valco, Houston, TX, USA) that was allowed to flow to the Z-spray TOF instrument (Micromass, Manchester, UK). The eluent stream was compatibilised with a NaI solution (10 \(\mu\)l/min of a 250 \(\mu\)M NaI solution in THF/iso-propylalcohol). The remaining eluent was eluted through an UV diode array detector model 1000S (Applied Biosystem, San Jose, Ca, USA).

5.2.3. Materials

The unstabilised THF was obtained from Fluka (Buchs, Switzerland). The polymers used for this study are the homopolymesters poly(di-propoxylated bisphenol-A/adipic acid) and poly(di-propoxylated bisphenol-A/isophthalic acid) and the copolyester poly(di-propoxylated bisphenol-A/adipic acid/isophthalic acid). These polymers were a gift from Oc\'e Technologies (Venlo, The Netherlands) and are from the same batch as the polymesters described in a previous publication. The structures are shown in table 5.1. Di-propoxylated bisphenol-A, adipic acid and isophthalic acid will be abbreviated by D, A and I throughout the chapter, respectively. The copolyesters studied have the composition DAI31, DAI21, DAI11, DAI12 and DAI13 where the numbers denote the molar ratio of adipic acid and isophthalic acid. DAI31, DAI21, DAI11, DAI12 and DAI13 contain a molar ratio D:A:I of 0.5:0.37:0.13, 0.51:0.33:0.16, 0.5:0.26:0.24, 0.51:0.16:0.33 and 0.51:0.12:0.37, respectively as was determined with NMR. Since identification of the oligomers was not performed using mass spectrometry, the GPEC experiments were repeated with online ESI TOF for identification.
5.2.4. Copolymerisation models

The copolymerisation models and their link with mass spectrometry have first been used by Montaudo and coworkers. In their work the entire mass spectrum is used to determine the composition and ‘blockiness’ of the copolymers. Here, we investigate the copolymer composition for each endgroup class separately and subsequently as a function of the degree of polymerisation and charge state. For example, the composition of the pentamer with two alcohol endgroups in charge state 2 was studied by searching for the best fit of the experimentally observed intensity profile with the chain statistical models. This allows studying the chemical inhomogeneity as a function of the degree of polymerisation, endgroup class and charge state. The Markovian and Bernoullian models used were implemented in Matlab 5.3 (The MathWorks, Inc.) and are described below.

5.2.4.1. Markovian chain statistical model

Consider a system composed of linear copolymers constructed from \( N \) different types of monomers, which we will label by the letters \( \tau = A, B, \ldots \). Each polymer molecule consists of \( M = 1, 2, 3, \ldots \) monomers whose position within the chain is labelled by lowercase letters \( m, k, l, n, \ldots \). Note that we allow bare monomers as ‘chains’ of length \( M = 1 \). We denote the probability for an \( M \)-mer to have a specific monomer sequence by \( P^{(M)}(\tau_1, \tau_2, \ldots, \tau_M) \). Let us further define the probability of finding a certain initial segment of length \( m \) in a polymer of total length \( M \)

\[
P_m^{(M)}(\tau_1, \tau_2, \ldots, \tau_m) = \sum_{\tau_{m+1}=\tau_M} P^{(M)}(\tau_1, \tau_2, \ldots, \tau_M)
\]  

(5.1)

The Markovian assumption now states that the conditional probability of finding the next monomer \( \tau_{m+1} \) of a chain to be of a certain type only depends on the type of the immediately preceding monomer \( \tau_m \), the penultimate effect, i.e.

\[
P^{(M)}_{m+1}(\tau_{m+1}|\tau_1, \tau_2, \ldots, \tau_m) = \frac{P^{(M)}(\tau_1, \tau_2, \ldots, \tau_m, \tau_{m+1})}{P^{(M)}(\tau_1, \tau_2, \ldots, \tau_m)} = P(\tau_m \rightarrow \tau_{m+1})
\]  

(5.2)
where we adopt the arrow notation to stress the order of the two monomers in the sequence. Each probability \( P(\tau_m \rightarrow \tau_{m+1}) \) corresponds to a matrix element \( P_{ij} \) from the probability matrix \( P \) as described by Montaudo and coworkers.\(^{119,120}\) Note that

\[
\sum_{\tau'} P(\tau \rightarrow \tau') = 1 \quad (5.3)
\]

Under this assumption we find that

\[
P^{(M)}_M(\tau_1, \tau_2, ..., \tau_{M-1}, \tau_M) = P^{(M)}_{M-1}(\tau_1, \tau_2, ..., \tau_{M-1}) \cdot P(\tau_{M-1} \rightarrow \tau_M) \quad (5.4)
\]

which upon iteration yields

\[
P^{(M)}_M(\tau_1, \tau_2, ..., \tau_{M-1}, \tau_M) = P^{(M)}_1(\tau_1) \cdot \prod_{m=2}^{M} P(\tau_{m-1} \rightarrow \tau_m) \quad (5.5)
\]

The probability \( P^{(M)}_1(\tau_1) \) is the probability that a polymer molecule starts with \( \tau_1 \) (an A, B, ...). However, since the choice of the start of a chain is arbitrary, one could just as well start at the other end to obtain

\[
P^{(M)}_M(\tau_1, \tau_2, ..., \tau_{M-1}, \tau_M) = P^{(M)}_M(\tau_M, \tau_{M-1}, ..., \tau_2, \tau_1) \\
= P^{(M)}_{M-1}(\tau_M, \tau_{M-1}, ..., \tau_2) \cdot P(\tau_2 \rightarrow \tau_1) \quad (5.6)
\]

or

\[
P^{(M)}_M(\tau_1, \tau_2, ..., \tau_{M-1}, \tau_M) = P^{(M)}_1(\tau_M) \cdot \prod_{m=2}^{M} P(\tau_m \rightarrow \tau_{m-1}) \quad (5.7)
\]

Next, we also assume the probability that a chain starts with a specific type of monomer does not depend on the length of the chain i.e. \( P^{(M)}_1(\tau_1) = P_1(\tau_1) \) so that equation (5.6) simplifies to

\[
P^{(M)}_M(\tau_1, \tau_2, ..., \tau_{M-1}, \tau_M) = P^{(M)}_{M-1}(\tau_M, ..., \tau_3, \tau_2) \cdot P(\tau_2 \rightarrow \tau_1) \quad (5.8)
\]
Note that by definition
\[ \sum_{\tau} \rho_{i}(\tau) = \sum_{\tau} \rho_{i}(\tau_{M}) = 1 \quad (5.9) \]

This relation can be used to obtain a self-consistency condition on \( \rho_{i}(\tau_{1}) \) (or \( \rho_{i}(\tau_{M}) \)) as follows

\[ \rho_{i}(\tau_{1}) = \sum_{\tau_{2}, \ldots, \tau_{M}} \rho_{i}^{(M)}(\tau_{M}, \tau_{M-1}, \ldots, \tau_{2}, \tau_{1}) \]
\[ = \sum_{\tau_{2}, \ldots, \tau_{M}} \rho_{i}^{(M-1)}(\tau_{M}, \tau_{M-1}, \ldots, \tau_{2}) \cdot P(\tau_{2} \to \tau_{1}) \]
\[ = \sum_{\tau_{2}, \tau_{3}, \ldots, \tau_{M}} \rho_{i}^{(M-1)}(\tau_{M}, \tau_{M-1}, \ldots, \tau_{2}) \cdot P(\tau_{2} \to \tau_{1}) \]
\[ = \sum_{\tau_{2}} \rho_{i}(\tau_{2}) \cdot P(\tau_{2} \to \tau_{1}) \quad (5.10) \]

This equation together with the two normalisation constraint equations (5.9) and (5.3) allow \( \rho_{i}(\tau_{1}) \) to be expressed in terms of the transition probabilities \( P(\tau_{m} \to \tau_{m-1}) \). Consider a copolymer made of monomers A and B. The probability a sequence starts with an A is given by equation (5.10) or

\[ P_{i}(A) = \sum_{\tau_{2}} \rho_{i}(\tau_{2}) \cdot P(\tau_{2} \to \tau_{A}) = P_{i}(A) \cdot P(A \to A) + P_{i}(B) \cdot P(B \to A) \quad (5.11) \]

Using the notation of Montaudo and coworkers, equation (5.11) becomes
\[ S_{A} = S_{A}P_{AA} + S_{B}P_{BA}. \]

Note that equation (5.8) is the basis of the Markovian model applied to copolymer mass spectra. By correlating this equation to the measured intensity \( I_{M}^{(M)}(\tau_{1}, \tau_{2}, \ldots, \tau_{M}) \) in a mass spectrum, equation (5.8) becomes

\[ I_{M}^{(M)}(\tau_{1}, \tau_{2}, \ldots, \tau_{M-1}, \tau_{M}) = \]
\[ = IE^{(M)}(\tau_{1}, \tau_{2}, \ldots, \tau_{M-1}, \tau_{M}) \cdot P_{i}^{(M)}(\tau_{1}) \cdot \prod_{m=2}^{M} P(\tau_{m-1} \to \tau_{m}) \quad (5.12) \]
where \( IE^{(M)}(\tau_1, \tau_2, ..., \tau_{M-1}, \tau_M) \) is the ionisation efficiency of the co-polymer with monomer composition and sequence \((\tau_1, \tau_2, ..., \tau_{M-1}, \tau_M)\). For example, the mass spectrometric intensity for the specific oligomer with sequence AABBA is given by

\[
I_s^{(5)}(A, A, B, B, A) = IE^{(S)}(A, A, B, B, A) \cdot P(A \rightarrow A) \cdot P(A \rightarrow B) \cdot P(B \rightarrow B) \cdot P(B \rightarrow A) \tag{5.13}
\]

The ionisation efficiency \( IE^{(M)}(\tau_1, \tau_2, ..., \tau_{M-1}, \tau_M) \) has until now always assumed to be 1. This chapter will demonstrate that this assumption is not valid.

The models used by Montaudo and coworkers assume that the probability that a chain starts with \( \tau_1 \) is proportional to the molar fraction of the monomer in the copolymer. Here we provide a simple proof of this assertion based on the Markovian assumption itself. Consider thereto the probability \( P_m(\tau_m) \) that a given monomer at position \( 1 \leq m \leq M \) within the chain is of a certain type

\[
P_m(\tau_m) = \sum_{\tau_1, \tau_2, ..., \tau_{M-1}} P^{(M)}(\tau_1, \tau_2, ..., \tau_{M-1}, \tau_M)
\]

\[
= \sum_{\tau_1, \tau_2, ..., \tau_{M-1}} \sum_{i=1}^{M-1} P_1(\tau_1) \prod_{i=1}^{M-1} P(\tau_i \rightarrow \tau_{i+1})
\]

\[
= \sum_{\tau_1, \tau_2, ..., \tau_{M-1}} P_1(\tau_1) \prod_{i=1}^{M-1} P(\tau_i \rightarrow \tau_{i+1}) \sum_{\tau_{m+1}, \tau_{m+2}, ..., \tau_M} \prod_{j=m}^{M-1} P(\tau_j \rightarrow \tau_{j+1})
\]

\[
= \sum_{\tau_1, \tau_2, ..., \tau_{M-1}} P_1(\tau_2) \prod_{i=1}^{M-1} P(\tau_i \rightarrow \tau_{i+1}) \sum_{\tau_{m+1}, \tau_{m+2}, ..., \tau_M} \prod_{j=m}^{M-2} P(\tau_j \rightarrow \tau_{j+1})
\]

where we repeatedly employ Eqs. (5.3) and (5.10). As the position \( m \) is arbitrary we can simply define the probability that an arbitrary monomer has a given type to be \( P(\tau) = P_m(\tau) = P_1(\tau) \).
5.2.4.2. Bernoullian chain statistical model

In case of Bernoullian chain statistics the addition of a new monomer does not depend on the nature of the previous monomer and it is assumed that it also does not depend on the chain length

\[ P(\tau_m \rightarrow \tau_{m-1}) = P(\tau_m) \]  \hspace{1cm} (5.15)

The intensity of an ion in the mass spectrum becomes

\[ I_M^{(\tau)}(\tau_1, \tau_2, \ldots, \tau_{M-1}, \tau_M) = IE^{(\tau)}(\tau_1, \tau_2, \ldots, \tau_{M-1}, \tau_M) \cdot \prod_{m=1}^{M} P(\tau_m) \] \hspace{1cm} (5.16)

or for the oligomer with sequence AABBA

\[ I_5^{(A)}(A, A, B, B, A) = IE^{(A)}(A, A, B, B, A) \cdot P(A)^3 \cdot P(B)^2 \] \hspace{1cm} (5.17)

where P(A) and P(B) are the molar fractions of A and B, respectively. The Bernoullian model (random copolymerisation) uses the molar fraction as determined by NMR and the degree of polymerisation as input. The output of the model is the theoretical intensity profile. The Markovian model (non-random copolymerisation) has as input the mass spectrometric relative intensity profile for an oligomer intensity distribution with a degree of polymerisation \( n \). The program searches for the best fit using two variables: the molar fraction of the monomers and the probability to find two monomers A after each other. The probability to find two monomers A after each other can be used to calculate the 'blockiness' of the copolymer.
5.3. Results and discussion

5.3.1. ESI FT-ICR MS spectra of copolyesters

The ESI FT-ICR MS spectrum of the copolyester poly(di-propoxylated bisphenol-A/adipic acid/isophthalic acid) DAI11 is presented in figure 5.1. The polymer molecules observed are sodium cationised and contain two alcohol endgroups \((\text{DA})_m(\text{DA})_m\), one alcohol and one acid endgroup \((\text{DI})_n(\text{DA})_m\) or two acid endgroups \((\text{DI})_n(\text{DA})_m\). Cyclic molecules, \((\text{DA})_n(\text{DA})_m\), were also observed. \(D\) denotes the dialcohol di-propoxylated bisphenol-A, \(A\) and \(I\) the diacids adipic acid and isophthalic acid, respectively, \(n\) and \(m\) the degree of polymerisation and \(c\) denotes cyclic structure of the molecules.

![Figure 5.1](image)

**Figure 5.1** ESI FT-ICR MS spectrum of DAI11 (1 mg/ml, 5 mM NaI, sprayed in THF). The insert shows the intensity profile of the tetramer with one alcohol and one acid endgroup.

The insert of figure 5.1 shows a typical intensity profile of the composition of the tetramer with one alcohol and one acid endgroup. Bernoullian and Markovian chain statistics were used to fit the oligomer intensity profile in the mass spectra using the model described earlier and first applied to copolymer mass spectra by Montaudo et al. Here we have determined separately the
copolymers as a function of the degree of polymerisation and the charge state for each endgroup class.

5.3.2. Copolymerisation statistics applied to mass spectra

The experimentally observed intensities, denoted by an open square, of the tetramer with two acid endgroups electrosprayed in acetone is plotted in figures 5.2a-c for three copolymers with different molar ratios of adipic and isophthalic acid. The intensity profiles in figures 5.2a-c originate from copolymers containing a molar ratio of 3:1, 1:1 and 1:3 adipic(A):isophthalic acid(I) denoted by DAI31, DAI11 and DAI13, respectively. The oligomer intensities presented in figures 5.2a-c correspond with A(DA)_3, A(DA)_2(DI), A(DA)(DI)_2, A(DI)_3 and I(DI)_3. The measured m/z values and normalised intensities are presented in table 5.2. Random copolymerisation (Bernoullian) chain statistics were used to calculate the theoretical intensity profile (denoted by a closed circle •). The molar fraction of adipic acid and isophthalic acid in the bulk determined by NMR were used for the calculations. The results of the Bernoullian calculations are presented in table 5.2. It can be seen from figures 5.2a-c that the intensity profile (open squares) observed with mass spectrometry and the intensity profile as calculated by random copolymerisation statistics • do not agree. In all cases the experimental intensity profiles are shifted in favour of adipic acid. A better agreement between experiment and the Bernoullian intensity profile for copolymer DAI11 is obtained when ~10% more adipic acid (A) is used in the calculation. The calculated molar fractions of adipic acid and isophthalic acid P(A) and P(I), presented in table 5.2, suggest that the copolymers contain more adipic acid than determined by NMR. The disagreement between the intensity profiles obtained with mass spectrometry and the Bernoullian calculation is more prominent for copolymers with a high molar ratio of isophthalic acid.
Figure 5.2  Experimental relative intensity profiles (open square with dotted line) of the tetramers with two acid endgroups for the copolyesters DAI31 (a), DAI11 (b) and DAI13 (c) sprayed in acetone. The numbers denote the molar ratio of adipic acid and isophthalic acid. The figures also display the theoretical Bernoullian (○) and Markovian intensity profiles (▲).

A better agreement between experiment and theory is obtained using Markovian chain statistics, denoted in figure 5.2 by a closed triangle ▲, resulting in a calculated molar fraction of adipic acid \( P(A) \) that is ~10% higher as determined by NMR\(^{213,214}\) for the copolymer DAI11. This is similar to the results of the Bernoullian model, but with a slight block formation of the monomers. The calculated molar fraction adipic acid and isophthalic acid with the Markovian chain statistics is presented in Table 5.2. Oligomers with another degree of polymerisation and with other endgroups give similar results compared to those obtained from the analysis of the tetramer with two acid endgroups. When the ESI experiments are performed in THF under the same experimental conditions approximately 5% more adipic acid is found with the Markovian calculation (results not shown).

The average molar fraction of adipic acid determined by NMR is plotted in figures 5.3a-b as a function of the molar fraction determined by a Markovian fit of the ESI FT-ICR MS spectrum (sprayed in THF). The curves represent the oligomers of five copolyesters with two alcohol endgroups in charge states 1 and 2, respectively. The molar fraction of adipic acid as calculated with the Markovian
Ionisation efficiency in Electrospray Ionisation model increases with increasing charge state and decreases with increasing degree of polymerisation.

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<th>A(DI)_3</th>
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Table 5.2  Experimentally observed m/z (open square) and intensity profile for the tetramer with two acid endgroups. The experimentally observed intensity profile has been used for the calculation of the molar ratio of adipic P(A) and isophthalic acid P(I) with Bernoullian (+) and Markovian (▼) chain statistical models. The molar ratio of adipic and isophthalic acid determined by NMR has also been used for the calculation of the Bernoullian theoretical intensity profile.

The ~10% higher molar fraction of adipic acid as calculated with the Bernoullian and Markovian models for the copolysters can be explained in two ways. One possibility is that the molecular weight distribution is chemically inhomogeneous, a feature that cannot be determined by NMR since it is impossible to make a distinction between the different polymer molecules. In that case the low part of the MWD would contain more adipic acid than isophthalic acid, while the high part of the MWD would contain more isophthalic acid than adipic acid. Another explanation is that the ionisation efficiency of adipic acid is higher than isophthalic acid in the ESI process. This will result in a shift of the intensity profile in favour of adipic acid.
Figure 5.3 Molar fraction of adipic acid determined by NMR versus molar fraction as determined by the Markovian fit of the ESI FT-ICR MS intensity profiles of the oligomers with two alcohol endgroups sprayed in THF for charge states 1 (a) and 2 (b). Degrees of polymerisation shown are n=3 (Δ), 4 (×), 6 (●) and 7 (●).

The chemical inhomogeneity of the copolymer MWD can only be studied quantitatively with ESI FT-ICR MS if the relative ionisation efficiency of the individual components in the MWD is known. Therefore the remaining part of this chapter will focus on the relative ionisation efficiency of adipic and isophthalic acid in the ESI process. Quantification of the oligomers with Gradient polymer elution chromatography (GPEC) is required to test for the presence of relative ionisation efficiency differences.

5.3.3. Polyester quantification with GPEC

GPEC was applied using an UV diode array detector for quantification and online ESI TOF for identification of the oligomers in the MWD. GPEC is based on a combination of precipitation/redissolution, sorption and exclusion processes. The technique requires a gradient LC system with at least two solvents. One of the solvents, which is often a bad solvent for the polymer of interest, promotes the precipitation of the polymer after injection on the column. The other solvent is a good solvent that enables the redissolution of the polymer. Because most components from the MWD have different solubilities, the components will not redissolve at the same time. The LC column enhances the separation of the eluting molecules. Oligomers of various homo-polyesters were endgroup separated with this technique up to the 7-mer (~3500 Da). The technique fails to separate larger homo-oligomers due to smaller differences in
solubility and hydrodynamic volumes. Quantification of copolymers with GPEC is difficult because the number of oligomers with different compositions increases dramatically with increasing degree of polymerisation \( n \), which results in co-elution.

The GPEC chromatogram of DAI31 is presented in figure 5.4a. The oligomers are endgroup separated to \( n=2 \). This limited amount of information does not allow the quantification of the oligomers in the MWD, which is a requirement for the determination of the relative ionisation efficiency (IE) in the ESI process. Therefore, we did not further consider the GPEC separations of the copolyesters.

The homopolymesters poly(di-propoxylated bisphenol-A/adipic acid) (polyDA) and poly(di-propoxylated bisphenol-A/isophthalic acid) (polyDI) are better candidates for IE studies because the low molecular weight part of the MWD can be separated with GPEC (see figure 5.4b and c). The oligomers in the MWD are endgroup separated up to \( n=6 \) and \( n=7 \) for polyDA and polyDI, respectively, providing quantitative information.

Figure 5.4  Gradient polymer elution chromatograms of polyDAI (a), polyDA (b) and polyDI (c).
5.3.4. Electrospray ionisation efficiency (IE)

Mixtures of the homopolyesters were analysed to determine whether differences in IE between the adipic acid and isophthalic acid structures occur in ESI. The concentration of one of the polymers was held constant while the concentration of the other one was increased. The intensity ratio of oligomers from polyDA:polyDI in the mass spectra should vary proportionally with the molar ratio of homopolyesters present in the sample, when differences in IE are absent. Differences in the relative IE between adipic acid and isophthalic acid containing polyesters should become apparent when comparing structurally similar oligomers. For example, (DA)$_3$ and (DI)$_3$ are of the same degree of polymerisation and both have one acid and one alcohol endgroup. If we compare (DA)$_3$ and (DI)$_3$ the relative IE is only influenced by the adipic and isophthalic acid structures. It is assumed that the difference between the acid endgroups does not influence the IE.

The ESI FT-ICR MS spectra of polyDA and polyDI show polymer molecules up to a degree of polymerisation of 14 in charge states 1-4 (spectra not shown). The polymer molecules are isotopically resolved with a resolution of 20,000 at m/z 1.000 (128kB data points, bandwidth 500 kHz). The $M_w$ measured with SEC is approximately 3000 Da higher than the $M_w$ measured with ESI. This demonstrates that the low part of the MWD is preferably ionised resulting in a discrimination against the higher molecular weight polymer molecules (see chapter 3). The comparison of the intensity of an oligomer with, for example, $n=2$ with an oligomer of $n=5$, is therefore not performed because they are ~1500 Da separated, for the polymers studied in this chapter. A difference in the response between these oligomers will be influenced by the size of the molecule and the chemical composition. The influence of the size of the oligomers is negligible if we assume that the intensity of an oligomer of $n=3$ of polyDA can be compared with an oligomer of $n=3$ of polyDI since they are separated by only 3·19.97 Da. Note that the $M_w$ measured with SEC is based on a calibration of the SEC system with polystyrene samples. The real $M_w$ is approximately 5000 Da.

The peak intensities in the ESI FT-ICR MS spectra will correlate with the number fraction $n(M)$ of a certain oligomer with molar mass M if all oligomers have the same IE. The number fraction according to mass spectrometry is $n(M)_{DI}=I_{DI}/I_{total}$, in which $I_{DI}$ and $I_{total}$ are the peak intensity of oligomer DI and the integrated intensity of the MWD, respectively. However, the relative IE of the different oligomers during ESI is not known and therefore $n(M)_{DI}$ has only a limited meaning. The ratio $N(M)$ is introduced which allows the comparison of the relative IE of structurally similar oligomers. This ratio is given for the trimer with one acid and one alcohol endgroup by $N(M)_{(DA)3}=I_{(DA)3}/(I_{(DI)3}+I_{(DA)3})$. 

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The GPEC analysis are used to determine the weight fraction \( w(M) \) of a certain oligomer e.g. for DI: \( w(M)_{\text{DI}} = A_{\text{DI}} / A_{\text{total}} \), in which \( A_{\text{DI}} \) and \( A_{\text{total}} \) are the peak areas of DI and of the entire molecular weight distribution (MWD), respectively. The number ratio \( N(M) \) determined with ESI FT-ICR MS must be converted into the weight ratio \( W(M) \) to compare the ESI results with GPEC. The weight ratio is calculated for ESI FT-ICR MS by

\[
W(M)_{\text{ESIFTMS}} = \frac{I_{(DA)n} \cdot M_{(DA)n}}{P(n)_{(DA)n}} \cdot \frac{I_{(DI)n} \cdot M_{(DI)n}}{P(n)_{(DI)n}}
\]

(18)

For GPEC, \( W(M)_{\text{GPEC}} \) is given by

\[
W(M)_{\text{GPEC}} = \frac{w(M)_{(DA)n} \cdot m_{\text{polyDA}}}{w(M)_{(DA)n} \cdot m_{\text{polyDA}} + w(M)_{(DI)n} \cdot m_{\text{polyDI}}}
\]

(19)

where \( m_{\text{polyDA}} \) and \( m_{\text{polyDI}} \) are the amounts of homopolyesters in the mixtures (in gram). In figure 5.5a a plot is presented of \( W(M)_{\text{ESIFTMS}} \) (sprayed in acetone) as a function of \( W(M)_{\text{GPEC}} \) for oligomers with one acid and one alcohol endgroup (n=1-3). Note that each series of peaks corresponds to the concentration dependence (by changing the mixing ratio polyDA:polyDI) of two structurally similar oligomers e.g. DA\(_3\) and DI\(_3\). In the absence of differences in IE a linear relation between \( W(M)_{\text{ESIFTMS}} \) and \( W(M)_{\text{GPEC}} \) with a slope of 1 and intercept of 0 should be obtained (see dotted line in figure 5.5a). However, more adipic acid is detected with ESI FT-ICR MS than is present in the mixture as can be seen by the curved lines in figure 5.5a that all lie above the dotted line. Similar but less dramatic results were observed when THF was used as solvent. This is probably due to the lower permittivity of THF (compared with acetone) resulting in ESI generated droplets with a smaller radius and higher surface area from which ions can desorb more efficiently. These results demonstrate that oligomers with adipic acid are more efficiently ionised than the oligomers with isophthalic acid.
The relative difference in IE has not been quantified here because too many parameters influence the IE.

The relative difference in IE can be attributed to a difference in solvation energy of polyDA and polyDI. If PolyDA has lower solvation energy than polyDI, this will lead to a relatively high surface activity and therefore an efficient ion desorption from the surface of an electrosprayed droplet. Unfortunately the solvation energies of the molecules studied in this work are unknown and an explanation based on a difference in the solvation energy will remain an inference. Several authors have shown a large influence of the surface activity on IE. An additional indication that the solvation energy of polyDA is relatively low is seen by comparison of different charge states. The intensity of PolyDI in charge state 2 has a negligible abundance compared to the intensity of polyDA. PolyDI has not been observed in charge states 3 and 4, although polyDA has been observed in charge states 1-4. This is probably because most charges are located on the surface of the droplets together with polyDA making it more probable for polyDA to be multiply charged than polyDI.

The $W(M)^{ESIFTMS}$ values of the oligomers with two alcohol and two acid endgroups are plotted as a function of $W(M)^{SPEC}$ in figures 5.5b-c, respectively. The results demonstrate that relative differences in IE become larger with an increasing number of acid endgroups. The intensity of the isophthalic acid containing oligomers has almost become zero for the oligomers with two acid endgroups. Differences in IE for the cyclic oligomers are in-between that of the oligomers with two acid endgroups and oligomers with one acid and one alcohol endgroup. The difference in IE increases in favour of adipic acid with increasing degree of polymerisation $n$ as can be seen in figures 5.5a-b.

The increasing difference in IE with increasing number of acid endgroups can be explained by a higher sodium affinity of adipic acid compared to isophthalic acid. Oligomers with two adipic acid endgroups will have a higher chance to interact with the sodium cation than oligomers with one acid and one alcohol endgroup.

The results presented in figures 5.5a-c were performed at relatively high polyDA concentration. To exclude possible concentration effects induced by the high polyDA concentration a new series of experiments was performed. Dilutions of a 1:1 mixture of polyDA and polyDI (w/w) were made in acetone and THF to which 5 mM NaI was added. The stock solution contained 2 mg/ml polyDA and 2 mg/ml polyDI and was diluted to samples with 0.01 – 2 mg/ml of each polymer.
Ionisation efficiency in Electrospray Ionisation

Figure 5.5 $W(M)^{ESI\ FT-MS}$ sprayed in acetone is plotted as a function of $W(M)^{GPEC}$ for the oligomers with one acid and one alcohol endgroup (a), for oligomers with two alcohol endgroups (b) and for oligomers with two acid endgroups (c). Degrees of polymerisation shown are $n=1$ (○), 2 (■), 3 (▲) and 4 (×). The blends contain 0.5-2 mg/ml polymer.

Figure 5.6 shows the molar ratio polyDA:polyDI for the structurally similar dimers observed in the FT-ICR MS spectra sprayed in THF. A molar ratio of 1 indicates that the oligomers in polyDA and polyDI are present in equimolar amounts. The differences in relative IE show similar effects as observed in figures 5.5a-c. As can be seen clearly, the effects decrease significantly at low homopolyester concentration, which points to concentration effects. The measured molar ratio has become lower than one for most of the oligomers at the detection limit of the experiment. This indicates that polyDI is more efficiently ionised at low concentration. Note that this effect can have a different offset when the same mixture is measured with other mass spectrometers. The detection limit was approximately 3 fmol for A(DA)$_2$ ($m/z$ 1077) in the electrospray source not taking losses due to ion transport into account. The strongest concentration effects were observed for the oligomers with two acid endgroups A(DA)$_n$ and I(DI)$_n$ ($n=1-2$).
sprayed in acetone (results not shown). The molar ratio $A(DA)_n/I(DI)_n$ was 2.5 at 0.01 mg/ml and increased to 60 at 2 mg/ml. It is unclear why the relative IE becomes larger for polyDI, or lower for polyDA, at concentrations near the detection limit.

![Graph showing molar ratio of polyDA to polyDI for different concentrations](image)

Figure 5.6  Molar ratio polyDA and polyDI for the structurally similar dimers observed in the spectra sprayed in THF. The mixtures contain 1:1 polyDA and polyDI (w/w). The 1 on the x-axis corresponds to a mixture of 1 mg/ml polyDA and 1 mg/ml polyDI. The symbols denote $(DA)_2/(DI)_2$ (●), $(DA)_2D/(DI)_2D$ (■), $A(DA)_2/I(DI)_2$ (▲) and cyclics $(DA)_3/(DI)_2$ (※).

### 5.4. Conclusions

Our results demonstrate that differences in IE between the monomeric structures of copolymers can influence the copolymer composition as measured with electrospray ionisation mass spectrometry. The concentration and nature of the solvent influence the relative IE of the oligomer ions in the ESI process. Other important parameters that may influence the ionisation efficiency of co-oligomers are the chemical composition (i.e. the number of A’s and B’s in the oligomers), the sequence of the monomers, the polydispersity (D) but also spray conditions (SC) and instrumental parameters (IP). To deal with some of these variables, the ionisation efficiency $IE^{(M)}(\tau_1, \tau_2,...,\tau_{M-1}, \tau_M)$ was introduced in equation (5.12). When all parameters are taken into account that can influence the ionisation efficiency, the ionisation efficiency should in principle be written as
Ionisation efficiency in Electrospray Ionisation

\[ IE^{(M)}(D,SC,IP)(\tau_1,\tau_2,\ldots,\tau_{M-1},\tau_M) \]

It will however be very difficult to determine the \[ IE^{(M)}(D,SC,IP)(\tau_1,\tau_2,\ldots,\tau_{M-1},\tau_M) \] for each component separately but should be performed for a good characterisation of the copolymer chemical composition and sequence distribution using ESI (FT-ICR) MS.

Differences in IE have also been demonstrated for the copolymer poly(ethylene glycol/propylene glycol) studied with MALDI by Chen and coworkers. The study of Chen and the work presented here demonstrate that the statistical copolymerisation models can only be used to model the mass spectra successfully when differences in IE have been quantified. Hence, earlier published copolymer sequence studies have become questionable.