Quantitative Analysis of Copolymers: Influence of the Structure of the Monomer on the Ionization Efficiency in Electrospray Ionization FTMS

Sander Koster,[†,‡] Bela Mulder,[†] Marc C. Duursma,[†] Jaap J. Boon,[†] Harry J. A. Philipsen,[§] Jan W. v. Velde,[∥] Michel W. F. Nielen,[⊥] Chris G. de Koster,[#,○] and Ron M. A. Heeren*[,†]

Unit for Macromolecular Mass Spectrometry, FOM-Institute for Atomic and Molecular Physics, Kruislaan 407, 1098 SJ, Amsterdam, The Netherlands; Océ Technologies, P.O. Box 101, 5900MA Venlo, The Netherlands; Akzo Nobel Chemicals Research Arnhem, P.O. Box 9300, 6800SB Arnhem, The Netherlands; RIKILT, P.O. Box 230, 6700AE Wageningen, The Netherlands; and DSM Research, P.O. Box 18, 6160MD Geleen, The Netherlands

Received July 16, 2001; Revised Manuscript Received March 26, 2002

ABSTRACT: The influence of the ionization efficiency on the measured copolymer sequence distribution is presented. Large differences in ionization efficiency were observed for mixtures of homopolyesters 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 end groups in favor of adipic acid containing oligomers. The ESI response of the oligomers depends on the polymer concentration in the sprayed mixture. This makes it impossible to correct for the ionization efficiency necessary for copolymer analysis. If differences in ionization 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 cationization techniques like MALDI and FAB.

Introduction

Copolymers consist of a complex mixture of molecules that differ in size, chemical composition and sequence. Complete characterization of such complex materials demands new analytical approaches. A technique that has become increasingly popular for copolymer analysis during the last 10 years is mass spectrometry. Soft ionization techniques like matrix-assisted laser desorption ionization (MALDI) and electrospray ionization (ESI) have been successfully used to ionize intact oligomers,[1−6] which made it possible to study the chemical composition distribution of the copolymer directly from the mass spectrum.[7−11] Accurate end group and monomer masses have been obtained using MALDI and ESI coupled to TOF and FTICR-MS instruments.[12−15]

Two mass spectrometric methods are being used to obtain information about the copolymer sequence. The first method is based on MS/MS experiments, which allows determining whether the copolymer has a random or block sequence.[16−19] This approach fails for oligomers with a low degree of polymerization because of the absence of sequence specific fragments in the MS/MS spectra.[18] A further drawback of this approach is that only block and random sequences can be distinguished. It is not possible to demonstrate the partial block/partial random structure of statistical copolymers.

The second method for copolymer sequence analysis overcomes this drawback and provides information about random, block, and partially block/partially random sequences. This method is based on Bernoullian and Markovian chain statistics applied to the intensity profile of the oligomer distribution.[20−23] The Bernoullian chain statistics are used to model random copolymerization reactions. Markovian statistics can be used if the deviation between the Bernoullian statistics and experimental oligomer distribution is large. Markovian statistics allow the modeling of both random and nonrandom copolymerization reactions. Nonrandom polymerization reactions in step (radical) polymerization normally result in the formation of block copolymers.

The chain statistical models rely on a uniform response i.e., an equal ionization 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 ionization 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 ionized less easily.[24−30] Other effects that influence the ionization efficiency of an analyte are the polarity of the solvent, the structure of the ion, i.e., the organic functionalities in the molecule and the flexibility of the chain for example cyclic vs linear oligomers, pH,

* Corresponding author. E-mail: heeren@amolf.nl. Fax: (+31) 20 6684106.
[†] FOM-Institute for Atomic and Molecular Physics.
[‡] Present address, Institute of Microtechnology, University of Neuchâtel, Rue Jacquet-Droz 1, P.O. Box 3, CH-2007, Neuchâtel, Switzerland.
[§] Océ Technologies.
[∥] Akzo Nobel Chemicals Research Arnhem.
[⊥] RIKILT.
[#] DSM Research.
[○] Present address, University of Amsterdam, Swammerdam Institute Of Life Sciences, Nieuwe Achtergracht 129, 1018WS Amsterdam, The Netherlands.

10.1021/ma011234l CCC: $22.00
Published on Web 05/24/2002

and the nature of the cation,[31,32] charge state, counterion effects,[33] drying gas flow rate, and the ESI source temperature (selective nozzle skimmer ion activation).[34−39] Several studies have been reported in the literature in which ionization efficiencies of mixtures of different cyclic compounds like crown ethers and peptides with a variety of cations were studied using FABMS,[40,41] MALDIMS,[42] and ESIMS.[26,32,42−48] Most studies indicate that a good correlation exists between the selectivity of analytes toward metal ions in the liquid phase and the intensity in the mass spectrum which allows a correction for differences in ionization efficiency. Synthetic polymer mass spectrometric studies carried out sofar do not take the ionization 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 difficult to quantify with techniques like NMR and LC. Besides, the determination of the ionization efficiency of all individual oligomers would be a very laborious process.

In this paper Bernoullian and Markovian chain statistics are applied to the ESI FTMS spectra of the copolyester poly(dipropoxylated bisphenol A/adipic acid/isophthalic acid). The copolymer composition determined with the chain statistics will be different from the real copolymer composition if differences in ionization 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(dipropoxylated bisphenol A/adipic acid) and poly(dipropoxylated 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 FTMS.

Materials and Methods

ESI FTMS Analysis. The ESI FTICR-MS (Bruker-Spectrospin APEX 7.0e, Fällanden, Switzerland) used in this work has been described earlier.[15,49,50] The cell is an in-house constructed open cell.[51] The electrospray generated ions were trapped for 2−4 s. Argon gas is introduced through a pulsed valve to enhance trapping ($P_{Ar} = 5 \times 10^{-6}$ mbar). Only the total ion intensity was influenced by the trapping time. The relative intensity profile of the ions observed in the mass spectra was not affected.

The monoisotopic peaks of the oligomers were used throughout this paper in the copolymerization models and figures. All intensities of the monoisotopic peaks were corrected for the number of carbon atoms described in eq 18 and ref 11 because the intensity of the monoisotopic peak decreases with increasing number of carbon atoms.

GPEC ESI TOF Measurements. GPEC measurements were performed at Akzo Nobel Chemicals Research as described elsewhere.[52] The Hewlet Packard LC system model 1050 (Palo Alto, CA) was operated with an autoinjector model Basic Marathon (Spark Holland, Emmen, The Netherlands) with a loop volume of 10 μL. Solution A contained unstabilized THF:H_2O 5:95 and solution B unstabilized THF:H_2O 95:5 (v/v). To both solutions was added 0.1% glacial acetic acid. The applied gradient started with 45% of solution A at $t = 0$ to 10% of solution A at $t = 35$ min with a flow of 1 mL/min. The column was a Waters Symmetry C18 (5 μm particle size), 150 mm × 4.6 mm i.d.. Approximately 30 μL/min of the eluent from the column was split with a 0.01 in. i.d. stainless steal T-piece (Valco, Houston, TX) that was allowed to flow to the Z-spray TOF instrument (Micromass, Manchester, UK). The eluent stream was compatibilised with a NaI solution (10 μL/min of a 250 μM NaI solution in THF/isopropyl alcohol). The remaining eluent was eluted through the UV diode array detector model 1000S (Applied Biosystem, San Jose, CA).

Materials. The unstabilized THF was obtained from Fluka (Buchs, Switzerland). The polymers used for this study are the homopolyesters poly(dipropoxylated bisphenol A/adipic acid) and poly(dipropoxylated bisphenol A/isophthalic acid) and the copolyester poly(dipropoxylated bisphenol A/adipic acid/isophthalic acid). These polymers were a gift from Océ Technologies (Venlo, The Netherlands) and are from the same batch as the polyesters described in a previous publication.[53] Dipropoxylated bisphenol A, adipic acid, and isophthalic acid will be abbreviated by D, A, and I throughout the paper, respectively. The copolyesters studied have the compositions DAI31, DAI11, and DAI13, where the numbers denote the molar ratio of adipic acid and isophthalic acid. DAI31, DAI11, and DAI13 contain molar ratios D:A:I of 0.5:0.37:0.13, 0.5:0.26:0.24, and 0.51:0.12:0.37, respectively as was determined with NMR. These results correspond to the molar ratios of the monomers used for the preparation of the polymers. Since identification of the oligomers was not performed using mass spectrometry,[53] the GPEC experiments were repeated with online ESI TOF for identification.

Copolymerization Models. The copolymerization models and their links with mass spectrometry have first been used by Montaudo and co-workers.[20,21] 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 end group class separately and subsequently as a function of the degree of polymerization and charge state. For example, the composition of the pentamer with two alcohol end groups 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 polymerization, end group class and charge state. The Markovian and Bernoullian models used were implemented in Matlab 5.3 (The MathWorks, Inc.) and are described below.

(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 $\tau = A, B, ...$ Each oligomer consists of $M = 1, 2, 3, ...$ monomers whose position within the chain is labeled by lowercase letters $m, k, l, n, ...$ 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 $P^{(M)}(\tau_1,\tau_2,...,\tau_M)$. Let us further define the probability of finding a certain initial segment of length m in a polymer of total length M.

$$P_m^{(M)}(\tau_1,\tau_2,...,\tau_m) = \sum_{\tau_{m+1},...,\tau_M} P^{(M)}(\tau_1,\tau_2,...,\tau_M) \qquad (1)$$

The Markovian assumption now states that the conditional probability of finding the next monomer τ_{m+1} of a chain to be of a certain type only depends on the type of the immediately preceding monomer τ_m, i.e.

$$P_{m+1}^{(M)}(\tau_{m+1}|\tau_1,\tau_2,...,\tau_m) = \frac{P_{m+1}^{(M)}(\tau_1,\tau_2,...,\tau_m,\tau_{m+1})}{P_m^{(M)}(\tau_1,\tau_2,...,\tau_m)} = P(\tau_m \rightarrow \tau_{m+1}) \qquad (2)$$

where we adopt the arrow notation to stress the order of the two monomers in the sequence. Each probability $P(\tau_m \rightarrow t_{m+1})$ corresponds to a matrix element P_{ij} from the probability matrix **P** as described by Montaudo and co-workers.[20,21] Note that

$$\sum_{\tau'} P(\tau \rightarrow \tau') = 1 \qquad (3)$$

Under this assumption, we find that

$$P_M^{(M)}(\tau_1,\tau_2,...\tau_{M-1},\tau_M) = P_{M-1}^{(M)}(\tau_1,\tau_2,...,\tau_{M-1})P(\tau_{M-1}\rightarrow\tau_M) \quad (4)$$

which upon iteration yields

$$P_M^{(M)}(\tau_1,\tau_2,...\tau_{M-1},\tau_M) = P_1^{(M)}(\tau_1)\prod_{m=2}^{M}P(\tau_{m-1}\rightarrow\tau_m) \quad (5)$$

The probability $P_1^{(M)}(\tau_1)$ is the probability that an oligomer starts with τ_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

$$\begin{aligned}P_M^{(M)}(\tau_1,\tau_2,...\tau_{M-1},\tau_M) &= P_M^{(M)}(\tau_M,\tau_{M-1},...\tau_2,\tau_1)\\ &= P_{M-1}^{(M)}(\tau_M,\tau_{M-1},...,\tau_2)P(\tau_2\rightarrow\tau_1)\end{aligned} \quad (6)$$

or

$$P_M^{(M)}(\tau_1,\tau_2,...\tau_{M-1},\tau_M) = P_1^{(M)}(\tau_M)\prod_{m=2}^{M}P(\tau_m\rightarrow\tau_{m-1}) \quad (7)$$

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., $P_1^{(M)}(\tau_1) = P_1(\tau_1)$ so that eq 6 simplifies to

$$P_M^{(M)}(\tau_1,\tau_2,...\tau_{M-1},\tau_M) = P_{M-1}^{(M-1)}(\tau_M,...,\tau_3,\tau_2)P(\tau_2\rightarrow\tau_1) \quad (8)$$

Note that by definition

$$\sum_\tau P_1(\tau_1) = \sum_\tau P_1(\tau_M) = 1 \quad (9)$$

This relation can be used to obtain a self-consistency condition on $P_1(\tau_1)$ (or $P_1(\tau_M)$) as follows

$$\begin{aligned}P_1(\tau_1) &= \sum_{\tau_2,...,\tau_M} P_M^{(M)}(\tau_M,\tau_{M-1},...,\tau_2,\tau_1)\\ &= \sum_{\tau_2,...,\tau_M} P_{M-1}^{(M-1)}(\tau_M,\tau_{M-1},...,\tau_2)P(\tau_2\rightarrow\tau_1)\\ &= \sum_{\tau_2}\sum_{\tau_3,...,\tau_M} P_{M-1}^{(M-1)}(\tau_M,\tau_{M-1},...,\tau_2)P(\tau_2\rightarrow\tau_1)\\ &= \sum_{\tau_2} P_1(\tau_2)P(\tau_2\rightarrow\tau_1)\end{aligned} \quad (10)$$

This equation together with the two normalization constraints shown in eqs 9 and 3 allows $P_1(\tau_1)$ to be expressed in terms of the transition probabilities $P(\tau_m\rightarrow\tau_{m-1})$.

Consider a copolymer made of monomers A and B. The probability a sequence starts with an A is given by eq 10 or

$$P_1(A) = \sum_{\tau_2} P_1(\tau_2)P(\tau_2\rightarrow\tau_A) = P_1(A)P(A\rightarrow A) + P_1(B)P(B\rightarrow A) \quad (11)$$

Using the notation of Montaudo and co-workers, eq 11 becomes $S_A = S_A P_{AA} + S_B P_{BA}$.

Note that eq 8 is the basis of the Markovian model applied to copolymer mass spectra. By correlating this equation to the measured intensity $I_M^{(M)}(\tau_1,\tau_2,...\tau_{M-1},\tau_M)$ in a mass spectrum, eq 8 becomes

$$I_M^{(M)}(\tau_1,\tau_2,...\tau_{M-1},\tau_M) = \mathrm{IE}^{(M)}(\tau_1,\tau_2,...\tau_{M-1},\tau_M)P_1^{(M)}(\tau_1)\prod_{m=2}^{M}P(\tau_{m-1}\rightarrow\tau_m) \quad (12)$$

where $\mathrm{IE}^{(M)}(\tau_1,\tau_2,...\tau_{M-1},\tau_M)$ is the ionization efficiency of the co-oligomer 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

$$I_5^{(5)}(A,A,B,B,A) = \mathrm{IE}^{(5)}(A,A,B,B,A)P_1^{(5)}(A)P(A\rightarrow A)P(A\rightarrow B)P(B\rightarrow B)P(B\rightarrow A) \quad (13)$$

The ionization efficiency $\mathrm{IE}^{(M)}(\tau_1,\tau_2,...\tau_{M-1},\tau_M)$ has until now always assumed to be 1.[20,21] This paper will demonstrate that this assumption is not valid.

The models used by Montaudo and co-workers assume that the probability a chain starts with τ_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 $1 \le m \le M$ within the chain is of a certain type

$$\begin{aligned}P_m(\tau_m) &= \sum_{\tau_1,\tau_2,...,\tau_{m-1}\tau_{m+1},\tau_{m+2},...,\tau_M}\ \sum P^{(M)}(\tau_1,\tau_2,...,\tau_M)\\ &= \sum_{\tau_1,\tau_2,...,\tau_{m-1}\tau_{m+1},\tau_{m+2},...,\tau_M}\ \sum P_1(\tau_1)\prod_{i=1}^{M-1}P(\tau_i\rightarrow\tau_{i+1})\\ &= \sum_{\tau_1,\tau_2,...,\tau_{m-1}} P_1(\tau_1)\prod_{i=1}^{m-1}P(\tau_i\rightarrow\tau_{i+1}) \times \sum_{\tau_{m+1},\tau_{m+2},...,\tau_M}\prod_{j=m}^{M-1}P(\tau_j\rightarrow\tau_{j+1})\\ &= \sum_{\tau_2,\tau_3,...,\tau_{m-1}} P_1(\tau_2)\prod_{i=1}^{m-1}P(\tau_i\rightarrow\tau_{i+1}) \times \sum_{\tau_{m+1},\tau_{m+2},...,\tau_{M-1}}\prod_{j=m}^{M-2}P(\tau_j\rightarrow\tau_{j+1})\\ &= \{\text{repeat the same steps}\}\\ &= P_1(\tau_m)\end{aligned} \quad (14)$$

where we repeatedly employ eqs 3 and 10. As the position *m* is arbitrary we can simply define the probability that an arbitrary monomer has a given type to be $P(\tau) = P_m(\tau) = P_1(\tau)$

(2) Bernoullian Chain Statistical Model. In the 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

$$\boldsymbol{P}(\tau_m\rightarrow\tau_{m-1}) = P(\tau_m) \quad (15)$$

The intensity of an ion in the mass spectrum becomes

$$I_M^{(M)}(\tau_1,\tau_2,...\tau_{M-1},\tau_M) = \mathrm{IE}^{(M)}(\tau_1,\tau_2,...\tau_{M-1},\tau_M)\prod_{m=1}^{M}P(\tau_m) \quad (16)$$

or for the oligomer with sequence AABBA

$$I_5^{(5)}(A,A,B,B,A) = \mathrm{IE}^{(M)}(A,A,B,B,A)P(A)^3P(B)^2 \quad (17)$$

The Bernoullian model (random copolymerization) uses the molar fraction as determined by NMR and the degree of polymerization as input. The output of the model is the theoretical intensity profile. The Markovian model (nonrandom copolymerization) has as input the mass spectrometric relative intensity profile for an oligomer intensity distribution with a degree of polymerization *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.

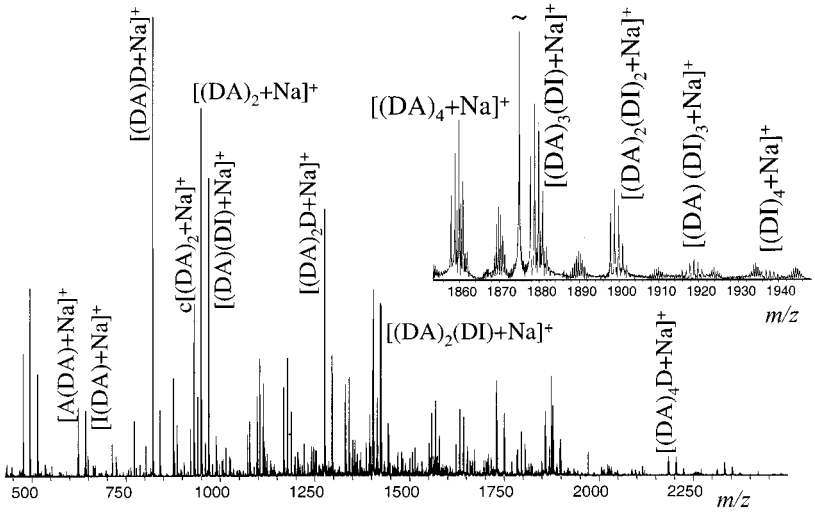

Figure 1. ESIFTMS spectrum of DAI11 (1 mg/mL, 5 mM NaI, sprayed in THF). The inset shows the intensity profile of the tetramer with one alcohol and one acid end group. ~ denotes electronic noise.

The probability to find two monomers A after each other can be used to calculate the "blockiness" of the copolymer.[20,21]

Results and Discussion

ESI FTMS Spectra of Copolyesters. The ESI FTMS spectrum of the copolyester poly(dipropoxylated bisphenol A/adipic acid/isophthalic acid) DAI11 is presented in Figure 1. The oligomers observed are sodium cationized and contain two alcohol end groups $(DI)_n(DA)_mD$, one alcohol and one acid end group $(DI)_n(DA)_m$ or two acid end groups $A(DI)_n(DA)_m$, $I(DI)_n(DA)_m$. Cyclic oligomers, $c(DI)_n(DA)_m$, were also observed. D denotes the dialcohol dipropoxylated bisphenol A, A and I denote the diacids adipic acid and isophthalic acid, respectively, *n* and *m* denote the degree of polymerization, and c denotes the cyclic structure of the oligomers.

The inset of Figure 1 shows a typical intensity profile of the composition of the tetramer with one alcohol and one acid end group. 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.[20,21] Here we have determined separately the copolymer composition as a function of the degree of polymerization and the charge state for each end group class.

Copolymerization Statistics Applied to Mass Spectra. The experimentally observed relative intensities, denoted by a closed triangle (▲), of the tetramer with two acid end groups electrosprayed in acetone is plotted in Figure 2, parts a−c for three copolymers with different molar compositions of adipic and isophthalic acid. The intensity profiles in Figure 2, parts a−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 Figure 2, parts a−c correspond to $A(DA)_3$, $A(DA)_2(DI)$, $A(DA)(DI)_2$, $A(DI)_3$, and $I(DI)_3$. The measured *m*/*z* values and normalized intensities are presented in Table 1. Random copolymerization (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 1. It can be seen from Figure 2, parts a−c that the intensity profile (▲) observed with mass spectrometry and the intensity profile as determined by random copolymerization statistics (●) do not agree at all. In all cases, the experimental intensity profiles are shifted in favor 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 1, 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.

A better agreement between experiment and theory is obtained using Markovian chain statistics, denoted by a closed square (■), resulting in a calculated molar fraction of adipic acid *P*(A) that is ~10% higher as determined by NMR 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 are presented in Table 1. Oligomers with another degree of polymerization and with other end groups give similar results compared to those obtained from the analysis of the tetramer with two acid end groups. 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 Figure 3, parts a and b as a function of the molar fraction determined by a Markovian fit of the ESI FTMS spectrum (sprayed in THF). The curves represent the oligomers of five copolyesters with two alcohol end groups in charge states 1 and 2, respectively. The molar fraction of adipic acid as calculated with the Markovian model increases with increasing charge state and decreases with increasing degree of polymerization.

The ~10% higher molar fraction of adipic acid as calculated with the Bernoullian and Markovian models for the copolyesters (sprayed in acetone) can be explained in two ways. One possibility is that the molec-

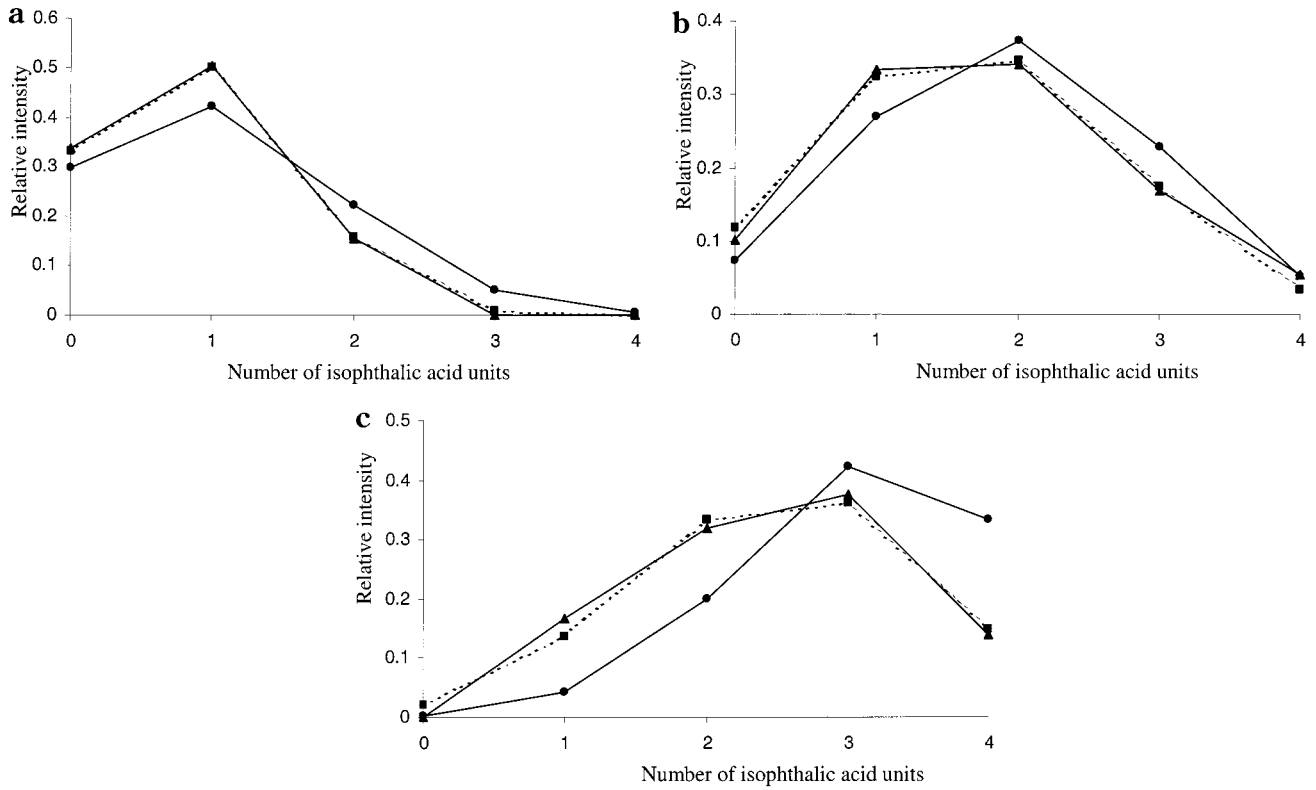

Figure 2. Experimental relative intensity profiles (▲) of the tetramers with two acid end groups for the copolyesters DAI31 (a), DAI11 (b), and DAI13 (c) sprayed in acetone. The numbers denote the molar ratio of adipic acid and isophthalic acid. The figures also display the Bernoullian (●) and Markovian theoretical intensity profiles (■ with dotted line).

Table 1. Experimentally (▲) Observed *m/z* and Intensity Profile for the Tetramer with Two Acid End Groups[a]

		m/z					calcd molar fraction	
		$A(DA)_3$	$A(DA)_2(DI)$	$A(DA)(DI)_2$	$A(DI)_3$	$I(DI)_3$	*P*(A)	*P*(I)
measured		1531.754	1551.723	1571.691	1591.660	1611.629		
NMR	DAI31						0.74	0.26
	DAI11						0.52	0.48
	DAI13						0.24	0.76
experimental (▲)	DAI31	0.339	0.505	0.156	0.000	0.000		
	DAI11	0.101	0.335	0.342	0.168	0.054		
	DAI13	0.000	0.166	0.320	0.376	0.138		
Bernoullian theory with NMR data (●)	DAI31	0.300	0.421	0.222	0.052	0.005		
	DAI11	0.073	0.270	0.374	0.230	0.053		
	DAI13	0.003	0.042	0.200	0.421	0.334		
Bernoullian fit with ~10% more A	DAI31	0.355	0.420	0.186	0.037	0.003	0.772	0.228
	DAI11	0.113	0.328	0.356	0.172	0.031	0.580	0.420
	DAI13	0.022	0.140	0.336	0.359	0.144	0.384	0.616
Markovian fit (■)	DAI31	0.333	0.501	0.159	0.007	0.000	0.790	0.210
	DAI11	0.119	0.324	0.348	0.174	0.034	0.580	0.420
	DAI13	0.021	0.136	0.333	0.362	0.148	0.380	0.620

[a] The experimentally observed intensity profile has been used for the calculation of the molar ratio of adipic and isophthalic acid 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.

ular weight distribution is chemically inhomogeneous, a feature that cannot be determined by NMR since it is impossible to make a distinction between the different oligomers. 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 ionization efficiency of adipic acid is higher than isophthalic acid in the ESI process. This will result in a shift of the intensity profile in favor of adipic acid.

The chemical inhomogeneity of the copolymer MWD can only be studied quantitatively with ESI FTMS if the relative ionization efficiency of the individual components in the MWD is known. Therefore, the remaining part of this paper will focus on the relative ionization efficiency of adipic and isophthalic acid in the ESI process. Quantification of the oligomers with gradient polymer elution chromatography (GPEC) is required to test the ionization efficiency.

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.[53,54] 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, results in a precipitation of the polymer after injection

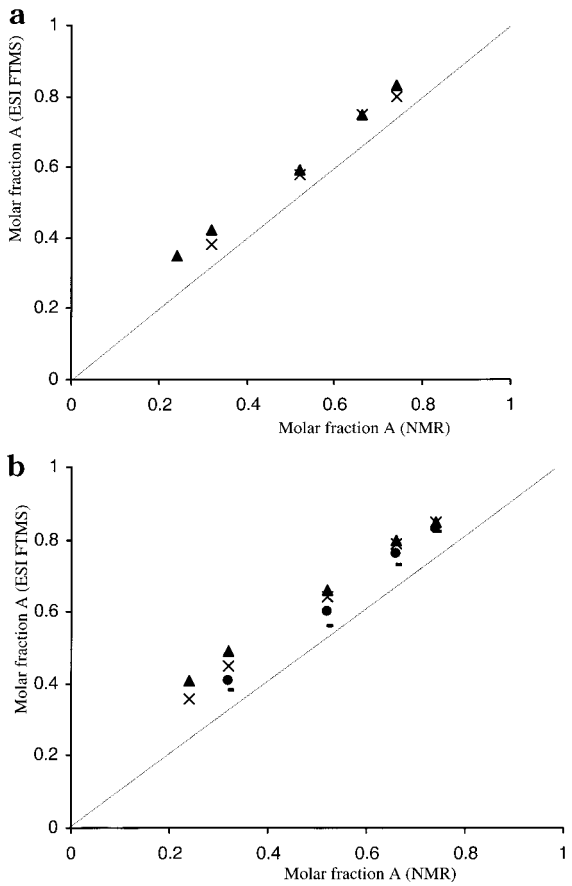

Figure 3. Molar fraction of adipic acid determined by NMR vs molar fraction as determined by the Markovian fit of the ESI FTMS intensity profiles of the oligomers with two alcohol end groups sprayed in THF for charge states 1 (a) and 2 (b). Degrees of polymerization shown are $n = 3$ (▲), 4 (×), 6 (●), and 7 (-).

on the column. The other solvent is a good solvent that enables the redissolvation of the polymer. Because most components from the MWD have different solubilities, the components will not redissolve at the same time while the LC column enhances the separation of the eluting molecules. Oligomers of various homopolyesters were end group separated with this technique up to the 7-mer (~3500 Da).[53] The technique fails to separate larger homooligomers 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 polymerization n, which results in coelution.

The GPEC chromatogram of DAI31 is presented in Figure 4a. The oligomers are end group separated to $n = 2$. This limited amount of information does not allow the quantification of the oligomers in the low molecular weight part of the MWD, which is a requirement for the determination of the relative ionization efficiency (IE) in the ESI process. Therefore, we did not further consider these GPEC measurements.

The homopolyesters poly(dipropoxylated bisphenol A/adipic acid) (polyDA) and poly(dipropoxylated 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 4, parts b and c). The oligomers in the MWD are end group separated up to $n = 6$ and $n = 7$ for polyDA and polyDI, respectively, providing quantitative information.

Electrospray Ionization Efficiency (IE). Mixtures of the homopolyesters were analyzed 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 polymerization and both have one acid and one alcohol end group. If we compare $(DA)_3$ and $(DI)_3$, the relative IE is only influenced by the adipic acid and isophthalic acid structures.

The ESI FTMS mass spectra of polyDA and polyDI show oligomers up to a degree of polymerization of 14 in charge states 1−4 (spectra not shown). The oligomers are isotopically resolved with a resolution of 20000 at m/z 1000 (128kB data points, bandwidth 500 kHz). The M_w measured with size exclusion chromatography is approximately 3000 Da higher than the M_w measured with ESI. This demonstrates that the low part of the MWD is preferably ionized resulting in a discrimination against the higher molecular weight oligomers and/or the detection efficiency is in favor of the lower masses. 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 paper. 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 $n \times 19.97$ Da.[48]

The peak intensities in the ESI FTMS spectra will correlate with the number fraction $n(M)$ of a certain oligomer if all oligomers have the same IE. The number fraction is $n(M)_{DI}=I_{DI}/I_{total}$, in which I_{DI} and I_{total} are the peak intensity of oligomer DI and the total 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 end group by $N(M)_{(DI)_3} = I_{(DI)_3}/(I_{(DI)_3}+I_{(DA)_3})$.

The GPEC measurements are used to determine the weight fraction $w(M)$ of a certain oligomer e.g. for DI: $w(M)_{DI} = A_{DI}/A_{total}$, in which A_{DI} and A_{total} are the peak areas of DI and of the entire molecular weight distribution (MWD), respectively. The number ratio $N(M)$ determined with ESI FTMS must be converted into the weight ratio $W(M)$ to compare the ESI results with GPEC. The weight ratio is calculated for ESI FTMS by $W(M)^{ESIFTMS}_{(DA)_3} = I_{(DA)_3}M_{(DA)_3}/(I_{(DA)_3}M_{(DA)_3} + I_{(DI)_3}M_{(DI)_3})$. However the intensity of the monoisotopic peak decreases with increasing number of carbon atoms. Therefore, the peak intensity in the mass spectrum should be corrected for this effect with $P(n)_{(DA)_n}$ and $P(n)_{(DI)_n}$.[11]

$$W(M)^{\mathrm{ESIFTMS}}_{(\mathrm{DA})_n} = \frac{\dfrac{I_{(\mathrm{DA})_n} M_{(\mathrm{DA})_n}}{P(n)_{(\mathrm{DA})_n}}}{\dfrac{I_{(\mathrm{DA})_n} M_{(\mathrm{DA})_n}}{P(n)_{(\mathrm{DA})_n}} + \dfrac{I_{(\mathrm{DI})_n} M_{(\mathrm{DI})_n}}{P(n)_{(\mathrm{DI})_n}}} \quad (18)$$

For GPEC, $W(M)^{\mathrm{GPEC}}_{(\mathrm{DA})_n}$ is given by

$$W(M)^{\mathrm{GPEC}}_{(\mathrm{DA})_n} = \frac{w(M)_{(\mathrm{DA})_n} m_{\mathrm{polyDA}}}{w(M)_{(\mathrm{DA})_n} m_{\mathrm{polyDA}} + w(M)_{(\mathrm{DI})_n} m_{\mathrm{polyDI}}} \quad (19)$$

where m_{polyDA} and m_{polyDI} are the amounts of homopolyesters in the mixtures (in gram). In Figure 5a, a plot is presented of $W(M)^{\mathrm{ESIFTMS}}_{(\mathrm{DA})_n}$ (sprayed in acetone) as a function of $W(M)^{\mathrm{GPEC}}_{(\mathrm{DA})_n}$ for oligomers with one acid and one alcohol end group ($n = 1-3$) observed in charge state 1. Note that each series of peaks corresponds to the concentration dependence (by changing the mixing ration 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 $W(M)^{\mathrm{ESIFTMS}}$ and $W(M)^{\mathrm{GPEC}}$ with a slope of 1 and intercept of 0 should be obtained (see dotted line in Figure 5a). However, more adipic acid is detected with ESI FTMS than is present in the mixture as can be seen by the curved lines in Figure 5a, which all lie above the dotted line. Similar but less dramatic results were observed in THF. 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, with a different nature, can desorb more efficiently.[55,56] These results demonstrate that oligomers with adipic acid are more efficiently ionized than the oligomers with isophthalic acid. The relative difference in IE has not been quantified here because too many parameters influence the IE (see also further).

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. The solvation energies of the molecules studied in this work are unfortunately 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.[24−29] 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 $W(M)^{\mathrm{ESIFTMS}}$ values of the oligomers with two alcohol and two acid end groups observed in charge state 1 are plotted as a function of $W(M)^{\mathrm{GPEC}}$ in Figure 5, parts b and c, respectively. The results demonstrate that relative differences in IE become larger with an increasing number of acid end groups. The intensity of the isophthalic acid containing oligomers has almost become zero for the oligomers with two acid end groups. Differences in IE for the cyclic oligomers are between that of the oligomers with two acid end groups and oligomers with one acid and one alcohol end group. The difference in IE increases in favor of adipic acid with increasing degree of polymerization n as can be seen in Figure 5, parts a and b.

The increasing difference in IE with increasing number of acid end groups can be explained by a higher sodium affinity of adipic acid compared to isophthalic acid. Oligomers with two adipic acid end groups will have a higher chance to interact with the sodium cation than oligomers with one acid and one alcohol end group as was observed experimentally.

The results described in Figure 5, parts a−c, at low polyDI concentration were performed at relatively high polyDA concentration since the concentration of polyDA was held constant in the experiments. To exclude possible concentration effects induced by the high polyDA concentration a new series of experiments was performed. A series of dilutions of a 1:1 mixture of polyDA and polyDI (w/w) was 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. Figure 6 shows the molar ratio polyDA:polyDI for the structurally similar dimers observed in the FTMS 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 Figure 5, parts a−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 ionized at low concentration. 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 end groups $A(DA)_n$ and $I(DI)_n$ ($n = 1-2$) sprayed in acetone (results not shown). The molar ratio $A(DA)_n/I(DI)_n$ at 0.01 mg/mL was 2.5 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.

Copolymer Sequence Quantification with Mass Spectrometry: A Comment. Our results demonstrate that differences in IE between the monomeric structures of copolymers can influence the copolymer composition as measured with electrospray ionization 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 ionization 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 these variables, the ionization efficiency $\mathrm{IE}^{(M)}(\tau_1,\tau_2,...\tau_{M-1},\tau_M)$ was introduced in eq 12. When all parameters are taken into account that can influence the ionization efficiency, the ionization efficiency should in principle be written as $\mathrm{IE}^{(M)}(D,\mathrm{SC},\mathrm{IP})(\tau_1,\tau_2,...\tau_{M-1},\tau_M)$. It will, however, be very difficult to determine the $\mathrm{IE}^{(M)}(D,\mathrm{SC},\mathrm{IP})(\tau_1,\tau_2,...\tau_{M-1},\tau_M)$ for each component separately but should be performed for a good characterization of the copolymer chemical composition and sequence distribution. Better results

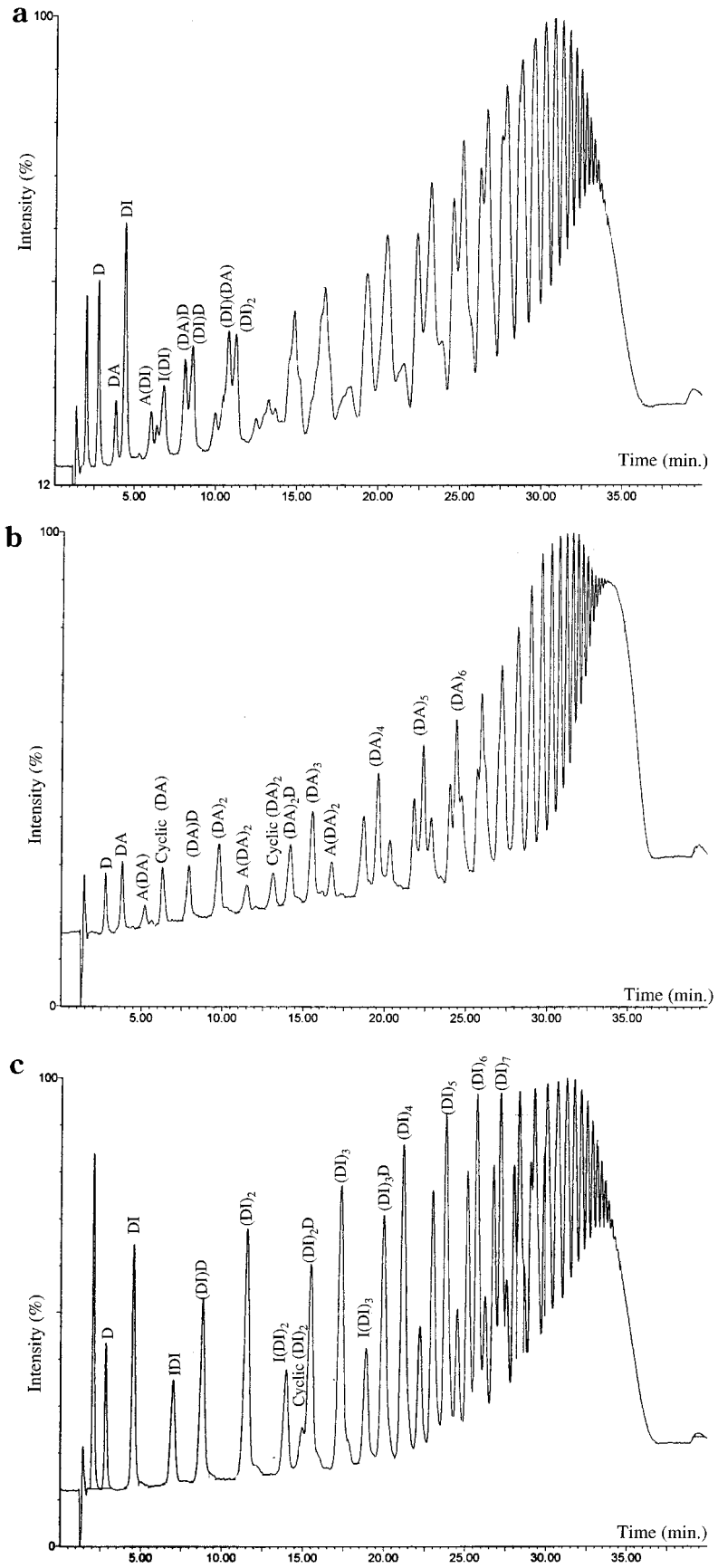

Figure 4. Gradient polymer elution chromatograms of polyDAI (a), polyDA (b), and polyDI (c).

may be obtained by using nanospray as demonstrated by others for a mixture of polydimethylsiloxane and poly(ethylene glycol).[30]

Differences in IE have also been demonstrated for the copolymer poly(ethylene glycol/propylene glycol) studied with MALDI by Chen and co-workers.[57] The study of

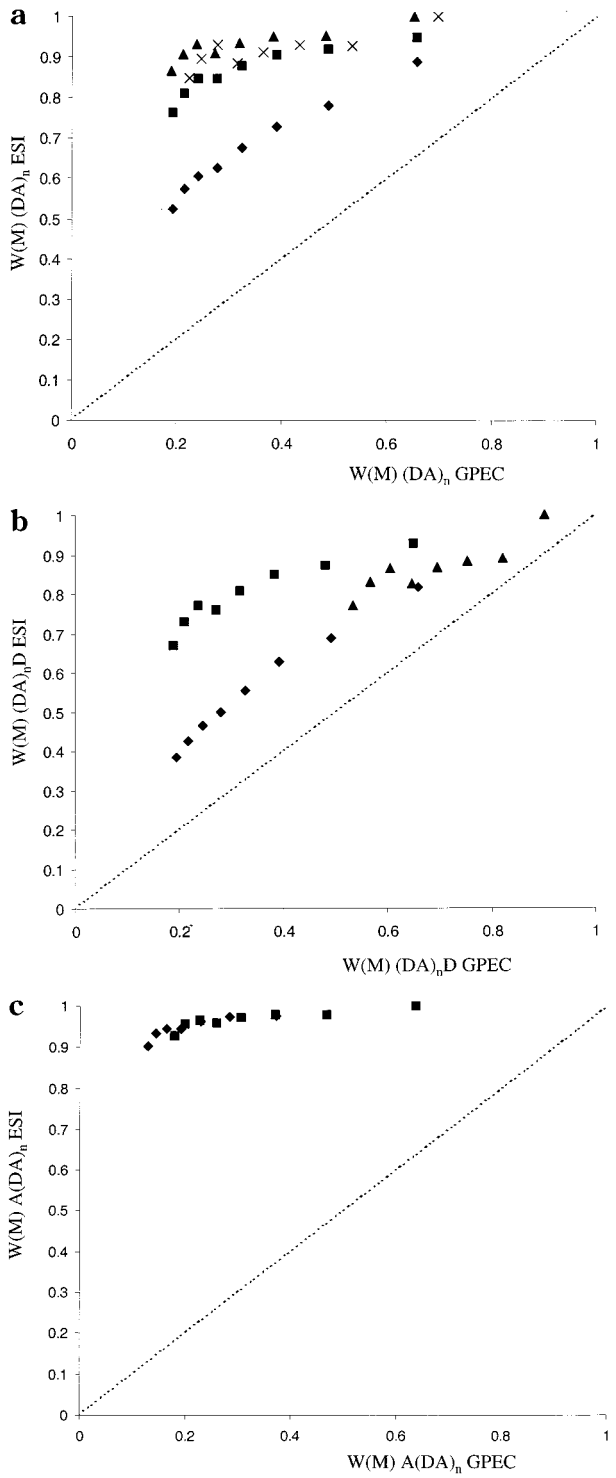

Figure 5. $W(M)^{ESIFTMS}$ sprayed in acetone plotted as a function of $W(M)^{GPEC}$: (a) oligomers with one acid and one alcohol end group; (b) oligomers with two alcohol end groups; (c) oligomers with two acid end groups observed in charge state 1. Degrees of polymerization shown are $n = 1$ (◆), 2 (■), 3 (▲), and 4 (×).

Chen and the work presented here demonstrate that the statistical copolymerization models can only be used to model the mass spectra successfully when differences in IE have been quantified.

Conclusions

Bernoullian and Markovian chain statistics were applied to analyze ESI FTMS spectra of the copolyester

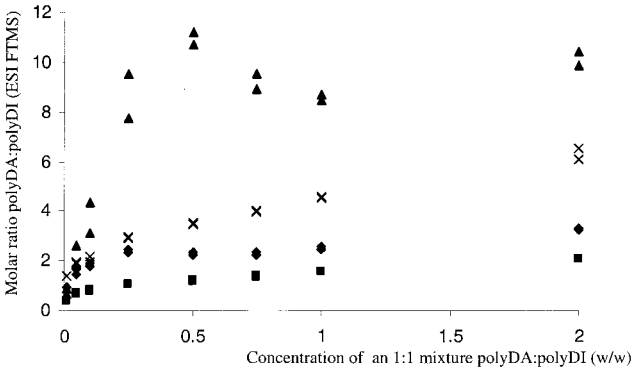

Figure 6. Molar ratio polyDA and polyDI for the structurally similar dimers observed in the spectra sprayed in THF. The mixtures contain 1:1 polyDA and polyDI (w/w). The 1 on the *x*-axis corresponds to a mixture of 1 mg/mL polyDA and 1 mg/mL polyDI. The symbols denote $(DA)_2/(DI)_2$ (◆), $(DA)_2D/(DI)_2D$ (■), $A(DA)_2/I(DI)_2$ (▲), and cyclics $(DA)_2/(DI)_2$ (×).

poly(dipropoxylated bisphenol A/adipic acid/isophthalic acid), but the calculated molar fraction of adipic acid was much higher than that determined with NMR. This discrepancy can be explained by a chemically inhomogeneous MWD or by differences in the ionization efficiency (IE) between the adipic and isophthalic acid structure. An inhomogeneous MWD can only be studied if the relative IE of the oligomers is known. Therefore, the weight fractions of the various oligomers in the MWD of the homopolyesters poly(dipropoxylated bisphenol A/adipic acid) and poly(dipropoxylated bisphenol A/isophthalic acid) were quantified with gradient polymer elution chromatography (GPEC). Quantification of the copolyester in this way is not possible. Still this quantification is required to determine the origin of the high molar fraction of adipic acid predicted by the Bernoullian and Markovian chain statistical models. Differences in the IE were studied by analyzing mixtures of homopolymers with ESI FTMS.

Large differences in IE were indeed observed between adipic and isophthalic acid containing homooligomers. On the basis of these results, we can conclude that this will occur for copolyesters as well. The same phenomena have been observed for MALDI[57] and will probably be true for other ionization techniques like FAB. The relative difference in IE has not been quantified here because too many parameters influence the IE (solvent, charge state, end group, polymer concentration). If differences in IE are not corrected, the ion intensities in the copolymer mass spectra will show large deviations from the actual composition, and no conclusions can be drawn about a random or block structure or even the chemical (in)homogeneity of the copolymer using chain statistical models. It is clear from our measurements that the relative IE in the mass spectra has to be taken into account when polymer mass spectral distributions are described using statistical models.

Acknowledgment. This work is part of the research program No. 28 "Mass Spectrometry of Macromolecular systems" of the "Stichting voor Fundamenteel Onderzoek der Materie (FOM)" a subsidiary of the "Nederlandse organisatie voor Wetenschappelijke Onderzoek (NWO)". This project is financially supported by FOM, DSM Research (Geleen, The Netherlands) and Akzo Nobel Chemicals Research Arnhem (Arnhem, The Netherlands).

References and Notes

(1) Danis, P. O.; Karr, D. E.; Mayer, F.; Holle, A.; Watson, C. H. *Org. Mass Spectrom.* **1992**, *27*, 843−846.
(2) Lloyd, P. M.; Suddaby, K. G.; Varney, J. E.; Scrivener, E.; Derrick, P. J.; Haddleton, D. M. *Eur. Mass Spectrom.* **1995**, *1*, 293−300.
(3) Jackson, C.; Larsen, B.; McEwen, C. *Anal. Chem.* **1996**, *68*, 1303−1308.
(4) Wong, S. F.; Meng, C. K.; Fenn, J. B. *J. Phys. Chem.* **1988**, *92*, 546−550.
(5) O'Connor, P. B.; McLafferty, F. W. *J. Am. Chem. Soc.* **1995**, *117*, 12826−12831.
(6) Nielen, M. W. F. *Rapid Commun. Mass Spectrom.* **1996**, *10*, 1652−1660.
(7) Wilczek-Vera, G.; Danis, P. O.; Eisenberg, A. *Macromolecules* **1996**, *29*, 4036−4044.
(8) Wilczek-Vera, G.; Danis, P. O.; Eisenberg, A. *Polym. Prepr.* **1996**, *37*, 294−295.
(9) Yoshida, S.; Yamamoto, S.; Takamatsu, T. *Rapid Commun. Mass Spectrom.* **1998**, *12*, 535−544.
(10) Wilczek-Vera, G.; Yu, Y.; Waddell, K.; Danis, P. O.; Eisenberg, A. *Rapid Commun. Mass Spectrom.* **1999**, *13*, 764−777.
(11) van Rooij, G. J.; Duursma, M. C.; de Koster, C. G.; Heeren, R. M. A.; Boon, J. J.; Schuyl, P. J. W.; van der Hage, E. R. E. *Anal. Chem.* **1998**, *70*, 843−850.
(12) de Koster, C. G.; Duursma, M. C.; van Rooij, G. J.; Heeren, R. M. A.; Boon, J. J. *Rapid Commun. Mass Spectrom.* **1995**, *9*, 957−962.
(13) van Rooij, G. J.; Duursma, M. C.; Heeren, R. M. A.; Boon, J. J.; de Koster, C. G. *J. Am. Soc. Mass Spectrom.* **1996**, *7*, 449−457.
(14) Nielen, M. W. F. *Rapid Commun. Mass Spectrom.* **1999**, *13*, 826−827.
(15) Koster, S.; Duursma, M. C.; Boon, J. J.; Heeren, R. M. A. *J. Am. Soc. Mass Spectrom.* **2000**, *11*, 536−543.
(16) Jackson, A. T.; Scrivens, J. H.; Simonsick, W. J.; Green, M. R.; Bateman, R. H. *Proceedings 47th ASMS conference and allied topics, Dallas, Texas, June 13−17 1999*; 1999; pp 713−714.
(17) Urakami, K.; Akimoto, N.; Nishijima, K.; Kitanaka, Y.; Echigoya, M.; Hashimoto, K. *Chem. Pharm. Bull.* **1999**, *47*, 1068−1072.
(18) Koster, S.; Duursma, M. C.; Boon, J. J.; Nielen, M. W. F.; de Koster, C. G.; Heeren, R. M. A. *J. Mass Spectrom.* **2000**, *35*, 739−748.
(19) Scrivens, J. H.; Jackson, A. T. *Int. J. Mass Spectrom.* **2000**, *200*, 261−276.
(20) Montaudo, M. S.; Ballistreri, A.; Montaudo, G. *Macromolecules* **1991**, *24*, 5051−5057.
(21) Montaudo, M. S.; Montaudo, G. *Macromolecules* **1992**, *25*, 4264−4280.
(22) Chen, G.; Cooks, R. G.; Jha, S. K.; Oupicky, D.; Green, M. M. *Int. J. Mass Spectrom. Ion. Processes* **1997**, *165/166*, 391−404.
(23) Shard, A. G.; Volland, C.; Davies, M. C.; Kissel, T. *Macromolecules* **1996**, *29*, 748−754.
(24) Cole, R. B.; Harrata, A. K. *J. Am. Soc. Mass Spectrom.* **1993**, *4*, 546−556.
(25) Wang, G.; Cole, R. B. *J. Am. Soc. Mass Spectrom.* **1996**, *7*, 1050−1058.
(26) Blair, S. M.; Kempen, E. C.; Brodbelt, J. S. *J. Am. Soc. Mass Spectrom.* **1998**, *9*, 1049−1059.
(27) Enke, C. G. *Anal. Chem.* **1997**, *69*, 4885−4893.
(28) Tang, L.; Kebarle, P. *Anal. Chem.* **1993**, *65*, 3654−3668.
(29) Iribarne, J. V.; Thomson, B. A. *J. Chem. Phys.* **1976**, *64*, 2287−2294.
(30) Maziarz III, E. P.; Baker, G. A.; Mure, J. V.; Wood, T. D. *Int. J. Mass Spectrom.* **2000**, *202*, 241−250.
(31) Kelly, M. A.; Vestling, M. M.; Fenselau, C. C.; Smith, P. B. *Org. Mass Spectrom.* **1992**, *27*, 1143−1147.
(32) Chapeaurouge, A.; Bigler, L.; Schafer, A.; Bienz, S. *J. Am. Soc. Mass Spectrom.* **1995**, *6*, 207−211.
(33) Wang, G.; Cole, R. B. *Proceedings 44th ASMS conference and allied topics, 44th ASMS, Portland, OR, May 12−16, 1996*; ASMS: 1996; p 1016.
(34) Loo, J. A.; Udseth, H. R.; Smith, R. D. *Rapid Commun. Mass Spectrom.* **1988**, *2*, 207−210.
(35) Cody, R. B.; Tamura, J.; Musselman, B. D. *Anal. Chem.* **1992**, *64*, 1561−1570.
(36) McEwen, C. N.; Simonsick, J. W. J.; Larsen, B. S.; Ute, K.; Hatada, K. *J. Am. Soc. Mass Spectrom.* **1995**, *6*, 906−911.
(37) Sherrard, K. B.; Marriott, P. J.; McCormick, M. J.; Colton, R.; Smith, G. *Anal. Chem.* **1994**, *66*, 3394−3399.
(38) Jasieczek, C. B.; Buzy, A.; Haddleton, D. M.; Jennings, K. R. *Rapid Commun. Mass Spectrom.* **1996**, *10*, 509−514.
(39) Hunt, S. M.; Sheil, M. M.; Belov, M.; Derrick, P. J. *Anal. Chem.* **1998**, *70*, 1812−1822.
(40) Johnstone, R. A.; Lewis, I. A. S.; Rose, M. E. *Tetrahedron* **1983**, *39*, 1597.
(41) Langley, G. J.; Hamilton, D. G.; Grossel, M. C. *J. Chem. Soc., Perkin Trans.* **1995**, *2*, 929−933.
(42) Goolsby, B. J.; Brodbelt, J. S.; Adou, E.; Blanda, M. *Int. J. Mass Spectrom.* **1999**, *193*, 197−204.
(43) Leize, E.; Jaffrezic, A.; van Dorsselaer, A. *J. Mass Spectrom.* **1996**, *31*, 537−544.
(44) Young, D.-S.; Hung, H.-Y.; Liu, L. K. *J. Mass Spectrom.* **1997**, *32*, 432−437.
(45) Young, D.-S.; Hung, H.-Y.; Liu, L. K. *Rapid Commun. Mass Spectrom.* **1997**, *11*, 769−773.
(46) Kempen, E. C.; Brodbelt, J. S.; Bartsch, R. A.; Jang, Y.; Kim, J. S. *Anal. Chem.* **1999**, *71*, 5493−5500.
(47) Brodbelt, J. S.; Kempen, E.; Reyzer, M. *Struct. Chem.* **1999**, *10*, 213−220.
(48) Cech, N. B.; Enke, C. G. *Anal. Chem.* **2000**, *72*, 2717−2723.
(49) Heeren, R. M. A.; Boon, J. J. *Int. J. Mass Spectrom. Ion Processes* **1996**, *157/158*, 391−403.
(50) Heeren, R. M. A.; de Koster, C. G.; Boon, J. J. *Anal. Chem.* **1995**, *67*, 3965−3970.
(51) Heeren, R. M. A.; Duursma, M. C.; Drahos, L.; Vekey, K. *Proceedings 48th ASMS conference and allied topics, Long Beach, CA, June 11−15, 2000*; ASMS: 2000; pp 804−805.
(52) Nielen, M. W. F.; Buijtenhuijs, F. A. *Anal. Chem.* **1999**, *71*, 1809−1814.
(53) Philipsen, H. J. A.; Wubbe, F. P. C.; Klumperman, B.; German, A. L. *J. Appl. Polym. Sci.* **1999**, *72*, 183−201.
(54) Cools, P. J. C. H.; van Herk, A. M.; German, A. L.; Staal, W. *J. Liqid Chromatogr.* **1994**, *17*, 3133−3143.
(55) De la Mora, J. F. *J. Fluid Mech.* **1992**, *243*, 561−574.
(56) Cole, R. B. *Electrospray Ionization mass spectrometry: Fundamentals, Instrumentation, and applications*, 1st ed.; John Wiley & Sons: New York, 1997.
(57) Chen, R.; Zhang, N.; Tseng, A. M.; Li, L. *Rapid Commun. Mass Spectrom.* **2000**, *14*, 2175−2181.

MA011234L