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Structural Characterization of Multicomponent Copolyesters by Mass Spectrometry

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ABSTRACT: The structural characterization and composition of five random copolyesters, originating from 1,4-butanediol and mixtures of succinic, adipic, sebacic, and terephthalic acids, were obtained by analysis of their fast atom bombardment (FAB) and their matrix-assisted laser desorption ionization (MALDI) mass spectra. Multicomponent condensation copolymers may sometimes prove difficult to characterize by conventional techniques, whereas mass spectrometry is able to handle them. Once the choice between Bernoullian or Markoffian models has been made, the determination of copolymer composition, number-average sequence length, and related quantities can be achieved by applying well-defined analytical equations. The theoretical mass spectra of multicomponent copolymers are remarkably simple, and the number of peaks appearing in each copolymer mass spectrum is easily predictable. Different kinds of spectral fitting algorithms may help in the actual computations, and it has been shown that the apparent complexity of mass spectra of copolymers is due to the presence of mass series bearing different end groups. By selecting a single mass series, one obtains an experimental spectrum immediately comparable to the theoretical one. Detailed examples, together with a discussion on the reliability of results, are given to apply the computation procedures and to gain proper understanding of the concepts involved.

1. Introduction

A variety of materials based on multicomponent copolymers are produced and used more and more nowadays, posing the general problem of their structural characterization.

The determination of sequence and composition in copolymers having large comonomer subunits can be achieved[1−14] by mass spectrometry (MS), which is able to look at the mass of individual molecules in a mixture of homologues and complements NMR, which until recently had been the only method able to perform this kind of analysis.[15]

A very limited number of mass spectra of multicomponent condensation copolymers have appeared in the literature, and we intend to extend this subject.

We report here the fast atom bombardment (FAB) and the matrix-assisted laser desorption ionization (MALDI) mass spectra of some multicomponent copolyesters listed in Table 1 (these copolymers were synthesized by condensation polymerization, and therefore a random distribution of sequences is expected), together with the theoretical mass spectra corresponding to these copolymers and to the best matches of the experimental spectra with those theoretically calculated.

The problem of decoding the intensity of peaks appearing in the mass spectra of copolymers and of relating them to the comonomer sequence has already been approached, providing a new method for deducing the sequence distributions and composition of comonomers in copolymers.[1−4]

Chain statistics (Bernoullian and first- or second-order Markoffian) allow one to generate any arrangement of comonomer units along the chain. Starting from any sequence, a theoretical mass spectrum can be generated, based on the assignment of each spectroscopic peak to a set of sequencial arrangements of monomers.[2]

Table 1. Structure and Properties of the Copolyesters Analyzed

sample[a]	comp[b]	η_{inh}[c]	M_{SEC}[d]
PBSu/PBA	50/50	0.19	14 200
PBSu/PBA/PBT	35/40/25	0.17	13 200
PBSu/PBA/PBSe	32/34/34[e]	0.17	8 400
PBA/PBT/PBSe	25/34/41	0.17	10 500
PBSu/PBA/PBT/PBSe	17/21/18/44	0.23	17 000

[a] PBA = poly(butylene adipate), PBSe = Poly(butylene sebacate), PBSu = poly(butylene succinate), PBT = poly(butylene terephthalate). [b] Molar ratio of monomers measured by FAB mass spectrometry. [c] Inherent viscosity values were obtained in THF at 30 ± 0.1 °C. [d] Molecular mass computed using the universal calibration curve obtained in THF (see the Experimental Section). [e] Molar ratio of monomers measured by MALDI mass spectrometry.

When copolymers are looked at by MS, the relative abundance of all of the co-oligomers of a defined chain length reflects the composition and sequence present in the copolymer.[2] This means that one has the possibility to build a theoretical mass spectrum for any given copolymer sequence, to be compared with the experimental mass spectrum corresponding to the copolymer sample being investigated.

Since each series of oligomers (dimers, trimers, etc.) allows the calculation of the copolymer composition and sequence distribution and since the mass spectra often provide information up the decamers and beyond, the MS method provides an excellent way to evaluate these two quantities with good precision.[1,2]

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In the original approach[2] to the general problem of decoding the intensity of copolymer mass peaks, besides the above-mentioned chain statistics, we also developed an alternative procedure to be used in the composition calculations, which was named "composition estimates".

The determination of composition in binary copolymers has become widespread,[1−19] and some authors[16−19] have actually used the latter procedure, which provides correct results in many cases.

Quantities that can be determined from the mass spectral intensities in copolymers are copolymer composition, average length of long blocks of like monomers, degree of randomness, reactivity ratios, and the compositional heterogeneity index, which indicates if the composition changes as the length of the macromolecular chain grows. However, contrary to the chain statistics, in some cases, the composition estimates procedure may fail. In the Appendix we discuss further this matter.

2. Mass Spectrometry of Polymers

The technique of FAB has been applied to study the structure of numerous synthetic and biological copolymers.[4]

FAB belongs to the wide range of desorption techniques[20−23] that minimize the fragmentation processes of molecular ions, and this is very useful for the analysis of oligomer mixtures encountered in the MS analysis of polymers.

The mechanism of ionization occurring in FAB has been investigated, and several studies agree in indicating that the species emitted by the liquid matrix are those which do not collide directly with the fast atoms beam.[4,20,21]

The assumption on which the MS analysis of copolymers is based is that ions detected in FAB-MS do originate from species already present in the polymeric sample and not from the rupture of the macromolecular chains.[4] An additional assumption is that the ion fragmentation eventually produced by fast atom bombardment is independent from the chain length. This condition is seldom entirely satisfied in the FAB mode, but the calculation methods[1−4] actually compute peak intensity ratios of each series of oligomers (dimers, trimers, etc.) and this greatly reduces the problem.

FAB spectra cannot yield direct structural information on intact macromolecules because of mass range limits, but the polymer structure can be inferred from the analysis of the relatively low molar mass species (oligomers) eventually contained in the polymer. To obtain information about the polymer structure, a degradative method has to be applied to produce low molar mass species.[4,8]

Partial degradation (methanolysis in the present case), followed by FAB analysis, allows the identification of the oligomeric species formed and affords therefore the structural characterization of homopolymers and copolymers.[8,9]

In principle, the composition and sequence of multicomponent copolymers can be deduced by suitable mass spectra obtained by any ionization technique (FAB, SIMS, MALDI, etc.).

In the present paper, we have used FAB spectra because they allow the chain statistics analysis (see below) to be performed by making use of dimers, trimers, and tetramers, which are the simplest oligomers.

Table 2. Theoretical Number of Peaks[a] Corresponding to Oligomers that Can Be Observed in the Mass Spectrum of a Copolymer

	AB	ABC	ABCD
dimers	3	6	10
trimers	4	10	20
tetramers	5	15	35
pentamers	6	21	56
hexamers	7	28	84
heptamers	8	36	120
octamers	9	45	165
nonamers	10	55	220
decamers	11	66	286
11-mers	12	78	364
12-mers	13	91	455
13-mers	14	105	560
14-mers	15	120	680
15-mers	16	136	816
16-mers	17	153	969

[a] The equations that allow us to compute the number of peaks are

$$AB = 1 + N; \quad ABC = 1 + (3/2)N + (1/2)N^2; \quad ABCD = 1 + (11/6)N + N^2 + (1/6)N^3$$

where N is the size of the oligomer.

If one uses MALDI, instead, these oligomers are usually covered by the matrix clusters, and the analysis is made using higher oligomers (usually decamers and higher co-oligomers).

The use of MALDI for two-component copolymer analysis has been demonstrated.[24−26] MALDI has the advantage of avoiding the step of partial degradation of the copolymer sample, since the technique can be applied to the intact polymer.

Here we have used MALDI to demonstrate its applicability in the case of a three-component copolymer (Table 1).

3. Theoretical Mass Spectra of Copolymers

The intensities of MS peaks depend both on the copolymer composition and on the type of unit distribution along the chain (i.e., Bernoullian or Markoffian). Therefore, assuming a theoretical distribution model and then fitting the calculated oligomer abundance with the experimental peak intensities, the copolymer composition can be determined. Opportune algorithms to generate the theoretical mass spectra of copolymers and to match the experimental mass spectra to those theoretically calculated are already available (MACO4 program).[2]

Copolymers with two, three, and four components contain co-oligomers of the type A_mB_n, $A_mB_nC_p$, and $A_mB_nC_pD_q$, respectively, where m, n, p, and q are the number of units in the molecule. The theoretical number of peaks which can appear in the mass spectra of these types of copolymers can be calculated a priori, and it increases with the number of components, as shown in Table 2.

The peak intensity (I) of the three types of copolymers is obtained using the following formulas:

$$I[A_mB_n] = g_{AB}(c_A)^m(c_B)^n \tag{1}$$

$$I[A_mB_nC_p] = g_{ABC}(c_A)^m(c_B)^n(c_C)^p \tag{2}$$

$$I[A_mB_nC_pD_q] = g_{ABCD}(c_A)^m(c_B)^n(c_C)^p(c_D)^q \tag{3}$$

where c_A, c_B, c_C, c_D are the molar fractions of A, B, C,

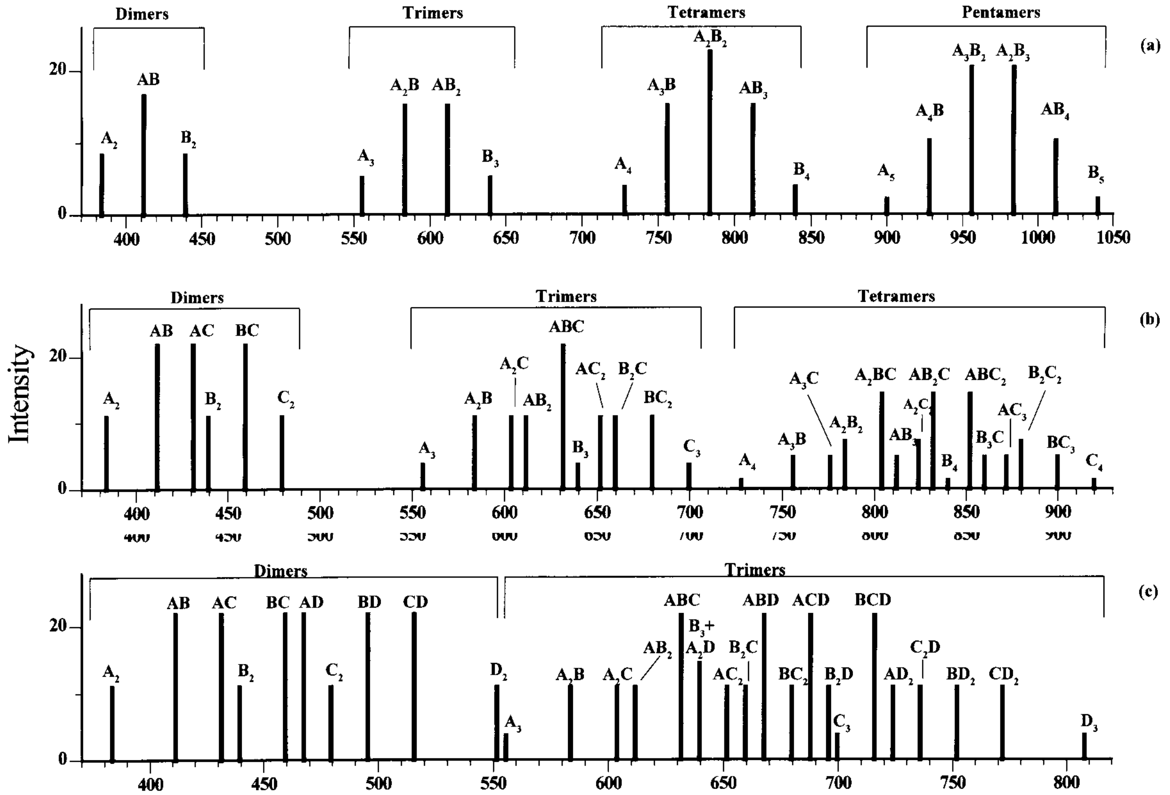

Figure 1. Theoretical mass spectra (a) for an AB random copolymer which possesses a 50/50 composition and repeat units of 172 and 200 g/mol, (b) for an ABC random copolymer which possesses a 33/33/33 composition and repeat units of 172, 200, and 220 g/mol, and (c) for an ABCD random copolymer which possesses a 25/25/25/25 composition and repeat units of 172, 200, 220 and 256 g/mol.

and D units in the copolymers and $g_{AB} = (m + n)!/[m!n!]$, $g_{ABC} = (m + n + p)!/[m!n!p!]$, and $g_{ABCD} = (m + n + p + q)!/[m!n!p!q!]$.

Equations 1−3 allow us to generate theoretical mass spectra which must then be compared with the experimental ones.

In Figure 1a−c are shown the theoretical mass spectra (up to molar mass 1100) for equimolar copolymers with two, three, and four components which follow Bernoullian chain statistics. The theoretical mass spectrum for a two-component copolymer (Figure 1a) consists of 3 dimers, 4 trimers, 5 tetramers, and 6 pentamers, and the one for a three-component copolymer (Figure 1b) consists of 6 dimers, 10 trimers, and 15 tetramers. The theoretical mass spectrum for a four-component copolymer (Figure 1c) shows exclusively dimers and trimers, since the number of tetramers is too high (Table 2).

4. Experimental Part

4.1. Materials. Dimethyl succinate, dimethyl adipate, dimethyl sebacate, and dimethyl terephthalate were purchased from Sigma−Aldrich (Mi, Italy) whereas 1,4-butanediol was purchased from Jansen Chimica. Dimethyl terephthalate was purified by crystallization from *n*-hexane, whereas the other reagents were purified by vacuum distillation before use.

4.2. Copolyesters Synthesis. Homopolyesters and copolyesters were synthesized by melt polymerization starting from stoichiometric amounts of dimethylesters and 1,4-butanediol in the presence of a mixture of $Zn(COOCH_3)_2$ and Sb_2O_3 (80/20, w/w) as transesterification catalyst. An equimolar mixture of two, three, or four dimethyl esters was reacted with 1,4-butanediol to obtain two, three-, and four-component copolymers. The reaction was carried out at 180 °C for 2 h and at 230 °C for 5 h under reduced pressure (1.5 Torr) to eliminate the methanol formed in the reaction. In the following the synthesis of poly(butyleneadipate-*co*-butylene sebacate) is described as an example. A total of 1.51 g (0.0088 mol) of dimethyl adipate was poured in a flask together with 2.024 g (0.0088 mol) of dimethyl sebacate, with 1.58 g (0.0175 mol) of 1,4-butanediol and with 3.95 mg (0.25% of the diol weight) of catalyst. The temperature of the mixture was gradually raised to 180 °C and kept at this value for 2 h, with stirring. Thereafter, the pressure was reduced to 1.5 Torr, and the temperature was gradually increased up to 230 °C and kept at this value for 5 h. To remove the residual catalyst from the reaction mixture, the crude homopolymers and copolymers were dissolved in the minimum amount of $CHCl_3$, filtered, and precipitated into methanol. The solid materials were filtered, washed several times with methanol, dried at 50 °C under vacuum, and characterized by viscosimetry, size-exclusion chromatography (SEC), NMR, and MS.

4.3. SEC. The SEC analyses were performed in THF or $CHCl_3$ on a Waters 6000A apparatus equipped with four ultrastyragel columns (in the order 1000, 500, 10 000, and 100 Å pore size) attached in series, using a Waters R401 differential refractometer. A polymer solution (60 μL, 15 mg/mL) was injected and eluted at a flow rate of 1 mL/min.

4.4. Methanolysis. To obtain low molar mass oligomers suitable of FAB-MS analysis, the copolyesters synthesized (Table 1) were partially degraded by methanolysis in a $CHCl_3$ solution at room temperature for 20 and 48 h for the copolyesters which contain poly-

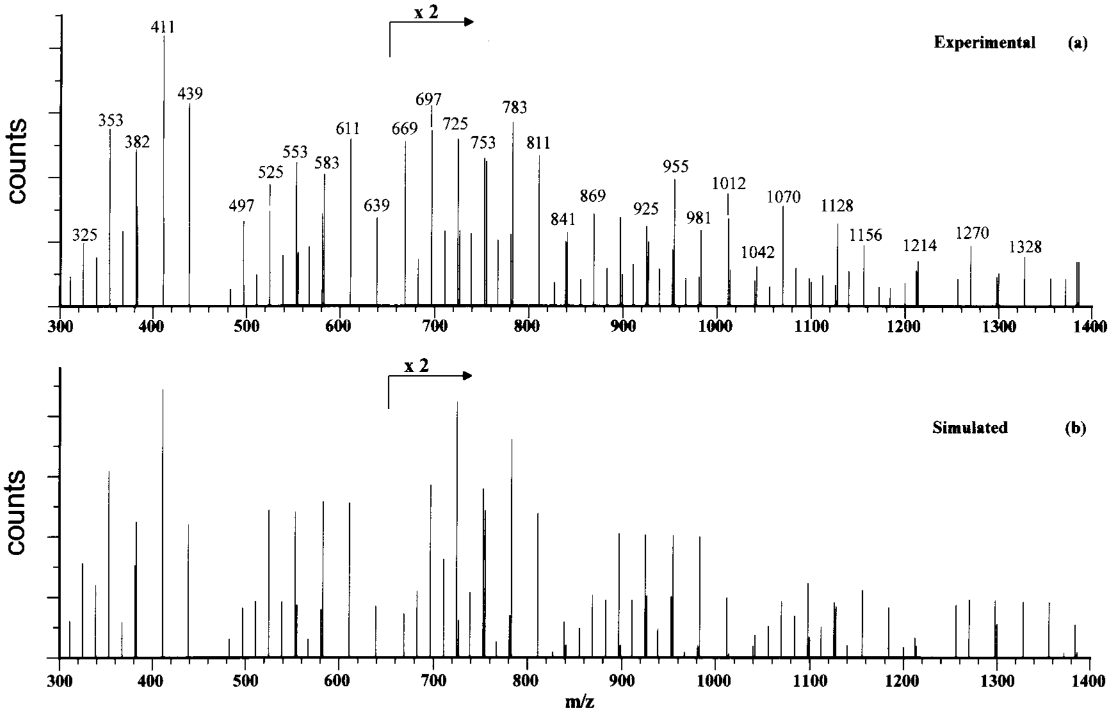

Figure 2. Mass spectra for the sample obtained by subjecting PBSu/PBA to partial degradation: (a) experimental FAB mass spectrum; (b) simulated mass spectrum.

(butylene terephthate) units. In a typical procedure 20 mg of a copolyester was dissolved in 5 mL of $CHCl_3$, and 0.4 mL of a freshly prepared 1 N solution of HCl in methanol was added. The mixture was allowed to react at room temperature for 20 or 48 h, and then the solvent was evaporated and the residue dried under vacuum at 45 °C.

4.5. M.S. FAB mass spectra were obtained on a Kratos MS50 double-focusing mass spectrometer equipped with a cesium gun operating at 20–21 kV and an Eclipse 120 (Data General) data system with DS90 software. The instrument was scanned from *m*/*z* 5000 to 100 at a scan rate of 10 s/decade and an accelerating voltage of 8 kV. A mixture of cesium and rubidium iodide (50/50, w/w) was used for computer calibration. The resolution was about 2000. A small amount of sample was placed on the end of the FAB probe and mixed with 3-nitrobenzyl alcohol (TNBA) as a matrix doped with LiCl. Peak intensity values shown in the mass spectrum were computed after subtraction of the contribution from the matrix. MALDI mass spectra were obtained on a Perspetive Biosystems (MA) model Voyager instrument (acc. pot 25 000 V, grid 1%, extraction delay 500 ns). The matrix used in the sample preparation was 2-[(4-hydroxyphenyl)azo]benzoic acid (HABA), whereas the solvent was tetrahydrofuran. The probe tip was loaded with 300 pmol of copolymer and 300 nmol of matrix. No cationization agent was added.

4.6. Molar Mass Calculations. The molar masses of the copolyester samples were calculated with the universal calibration curve obtained by using a set of 12 poly(methyl methacrylate) and 14 polystyrene well-characterized samples (purchased from Polymer Labs) having a narrow molar mass distribution ($M_w/M_n < 1.1$). We measured the viscosity and the elution volume at which the SEC trace takes its tallest value (V_e) and plotted log(M) versus V_e. The resulting plot was the universal calibration line for our set of SEC columns, and it fits with the following equation: $\log(M) = 0.016(V_e)^2 - 1.76V_e + 22.7$. Molar mass data (Table 1) were calculated considering the viscosity of unfractionated copolymer samples and the elution volume at the maximum of the SEC curves.

4.7. Viscometry. Inherent viscosities ($\eta_{inh} = \ln \eta_R / C$, where $C = 0.5$ g/dL) were measured in a Desreux–Bishoff suspended-level viscometer at 30 ± 0.1 °C. Copolyester data are reported in Table 1.

4.8. MACO4 Calculations. The intensities of mass spectra of copolyesters were used to determine the sequence distribution of the copolymer samples by using the MACO4 computer program.[2] It was assumed that the samples follow Bernoullian statistics and that the sequence distribution of the partially degraded polyester reflects the sequence distribution of the undegraded sample. Theoretical MS intensities were computed using eqs 1–3. The theoretical intensities were then compared with the experimental ones. To perform the minimization, the program uses MINPACK, which belongs to the Argonne library.

MINPACK computes the Euclidean norm of the residual vector. It gives as an output the compositional values for which a minimum occurs, the norm and the covariance matrix. MACO uses the norm to compute the agreement factor[2] and the elements on the principal diagonal of the covariance matrix to compute the error. The overall theoretical intensity of a peak is then spread to all of the isotopes which are actually seen in the spectrum. Thresholding to eliminate peaks with insignificant intensity (below 3 ppt) was never applied except for preliminaries. Finite resolution was taken into account in the standard manner.[27]

5. Results and Discussion

5.1. Fab Mass Spectra. The molar masses of the copolyesters in Table 1 are too high to produce FAB-MS spectra, and therefore a controlled partial degradation (methanolysis, in this case) has been performed in

Table 3. Identification of the Peaks Observed in the FAB Mass Spectrum of Sample PBSu/PBA

assign[a]	obs[b]	obs[c]	obs[d]
A_2	383	325	311
AB	411	353	339
B_2	439	382	368
A_3	555	497	483
A_2B	583	525	511
AB_2	611	553	539
B_3	639	581	567
A_4	727	669	
A_3B	755	697	683
A_2B_2	783	725	711
AB_3	811	753	739
B_4	839	781	767
A_5	899	841	827
A_4B	927	869	855
A_3B_2	955	897	883
A_2B_3	983	925	911
AB_4	1012	953	939
B_5	1040	981	967
A_6	1072	1014	
A_5B	1100	1042	
A_4B_2	1128	1070	1056
A_3B_3	1156	1098	1084
A_2B_4	1194	1126	1112
AB_5	1212		1140
B_6			
A_7			
A_6B		1214	1200
A_5B_2	1300		
A_4B_3	1328	1270	1256
A_3B_4	1356	1298	
A_2B_5	1384		
AB_6	1412		
B_7			

[a] Acid groups in the oligomer backbone. A = succinate. B = adipate. [b] Observed mass of the ion. Ions of the type $HO(CH_2)_4O/.../OCH_3...Li^+$. [c] Observed mass of the ion. Ions of the type $CH_3O/.../OCH_3...Li^+$. [d] Observed mass of the ion. Ions of the type $CH_3O/.../COOH...Li^+$.

Table 4. Identification of the Peaks Observed in the FAB Mass Spectrum of Sample PBSu/PBA/PBT

assign[a]	obs[b]	obs[c]
A2	383	325
AB	411	353
AC	431	373
B2	439	381
BC	459	401
C2	479	421
A3	555	497
B3	639	581
C3	699	641
A2B	583	525
A2C	603	545
ABC	631	573
AB2	611	553
AC2	651	593
B2C	659	601
BC2	679	621
A4		
B4	839	781
C4		
A3B	755	697
A3C	775	717
A2B2	783	725
A2C2	823	765
A2BC	803	745
AB2C	831	773
ABC2	851	793
AB3	811	753
AC3		
B3C	859	801
B2C2	879	821
BC3	899	
A3B2	955	897
A3C2	995	937
A2B3	983	925
A2C3	1043	985
A3BC	975	917
A2B2C		945
A2BC2		965
AB4	1011	1069
AC4	1091	1149
AB3C	1031	973
AB2C2	1051	993
ABC3	1071	1013
B4C	1059	1001
B3C2	1079	1021
B2C3	1099	1041
BC4	1119	1061

[a] Acid groups in the oligomer backbone. A = succinate. B = adipate. C = terephthalate. [b] Observed mass of the ion. Ions of the type $HO(CH_2)_4O/.../OCH_3...Li^+$. [c] Observed mass of the ion. Ions of the type $CH_3O/.../OCH_3...Li^+$.

order to yield oligomers with suitable molar masses (MM, see the Experimental Section).

Figure 2a reports the FAB mass spectrum of the oligomers obtained by partial methanolysis of a PBSu/PBA sample.

In Table 3 are reported the structural assignment of the 78 peaks corresponding to litiated molecular ions of dimers, trimers, tetramers, and heptamers which are terminated with three different kinds of end groups, namely, hydroxy, methyl ester, and carboxylic acid ($HO/.../OCH_3$, $CH_3O/.../OCH_3$, and $CH_3O/.../COOH$). Peaks at masses 441, 469, 497, 613, 641, 669, 697, 785, 813, 841, 869, and 897 Da (due to oligomers terminated by butanediol on both sides) are absent (with few exceptions). This is not surprising, since peaks due to these types of oligomers are usually[28,29] very weak in FAB spectra of polyesters.

The intensities of the 78 peaks shown in the mass spectrum in Figure 2a were given as an input to MACO4. The iterative process converged to a well-defined minimum corresponding to a copolymer composition of 50/50 (succinate/adipate) with an agreement factor AF = 30%, and the error associated with this composition value is ±0.02 mol. The results of this calculation, namely, the simulated mass spectrum, are shown in Figure 2b.

An inspection of the assignments (Table 3) shows that the spectrum in Figure 2a is generated by 28 distinct oligomers which bear different end groups (Table 3), and therefore chains with the same macromolecular backbone appear more than once in the spectrum in Figure 2a. This implies that the same information can be recovered in three different ways. For instance, the ratio between the abundance of pentamer A_5 and pentamer B_5 can be obtained by taking the ratio between the intensities of three different couples of peaks, namely, peaks at m/z 899 and 1040, peaks at m/z 841 and 981, and peaks at m/z 827 and 967. Since the macromolecular backbone is the same, the three ratios are equivalent. In practice, the three ratios display some small differences.

If peaks belonging to a single mass series can be neglected without introducing changes in the calculated composition, one should be able to recover the correct composition value by considering only a fraction of the mass spectral peaks.

We tested this hypothesis on the mass spectrum in Figure 2. We assumed that mass spectral peaks corresponding to oligomers of the type $CH_3O/.../OCH_3$ and

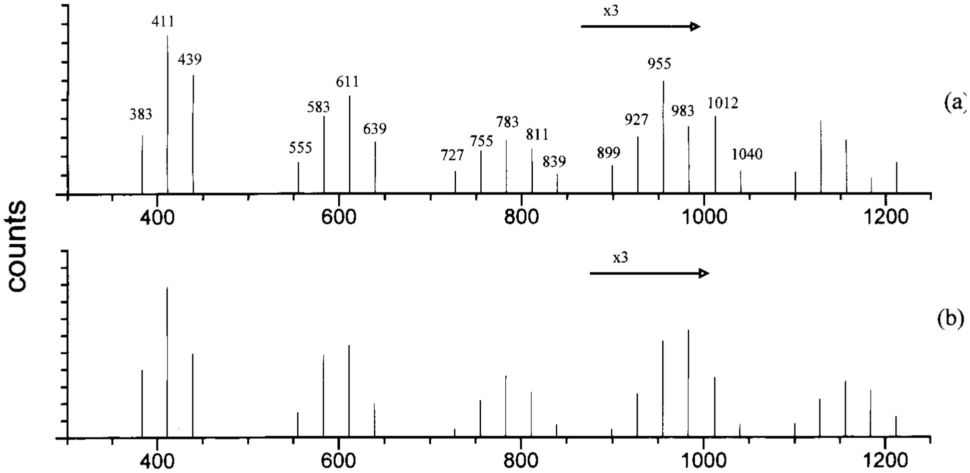

Figure 3. Mass spectra for peaks belonging to mass series HO/.../OCH$_3$ for the sample obtained by subjecting PBSu/PBA to partial degradation: (a) experimental FAB mass spectrum; (b) simulated mass spectrum.

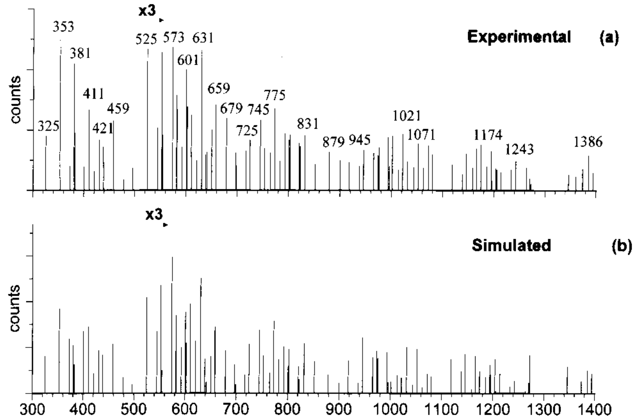

Figure 4. Mass spectra for the sample obtained by subjecting PBSu/PBA/PBT to partial degradation: (a) experimental FAB mass spectrum; (b) simulated mass spectrum.

CH$_3$O/.../COOH are surplus peaks which can be neglected without changes in the composition. The peaks which remain are the peaks belonging to a single mass series (i.e., corresponding to oligomers of the type HO/.../OCH$_3$). They were torn away from the other peaks and considered separately. This plot (Figure 3a) is undoubtedly less complex than the previous one, and it resembles closely the theoretical mass spectrum calculated for an equimolar AB copolymer, reported in Figure 1a.

The intensity of this group of peaks was given as an input to the MACO4 program[2] assuming Bernoullian chain statistics. The iterative process converged to a well-defined minimum corresponding to a copolymer composition of 47/53 (succinate/adipate) with an agreement factor AF = 20% and an error of ±0.02 mol. The simulated mass spectrum relative to this case is reported in Figure 3b. Therefore, the process of reducing the number of peaks has not affected drastically the compositional value.

Contrary to the large number of mass spectra of binary copolymers that appeared in recent years, mass spectra of terpolymers are scarce in the literature.

Figure 4a shows the FAB mass spectrum of the oligomers obtained after partial methanolysis of the PBSu/PBA/PBT copolymer. The peaks appearing in the mass spectrum can be assigned to litiated molecular ions of oligomers (up to heptamers) terminated with two different kinds of end groups, namely, hydroxyl and methyl ester (HO/.../OCH$_3$ and CH$_3$O/.../OCH$_3$) (Table 4). The intensity of the overall mass spectral data displayed in Figure 4a was given as an input to MACO4, and the results were the following: a copolymer composition of 35/40/25 (succinate/adipate/terephthalate) with an AF value of 35%, corresponding to an error of ±0.02 mol for each comonomer composition.

Figure 4b reports the overall simulated mass spectrum for this case.

An inspection of the table of assignments (Table 4) shows that the spectrum in Figure 4a is generated by a small number of distinct oligomers which bear different end groups, and therefore chains with the same macromolecular backbone appear more than once in the spectrum. As a consequence, the spectrum in Figure 4a contains a number of peaks almost doubled with respect to the corresponding theoretical spectrum for a three-component random copolymer (Figure 1b). To test the above hypothesis and to achieve a better insight of the mass spectral properties, the composition of the copolymer was computed by using the intensity of peaks belonging to the mass series CH$_3$O/.../OCH$_3$ (Figure 5a). A value of 34/42/23 (succinate/adipate/terephthalate) with an AF of 40% was obtained in this case. The simulated spectrum is reported in Figure 5b.

Figure 6a reports the FAB mass spectrum of the sample obtained by subjecting PBA/PBT/PBSe copolymer to partial methanolysis. The overall number of peaks is higher than the spectrum in Figure 4a and can be assigned (Table 5) to oligomers bearing two types of end groups, namely, HO/.../OCH$_3$ and CH$_3$O/.../OCH$_3$. The peak intensity of the whole spectrum was fed as an input to MACO4, and a deep minimum (AF = 30%) for a composition of 25/34/41 (adipate/terephthalate/sebacate) has been found. The error associated with this compositional value was ±0.02 mol. The composition differs from that expected (33/33/33) from the equimolar feed. However, PBA/PBT/PBSe has been analyzed by FAB only after methanolysis. It has been established[30] that the methanolysis of the sebacic unit is slower than that of the succinic, terephthalic, and adipic units. Therefore, the resulting polymer is richer in sebacic unit with respect to the terephthalic and adipic ones. In Figure 6b the simulated mass spectrum is reported.

Figure 7a reports the FAB mass spectrum of the sample obtained by subjecting PBSu/PBA/PBT/PBSe to partial methanolysis. Peaks are due to chains of the

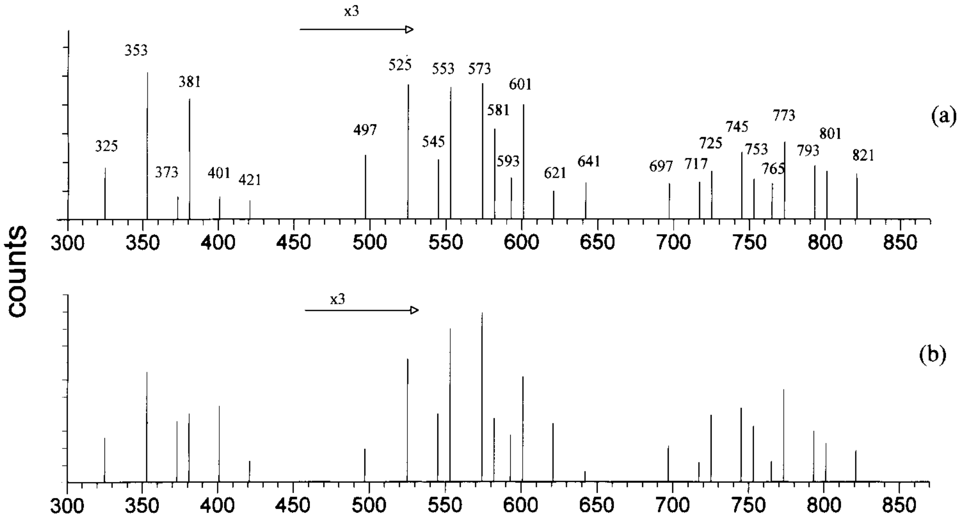

Figure 5. Mass spectra for peaks belonging to mass series H_3CO/.../OCH_3 for the sample obtained by subjecting PBSu/PBA/PBT to partial degradation: (a) experimental FAB mass spectrum; (b) simulated mass spectrum.

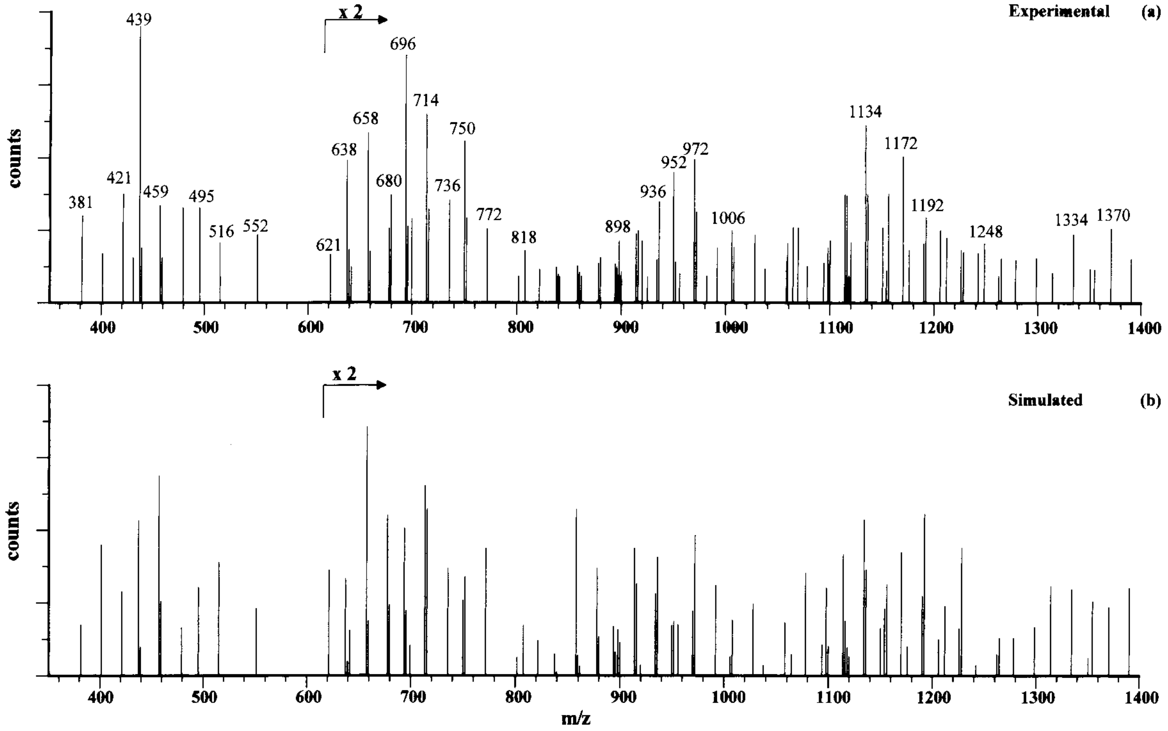

Figure 6. Mass spectra for the sample obtained by subjecting PBA/PBT/PBSe to partial degradation: (a) experimental FAB mass spectrum; (b) simulated mass spectrum.

type HO/.../OCH_3 and CH_3O/.../OCH_3 (see Table 6). For oligomers of the type HO/.../OCH_3, the number of dimers coincides with the number of possible ABCD oligomers (see Table 1), whereas the number of trimers is lower. For what concerns oligomers belonging to the other mass series, all dimers are seen and most of the trimers are present. The peak intensities were fed as an input to the program MACO4. A minimum (AF = 40%) was found, associated with a copolymer composition of 17/21/18/44 (succinate/adipate/terephthalate/sebacate). The error associated with this composition was ±0.03 mol. In Figure 7b is shown the simulated mass spectrum for this copolymer.

To evidence the analogies between the spectrum in Figure 7a with the theoretical spectrum for a four-component random copolymer (see Figure 1c), peaks due to dimers and trimers of the type HO/.../OCH_3 were considered separately (Figure 8a). The peak intensities were fed as an input to the program MACO4 and compared with the theoretical spectrum generated by eq 3. The resulting composition was 17/22/21/40. Figure 8b reports the simulated spectrum. This value of composition comes very close with the copolymer composition obtained in the case of the single mass series in Figure 7a,b.

5.2. MALDI Mass Spectra. MALDI spectra of three binary copolymers have already been reported.[24−26] Here we attempt the MALDI analysis of a terpolymer. Figure 9 reports the MALDI-TOF mass spectrum of the sample PBSu/PBA/PBSe. It displays over 200 peaks in the mass range 2000−9000 Da. The signals at low masses (range 2000−3000 Da) are due to 11-mers, 12-mers, 13-mers, and up to 16-mers, whereas the signals at high masses (range 8000−9000 Da) are due to 35-mers, 36-

Table 5. Identification of the Peaks Observed in the FAB Mass Spectrum of Sample PBA/PBT/PBSe

assign[a]	obs[b]	obs[c]
A2	439.4	381.4
AB	459.4	401.4
AC	495.5	437.5
B2	479.4	421.4
BC	515.5	457.5
C2	551.6	
A3	639.6	
B3	699.6	641.6
C3	807.9	749.9
A2B	659.6	
A2C	695.7	637.7
ABC	715.7	657.7
AB2	679.6	
AC2	751.8	693.8
B2C	735.7	677.7
BC2	771.8	714
A4	839.8	781.8
B4	919.8	861.8
C4	1064.2	1006
A3B	859.8	801.8
A3C	895.6	837.9
A2B2	879.8	821.8
A2C2	952	894
A2BC	916	858
AB2C	936	878
ABC2	972	914
AB3	900	922
AC3	1008	950
B3C	956	898
B2C2	992	934
BC3	1028	970
A3B2		1022
A3C2		1094
A2B3	1100	1042
A2C3	1208	1150
A3BC	1084	1058
A2B2C	1136	1078
A2BC2	1172	1134
AB4	1120	1062
AC4	1264.4	1206.3
AB3C	1156.1	1098
AB2C2	1192.1	1134
ABC3	1128.1	1170
B4C	1176	1118
B3C2	1212	1154
B2C3	1248	1190
BC4	1284	1226
A4B2		1222
A4C2		1294
A2B3C	1356	
A2B2C2		1334.4
A2BC3	1428.2	1370.4
AB5		
AB4C	1376.3	
AB3C2	1412.3	1354.4

[a] Acid groups in the oligomer backbone. A = succinate. B = terephthalate. C = sebacate. [b] Observed mass of the ion. Ions of the type $HO(CH_2)_4O/.../OCH_3...Li^+$. [c] Observed mass of the ion. Ions of the type $CH_3O/.../OCH_3...Li^+$.

mers, 37-mers, and up to 44-mers. The MS peaks seen at 2699, 2728, 2756, 2785, 2812, 2840, 2869, 2898, 2930, 2957, 2985, and 3013 Da in Figure 9 were assigned to oligomers terminated by $H_3CO/.../OCH_3$ cationized with lithium (see Table 1S in Supporting Information). These peaks have low enough masses to be unequivocally assigned (i.e., there are very few alternative structures, corresponding to these oligomers, which can interfere or superpose in the assignment). The molecular species present in the terpolymer and the cation (Li) turned out to display a close analogy with a sample of PBA/PBSe previously analyzed.[26] The other peaks in Figure 9 possess higher masses, and they cannot be assigned as easily as those at low masses. We made a series of attempts generating theoretical spectra corresponding to mixtures made of chains of the type $HO/.../OCH_3$ and of chains of the type $H_3CO/.../OCH_3$. The attempt which gave the best results was that where the oligomers are terminated only by $H_3CO/.../OCH_3$ cationized with lithium (see Table 1S in Supporting Information). The observed mass numbers turned out to be almost exactly coincident with the expected ones. Alternative attempts failed.

The mass range in Figure 9 is substantially higher than that in the previous mass spectra, and this deserves some comment. At small oligomer sizes, peaks due to oligomers having size *n* appear before peaks due to oligomers having size $n + 1$, whereas at large oligomer sizes, peaks due to *n*-mers and to $(n + 1)$-mers are close to each other. For instance, 29-mers, 30-mers, and 31-mers appear in the mass ranges 5000−7400, 5100−7600, and 5300−7900 Da, respectively. In practice, the envelope of peaks due to 29-mers overlaps strongly (over 2000 masses) with the envelope due to 30-mers and superposes strongly (over 2200 masses) above the envelope due to 31-mers.

Table 1S (Supporting Information) reports the peak assignment, and it shows the peculiar feature that each mass spectral peak has more than one assignment. This is in contrast with the previous cases (see Tables 3 and 4), where a one-to-one correspondence can be found between the peak and assignment. Each assignment represents an oligomer. The contribution of each oligomer is summed in the peak intensity. Data in Table 1S were used by us to estimate the average number of oligomers which contribute to a single peak, which turned out to be about 11 contributions. Some of the oligomers which contribute to a peak possess slightly different masses. Therefore, if an instrument with a higher resolving power is used, the individual contributions can be resolved. However, some of the oligomers which contribute to a peak are isomers; i.e., they possess the same chemical formula and therefore have exactly the same mass. In this case even a higher resolving power cannot discern the individual contributions. Let us consider, for instance, the four oligomers $(B)_{14}(Su)_7(Ad)_2(Se)_6$, $(B)_{14}(Su)_5(Ad)_5(Se)_5$, $(B)_{14}(Su)_3(Ad)_8(Se)_4$, and $(B)_{14}(Su)_1(Ad)_{11}(Se)_3$, where B is butanediol (they are all 15-mers). They possess the same formula, namely, $C_{158}O_{60}H_{258}...Li$. As a consequence, they contribute to a single mass spectral peak, namely, the peak at *m*/*z* 3125.

MALDI-TOF spectra have never been used to determine terpolymer composition. The about 200 mass spectral peaks in Figure 9 were given as an input to the MACO4 program. The program converged toward a sharp minimum (AF = 8%), corresponding to a composition of 32/34/34 (succinate/adipate/sebacate).

This represents a successful result and demonstrates that it is possible to apply the chain statistics to MALDI spectra of three-component copolymers. Figure 10 reports the simulated mass spectrum. It consists of about 1000 peaks. Nevertheless, some peaks are still overlapped since they have the same formula and cannot be resolved. This demonstrates the need for a procedure such as MACO4, which deconvolutes overlapping peaks.

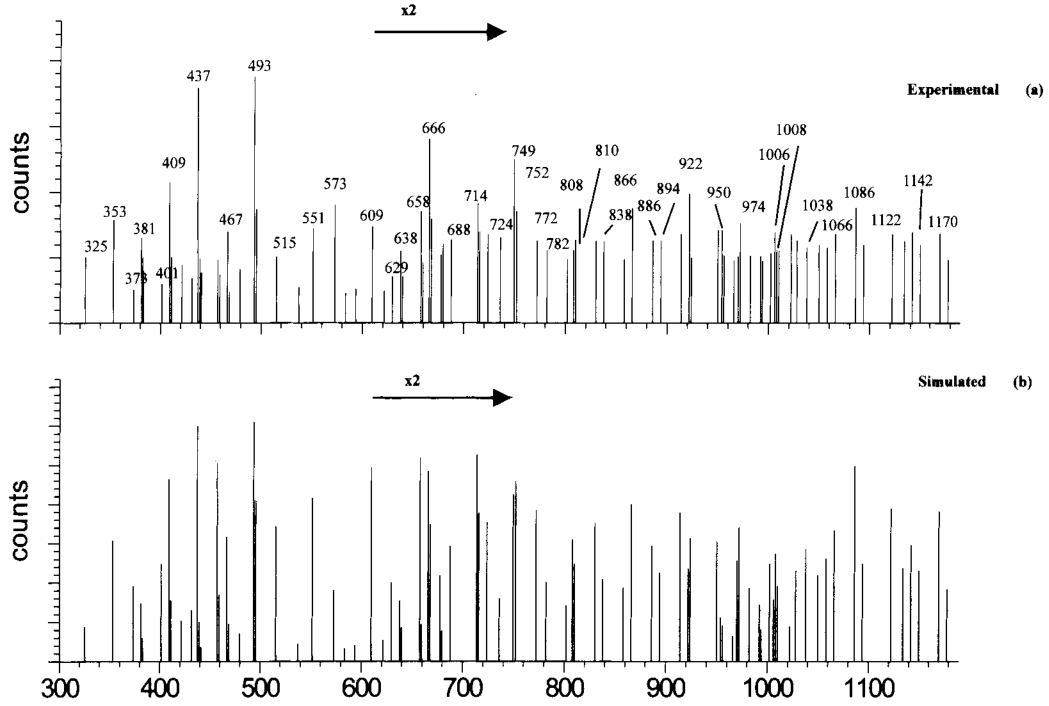

Figure 7. Mass spectra for the sample obtained by subjecting PBSu/PBA/PBT/PBSe to partial degradation: (a) experimental FAB mass spectrum; (b) simulated mass spectrum.

Table 6. Identification of the Peaks Observed in the FAB Mass Spectrum of Sample PBSu/PBA/PBT/PBSe

assign[a]	obs[b]	obs[c]	assign[a]	obs[b]	obs[c]	assign[a]	obs[c]
A2	383	325	C3	641		C2D2	934
AB	411	353	A3B		698	CD3	976
AC	431	373	A3C		718	ABCD	830
AD	467	409	A3D		754	A2BD2	1038
B2	439	381	A2B2		726	AD3C	1142
BC	459	401	A2BC		746	AD4	1178
BD	495	437	A2C2		766	A2CD2	1058
C2	479	421	A2CD		802	AB2D2	1066
CD	515	457	A2D2		837	ABCD2	1086
D2	551	493	AB3		754	ABD3	1122
A3		497	AB2C		778	ACD3	1142
A2B	583	525	AB2D		810	BCD3	1170
ABC		573	ABC2		794	BC2D2	1134
ABD	667	609	AC3		866	B2D3	1150
ACD	687	629	AD3		814	B3D2	1094
AB2		553	AC2D		922	CD4	1086
AC2		593	AC2D		818	BD4	1066
AD2	724	665	ACD2		886	B2D3	1010
B2C	660		B3C		802	ACD3	1142
BCD	716	657	B2C2		842	A2BC2	966
BC2	680	621	B2D2		858	A2B2D	982
BD2	752	693	BC3		894	AB2C2	994
C2D	736	677	BC2D		846	AB3D	1010
CD2	772	714	BCD2	972	914	A2BD2	1006
D3	808	749	BD3	1008	950	A3BD	954
B3		582	C3D	956	898	ABC2D	1050

[a] Acid groups in the backbone. A = succinate. B = adipate. C = terephthalate. D = sebacate. [b] Observed mass of the ion. Ions of the type $HO(CH_2)_4O/.../OCH_3...Li^+$. [c] Observed mass of the ion. Ions of the type $CH_3O/.../OCH_3...Li^+$.

6. Conclusions

Contemporary MS analysis appears to be a significant addition to the methods currently used in the structural analysis of synthetic copolymers.

Multicomponent condensation copolymers may sometimes prove difficult to characterize by conventional techniques, whereas MS is able to handle them. Once the choice between Bernoullian or Markoffian models has been made, the determination of copolymer composition, number-average sequence length, and related quantities can be achieved by applying well-defined analytical equations.[2]

The theoretical mass spectra (Figure 1) of multicomponent copolymers are remarkably simple, and the number of peaks appearing in each copolymer mass spectrum is easily predictable (Table 2). Different kinds of spectral fitting algorithms may help in the actual computations, and it has been shown that the apparent complexity of mass spectra of copolymers is due to the presence of mass series bearing different end groups. By selecting a single mass series, one obtains an experimental spectrum immediately comparable to the theoretical one.

7. Appendix

In this appendix we discuss the methods used to extract information on mass spectra of copolymers. Quantities to be determined from the mass spectral intensities in copolymers are copolymer composition, average length of long blocks of like monomers, the degree of randomness, the reactivity ratios, and the compositional heterogeneity index, which indicates if the composition changes as the length of the macromolecular chain grows.

In the minimization program based on chain statistics (MACO4),[2] the composition is derived from the value at the minimum and one recovers also the number-average lengths and the reactivity ratios (once the monomer feed is known).

$$\min(\|r\|/\|e\|) \rightarrow \{c_A c_B n_A n_B r_A r_B\}$$

where $\|r\|$ and $\|e\|$ are euclidean norms. The compositional heterogeneity index can be recovered too, although it requires specific calculations.[2]

As an alternative to the chain statistics, other procedures exist, i.e., the composition estimates, a linear combination of mass spectral intensities that yields

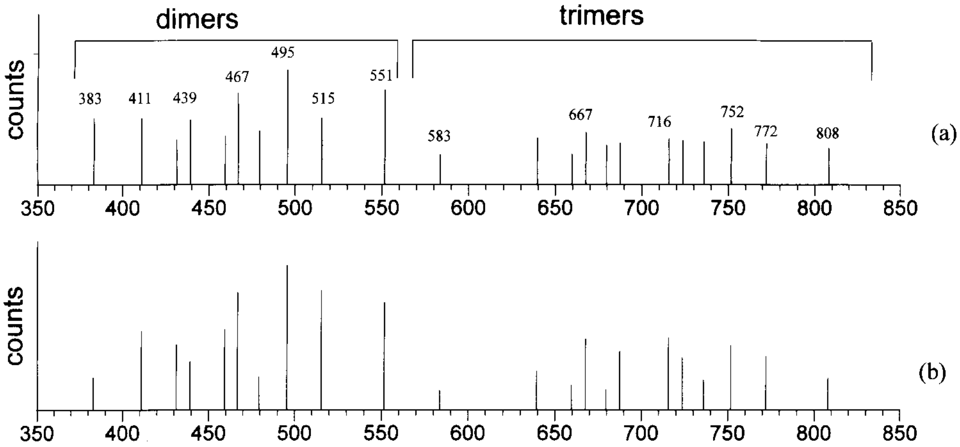

Figure 8. Mass spectra for peaks belonging to mass series HO/.../OCH$_3$ for the sample obtained by subjecting PBSu/PBA/PBT/PBSe to partial degradation: (a) experimental FAB mass spectrum; (b) simulated mass spectrum.

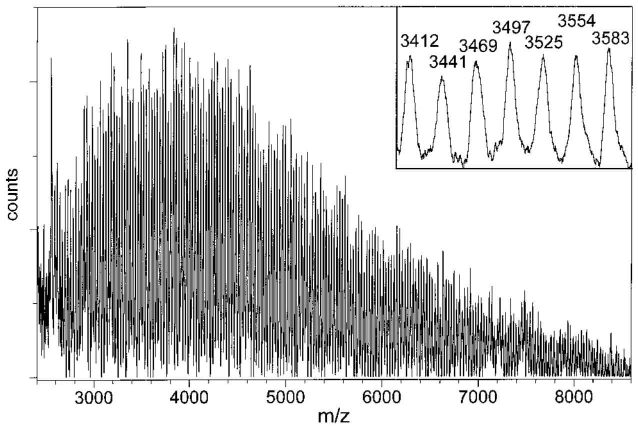

Figure 9. MALDI-TOF mass spectrum for sample PBSu/PBA/PBSe. The inset reports an expansion of the mass range 3400–3800 cm^{-1}.

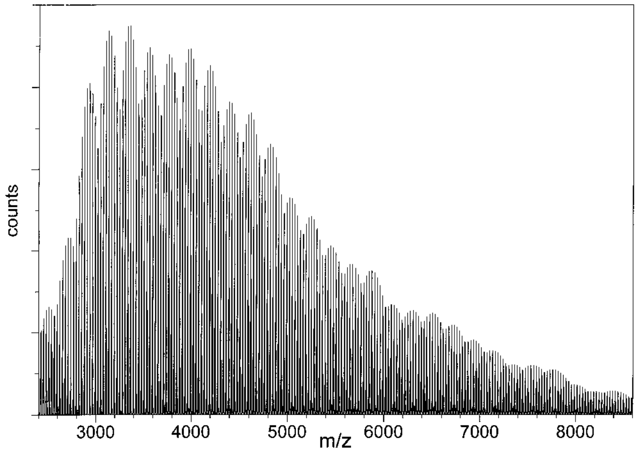

Figure 10. Simulated mass spectrum for sample PBSu/PBA/PBSe.

copolymer composition.[2] A slightly different combination gives the number-average lengths.[6] If the feed value is known, the composition estimates yield the reactivity ratios.[31]

Both chain statistics and composition estimates procedures have the same finality and are both used.[1−19]

The method of composition estimates has the clear advantage of making no assumptions on the comonomer units distribution, whereas the statistical method should perform better in the case of convoluted peaks (i.e., peaks corresponding to different comonomers which have the same mass), since it performs the peak deconvolution procedure.

We have made computations, using both methods, on some mass spectra of copolymers which appeared in the literature[6,12,19] and have compared the results.

We considered a FAB mass spectrum[12] of a copolymer sample containing units of β-hydroxybutyrate and β-hydroxyvalerate (HB/HV), with total composition 87/13. The sample is a mixture of an HB homopolymer and an HB/HV copolymer, in which the composition of the second component (HB/HV) is known to be 72/28.

When the estimates formula is applied, the result was a second component of the mixture HB/HV = 63/37. When the chain statistics is applied, the result was a second component of the mixture HB/HV = 74/26. Therefore, the estimates formula gives an erroneous result.

A possible explanation is that the composition obtained by estimates for copolymer mixtures may be affected by error, since the mass spectral peaks of the mixture originate from two different contributions and the estimates procedure is not able to deconvolute the contribution of interest.

We considered the FAB mass spectrum[6] of a copolymer sample containing units of ethylene terephthalate (ET) and ethylene adipate (EA), obtained by attacking the ET/EA copolymer with piperidine for 20 min.

We computed the average length of adipate and terephthalate blocks before the degradation, applied the estimates formula, and obtained n_{EA} before degradation = 1.6 and n_{ET} before degradation = 2.

We applied the chain statistics, and the result was n_{EA} before degradation = 7.1 and n_{ET} before degradation = 2.

The value of n_{ET} is identical in the two cases, whereas the n_{EA} value is affected by a huge error, which makes the result unacceptable. The explanation of this result is that the piperidine attack reduces the average length of the chains. Since the average length of long terephthalate blocks is small, it is negligibly influenced by degradation. On the other hand, the average length of long adipate blocks is high, and it is strongly influenced by degradation.

We then considered a group of three mass spectra[19] of a copolymer containing methyl methacrylate (MMA) and butyl acrylate (BA) units.

The samples were polymerized using different MMA/BA molar ratios in the feed,[19] namely, 86.4/13.6, 73.8/26.2, and 62.1/37.9.

We entered the mass spectral intensities into the MACO program to compute the reactivity ratios which gave as output reactivity ratios $r_{MMA} = 2.1$ and $r_{BA} = 0.7$.

We computed the composition estimates, which were used to compute the reactivity ratios using the method[25] of Polic et al. The result was reactivity ratios $r_{MMA} = 1.6$ and $r_{BA} = 0.82$.

This difference may be due to the fact that the information contained in the mass spectrum (composition and sequence) is compacted in a single value (the composition). This causes a loss of information which in turn causes an error in reactivity ratio computation.

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Supporting Information Available: Table 1S reports the assignments for the peaks in the MALDI mass spectrum of sample PBSu/PBA/PBSe (5 pages). Ordering and Internet access information is given on any current masthead page.

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