Macromolecules 1991, *24*, 1231–1236

Microstructure of Bacterial Poly(β-hydroxybutyrate-*co*-β-hydroxyvalerate) by Fast Atom Bombardment Mass Spectrometry Analysis of the Partial Pyrolysis Products

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Received April 11, 1990; Revised Manuscript Received July 2, 1990

ABSTRACT: The microstructure of poly(β-hydroxybutyrate-*co*-β-hydroxyvalerate) (P(HB-*co*-HV)) has been studied by mass spectrometry. We describe a novel method for the structural analysis of P(HB-*co*-HV) copolymers, based on the direct FAB-MS analysis of the partial pyrolysis products. The FAB spectra obtained allow the identification of the copolymer microstructure up to the hexads level, and a comparison between the experimental and calculated peak intensities makes it possible to discern clearly if the sample is made of a single random copolymer or of a mixture of two random copolymers. In addition, an algorithm has been developed that also allows the determination of both the amount of the random copolymers in the binary P(HB-*co*-HV) mixtures, as well as the copolymer composition of the individual copolymers. The highly discriminating power of the method developed here is due to the possibility of fitting the calculated statistical abundances with the MS experimental values corresponding to higher oligomers, i.e., hexamers as compared to diad and triad monomer sequences seen by ^{13}C NMR.

Introduction

The microstructure of poly(β-hydroxybutyrate) (PHB) and poly(β-hydroxybutyrate-*co*-β-hydroxyvalerate) (P(HB-*co*-HV)) has been recently studied.[1-5]

These polymers have attracted much attention because of their microbial origin and of their unique properties.[6-8]

The sequence distribution of P(HB-*co*-HV) copolymers has been deduced from diad and triad analysis by ^{13}C NMR spectrometry, and it has been concluded that some of the bacterially synthesized P(HB-*co*-HV) samples are random copolymers of HB and HV units, but others are mixtures of P(HB-*co*-HV) random copolymers with different compositions.[5]

However, NMR can hardly discern beyond the triad level, and the distinction between a single copolymer and a mixture of copolymers is sometimes too difficult.[5]

Mass spectrometry is able to look at the mass of individual molecules in a mixture, and it is therefore an interesting alternative to NMR.

We have described how such copolyesters can be analyzed for sequence distribution by fast atom bombardment mass spectrometry (FAB-MS) of the oligomers formed by partial methanolysis of the copolymers.[4]

In this report we describe a novel method for the structural analysis of P(HB-*co*-HV) copolymers, based on the direct FAB-MS analysis of the partial pyrolysis products.

The FAB spectra obtained allow the identification of the copolymer microstructure up to the hexads level, and a comparison between the experimental and calculated peak intensities makes it possible to discern clearly if the sample is made of a single random copolymer or of a mixture of two random copolymers.

The highly discriminating power of the method developed here is due to the possibility of fitting the calculated statistical abundances with the experimental values corresponding to higher oligomers (hexamers as compared to diads and triads, which are seen by ^{13}C NMR).

We have recorded the negative FAB mass spectra of residues from partial pyrolysis of PHB and P(HB-*co*-HV), using a standard thermogravimetric (TG) apparatus to monitor the pyrolysis and to stop it at a predetermined weight-loss level.

Once the partial pyrolysis is performed, the residue consists of a mixture of oligomers, and FAB-MS provides an excellent means to detect intense molecular ions corresponding to the higher oligomers present in the mixture. This method is fast and accurate. Its advantage being that it simply requires one TG run followed by one FAB mass spectrum, thus avoiding the time-consuming partial methanolysis and HPLC fractionation described previously.[4]

Experimental Section

Materials. PHB (MW 800 000), P(HB-*co*-19%HV) (MW 800 000, code 28,248-0, lot no. 1034560, catalog 1986-87) and P(HB-*co*-28%HV) (code 28,248-0, lot no. 0728469, catalog 1988-89) were obtained from Aldrich Chemical. Co. P(HB-*co*-6%HV) (MW 750 000) and P(HB-*co*-13%HV) (MW 750 000) were obtained from Marlborough Biopolymers Ltd. (Biopol).

The 250-MHz ^{1}H NMR spectra of each sample gave the composition indicated above on the basis of the ratio of the methyl triplet at 0.895 ppm and the methyl doublet at 1.274 ppm. The peak assignments are according to the literature.[1,3] Manufacturer compositions and compositions determined from NMR and from FAB mass spectra (see below) are indicated in Table I. The mixtures indicated in Table I were obtained by dissolving

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Table I
Composition and Melting Points of P(HB-*co*-HV) Samples Investigated

sample	HB/HV[a]	HB/HV[b]	HB/HV[c]	mp, °C
1	100/0	100/0	100/0	180
2	94/06	93/07	91/09	165
3	87/13	85/15	80/20	145
4	81/19	78/22	70/30	120
5	72/28	72/28	70/30	110
6	86/14[d]			110–180
7	83/17[e]			110–165

[a] Obtained from ^{1}H NMR spectrum (mol %). [b] Obtained from FAB mass spectrum (mol %). [c] Manufacturer composition (mol %). [d] Mixture (50% sample 1 + 50% sample 5). [e] Mixture (50% sample 2 + 50% sample 5).

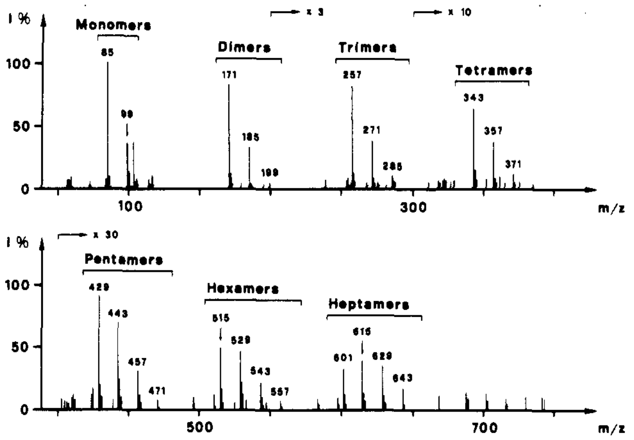


Figure 1. Negative ion FAB mass spectrum of the TG residue from the partial pyrolysis of P(HB-*co*-15%HV) (sample 3).

the two components, in $CHCl_3$. $CHCl_3$ was then evaporated at 50 °C under vacuum.

Partial Pyrolysis. Partial pyrolyses of microbial polyesters were performed with a Perkin-Elmer TGS/2 thermogravimetric (TG) apparatus, in a nitrogen atmosphere (60 mL/min) at a heating rate of 10 °C/min, up to a temperature at which the weight loss was 2, 5, 10, 20, 40, and 60%. At this temperature the TG apparatus was set to a rapid cooling mode (100 °C/min); the residue was recovered at room temperature. This residue was found to be completely soluble in chloroform.

DSC Measurements. A DSC Mettler TA 3000 was used to determine the melting points of the samples at a heating rate of 10 °C/min. These measurements refer to first heatings.

Negative Ion FAB Mass Spectra. A double-focusing Kratos MS 50 S mass spectrometer, equipped with the standard FAB source and a DS 90 data system, was used to obtain mass spectra. The FAB gun (Ion Tech) was operated in negative mode with a 7–8-keV xenon beam. The instrument was scanned from m/z 2200 to 60, with a scan rate of 10 s/decade. The accelerating voltage was 6–8 kV. Cesium and rubidium iodides (50:50 by weight) were used for computer calibration. The resolution was approximately 3000.

Samples obtained from partial pyrolysis of the polymers in TG experiments were placed on the copper target of the direct insertion probe and mixed with 3-nitrobenzyl alcohol as a matrix to dissolve the oligomers. Peak intensity values shown in mass spectra or in tables represent the average of three separate mass spectra. No significant differences were observed between the results obtained from the partial pyrolysis of the same sample, stopped at different weight losses, if the calculations (see below) were performed on the dimers, trimers, and tetramers. However, more reproducible results were observed from samples pyrolyzed up to 60% weight loss, when the calculations were performed also on the pentamers and hexamers. Deviations in the values of the relative peak intensities were found to be below 3%.

The peaks corresponding to heptamers were also detected in the spectra, but their intensities proved too low and not as reproducible as those corresponding to lower oligomers. Therefore they were not considered in the calculations.

Table II
Identification of the Pyrolysis Products from P(HB-*co*-HV) Samples by FAB-MS Analysis

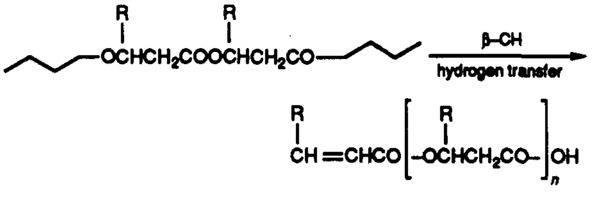
oligomers[a]	m/z (M − H)$^-$	oligomers[a]	m/z (M − H)$^-$
B	85	B_5	429
V	99	B_4V	443
B_2	171	B_3V_2	457
BV	185	B_2V_3	471
V_2	285	B_6	515
B_3	257	B_5V	529
B_2V	271	B_4V_2	543
BV_2	285	B_3V_3	557
B_4	343	B_2V_4	571
B_3V	357	B_7	601
B_2V_2	371	B_6V	615
BV_3	385	B_5V_2	629
		B_4V_3	643

[a] The pyrolysis products contain variable amounts of the following monomer units: $RCH{=}CHCO[-OCRHCH_2CO-]_nO^-$, where R = $-CH_3$ (B), $-CH_2CH_3$ (V).

The experimental peak intensities corresponding to monomers were also excluded from the calculations (see below). In fact, for each set of oligomers, from dimers to hexamers, the volatility difference in the TG experiments can be assumed negligible. Instead, for the two monomers HB and HV the difference in volatility cannot be neglected, and thus their peak intensity does not exactly reflect the copolymer composition.

Results and Discussion

The thermal decomposition of PHB and of P(HB-*co*-HV) has been investigated,[9-12] and the results indicate that it occurs quite selectively by a β-hydrogen transfer process, with formation of oligomers bearing carboxyl and olefin end groups (eq 1, R = $-CH_3$ (B); $-C_2H_5$ (V)).



$$\sim\!\sim\mathrm{O}\overset{R}{\overset{|}{\mathrm{C}}}\mathrm{HCH_2COO}\overset{R}{\overset{|}{\mathrm{C}}}\mathrm{HCH_2CO}\sim\!\sim \xrightarrow[\text{hydrogen transfer}]{\beta\text{-CH}} \overset{R}{\overset{|}{\mathrm{C}}}\mathrm{H{=}CHCO}\left[-\mathrm{O}\overset{R}{\overset{|}{\mathrm{C}}}\mathrm{HCH_2CO}-\right]_n\mathrm{OH} \qquad (1)$$

Furthermore it has been established[12] that the time-dependent changes in molecular weights during the thermal degradation follow the kinetic model of random chain scission at the ester group and that the rates of random chain scission were independent on the type of the compositions of the copolyesters.

The partial pyrolysis of PHB and P(HB-*co*-HV) samples was performed using a standard thermogravimetric apparatus (TG) to monitor the pyrolysis and to stop it at a predetermined weight loss level (typically 2, 5, 10, 20, 40, and 60%).

Once the partial pyrolysis is performed, the residue consists of a mixture of oligomers having the end groups described in eq 1, and FAB-MS provides an excellent means to detect intense molecular ions corresponding to the higher oligomers present in the mixture.

As these acid compounds can easily lose one proton when they are bombarded in a FAB source, we have recorded the spectra in negative FAB mode, revealing them as carboxylate ions.

The negative FAB mass spectrum of the pyrolate of a P(HB-*co*-15%-HV) copolymer (sample 3, Table I) at 60% weight loss is shown in Figure 1. It consists essentially of the (M − H)$^-$ pseudomolecular ions corresponding to oligomers of the structure shown in eq 1 (the matrix peaks

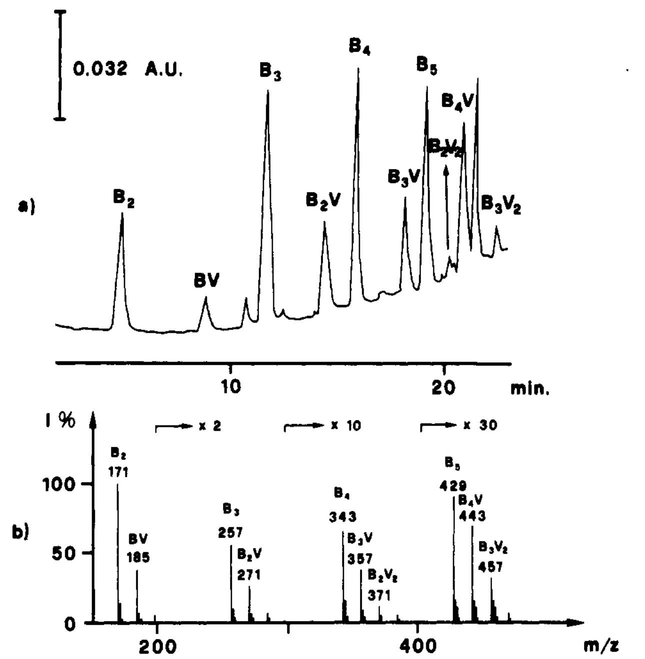


Figure 2. HPLC separation of the partial methanolysis products (a) and negative ion FAB mass spectrum of the partial pyrolysis products (b) of P(HB-*co*-15%HV) (sample 3).

Table III
Experimental[a,b] and Calculated[c] Relative Amounts of the Oligomeric Degradation Products from Sample 3 by HPLC and TG FAB/MS Analyses

oligomers	HPLC[a]	TG FAB-MS[b]	calcd[c] for B/V 87/13	85/15
B_2	74	72	77	74
BV	26	28	23	26
B_3	72	68	69	65
B_2V	28	32	31	35
B_4	62	57	59	53
B_3V	31	33	34	37
B_2V_2	7	10	7	10
B_5	53	48	51	46
B_4V	40	36	38	40
B_3V_2	7	16	11	14

[a] Normalized HPLC areas of the oligomeric species produced by methanolysis (see Figure 2). [b] Normalized intensities of $(M - H)^-$ ions of the pyrolysis products in the FAB mass spectrum. [c] Relative intensities of the degradation products, calculated by eq 2 for two copolymer compositions.

have been subtracted). The assignments for the oligomers identified are reported in Table II.

The spectrum in Figure 1 shows little evidence of the molecular ions fragmentation and might therefore be used for quantitative analysis, i.e., to estimate the copolymer composition and sequence distribution.

To test this hypothesis, we have compared the above FAB-MS data with those obtained in the HPLC analysis of the partial methanolysis products of the same copolymer sample, as shown in Figure 2.

In Figure 2a a section of the HPLC trace is reproduced, which was previously obtained from the methanolysis products of the P(HB-*co*-15%-HV) copolymer,[4] and the corresponding section of the TG-FAB/MS spectrum is shown in Figure 2b (which is a portion of Figure 1).

The relative amounts for each set of the oligomeric products, from dimers to pentamers, obtained by the two partial degradation methods, are compared in Table III. It can be noticed that the level of agreement can be regarded as satisfactory, considering that the oligomers V_2, BV_2, BV_3, and B_2V_3 are present in very low quantity and are not seen in the HPLC trace. Furthermore, by comparing the two experimental results with the calculated ones, it can be seen that the best fit value is 87/13 for the HPLC and 85/15 for the TG-FAB/MS analysis. Therefore, the results are not very far from each other. Similar results were found by comparing HPLC traces with the corresponding TG-FAB/MS spectra for the other samples investigated here.

Table IV
Experimental[a] and Calculated[b] Normalized Abundances of the Partial Pyrolysis Products from FAB-MS Analysis of P(HB-*co*-HV) Sample 3

oligomers	exptl[a]	calcd[b] 85/15	80/20	90/10
B_2	69	73	64	81
BV	27	25	32	18
V_2	4	2	4	1
B_3	64	61	52	73
B_2V	30	33	38	24
BV_2	6	6	10	3
B_4	55	52	41	66
B_3V	32	37	41	29
B_2V_2	10	10	16	5
BV_3	3	1	2	
B_5	45	45	33	60
B_4V	35	39	41	33
B_3V_2	16	14	21	7
B_2V_3	4	2	5	
B_6	40	38	27	53
B_5V	38	40	40	36
B_4V_2	17	18	25	10
B_3V_3	5	4	8	1

[a] Normalized intensities of the peaks in the FAB mass spectrum. [b] Normalized abundances of partial pyrolysis products calculated by eq 2.

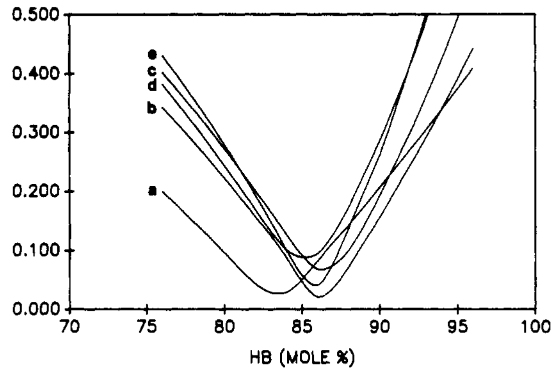


Figure 3. Agreement factor (AF) versus HB content (mol %) corresponding to each set of peak intensities for (a) dimers, (b) trimers, (c) tetramers, (d) pentamers, and (e) hexamers from sample 3.

Assuming that the same response factor to FAB-MS can be used for each set of oligomers produced in the partial pyrolysis, then the normalized intensities of the peaks corresponding to dimers, trimers, tetramers, pentamers, and hexamers ought to reflect the copolymer composition; this can be calculated on the assumption of a random distribution of the HB and HV units in the copolymer.[13]

The statistical probability $P_{x,y}$ of finding a given B_xV_y sequence, assuming Bernoullian (random) statistics for each oligomer, is given by

$$P_{x,y} = \binom{x+y}{y} P_B{}^x P_V{}^y \qquad (2)$$

where P_B and P_V are the molar fractions of HB and HV in the copolymer. The binomial coefficient in this equation is the number of possible sequence arrangements of the

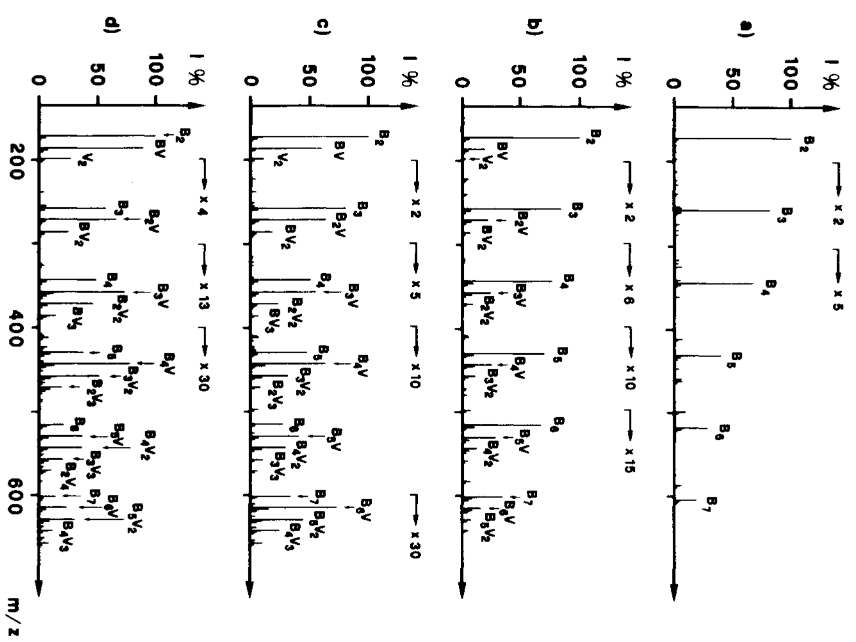


Figure 4. Negative ion FAB mass spectra of the TG residues from the partial pyrolysis of samples 1 (a), 2 (b), 4 (c), and 5 (d) (Table I).

B_xV_y oligomers. Starting with an arbitrary set of P_B and P_V values, it is possible to calculate the statistical abundances, $P_{x,y}$, for the oligomers actually present in the pyrolized copolymer. The best match between the experimentally observed oligomer distributions and the calculated statistical abundances identifies the copolymer composition.

The results of these calculations are reported in Table IV. The experimental data used are the normalized intensities of the oligomeric species appearing in the FAB spectrum in Figure 1. The agreement between the observed and calculated values was a function of the P_B/P_V ratio, and the best fit was for a ratio 85/15 (Table IV), i.e., almost the same value (86/14) obtained for the same sample when FAB mass spectra were obtained from lyophilized fractions of the methanolysis products.

The difference between observed and calculated values can be expressed in terms of error by means of the Hamilton[14] agreement factor (AF):

$$AF = [\sum_i (I_{\exp_i} - I_{\mathrm{calcd}_i})^2 / \sum_i I_{\exp_i}{}^2]^{1/2} \tag{3}$$

where $I_{\exp_i}$ and I_{calcd_i} are the normalized experimental and calculated abundances of partial pyrolysis products.

Figure 3 illustrates the calculated AF curves as a function of the copolymer composition for sample 3. Each curve corresponds to a specific set of oligomers (dimers, trimers, etc.), and it can be noticed that the minima indicate almost the same composition of about 15 mol % HV, whereas the AF value at the minimum is roughly constant (below 10%).

In Figure 4 are shown the partial pyrolysis FAB mass spectra of the remaining three P(HB-*co*-HV) copolymers investigated, together with the spectrum of the pure PHB homopolymer. Careful inspection of the spectra in Figure

Table V
Experimental and Calculated Normalized Abundances of Partial Pyrolysis Products for P(HB-*co*-HV) Samples

sample		copolym[a] compn B/V	B_2	BV	V_2		B_3	B_2V	BV_2		B_4	B_3V	B_2V_2	BV_3		B_5	B_4V	B_3V_2	B_2V_3		B_6	B_5V	B_4V_2	B_3V_3	B_2V_4		mixture compn A/B/X
2	exptl[b]		84	15	1		79	18	3		75	22	3			70	23	6			65	26	9				
	calcd[c]	93/07	86	13	1		80	18	2		75	20	5			70	26	4			65	29	6				
	AF[d]					0.03				0.02					0.04					0.05						0.06	
3	exptl[b]		69	27	4		64	30	6		55	32	10	3		45	35	16	4		40	38	17	5			
	calcd[c]	85/15	73	25	2		61	31	6		58	34	7	1		45	39	14	2		43	39	15	3			
	AF[d]					0.06				0.06					0.08					0.08						0.07	
4	exptl[b]		59	35	6		49	40	11		38	40	17	5		32	42	20	6		24	39	27	10			
	calcd[c]	78/22	61	34	5		48	41	11		37	42	17	4		29	41	23	7		23	39	27	11			
	AF[d]					0.03				0.02					0.04					0.08						0.03	
5	exptl[b]		49	41	10		39	44	17		28	40	25	7		21	41	28	10		18	30	30	15	7		
	calcd[c]	72/28	52	40	8		38	45	17		27	42	24	6		20	39	30	11		14	32	32	16	5		
	AF[d]					0.06				0.02					0.05					0.06						0.10	
6	exptl[b]		70	24	6		65	25	10		60	22	14	4		54	22	16	8		56	16	19	9			1/0.74/0.4[h]
	calcd[c]	86/14[f]	74	24	2		64	31	5		55	35	9	1		47	38	12	2		40	40	16	3			
	AF[d]					0.07				0.11					0.22					0.31						0.50	n
	calcd[e]		76	20	4		69	22	9		64	21	12	3		60	19	15	6		59	17	16	8			1/0.72/0.5[g]
	AF[d]					0.09				0.07					0.07					0.11						0.07	
7	exptl[b]		66	30	4		59	30	11		48	31	18	3		42	32	18	8		39	31	20	10			0.95/0.73/0.4[h]
	calcd[c]	83/17[f]	69	28	3		58	35	7		48	39	12	1		40	40	17	3		33	41	21	5			
	AF[d]					0.05				0.08					0.16					0.15						0.22	
	calcd[e]		69	27	4		60	31	9		51	32	14	3		45	32	17	6		40	32	19	9			0.93/0.72/0.5[g]
	AF[d]					0.06				0.04					0.08					0.06						0.04	

[a] B/V best-fit values calculated by eqs 2 and 3. [b] Relative intensities of the peaks in FAB mass spectrum. [c] Relative intensities calculated by eqs 2 and 3 for the B/V best-fit composition values. [d] Agreement factor calculated by eq 3. [e] Relative intensities calculated by eqs 4–8 using the known values of *A*, *B*, and *X* from Table I. [f] B/V content (mol %) in the mixture from Table I. [g] *A*, *B*, and *X* known values. [h] *A*, *B*, and *X* best-fit values calculated with algorithm described in the text.

4 reveals that the relative intensities of the peaks within each cluster change sensibly with the HV content in the copolymer.

In Table V are reported the experimental and calculated normalized abundances of the partial pyrolysis products for the samples investigated and the corresponding AF for the best fit values.

From Table V it can be seen that for the pure copolymers (samples 2–5) the best-fit copolymer compositions, calculated by eqs 2 and 3, are in excellent agreement with those determined by ^{1}H NMR (reported in Table I).

In Figure 5b,c the FAB mass spectra are shown for the partial pyrolysis products of two physical mixtures of P(HB-*co*-HV), having an overall B/V composition very similar to that of the pure copolymer shown in Figure 5a.

Significant differences in the relative intensities of the peaks are discernible among the three spectra in Figure 5. When we tried to calculate the average copolymer compositions by means of eq 2, the AF values for the two mixtures (samples 6 and 7 in Table V) revealed an increasing error level going from lower to higher oligomers. This is shown in Figure 6, where the AF values calculated according to eq 3 for sample 6, are plotted as a function of the copolymer composition.

Contrary to the case reported in Figure 3, which pertains to a pure copolymer (sample 3, Table I), for the mixture in Figure 6 the minimal AF values show a drastic increment going from lower to higher oligomers, and furthermore the minima do not fall at the same composition value. This is because eq 2 assumes a Bernoullian (random) comonomer distribution, whereas sample 6 is actually a mixture of two polymers. Evidently, higher oligomers are more sensitive in discriminating a pure copolymer from a mixture because they carry more specific sequential information. This example indicates the high discriminating power of the present MS method, which is capable of determining the relative abundances of oligomers up to the hexamers.

Furthermore, we have verified the absence of ester exchange processes in several poly(β-lactones) and in P(HB-*co*-HV) mixtures, when they are heated in a TG experiment, and have concluded that the rate of the hydrogen-transfer process described in eq 1 is much higher than that of the transesterification reaction, below 220 °C, for poly(β-lactones).[15]

Determination of the Copolymer Composition in the Case of Two-Component Mixtures

Having developed a rapid method for determining the composition of a random copolymer and for discriminating a pure copolymer from a mixture of copolymers, we wish now to discuss an algorithm for determining the composition of the two copolymers that constitute the blend.

Given a mixture of two Bernoullian random P(HB-*co*-HV) copolyesters, let A be the molar fraction of HB in the copolyester 1, B the molar fraction of HB in the copolyester 2, and X the molar fraction of copolyester 1 in the mixture. Then the following equations can be written for each set of oligomers:

dimers

$$F_{B_2} = A^2X + B^2(1 - X)$$

$$F_{BV} = 2[A(1 - A)X + B(1 - B)(1 - X)]$$

$$F_{V_2} = (1 - A)^2X + (1 - B)^2(1 - X) \tag{4}$$

trimers

$$F_{B_3} = A^3X + B^3(1 - X)$$

$$F_{B_2V} = 3[A^2(1 - A)X + B^2(1 - B)(1 - X)]$$

$$F_{BV_2} = 3[A(1 - A)^2X + B(1 - B)^2(1 - X)]$$

$$F_{V_3} = (1 - A)^3X + (1 - B)^3(1 - X) \tag{5}$$

tetramers

$$F_{B_4} = A^4X + B^4(1 - X)$$

$$F_{B_3V} = 4[A^3(1 - A)X + B^3(1 - B)(1 - X)]$$

$$F_{B_2V_2} = 6[A^2(1 - A)^2X + B^2(1 - B)^2(1 - X)]$$

$$F_{BV_3} = 4[A(1 - A)^3X + B(1 - B)^3(1 - X)]$$

$$F_{V_4} = (1 - A)^4X + (1 - B)^4(1 - X) \tag{6}$$

pentamers

$$F_{B_5} = A^5X + B^5(1 - X)$$

$$F_{B_4V} = 5[A^4(1 - A)X + B^4(1 - B)(1 - X)]$$

$$F_{B_3V_2} = 10[A^3(1 - A)^2X + B^3(1 - B)^2(1 - X)]$$

$$F_{B_2V_3} = 10[A^2(1 - A)^3X + B^2(1 - B)^3(1 - X)]$$

$$F_{BV_4} = 5\,[A(1 - A)^4X + B(1 - B)^4(1 - X)]$$

$$F_{V_5} = (1 - A)^5X + (1 - B)^5(1 - X) \tag{7}$$

hexamers

$$F_{B_6} = A^6X + B^6(1 - X)$$

$$F_{B_5V} = 6[A^5(1 - A)X + B^5(1 - B)(1 - X)]$$

$$F_{B_4V_2} = 15[A^4(1 - A)^2X + B^4(1 - B)^2(1 - X)]$$

$$F_{B_3V_3} = 20[A^3(1 - A)^3X + B^3(1 - B)^3(1 - X)]$$

$$F_{B_2V_4} = 15[A^2(1 - A)^4X + B^2(1 - B)^4(1 - X)]$$

$$F_{BV_5} = 6[A(1 - A)^5X + B(1 - B)^5(1 - X)]$$

$$F_{V_6} = (1 - A)^6X + (1 - B)^6(1 - X) \tag{8}$$

where $F_{B_nV_m}$ is the normalized intensity of the MS peak corresponding to each B_nV_m oligomer. The normalization factor is the sum of intensities within each set of equations.

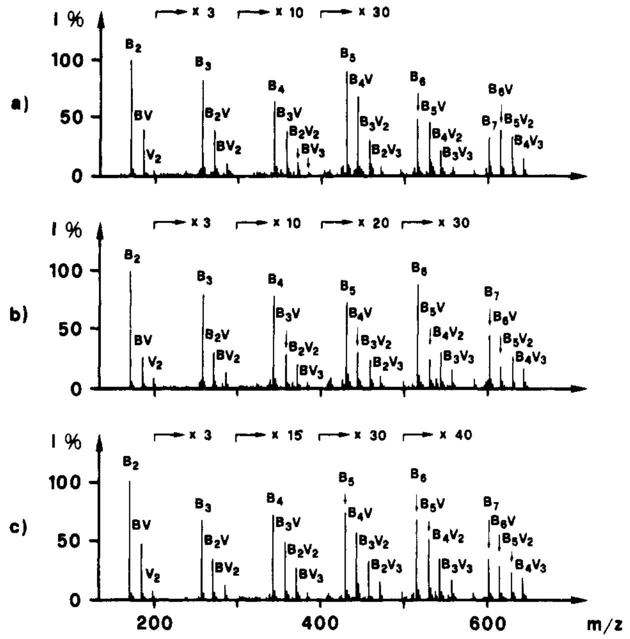


Figure 5. Negative ion FAB mass spectra of the TG residues from the partial pyrolysis of samples 6 (a), 7 (b), and 3 (c) (Table I).

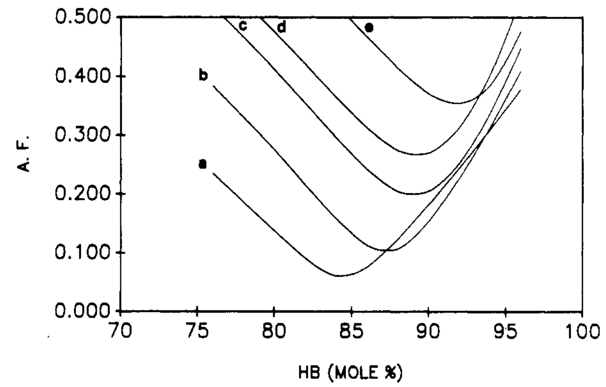


Figure 6. Agreement factor (AF) versus HB content (mol %) corresponding to each set of peak intensities for (a) dimers, (b) trimers, (c) tetramers, (d) pentamers, and (e) hexamers from sample 6.

For each term (A, B, X), these equations yield 25 values. The composition of the copolymer mixture is that which gives 25 values closest to the experimental data.

A FORTRAN-77 best-fit program named Mass Analysis of Copolymers (MACO) has been written that runs on a Microvax computer (MicroVMS4.2). The program finds the composition (A, B, X) that best fits the 25 experimental normalized intensities and employs the minimization routine MINPACK1 belonging to Argonne Library.[16]

In Table V the best-fit values of A, B, and X are reported for the two mixtures analyzed.

In Table V are also reported the normalized oligomer abundances calculated by using eqs 4–8 with the known values A, B and X from Table I for each mixture.

It can be seen that these calculated relative intensities are in good agreement with the experimental ones, confirming that the FAB-MS is a suitable technique for determining the relative amounts and copolymer compositions of binary P(HB-*co*-HV) mixtures.

Acknowledgment. Partial financial support from the Italian Ministry for University and for Scientific and Technological Research (MURST) and from National Council of Research (CNR) (Rome), Finalized Project of Fine and Secondary Chemistry, is gratefully acknowledged.

References and Notes

(1) Bloembergen, S.; Holden, D. A.; Hamer, G. K.; Bluhm, T. L.; Marchessault, R. H. *Macromolecules* **1986**, *19*, 2865; **1989**, *22*, 1663.
(2) Bluhm, T. L.; Hamer, G. K.; Marchessault, R. H.; Fyfe, C. A.; Veregin, R. P. *Macromolecules* **1986**, *19*, 2871.
(3) Doi, Y.; Kunioka, M.; Nakamura, Y.; Soga, K. *Macromolecules* **1986**, *19*, 2860.
(4) Ballistreri, A.; Garozzo, D.; Giuffrida, M.; Impallomeni, G.; Montaudo, G. *Macromolecules* **1989**, *22*, 2107.
(5) Kamiya, N.; Yamamoto, Y.; Inoue, Y.; Chujo, R.; Doi, Y. *Macromolecules* **1989**, *22*, 1672; **1989**, *22*, 3800.
(6) Holmes, P. A. *Phys. Technol.* **1985**, *16*, 32.
(7) King, P. P. *J. Chem. Technol. Biotechnol.* **1982**, *32*, 2.
(8) Holland, S. J.; Jolly, A. M.; Yesin, M.; Tighe, B. *J. Biomater.* **1987**, *8*, 289.
(9) Morikawa, H.; Marchessault, R. H. *Can. J. Chem.* **1981**, *59*, 2306.
(10) Grassie, N.; Murray, E. J.; Holmes, P. A. *Polym. Deg. Stab.* **1984**, *6*, 127.
(11) Helleur, R. *J. Polym. Prepr.* (*Am. Chem. Soc., Div. Polym. Chem.*) **1988**, *29*, 609.
(12) Kunioka, M.; Doi, Y. *Macromolecules* **1990**, *23*, 1933.
(13) Randall, J. C. *Polymer Sequence Determination*; Academic Press: New York, 1977; Chapter 4.
(14) Hamilton, W. C. *Acta Crystallogr.* **1965**, *18*, 502.
(15) To be published.
(16) The program listing is available on request.

Registry No. (HB)(HV) (copolymer), 80181-31-3.