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Bulk and Surface Quantification of a Biodegradable and -medical Copolyester

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ABSTRACT: A multitechnique analysis approach has been used to study the bulk and surface properties of a biodegradable copolymer produced from agricultural feedstocks. The sample analyzed is a thermoplastic copolyester commercialized under the trademark Biopol that contains β-hydroxybutyrate (HB) and β-hydroxyvalerate (HV) units incorporated randomly throughout the polymer chain. The techniques applied are gas chromatography mass spectrometry (GC-MS), nuclear magnetic resonance spectroscopy (NMR), differential scanning calorimetry (DSC), time-of-flight secondary ion mass spectrometry (TOF-SIMS), and X-ray photoelectron spectroscopy (XPS). Special emphasis is put on the quantitative determination of the surface composition of the copolyester by TOF-SIMS and XPS as well as the analysis of the molar bulk fraction by NMR and DSC.

Introduction

The current interest in the poly(β-hydroxybutyrate-*co*-β-hydroxyvalerate) (P(HB-*co*-HV), tradename Biopol) copolymer system is largely based on its biodegradability as a natural polyester. Generally PHB polymer derivatives are produced by fermentation of sugar with a specific microorganism Alcaligenes eutrophus.[1] Up to 80% of the dry weight of this bacteria consists of the polymeric feedstock from which a polymer granule can be extracted. The addition of propionic acid to the nutrient feedstock supplied to the bacteria yields a statistic[2,3] copolymer consisting of up to 30% of HV (see formula in Figure 1). Depending on the valerate content (HV) of the copolymer, the melting point of Biopol can be lowered to 90 °C.[2,3] This is favorable for the melting process during manufacturing to avoid thermal degradation of the product. Up to now thermoplastic biopolymers have attracted industrial attention as a candidate for large-scale biotechnological production and have been introduced in the market by Imperial Chemical Industries (ICI) in the early 1980s.[4] Due to their industrial availability, a variety of applications were studied such as the use as a biodegradable packaging material, which according to Hocking and Marchessault[5] could lead to applications in food packaging or as a replacement of PET for plastic beverage bottles. In addition, Biopol represents a promising biocompatible material[5] for controlled drug release, tissue engineering, etc.

The present paper is focused on the comparison between bulk and surface quantifications of the monomer units (valerate and butyrate) from a commercial Biopol-foil. Nuclear magnetic resonance (NMR) is an established technique for determining the bulk monomer distribution of Biopol materials.[2,6] Melting point diagrams as obtained by differential scanning calorimetry (DSC) for different P(HB-*co*-HV) compositions[2] are useful in determining the stoichiometry of Biopol by means of melting point endotherms.

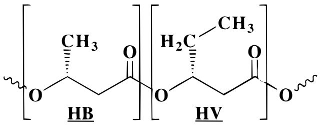

Figure 1. Chemical formula of a Biopol copolymer containing HB [β-hydroxybutyrate] and HV [β-hydroxyvalerate] units.

Up to now surface sensitive information by secondary ion mass spectrometry (SIMS) or X-ray photoelectron spectroscopy (XPS) for this system[7−9] was regarded to be essentially qualitative. A first approach to quantitative time-of-flight SIMS (TOF-SIMS) analysis (copolymer composition) was presented in a previous study,[10] however limited to the positive mass range. In the present paper a detailed TOF-SIMS analysis is demonstrated for the positive and negative mass ranges, including the identification of ion fragments and the discussion of proposed constitution formulas. The mass fragments originating from Biopol are classified with respect to the monomer units (valerate and butyrate), and corresponding secondary ion intensities are evaluated for the surface quantification of the copolymer composition. In addition, the chemical information of XPS measurements from the Biopol surface and the utility of XPS data in determining the copolymer composition are studied. The surface composition of the investigated Biopol sample is discussed with respect to

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the bulk composition obtained from NMR and DSC measurements.

Experimental Section

Material (Sample Preparation). The P(HB-*co*-HV) sample used in this work was a technical grade Biopol (ZENECA Bioproducts) manufactured to semicrystalline packaging foil by Safta (Piacenza, Italy). These materials are introduced in the market as nonfood containers, compostable garbage bags, transparent office folders, etc.

Nuclear Magnetic Spin Resonance Spectrometry. Solution ^1H spectra were recorded on a Bruker AMX 500 spectrometer operating at 500.135 MHz at room temperature. Samples were prepared in deuterated chloroform (10−20 mg/mL) containing tetramethylsilane (TMS, Me_4Si, $\delta = 0$) as an internal chemical shift standard.

Differential Scanning Calorimetry. Thermal data were recorded on a Mettler TA4000 system. Samples (5 mg) were heated from room temperature to 250 °C with a heating rate of 10 °C/min. To use the melting diagram of Bluhm et al. [2] (determination of the copolymer composition), the melting point of the higher temperature endotherm was considered the true melting point.

Gas Chromatography−Mass Spectrometry. A simple polymer extract was injected into a Carlo Erba HRGC 5300 Mega series gas chromatograph equipped with a 60 m DB-Dioxin bonded nonpolar column (internal diameter of 0.25 mm). The GC column temperature program was started at 110 °C with a heating rate of 15 °C/min up to a final maintained temperature of 240 °C. The mass-spectrometer detector consisted of a Hewlett-Packard 5970 series quadrupole MS with a mass range of up to 1000 *m*/*z*. Typical spectra were acquired up to a mass of 500 *m*/*z* using a sweep rate of 0.75 [*m*/*z*]/s.

Time-of-Flight Secondary Ion Mass Spectrometry. Static TOF-SIMS surface analysis was performed on a commercially available apparatus (PHI-EVANS TRIFT I) described in detail elsewhere.[11,12] Positive spectra were measured with an 8 kV and negative spectra with a 14 kV primary Cs^+ ion beam, respectively. The SIMS TOF design (“TRIFT”) includes three electrostatic analyzers (ESA) in series (i.e., the secondary ion trajectories display a 270° loop) and establishes kinetic energy compensation for improved mass resolution as well as imaging capabilities of the analyzed sample area. The secondary ions are post accelerated toward a stack of microchannel plates (MCP), and particle detection is made by means of single ion counting using a single-stop time-to-digital converter (1-stop TDC) with a time resolution of 156 ps. To improve the total secondary ion yield, the estimated ion dose was chosen to be $(3-5) \times 10^{13}$ ions/cm^2 (upper limit) slightly above the static SIMS limit.[13,14] To minimize charging of the nonconductive plastic material, a low-energy (about 20 eV) electron flood gun was operated in a pulsed mode on an unbiased sample. To establish charge compensation, the sample bias voltage had to be switched off (from 3 kV with respect to the grounded extraction electrode) to avoid electron beam damage of the polymer material.

X-ray Photoelectron Spectrometry. XPS spectra were obtained using a Perkin-Elmer Phi 5500 XPS system employing Mg $K\alpha$ ($h\nu = 1253.6$ eV) X-rays. Electron energy distributions $N(E)\,dE$ were obtained in the retarding field mode operated at a constant pass energy of 23.5 eV at a step width of 0.1 eV. Typical XPS spectra were acquired at a take off angle of 45°, for about 5 min. A low-energy electron flood gun (about 20 eV) was used in order to minimize bombardment-induced surface charging during XPS data acquisition. More detailed information on the XPS setup is given elsewhere.[15] Spectra were corrected for sample charging (referenced to C−H/C−C at 285 eV in the C 1s spectrum).

Results

Identification of Polymer Additives. To identify the presence of additives, a simple chloroform/hexane extract was injected into a GC-MS. Two GC signals originating from plasticizers were detected. From these eluded GC fractions, electron impact mass spectra (EIMS) were recorded, as displayed in Figure 2. The presence of the triacetin (glyceryl triacetate, $C_9H_{14}O_6$, 216 *m*/*z*) and citroflex (acetyl tributyl citrate, $C_{20}H_{34}O_8$, 402 *m*/*z*) was identified. Both substances are commercial additives known as nontoxic biodegradable plasticizers.[16] Possible MS peak assignments are illustrated directly within the mass spectra.

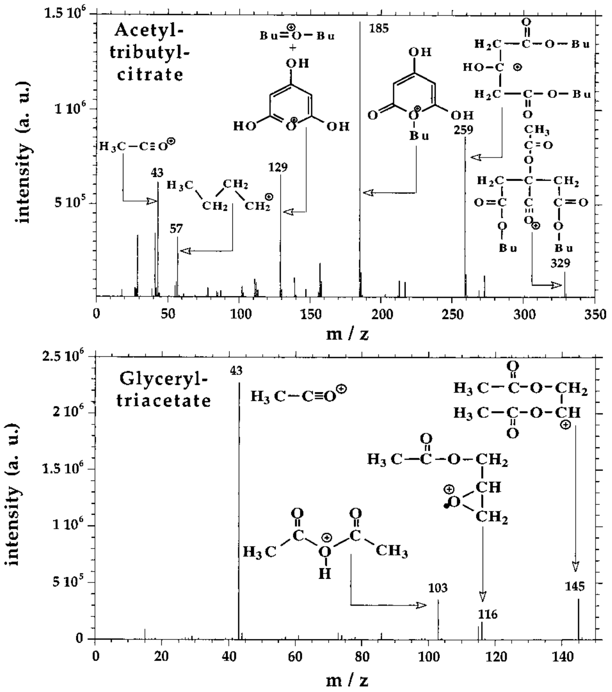


Figure 2. Electron impact mass spectra recorded by a GC-MS of the biopolymer plasticizers triacetin and citroflex.

Nuclear Magnetic Spin Resonance Spectrometry. The ^1H NMR spectrum displayed in Figure 3 of the dissolved copolymer was found to be consistent with spectra obtained from the literature.[2,6] The mole fractions of the two monomeric units were determined from the intensity ratio of the doublet CH_3−proton resonance of butyrate at 1.27 ppm to the triplet CH_3−proton resonance of valerate at 0.89 ppm. Further quantification was possible using the intensity ratio of the sextet CH−proton resonance of butyrate at 5.25 ppm to the quintet CH−proton resonance of valerate at 5.185 ppm. From these ratios a polymer butyrate content of $c_b(B) = 89.8\%$ and a valerate content of $c_b(V) = 9.2\%$ with a standard deviation of 0.6% were determined.

In addition the presence of triacetin and citroflex additives as identified by GC-MS were confirmed by NMR. From specific signal intensities, the additive mole fraction was obtained for acetyl tributyl citrate as $x_A(C) = 1.2\%$ and for triacetin as $x_A(T) = 0.5\%$. The relative error of the additive content is estimated to be 10%. Note that in this case the polymer mole fraction was calculated on the basis of the molecular weight of its monomer repetition units to give a total of $x_p(B+V) = 98.3\%$.

Differential Scanning Calorimetry. The DSC measurement in Figure 4 displays in the 150 °C region relatively sharp melting endotherms. From the total peak integral, an enthalpy of fusion ΔH_f of 18.3 ± 3 cal/g [2,3] was computed. In addition, two melting points at 142 and 154 °C were observed for the valerate contain-

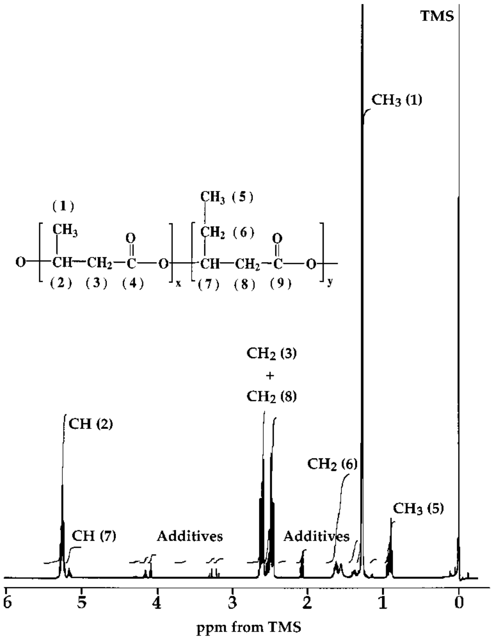


Figure 3. ^{1}H NMR spectrum at 500 MHz of the P(HB-*co*-HV) bio-polyester containing β-hydroxybutyrate and β-hydroxyvalerate units at room temperature in deuterated chloroform. Chemical shifts are in parts per million downfield from TMS (Me_4Si).

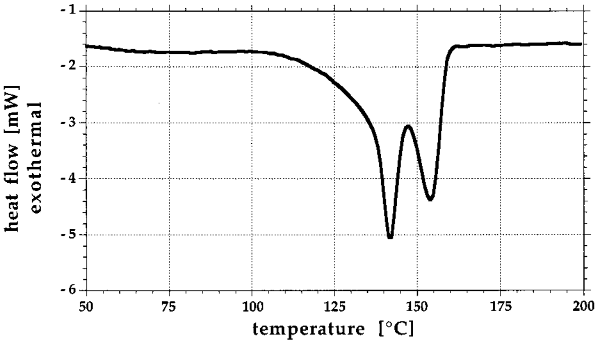


Figure 4. Differential scanning calorimetry of the P(HB-*co*-HV) bio-polyester. The higher temperature endotherm at 154 °C was taken as the true melting point.

ing PHB biopolymer. From the higher melting point a bulk composition of $c_b(V) = 12\%$ with an estimated error of 3% was deduced using the melting diagram of Bluhm et al.[2] It is important to note that this value only represents an upper limit for the HV content, since present additives are lowering the melting point T_m. Using the Flory eq 1,[17,18] the melting point depression

$$1/T_m - 1/T_m{}^\circ = -[R \ln(1 - x_A)]/\Delta H_u \qquad (1)$$

can be estimated. Here ΔH_u denotes the enthalpy of fusion per repeating units, R is the universal gas constant, T_m is the observed melting temperature, and $T_m{}^\circ$ is the corrected melting point of the copolymer. x_A represents the total mole fraction of additive, which was shown by NMR to be in the range of 1.7%. An estimation for ΔH_u of 1600 cal/mol was computed from the obtained ΔH_f and the expected averaged molar mass (between 10 and 20% HV) of the Biopol repetition unit. It should be noted the ΔH_f is used here as a rough estimation for the enthalpy of fusion of the polymer without any additives.

Applying eq 1, the observed melting point was changed to 158 °C and with the melting diagram of Bluhm et al.[2] a corrected valerate content $c_b(V) = 10 \pm 3\%$ is found.

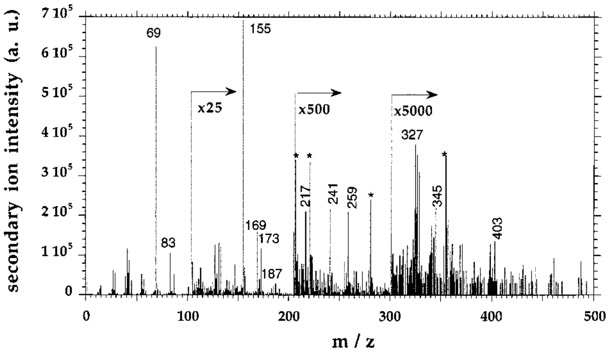


Figure 5. Positive mode secondary ion mass spectrum (8 kV Cs^+) of the P(HB-*co*-HV) bio-polyester.

Time-of-Flight Secondary Ion Mass Spectrometry. In Figure 1, the symbol B denotes butyrate (the molecule $C_4H_6O_2$ at mass 86.04 *m/z*) and V stands for valerate (the molecule $C_5H_8O_2$ at mass 100.05 *m/z*). The TOF-SIMS quantification of such a statistical copolymer is based on the assumption that matrix effects and recombination in a vacuum are negligible.[19] Considering the very similar chemical structure of both monomers HB and HV, this approach is acceptable for related ion fragments.

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

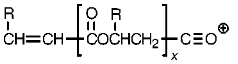
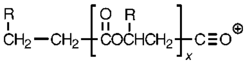
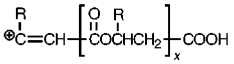
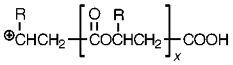
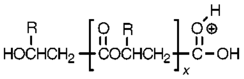
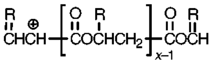
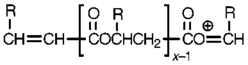
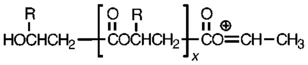
In the present case the statistical probability of formation of an oligomer fragment B_xV_y is given by eq 2, where P_B and P_V are the molar fractions of HB and HV units in the copolymer. $P_{x,y}$ is the probability of finding a given B_xV_y sequence assuming Bernoullian (random) statistics. The binomial coefficient $\binom{x+y}{y}$ represents the number of possible sequence arrangements of the B_xV_y polymer fragments. Starting with a set ($x + y =$ constant) of statistical abundances $P_{x,y}$ which correspond to a series of normalized TOF-SIMS signal intensities from characteristic polymer ion fragments, it is possible to calculate the molar fraction P_B and P_V of the copolymer units HB and HV.

Positive TOF-SIMS Spectra. A typical positive secondary ion TOF-SIMS mass spectrum of Biopol obtained under 8 kV Cs^+ bombardment is displayed in Figure 5. Tetramer ions are assigned to the masses 345.15 *m/z* for B_4H^+, 341.16 *m/z* for $B_3V(-OH)^+$, and 327.14 *m/z* for $B_4(-OH)^+$. Denotations such as (−OH) or (−H) indicate the loss of an OH group or of a proton, respectively.

Other heavy secondary ions marked with an asterisk (∗) in Figure 5 at masses 207.03, 221.08, 281.05, 355.07, and 487.08 *m/z* are contributions of fragments from lubricant PDMS (poly(dimethylsiloxane)). Mass 403.23 *m/z* consists of the protonated molecular ion peak from the acetyl tributyl citrate plasticizer (identified by GC-MS), which shows thus that it is also present at the polymer surface. Principal structures of ion fragments containing monomer repetition units and their corresponding masses are summarized in Table 1.

The ion fragment $M_{x+1}H^+$ of the protonated principal repetition unit was chosen to illustrate the variety of possible constitution formulas, which may be associated with SIMS data. All the structures sketched in Figure 6 are equal in mass and therefore cannot be distin-

Table 1. Major Positive Fragment Ions of P(HB-*co*-HV) Bio-polyester[a]

2		1			0		repetition units *x*	
VB_2	B_3	V_2	VB	B_2	V	B	positive ion structure	sum formula
255	241	183	169	155	83	69		$M_{x+1}(-OH)^+$
257	243	185	171	157	85	71		$M_{x+1}H(-O)^+$
		199	185	171	99	85		$M_{x+1}(-H)^+$
273	259	201	187	173	101	87		$M_{x+1}H^+$
				191		105		$M_{x+1}H_3O^+$
	197	139	125	111	–	–		$M_xC_2H^+$
	199	141	127	113	–	–		$M_xC_2H_3^+$
	303		231	217	145	131		$M_{x+1}OC_2H_5^+$

[a] B and V denote the butyrate and the valerate monomer units, respectively. R ascribes the alkyl rest which is either a methyl (B) or an ethyl (V).

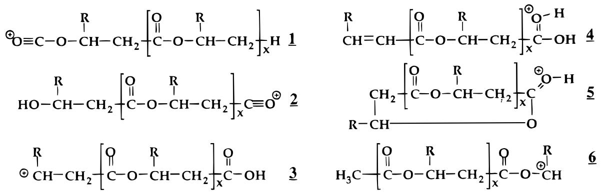


Figure 6. Possible structure assignments for $M_{x+1}H^+$.

Table 2. Summary of Positive Mode SIMS Data Evaluated for Quantification of the Surface Concentration c_s of Butyrate (B) and Valerate (V)[a]

mass *m*/*z*	assigmt	intens (counts)	expt (%)	calc (%)	c_s (%)
69	$B(-OH)^+$	626 910	85.6	**88.8**	85.6 *B*
83	$V(-OH)^+$	105 544	14.4	**11.2**	14.4 *V*
155	$B_2(-OH)^+$	27 709	79.7	78.9	89.4 *B*
169	$VB(-OH)^+$	6 401	18.4	19.9	
183	$V_2(-OH)^+$	661	1.9	1.2	10.6 *V*
173	B_2H^+	5 064	77.6	78.9	88.6 *B*
187	VBH^+	1 241	19.0	19.9	
201	V_2H^+	222	3.4	1.2	11.4 *V*
241	$B_3(-OH)^+$	466	71.5	70.0	88.3 *B*
255	$VB_2(-OH)^+$	186	28.5	26.5	
269	$V_2B(-OH)^+$	noise			
283	$V_3(-OH)^+$	noise			11.7 *V*
259	B_3H^+	439	79.8	70.0	92.2 *B*
273	VB_2H^+	111	20.2	26.5	
287	V_2BH^+	noise			
301	V_3H^+	noise			7.8 *V*

[a] Here expt (%) denotes the signal intensity in percent obtained directly from the experimental values (intensity in counts). Calc (%) denotes the signal intensity in percent obtained by calculation from the average surface concentration c_s (%). c_s (%) was obtained with the best fit for the experimental data. The biopolymer mean composition used for statistical calculations is $c_s(B) = 88.8\%$, $c_s(V) = 11.2\%$.

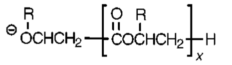
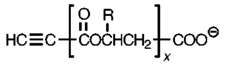
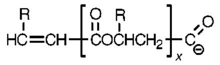
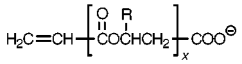
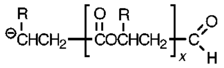
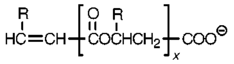
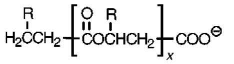
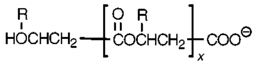
guished by TOF-SIMS. Structures **1**−**4** represent general ester cleavage products. For the present investigated system, Davies et al.[9] proposed configuration **3**. Following conventional mass spectrometry (EIMS) rules[20] for ester bonds with long alcohol chain residues, a favored double hydrogen rearrangement is proposed, leading to a structure of type **4**. The configuration formula **4** consists of an olefinic elimination product protonated at its acid functional group. A lactone-like structure such as configuration **5** was proposed by Davies et al.[7] for a related polyester system poly(lactic acid) (PLA). In the formation process of the positive fragment ion **6**, a McLafferty type rearrangement reaction may be involved. To distinguish structures with identical molecular masses and finally to confirm proposed ion fragments, additional tandem mass spectrometry (MS-MS)[21] measurements should be carried out, as already proposed in the literature.[22]

Table 2 summarizes the assignments of some lower-mass secondary ion peaks, which were selected for data evaluation, to quantify the (relative) surface concentrations c_s of butyrate (B) and valerate (V). Especially, five independent SIMS quantification attempts were made, using the monomer signals $M(-OH)^+$ at masses 69.03 and 83.05 *m*/*z*, the dimer signals $M_2(-OH)^+$ at masses 155.07, 169.09, and 183.10 *m*/*z*, the dimer signals M_2H^+ at masses 173.08, 187.10, and 201.11 *m*/*z*, the trimer signals $M_3(-OH)^+$ at masses 241.11 and 255.12 *m*/*z*, and the trimer signals M_3H^+ at masses 259.12 and 273.13 *m*/*z*. Note that only the two most dominant trimer ions, M_3H^+ and $M_3(-OH)^+$, could be detected, and therefore the calculation of the experimental surface concentration was based on the secondary ion intensities of these mass fragments only.

The experimentally obtained abundance distribution (expt (%)) can be directly compared with corresponding predictions (calc (%)) by standard statistical (Bernouillian) combination theory.[23] Since such predictions depend on the surface stoichiometry, Table 2 shows with (calc (%)) a corresponding abundance distribution which has been calculated in order to get the best fit with the experimental data. The mean values are $c_s(B) = 88.8\%$, $c_s(V) = 11.2\%$, respectively. The standard deviation of $c_s(V)$ is 2.1%.

However, the signal intensities directly taken from the monomer fragments HB^+ at 87.04 *m*/*z* with 51 773

Table 3. Major Negative Fragment Ions of P(HB-*co*-HV) Bio-polyester[a]

2		1			0		repetition units *x*	
VB_2	B_3	V_2	VB	B_2	V	B	negative ion structure	sum formula
		173	159	145	73	59	 $^{\ominus}OCH(R)CH_2-[C(=O)OCH(R)CH_2]_x-H$	$M_{x+1}H(-CO)^-$
255	241	–	169	155	–	69	 $HC\equiv C-[C(=O)OCH(R)CH_2]_x-COO^{\ominus}$	$M_{x+1}(-RH)(-H)^-$
255	241		169	155	83	69	 $HC(R)=CH-[C(=O)OCH(R)CH_2]_x-C^{\ominus}=O$	$M_{x+1}(-OH)^-$
257	243	–	171	157	–	71	 $H_2C=CH-[C(=O)OCH(R)CH_2]_x-COO^{\ominus}$	$M_{x+1}(-R)^-$
257	243		171	157	85	71	 $^{\ominus}CH(R)CH_2-[C(=O)OCH(R)CH_2]_x-C(=O)H$	$M_{x+1}H(-O)^-$
	257	199	185	171	99	85	 $HC(R)=CH-[C(=O)OCH(R)CH_2]_x-COO^{\ominus}$	$M_{x+1}(-H)^-$
	259	201	187	173	101	87	 $H_2C(R)CH_2-[C(=O)OCH(R)CH_2]_x-COO^{\ominus}$	$M_{x+1}H^-$
		217	203	189	117	103	 $HOCH(R)CH_2-[C(=O)OCH(R)CH_2]_x-COO^{\ominus}$	$M_{x+1}OH^-$

[a] B and V Denote for the butyrate and the valerate monomer units, respectively. R ascribes for the alkyl rest which is either a methyl (B) or an ethyl (V).

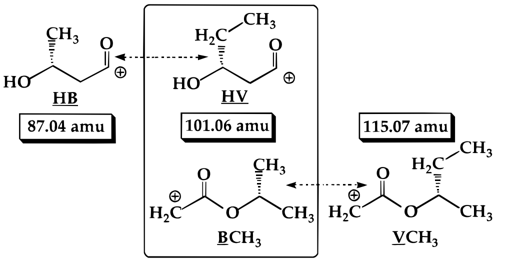


Figure 7. Possible contributions at mass 101.06 *m*/*z* originating from VH^+ and BCH_3^+ ions.

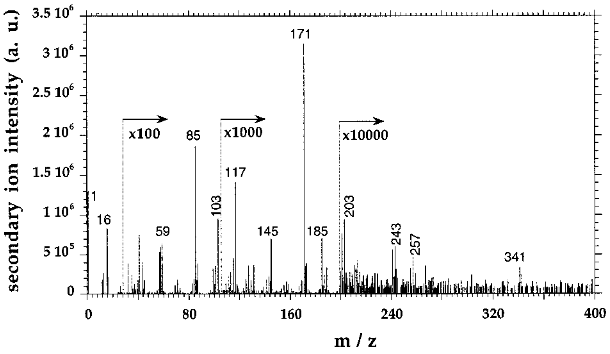


Figure 8. Negative mode secondary ion mass spectrum (14 kV Cs^+) of the P(HB-*co*-HV) bio-polyester.

counts and HV^+ at 101.06 *m*/*z* with 19 361 counts lead to a hypothetical $c_s(V)$ content of 27.2%. This indicates that a direct data evaluation cannot be used in the case of these principal monomer fragments. The unexpected high signal intensity at mass 101.06 *m*/*z* can be explained by the contribution of two fragment ions, originating from VH^+ and BCH_3^+, as indicated in Figure 7. Taking into account the signal intensity of the VCH_3^+ fragment (1285 counts), one obtains a corrected $c_s(V)$ content of 8.0%.

Negative TOF-SIMS Spectra. The negative ion TOF-SIMS spectrum shown in Figure 8 contains in the lower mass range ions at 12.00, 13.01, 16.00, 17.00, 24.00, 25.01, 41.00, 43.01, and 45.00 *m*/*z* which may be assigned to C^-, CH^-, O^-, OH^-, C_2^-, C_2H^-, C_2HO^-, $C_2H_3O^-$, and CHO_2^-. In the mass range up to 400 *m*/*z*, the highest masses could be identified as the deprotonated tetramers $B_4(-H_2)^-$, $B_4(-H_3)^-$ at masses 342.13 and 341.12 *m*/*z* as well as trimers $B_3(-H)^-$, $B_3H(-O)^-$/$B_3(-CH_3)^-$ at masses 257.10 and 243.12/243.09 *m*/*z*. Other typical negative ion fragments are listed up in Table 3. Only masses observed in the recorded spectra are represented and aligned analogously, as in Table 1 according to their incorporated repetition units. Within the negative TOF-SIMS data, various ion ratios were used for the estimation of the butyrate and valerate contents. Suitable monomer signals were found to consist of $MH(-CO)^-$ at masses 59.05 and 73.07 *m*/*z*, as well as of $M(-H)^-$ at 85.03 and 99.05 *m*/*z* and of MOH^- at 103.04 and 117.06 *m*/*z*. It should be mentioned that the mass peaks of $M(-H)^-$ may contain also contributions from other mass fragments such as $VH(-O)^-$ at mass 85.07 *m*/*z* (see Table 3) and BCH^- at mass 99.05, which could lead to an inaccurate estimation of the valerate content.

Among the dimer signal ratios, $M_2H(-CO)^-$ at masses 145.09, 159.10, and 173.12 *m*/*z*, as well as $M_2(-H)^-$ at masses 171.07, 185.08, and 199.10 *m*/*z* and M_2OH^- at masses 189.08, 203.09, and 217.11 *m*/*z* were found to be useful. However the superposition at mass 173 *m*/*z* caused by B_2H^- (173.08 *m*/*z*) and $V_2H(-CO)^-$ (173.12 *m*/*z*) did not allow consideration of this mass peak for the data evaluation of the surface valerate content.

Table 4 is structured like Table 2. Using the set of negative ion fragments, the mean surface stoichiometry from the c_s (%) values was determined as $c_s(B) = 89.0\%$ and $c_s(V) = 11.0\%$ with a standard deviation of $c_s(V)$ of 2.3%.

X-ray Photoelectron Spectrometry. As a complementary technique to TOF-SIMS measurements, XPS was used to characterize the surface of the Biopol polymer. In XPS, the information includes a maximal depth region of up to 10 nm as calculated by the equation of Seah and Dench.[24]

Table 4. Summary of Negative Mode SIMS Data Evaluated for Quantification of the Surface Concentration c_s of butyrate (B) and Valerate (V)[a]

mass m/z	assigmt	intens (counts)	expt (%)	calc (%)	c_s (%)
59	$BH(-CO)^-$	6 301	90.0	**89.0**	90.0 *B*
73	$VH(-CO)^-$	703	10.0	**11.0**	10.0 *V*
85	$B(-H)^-$	18 559	85.5	**89.0**	85.5 *B*
99	$V(-H)^-$	3 152	14.5	**11.0**	14.5 *V*
103	BOH^-	9 397	87.2	**89.0**	87.2 *B*
117	VOH^-	1 382	12.8	**11.0**	12.8 *V*
145	$B_2H(-CO)^-$	667	86.4	79.3	92.7 *B*
159	$BVH(-CO)^-$	105	13.6	19.5	
173	$V_2H(-CO)^-$	superposition			7.3 *V*
171	$B_2(-H)^-$	3 115	80.7	79.3	89.9 *B*
185	$BV(-H)^-$	691	17.9	19.5	
199	$V_2(-H)^-$	53	1.4	1.2	10.1 *V*
189	B_2OH^-	311	80.0	79.3	88.9 *B*
203	$BVOH^-$	78	20.0	19.5	
217	V_2OH^-	noise			11.1 *V*

[a] Here exp (%) denotes signal intensity in percent obtained directly from the experimental values (intensity in counts), calc (%) denotes the signal intensity in percent obtained by calculation from the average surface concentration c_s (%). c_s (%) was obtained with the best fit for the experimental data. The biopolymer mean composition used for statistical calculations is $c_s(B) = 89.0\%$, $c_s(V) = 11.0\%$.

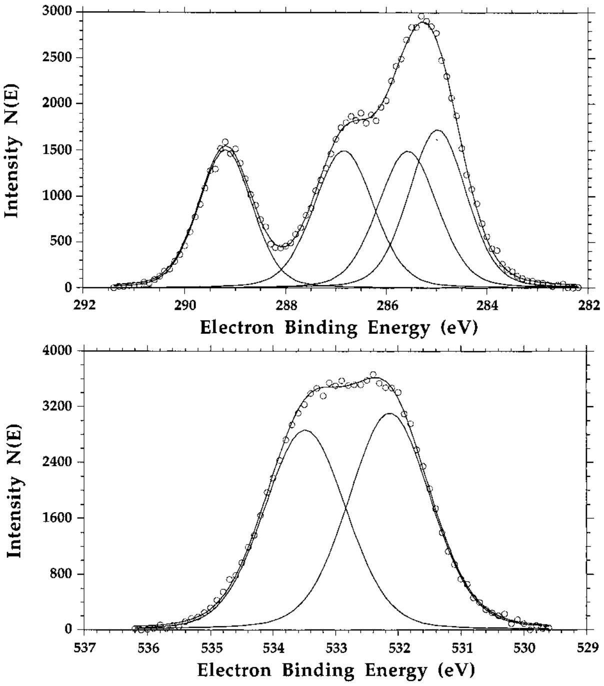


Figure 9. Carbon 1s and oxygen 1s XPS spectra of the P(HB-*co*-HV) bio-polyester.

Table 5. Bulk and Surface Valerate Contents c_b(V) and c_s(V) within P(HB-*co*-HV) Determined by Various Techniques[a]

bulk anal	c_b(V) valerate (%)	surf anal	c_s(V) valerate (%)
^{1}H NMR	9.2 ± 0.9	TOF-SIMS (+)	11.2 ± 2.1
DSC (T_m)	12 ± 3	TOF-SIMS (−)	11.0 ± 2.3
DSC $(T_m)_{corr}$	10 ± 3	XPS from C 1s	18 ± 7
		XPS from C/[C + O]	32 ± 20

[a] ^{1}H NMR denotes nuclear magnetic proton resonance spectrometry. For the differential scanning calorimetry (DSC), different values were obtained, depending whether the melting temperature T_m was corrected for the plasticizer content or not.

The chemical shifts in C 1s and O 1s core level spectra for P(HB-*co*-HV) shown in Figure 9 are in good agreement with the molecular structure of P(HB-*co*-HV). The observed electron binding energy shifts to 289.2 eV for ($-\boldsymbol{C}OO-$), to 286.9 eV for ($COO\boldsymbol{C}H-$) and to 285.6 eV for ($-\boldsymbol{C}H_2-COO$) correspond well to values found in the literature.[7,9,25] These signals belong to the functional ester group of valerate and butyrate and therefore, when fitted with a Gaussian−Lorentzian distribution curve, yield similar peak areas (in percent based on the total carbon signal intensity): *22.4* ($-\boldsymbol{C}OO-$); *24.9* ($COO\boldsymbol{C}H-$); *25.0* ($-\boldsymbol{C}H_2-COO$).

The valerate content can be calculated by dividing the concentration of the hydrocarbon component at 285.0 eV with the total contribution of the C 1s spectrum. Here, the pure PHB shows a theoretical ratio of 1:4, whereas the ratio of PHV corresponds to 2:5. A valerate concentration c_s(V) of 18% is obtained for the investigated copolymer system using the hydrocarbon component of 27.7% at 285.0 eV and neglecting the influence of possible surface hydrocarbon contaminations. It should be noted that the uncertainty of this value is high due to the variation of the fitting results. The relative error is estimated to be on the order of 40%.

The oxygen 1s spectrum shows two similar shaped peaks; the one at lower electron binding energy is assigned to $O-C{=}\boldsymbol{O}$ at 532.2 eV and the higher-energy oxygen peak is attributed to the $\boldsymbol{O}-C{=}O$ group at 533.5 eV.[25]

The carbon and oxygen contents deduced from the integrals of the C 1s and O 1s spectra were found to be 68.2 and 31.8% using the relative sensitivity factors $f_C = 0.296$ and $f_O = 0.711$. Here, the pure PHB represents a C/[C + O] ratio of 4:6, whereas the pure PHV shows a theoretical ratio of 5:7. From the present carbon and oxygen contents, a valerate concentration c_s(V) of 32% can be deduced. However, an error of only 1% of the carbon and the oxygen contents leads to a valerate concentration of 53 or 11%. This indicates that the uncertainty of this ratio is too high to estimate a precise valerate concentration c_s(V).

General Discussion and Conclusions

The copolyester Biopol consisting of randomly distributed hydroxybutyrate (HB) and hydroxyvalerate (HV) monomer units has been studied by bulk (DSC, NMR) and surface (TOF-SIMS, XPS) analysis techniques. Quantification of the valerate content in the investigated Biopol sample has been carried out and results are summarized in Table 5.

The bulk valerate content c_b(V) deduced from ^{1}H NMR measurements was found to be 9.2%. The calculations are based on the intensity ratios of characteristic proton signals originating from the valerate and butyrate units. The melting temperature as obtained by DSC was corrected for the detected polymer additives and then associated in the melting diagram of Bluhm et al.[2] with a valerate content of 10%. The bulk valerate content obtained by both methods is comparable within the given errors.

The surface analysis with TOF-SIMS revealed in the positive and negative mass range specific fragment ions, which allowed the proper identification of the polymer and its constituting units. The TOF-SIMS quantification is based on Bernoullian probability calculations, using in the positive mode five fragment series and in the negative mode six fragment series which provided a good reliability to the calculated surface valerate content of 11.2 and 11.0% (mean values). The error of

2.1 and 2.3% associated with the TOF-SIMS quantification in Table 5 represents the standard deviation calculated from the data of the positive and negative fragment series. TOF-SIMS measurements provide good quantification results with respect to the standard deviations and the comparability between data obtained from the positive and the negative mass ranges. However, as it was shown for the protonated monomer fragment MH^+, care has to be taken when using only one intensity ratio from characteristic butyrate and valerate fragments for direct surface quantification. The interference between BCH_3^+ and VH^+ at mass 101.06 m/z (see Figure 7) should be taken into account to achieve a precise quantification from MH^+ ions. The number of consistent data obtained using Bernoullian probability calculations gives confidence on the results on one sample only, while quantification methods based on one fragment or one ratio must often be confirmed by analyzing a copolymer range.

XPS measurements confirmed the chemical composition of the Biopol surface. The quantification of XPS data was based either on the elemental C/[C + O] ratio or on the relation between the total contribution of the C 1s spectrum and the amount of aliphatic hydrocarbon residues ($-CH_2-R$), obtained from the C 1s core level spectra fitting. However, an accurate estimation of the surface stoichiometry of the repetition units cannot be achieved. This shortcoming can be explained by the hydrocarbon contamination and the probable X-ray damage of the polymer. XPS data provide only a rough estimate of the surface composition, contrary to TOF-SIMS measurements, which in the present study allowed an appropriate quantification of the molar surface fractions of the polymer repetition units HB and HV.

The surface concentration deduced from TOF-SIMS measurements are with 11.2 ± 2.1 and 11.0 ± 2.3% in the same range as values found by NMR (9.2 ± 0.9%) and by DSC (10 ± 3%) for the bulk material. It is not surprising that the surface composition exhibited by the method of quantification reflects the bulk composition as Biopol is a random copolymer. In terms of the performance of the analyzed material as a biomaterial for which the surface properties are of considerable importance, results described in this work ensure that no hazardous reaction related to surface segregation effect occurs. Moreover, the method of quantification proposed here can be used to confirm the similarity of bulk and surface compositions over a copolymer range of the P(HB-*co*-HV) system.

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