Time-of-Flight Secondary Ion Mass Spectrometry Studies of in Vitro Hydrolytic Degradation of Biodegradable Polymers

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ABSTRACT: The in vitro hydrolytic degradation at the surface of six biodegradable polymers, namely polyglycolic acid (PGA), poly(lactic acid) (PLA), random copolymer poly(lactic-*co*-glycolic) acid (PLGA), poly(sebacic acid) (PSA), and two random copolymer poly(fumaric-*co*-sebacic) acid (PFS) of different compositions, has been studied using time-of-flight secondary ion mass spectrometry (ToF SIMS). A distribution of hydrolysis products, namely oligomer molecules, were observed as a series of intact molecular ions in the ToF SIMS spectra for all polymers studied. In most cases, the molecular ion peak in each repeating pattern is the most intense peak. Analysis of the intensities of the molecular ions in the distribution (e.g., approaching M_n) allows chemical kinetic information to be obtained from the ToF SIMS spectra of hydrolyzed samples. In the case of polyglycolic acid, a maximum in the distribution of the molecular ion peaks is observed which changes with respect to the hydrolysis time. For PGA, the average molecular weight of the hydrolysis products can be calculated from the ToF SIMS spectra, and a good linear relationship between the molecular weight and hydrolysis time was obtained. In the case of polyanhydrides, molecular ion peak intensities of the hydrolysis products decrease exponentially with respect to the molecular weight of the hydrolysis products. The slope of log peak intensity versus the molecular weight of hydrolysis products was found to vary with respect to the hydrolytic degradation rates of the polymer. The use of such in vitro kinetic information may be possible to estimate the degradation rate of the surface of the polymer. These data could be used for rapid screening of formulations and preparation of new materials.

Introduction

Synthetic biodegradable polymers have been used in clinical applications for decades. Some relevant applications include surgical implants, wound healing materials, absorbable sutures, and drug delivery devices.[1,2] Among important issues in developing biomedical applications based on polymer biodegradability are the properties of degradation (i.e., rate, mechanism, byproducts, etc.) of the polymer material. The study of hydrolytic degradation of biodegradable polymers has been a research focus in the past few decades.[2] In vivo investigations of biopolymer implants have been the major clinical investigation method.[3−5] Direct monitoring of the weight loss of polymer implants and histological observations provides macroscopic information on the hydrolytic degradation. A drawback to this approach is that it is very time-consuming. The observation has to be conducted in full course of the hydrolytic degradation of polymer implants. For example, the observation of the degradation of poly(caprolactone) implant in rabbits, reported by Pitt,[6] spanned over 200 weeks. Although in vivo degradation studies will always be necessary for biomedical materials, developing rapid screening and investigation techniques is much needed especially for consideration of new formulation, morphologies and preparations, and new materials.

Many bulk characterization techniques have been employed for in vitro investigation of hydrolytic degradation of biodegradable polymers. Measurements of tensile strength[7−12] have been used to monitor the hydrolysis of biodegradable polymers. It has been realized that the thermal properties such as glass transition temperature and crystallinity are closely related to the hydrolysis rate of polymers. Hence, thermal analysis techniques such as differential scanning calorimetry (DSC)[9,10,13−19] and gravimetry[10,15] measurements have been widely involved in studying the hydrolytic degradation of synthetic biopolymers. Techniques for direct quantitative monitoring of the degradation of biodegradable polymers include measuring the weight loss[20−23] and the decreases in molecular weight by gel permeation chromatography (GPC) and size exclusion chromatography (SEC).[10,15,17,21,23−26] Other techniques involved in the study of hydrolytic degradation of biodegradable polymers include FTIR,[13,27] NMR,[28,29] X-ray diffraction,[14,27,28] and laser diffractometry.[30]

The surface of biomedical devices provides the initial point of contact with a biological milieu or cell/tissue interaction. Surface properties of biodegradable polymers can be of primary importance where drug delivery is a dominant interaction. For example, some polymers that are important for drug delivery applications such as polyanhydrides degrade mainly by surface erosion.[31] Vert and co-workers[32] found that poly(lactic acid) copolymers samples in the submillimetric range are dominated by homogeneous degradation. In these cases, the surface degradation rate and mechanism are very important. Thus, the present paper explores a new method for investigating these properties. Because of the importance of surface interactions between biomedical devices and biological environments, surface-sensitive microscopic and spectroscopic techniques have become an important means in the study of polymeric biomaterials and, in particular, biodegradable polymeric materials. Of all surface methods, scanning electron spectroscopy (SEM) has been one of the most widely used surface-sensitive techniques[16−18,20,24,26,33,34] for both

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in vitro and in vivo investigation of the degradation of polymeric biomaterials. It produces images of the morphological changes for visualization during hydrolytic degradation. Atomic/scanning force microscopies (AFM/SFM) have become powerful tools in the characterization of polymer degradation.[31,35−38] The capability of acquiring data directly in hydrolysis solution offers a unique advantage in that the monitoring of polymer hydrolytic degradation can be carried out in situ.[36−38]

These surface microscopy techniques, however, do not provide chemical composition or structural information. Static SIMS and XPS have been the workhorse methods for surface chemical composition and structure analysis of polymeric materials. Davies and co-workers[39−44] conducted surface characterization on a series of biodegradable polymers for chemical composition and structures at surfaces using static SIMS (quadrupole SIMS and ToF SIMS) and XPS, in combination with other techniques such as AFM and SEM. In particular, Leadley et al.[45] employed ToF SIMS, XPS, and AFM in the study of hydrolysis mechanisms of poly(ortho esters). The SIMS results suggested that the preferred mechanism for hydrolysis was via the cleavage of an exocyclic alkoxy bond in poly(ortho ester)s. We have reported[46] preliminary results using ToF SIMS in the study of the hydrolytic degradation of poly(glycolic acid) (PGA). It was demonstrated that the hydrolytic degradation products of PGA could be directly observed in ToF SIMS spectra. The low molecular weight oligomers, which are hydrolysis products, were observed as intact molecular ions, and the apparent M_w and M_n changed systematically with the hydrolysis time. It will be demonstrated in the present paper that the observation of hydrolytic degradation products in the form of intact molecular ions in ToF SIMS is not a special case for PGA, but a general phenomenon for different classes of biodegradable polymers. ToF SIMS could be a powerful tool for screening new biodegradable polymers and studying the degradation kinetics at the surface of biodegradable polymers. Polymers studied in the present paper include both homopolymers and random copolymers of polyesters and polyanhydrides, in particular polyglycolic acid (PGA), poly(lactic acid) (PLA), and their copolymer poly(lactic-*co*-glycolic) acid (PLGA) in the polyester category, and poly(sebacic acid) (PSA) and its copolymers with fumaric acid (PFS) of different compositions in the polyanhydride category. The molecular weight of the hydrolysis products observed using ToF SIMS is a function of the hydrolysis time. (Details of kinetics studies are reported in a separate paper.[47])

Experimental Section

Poly(glycolic acid) (PGA) was received as a donation from Dr. Peter Jarrett of Davis & Geck Division of American Cyanamid Co. (One Casper St., Danbury, CT). Poly(L-lactic acid) (PLA) (weight-average molecular weight 93 000) and poly(DL-lactic-*co*-glycolic acid) (PLGA) (50:50, molecular weight 50 000−75 000) were purchased from Sigma Chemical Co. (St. Louis, MO). Poly(fumaric-*co*-sebacic acid) (PFS) (50:50, average molecular weight 3046, 20:80, average molecular weight 6500) and poly(sebacic acid) (PSA, average molecular weight 12 000) were supplied by Professor Edith Mathiowitz of Brown University.[48] The physiological solution ISOTON II was purchased from Coulter Diagnostics (a division of Coulter Electronics, Inc., Hialeah, FL).

PGA, PLA, and PLGA samples were prepared by melt-pressing on aluminum foil. Prior to each melt-press, thick PGA plates were cut to small pieces and washed with an ultrasonic cleaner (Branson Cleaning Equipment Co., model B-52) in hexane and chloroform for 10 min each. Aluminum foil was precleaned with chloroform. PLA and PLGA were used as received. Samples were pressed at about 200 °C to ca. 1 mm of thickness. The aluminum foil was peeled off, and hydrolysis treatment was conducted immediately. Polyanhydride samples were prepared by melting polymers on aluminum foil at their melting temperatures. Polymer samples were precleaned in hexanes and vacuum-dried prior to melt. The sample thickness was ca. 1 mm. The aluminum foil was peeled off for PSA samples.

Table 1. Initial Molecular Weight and Time of Hydrolysis Reactions

polymer	mol wt	hydrolysis time (h)	polymer	mol wt	hydrolysis time (h)
PGA	NA	1	PSA	12K	24
PLA	93K	30	PFS 20:80	6.5K	3
PLGA	50−75K	24	PFS 50:50	3K	2

The hydrolytic degradation of all polymers was carried out in a physiological buffer solution, ISOTON II (pH = 7.4), at 37.0 °C. Each sample was immersed in a separate vial prefilled with 14 mL of ISOTON II solution and sealed airtight. The reaction vials were immersed in a temperature bath for predetermined periods depending on the sample's sensitivity to hydrolytic degradation and other properties such as molecular weight and hydrophobicity of the polymer. Because the objective of this paper is to demonstrate the capability of ToF SIMS in exploring reaction properties of biodegradable polymers at surfaces, the hydrolysis time for each polymer was chosen so that the molecular ions of hydrolysis products were observed with good peak intensity. At least three measurements were made on each sample to ensure good data reproducibility. The hydrolysis times chosen for each polymer sample reported in this paper are listed in Table 1. Detailed information on studies of full course biodegradation will be reported separately.[47] Samples after the hydrolysis treatment were washed in distilled water without any agitation or cavitation. This should minimize adsorbed salts from buffer solution. Samples were then vacuum-dried and stored in sealed vials filled with argon under vacuum to eliminate further degradation during storage. For all samples hydrolyzed, ToF SIMS analysis was conducted in no more than 7 days after the hydrolysis experiment.

ToF SIMS analysis was conducted on a Physical Electronics 7200 time-of-flight secondary ion mass spectrometer equipped with a pulsed cesium ion gun and a channel plate detector. The primary ion gun was operated at 8 keV in all spectral acquisitions. The static mode was used in all acquisitions with the primary ion current of 0.3 pA. The pulse width of primary ion current was 1.0 ns. The total ion dosage in each spectral acquisition did not exceed 1×10^{11} ions/cm^2. An electron neutralizer was operated during all spectral acquisitions in pulse mode at low electron energy with a target current under 1 μA for charge compensation. A time resolution of 1.25 ns per step was used for good signal-to-noise ratio at high m/z range. Data reduction was performed using Physical Electronics TOFPak software (version 2.0).

Results

ToF SIMS of Hydrolyzed Samples. Ions from polymer chain fragments, with exponentially decreasing intensity, are normally observed in the high mass range ToF SIMS spectra of thick film polymer samples, unless sample films are prepared as monolayers deposited on metal substrates.[46] Oligomeric ion distributions are not normally observed for thick films due to the entanglement of the long-chain molecules in polymer samples. Random chain scission occurs when samples are bombarded by primary ions, which transfers energy to polymer chains near the surface. This process generates fragment ions in ToF SIMS spectra with the characteristic of exponentially decreased intensity with few

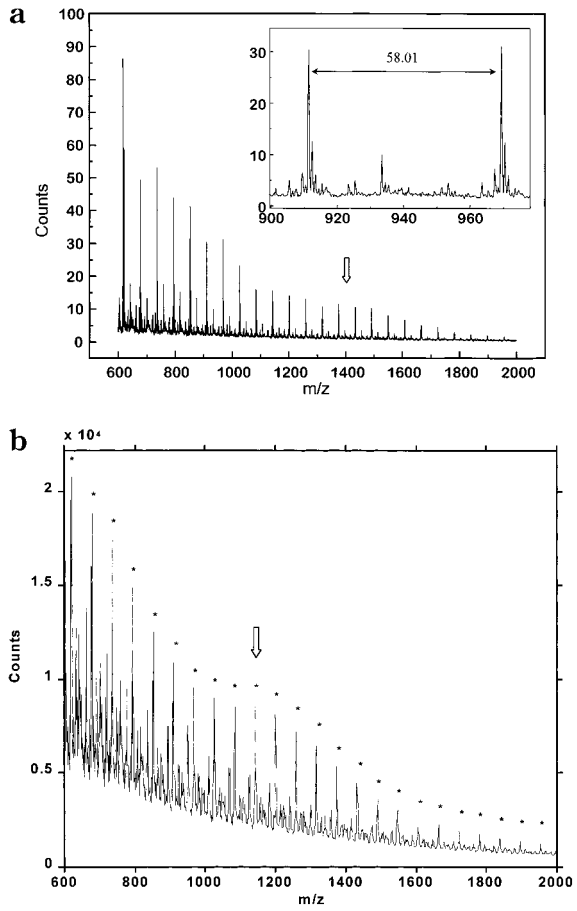


Figure 1. (a) High mass portion of ToF SIMS spectra of hydrolyzed PGA for 1 h. (b) High mass portion of ToF SIMS spectra of hydrolyzed PGA for 4 h. The maximum in molecular peak distribution is shown as marked.

meaningful peaks due to the decreased possibility of producing high mass fragments. Therefore, the ToF SIMS spectra of polymers before hydrolysis are not shown in this paper unless necessary. The previous preliminary report[46] did report results on the hydrolysis of PGA.

Upon hydrolysis, polymer chain lengths are reduced gradually until the oligomers become small enough to desorb from the polymer surface and dissolve in the surrounding liquid phase, where they continue to hydrolyze, yielding monomers as the ultimate reaction products. On the surface of a degraded polymer, the entanglement of the degradation-generated oligomers is greatly reduced because of the decrease in molecular chain length. During ToF SIMS measurements, low molecular weight oligomers become easier to desorb from the sample surface upon the bombardment of primary ions. Therefore, when degradation occurs at polymer sample surfaces, intact molecular ions of the degradation products can be observed in relatively high mass range of ToF SIMS spectra.

ToF SIMS of Hydrolyzed PGA. Figure 1 shows the high mass portion (600−2000 Da) of ToF SIMS spectra of PGA hydrolyzed for 1 h. Before the hydrolysis treatment, essentially nothing can be observed in this range[46] except a noisy background. Upon hydrolysis, a peak pattern characterized by the differences due to the mass of the repeat unit of PGA was observed.

All the ions of the major peaks in Figure 1 have the structure of $[nG + H_2O + Na]^+$, where G stands for the repeat unit of PGA. This indicates that the ions detected from the hydrolyzed PGA sample are in the intact molecules of the hydrolytic degradation products. The sodium ion comes from the hydrolysis solution and acts as an ionization assisting agent. It will be seen that in all samples studied sodium plays an important role in the ionization process, and all species detected in this series contain one sodium ion each. It was also observed that when the concentration of potassium ions is high enough, a set of molecular ion peaks associated with a potassium ion could be present simultaneously with the series of peaks associated with a sodium ion. This will be discussed below.

Table 2. Hydrolysis Products of PGA and PLA Observed in ToF SIMS Spectra[a]

no. of monomers	*m/z* PGA[b]	*m/z* PLA[c]	no. of monomers	*m/z* PGA[b]	*m/z* PLA[c]
1	99	113	24	*1433*	*1769*
2	157	185	25	*1491*	*1841*
3	215	257	26	*1549*	
4	273	329	27	*1607*	
5	331	401	28	*1665*	
6	389	473	29	*1723*	
7	447	*545*	30	*1781*	
8	505	*617*	31	*1839*	
9	563	*689*	32	*1897*	
10	*621*	*761*	33	*1955*	
11	*679*	*833*	34	2013	
12	*737*	*905*	35	2071	
13	*795*	*977*	36	2129	
14	*853*	*1049*	37	2187	
15	*911*	*1121*	38	2245	
16	*969*	*1193*	39	2303	
17	*1027*	*1265*	40	2361	
18	*1085*	*1337*	41	2419	
19	*1143*	*1409*	42	2477	
20	*1201*	*1481*	43	2535	
21	*1259*	*1553*	44	2593	
22	*1317*	*1625*	45	2651	
23	*1375*	*1697*			

[a] Peaks in italics are shown in Figures 1 and 2. [b] Composition nG + OH + H + Na. [c] Composition nL + OH + H + Na.

Molecular ions of up to 33 repeat patents were observed in ToF SIMS spectra of some samples of hydrolyzed PGA. Small oligomers detected in this study can be traced down to the final hydrolysis product, i.e., the single glycolic acid molecule associated with one sodium ion. Table 2 lists the full range of molecular ions have been detected in this study, with the ions shown in Figure 1 listed in shaded cells.

In addition to the wide distribution of molecular ion peaks, a maximum in the molecular ion peak distribution can also be seen from 1000 to 1500 Da in the ToF SIMS spectrum of PGA hydrolyzed for 4 h (Figure 1b). These maxima can be followed from the 1 h hydrolysis sample spectra (Figure 1a) at 1400−1500 Da, and shifts to lower mass ranges gradually as the hydrolysis time increased. This effect was also reported in the previous paper.[46] It is reasonable to assume that the maxima represent the most probable molecular weights of hydrolysis products at the particular reaction time. While this general visual result can be observed directly, a detailed examination of M_n and M_w is used to follow the hydrolytic degradation kinetics using the data from the ToF SIMS analysis. (The kinetics study of PGA will be reported in a separate paper.[47])

ToF SIMS of Hydrolyzed PLA. PLA has the same mainchain backbone as PGA plus a methyl group as a side chain. However, the presence of the methyl group

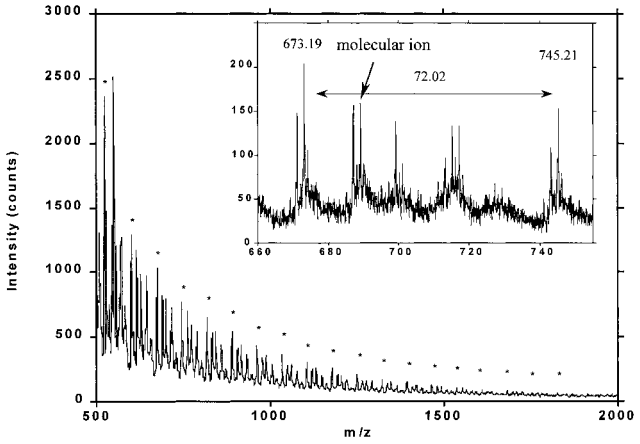


Figure 2. High mass portion of the ToF SIMS spectra of hydrolyzed PLA.

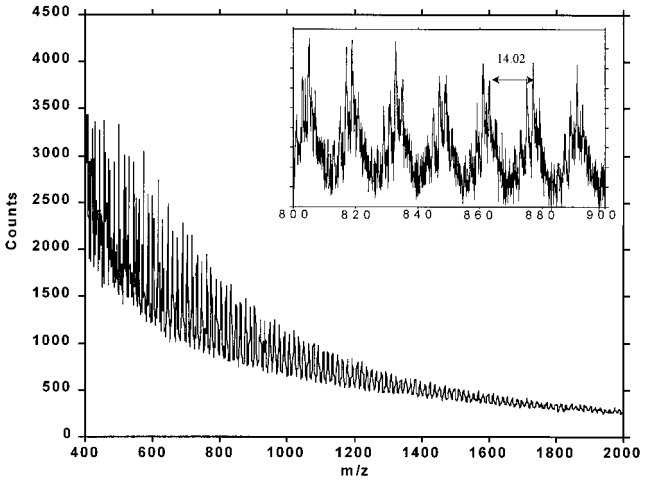


Figure 3. High mass portion of the ToF SIMS spectra of hydrolyzed PLGA 50:50 copolymer.

significantly changes the properties of the ester carbon as well as the bulk polymer properties such as morphology and hydrophobicity. These changes are reflected in the characteristic rates of hydrolytic degradation and consequently in the ToF SIMS spectra of hydrolyzed samples. Figure 2 shows the high mass portion (from 500 to 2000 Da) of ToF SIMS spectra of PLA disk samples hydrolyzed for 30 h. The star marked peaks are the most intense peak in each repeat pattern. As in the spectra of PGA, each repeat pattern corresponds to one repeat unit of PLA. The intervals between each of the star marked peaks are 72.02, exactly the mass of one PLA repeat unit. However, different from the PGA spectrum shown in Figure 1, the intensities of fragment peaks are significantly more intense. In fact, the intensity of the molecular ion peak is the second most intense peak in every repeat pattern. The most intense peak is the fragment ion assigned to a structure resulted from the loss of one oxygen atom from the molecule of the hydrolysis product ($[nL - O + Na]^+$).

Molecular ion peaks are observed from the surface of hydrolyzed PLA from the final hydrolysis product (i.e., the single lactic acid molecule) up to the oligomer with 25 repeat units of PLA. The intensity of the low mass species (not shown in the figures) becomes lower and lower quickly; this is likely due to the increased diffusibility of the low mass hydrolysis products from the solid sample surface to the hydrolysis solution and the increased solubility as the molecular weight is reduced.

It is well-known that one remarkable difference between PLA and PGA is the hydrolytic degradation rate.[49] The spectra shown in Figure 2 are PLA hydrolyzed for 30 h under the same hydrolysis conditions as that for PGA. For PLA samples hydrolyzed in shorter times, good peak patterns of hydrolysis products were not observed. In addition, there is no peak maximum observed at this hydrolysis time, as seen in the ToF SIMS spectra of hydrolyzed PGA (Figure 1). The intensity of molecular ion peaks is exponentially decreasing as the *m*/*z* increases.

ToF SIMS of Hydrolyzed PLGA. Figure 3 shows the high mass portion (400−2000 Da) of the ToF SIMS spectrum of PLGA 50:50 random copolymer hydrolyzed for 24 h. The pattern of the spectra is obviously more complicated than both the PGA and PLA spectra. Each of the peaks in Figure 3 consists of a group of peaks (see inset of Figure 3). The intervals between the groups are essentially 14 Da, indicating the repeat pattern is governed by the structural difference between PLA and PGA. In addition, the most intense peak in each group shifts gradually toward lower *m*/*z* so that the overall interval of the repeat pattern is not the exact *m*/*z* of one CH_2 group over the full range shown in Figure 3. This complicated pattern can be understood by considering all possible compositions of hydrolysis products of this random copolymer. Table 3 tabulates the *m*/*z* values of all possible molecular ion compositions in terms of *m*/*z* for low molecular weight oligomers of the hydrolysis products up to 15 PLA repeat units and 12 PGA repeat units. These ions represent the structure of intact molecules, which would be then cationized with one sodium ion each. All molecular ions listed in Table 3 are observed in the ToF SIMS spectra except those with high composition ratios of PGA over PLA repeat units. This is due likely to the faster hydrolysis rate of PGA than PLA. To illustrate this, ions in the framed cells (oligomer molecular ions from 800 to 1000 Da) are shown and marked with *m*/*z* values in Figure 4. It shows that most peaks shown in the spectra are molecular ion peaks with the most intense peak shifts to the left in each cluster due to the composition of the molecular ions. In fact, the most intense peak in each cluster is always corresponding to the ion with the smallest number of glycolic acid repeat units and the largest number of lactic acid repeat units. The peak intensities for the ions in the shaded cells of Table 2, consisting of a larger ratio of glycolic acid repeat units than the ions to their left, become too low to be detected.

The relative intensity of molecular ion peaks also serves as an indication of the relative hydrolysis rates of the two components of the copolymer. PGA hydrolyzes faster than PLA as observed in the homopolymers of PGA and PLA; therefore, fewer PGA repeat units remain in the hydrolysis products. No oligomers with only PGA repeat units were observed while oligomers with pure PLA were observed as the most intense peak in its group (peaks of 833, 905, and 977 Da in Figure 4, for example).

Figure 5 exemplifies the composition of the molecular ion peaks by comparing one of the peak groups with the theoretically calculated spectra. Figure 5a is a small portion of the spectrum shown in Figure 3, and Figure 5b is the corresponding molecular ion peak region theoretically calculated using an isotopic mass calculator within the Googly[50] software. The match in peak position and relative intensity between the experimen-

Table 3. Theoretically Predicted PLGA Oligomers of Hydrolysis Products[a]

	G[b]												
L[b]	0	1	2	3	4	5	6	7	8	9	10	11	12
0	41	99	157	215	273	331	389	447	505	563	621	679	737
1	113	171	229	287	345	403	461	519	577	635	693	751	**809**
2	185	243	301	359	417	475	533	591	649	707	765	**823**	**881**
3	257	315	373	431	489	547	605	663	721	779	*837*	**895**	**953**
4	329	387	445	503	561	619	677	735	793	*851*	*909*	**967**	1025
5	401	459	517	575	633	691	749	*807*	*865*	*923*	*981*	1039	1097
6	473	531	589	647	705	763	*821*	*879*	*937*	*995*	1053	1111	1169
7	545	603	661	719	*777*	*835*	*893*	*951*	1009	1067	1125	1183	1241
8	617	675	733	791	*849*	*907*	*965*	1023	1081	1139	1197	1255	1313
9	689	747	*805*	*863*	*921*	*979*	1037	1095	1153	1211	1269	1327	1385
10	761	*819*	*877*	*935*	*993*	1051	1109	1167	1225	1283	1341	1399	1457
11	*833*	*891*	*949*	1007	1065	1123	1181	1239	1297	1355	1413	1471	1529
12	*905*	*963*	1021	1079	1137	1195	1253	1311	1369	1427	1485	1543	1601
13	*977*	1035	1093	1151	1209	1267	1325	1383	1441	1499	1557	1615	1673
14	1049	1107	1165	1223	1281	1339	1397	1455	1513	1571	1629	1687	1745
15	1121	1179	1237	1295	1353	1411	1469	1527	1585	1643	1701	1759	1817

[a] The hydrolysis products are tabulated in *m*/*z* with the ion composition of $[xL + yG + H_2O + Na]^+$, where L and G represent the monomer of lactic acid or glycolic acid, respectively. Ions in italics are shown in Figure 4. Ions in boldface become too low in intensity. [b] The number of lactic acid monomers in each oligomer molecule is shown in rows and the number of glycolic acid monomers is shown in columns.

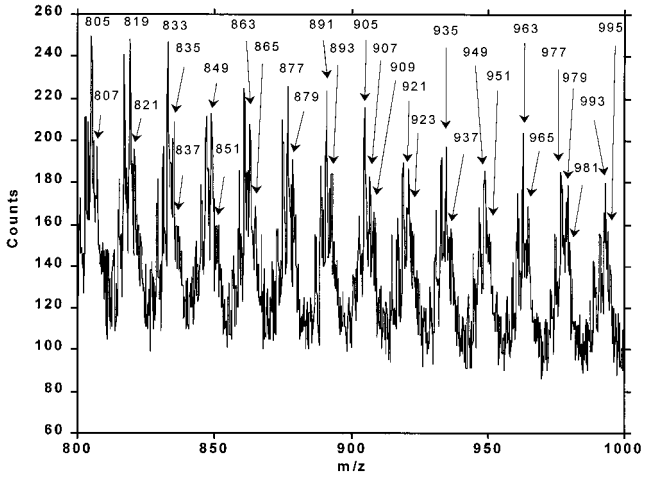
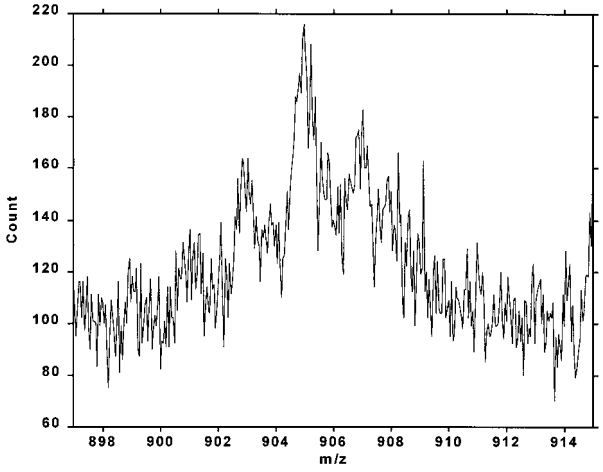


Figure 4. ToF SIMS spectra of hydrolyzed PLGA from 800 to 1000 Da. Number marked peaks are molecular ion peaks.



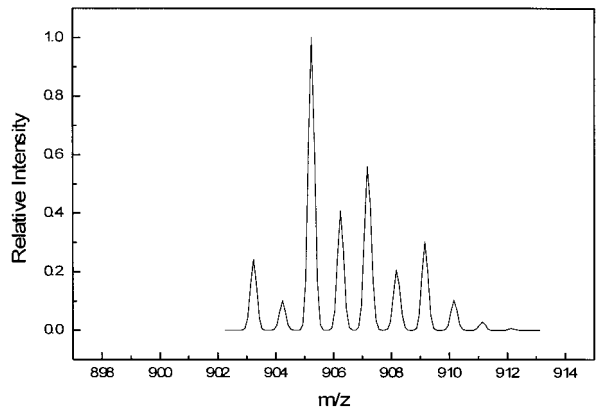


Figure 5. Comparison of a group of molecular ion peaks in the ToF SIMS spectra with the theoretically calculated mass spectra.

tally recorded spectra and the theoretically predicted one supports the assignment of the peaks. Figure 4 indicates that all major peaks in the ToF SIMS spectra of hydrolyzed PLGA copolymer are intact molecular ions of the hydrolysis products.

ToF SIMS of Hydrolyzed PSA. Polyanhydrides are significantly different from polyesters in that the anhydride linkage in the backbone is more vulnerable to attack by water than the ester bond. This leads to faster hydrolysis rates for polyanhydrides and causes a narrow molecular weight distribution of the hydrolysis products. Figure 6 shows the ToF SIMS spectra of hydrolyzed PSA from 400 to 1200 Da. Molecular ion peaks are the most dominant one in each repeat pattern, indicating that the anhydride bond is far easier to break than the alkyl chain. Intact molecular ions observed are from the single sebacic acid molecule up to the oligomer of six PSA repeat units. The single sebacic acid molecule, which is the first member in the series of hydrolysis products, however, is small enough to be dissolved in the hydrolysis solution; hence, very few of them stay on the surface of samples after the hydrolysis experiments. Therefore, the first significant molecular ion peak is the one consisting of two PSA repeat units. All observed oligomers of the hydrolysis products are listed in Table 4 in the form of actually observed ions, consisting of the intact molecules attached with a sodium ion.

Remarkably different from the polyesters, the intensity of molecular ion peaks drops quickly and exponentially. It can be seen from the spectra in Figure 6 that there would be no species larger than the six-repeat-unit oligomer detectable on the sample surface.

ToF SIMS of Hydrolyzed PFS Copolymers. Two random copolymers of PFS have been studied, in

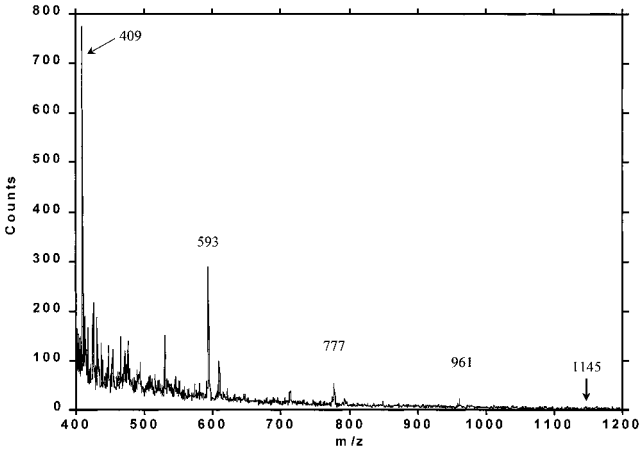


Figure 6. High mass portion of the ToF SIMS spectra of hydrolyzed PSA.

Table 4. Molecular Ion Peaks Observed in Hydrolysis Products of Polyanhydrides

no. of repeat unit	*m/z*		
	PSA	PFS 20/80	PFS 50/50
1[a]	225	225	225
2	409	409	409
3	593	593	593
4	777	777	777
5	961	961	
6	1145		

[a] Molecular ion peaks of the final products are very low intensity, not shown in the figures.

particular 50:50 and 20:80 by weight percentage of fumaric acid to sebacic acid. The initial molecular weight of 50:50 PFS sample is about 3000 by number-average molecular weight, and that of 20:80 PFS sample is about 6000.[48] Considering the molecular weights of the repeat units being 98 and 184 for fumaric acid and sebacic acid, respectively, the degree of polymerization is quite low for both copolymers, approximately 20 for the 50:50 copolymer and 36 for the 20:80 copolymer. It was expected that unreacted oligomers might be detected in the medium mass range of ToF SIMS spectra of unhydrolyzed samples. Figure 7 shows the ToF SIMS spectra of 20:80 copolymer before hydrolysis from 350 to 1050 Da. As it is expected, significant ion series were detected up to 1000 Da. The ion sequence of 467, 651, 835, and 1019 Da (framed number marked in Figure 7) has the composition of $[F + nS + H]^+$, in which F and S represent the repeat unit of fumaric acid and sebacic acid, respectively. However, in addition to the fragment ion peaks, the molecular ion peak series of 409, 593, 777, and 961 Da are also present. The composition of this series conforms the ion structure $[nS + H_2O + Na]^+$, indicating that the copolymer has already partially hydrolyzed during storage. Note that the relative intensity of the two series changes as the *m/z* increases. The fact that the relative intensity of the molecular ion peak series to the fragment series increases as the *m/z* increase indicates that the distribution of molecular oligomer ions is independent from the fragment ion distribution.

Upon hydrolysis treatment, the fumaric acid repeat unit could not be detected from any hydrolysis products. Figure 8 shows the ToF SIMS spectra of 20:80 copolymer from 400 to 1200 Da. The spectra of hydrolyzed PFS copolymer are almost the same as that of hydrolyzed PSA, indicating that the fumaric acid component in the

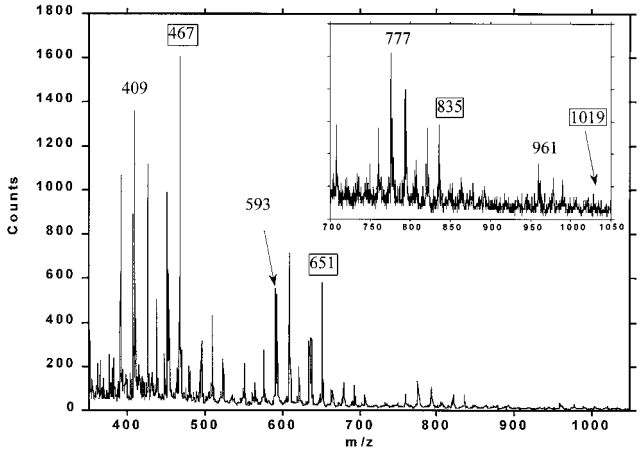


Figure 7. High mass portion of the ToF SIMS spectra of PFS 20:80 copolymer before hydrolysis treatments.

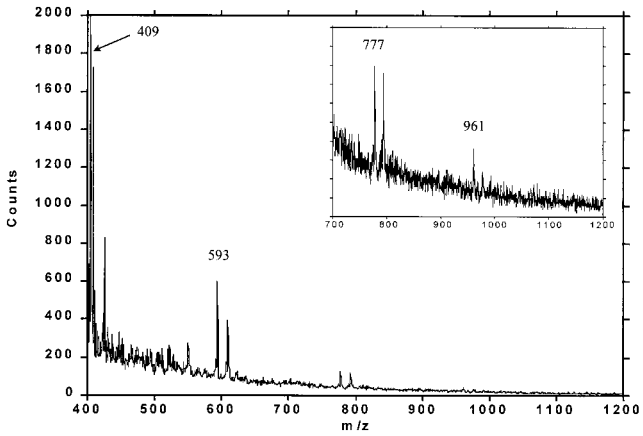


Figure 8. High mass portion of the ToF SIMS spectra of hydrolyzed PFS 20:80 copolymer.

random copolymer chain sequence is far more sensitive to hydrolysis environment and hydrolyzes faster than the PSA sequences. The marked peaks in Figure 8 have exactly the same ion composition as the hydrolysis products of PSA. The difference between this spectrum and that of the product of hydrolyzed PSA is that the largest molecule detected in the 20:80 PFS copolymer has five sebacic acid repeat units while the largest molecule detected in PSA has six sebacic acid repeat units, despite the shorter hydrolysis time for the copolymer samples. This is an additional indication of the faster hydrolysis property of the PFS copolymer compared to that of the homopolymer of PSA.

Similar results were observed for the 50:50 PFS copolymer. Figure 9 shows the ToF SIMS spectra of the hydrolyzed sample from 400 to 1200 Da. Similar to the 20:80 sample, only low molecular weight oligomers of PSA were observed, and products are more narrowly distributed to lower molecular weights. The largest oligomer molecule observed in the 2 h hydrolyzed sample of this copolymer has only four sebacic acid repeat units. In addition, the intensity of the molecular ion peaks decreases much faster than the 20:80 copolymer and the homopolymer of PSA, indicating higher hydrolysis rate is associated with higher fumaric acid content. More discussion regarding this will be given in the next section.

Although there was no fumaric acid units detected in the molecular ions of hydrolysis products of the two PFS copolymers, fragment ions from fumaric acid units

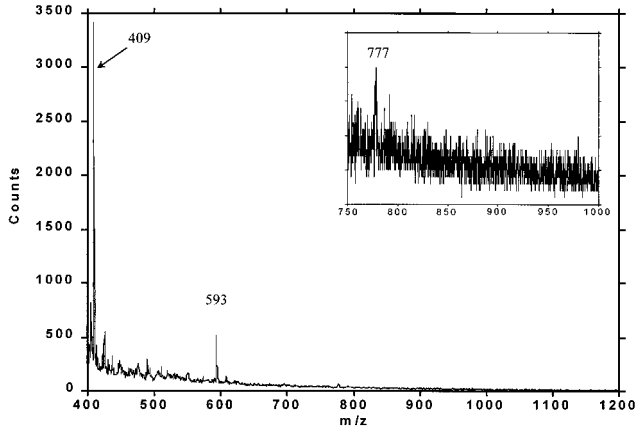


Figure 9. High mass portion of the ToF SIMS spectra of hydrolyzed PFS 50:50 copolymer.

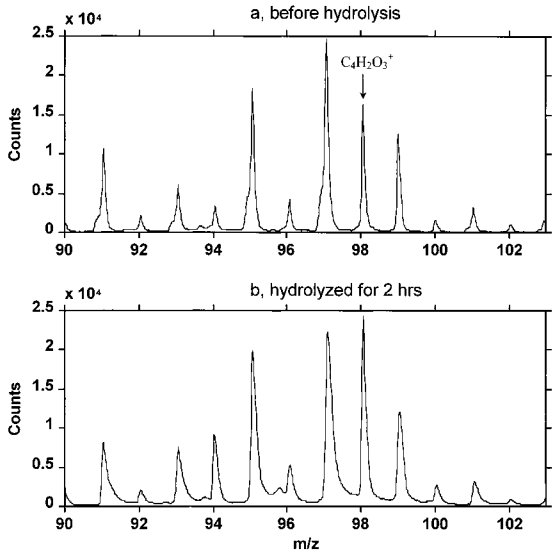


Figure 10. Comparison of the low mass portion (90−103 Da) of the ToF SIMS spectra of PFS copolymer before and after hydrolysis.

were indeed detected in all cases, indicating that the fumaric acid repeat unit is released from the polymer chain sequence as whole units during the hydrolytic degradation, and fumaric acid more easily undergoes hydrolysis as well. Figure 10 shows the fragment peaks of fumaric acid repeat unit before and after hydrolysis for 2 h. Peaks of 97, 98, and 99 Da are attributed to ions of $[F - H]^+$, F^+, and $[F + H]^+$, respectively. The intensity of the F^+ peak increased from less intense than the $[F - H]^+$ peak before the hydrolysis to more intense after the hydrolysis. Since the back-bond linkage at fumaric acid is more sensitive to water, the hydrolysis treatments cause this bond to cleave more easily than before hydrolysis treatments. The detection of fumaric acid fragments in samples before hydrolysis indicates that the samples have been undergoing hydrolysis at room temperatures during storage.

Discussion

Polyesters, in particular homopolymers and copolymers, consisting of LA and GA have been intensively studied in the past years, due to their importance as polymeric biomaterials. Vert and co-workers conducted extensive studies on the hydrolytic degradation of PGA/PLA and copolymers in vivo and in vitro.[51−54] The hydrolytic degradation of PLA/GA polymer devices were found to be rather complex, especially for massive devices. The degradation of the polymer matrix inside and outside of the device can be significantly different due to the difference in mass transfer. Many factors may have significant influences on the degradation of biomedical devices such as morphology and crystallinity of the material and formulation. Therefore, the ToF SIMS approach described in this paper provides a surface point of view in the study of these complicated processes, which may help a better understanding of the mechanism and kinetics of biodegradation of polymers, in cases where surface properties are important, even if not dominant. The actual ability to predict the performance from these data will depend on the importance of the surface interaction of the application. A further unrevealed issue in the present study is the role of bulk crystallinity on surface and bulk degradation rates. We are presently studying the direct comparison of change in crystallinity vs the surface degradation rate as determined by ToF SIMS.[55] The role of the amorphous fraction of a polymer, which is assumed from AFM[39−44] to be surface segregated, on the ToF SIMS degradation rate will be explored in this new work.

Polyesters. PGA is a highly crystalline and hydrophilic polyester. Each of the carbon atoms in this polymer is connected to at least one oxygen atom. These properties influence not only its hydrolytic degradation property but also the fragmentation process upon the bombardment of the primary ions. As seen in Figure 1, intact molecular ion peaks are overwhelmingly dominant in the ToF SIMS spectra of hydrolyzed PGA over the medium to high mass range.

PLA is only one methyl group different from the structure of PGA, which presents as the side chain on the α-carbon. However, this methyl group causes a remarkable change in its properties from PGA, in addition to the formation of two monomeric enantiomeric structures, and copolymers of different tacticities. For example, the crystallinity of both P(d)LA and P(l)LA is lower than that of PGA. The hydrophilicity of PLA is also much lower than that of PGA, and the solubility in most common organic solvents is significantly higher than that of PGA. In fact, PGA does not dissolve in any of the common organic solvents. The presence of this methyl group significantly decreases its reactivity toward nucleophilic reagents due to the electron-donating effect of the methyl group. More importantly, this methyl group may have a substantial weakening effect on the bond between the carboxylic carbon and the ester oxygen and stabilizing the carboxylic carbon after the cleavage of this bond.[56] This might be the reason that the most intense peak in the pattern of ToF SIMS spectra of hydrolyzed PLA is not the intact molecular ion peak, but the structure of one oxygen atom less than intact molecules.

The difference in hydrolytic degradation rates between PLA and PGA can also be observed from the ToF SIMS spectra of the hydrolyzed PLGA 50:50 copolymer. As shown in Figure 4, peaks consisting of more PLA repeat units and less PGA repeat units are always more intense than those consisting of more PGA repeat units, indicating that PLA segments are more stable than PGA segments. The overall trend of molecular ion peak intensities of the hydrolyzed PLGA copolymer is similar to that of hydrolyzed PLA spectra (Figure 2).

Polyanhydrides. Both PSA and PFA (poly(fumaric acid)) are highly crystalline materials. It has been

determined in previous work[57,58] that the crystallinity of homopolymers of PSA and PFA are 66% and 60%, respectively. The crystallinity of their copolymers decreases, depending on the composition of the copolymer, but it is no lower than 38% for all compositions.[57] The samples of the polyanhydrides studied in this work, therefore, were made by melt-cast. One of the concerns for the melt-cast sample preparation procedure is the possibility of oxidation or cross-linking of the double bond in fumaric acid at elevated temperatures. There is no evidence found, however, that the double bond in fumaric acid has been severely changed. The intense fragments of fumaric acid at 97, 98, and 99 Da, corresponding to $[F - H]^+$, F^+, and $[F + H]^+$, are evidence of the existence of an abundance of unreacted fumaric acid structures (Figure 10a). This structure was also detected in high intensity after the hydrolysis treatments (Figure 10b), suggesting the basic repeat units of fumaric acid were not changed during the hydrolysis reaction either.

However, fragment ions containing multiple fumaric acid repeat units were never detected in this study. In the ToF SIMS spectra of PFS samples without hydrolysis treatments, only one fumaric acid repeat unit was detected in fragment sequences containing fumaric acid, whereas fragments with up to five sebacic acid repeat units were detected. The molecular ion peak series of hydrolysis products were found in both spectra of 20:80 and 50:50 copolymer samples before hydrolysis treatments. These molecular ion peak sequences consist of only sebacic acid monomers, suggesting that the anhydride bond of fumaric acid is more sensitive to hydrolysis than the anhydride bond of sebacic acid. There are two factors that may play an important role in this issue. One is the hydrophilicity. Sebacic acid contains a highly hydrophobic aliphatic structure while the structure of fumaric acid is highly hydrophilic. This may result in the hydrolytic degradation during storage to occur selectively at the fumaric acid sequence. The other factor is the conjugative property of the fumaric acid structure. With two carboxylic acid groups bridged by a double bond, fumaric acid forms a conjugated structure. This conjugated system increases the reactivity of the carboxylic acid carbon toward nucleophilic reactions.[59] Further investigation is needed to find out relative hydrolysis rates among FA−FA, FA−SA, and SA−SA linkages.

The differences in hydrolytic degradation rates among the three polyanhydrides can be seen by the hydrolysis time and the hydrolysis products illustrated in the ToF SIMS spectra. Table 4 lists all molecular ions detected in the hydrolyzed samples of all three polyanhydrides. The largest molecule of PSA hydrolysis products has six sebacic acid repeat units while the 20:80 copolymer has five and the 50:50 copolymer has four, although shorter hydrolysis times were used for the PFS copolymers. Furthermore, the intensities of the most intense molecular ion peaks are about 3500, 2000, and 800 for 50:50, 20:80, and the homopolymer of PSA, respectively. Therefore, the molecular ion peaks decrease faster for copolymers that have higher fumaric acid content.

Information for Kinetics Analysis. As mentioned above in the discussion of PGA hydrolysis results, a maximum of the molecular ion peaks exists which increase and moves toward the lower mass as the hydrolysis time increased. Under the assumption that this maximum represents the most probable molecular weight distribution of the hydrolysis products, the average molecular weight of the hydrolysis products can be calculated from the ToF SIMS spectra. The average molecular weight obtained from ToF SIMS is a function of hydrolysis time. A linear relationship between the apparent molecular weight and the hydrolysis time has been observed in our previous work.[46b] This observation suggested that the ToF SIMS spectra of hydrolyzed samples carry information about the hydrolytic degradation process of the polymer, which can be used in kinetics and mechanism analysis of hydrolytic degradation of the polymer. On the basis of this assumption, ToF SIMS studies of the degradation kinetics have been carried out, which is reported in a separate paper.[47]

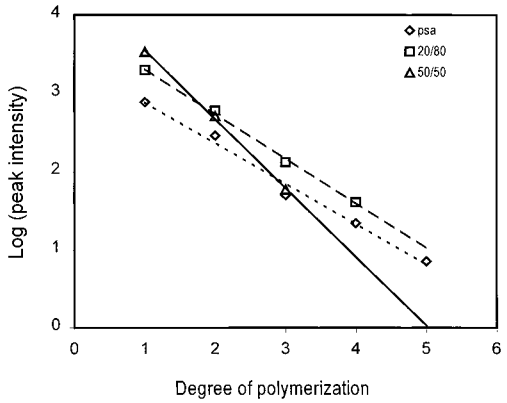


Figure 11. Plot of log molecular ion peak intensity of hydrolyzed polyanhydrides versus degree of polymerization of the hydrolysis products.

As discussed in the previous section, the size of the largest molecule of the hydrolysis products of polyanhydrides decreases as the content of fumaric acid in the copolymer increases, and the molecular ion peak intensity decreases faster accordingly. Obviously, this is directly related to the hydrolytic degradation property of the polymer. It is not a surprise that the intensity of the molecular ion peaks decreases exponentially. When the logarithm of the intensity of the molecular ion peak is plotted versus the degree of polymerization, a good linear relationship exists, as shown in Figure 11. It should be noted that there are only three and four points in the plot (Figure 11) for the 50:50 and 20:80 copolymers, respectively, due to the fast degradation rates of these copolymers. However, it is important to notice that the slope of the plots reflects the decreasing rate of the molecular ion peak intensities with respect to molecular weights, and the intersection point of the plot with *x*-axis indicates the limiting size of molecules can be detected on sample surfaces. As observed in this study, the slope of the straight lines increases with increasing of the hydrolytic degradation rates. The utilization of this trend in quantitative description of hydrolytic degradation rates is under investigation.

Conclusion

The hydrolytic degradation of six biodegradable polymers, involving two important classes of biodegradable polymers, in particular polyesters and polyanhydrides, and both homopolymers and random copolymers, has been studied using ToF SIMS. It has been demonstrated that, upon the hydrolytic degradation of biodegradable polymers, low molecular weight oligomers generated during the hydrolytic degradation can be directly detected in ToF SIMS spectra for all biodegradable polymers. The oligomers desorb from the sample surface

upon the bombardment and ionization, in the form of intact molecules, usually attached with an alkali metal ion. In most cases, the molecular ion peak is the most intense peak in each repeat pattern of ToF SIMS spectra of hydrolyzed polymers.

On the basis of the observations demonstrated in this work, it can be concluded that the direct observation of hydrolytic degradation products using ToF SIMS is common to hydrolytic degradation of many biodegradable polymers. Further studies of the ToF SIMS spectra have shown (reported separately) that the ToF SIMS data from hydrolyzed polymers provide the information for kinetics analysis of hydrolytic degradation at the surface of a material. The study of biodegradation of polymeric materials using ToF SIMS provides a fast and direct access to the degradation products. The time scale of each observation in the ToF SIMS study of the degradation of biodegradable polymers is reduced to hours from months with the conventional techniques, which may be useful and of importance where surface properties and surface etching rates are important.

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