Enzymatic Degradation of Hyperbranched Polyesters

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ABSTRACT: In this work, the enzyme-catalyzed degradation of hyperbranched polyesters (HBPEs) was investigated. Enzymatic degradation experiments were performed in a phosphate buffer in the presence of the lipases *Candida cylindracea*, *Pseudomonas cepacia*, Novozym 388, Amano CE, Lipomod 34P, and Cal-B, whereas control experiments were performed in the same system without lipases. The extent of polymer degradation was determined by quantification of the released free fatty acids by gas chromatography. The influence of the alkane chain length and the number of alkane chain end groups on the lipase-catalyzed hydrolysis of esterified HBPEs was investigated systematically. It was found that the increase in the alkane chain length of the end groups diminished the enzymatic degradation of the polymer, whereas the number of end groups had no influence on the degradation rate. The effect of temperature on the rate of degradation was also described. Surface morphological changes that occurred during the degradation were assessed with reflected electron microscopy. The changes in the crystallinity of the polymers after they were subjected to degradation were qualitatively determined with differential scanning calorimetry through the quantification of the enthalpy of melting. The enthalpy of melting of one HBPE sample increased from 79 to 90 and 94 J/g with and without the action of Lipomod 34P, respectively, in 7 days, showing the changes in the crystallinity of the polymer. The results prove that modified HBPEs are an important new class of biodegradable materials with a predictable degradation mechanism, and the degradation can be adjusted on the basis of the molecular engineering. J Appl Polym Sci 112: 1873–1881, 2009

Key words: biodegradable; enzymes; hyperbranched; polyesters

INTRODUCTION

In view of the growing interest in biodegradable polymers in the field of life sciences, especially in pharmaceutical applications, it is important to know the degradation behavior of such polymers under different conditions. Among the existing polymers for pharmaceutical and biomedical applications, polyesters offer the advantage of being hydrolyzed in the presence of enzymes.[1–3] The biomedical and ecological applications of biodegradable linear synthetic polyesters, such as poly(lactic acid), poly(glycolic acid), polycaprolactone, and their copolyesters, have been intensively studied.[4–7] The enzymatic degradation of these and other linear synthetic polyesters has also been reported.[8–14]

In recent years, hyperbranched polymers, especially hyperbranched polyesters (HBPEs), have been receiving increasing attention in the field of life sciences, particularly as drug delivery materials, because of their attractive properties, such as nontoxicity, biocompatibility, and large numbers of functional groups. Hyperbranched polymers are highly branched, polydisperse macromolecules with a treelike topology carrying a large number of functional end groups. The biomedical applications of Boltorn H30, a commercially available HBPE, have been reported in the literature.[15] It has been proved experimentally that the polymer contains 12 hydroxyl groups on its periphery and that they can be modified for the end use of the polymer.[16,17] The influence of the end groups on the properties of hyperbranched polymers has been investigated intensively by many authors.[18–24] Suttiruengwong et al.[25] employed HBPEs and poly(ester amide)s for the controlled release of acetaminophen. Gao et al.[26] synthesized a water-soluble hyperbranched polymer for drug delivery applications. The use of HBPEs as dental composite materials was investigated by Klee.[27] Recently, hyperbranched poly(ester amide)s were synthesized with gallic acid

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and amino acids, and their degradation was studied by Li et al.[28] Haag and Kratz[29] synthesized hyperbranched polyglycerol and demonstrated its potential as a candidate for the delivery of anticancer drugs. Kainthan and coworkers[30–33] investigated the *in vitro* and *in vivo* biological evolution of low- and high-molecular-weight hyperbranched polyglycerols and reported that the polymers are highly biocompatible and nontoxic. The polymers could be used in various applications in nanobiotechnology and nanomedicine. Along with the growing potential pharmaceutical applications of hyperbranched polymers, they also have a wide array of applications in the field of chemical engineering, as recently reviewed by Seiler.[24]

The enzyme-catalyzed degradation of polyesters is a complex process. To reach the surface, the enzyme must be soluble and undergo a conformational change to fix itself at the substrate surface.[2] In the first step, the binding sites of an enzyme make bonds with the substrate through noncovalent bonds such as hydrophobic interactions; in the second step, the catalytic domains facilitate the hydrolysis process at the site of action. The structural dependence of aliphatic polyesters on the enzymatic degradation was investigated by Mochizuki and Hirami.[34] They found that the enzymatic degradation of polyesters depends on the chemical structure of the polymer, crystallinity, hydrophilic–lipophilic balance, and presence of functional groups on the polymer surface.

The synthesis and degradable aspects of poly (3-hydroxybutyrate) (PHB), which is a natural polymer, were reviewed by Lenz and Marchessault.[35] The enzymatic degradation kinetics of PHB were intensively studied by Timmins et al.[36] and Mukai et al.[37] They demonstrated that the enzymatic degradation of PHB is a heterogeneous process and that the degradation rates do not follow the classical Michaelis–Menten equations, and they proposed a new kinetic model for the degradation of PHB.

A common method for the determination of the biodegradation of polymers is based on weight-loss measurements. Even though it is a fast and convenient method for getting an initial idea about the degradation of polymeric materials, it is hard to obtain precise time-dependent degradation kinetics with this method.[10] Depending on the substrate nature and the types of degradation products that form during the degradation process, several methods have been developed for obtaining the time-dependent degradation kinetics of polymers. The most common methods are turbidimetric and titrimetric methods,[36,38,39] spectrophotometry,[28] light scattering,[5,40] and monitoring of the changes in the molecular weight distribution.[14]

However, a systematic study of the enzymatic degradation of HBPEs has not yet been reported in the literature. The goal of this work is to investigate the enzymatic degradation of different HBPEs esterified with fatty acids. The influence of the number and type of end groups on the enzymatic degradation of HBPEs is investigated, and parameters such as the type of lipase, its concentration, and the temperature are evaluated.

EXPERIMENTAL

Materials

The aliphatic HBPE Boltorn H30 is a commercial product of Perstorp AB (Sweden). It was esterified with fatty acids by a method similar to that described by Teng et al.[20] The polymers were designated as HBPE-I to HBPE-IV, and their properties are given in Table I. Lipases from *Candida cylindracea* (Lipase CC) and *Pseudomonas cepacia* (Lipase PC) were purchased from Sigma–Aldrich Chime GmbH (Steinheim, Germany). Lipomod 34P, Cal-B, Novozym 388, and Amano CE were kindly provided by Evonik Goldschmidt GmbH (Essen, Germany). The activities of the lipases, as measured with tributyrin, are given in Table II. Ammonium phosphate salts used for the preparation of the buffer solution were obtained from Merck (Darmstadt, Germany). All solvents used in this study were analytical-grade and were used as received. The total amount of fatty acids

TABLE I
Properties of the HBPEs

Polymer	Molecular weight (g/mol): a/b/c[a]	Degree of esterification (%)	Type of fatty acids	Fatty acid (wt %): a/b/c[a]	Melting temperature (°C)[b]	Enthalpy of melting (J/g)[b]
HBPE-I	7500/2830/2924	50	C16/C18	57.7/57.2/55.5	41	58.75 ± 0.59
HBPE-II	8700/3840/3934	80	C16/C18	79.6/67.5/66.0	41	72.92 ± 0.73
HBPE-III	9000/4097/4187	95	C16/C18	90.3/75.3/73.6	44	79.01 ± 0.79
HBPE-IV	12100/4714/4804	90	C20/C22	77.7/74.8/73.4	61	78.31 ± 0.78

[a] Molecular weight (a) was calculated on the basis of the theoretical core-to-monomer ratio, (b) was calculated with the core polymer molar mass determined by the vapor pressure osmometry (data of Burgath et al.[41]), and (c) was calculated with the core polymer molar mass determined by the size exclusion chromatography with detection by multiangle light scattering data of Žagar and Žigon.[16,17]

[b] Determined by DSC.

TABLE II
Details of the Lipases

Name of the lipase	Activity (U/mg)[a]
Lipase *C. cylindracea*	30
Lipase *P. cepacia*	50
Lipomod 34P	115
Amano CE	170
Novozym 388	15[b]
Cal-B	7[b]

[a] 1 U corresponds to the amount of enzyme that liberates 1 μmol of butyric acid/min (tributyrin as the substrate at 40°C at pH 7).
[b] These lipases were supplied in a liquid state, and here, the measured activity was given as U/μL.

present on the polymer surface was calculated by multiplication of the degree of esterification by the total number of hydroxyl groups.

Enzymatic degradation procedure

In this study, a method based on gas chromatography (GC) analysis was used for the quantification of fatty acids. The enzymatic degradation of fatty-acid-modified HBPEs was carried out in a phosphate buffer solution of pH 5 in the presence of a lipase. A schematic representation of the enzyme attack at the ester bonds of HBPE-I is depicted in Figure 1. Just before the experiment, the lipase solution was prepared by the dissolution of lipase in the phosphate buffer. Five milliliters of this solution was placed in a 20-mL screw-capped glass bottle, and 75 mg of hand-milled HBPE powder with a diameter less than 90 μm was added. The contents were mixed vigorously by manual shaking for 2 min. Then, the samples were incubated in a water bath at 37 ± 0.1°C. Nonenzymatic degradation of modified HBPEs was determined by a control sample under identical conditions but without a lipase, that is, in a pure buffer solution. The amount of degradation of HBPE was determined by quantification of the amount of the released free fatty acids. The GC procedure for the analysis of fatty acids is described in detail in the following section. For the experiments with different lipases, the relative concentration of each lipase was maintained at 57.5 U/mL of buffer. The absolute concentrations needed for the degradation experiments were calculated from the activities of the corresponding lipases, which are given in Table II.

GC procedure for the quantification of fatty acids

After a specified time, the samples were removed from the thermostat and cooled to room temperature. Then, 5 mL of heptane was added to the entire amount of each sample, and the components were mixed properly by manual shaking for 2 min; then, 10 min was allowed for phase separation. After the two phases had separated, 1 mL of the upper phase (heptane-rich phase) was taken, and 0.3 g of $CaCl_2$ was added to remove the traces of water remaining in the sample. $CaCl_2$ was removed from the sample by ultracentrifugation at 14,000 rpm for 2 min (type Z 160 M, Hermle Labortechnik, Heidelberg, Germany). Two hundred fifty microliters of pyridine containing a 0.33 wt % concentration of an internal standard was added to 500 μL of this sample, and the contents were mixed by manual shaking and centrifuged once again for the removal of some traces of $CaCl_2$ salt. The silylation reaction was carried out by the addition of 100 μL of *N*-methyl-*N*-trimethylsilyl trifluoracetamide to half of the aforementioned solution, and the reaction

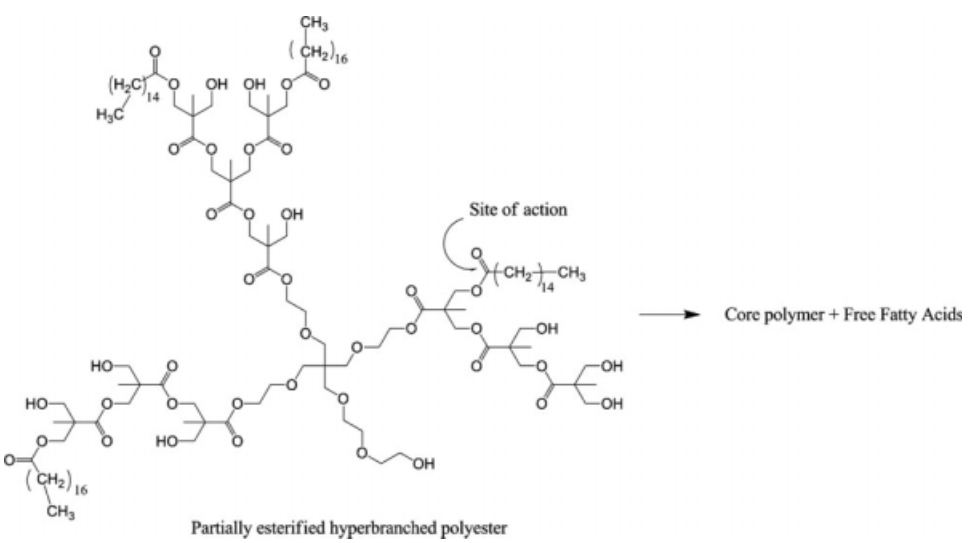

Figure 1 Schematic representation of the enzymatic degradation of HBPE-I containing C16 and C18 fatty acid ester end groups.

was performed at 105°C for 30 min. Then, the sample was injected into the GC apparatus (5890 series II gas chromatograph with a 7673A automatic injector, Hewlett–Packard) [Germany] and the amount of free fatty acids was determined.

Thermal analysis

The thermal behavior of the pure polymers and degraded polymers was obtained from the differential scanning calorimetry (DSC) analysis. The experiments were performed with a Netzsch (Selb, Germany) DSC 200F3 Maio analyzer. An accurately weighed sample amount of 6–8 mg was placed in hermitically sealed aluminum pans. The thermograms were measured from −50 to 100°C at a heating rate of 10°C/min in a nitrogen atmosphere.

High-performance liquid chromatography (HPLC)

HPLC was used for the quantitative analysis of 2,2-bis(hydroxymethyl) propionic acid (bis-MPA) released during the degradation of the core polymer (Boltorn H30). An instrument from Waters Corp. (Frechen, Germany) was employed and consisted of a Waters autosampler with a Shimadzu SPD-10AVP UV detector (Ouisburg, Germany). The following conditions were used:

Column: ET 250/8/4 Nucleosil 100-5 SA.
Eluent: 4.6 g of $(NH_4)H_2PO_4$/L of H_2O with the pH adjusted to 3 with H_3PO_4.
Flow rate: 0.6 mL/min.
Injection volume: 20 μL.
Temperature: 25°C.
UV detection wavelength: 208 nm

Reflected electron microscopy (REM)

REM (model 1810, Amray, Inc.) [Bedford, MA, USA] was used to obtain information about the surface morphology of the microparticles. To make the polymer particles conductive, they were coated with a thin layer of gold.

X-ray diffraction (XRD) measurements

To examine the crystalline structure of the investigated HBPEs, XRD measurements were performed with Cu Kα1 radiation with a Philips X'pert MPD PW 3040 diffractometer (Philips, The, Netherlands).

RESULTS AND DISCUSSION

Comparison of different lipases

As a first step, the influence of different enzymes on the degradation was studied with several commercially available lipases. All experiments were carried out with HBPE-I for 24 h, the enzyme concentration being maintained at 57.5 U/mL. After 24 h, the samples were analyzed for the free fatty acid concentration. The corresponding gas chromatograms of released free fatty acids from the degradation of HBPE-I in a buffer and in a lipase solution together with pure fatty acids are presented in Figure 2.

HBPE-I was esterified with a mixture of palmitic and stearic acids, so it contained C16 and C18 fatty acids as end groups linked by ester bonds. Obviously, one can expect the degradation products to be palmitic and stearic acids and monomeric units if any degradation of the core material takes place. Indeed, the peaks at about 15 and 18 min, labeled as free fatty acids 16 and 18, correspond to palmitic and stearic acids, respectively, as proven by the injection of the pure acids under the same conditions. The calibration was done with *n*-tetradecane as the internal standard (peak IS). Theoretically, 57 wt % of the fatty acids were present in HBPE-I. This was calculated from the known degree of esterification of the polymer and the known total number of end groups under the assumption of equal amounts of palmitic and stearic acids. This value was set to 100% for the degradation experiments, and the relative amount of fatty acid released by hydrolysis or enzymatic degradation was calculated from this value. It is worth mentioning that several research groups have reported discrepancies associated with the experimentally determined number of end groups, number-average molecular weights, and degree of branching of hyperbranched polymers and the theoretical values.[17,18,41] For the calculation of the amount of fatty acids present in the polymer, the experimental values of the molar mass of the core polymer and the number of hydroxyl groups from Žagar and Žigon[16,17] were considered.

The amount of fatty acids released in the presence of various lipases is presented in Figure 3. From the results, it is obvious that the catalytic activity toward the HBPEs strongly depends on the source of the lipase. The lipase from *C. cylindracea* (Lipase CC) showed the strongest activity by cleaving 23% of the fatty acid groups (scaled to 100% as mentioned previously). The difference in the catalytic performance of the lipases toward HBPE-I could be explained by the different binding capacities of the lipases because it is known that each lipase has the same catalytic behavior but the binding capacity to the substrate varies from lipase to lipase, depending on the microbial source of the enzyme.[34] Also, here the lipases were acting on an unusual solid substrate; this is different from their normal mode of action at oil/water interfaces for hydrolyzing triglycerides. Nevertheless, significant amounts of hydrolysis were observed. On the basis of these results, further

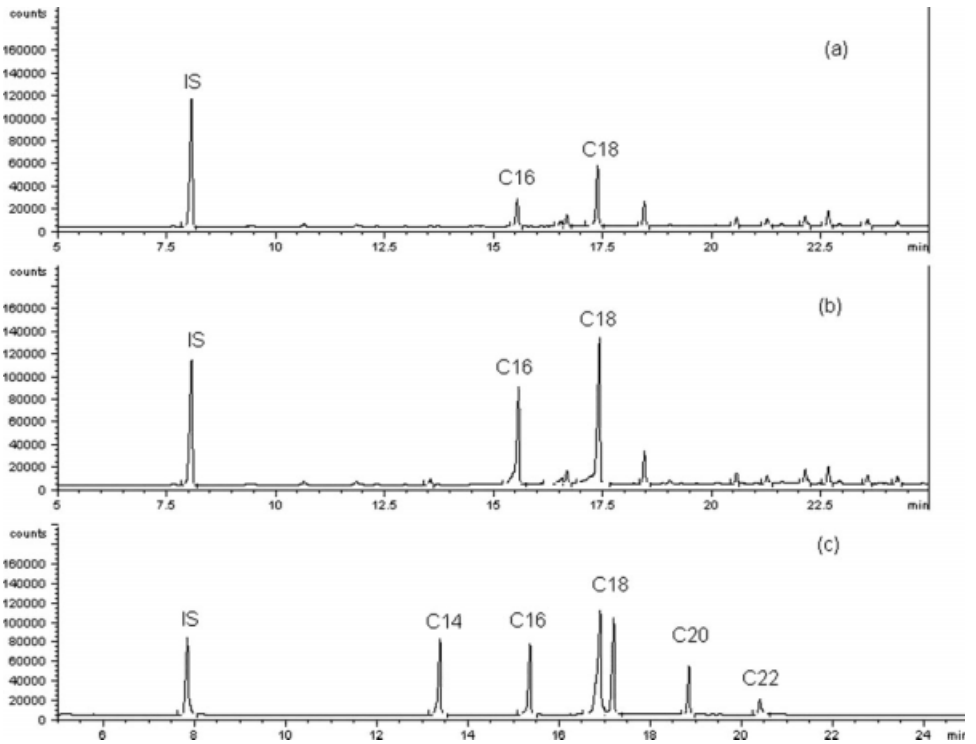

Figure 2 Gas chromatograms of released free fatty acids from the degradation of (a) HBPE-I in a buffer solution, (b) HBPE-I in a lipase solution, and (c) pure fatty acids.

experiments were performed with the lipase Lipomod 34P because of its good availability.

Time dependence of the enzymatic degradation

The degradation of HBPE-I was investigated in the presence of Lipomod 34P for a period of 1 week through the monitoring of the amount of free fatty acids in solution. The amount of the released free fatty acids as a function of time is presented in Figure 4. All points are the averages of three measurements, the standard deviation given in the figure being less than 5% of the absolute concentration at any particular time. The time course of the enzymatic degradation of HBPE-I can clearly be seen, and 33% of the fatty acids were released after 7 days. The amount of nonenzymatic degradation was negligible during the 1-week period.

It is known from the literature that the surface erosion mechanism would be the predominant mechanism in the enzymatic degradation of insoluble hydrophobic substrates.[3,34] It is assumed that the same mechanism might be involved in the enzyme-

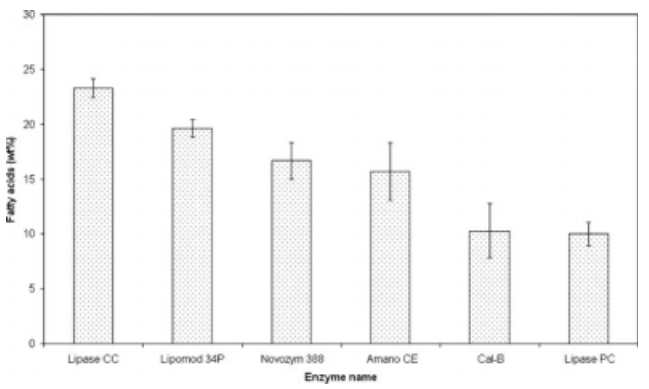

Figure 3 Enzymatic degradation of HBPE-I with the action of various lipases (mean ± standard deviation, $n = 3$) in a pH 5 buffer solution at 37°C after 24 h (enzyme activity = 57.5 U/mL).

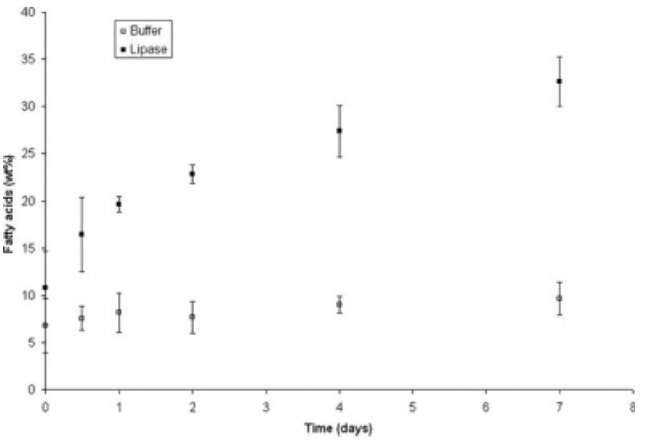

Figure 4 Degradation of HBPE-I with the action of Lipomod 34P in a pH 5 buffer solution at 37°C over 1 week (mean ± standard deviation, $n = 3$).

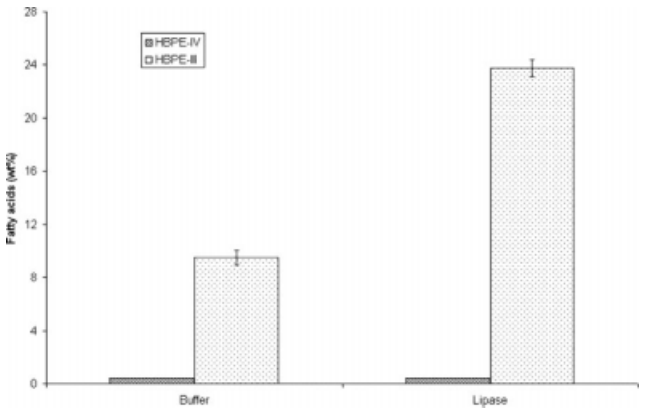

Figure 5 Enzymatic degradation of HBPE-III and HBPE-IV (comparable degrees of esterification but different end groups) in the presence of Lipomod 34P for 24 h at 37°C and pH 5 (mean ± standard deviation, $n = 3$).

catalyzed degradation of these modified HBPEs. As the degradation process proceeds, fatty acids are released from the polymer, leaving the core polymer with hydroxyl groups as end groups. The core material is a hydrophilic polymer, and the enzyme may not bind to its surface because the hydrophilic and lipophilic balance of the substrate should be at its optimum level to achieve good enzyme activity.

Effect of the end-group type

The effect of the type of end group on the enzymatic degradation of HBPE was investigated by the selection of HBPE-III and HBPE-IV with C16/C18 and C20/C22 fatty acid esters as end groups, respectively (Fig. 5). The degree of functionalization of the polymers (i.e., the number of peripheral hydroxyl groups of Boltorn H30 esterified with the fatty acids) is given in Table I; although the degree of functionalization of the two polymers differed by 5%, the degradation results were compared. In both cases, the substrate and lipase concentrations were the same, and the experiments were carried out under identical conditions, as described in the Experimental section. The degradation of HBPE-IV was extremely low compared to that of HBPE-III. Just by an increase in the chain lengths of the fatty acid ester end groups by four methylene groups on the surface of the HBPE (C20/C22 versus C16/C18), the whole degradation behavior of the HBPE was changed, both in the buffer and in the presence of a lipase. Only 0.5 wt % of the fatty acids was released from the enzymatic degradation of HBPE-IV in 24 h, and nearly the same amount was observed without the action of an enzyme. A possible reason for the slow degradation of HBPE-IV is the conformational arrangement of the polymer molecule, which seems to be not suitable to fit into the active site of an enzyme because of the presence of more bulky groups on the surface. It is known that some lipases are so specific for a given substrate that they do not tolerate any structural or configurational changes in the substrate molecule.[34] The polymer structure must be flexible enough to mold itself to fit into the active site of the enzyme, and any small changes in the polymer structure could affect the enzyme attack adversely. The increase in the fatty acid chain length may cause a change in the structural or crystalline behavior of the solid polymer. The qualitative measurement of the amount of crystallinity has revealed that the two polymers are similar in terms of crystallinity, so the increase in the alkane chain length could possibly decrease the adsorption of the enzyme onto the polymer surface.

Influence of the degree of esterification

To investigate the influence of the number of alkane chains on the enzymatic degradation, the degradation of three products with different degrees of esterification—HBPE-I, HBPE-II, and HBPE-III—was compared (Fig. 6). All the polymers contained C16 and C18 fatty acid esters as end groups, but the numbers of esterified end groups were different (50, 80, and 95%, respectively; cf. Table I). HBPE-III showed slightly higher degradation than HBPE-I with and without the action of Lipomod 34P, whereas HBPE-II showed less degradation versus the other two polymers. The results suggest that there is no obvious relationship between the degree of esterification and the amount of degradation.

Influence of the lipase concentration on the enzymatic degradation

The influence of the concentration of the enzyme on the degradation of HBPE was investigated for

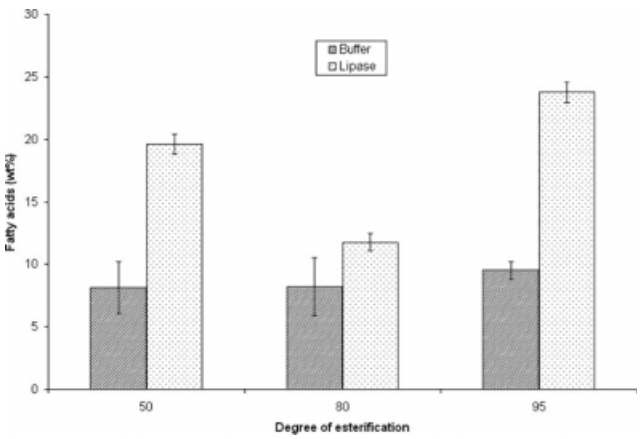

Figure 6 Degradation of HBPEs containing different amounts of C16 and C18 fatty acid esters as end groups at 37°C, pH 5, and 24 h (mean ± standard deviation, $n = 3$).

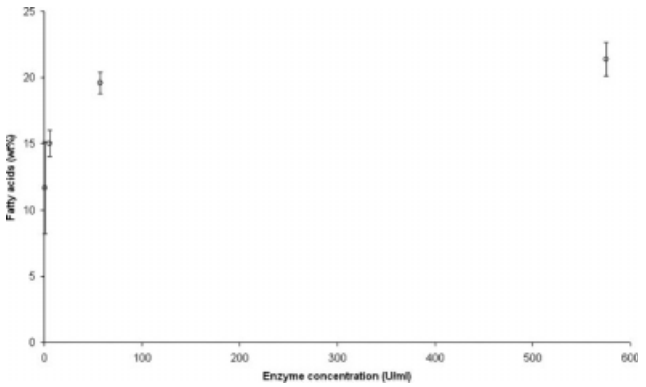

Figure 7 Influence of the lipase concentration on the enzymatic degradation of HBPE-I (mean ± standard deviation, $n = 3$) at 37°C, pH 5, and 24 h.

HBPE-I in the presence of Lipomod 34P at different concentrations ranging from 0.575 to 575 U/mL, and the results are presented in Figure 7.

A nonlinear increase in the degradation of HBPE-I was observed with an increase in the enzyme concentration in the solution. First, the degradation of the polymer took place very quickly up to an enzyme concentration of 57.5 U/mL. After this, even a 10-fold increase in the enzyme concentration did not result in any further increase of the degradation. For a given specific surface area of the substrate, only a specific amount of the enzyme was adsorbed onto the substrate, and the rest of the enzyme was no more involved in the hydrolysis of ester bonds. This is in good agreement with the results reported in the literature for linear polyesters.[10]

Effect of temperature

The influence of the temperature on the enzymatic degradation of HBPE-I is presented in Figure 8. As the temperature increased, the rate of degradation also increased in the presence of a lipase up to 37°C; this was due to the increased activity of Lipomod 34P. At 37°C, large differences in the degradation of HBPE-I with and without the action of Lipomod 34P were observed. In the case of the pure buffer, the maximum degradation was observed at 28°C. At higher temperatures, 45 and 50°C, a large decrease in the enzymatic degradation was observed. Even though Lipomod 34P is more active in the temperature range of 40–55°C (provided by the manufacturer), above 41°C, the polymer starts to melt, and this causes the polymer to form a large droplet, which inherently reduces an enzyme's chances of reaching the polymer surface. As a result, a lower rate of enzymatic degradation of the polymer was observed at the higher temperatures.

Surface morphology

The structural and morphological changes were investigated with REM and XRD methods, respectively. The obtained REM pictures of the surface of HBPE-I microparticles before and after enzymatic degradation are presented in Figure 9.

From the surface analysis, one can see the clear difference in the particle surface after the polymer was subjected to degradation. Before the degradation, the polymer surface was quite smooth [Fig. 9(a)], and after incubation in a buffer solution, the surface hardly changed [Fig. 9(b)], whereas after the enzymatic degradation, the surface became very rough [Fig. 9(c)]. The results qualitatively suggest that the polymers were truly enzymatically degraded. The changes in crystallinity can be observed by XRD measurements. X-ray diffractometer scans of pure HBPEs are presented in Figure 10. The results showed that the crystallinity of the polymers did not change significantly either with the degree of esterification of the polymer or with the type of fatty acids. The measurements with the polymers after subjection to the degradation were not successful because the polymer concentration (15 mg/mL) in the suspension was too low to perform the XRD experiments. Alternatively DSC analysis was applied to characterize the changes in the crystallinity of the polymers because it requires only a small amount of a sample (5–6 mg). The enthalpy of fusion or melting, which indicates the crystallinity of polymers, changed significantly with the time of degradation. The enthalpy of melting of HBPE-III increased from 79 to 90 and 94 J/g with and without the action of Lipomod 34P, respectively, in the 7-day period (Table III). A possible reason is that the crystalline region might have increased with the extent of degradation because of the increased space for the rearrangement of the remaining alkane chains.

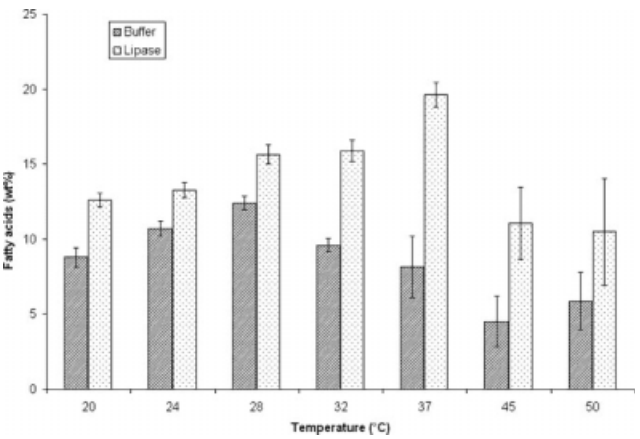

Figure 8 Influence of the temperature on the degradation of HBPE-I, with and without Lipomod 34P, in a pH 5 buffer after 24 h (mean ± standard deviation, $n = 3$).

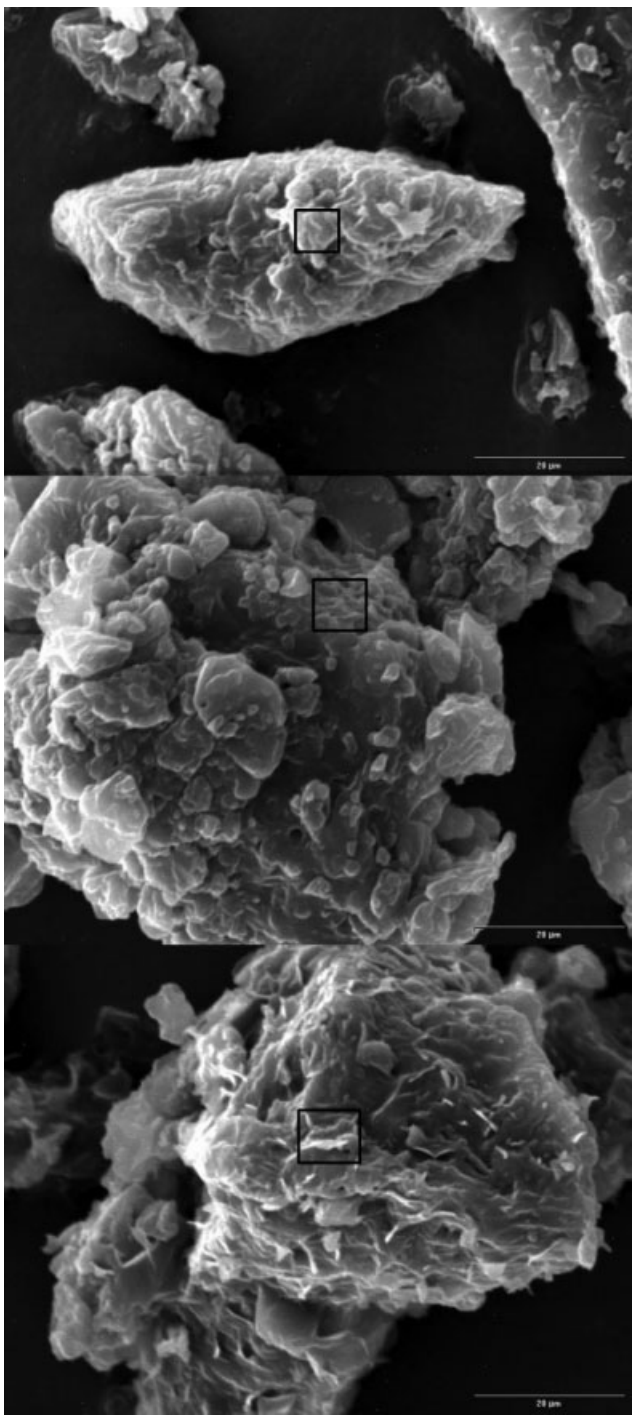

Figure 9 REM pictures of HBPE-III under different conditions after 24 h (a) before degradation (as received), (b) subjected to degradation in a buffer solution, and (c) subjected to degradation in a lipase solution.

This could be due to the fact that the enzymatic degradation preferably took place in the amorphous region, as in the case of linear biodegradable polymers. It is also widely accepted that the less orderly arranged amorphous region is more easily accessible by the enzyme versus the highly ordered crystalline region. The melting temperature of the polymers did not change after the degradation process. The error in the determination of the enthalpy of fusion was around 1% in the absolute amount.

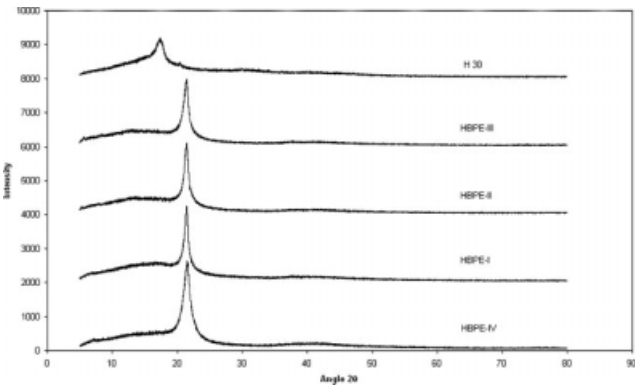

Figure 10 X-ray diffractometer patterns of HBPEs.

Enzymatic degradation of the core material

Boltorn H30 was the core material for HBPE-I to HBPE-IV and consists of the polyester of the dihydroxycarboxylic, acid bis-MPA. The polymer contains 12 hydroxyl groups on its surface[17] and is considerably hydrophilic in nature. Enzymatic degradation of this core molecule was investigated to see whether it could also act as a substrate for the enzymes. It was subjected to the same degradation conditions described in the Experimental section. The amount of degradation was determined by the monitoring of the concentration of the monomer (bis-MPA) in the supernatant solution by HPLC. Figure 11 shows the results of the enzymatic degradation of Boltorn H30 with the action of Lipomod 34P for a period of 2 days. The polymer was found to be not degraded by the lipase action. The amount of bis-MPA at the beginning was about 4.8 wt %, and after 48 h, very little difference was observed with and without the action of Lipomod 34P. The reason for the poor lipase

TABLE III
Changes in the Enthalpy of Fusion and Melting Temperature of HBPE-III With and Without the Action of Lipomod 34P

	Enthalpy of fusion (J/g)[b]		Peak melting temperature (°C)	
Time (h)	Buffer	Lipomod 34P	Buffer	Lipomod 34P
0[a]	79.01 ± 0.79	79.01 ± 0.79	43.8	43.8
24	86.61 ± 0.86	86.49 ± 0.86	42.7	42.7
48	84.28 ± 0.84	87.39 ± 0.87	42.9	42.5
96	88.33 ± 0.88	88.81 ± 0.88	42.6	42.3
168	90.43 ± 0.91	94.50 ± 0.94	42.0	42.5

[a] Polymer as received.
[b] The error is shown as mean ± standard deviation ($n = 3$).

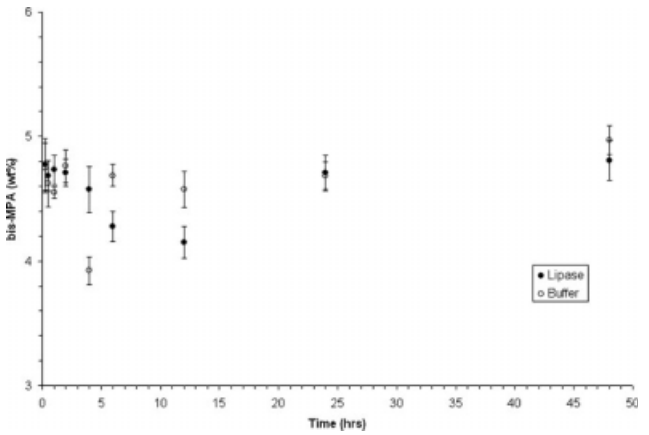

Figure 11 Degradation of Boltorn H30 in a pH 5 buffer at 37°C.

activity was either the absence of hydrophobic binding interaction sites on the polymer surface or the steric hindrance of the polymer.

CONCLUSIONS

In this work, the lipase-catalyzed degradation of four HBPEs esterified with long-chain fatty acids was studied. It was found that the source of the enzyme had a large influence on the degradation of HBPEs. Two enzymes, the lipase from *C. cylindracea* and Lipomod 34P, showed the highest activity toward the degradation of the HBPEs among the six investigated lipases. The concentration of the lipase showed a significant influence on the enzymatic degradation of HBPE-I, which increased with the increase in the lipase concentration until the saturation was reached. It was found that, in the presence of Lipomod 34P, the degree of enzymatic degradation increased with the temperature increasing up to 37°C. In a pure buffer solution, the maximum degradation was observed at 28°C. The maximum difference in the degree of degradation between the lipase and buffer was observed at 37°C for HBPE-I. For the first time, the results showed that the types of alkane chains on the HBPE surface could influence the behavior of the lipase-catalyzed hydrolysis. Furthermore, it was proven that in comparison with the hydrolysis of the esterified polymers, the hydrolysis of the core material (Boltorn H30) was negligible. Thus, one may be able to tailor the rate of degradation of HBPEs by tailoring the molecular architecture. The reproducibility of the experiments was remarkably good, and the standard deviation was less than 5% in all cases. These results, for the first time, confirm the biodegradability of modified HBPEs and open up the possibility of using them in biomedical applications, such as the delivery of bioactive agents by an enzyme-triggered process.

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