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MECHANISM AND KINETICS OF THE HYDROLYTIC DEGRADATION OF AMORPHOUS POLY(3-HYDROXYBUTYRATE)

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ABSTRACT

Amorphous poly(3-hydroxybutyrate) films prepared by compression molding and solvent casting were degraded in aqueous media at different pH values. The time dependence of degradation was monitored by the measurement of weight loss, the extraction of the degradation products from the degrading sample, as well as by UV-Vis spectrophotometry and HPLC analysis of the aqueous solution. The results proved that degradation takes place mainly in the bulk of the samples and not on their surface. The overall rate of degradation depends strongly on pH; it increases with increasing pH values. Metabolite extraction and chromatography proved that degradation does not occur randomly, but with larger frequency at the end of the chains. By assuming that the hydrolysis of PHB is a S_N2 type nucleofil substitution reaction, a kinetic model was proposed which describes the formation of various degradation products. The diffusion of metabolites was also accommodated into the model thus the concentration in the aqueous solution could also be predicted well. The correlation between prediction and experimental results is excellent. The model can be extended also for the description of the hydrolytic degradation of other aliphatic polyesters.

1 INTRODUCTION

Poly(3-hydroxybutyrate) (PHB) is one of the most important biopolyesters from the family of polyhydroxyalkanoates which are produced by microorganisms from renewable resources [1-7]. Unlike in the synthetic polymerization of PHB, the production of the biopolymer by microbial fermentation excludes the presence of toxic products [8-10] and the hydrolytic degradation of PHB leads mainly to the monomer D-3-hydroxybutyric acid. This acid is a normal component of blood and is one of the three ketones which are

produced endogenously by ketogenesis [11].

Since neither the production, nor the hydrolytic degradation of this polymer yields toxic metabolites, PHB is a potential biopolymer for the production of ecofriendly commercial products, like plastic bags, films, implants, etc. [12,13]. In spite of the widening range of possibilities provided by the production and application of PHB and its copolymers, their market share is still small [11]. Several works focusing on the comparison of the mechanical properties of the most important biopolymers [poly(lactic acid), PLA; poly(glycolic acid), PGA; poly(lactic-co-glycolic acid), PLGA, PHB, poly(hydroxybutyrate-co-valerate), PHB-HV] demonstrated that PHB and its copolymers are generally less suitable for applications in which the mechanical characteristics of the polymer are important, than for example PLA [14-16]. To overcome the problem of inferior mechanical properties and the often encountered processing difficulties, PHB is often modified by copolymerization usually to produce PHB-HV copolymers [21-26]. In spite of these deficiencies the fermentation of the 3-hydroxybutyrate polymer family offers a considerable industrial potential, mainly because PHB and other members of its family turned out to be excellent matrix polymers in the field of controlled drug release [27-31].

Because of the increasing interest in biopolymers and the potentials of PHB, a considerable number of studies have been dedicated to the investigation of the hydrolytic degradation of this polymer [32-34]. Although these studies are rather diverse, they agree that the degradation of PHB in aqueous media is a base catalyzed hydrolytic reaction, in which the rate of the reaction is primarily determined by the concentration of hydroxide ions [32-34]. Unfortunately, mainly because of the different methods applied for the production of PHB films and pellets (film casting [32,33], microencapsulation followed by cold pressing [32], injection molding [35]) in these studies, even when two research teams used the same polymer or copolymer, their results are quite difficult to compare.

Moreover, none of the papers cited above attempted to define the mechanism and to describe the kinetics of the hydrolytic degradation of the biopolymers studied. The theoretical background of polyester hydrolysis has been already described by Flory [36], who established that it is a reversible reaction, which is expected to reach equilibrium eventually in a given period of time. However, the formation and the hydrolysis of polyester macromolecules differ from each other. Based on the analysis of his experimental results Flory [36] concluded that the hydrolytic fragmentation of polyesters occurs with larger probability at ester groups located close to the end of the polymer chain with the consequence that metabolites with smaller molecular mass are expected to form with larger probability. Accordingly, the most probable product of chain fragmentation is the monomer and the amount of other oligomers decreases with increasing molecular weight. Position dependent hydrolysis rates were needed to account for and describe quantitatively the inhomogeneous distribution of metabolites [36]. Even though none of the polymers mentioned and investigated by Flory [36] was a microbial polyester, we might assume that also the hydrolysis of PHB follows the characteristics described above.

The theoretical treatment of Flory [36] was based on the assumption of homogeneous reaction; hydrolytic degradation was carried out in solution. However, under practical conditions, the degradation of biopolymers occurs heterogeneously both in composting and in vivo, like in the case of implants. Accordingly the goal of this study was to investigate the hydrolytic degradation of poly(3-hydroxybutyrate) under heterogeneous conditions, on films prepared by compression molding and solvent casting. We intended to check the prediction of Flory [36] on the position dependent cleavage of the polymer chains. Assuming that hydrolysis is a nucleophile substitution reaction, a kinetic model was proposed which considers the heterogeneous nature of degradation and accounts for the diffusion of the main components. Rate constants were determined by the fitting of the

model to the experimental data, which have not been published before and are not available otherwise.

2 EXPERIMENTAL

2.1 Materials

Poly(3-hydroxybutyrate) granules were obtained from Metabolix Ltd. (Mirel M2100, ≥99.5 % purity) with an approximate crystallinity of 60 %. The aqueous media used to degrade the PHB films consisted of technical grade NaOH (Molar Chemicals Ltd.) and distilled water, while the HPLC eluent was a H_3PO_4/KH_2PO_4 phosphate buffer consisting of components purchased from Molar Chemicals Ltd. (H_3PO_4) and Fluka GmbH (KH_2PO_4), respectively. Technical grade chloroform stabilized with 1 % EtOH (Molar Chemicals Ltd.) and laboratory grade acetonitrile (Promochem Ltd.) were used for the extraction of metabolites from degraded polymer films. The 3-hydroxybutyric acid with a purity of ~95% used for the calibration of the HPLC detector and the UV-VIS spectrophotometer was supplied by Sigma-Aldrich.

2.2 Preparation of PHB films

Amorphous poly(3-hydroxybutyrate)films were prepared by compression molding and solvent casting, respectively. Films of 100 μm thickness were compression molded using a Fontijne SRA 100 machine at 120 kN, 3 min, 220 °C and at a cooling rate of about 30 °C/min. Films were cast onto a glass surface from a chloroform solution of 2 m/m% of the polymer and subsequently kept at constant temperature (25 °C) and relative humidity (50 %). Compression molding produced amorphous films with an approximate thickness of 100 μm and a surface area of 192 cm^2. The surface area of the solvent cast films was the same, but their average thickness was much smaller (~10 μm).

2.3 Characterization, measurements

The hydrolytic degradation of PHB was monitored quantitatively by four independent techniques. Two of them, gravimetric analysis and metabolite extraction, were applied in order to characterize the polymer phase during hydrolysis, while the other two, UV-VIS spectrophotometry and high performance liquid chromatography (HPLC), was employed to provide information on the quality and quantity of metabolites present in the aqueous phase.

For gravimetric analysis amorphous PHB films were prepared, weighed, and subsequently placed into a 100 ml flask containing aqueous media with the pH value of 13.0, 12.5, 12.0 and 7.0, respectively. The flasks were sealed and then opened after a given degradation time (7, 14, 21 and 28 days). The degraded films were washed, dried and kept in a room of constant temperature (25 °C) and relative humidity (50 %).

To determine the metabolites present in the polymer, degraded PHB films were dissolved in 5 ml chloroform, which was later extracted with 20 ml of acetonitrile. The acetonitrile phase was centrifuged at 13000 rpm for 10 minutes in order to remove the precipitated PHB from the extracting solvent and then subsequently filtered with a PTFE syringe frit of 45 μm average pore size. The centrifuged and filtered extracting solvent was analyzed with a reverse phase liquid chromatograph (Merck-Hitachni LaChrom Elite) equipped with a LiChroChart 250-4 column. The column contained LiChrospher 100 RP-18 type end-capped silica with an average particle diameter of 5 μm and pore size of 100 nm. Laboratory grade acetonitrile was used as eluent with the isocratic and constant flow of 1 ml/min. The reverse phase LC system was equipped with a diode array (DAD) detector. The detector was set to record the wavelength range of 190-300 nm with a sampling time of 400 ms.

The composition of the aqueous phase was analyzed by UV-Vis spectroscopy using

a Thermo Scientific Unicam UV-500 instrument in the 190-400 nm wavelength range. Spectra were recorded in a quartz cuvette of 1 cm thickness on samples degraded for various length of times. The qualitative analysis of the aqueous media was performed with the HPLC system already mentioned, but using a H_3PO_4/KH_2PO_4 phosphate buffer of pH 3.0 at the concentration of 10 mmol/dm^3 as eluent. The pH of the buffer must be set to a low value to keep the metabolites in their protonated form which have significantly longer retention time than ions. The ionized metabolites of PHB degradation are practically inseparable when they are present in an aqueous media of large pH, i.e. in the degradation solution. For the sake of unbiased UV-detection, the phosphate acid and salt was applied at a relatively small concentration (10 mmol/dm^3) and the problem of low puffer capacity was overcome by adjusting the pH of the solutions to pH 7.0 just prior injection.

3 RESULTS

The results of the experiments are reported in several sections. The time dependence of hydrolytic degradation followed by various techniques is presented in the first. The composition of the degradation products is discussed in the next, followed by the presentation of the kinetic model used for the quantitative analysis of the results. Diffusion coefficients and rate constants are discussed in the next section including consequences for practice.

3.1 Degradation kinetics

The time dependence of hydrolytic degradation can be followed by various methods. One of the simplest and most often used one is gravimetric analysis, the determination of the decrease of weight as a function of time. Weight loss measured in alkali solutions of various pH values are plotted against time in **Fig. 1** for films prepared by solvent casting.

The figure highlights the most important features of the degradation: the rate of mass loss depends on the pH of the medium and the time dependence is distinctly nonlinear. The slightly accelerating rate indicates that the hydrolytic reaction occurs also inside the polymer film and not only on the surface, but this assumptions needs further verification. The most important difference between surface and bulk erosion is that in the former case reaction rate depends on the surface area of the sample and it is independent of its volume. In the case of bulk degradation, however, chain fragmentation occurs also inside the polymer and thus reaction rate depends on sample volume. Accordingly, one needs films with the same surface, but different thickness, i.e. volume, in order to determine reliably the character of degradation, to decide if it is mainly a surface or a bulk process. Contradictory statements have been published in the literature claiming either exclusive surface [32-34] or bulk degradation [37].

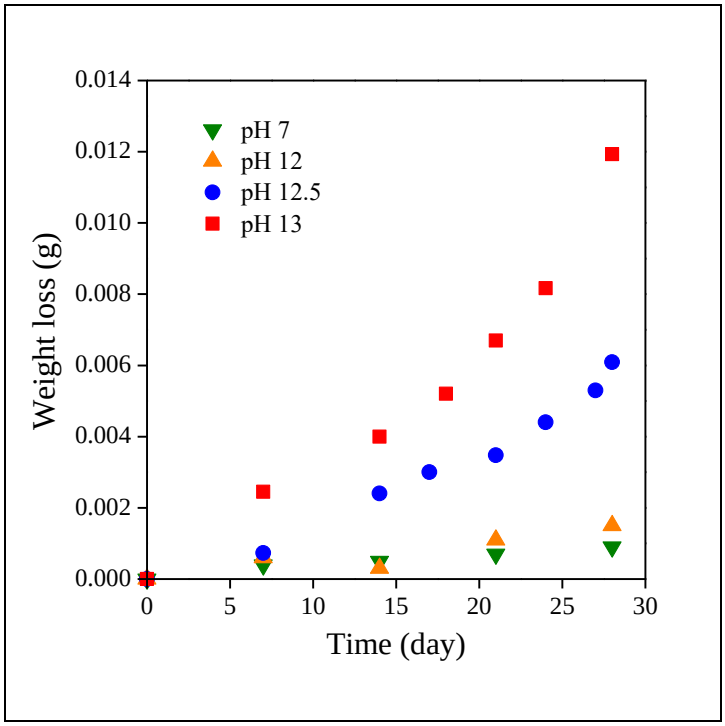

Fig. 1 Weight loss of solvent cast PHB films degraded in aqueous media of pH 13.0 (□), pH 12.5 (○), pH 12.0 (△), pH 7.0 (▽) plotted as a function of time.

The films used in our study satisfy this requirement, they have the same surface area, 192 cm^2, but different thicknesses, 10 and 100 μm for solvent cast and compression molded films, respectively. We assume that in the case of surface degradation, all reactions occur at the surface of the film and hydroxyl ions do not penetrate into the film. On the other hand, in the case of bulk degradation, reactions take place in the entire volume of the sample. We can talk about bulk degradation even in the case of the rather thin solvent cast film of 10 μm thickness, since the components of the degradation reaction (hydroxyl ion, monomer) are four to five orders of magnitude smaller (0.1 and 0.7 nm, respectively) than the thickness of the film.

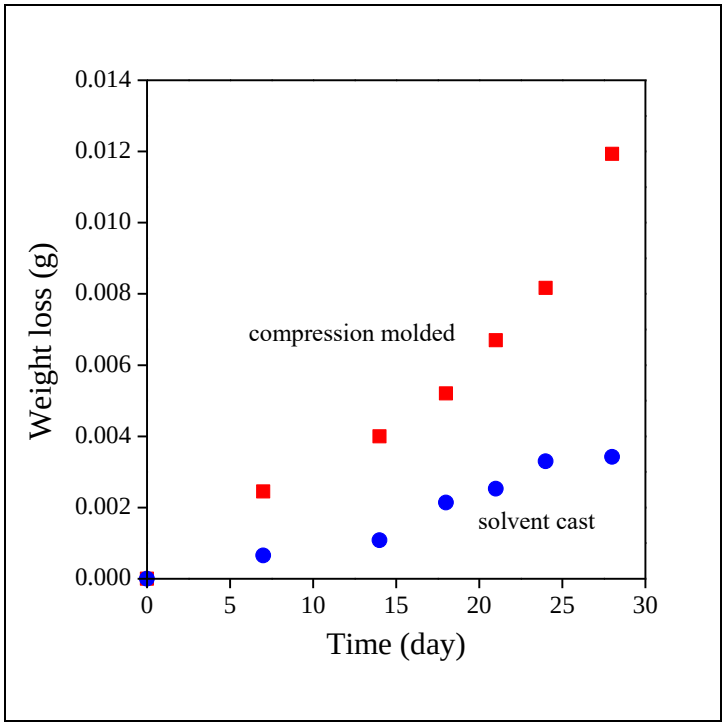

Fig. 2 Effect of thickness on the weight loss of compression molded (□) and solvent cast PHB films (○) with an average thickness of 100 and 10 μm, respectively. The samples were degraded in aqueous media of pH 13.0.

The degradation of films prepared by compression molding and solvent casting respectively is compared to each other in **Fig. 2**. The samples had the same surface area, but different thicknesses, 10 vs. 100 μm for solvent cast and compression molded samples, respectively. The comparison of the two functions clearly shows that degradation rate depends strongly on sample volume. The positive correlation together with the nonlinear time dependence of weight loss confirms that the hydrolytic degradation of PHB takes place mainly in the bulk of the sample and not on its surface.

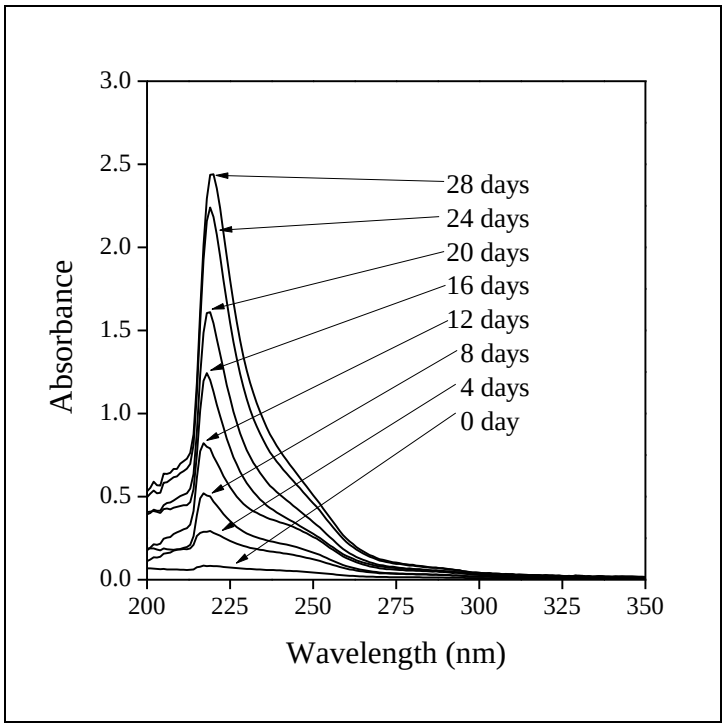

Fig. 3 UV-Vis spectra of aqueous degradation solutions recorded with 4 hour intervals. Compression molded film degraded at pH 13.0.

The measurement of the mass loss of the samples gives direct information about the kinetics of degradation. However, degradation kinetics can be followed also by the analysis of the degradation medium. Degradation products have a definite absorbance in the

UV-Vis spectrum in the range of 190 and 250 nm, thus changing intensity offers information about the amount of soluble components forming during degradation. UV-Vis spectra recorded on degradation media are presented in **Fig. 3**. A definite absorption peak appears on the spectra indeed, indicating the formation of degradation products and confirming that degradation kinetics can be followed in this way as well. However, the shape of the peaks indicate that more than one degradation product might be present in the solutions that complicates quantitative analysis. Accordingly, the peaks presented in **Fig. 3** might be the sum of absorptions resulting from the presence of more than one metabolite. To determine the quality and quantity of PHB chain fragments solved into the aqueous phase, the components must be separated first which can be done by liquid chromatography.

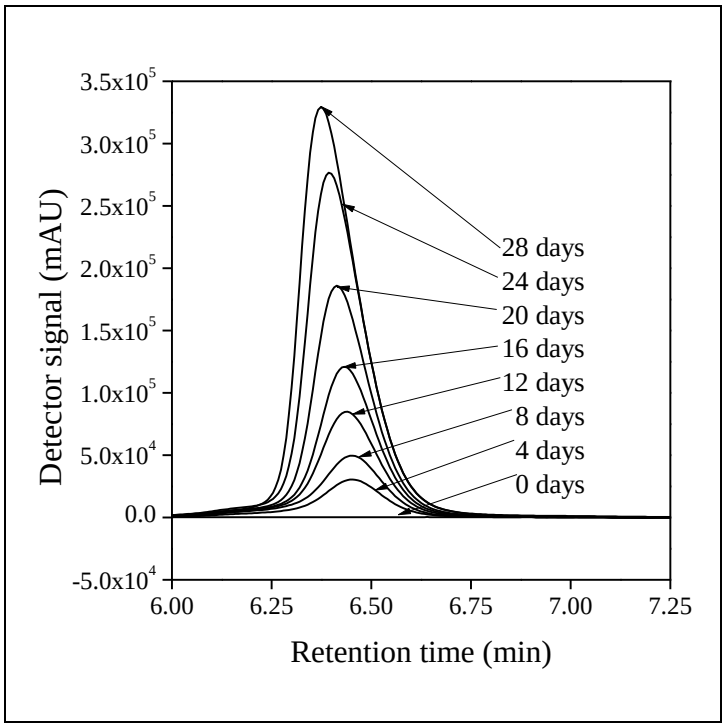

Fig. 4 HPLC chromatograms recorded on degradation solutions as a function of degradation time. Sampling frequency: 4 hour.

The chromatograms obtained are very simple. A small peak appears at around zero

retention time, which does not change with the time of degradation and obviously belongs to the ions present in the solutions. The intensity of the second peak, on the other hand, increases with degradation time as shown in **Fig. 4**. The time dependence of this second peak is very similar to that of the UV-VIS absorption peaks shown in **Fig. 3**. The lack of any other peak on the chromatogram of the degradation media obtained after various times of degradation indicates that only one degradation product, possibly the monomer, forms during degradation. The lack of other components may have several reasons.

The first one is closely related to the diffusion of possible metabolites. The diffusion coefficients of the 3-hydroxybutyric acid (monomer) and the 3-(3-hydroxybutanoyloxy) butanoate (dimer) in the PHB phase are unknown, but the dimer is expected to have a smaller diffusion coefficient, because of its larger size. It might not diffuse sufficiently fast to be detected in the aqueous phase. The other reason might be the strong basicity of the aqueous phase, in which the catalyst (hydroxide ions) is present in a relatively large concentration. Even if the dimer diffuses into the aqueous medium, it may hydrolyze immediately due to the large concentration of hydroxide ions.

The two methods, i.e. UV-Vis spectrophotometry and HPLC chromatography may supply different information about the products dissolved in the degradation solution. The intensity of the peaks detected by the two techniques is plotted against each other in **Fig. 5**. The correlation is very close with a small deviation at longer degradation times, probably because the concentration of the metabolite is too large for accurate detection. We may conclude that the two methods offer similar information about the degradation of PHB in aqueous medium under the effect of basic catalysis and can be used for the determination of degradation kinetics.

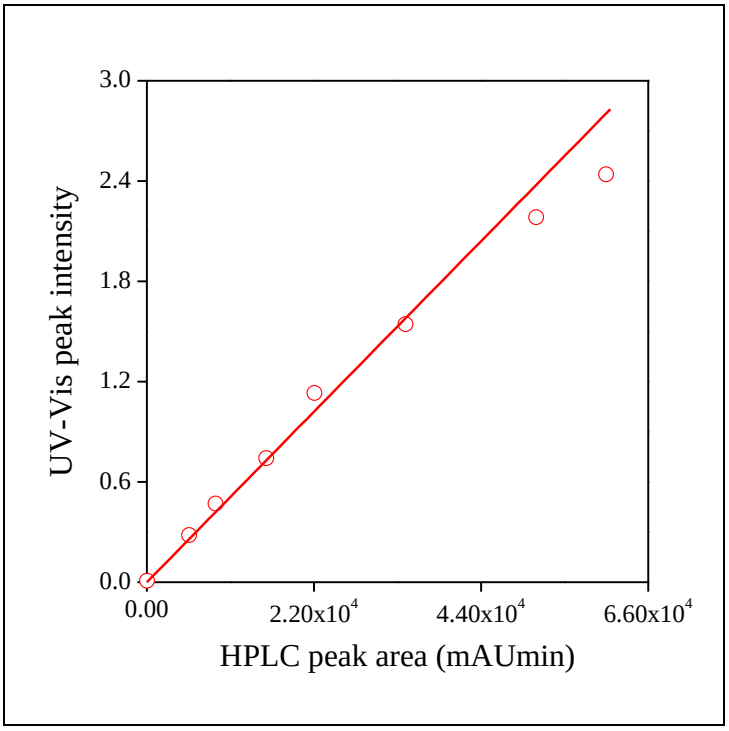

Fig. 5 Correlation between the intensity of UV-VIS absorption (peak height) and chromatographic peak area recorded on aqueous degradation solutions after

The area under the chromatographic peak recorded on the degradation solution after various length of times on compression molded and solvent cast films are plotted against the time of degradation in **Fig. 6**. The correlations correspond exactly to those determined by gravimetric analysis proving that both the measurement of the weight of the solid films or the analysis of the degradation solution reflects the same degradation kinetics. The acceleration of degradation and the difference in degradation rate for the two kinds of samples confirm that degradation occurs rather in the bulk of the sample than on its surface. Although the kinetics of degradation is clearly shown by these measurements, we can only guess the composition of the degradation products, which we assume to be mainly the monomer, but further experiments are needed to prove this assumption.

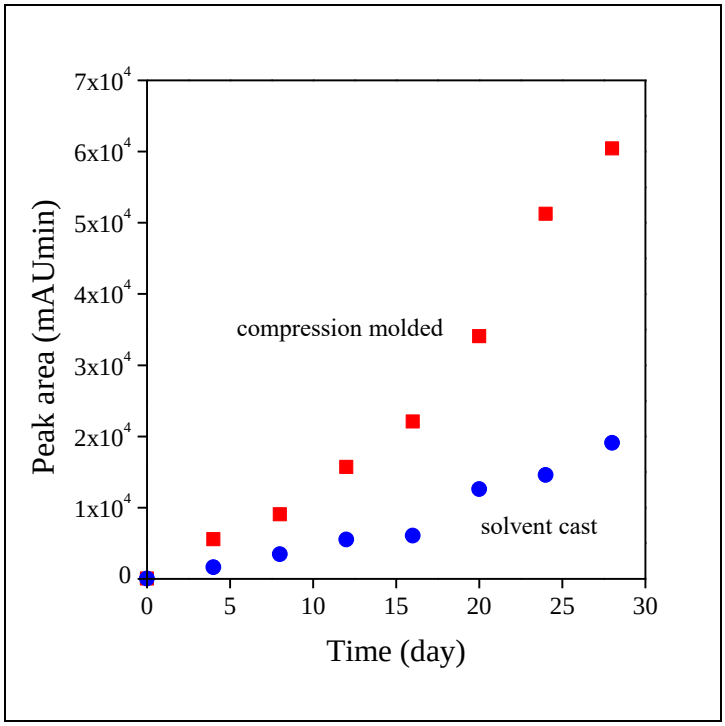

Fig. 6 Effect of the thickness on the kinetics of hydrolytic degradation of PHB films. Integrated peak area characterizing the monomer is plotted against time. Symbols: (□) compression molded, (○) solvent cast films. The samples were degraded in aqueous media at pH 13.0.

3.2 Composition of the degrading solid

The measurement of the weight loss of the samples during degradation and the analysis of the degradation solution revealed the mechanism and kinetics of the hydrolytic degradation of PHB. The first approach does not offer any information about the quality of the degradation products, while we can only speculate on them in the second case. To obtain further information about the composition of the degradation products and to determine the quality and quantity of the metabolites present inside the polymer, PHB films were dissolved in chloroform, then extracted by acetonitrile and subsequently the solution was analyzed by HPLC chromatography.

Chromatograms obtained on the extracting solution after various degradation times

are shown in **Fig. 7**. Three peaks appear in the chromatograms with different intensities and at different retention times. The peak at intermediate retention time belongs to chloroform dissolved in acetonitrile and its intensity is independent of the time of degradation. The first and third peaks, however, change intensity with degradation time thus they must belong to metabolites formed during degradation.

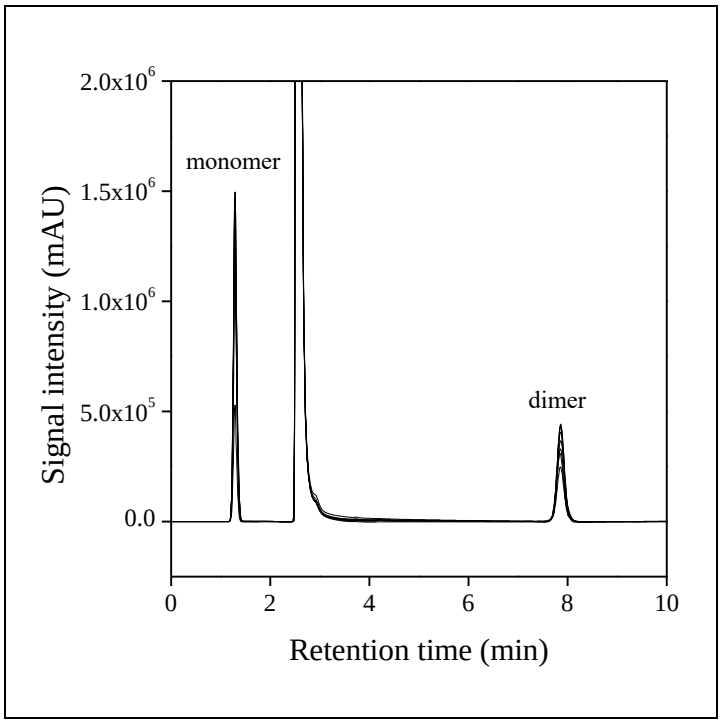

Fig. 7 Identification and quantitative analysis of degradation products extracted from degrading PHB films. Chromatograms recorded on acetonitrile solutions used for the extraction of metabolites from PHB films dissolved in chloroform.

The compound eluting at the shortest time was identified as the monomer, 3-hydroxybutyric acid by injecting the acetonitrile solution of the monomer purchased commercially onto the column. The resulting chromatogram exhibited a single peak located at exactly same retention time (1.2 minutes) as the first peak in **Fig. 7**. The identification of

the second compound eluted at longer time is more difficult. It is safe to assume that it is the dimer, but this compound [3-(3-hydroxybutanoyloxy)butanoate] is not available commercially and could not be applied as an internal standard.

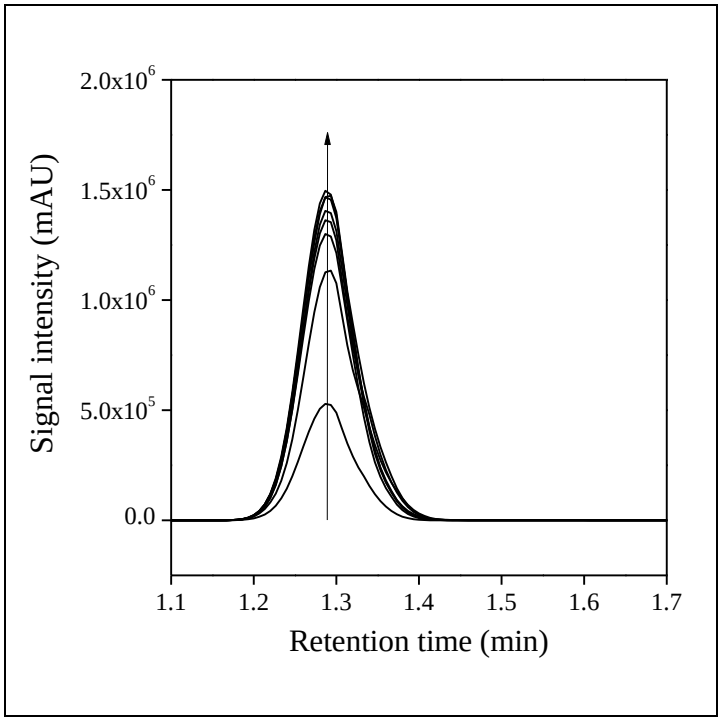

Fig. 8 Changing intensity of the chromatographic peak assigned to the monomer with degradation time. Chromatograms recorded on acetonitrile solutions.

The hydrolytic fragmentation of a polyester molecule is expected to occur with higher probability at ester groups located close to the end of the polymer chain [36]. Accordingly, metabolites with smaller molecular mass must form with higher probability and thus the most probable product of chain fragmentation is the monomer, while the second is the dimer. As the dimer has a smaller dipole moment than the monomer, it should elute from the column at longer times. There was some indication of a third peak at even longer retention times which might belong to the trimer, but the intensity of the peak was hardly larger than the stochastic noise of the measurement thus the identification of this compound

was impossible.

Accordingly, only the first two metabolites (monomer and dimer) were used for the quantitative characterization of the time dependence of hydrolytic degradation. The chromatographic peaks recorded after various degradation times are presented in **Fig. 8**. Unlike in gravimetric analysis (see **Fig. 1**), the amount of monomer extracted from the polymer phase appears to follow a saturation-like characteristic. A similar tendency can be observed for dimer molecules, which indicates that the concentration of metabolites in the polymer films does not increase exponentially as determined by the measurement of weight or the analysis of the degradation solution.

The area under the peaks belonging to the two degradation products was converted to concentration and this latter is plotted against time in **Fig. 9**. The amount of the two metabolites approaches a saturation value indeed. The concentration of the monomer and the dimer seems to reach its corresponding maximum value with different time constants; monomer concentration appears to converge faster to its plateau. We must consider here that the hydrolytic fragmentation of a polyester molecule occurs with higher probability at ester groups located closer to the end of the polymer chain [36], i.e. monomers form faster than dimers. Moreover, dimers may decompose to monomers with time yielding further monomer molecules. However, different time constants do not explain the saturation tendency observed. Here, one must consider also time dependent reaction rates and the diffusion of the metabolites into the degradation solution. Decomposition increases, while diffusion decreases their concentration and obviously an equilibrium is reached in the rate of the two processes as degradation proceeds. Since diffusion strongly influences the composition of the solid and the degradation solution, it must be taken into account in the development of a reliable kinetic model for the description of the hydrolytic degradation of PHB.

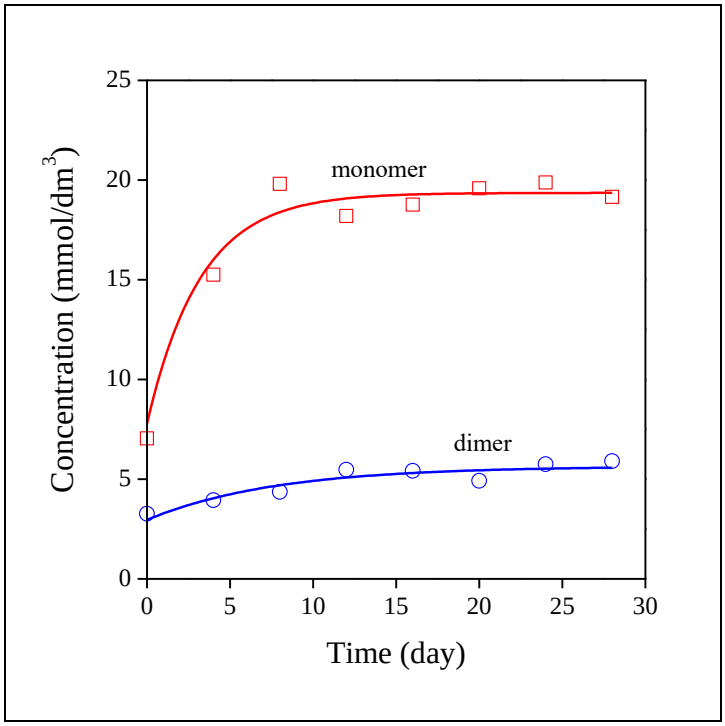

Fig. 9 Effect of degradation time on the concentration of metabolites in degrading PHB films. The films were prepared by compression molding and degraded at pH 13. Symbols: (□) monomer, (○) dimer. The solid lines represent correlations fitted according to the proposed model (see **Eqs. 4** and **5**).

3.3 The kinetic model

In order to describe the kinetics of degradation, we must know the mechanism of the reaction. Hydrolysis can be generally classified as bimolecular nucleophile substitution (S_N2), which begins with the attack of a nucleophile agent on the ester group. The attack results in the formation of an activated complex anion, which, in the presence of water, hydrolyzes immediately. The rate determining step is the formation of the activated complex thus the overall rate of ester hydrolysis depends only on the concentration of the hydroxide ions.

In our case, however, the fragmentation of the macromolecular chain alone would not result in the direct mass reduction of the PHB film, since the metabolites must leave the

polymer first. Accordingly, the overall rate of bulk degradation is determined by two parallel processes: the diffusion of the catalyst ions into the polymer and the diffusion of the metabolites into the solution. The diffusion coefficients of the catalyst ions and that of the metabolite molecules in the PHB phase must be known in order to identify the rate determining process. Unfortunately, these coefficients have not been published yet. Since the rate of diffusion depends on the size and dipole moment of the diffusing species, only the competition between the catalyst ions and the monomer must be considered. All other metabolites are much larger, consequently they diffuse much slower. All the above considerations result in the assumption that the diffusion of the hydroxide ion is considerably faster than that of the monomer thus the overall rate of weight loss is determined by the diffusion of the metabolites into the aqueous phase.

Since chain fragmentation is catalyzed by hydroxide ions, the rate of the degradation reaction is primarily determined by their concentration. Because their diffusion rate is considerably faster than that of any metabolite, their concentration is assumed to be constant from the very beginning of the degradation. Accordingly, the initial rate of hydrolysis is defined as

$$\frac{\mathrm{d}[m](t)}{\mathrm{d}t} = k_i\,[OH^-] \qquad (1)$$

where $[m]$ is the concentration of any arbitrary metabolite, k_i is its initial rate coefficient, while $[OH^-]$ is the nominal concentration of catalyst ions. Since the latter is assumed to be independent of time, it can be merged with the rate constant k_i to obtain k_i^*.

Polyester hydrolysis eventually reaches its equilibrium implying that the overall rate of the hydrolysis decreases with increasing amount of the reaction product, i.e.

$$\frac{\mathrm{d}[m](t)}{\mathrm{d}t} = k_i^* - k_m\,[m](t) \tag{2}$$

where k_m is the rate constant related to the time required to reach equilibrium. The results presented in **Section 3.1** indicated that two metabolites form in the polymer during degradation; **Eq. 2** must be specified for both of them.

Changes in the concentration of the monomer can be described by **Eq. 3**

$$\frac{\mathrm{d}[M](t)}{\mathrm{d}t} = k_{i,M}^* - k_M\,[M](t) \tag{3}$$

while the equation for the dimer takes the same form, but index *M* for the monomer changes to *D*.

As **Eq. 3** is a simple inhomogeneous, first order, linear differential equation, which can be solved analytically. The final solution gives the time dependence of monomer and dimer concentration in the following form

$$[M](t) = C_1\,e^{-k_M t} + \frac{k_{i,M}^*}{k_M} \tag{4}$$

$$[D](t) = C_2\,e^{-k_D t} + \frac{k_{i,D}^*}{k_D} \tag{5}$$

The analytical solutions presented above allow us to compare the prediction of the model to the measured values. After calibration the fitting of the model to the experimental results yielded the correlations shown by solid lines in **Fig. 9**. The agreement between the prediction and the measurements is excellent confirming the validity of our approach. It proves that degradation proceeds to equilibrium and that the rate of dimer formation is much

slower than that of the monomer. Although the calculated concentration values are valid for the solid phase and concentrations used for comparison were measured in the extract, their time dependence is certainly representative and can be applied to the calculation of time constants. The fitting procedure yielded the values of 0.32 1/day and 0.13 1/day for k_M and k_D, respectively. The values of the time constants indicate that the formation of monomer molecules reaches its equilibrium significantly faster than that of the dimer, indeed. The dependence of the rate of metabolite formation on the size of the molecule also confirms that the rate of hydrolysis depends on position along the chain [36].

Although the hydrolytic degradation of PHB is described by the model presented above, the decreasing mass of PHB films and the increasing concentration of the monomer in the aqueous phase has not been described kinetically yet. As mentioned above both is related to the diffusion of metabolites. Only monomers were detected in the aqueous phase, thus only the diffusion of the monomer will be considered in the kinetic treatment. Diffusion through a plane, through the surface of the PHB film in our case, can be described by Fick's first law

$$J = -D \frac{\partial c(x,t)}{\partial x} \tag{6}$$

where J is the diffusion flux, D the average diffusion coefficient, c concentration, while x is the spatial coordinate (position).

The analytical form of $c(t)$ was given above (see **Eqs. 4** and **5**). However, the metabolite concentration of the aqueous phase is also required in order to calculate the infinitesimal concentration change through the surface of the polymer film, $[\partial c(x,t)]$. Since the concentration of 3-hydroxybutyric acid remains rather small throughout the reaction, the

driving force of diffusion is expected to be constant. Therefore, the numeric value of the differential term in **Eq. 8** is assumed to be a linear function of the metabolite concentration inside the polymer given by Eqs**. 4** and **5**

$$J = -D^* \left[C_M \, e^{-k_M t} + \frac{k^*_{i,M}}{k_M} \right] \tag{7}$$

Eq. 7 defines the molar flux of monomer molecules through the surface of the polymer film with a size of a known value (A_s = 192 cm^2). Since the volume of the aqueous media is also known (V = 100 ml), the molar amount of metabolites can be converted immediately into concentration

$$J = \frac{1}{A_s} \frac{\mathrm{d}n_M(t)}{\mathrm{d}t} = \frac{1}{A_s} \, V \, \frac{\mathrm{d}c_M(t)}{\mathrm{d}t} \tag{8}$$

The substitution of **Eq. 7** into **Eq. 8** and rearrangement leads to the indefinite integral

$$c_M(t) = -D^* \, A_s \, \frac{1}{V} \int \left[C_M \, e^{-k_M t} + \frac{k^*_{i,M}}{k_M} \right] dt \tag{9}$$

which after integration gives the concentration of the metabolite as a function of time

$$c_M(t) = -D^* \, A_s \, \frac{1}{V} \left[\frac{C_M}{-k_M} \, e^{-k_M t} + \frac{k^*_{i,M}}{k_M} \, t \right] + C \tag{10}$$

To fit **Eq. 10** to the experimental data, detector signals must be converted into

concentration which was done by calibration. **Eq. 10** was then fitted to the experimental data using the Levenberg-Marquardt algorithm. The prediction of the model and the experimental data converted into concentration are compared to each other in **Fig. 10**. The agreement is excellent both for compression molded and solvent cast films showing that the kinetic model proposed describes properly the concentration of the monomer in the degradation solution and the kinetics of hydrolytic degradation generally. The fitting procedure allows now the determination of rate constants otherwise not available.

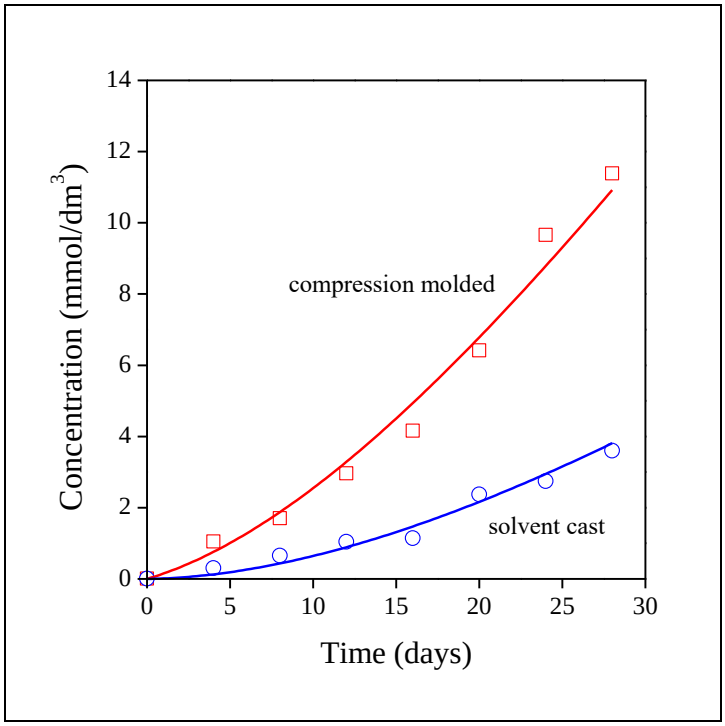

Fig. 10 Kinetics of the hydrolytic degradation of PHB films. Symbols: (□) compression molded, (○) solvent cast. The samples were degraded in aqueous media at pH 13.0. The solid lines represent correlations fitted according to the proposed model taking into account also the diffusion of the monomer into the degrading solution (see **Eq. 10**).

3.4 Parameters, consequences

The model contains altogether seven parameters. The first two are the surface area of the PHB films and the volume of the aqueous medium, and they are defined by the experimental conditions. The value of the kinetic coefficients (diffusion coefficient, rate constants) are determined primarily by the pH of the degradation medium and by the preparation method of the film used for the study. Parameter *C* is an integration constant, its value is defined by the initial condition of the process, by the concentration of the metabolite at the beginning of the degradation. If the aqueous media does not contain any degradation product at $t = 0$, then *C* equals to

$$C = D^* A_s \frac{1}{V} \frac{C_M}{-k_M} \tag{11}$$

The actual values of the parameters determined by the fitting procedure described above are compiled in **Table 1**. The first quantity, the pre-exponential factor contains several parameters, the diffusion coefficient of the monomer, the integration constant, C_M, and the time constant, k_M. The C_M/k_M ratio gives the concentration of metabolites produced by the initial, accelerating phase of the hydrolysis. Unfortunately diffusion rate cannot be determined separately from the model, we need independent measurements to obtain it. The time constant offers information about the deviation of kinetics from linearity during the accelerating phase of hydrolytic degradation, i.e. the curvature of the concentration vs. time function. Finally, the third quantity, the linear coefficient, gives the rate of the reaction at infinite time.

The table clearly shows that all values related to the rate of the reaction increase with increasing pH of the aqueous medium indicating that the main factor is the concentration of

the hydroxyl ions acting as catalyst. Although the diffusion coefficient cannot be determined separately, its dependence on the factors studied (pH, method of film preparation) can be estimated, since neither C_M nor k_M depend on them. The data of the table indicate that the rate of monomer diffusion increases with increasing pH, probably because of changes in local morphology as degradation proceeds. The importance of the structure of the films is shown also by the difference in the parameters determined for compression molded and solvent cast films. The two preparation methods result in films with different free volumes leading to dissimilar rates of diffusion. Obviously the molecules in films prepared by solvent casting are closer to equilibrium, have smaller free volume which leads to slower diffusion. With the help of the parameters determined, the time of degradation can be predicted reliably, if degradation conditions are known. With all probability the model can be used also for the description and prediction of the hydrolytic degradation of other aliphatic polyesters like PLA, but also of various copolymers.

4 CONCLUSIONS

The study of the hydrolytic degradation of PHB films prepared by compression molding and solvent casting, respectively, proved that degradation takes place mainly in the bulk of the samples and not on their surface. The overall rate of degradation depends strongly on pH, it increases with increasing pH values. The bulk-like nature of the degradation was also confirmed by the analysis of degradation products within the degrading polymer. Metabolite extraction and chromatography proved that degradation does not occur randomly, but with larger frequency at the end of the chains; basically only the monomer and the dimer was found in the degrading polymer. By assuming that the hydrolysis of PHB is a S_N2 type bimolecular nucleofil substitution reaction, a kinetic model was proposed which describes the formation of various degradation products. Weight loss

and the concentration of the metabolites in the aqueous phase depends also on the diffusion rate of the components, the diffusion of the monomer proved to be the rate determining step. Diffusion was accommodated into the model and thus the concentration of the monomer could be predicted also in the aqueous solution. The correlation between prediction and experimental results is excellent. The model can be extended for the description of the degradation of other aliphatic polyesters as well.

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Table 1 Numeric values of the parameters determined by the fitting of the model to the experimental results (see **Eq. 10**)

pH value	Preparation[a]	Pre-exponential factor ($D^*C_M/-k_M$)	Time constant (k_M)	Linear coefficient ($k_{i,M}{}^*/k_M$)
pH 13.0	CM	3.01E+05	0.0512	19200
	SC	2.32E+05	0.0434	9230
pH 12.5	CM	2.16E+05	0.0418	11300
	SC	9.53E+04	0.0225	6530
pH 12.0	CM	8.81E+04	0.0457	8040
	SC	4.27E+04	0.0368	2320
pH 7.0	CM	5.76E+04	0.0209	1960
	SC	4.70E+04	0.0162	2810

a) Method of film preparation; CM: compression molding, SC: film casting

CAPTIONS

Fig. 1 Weight loss of solvent cast PHB films degraded in aqueous media of pH 13.0 (□), pH 12.5 (○), pH 12.0 (△), pH 7.0 (▽) plotted as a function of time.

Fig. 2 Effect of thickness on the weight loss of compression molded (□) and solvent cast PHB films (○) with an average thickness of 100 and 10 μm, respectively. The samples were degraded in aqueous media of pH 13.0.

Fig. 3 UV-Vis spectra of aqueous degradation solutions recorded with 4 hour intervals. Compression molded film degraded at pH 13.0.

Fig. 4 HPLC chromatograms recorded on degradation solutions as a function of degradation time. Sampling frequency: 4 hour.

Fig. 5 Correlation between the intensity of UV-VIS absorption (peak height) and chromatographic peak area recorded on aqueous degradation solutions after various degradation times.

Fig. 6 Effect of the thickness on the kinetics of hydrolytic degradation of PHB films. Integrated peak area characterizing the monomer is plotted against time. Symbols: (□) compression molded, (○) solvent cast films. The samples were degraded in aqueous media at pH 13.0.

Fig. 7 Identification and quantitative analysis of degradation products extracted from degrading PHB films. Chromatograms recorded on acetonitrile solutions used for the extraction of metabolites from PHB films dissolved in chloroform.

Fig. 8 Changing intensity of the chromatographic peak assigned to the monomer with degradation time. Chromatograms recorded on acetonitrile solutions.

Fig. 9 Effect of degradation time on the concentration of metabolites in degrading PHB films. The films were prepared by compression molding and degraded at pH 13. Symbols: (□) monomer, (○) dimer. The solid lines represent

correlations fitted according to the proposed model (see **Eqs. 4** and **5**).

Fig. 10 Kinetics of the hydrolytic degradation of PHB films. Symbols: (□) compression molded, (○) solvent cast. The samples were degraded in aqueous media at pH 13.0. The solid lines represent correlations fitted according to the proposed model taking into account also the diffusion of the monomer into the degrading solution (see **Eq. 10**).