

Research Article

Intramolecular Lactonization of Poly(α -hydroxyacrylic acid): Kinetics and Reaction Mechanism

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Poly(α -hydroxyacrylic acid), PHA, is one of the few polymers with biodegradable properties used in mechanical pulp bleaching to stabilize hydrogen peroxide. A new method for the in situ follow-up of the lactone ring formation of PHA has been developed. The results have further been applied to describe the reaction kinetics of the lactonization and hydrolysis reactions through parameter estimation. In addition, the reaction mechanism is elucidated by multivariate data analysis. Satisfactory identification and semiquantitative separation of the lactone ring as well as the acyclic (carboxyl and hydroxyl groups) forms have been established by ¹H NMR in the pH range of 1–9. The lactonization reaction approaching equilibrium can be described by pseudo-first-order kinetics in the pH range of 1–6. The rate constants of the pseudo-first-order kinetic model have been estimated by nonlinear regression. Due to the very low rates of lactonization as well as the weak pH dependency of the reaction, an addition-elimination mechanism is proposed. Additionally, the presence of a transient reaction intermediate during lactonization reaction could be identified by subjecting the measurement data to multivariate data analysis (PCA, principal component analysis). A good correlation was found between the kinetic and the PCA models in terms of model validity.

1. Introduction

Poly(α -hydroxyacrylic acid), PHA (CAS 35326-33-1), is a weakly acidic polyelectrolyte which is used as stabilizing agent for hydrogen peroxide [1]. Polyelectrolytes are often known to have a poor biodegradability but the PHA molecule is biodegradable to a large extent which makes it attractive also elsewhere within chemical industry such as detergent applications as metal and fatty acid removing agent [2, 3].

Decomposition of hydrogen peroxide takes place both through radical reactions at elevated temperature and alkaline surroundings and catalytically by transition metals of which iron and manganese present the most destabilizing properties in the bleaching sequence [4, 5]. Furthermore, according to Ni et al., the catalytic effect of Mn³⁺ overrides that of Mn²⁺ in alkaline solution [6].

In order to avoid the metal-induced decomposition of hydrogen peroxide in the industrial applications, the concentration of the transition metals is suppressed as much as

possible. Traditionally, hydrogen peroxide in bleaching of mechanical pulp has been stabilized with sodium silicate. However, silicate increases the anionic load in filtrate circulations and causes precipitation on piping and equipment surfaces. PHA is able to replace sodium silicate as stabilizing agent in the bleaching of mechanical pulp.

The functional mechanism of PHA as a stabilizing agent for hydrogen peroxide is so far not completely elucidated. Previous studies have indicated plausible ionic interactions between PHA and the most detrimental divalent metals. Favorable conditions for its interaction with calcium, magnesium, and manganese have been defined to exist within the alkaline region, pH 6 to 10 [7]. Furthermore, calcium and manganese seem to interact with PHA more strongly than magnesium.

PHA can form intramolecular lactone rings between undissociated carboxyl and adjacent γ -hydroxyl group according to Figure 1 [8]. This feature affects its functional properties, such as dissociation behavior [9, 10].

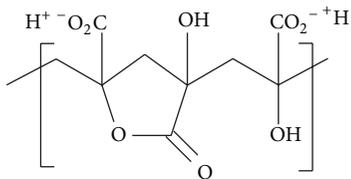


FIGURE 1: The chemical structure of poly(α -hydroxyacrylic acid), PHA, with an intramolecular lactone ring.

The build-up of lactone ring structures has an effect on the affinity of PHA towards metal species in stabilization of hydrogen peroxide. According to our previous study, it is, however, likely that nonionic interaction routes between PHA and species of, for example, manganese and iron prevail as well. In addition, some differences of preference, such as in favor of iron over manganese at acidic pH, have been noticed [7]. Despite the targeted high alkalinity of the mechanical pulp bleaching process, there are also strong local pH gradients especially in high consistency bleaching due to inefficient mass transfer. Depending, for example, on the chemical dosing sequence, the stabilizer may have a long residence time under process conditions well below the target pH. Therefore, it is of importance to study the kinetic behavior of the lactonization and hydrolysis reactions of PHA in more detail as a function of pH.

The goal of the present research was (1) to investigate the rate of the lactone ring build-up of poly(α -hydroxyacrylic acid) as a function of pH by means of ^1H NMR spectroscopy as well as other complementary methods of NMR and (2) to estimate the kinetic parameters for the lactonization rate expression in the following:



where

$$[\text{PHA}]_{\text{close}} + [\text{PHA}]_{\text{open}} = 1. \quad (2)$$

So far, the only published spectroscopic studies of the chemical structure of PHA consider the static reaction equilibrium results [8, 9]. Thus no kinetic data considering the structural dynamics of the reaction have been presented utilizing any kind of analytical method. The published acid-base titrations indicated extraordinary dissociation behavior in terms of large variation in the dissociation constants but left the reasoning without evidence in context of the corresponding simultaneous state of the chemical structure [7, 10]. A kinetic model covering the low pH region is essential for obtaining a better understanding of the lactone build-up mechanism (intramolecular versus intermolecular esterification reaction route). Moreover, knowledge of the time scale of the lactonization reaction provides valuable information to be utilized especially in the high-consistency bleaching processes as well as in gaining more information of the functionality of PHA in other chemical applications.

Spectroscopic methods (NMR and UV) have successfully been applied in many earlier kinetic studies also related to

lactone formation reactions within various compounds [11–14]. In addition, both qualitative and quantitative kinetic studies have been conducted to gain information on complex reacting multicomponent mixtures by utilizing NMR spectroscopy [15]. However, considering the nature of the polymeric analyte of the current study as well as the chemical environment of an electrolyte, the spectroscopic data is expected to spread out and have overlap between the observed components. Thus, to verify and elucidate the univariate approach of classic kinetic modeling by parameter estimation, multivariate data analysis has been applied.

2. Experimental Section

2.1. Analytical Methods. The propagation of the lactone ring formation reaction was detected and quantified by ^1H NMR spectroscopy (Bruker Avance II, 400 MHz) with D_2O as solvent and using a 5 mm BBO probe. Pulse delay was 2 s and width was 30° . The spectral width applied was 8 kHz.

2.2. Experimental Plan. The kinetic measurements covered samples in the pH range from 1 to 9 with the interval of one pH unit. In addition, one additional sample was tested at pH 4.5 to identify the possible turning point in terms of the reaction mechanism. All the measurements were conducted at room temperature ($T = 24.7 \pm 0.7^\circ\text{C}$).

2.3. Reagents. The aqueous solution of the sodium salt of poly(α -hydroxyacrylic acid) (30% NaPHA, $M_w = 30\,000$ g/mol) was provided by Kemira Oyj. The hydrochloric acid used was a commercial product of analytical grade and it was used as acquired at a proper dilution by D_2O without further purification.

2.4. Experimental Procedure. An aqueous stock solution containing 0.63 wt-% PHA polymer was prepared by dilution of the reagent solution (30% NaPHA, pH 9.5) with D_2O . The individual sample solutions were prepared by weighing approximately 7 g of the stock solution, followed by pH adjustment with hydrochloric acid (0.1, ..., 30% depending on the target pH) after which the ^1H NMR measurement was started immediately. The scans were recorded in the beginning of the measurement every five minutes until 20 minutes, after which the interval was increased to 10 minutes. Between one and four hours of measurement time, the scans were recorded every 30 minutes. The pH was measured in parallel with the scans using an identical reference solution. The pH values given in the present work for all individual measurements correspond to the initial values of each sample. The measurement was stopped when the reaction had reached the equilibrium. All the measurements fulfilled the condition $t_{\text{Eq}} > 10 \times t_{1/2}$, where t_{Eq} denotes the point in time when the equilibrium has been reached and $t_{1/2}$ denotes that when the conversion of the reaction is at half way [11].

2.5. Data Analysis. The parameter estimation, discussed in more detail later on, was conducted by Modest (MOdel ESTimation) software utilizing Matlab 6.1 interface and Fortran 77-based code. Modest program has been designed for

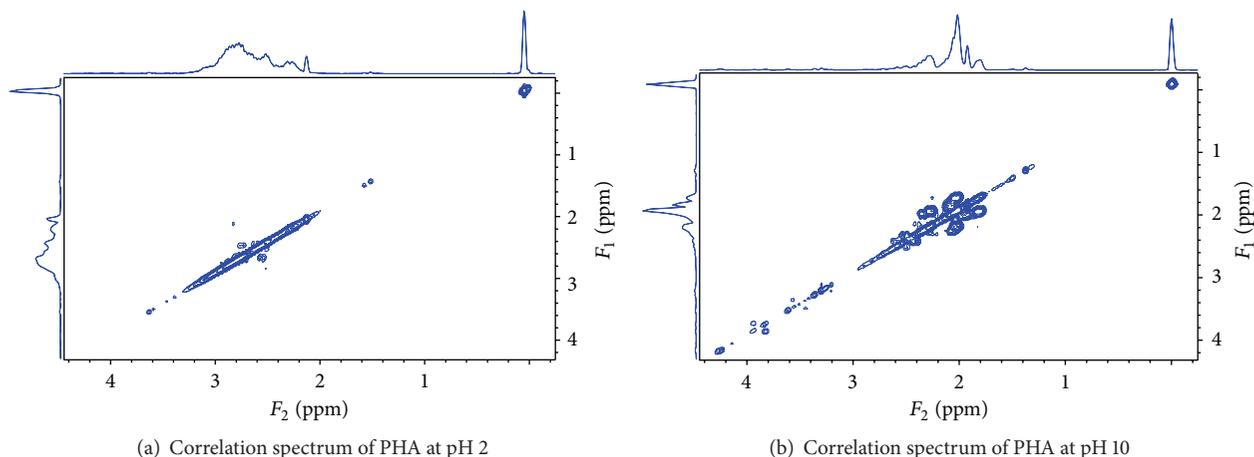


FIGURE 2: ^1H correlation spectra of PHA (a) at pH 2 and (b) at pH 10. The axes, F_1 and F_2 , represent the two frequency coordinates in chemical shifts (ppm).

parameter estimation of mathematical models as well as for experimental design, simulation, and optimization. Explicit algebraic, implicit algebraic (systems of nonlinear equations), and ordinary differential equations can be used to describe the reactions to be estimated [16].

Multivariate data analysis by principal component analysis (PCA) was applied to gain deeper insight into the spectral identification and the reaction mechanism during the propagation of the lactonization reaction.

PCA is a multivariate projection method designed to extract and display the systematic variation within a data matrix. The data in a multidimensional space is modeled as a plane or hyper plane, the axes of which are called principal components. This is done in order to reveal trends as well as relationships between the observations and variables or among variables themselves. Due to different numerical ranges of the original spectral data, preprocessing by means of mean-centering and scaling, that is, normalization to unit variance, is conducted to make the actual variance in the data attainable. If the amount of observations is large (as in the present case), averaging is conducted in order to reduce signal noise. The number of principal components needed to build a reliable model is determined by the degree of explanation of the model. For detailed theory of PCA, the reader is referred elsewhere [17].

3. Results and Discussion

3.1. NMR Analyses. The degree of polymerization of the PHA molecule causes the spectral bandwidths in the ^1H NMR spectrum to spread out in a significant manner in comparison to the case of monomeric analytes. Similar ^1H measurements of NaPHA have been conducted in an attempt to assign chemical structures to the broadened chemical shifts [8]. However, structural identification was judged as impossible by Yamazawa et al. due to the multiple electronic circumstances provided by the lactone rings to the methylene proton. The authors obtained a more resolved ^1H spectrum at elevated temperature, resulting in one central large peak

(racemo) and two small doublets (m1 and m2). They also stated that this type of spectrum has been observed for methylene protons of poly(methyl methacrylate) (PMMA) and poly(acrylic acid) (PAA) and interpreted as one central racemo peak and two meso peaks. Our own ^1H measurement (conducted at pH 10 and $T = 25^\circ\text{C}$) supports this conclusion. The m2 peak maximum is located at 1.8 ppm, the racemo peak maximum is located at 2.0 ppm, and the m1 peak maximum is located at 2.3 ppm. This area corresponds well with our ^1H measurements presented in Figures 4, 5, and 6, providing justification for the integration limits of the acyclic PHA form later on in the text. Additional verification to the assignment of the chemical shifts is provided by conducting two-dimensional ^1H correlation spectroscopy (COSY) as well as ^{13}C measurements (Figures 2 and 3).

The ^1H correlation spectroscopy measurement at pH 10 (Figure 2(b)) indicates at least three separate spin systems for the PHA molecule under alkaline conditions. The same number of separate signals is also seen in the ^{13}C spectrum at pH 10 (Figure 3(b)) within the methylene group signal. They originate from the possible variations between the orientations of the adjacent *n*-hydroxyl and carboxyl units. All the coupling constants, of approximately 15 Hz, represent typical geminal protons (H-C-H). Because of this, no interlinking between methylene groups is seen that would indicate exceptions from linear chain structure. ^1H correlation spectrum conducted at pH 2 (Figure 2(a)) presents a coupling between signals at 2.23 and 2.8 ppm, the justification of which is further elaborated by applying multivariate analysis of the data in Section 3.4 below.

The ^{13}C spectrum at pH 2 for lactonized PHA (Figure 3(a)) indicates signals in the range from 85 to 87 ppm, which supports earlier studies [8]. The chemical shift originating from the carboxylate carbon represents the intensity maximum. Additionally, the locations of the methylene carbon signal at approximately 50 ppm as well as the carbon connected to the free carboxylic acid group at ca. 78 ppm correspond to previous data [8].

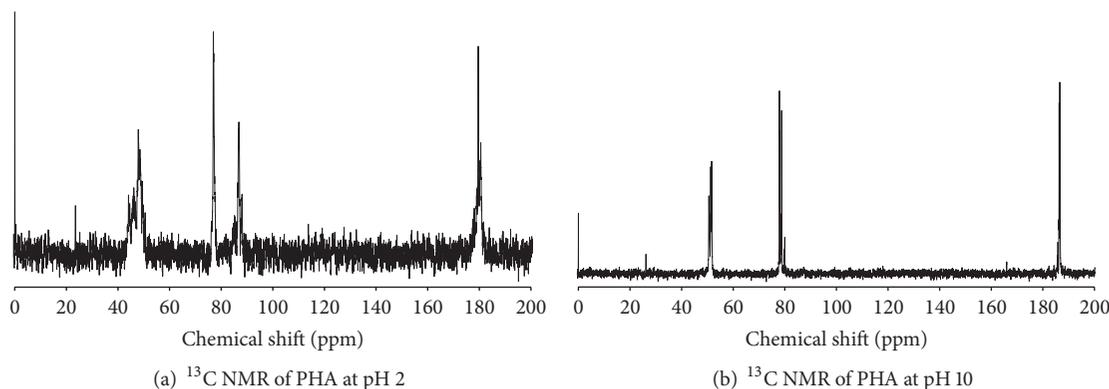


FIGURE 3: ^{13}C NMR spectra of PHA (a) at pH 2 and (b) at pH 10.

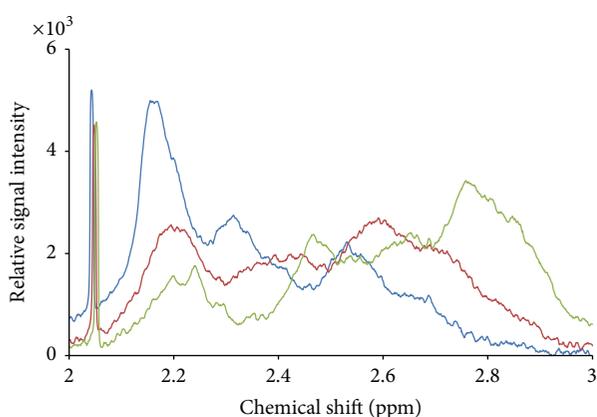


FIGURE 4: ^1H NMR spectrum of the lactone ring build-up reaction of PHA at pH 3 after 5 (blue line), 60 (red line), and 1600 (green line) minutes time from the beginning of the measurement.

According to the ^1H spectra, the lactonization reaction proceeds extensively at acidic conditions approximately below pH 5. This is depicted clearly in the ^1H measurements in Figure 4 at pH 3 by the decline and finally a substantial decrease of the original signals of the acyclic carboxylic structures and by the appearance of the new ones related to the lactone ring structures over the reaction time.

The decline of the signal intensities of the acyclic PHA at the lowest ppm level in Figure 5 (pH 1: ca. 2 ppm; at pH 4.5: <1.9 ppm) is indicative of the reaction propagation, as there is no overlapping with the signals arising from the lactonization reaction. The pH dependency of the signals occurring during the reaction is the most apparent and interesting phenomenon. This can be seen by studying, for example, the splitting of the broad signal of the acyclic PHA in the free acid regime appearing at 2.23 ppm at pH 1 (see Figure 5, single signal at 2.23 ppm).

In the measurements conducted at less acidic conditions (pH 3, Figure 4), this signal of acyclic PHA starts to split into two separate signals. At pH 4.5, there are two clearly separate signals at 2.20 ppm and 2.15 ppm (Figure 6).

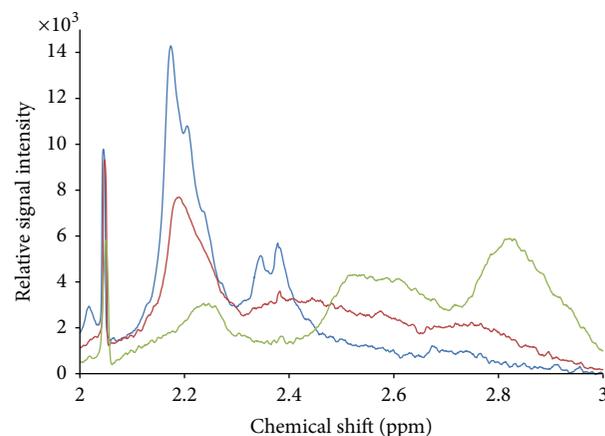


FIGURE 5: ^1H NMR spectrum of the lactone ring build-up reaction of PHA at pH 1 after 5 (blue line), 60 (red line), and 1600 (green line) minutes time from the beginning of the measurement.

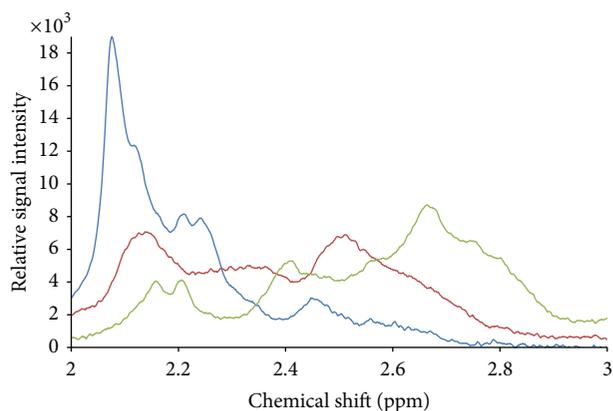


FIGURE 6: ^1H NMR spectrum of the lactone ring build-up reaction of PHA at pH 4.5 after 5 (blue line), 60 (red line), and 1600 (green line) minutes time from the beginning of the measurement.

The intensity maximum of the lactonized PHA at low pH values appears at 2.8 ppm. As pH increases, the intensity maximum shifts towards lower ppm values. Table 1 depicts the ^1H signal assignments as well as their average variation (ad) in measurements conducted in the pH range from 1 to 9.

TABLE 1: Assignment of the ^1H shifts (at intensity maximum) and their average deviations (ad) of the acyclic and lactonized structures of PHA at pH 1–9.

pH	Acyclic PHA ppm	ad	Lactonized PHA ppm	ad
1	2.18–2.26	0.04	n/a–2.84	n/a
2	2.17–2.24	0.04	n/a–2.79	n/a
3	2.18–2.24	0.03	2.53–2.78	0.13
4	2.10–2.22	0.06	2.47–2.68	0.11
4.5	2.07–2.20	0.07	2.44–2.67	0.12
5	2.03–2.13	0.05	2.47–2.62	0.08
6	1.98–2.00	0.01	2.42–2.46	0.02
7	1.97–1.99	0.01	n/a–n/a	n/a
8	1.97–1.98	0.01	n/a–n/a	n/a
9	1.97–1.98	0.01	n/a–n/a	n/a

Thus, as the pH value increases, the location of the intensity maximum is shifted to lower ppm values. This kind of pH dependent behavior is caused by the protonation and deprotonation of the carboxylic acid groups in the polymer chain at the reaction equilibrium and indicates the presence of free carboxylic acid structures. Similar behavior has been described by Zhang et al. for lactone ring formation of gluconic acid, where the displacement of C=O chemical shifts in ^{13}C NMR spectra towards higher frequencies with increasing pH values (pH region from 2 to 6) has been assigned to deprotonation of carboxylic groups [12]. This phenomenon is consistent also with previous work conducted on proteins [18].

The low numerical values of signal deviation (ad) during measurements conducted at neutral to basic environment (pH 7 to 9, acyclic PHA) indicate the domination of the deprotonated form and thus minor protonation occurrence. In this pH region, practically no lactone rings are present which is denoted by n/a in Table 1. Due to the slow lactone ring formation reaction at very low pH of 1 and 2, there are also no lactone rings present at the first point of measurements (5 min). Correspondingly, very low numbers will be shown for the measured rate constants for lactone ring formation at the same pH values in Table 2. Thus, the acyclic form (free hydroxyl and carboxyl groups) of PHA is approximated to be represented by the ^1H shifts in the range of 1.7–2.4 ppm. Studying the acidic solutions, the signals occurring in the range of 2.1–2.4 ppm can be assigned for the most part to free acid groups. Correspondingly, contributions to the area of 2.4–3.2 ppm are assumed to arise from the presence of lactone rings.

Choosing 2.4 ppm as the integration limit after evaluating all the individual spectra gives an acceptable semiquantitative representation of the overall lactone/free acid ratio for the kinetic parameter estimation. In addition, based on the pH dependent behavior described above, it can be concluded that PHA does not build lactone groups between all the available hydroxyl and carboxyl groups. This conclusion is also supported by the estimated equilibrium constant (K_{obs}) values

as well as the equilibrium conversion values (X_{eq}) calculated on the basis of the K_{obs} values for PHA lactonization as a function of pH (Table 2). However, when considering the calculated equilibrium conversion values, the semiquantitative nature of the spectra integration has to be kept in mind.

A careful study of the spectral data reveals also the presence of transient signals in the range of ca. 2.4–2.6 ppm, presumably by an unknown reaction intermediate (in Figure 4, the transient signal reaching an apparent maximum at 60 min at pH 3). Similar phenomenon can be noticed in all the measurements throughout the acidic pH range up to ca. pH 4.5–5. To get additional support for the assumption of the presence of a reaction intermediate, multivariate data analysis by assigning principal component analysis upon the spectral data has been applied (Section 3.4).

The impact of the deprotonation of the carboxylic groups on initiating a modification of the entire molecule conformation is raised in the context of explaining the transient signal. Earlier studies have reported this kind of behavior for isosaccharinic acid, where the deprotonation resulted in alterations in the hydrogen bonding environment of the hydroxyl groups attached to adjacent carbons [19]. The effect of hydrogen bonds on conformational changes is also addressed in an earlier paper studying the effects of the intramolecular hydrogen bonding between the different functional groups of a structurally close-related copolymer of reduced PLAC (poly(1-oxa-2-oxobutane-1,4:3,3-tetraol)), consisting of allyl alcohol, cyclic hemiacetal, and unreacted α -hydroxyacrylate segments [20, 21]. Based on the behavior of the different -OH groups in the reduced PLAC molecule, primary, hemiacetal, and tertiary, the conclusion drawn by the authors was the fact that intramolecular hydrogen bonds are formed not only between the allyl alcohol OH and the hemiacetal OH but also between the COO^- and the hemiacetal OH groups. The latter reaction was further confirmed by accomplishing a stable structure by ab initio calculation between the hemiacetal OH and the adjacent carboxyl group, stabilized by α -OH groups through their own hydrogen bonds to the carboxyl oxygen and the hemiacetal one.

3.2. Parameter Estimation. The rate profile of the lactone ring formation reaction can be described by the following pseudo-first-order equation [13, 22]:

$$\frac{d[\text{PHA}]_{\text{close}}(t)}{dt} = k(\text{close})[\text{PHA}]_{\text{open}}(t) - k(\text{open})[\text{PHA}]_{\text{close}}(t). \quad (3)$$

The observed equilibrium constant, K_{obs} , at a given pH value for the conversion of carboxylate to lactone, according to Figure 1 and (1), can be written as follows:

$$K_{\text{obs}} = \frac{[\text{PHA}]_{\text{close}}}{[\text{PHA}]_{\text{open}}} = \frac{k_{\text{close}}}{k_{\text{open}}}, \quad (4)$$

where $[\text{PHA}]_{\text{close}}$ and $[\text{PHA}]_{\text{open}}$ are the equilibrium concentrations of the ring-closed and ring-opened species, respectively.

TABLE 2: Effect of pH on the rate constants of lactone ring formation ($k(\text{close})$) and opening ($k(\text{open})$) and the relative standard error of the rate constants as well as the corresponding equilibrium conversion (X_{eq}) for PHA lactonization at $T = 24.7 \pm 0.7^\circ\text{C}$.

pH	$k(\text{close})$ s^{-1}	Standard error $k(\text{close})$ %	$k(\text{open})$ s^{-1}	Standard error $k(\text{open})$ %	K_{obs} $(k(\text{close}))/k(\text{open})$	X_{eq} %
1	1.14×10^{-4}	3.2	4.54×10^{-5}	5.2	2.51	71.5
2	1.09×10^{-4}	3.5	4.46×10^{-5}	6.8	2.44	70.9
3	1.91×10^{-4}	3.3	5.43×10^{-5}	4.8	3.52	77.9
4	1.71×10^{-4}	4.5	8.13×10^{-5}	6.6	2.10	67.7
4.5	1.78×10^{-4}	6.2	8.38×10^{-5}	9.5	2.12	67.9
5	8.92×10^{-5}	6.6	8.36×10^{-5}	8.7	1.07	51.7
6	4.52×10^{-5}	11.0	7.91×10^{-5}	13.4	0.57	36.3

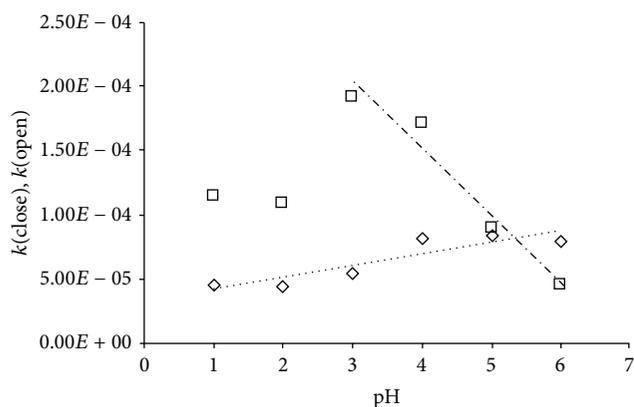


FIGURE 7: Rate constants of the hydrolysis ($k(\text{open})$, \diamond) and lactone ring formation ($k(\text{close})$, \square) as a function of pH at $T = 24.7 \pm 0.7^\circ\text{C}$.

The reaction rate expression of (3) forms an ordinary differential equation. The rate constants, $k(\text{close})$ and $k(\text{open})$, were both estimated for the lactonization and hydrolysis reactions, respectively (see Table 2 and Figure 7). The experimental data sets were fitted at individual pH values to the rate expression by least squares technique of minimizing the squared difference between the measured and calculated concentrations. Due to the unacceptable model validity (see Table 3), the results from measurements in the range of pH 7 to 9 are not shown in Table 2 and are thus excluded from further evaluations.

The lactone formation is clearly pH dependent in the pH region of 3 to 6. The lactonization rate is highest at pH 3 after which, when moving towards higher pH values, the rate is decreasing. The highest calculated equilibrium conversion, approximately 78% for the lactonization, is also seen at pH 3. At low pH values of 1 to 2, the lactonization rate is decreased again and seems to lose its pH dependency (see Figure 7). In the lower pH region (pH 1-2), presumably the increasing distance between the functional groups available for lactonization reaction affects the reaction rate significantly, leading to an inconsistent behavior of the reaction rates in that region.

The rate of hydrolysis increases slowly throughout the pH region of 1 to 3 after which a nonlinear stepwise increase in the rate is seen between pH 3 and 4. Between pH 4 and 6 the hydrolysis proceeds practically at a constant rate. The hydrolysis and lactonization reaction rates coincide approximately at pH 5.3.

TABLE 3: The correlation of the kinetic model to the measured values of the reaction conversion at pH 1-9.

pH	Model correlation
1	99.4
2	98.4
3	99.7
4	98.6
4.5	97.2
5	97.0
6	93.9
7	65.2
8	-1.1
9	69.9

In the neutral and alkaline pH regions the pseudo-first-order kinetic model loses its validity (see Table 3), most likely due to new reaction mechanisms like base-induced ester hydrolysis introduced in the system, and correspondingly the consistency of the rate constants is decreased. It is, however, evident that the hydrolysis rate is markedly increased between pH 7 and 9 and that the rate of lactonization approaches zero at high pH values. Alkaline-catalyzed hydrolysis reaction generally takes over at clearly alkaline surroundings. This reaction is irreversible since once the acid intermediate is formed it is immediately converted to the carboxylate anion, which is not further attacked by $[\text{OH}^-]$. As a result, the reaction goes to completion in the direction of hydrolysis with accelerated rate as alkalinity increases [23].

The conversion of the free acid form to lactone is thus dominating until the pH region of approximately 4 to 5, yet the rates are very low. No evident steric hindrance to explain the rate behavior (Table 2) can be seen. Hence, the low reaction rates in either direction together with the relatively weak pH dependency suggest an addition-elimination process (see Figure 8) as the dominating reaction mechanism [23]. The reaction is assumed to take place through a slow proton transition from hydroxyl to carboxylic oxygen via a dipolar intermediate followed by elimination of H_2O giving the final lactone. The simultaneously occurring hydrolysis and lactonization reactions hence give rise to an unstable equilibrium state and variation in the ionization degree locally along the polymer chain.

The rather reversible addition-elimination reaction is conventionally assumed to take place in the vicinity of neutral

TABLE 4: PCA parameters describing the dominating spectral characteristics in the ^1H NMR domain of lactone formation of PHA in the range of pH 1 to 5.

pH	Explained by	Explained by	Σ Explained	Chem.shift PC1	Chem.shift PC2	Time at max.	Time at max.
	PC1	PC2					
	%	%	%	ppm	ppm	min	min
1	88.5	8.1	96.6	2.767	2.442	1600	90
2	87.5	10.1	97.6	2.761	2.416	1720	90
3	82.6	12.4	95.0	2.742	2.580	1500	50
4	77.9	18.0	95.9	2.717	2.397	1600	60
4.5	79.4	17.5	96.9	2.636	2.335	1600	60
5	86.1	9.3	95.4	2.561	2.297	1600	90

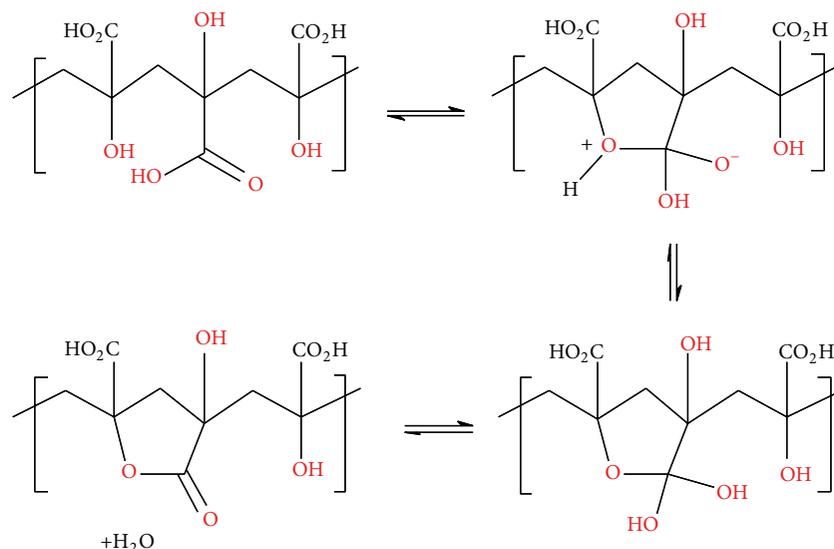


FIGURE 8: Addition-elimination lactonization mechanism suggested for PHA in the pH region 1 to 6.

pH region, with practically no free OH^- and H^+ participating in the reactions [23]. Hence, an acid catalyzed reversible lactonization would also be possible. In the studies considering camptothecin, both pH dependent as well as pH independent ring-closure reactions have been assumed to occur, the latter of which taking place at $\text{pH} > 6.5$ [11, 13]. The hydronium ion-dependent ring closing was reported to probably occur by acid-catalyzed reaction mechanism of the carboxylic acid at $\text{pH} < 3.5$. However, the reaction was in this case found strongly pH dependent which gave support to the acid-catalyzed mechanism. Yet, the presence of a possible transition state for the lactonization/hydrolysis was also assumed, where the α -hydroxyl group would be involved in one of the proton-transfer steps or stabilizing the transition state through hydrogen bonding ($-\text{O}-\text{H}-\text{OH}$) between the α -hydroxyl and another hydroxyl group before the completion of the lactone ring formation. In the present work, no strong pH dependency of reaction rates was seen but the rates measured are in good accordance with previous studies based on similar cyclic lactone structures of gluconic acid or its derivatives, thus supporting the addition-elimination approach [12, 24]. Furthermore, the formation of a transient tetrahedral intermediate, resulting from an analogous rate-limiting nucleophilic attack of hydroxide, has been discussed in the context of another related compound D-glucuno- δ -lactone [25].

3.3. Model Evaluation. The plots with measured lactone contents fitted to the model depict an acceptable fit in the pH region from 1 to 6. The correlation coefficients for the kinetic model are given in Table 3 and fits of the measurements at pH 1 and 2 are depicted as examples in Figure 9.

3.4. Multivariate Data Analysis by PCA. In the first stage, using principal component analysis on the spectral ^1H NMR data gives indication of the number and the relative contribution of the so-called principal components (PCs) needed to build the multivariate PCA model of the reaction studied. In the following stage, the dominating chemical shifts can be identified, thus promoting verification of the spectral assignment shown in Table 1. Incorporation of the time dimension enables also the comparison to the reaction rate parameters gained by parameter estimation.

The reliability of the PC analysis is described by the explanatory values of the principal component planes (see Table 4). In the cases of pH 1 to 5, PC1 and PC2 planes are able to capture 95% or more (Explained by PC1 + PC2 in Table 4) of the variance of these data set points, which represents an analysis of high reliability. Adding several components would not be meaningful since the predictive ability would not increase in a significant manner and no further correlation to the chemical behavior could be identified.

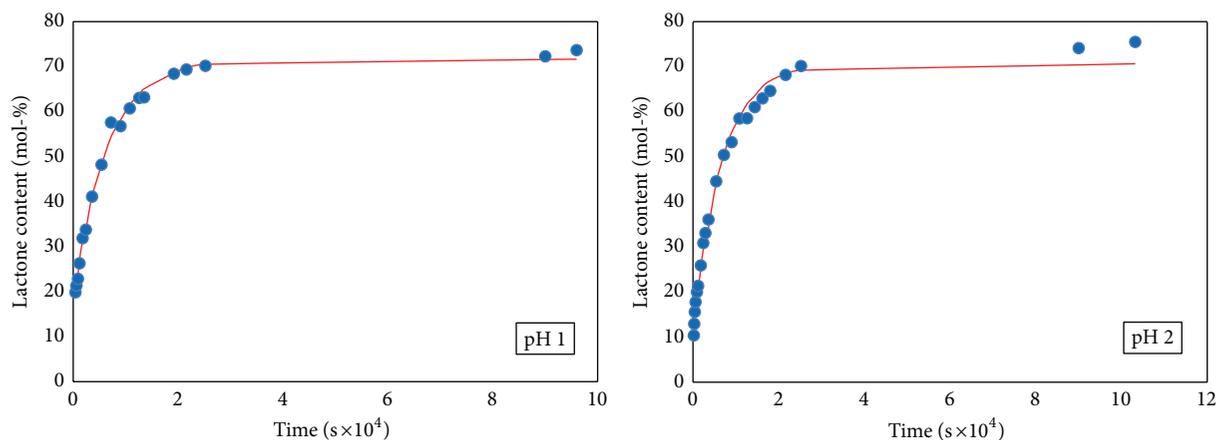


FIGURE 9: Experimental lactone conversion fitted to the kinetic model at pH 1 and 2.

Table 4 summarizes the essential characteristic values of the PCA model of the lactonization reaction.

Graphical illustration of the model serves as a tool to disclose the interdependences of the components. Out of the two components created, PC1 component is clearly dominating PC2 in terms of the degree of explanation of the model (Table 4, explained by PC1). In other words, PC1 describes to a large extent the total reaction propagation towards the evident end product in the spectral data, here the area covering the chemical shifts of lactone ring structures. Thus, it can well be concluded that PC1 represents the main chemical reaction of converting free carboxylic acid and hydroxyl groups into lactone ring structures. This is strongly supported by simultaneous inspection of the time stamps (Table 4, time at max. PC1) and the dominating chemical shifts (Table 4, chem. shift PC1) of the measurements, indicating that the maximum value of PC1 component is achieved at the end of the measurement with dominating ^1H NMR signals appearing in the range of 2.56–2.76 ppm depending on pH.

The corresponding evaluation of PC2 component indicates a strong linkage to a less dominating reaction taking place during the main one. The data analysis reveals that chemical shifts appearing at approximately 2.3–2.6 ppm (see Table 4, chem. shift PC2) are not directly contributed by the end product, lactone ring, but are also attributed to a temporary presence of an intermediate chemical structure approaching lactone ring formation. A clear pH dependency can again be identified to the degree of explanation, referring to increasing signal intensities with increasing pH. The maximum in the intermediate reaction is reached in the pH range from 4 to 4.5 with the corresponding degrees of explanation of 18.0 and 17.5%, respectively. The data analysis reinforces the earlier speculations about an intermediate structure appearance in the chemical shift range of ca. 2.3–2.6 (Table 4, chem. shift PC2), where a transient signal could be identified (see Section 3.1 NMR). Additionally, the time stamps (Table 4, time at max. PC2) for the maximal PC2 appearance coincide well with the visual inspections of the signal maxima in the ^1H NMR spectra.

The dominance of PC1 component can be seen to decrease as a function of pH until 4.5, while that of PC2 component increases. This is in good accordance with the earlier visual interpretations of the presence of a transient intermediate structure along the main lactone formation reaction.

Regardless of the temporary presence of the intermediate substance, the explanatory degrees of the PCA model and the kinetic model correlation values coincide well in the pH range from 1 to 5. This is probably due to the fact that the transient reaction intermediate is entirely converted into the end product and thus the net effect of the intermediate contribution is zero when the overall molar balance is calculated. The kinetic model correlation reaches still a satisfactory value at pH 6, while the explanatory degree of the PCA model drops already under 90%.

This study presents thus evidence on the presence of an earlier unknown reaction intermediate during the lactone formation reaction of PHA. Due to the transient and highly pH dependent nature of the polymeric substance appearance, a complete chemical identification of the substance is challenging. Prior studies on structurally related substances justify, however, the speculation on the structure, indicating the creation of a temporary stabilized lactone ring intermediate, where hydrogen bonding and the vicinity of α -hydroxide groups probably play a crucial role.

4. Conclusions

A new method based on in situ ^1H NMR spectroscopy was successfully developed for the follow-up of the lactone formation reaction of PHA. Kinetic parameter estimation of the experimental data was made by classic data fitting based on least squares method. In addition, PCA was successfully utilized in assigning chemical shifts to the reaction components and in the further reaction mechanism elucidation.

The pseudo-first-order reaction kinetic model suggested describes well the lactonization as well the hydrolysis reaction in the pH region from 1 to 6. The model validity is decreased significantly above pH 6 as more than one reaction mechanism begins to prevail simultaneously. Due to very low rates

of lactonization as well as the weak pH dependency of the reaction, an addition-elimination mechanism is proposed. The conversion of the free acid to the lactone is dominating until the pH range of approximately 4 to 5. Intermolecular ester formation could not be detected in significant extent.

The protonation and deprotonation of the carboxyl groups are inducing pH dependency to the chemical shifts in the ^1H NMR spectral domain. A possible linkage to altered molecule conformation and thus increased hydrogen bond creation between carboxylates and hydroxides and/or between hydroxide groups is raised, also due to indications of a transient reaction intermediate structure appearance in the ^1H NMR spectra. The presence of a reaction intermediate is further reinforced by PCA indicators, confirming also its maximal contribution to the reaction around pH 4.0–4.5. Furthermore, the maximal rate constant of the lactonization reaction appearing at pH 3 according to classic modeling coincides well with the PCA time stamp data. The central participation of the α -hydroxyl groups in the transition state formation through hydrogen bonding is addressed in related studies.

Reaction models described by classic kinetic modeling and independent multivariate principal component analysis coincide well in terms of model validity in the pH range from 1 to 5.

Considering the slow rate of lactone ring formation of PHA in terms of practical stabilization applications of hydrogen peroxide, the adverse effects are mainly viable in long-term stabilization below neutral pH domain. At the conventional alkaline pH range attributed to mechanical pulp bleaching, the dominating reaction indicated here appears to be hydrolysis, while the rate of lactonization seems to approach zero. However, considering the limited mass transfer during, for example, high-consistency bleaching, the effect of pH gradients occurring during long residence times should not be omitted when evaluating the performance of PHA in the stabilization of H_2O_2 .

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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