A Graphical Method for the Determination of the Mode of Hydrolysis of Biodegradable Polymers

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Purpose. To develop a qualitative method for the determination of the mode of scission in the hydrolysis of biodegradable polymers.
Methods. The method requires determination of the molar fraction of monomer (m_1) by ^{1}HNMR or HPLC, and the degree of polymer degradation (α) determined by ^{1}HNMR.
Results. If the scission of the backbone bonds is completely random, the molar fraction of the monomer must equal the square of the degree of degradation as predicted by Kuhn (1). If the degradation follows an exclusive chain-end "unzipping" mechanism then, $m_1 = \alpha$. Experimental data falling on the theoretical curves (m_1 vs. α) confirm the corresponding mode of scission. If the data fall between the two curves, it suggests a faster chain-end scission than random scission. When data fall below both of these curves, it suggests the chain-end bonds are less reactive than the internal bonds.
Conclusions. The acid catalyzed hydrolysis of a poly(ortho ester) and the base catalyzed hydrolysis of poly(D,L-lactide) (PLA) were by a random scission mechanism, while acid catalyzed hydrolysis of PLA demonstrated faster chain-end scission.

KEY WORDS: random scission; chain-end scission; hydrolysis; poly(D,L-lactide); poly(ortho ester)s.

INTRODUCTION

Biodegradable polymers are effective platforms for drug delivery (2). Although numerous publications have reported the application of these polymers (2), very few papers discussed the degradation mechanism (3–5). In these cases the mode of degradation was assumed to be random scission (6) where all backbone bonds were equally labile and the degradation can be expressed by a single rate constant. This assumption can not be accepted without verification. Bonds at the chain-ends have quite different electronic environments from the internal bonds, therefore the hydrolytic rate can be quite different. Hydrolysis of cellulose (7) and acid catalyzed hydrolysis of poly(D,L-lactide) (8) showed a faster chain-end scission while base catalyzed hydrolysis of polyglycine was slow at the chain-end but fast at the internal sites (7). Recognizing deviations from random scission kinetics may be obscured by mechanisms such as autocatalysis. A simple qualitative method, independent of degradation mechanism for discriminating the mode of scission is highly desirable. The present manuscript proposes a graphical method for such a purpose.

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MATERIALS AND METHODS

Dichloroacetic acid, 4-N,N-dimethylaminopyridine (DMP), p-dioxane-d_8 and sodium hydroxide (50%, carbonate-free) were obtained from Aldrich Chemical Company (Milwaukee, WI). Poly(D,L-lactide) was obtained from Medisorb (Cincinnati, OH). Poly(ortho ester), DETOSU-HD, was synthesized from hexane-1,6-diol (HD, Aldrich) and a diketene acetal (3,9-bis(ethylidene-2,4,8,10-tetraoxaspiro-[5,5]undecane; DETOSU, MRL, Rahway, NJ) using the procedure reported by Heller, et al. (9).

Hydrolysis of poly(D,L-lactide) Catalyzed by 4-N,N-dimethylpyridine

Poly(D,L-lactide) (21.5 mg) was placed in a one-ml volumetric flask. The polymer was dissolved in ca. 0.8 ml of p-dioxane-d_8. 4-N,N-dimethylaminopyridine (45.8 mg) and 0.1 ml of D_2O were added then filled to volume with p-dioxane-d_8. The solution was transferred into a screw-capped NMR tube and ^{1}HNMR spectra were taken hourly (Bruker ACE-500; probe temperature: 85°C) for fifty hours.

Hydrolysis of DETOSU-HD

DETOSU-HD (58.02 mg) was dissolved in ca. 1.7 ml of p-dioxane-d_8. To the solution was added 0.15 ml of 26.7 mM dichloroacetic acid solution in D_2O and 22.49 mg of heptane-1,7-diol (internal standard) before q.s. to 2 ml using p-dioxane-d_8. The solution was transferred into a screw-capped NMR tube, sealed and inserted in a 37°C water bath. The ^{1}HNMR spectra were taken periodically (Brucker ACE-200). Simultaneously, 30 μl of the reaction mixture were transferred into a vial and the reaction was stopped by addition of 1.5 ml of 0.5 N NaOH. High molecular weight polymer was allowed to precipitate before HPLC analysis for hexane-1,6-diol (10). The HPLC was equipped with a pump (Waters, M45), an autosampler (Waters, WISP), a PRP-1 column (Hamilton; 150 × 4.1 mm), a post column reaction system (Kratos; with a 0.1 ml Teflon delay coil) and a Dionex pulsed electrochemical detector (PED). The mobile phase was 8% acetonitrile in water (helium sparged) at a flow rate of 1 ml/min. The column effluent was mixed with sodium hydroxide (0.5 N, flow rate: 1.0 ml/min, helium sparged) and routed into PED. Pulsed amperometric detection mode was selected. The potential of the gold electrode was initially maintained at 0.1 V for 0.62 second and brought to 1.0 V for 0.2 second, followed by reduction to −0.8 V for an additional 0.32 second before reset to 0.1 V. The current between 0.52 and 0.62 second was integrated. The detector response was acquired and the peaks were integrated using equipments obtained from Nelson Analytic Company.

Hydrolysis of PLA in Neat State

PLA pellets (ca. 5 gm) were compressed (100°C) into a sheet using a Carver press (500 lb/in^2). Discs (8 mm diameter × 0.7 mm thickness) were punched from the sheet. The discs were weighed in 2-dram vials and placed in a constant humidity chamber (100% RH, 60°C). Periodically, 4 discs were retrieved and stored in a −4°C freezer before analysis. A

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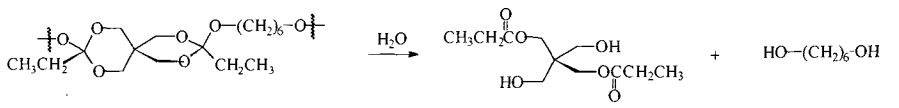


Scheme 1.

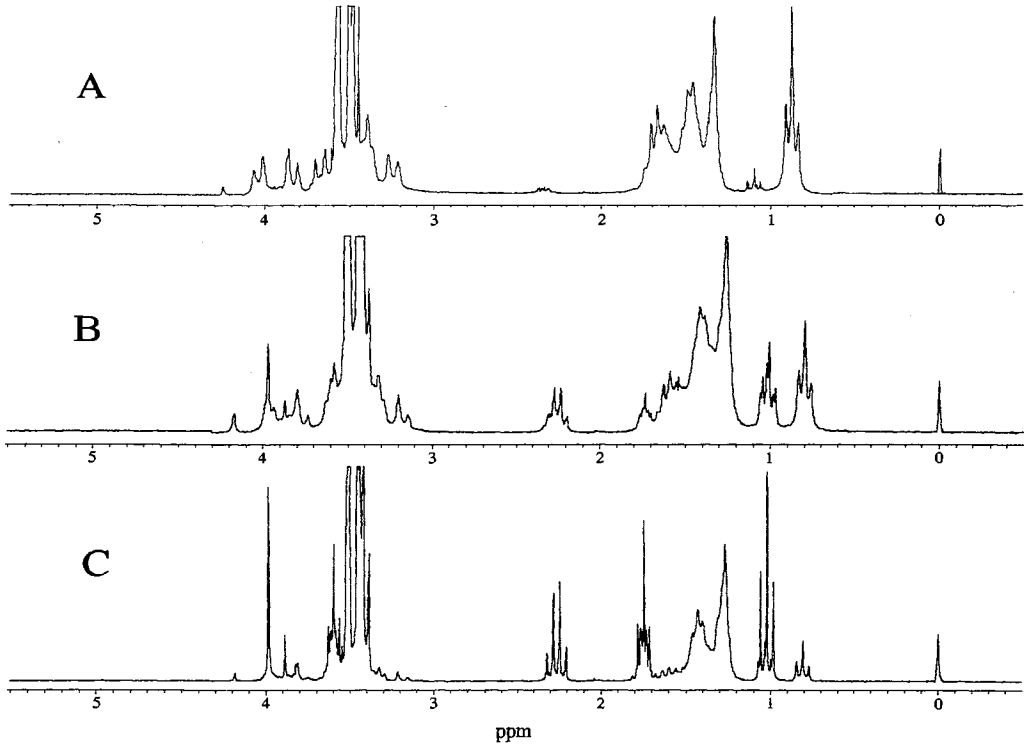


Fig. 1. ^{1}HNMR spectra of DETOSU-HD in D_2O/p-dioxane-d_8 (1:9) with dichloroacetic acid (0.03 M) at 37°C. Spectra taken when 95% (A), 59% (B) and 18% (C) of the ortho ester bonds remained.

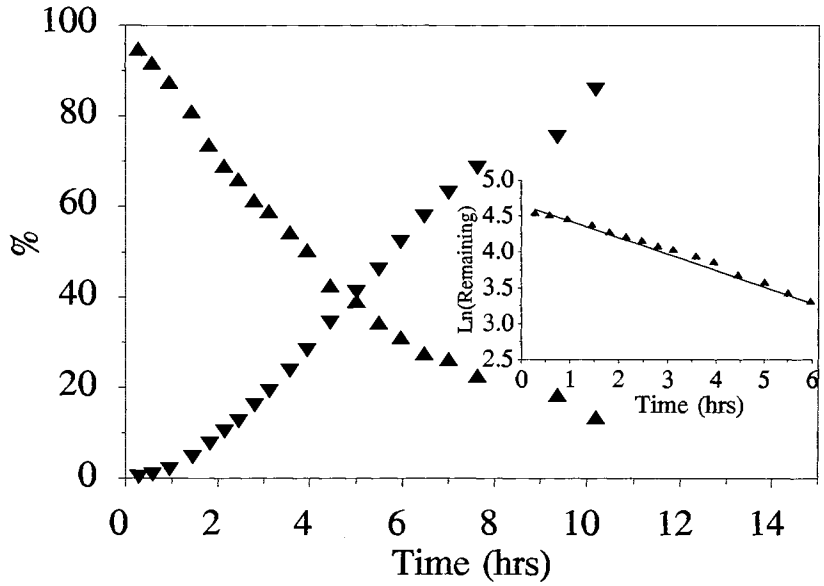
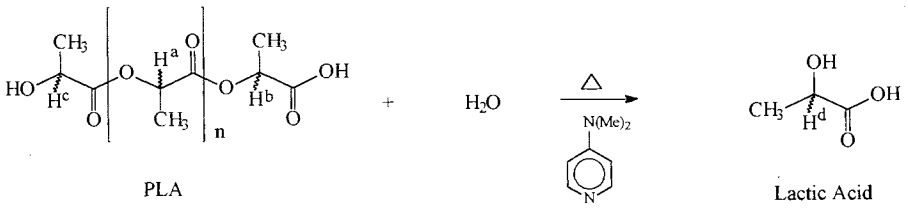


Fig. 2. Percentages of ortho ester bonds (▲) and hexanediol monomer (▼) during the hydrolysis of DETOSU-HD in D_2O/p-dioxane-d_8 (1:9) using dichloroacetic acid (30 mM) as a catalyst (37°C). The insert is the semilogarithm plot of the ortho ester bonds remaining vs. reaction time.



$H^a = M_1$, $H^b = M_2$,
$H^c = M_3$, $H^d = M_4$

Scheme 2.

disc was dissolved in ca. 1 ml of p-dioxane-d_8 which contained 0.12 mg/ml of 4-N,N-dimethylaminopyridine and the ^{1}HNMR spectrum was taken (Brucker ACE-200).

RESULTS

Acid Catalyzed Hydrolysis of a Poly(ortho ester)

Upon hydrolysis in acidic environments, poly(ortho ester)s are degraded into pentaerythritol dipropionate and a diol (Scheme 1). The progress of the hydrolysis was followed by ^{1}HNMR. Fig. 1 shows the spectra collected during hydrolysis of DETOSU-HD. The protons of the orthopropionate methyl group were triplets at δ0.79 (methyl; Me_1) and those of the methylene group were embedded in the multiplets between δ1.1 and δ1.8. The protons of the reaction product, propionic ester, were at δ1.00 (triplets; Me_2) and δ2.25 (quartet) for the methyl and methylene groups, respectively. These signals became progressively larger as the hydrolysis proceeded. The remaining ortho ester bonds were calculated from the areas corresponding to the methyl group ($Me_1/(Me_1 + Me_2)$). The loss of the ortho ester bonds followed first-order kinetics (Fig. 2). Also shown in Fig. 2 is the appearance of monomeric hexane-1,6-diol in the reaction mixture.

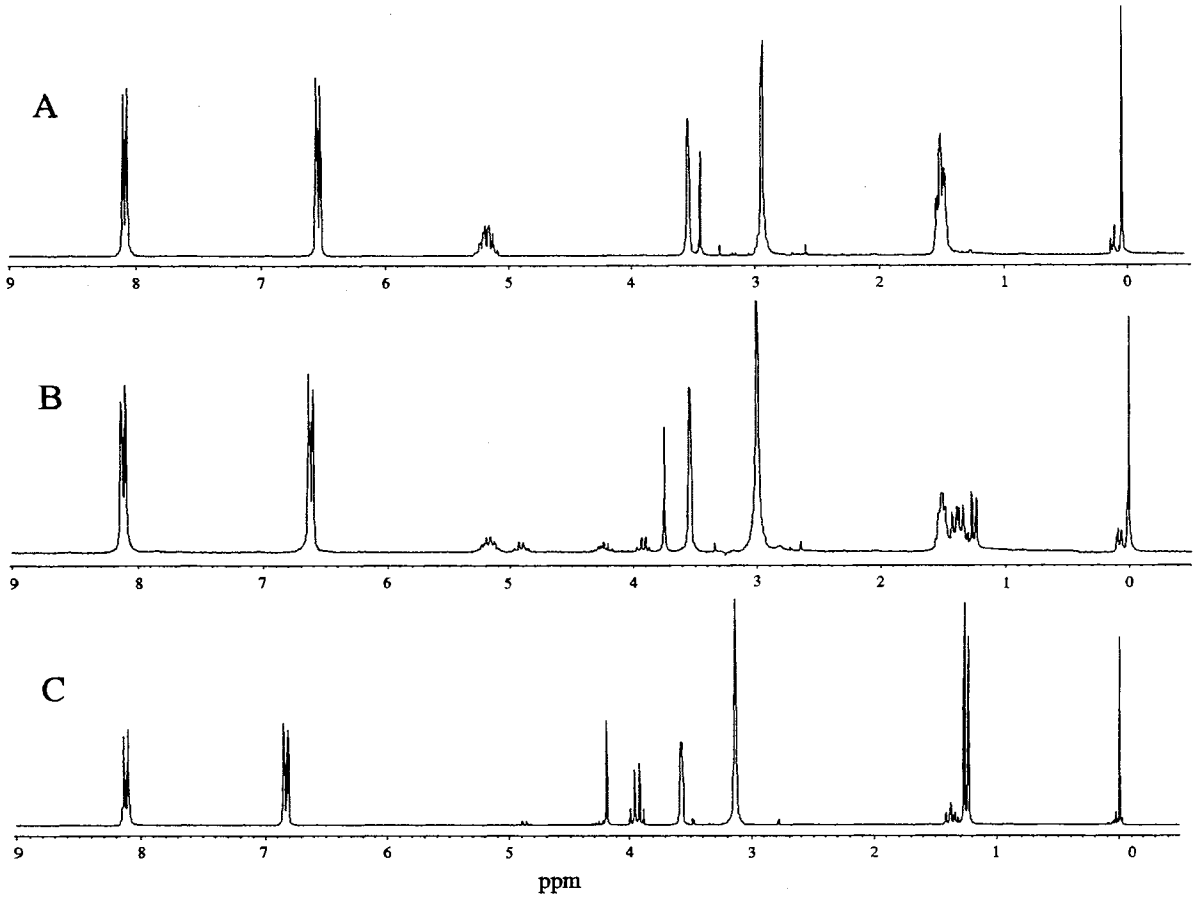


Fig. 3. ^{1}HNMR spectra of poly(D,L-lactide) in D_2O/p-dioxane-d_8 (1:9) with 4-N,N-dimethylaminopyridine (0.38 M) at 85°C. Spectra taken at t = 0 hr (A), 5 hrs (B) and 30 hrs (C).

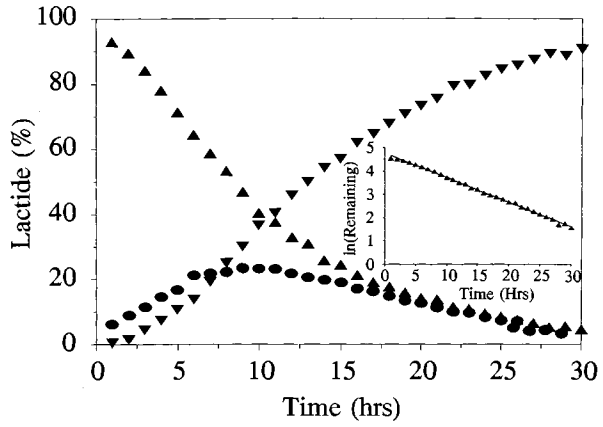


Fig. 4. Percentages of ester bonds (▲), chain-end lactide (●) and monomer (▼) during the hydrolysis of poly(D,L-lactide) in D_2O/p-dioxane-d_8 (1:9) using 4-N,N-dimethylaminopyridine (0.38 M) as a catalyst (85°C).

Hydrolysis of PLA Catalyzed by 4-N,N-dimethylaminopyridine

Hydrolysis of poly(D,L-lactide) (PLA) can be described by Scheme 2. Fig. 3 shows ^{1}HNMR spectra taken during hydrolysis of PLA catalyzed by 4-N,N-dimethylaminopyridine. Four types of methine protons (δ3.9–5.3) were identified. The multiplets at δ5.05–5.30 (M_1) that were present early in the polymer degradation were assigned to the methine of the internal lactides. Methines at δ4.85–5.03 (M_2) and at δ4.20–4.35 (M_3) were assigned to the lactides at the carboxylic and hydroxyl chain-ends, respectively. The methine of lactic acid appeared at δ4.00 (M_4) as a quartet. The fraction of unhydrolyzed ester bonds was calculated from the area ratios of $(M_1 + M_2)/(M_1 + M_2 + M_3 + M_4)$. The fraction of monomeric lactic acid was calculated from the area ratio of $M_4/(M_1 + M_2 + M_3 + M_4)$. The degradation profile is shown in Fig. 4. It is noted that the loss of ester linkages were *pseudo* first-order (Fig. 4, insert). In contrast, the degradation profile (Fig. 5, insert) of PLA in acidic solution reported previously (8), was nonlinear.

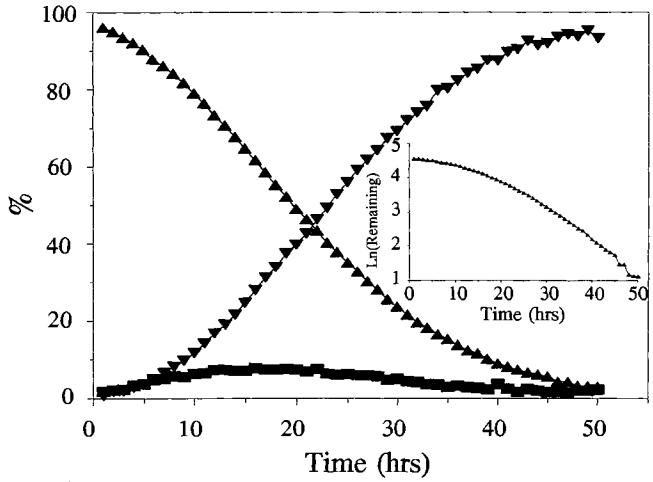


Fig. 5. Hydrolysis of poly(D,L-lactide) in D_2O/p-dioxane-d_8 (1:9) containing 0.118 M of DCl (60°C): loss of ester bonds (▲), formation of chain-end lactides (■), and formation of free lactic acid (▼) (8).

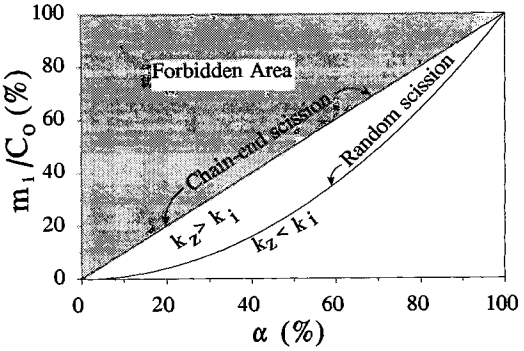


Fig. 6. Schematic diagram of monomer appearance as a function of the degree of degradation with scission rates at the internal (k_i) and chain-end sites (k_z).

DISCUSSION

Theory

Random scission assumes all backbone bonds are equally reactive and accessible. If the concentrations of water and catalyst are constant, a complete random scission mechanism would predict first-order kinetics as observed in the acid catalyzed hydrolysis of DETOSU-HD and the base catalyzed hydrolysis of PLA. In a previous report (8), a rapid chain-end scission in the acid catalyzed hydrolysis of PLA was concluded based on an argument that the maximum fraction of lactide at one of the chain-ends is less than the theoretical value of 0.25 assuring a random scission mechanism. Application of this method, however, requires monitoring of the degradation to >50%. Since the total number of units was unchanged (mass balance),

$$[C] + [E] + m_1 = C_o + E_o + m_1^o \qquad (1)$$

where [C], [E] and m_1 are the concentration of the intact backbone bonds, chain-end bonds, and the monomer, respectively, and C_o, E_o, m_1^o are the corresponding initial concentrations. The fraction of units at a chain-end, [E] can be expressed (8) as,

$$[E] = e^{-kt} \cdot [C_o - (C_o - E_o) \cdot e^{-kt}] \qquad (2)$$

and,

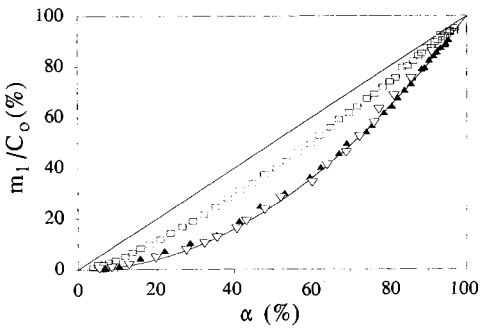


Fig. 7. Monomer (m_1/C_o) vs. degree of degradation (α) plots for the acid catalyzed hydrolysis of DETOSU-HD (▽), the hydrolysis of PLA catalyzed by 4-N,N-dimethylaminopyridine (□), and the hydrolysis of PLA catalyzed by DCl (▲). Data were taken from Figs. 2, 4 and 5.

$$[C] = C_o \cdot e^{-kt} \quad (3)$$

therefore,

$$m_1 = C_o + E_o + m_1^o - C_o \cdot e^{-kt} - e^{-kt} \cdot [C_o - (C_o - E_o) \cdot e^{-kt}] = C_o + E_o + m_1^o - 2C_o \cdot e^{-kt} + (C_o - E_o) \cdot e^{-2kt} \quad (4)$$

If the initial molecular weight is high, $m_1^o \approx 0$ and $E_o \approx 0$. Thus,

$$m_1/C_o = (1 - e^{-kt})^2 = \alpha^2 \quad (5)$$

where α ($= 1 - C/C_o$) is the degree of degradation. This result is identical to that calculated by Kuhn using a statistical method that was independent of the reaction mechanism (1). If the degradation proceeded exclusively by "unzipping", or chain-end scission, then,

$$m_1/C_o = \alpha \quad (6)$$

Both α and m_1 can be determined experimentally. If the degradation is a combination of both mechanisms, with the chain-end scission rate (k_z) faster than the scission at the internal sites (k_i), m_1 should be larger than the prediction of eq. 5 but less than that predicted by eq. 6. If $k_i > k_z$, m_1 must be less than that predicted by both eq. 5 and 6. This relationship is schematically shown in Fig. 6. Data presented in Figs. 2, 4, 5 were plotted in this manner and presented in Fig. 7. It is shown clearly that the hydrolysis of DETOSU-HD was completely by a random scission mechanism. The hydrolysis of PLA was random under basic conditions while the chain-end esters were significantly more labile than the internal esters under acidic conditions.

The proposed method is unambiguously applicable if the reaction occurs in a homogeneous mixture. This limits the application of this method to polymers which undergo bulk erosion. Data obtained from the hydrolysis of PLA in a constant humidity chamber (60°C) was treated by the current method. Fig. 8 showed that the appearance of lactic acid at any degree of degradation was higher than that predicted by eq. 5 suggesting that chain-end scission was faster than the scission of the internal bonds. Although many further experiments are warranted, this conclusion is consistent with the hydrolysis in acidic ($k_z > k_i$) solution described previously (8) as the internal environment of neat PLA is probably acidic.

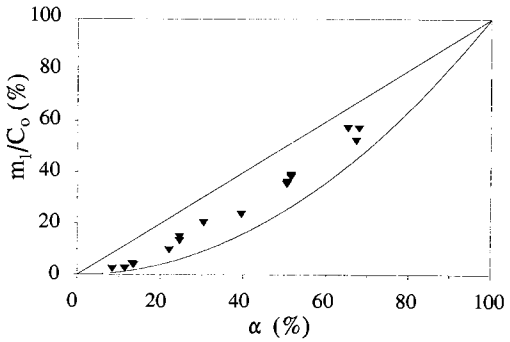


Fig. 8. Monomer (m_1/C_o) vs. degree of degradation (α) plot for the hydrolysis of PLA discs in a 100% RH chamber (60°C).

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