Quantification of Free Ketene Acetals and Determination of Cure Kinetics of Poly(ortho ester)s by a Facile Deuterium Labeling Method

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SYNOPSIS

A facile method was developed to quantify unreacted ketene acetals during polymerization of poly(ortho ester)s. The method was based on isotopic (deuterium) labeling of the unreacted ketene acetal groups arising from the diketene acetal monomer, 3,9-diethylidene-2,4,8,10-tetraoxaspiro[5,5]undecane (DETOSU). In deuterium oxide, free ketene acetals are hydrolyzed to an α-deuterated ester; ortho ester bonds are hydrolyzed to the nondeuterated analog. The relative abundance of the deuterated ester side chain can be quantified by gas chromatography-mass spectrometry (GC-MS). In the current method, aliquots of a diketene acetal/polyol(s) reaction mixture were dissolved (crosslinked polymers swollen) in methylene chloride and excess D_2O. The diketene acetal/polyol(s) reaction mixture was hydrolyzed under mildly acidic conditions to yield pentaerythritol dipropionate (PDP; hydrolysis product of DETOSU). PDP was extracted into an organic phase, silylated, and analyzed by GC-MS. Fragments corresponding to the $C_2H_5C{\equiv}O+$ ion (57 a.m.u.) and $C_2H_4DC{\equiv}O+$ ion (58 a.m.u.) were monitored and the quantity of free ketene acetal groups were calculated from the peak areas of the chromatograms. The precision of the method was ±0.1%. The accuracy, as compared to a parallel ^{1}H-NMR study, was within 5%. This method permits determination of the cure end-point of a poly(ortho ester) polymerization reaction to within ±0.25%. The curing kinetics agreed well with DSC branching/crosslinking measurements. © 1994 John Wiley & Sons, Inc.

INTRODUCTION

Poly(ortho ester) networks have been demonstrated as effective platforms for long-term drug therapy.[1,2] These networks were prepared by condensation polymerization of a diketene acetal (3,9-diethylidene-2,4,8,10-tetraoxaspiro [5,5] undecane (DETOSU)) and polyols. Both undercure (incomplete polymerization) and overcure (pyrolysis) could result in early erosion and irreproducible performance. Thus, the progress of the polymerization reaction, i.e., degree of cure, must be closely monitored. Cure has been followed by indirect methods such as differential scanning calorimetry (DSC),[3] dynamic mechanical analysis (DMA),[3] dielectrometry,[4] and conventional tensile testing.[5] However, by these methods, precise determination of the cure endpoint is difficult because of small changes in the polymer properties as the polymerization reaction approaches conclusion. The objective of this work was to develop a high precision analytical method for poly(ortho ester) cure, based on quantification of unreacted ketene acetal groups from the DETOSU monomer, that offers advantages over the indirect methods.

Ketene acetals have been monitored by UV spectrophotometry.[6] However, direct spectral measurements are limited by interference from additives (drugs, stabilizers, antioxidants, and other excipients typically included in a formulation) to the polymer. A chemical derivatization method has been reported for DETOSU[7] that is unsuitable for end-group analysis. The ketene acetal literature[8] offers few reactions that quantitatively yield stable derivatives. The present manuscript describes a novel hydrolytic labeling method where ketene acetals are

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converted into α-deuterated esters that are amenable to subsequent quantification by gas chromatography-mass spectrometry (GC-MS).

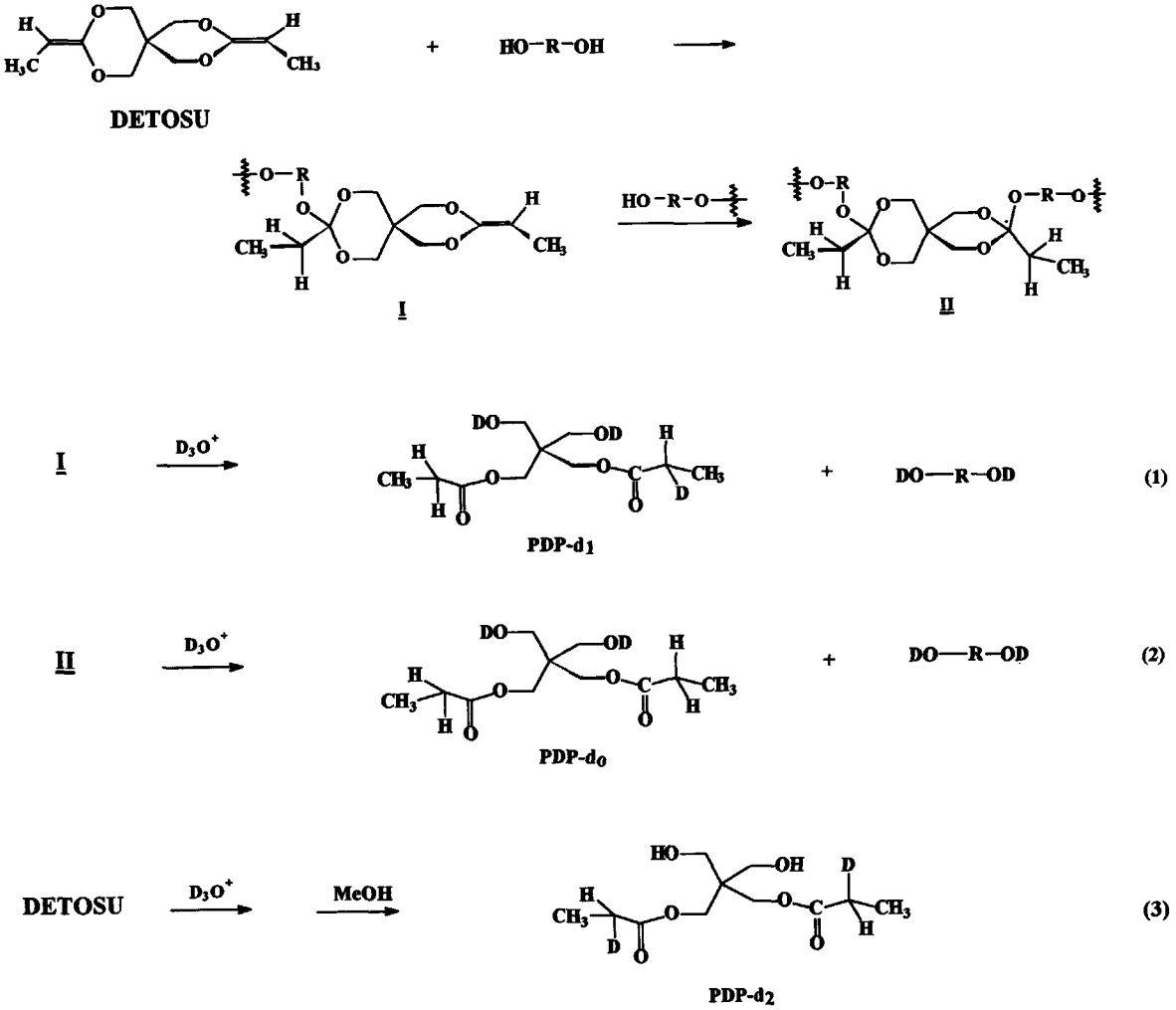
EXPERIMENTAL

Chemical

Deuterium oxide (99.95%D), trifluoroacetic acid-d_1 (99.5%D; 0.5 mL in ampules), pyridene, chlorotrimethylsilane, 1,1,1,3,3,3-hexamethyldisilazane, hexane-1,2,6-triol (HT), tetraethylene glycol (TTEG), and magnesium oxide (MgO) were purchased (Aldrich Chemical Company, Milwaukee, WI). Deuterium oxide was stored in sealed vials in a desiccator over silica gel. Hexane-1,6-diol (HD) was purchased (BASF, Williamsburg, VA). DETOSU and ivermectin were obtained from the Merck Research Laboratories (Rahway, NJ). Solvent grade methylene chloride was purchased (Baxter Healthcare Corp., Muskegon, MI). Exchangeable protons in methylene chloride due to trace HCl impurities were removed by triplicate extractions using D_2O (10 : 1, methylene chloride : D_2O) followed by storage under a layer of D_2O.

Synthesis of Poly(ortho ester)s

HD (5.96 g), HT (1.73 g), ivermectin (5.73 g), and MgO (0.717 g) were placed into a mixer (Helicone Model 2CV; Atlantic Research Corp. Gaisville, VA). The head space was purged with dry nitrogen (OM-1 cartridge, Supelco, Bellefonte, PA). Polymerization was initiated by addition of DETOSU (14.52 g) via a syringe. The reaction mixture was vigorously stirred at 60°C for 30 min then dispensed into tubing



Scheme1 Chemistry of the deuterium labeling method.

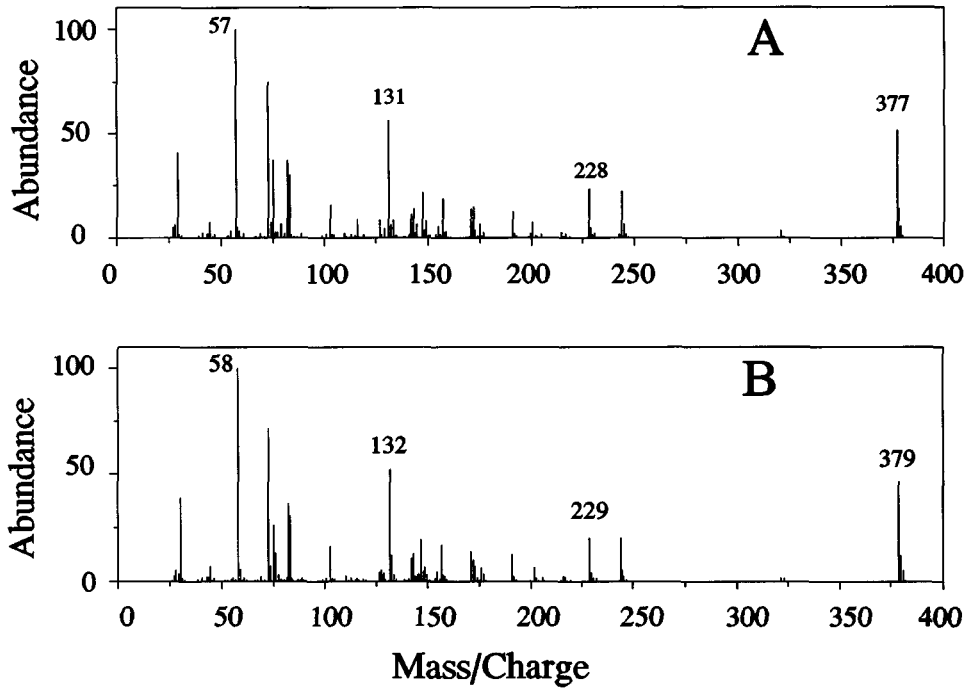


Figure 1 Mass spectra of PDP-d_0 (A) and PDP-d_2 (B).

made of fluorinated ethylene propylene copolymer (FEP). The samples were cured in an oven (60°C) for periods up to 100 h, after which the tubing was mechanically stripped to provide cylindrical poly(ortho ester) devices.

Preparation of α-Deuterated Pentaerythritol Dipropionate Standard (PDP-d_2)

DETOSU (2.1 g) was placed into an oven-dried (120°C) round bottom flask (100 mL) fitted with a

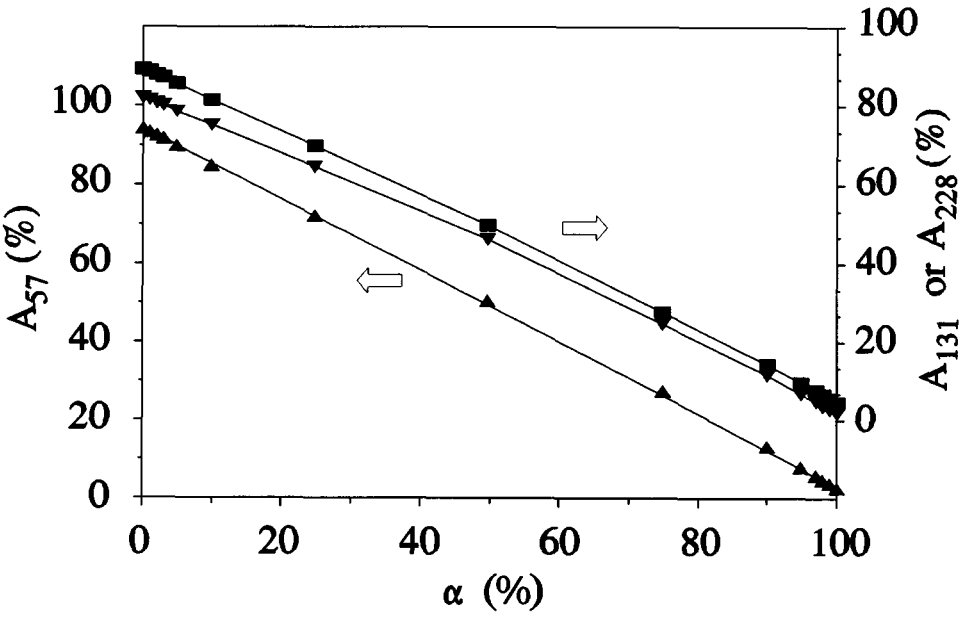


Figure 2 Calibration curves constructed from mixtures of PDP-d_0 and PDP-d_2 using 57 and 58 a.m.u. (▲), 131 and 132 a.m.u. (■; right axis), and 228 and 229 a.m.u. (▼; right axis).

Table I A_{57} (%) of Standard Solutions Prepared from PDP-d_0 and PDP-d_2

	PDP-d_0: PDP-d_2				
	A_{57} (%)				
	100 : 0	99.75 : 0.25	99.50 : 0.50	99.25 : 0.75	99 : 1
Sample #					
1	95.08	94.65	94.40	94.22	94.04
2	95.09	94.65	94.42	94.27	94.05
3	94.98	94.70	94.46	94.28	93.96
4	94.90	94.56	94.40	94.25	94.02
5	94.93	94.64	94.44	94.23	94.13
6	94.92	94.69	94.41	94.20	94.03
7	94.89	94.72	94.39	94.25	93.98
8	94.92	94.66	94.36	94.22	93.96
9	94.91	94.62	94.35	94.21	94.00
10	94.89	94.68	94.41	94.25	93.94
mean ± SD	94.95 ± 0.07	94.66 ± 0.05	94.41 ± 0.03	94.24 ± 0.03	94.01 ± 0.07

condenser. Methylene chloride (10 mL) and D_2O (5 mL) were added to the flask via dry syringes. The hydrolysis of the ketene acetal groups was initiated by addition of a trace amount of trifluoroacetic acid-d_1 (40 μL of a 1% solution in methylene chloride). The reaction mixture was stirred overnight (ca. 16 h) at room temperature. The solvents were then removed by vacuum distillation in a rotary evaporator. Exchangeable (i.e., alcoholic) deuterium atoms were replaced by hydrogen through repetitive (5 times) dissolution in methanol (20 mL) and evaporation. The residue was further purified by column chromatography (silica gel) using an ethyl acetate: methylene chloride (1:1) mobile phase. The solvent was removed under vacuum to yield white needles (mp: 25.9°C). Structure was confirmed by FTIR (Mattson Galaxy 5000) and ^{1}H-NMR (Bruker ACE-200). FTIR spectrum (THF): 1740 cm^{-1} ($\nu_{C=O}$). ^{1}H-NMR spectrum ($CDCl_3$): δ1.14 (d; 6H; CH_3), δ2.36 (q; 2H; CHD), δ3.18 (t; 2H; OH), δ3.60 (d; 4H; CH_2), and δ4.13 (s; 4H; CH_2).

Nondeuterated PDP was prepared similarly using nondeuterated reagents to yield white needles (mp: 28.3°C). FTIR spectrum (THF): 1740 cm^{-1} ($\nu_{C=O}$). ^{1}H-NMR spectrum ($CDCl_3$): δ1.15 (t; 6H; CH_3), δ2.38 (q; 4H; CH_2), δ3.19 (t; 2H; OH), δ3.60 (d; 4H; CH_2), and δ4.13 (s; 4H; CH_2).

Deuterium Labeling Method

A polymer sample (ca. 10 mg) was accurately weighed into a vial then methylene chloride (1 mL) and deuterium oxide (1 mL) were added. The mixture was vigorously shaken then stored at room temperature (ca. 50 min) to replace hydrogens on hydroxyl groups with deuterium. Deuterated trifluoroacetic acid (50 μL; neat) was then added to hydrolyze the polymer matrix (10 min) and to react with the free ketene acetals to form α-deuterated PDP derivatives. Sodium chloride crystals (ca. 350 mg) were added to salt-out PDP. An aliquot (0.5 mL) of the organic layer was transferred to a fresh vial and silylated with 1 mL of a silylating agent (pyridene:chlorotrimethylsilane:1,1,1,3,3,3-hexamethyldisilazane mixed 2:1:2).[9] The gas chromatograph (HP 5890 series II) was equipped with a split-splitless injector (230°C, split flow at 50 mL/min, septum purge flow at 3 mL/min), a DB-5 column

Table II Student *t*-test (one-tailed) comparison of Group A with Group B (data in Table I)

Difference	Group A PDP-d_0: PDP-d_2	Group B PDP-d_0: PDP-d_2	t^a (Calc.)
0.25%	100 : 0	99.75 : 0.25	10.62
0.25%	99.75 : 0.25	99.5 : 0.5	14.09
0.25%	99.5 : 0.5	99.25 : 0.75	12.39
0.25%	99.25 : 0.75	99 : 1	9.58
0.50%	100 : 0	99.5 : 0.5	21.17
0.50%	99.75 : 0.25	99.25 : 0.75	24.91
0.50%	99.5 : 0.5	99 : 1	16.13
1.00%	100 : 0	99 : 1	30.03

[a] Critical *t*-value = 2.7745 (95% confidence interval).

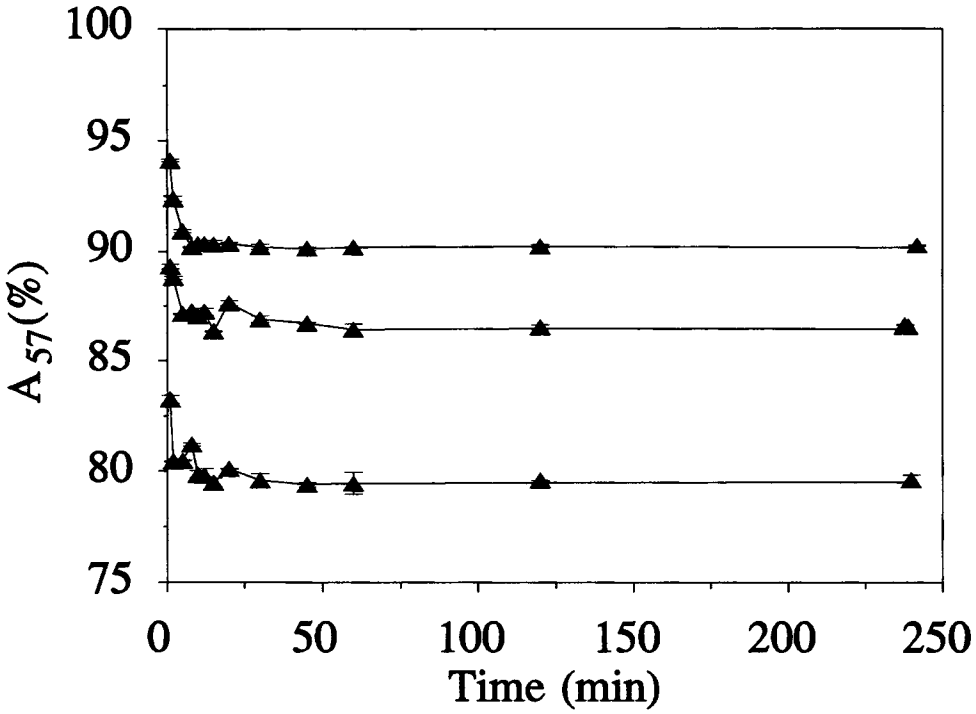


Figure 3 Deuterium exchange kinetics at various stages of the curing process.

(J&W, 15 m × 0.25 mm i.d., 0.25 μ film thickness) and a deactivated silica guard column (5 m × 0.25 mm). The oven was ramped (10°C/min) from 190°C to 235°C. Samples (0.15 μL) were autoinjected (HP 7673) with a 0.5 μL SGE syringe. The column effluents were introduced into a mass selective detector (HP 5970) through a capillary direct interface operated at 260°C. The detector was tuned daily (AUTOTUNE™) using perfluorotributylamine as the tune standard. Data from 1.2 to 3.5 min were acquired using a selective ion monitoring (SIM) mode. Ions at 57 and 58 a.m.u. were sampled with a 75 ms dwell time for each ion. This gave approximately 5.2 SIM cycles per second. The peak areas of PDP associated with these ions were integrated and the abundance of the 57 a.m.u. ion was calculated with respect to the total of the 57 and 58 a.m.u. ions.

Validation by ^{1}HNMR

TTEG (10.28 g) and MgO (0.558 g) were mixed (Helicone™ mixer) at 60°C under a nitrogen blanket. DETOSU (11.32 g) was added and mixing continued as the polymerization reaction proceeded. Periodically, two samples (ca. 10–20 mg each) were removed and one analyzed by the deuterium labeling method and the other analyzed by ^{1}H-NMR. The ^{1}H-NMR sample was dissolved in $CDCl_3$ (1 mL; previously percolated through a 10 cm column of silica gel to remove acidic impurities) and filtered through a sintered glass funnel (previously soaked in 1 *N* sodium hydroxide solution, rinsed with water and oven dried) to remove MgO particles prior to analysis. The filtrate was immediately analyzed (200 MHz NMR, Brucker ACE-200).

Kinetics of Deuterium Exchange

Samples (132 total; 44 from each of three batches) of partially cured poly(ortho ester)s (2.9, 6.4, and 11.5 h in a 60°C oven) were added to individual vials containing methylene chloride (1 mL) and D_2O (1 mL). The vials were sealed and allowed to stand at room temperature. Periodically, the swollen polymer samples (n = 4) were hydrolyzed by addition of trifluoroacetic acid-d_1 (50 μL; neat). The samples were silylated and analyzed by GC-MS as described previously.

DSC Analysis

A literature[3] DSC method was followed for determining degree of polymer cure. Polymer samples were periodically removed from the cure oven and stored in a freezer (−80°C). Prior to DSC analysis,

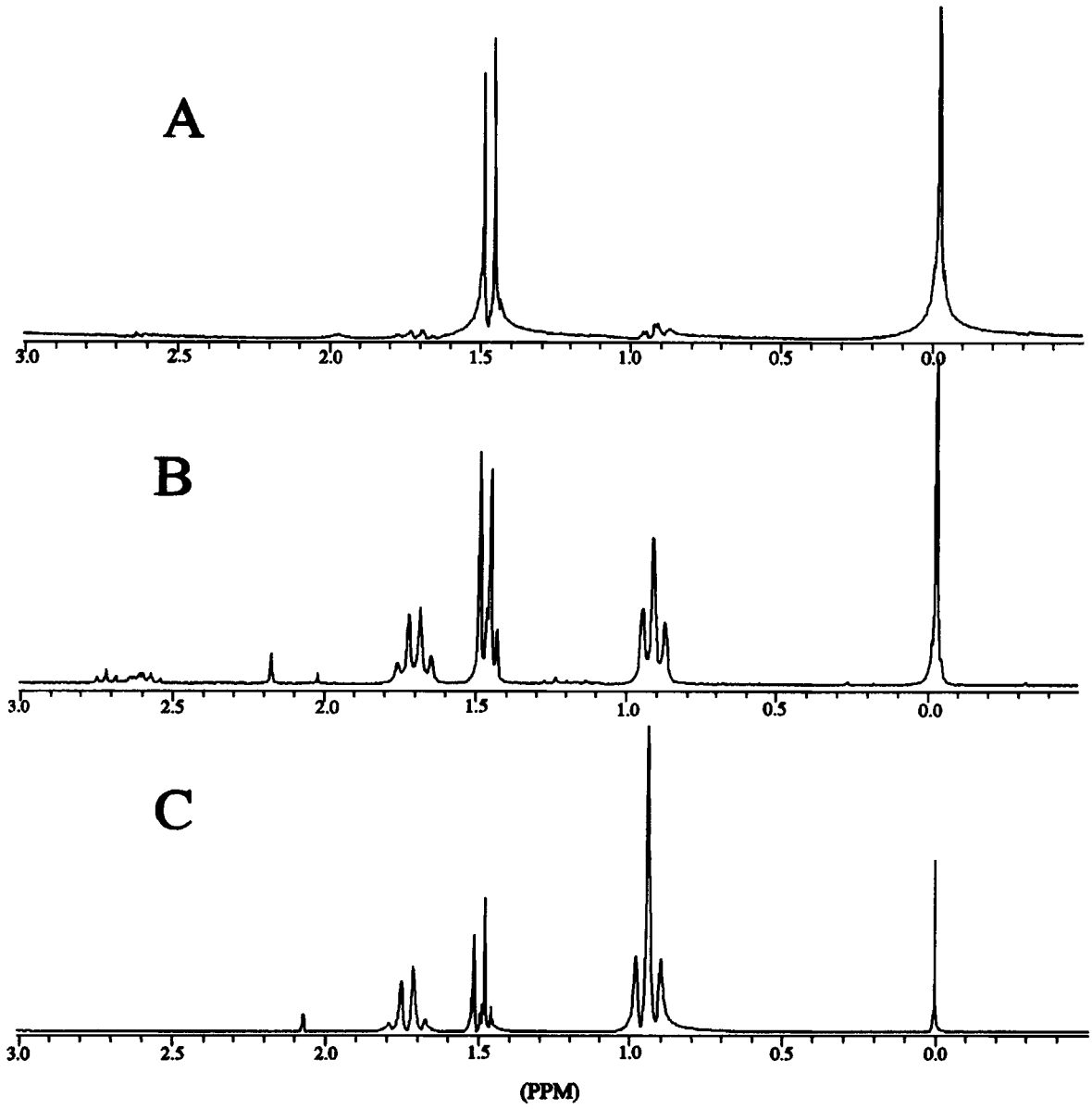


Figure 4 ^{1}H-NMR spectra ($CDCl_3$) during polymerization of DETOSU and TTEG: (A) 84.3%, (B) 50.6%, and (C) 18.6% ketene acetal remaining.

samples were equilibrated to room temperature, removed from the FEP tubing, accurately weighed (ca. 15 mg), sealed in aluminum pans, and immediately placed into the sample holder of the DSC (Perkin-Elmer; DSC-7). The sample was equilibrated at 25°C for 5 min followed by a ramp (500°C/min) to 120°C where the samples were maintained for 60 min. The exotherm was recorded. The sample was then cooled to 25°C then subjected to a second run using the identical temperature program. The second thermogram was subtracted from the first and the area under the resulting exotherm was integrated.

RESULTS AND DISCUSSION

Chemistry

Poly(ortho ester)s are prepared from the condensation reaction of the diketene acetal, DETOSU, with polyols (Scheme 1). Upon hydrolysis, free DETOSU, half-reacted DETOSU (one ketene acetal reacted to form an ortho ester bond), and fully reacted DETOSU yield pentaerythritol dipropionate (PDP).[10] Free ketene acetals hydrolyze in deuterated water to form α-deuterated propionates.[11] Free

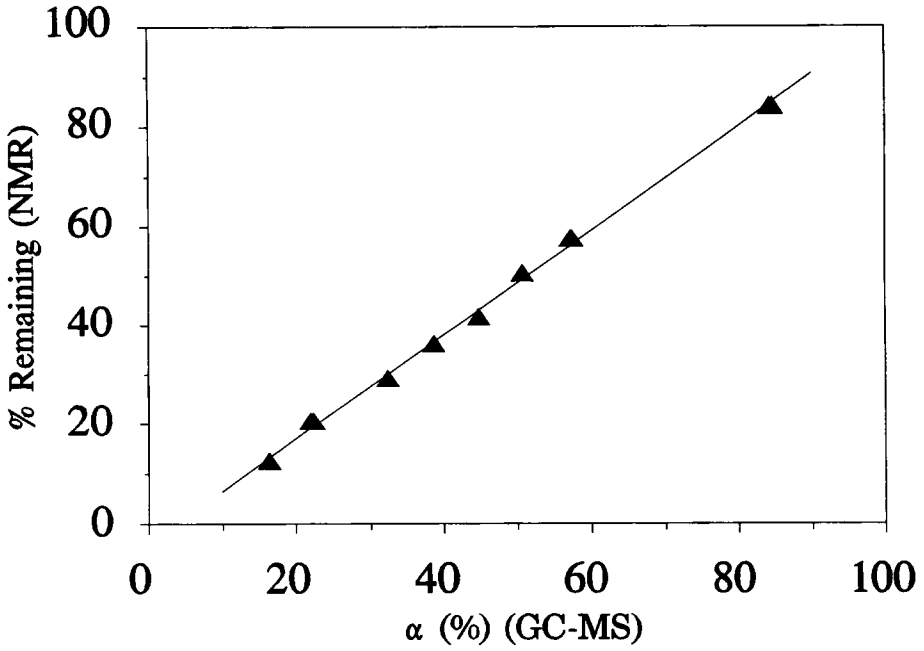


Figure 5 Correlation of data obtained by the ^{1}H-NMR method and the deuterium labeling method.

DETOSU monomer is hydrolyzed into PDP with a deuterated α-carbon on each propionate group (PDP-d_2). Half-reacted DETOSU, **I**, is hydrolyzed into PDP with a monodeuterated α-carbon on one propionate (PDP-d_1). Fully reacted DETOSU, **II**, is hydrolyzed into PDP with nondeuterated propionate side groups (PDP-d_0). Deuterated hydroxyls (— OD) formed during hydrolysis may be converted to normal hydroxyls (— OH) through repetitive hydrogen exchange reactions with methanol. The deuterated fraction of the propionate side chains of PDP was quantified by GC-MS.

Analytical Method

Direct analysis of PDP by GC was not possible due to poor peak shapes (tailing) and attendant irreproducibility. To improve peak shape and reproducibility, PDP produced during hydrolysis was salted-out of the aqueous phase into a methylene chloride layer where trimethylsilylation of the PDP was readily accomplished. The partition coefficients of the PDP standards (PDP-d_0 and PDP-d_2) between methylene chloride and a saturated aqueous sodium chloride solution were similar (37.9 ± 1.2 (n = 3) and 39.3 ± 1.0 (n = 3), respectively) and strongly favored the organic phase. Thus, sampling the organic phase would not create a bias toward one of the isotopic isomers. The mass spectra of silylated PDP-d_0 and PDP-d_2 are shown in Figures 1A and B, respectively. The molecular ion (392 or 394 a.m.u.) was absent from both spectra. The fragment of PDP-d_2 corresponding to the peak at 379 a.m.u. (molecular ion less a methyl group) was doubly deuterated because the corresponding fragment of PDP-d_0 was at 377 a.m.u. The abundance of these fragments was used to quantitate the free DETOSU monomer level in the polymer matrix. Similarly, fragments of PDP-d_2 at 58, 132, and 229 a.m.u. contained one deuterium as they were all one unit higher than the corresponding fragments of PDP-d_0. These ions can be used to calculate the level of unpolymerized ketene acetals (the total of unreacted and half-reacted DETOSU) in the polymer matrix. The 57 and 58 a.m.u. ions were assigned to the chemical formulas $C_2H_5C{\equiv}O+$ and $C_2H_4DC{\equiv}O+$, respectively. The chemical formulas of the 132 and 229 a.m.u. ions have not been assigned.

Quantitative analysis using fragments other than the molecular ion can be confounded by unknown contaminants. For example, a small peak at 57 a.m.u. was present in the spectrum of PDP-d_2 (Fig. 1B), while the corresponding ion (56 a.m.u.) in the nondeuterated sample was not detectable (Fig. 1A). The 58 a.m.u. peak may also contain unknown ions as the natural abundance contributed by ^{13}C and ^{2}H in C_3H_5O (3.4%)[12] was lower than the observed value (5.6%; Fig. 1A). An assumption was made that the

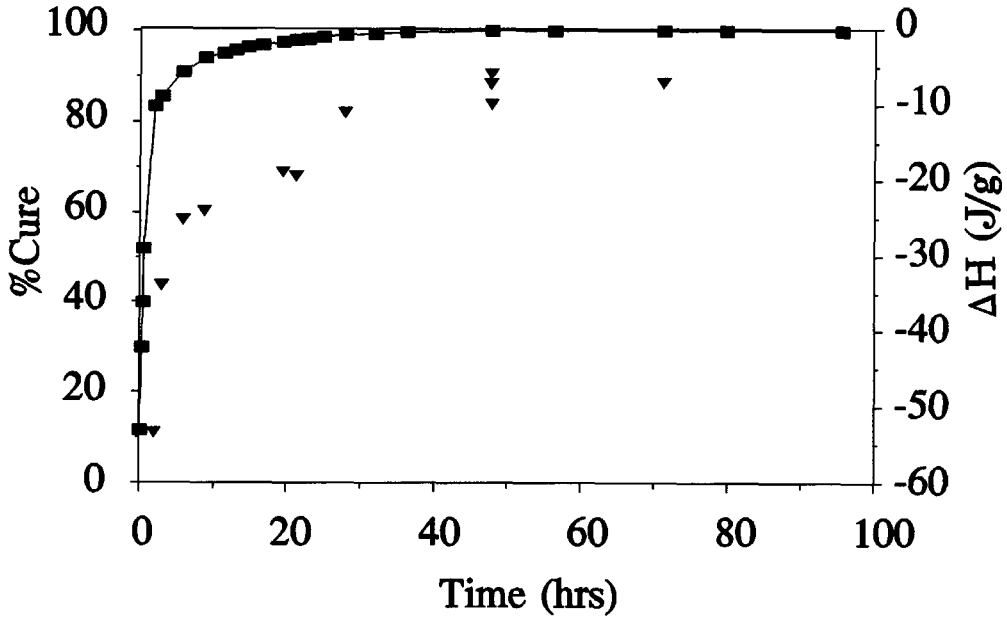


Figure 6 Polymerization/curing of a poly(ortho ester) network monitored by the deuterium labeling method (■) and DSC (▼).

relative amounts of unknown fragments were constant from sample to sample. A measurement of the abundance of ions at m a.m.u. normalized by the total of m and $m+1$ ions, A_m, is necessary to quantify the level of unpolymerized ketene acetals in a sample. A_m was expressed as:

$$A_m = C_m/(C_m + C_{m+1}) \quad (1)$$

where C_m and C_{m+1} were the intensities of the peaks at m and $m + 1$ a.m.u., respectively. Assuming the fraction of unpolymerized ketene acetal is α, C_m has contributions (Eq. 2) from the polymerized (nondeuterated) fraction ($N_m \cdot (1 - \alpha)$) and any impurity at m a.m.u. (I_m), where N_m was the natural abundance of the ion fragment of interest at m a.m.u. Likewise, C_{m+1} has contributions (Eq. 3) from the polymerized fraction ($N_{m+1} \cdot (1 - \alpha)$), the unpolymerized (deuterated) fraction ($N_m \cdot \alpha$), and any impurity at $m + 1$ a.m.u. (I_{m+1}), where N_{m+1} was the natural abundance of the $m + 1$ ion of the fragment at m a.m.u.

$$C_m = N_m \cdot (1 - \alpha) + I_m \quad (2)$$

$$C_{m+1} = [N_{m+1} \cdot (1 - \alpha)] + N_m \cdot \alpha + I_{m+1} \quad (3)$$

Substituting Eqs. 2 and 3 into Eq. 1 gives

$$A_m = \frac{[N_m \cdot (1-\alpha)] + I_m}{[N_m \cdot (1-\alpha)] + I_m + [N_{m+1} \cdot (1-\alpha)] + N_m \cdot \alpha + I_{m+1}} \quad (4)$$

The levels of impurities at m and $m + 1$ a.m.u. calculated with Eq. 4 using standards prepared from PDP-d_0 ($\alpha = 0$) and PDP-d_2 ($\alpha = 1$) were 0.0292 ± 0.0012 and 0.0200 ± 0.0012, respectively. The calibration curve (Fig. 2, A_{131} and A_{228} vs. α) based on Eq. 4 was convex and increased in curvature as N_{m+1} increased. For this reason, small fragments (57 and 58 a.m.u.) were selected for analysis to keep N_{m+1} and, hence, curvature of the calibration curve, small ($N_{58} = 0.034$). This resulted in experimental calibration curves that were essentially linear ($r^2 = 0.9998$; $n = 13$) (Fig. 2, A_{57} vs. α). The percent cure can be expressed as $[(1 - \alpha) \cdot 100]$, where $\alpha = 0$ when all ketene acetals have been polymerized.

Accuracy

The isotopic purities of the reagents and the PDP-d_2 standard would affect the accuracy of the method. The following precautions were taken to minimize exposure to extraneous moisture: D_2O was stored in a desiccator, methylene chloride was washed with D_2O and stored under D_2O, and trifluoroacetic acid-

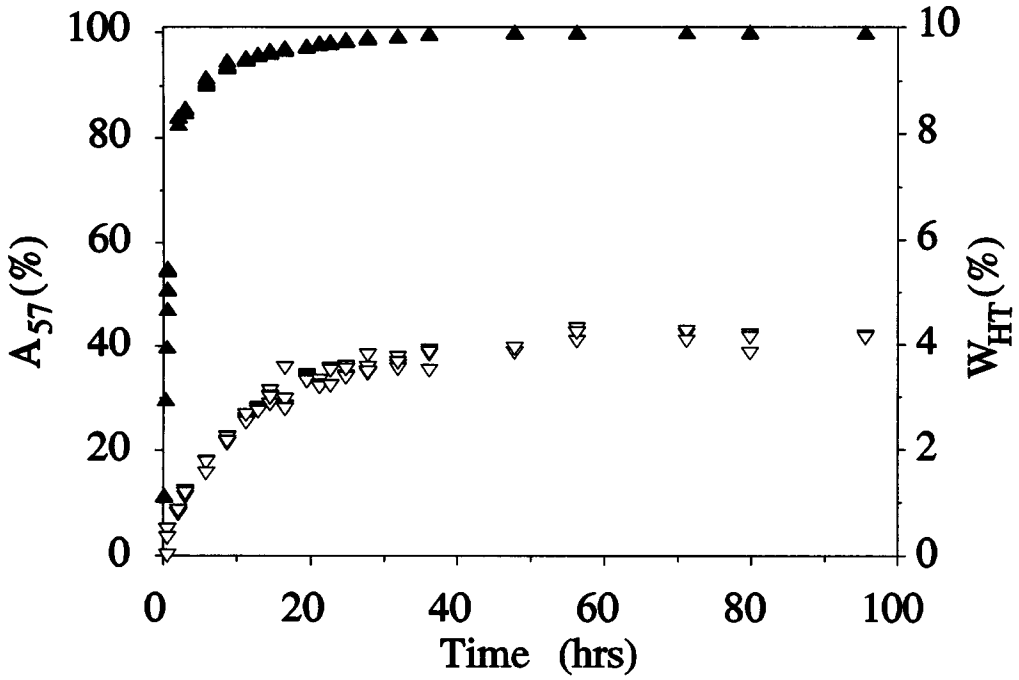


Figure 7 Polymerization/curing of a poly(ortho ester) network followed by the deuterium labeling method (▲) and a branching/crosslinking assay (W_{HT}; ▽).

d_1 was taken from freshly opened ampules. The isotopic purity of the PDP-d_2 standard was verified by ^{1}H-NMR. The absence of a triplet at δ1.15 suggested that the isotopic purity was > 98%. Further characterization of the PDP-d_0 and PDP-d_2 standards by chemical ionization (NH_3) mass spectrometry indicated that the isotopic purity of the PDP-d_2 standard was > 99%.

The accuracy would also be affected by free, unpolymerized hydroxyl groups in the polymerization mixture. These hydroxyls can react with free ketene acetal groups during the hydrolysis reaction and skew the results. Therefore, the exchangeable hydrogens on the free hydroxyls were exchanged for deuterium prior to hydrolysis. This exchange was accomplished by equilibration with excess (> 1,500-fold) D_2O. As shown in Figure 3, exchange was rapid (ca. 50 min for various stages of the curing process) and complete within the time for dissolution/swelling of the polymer matrices.

Another potential source of error was deuterium exchange of the α-hydrogen of PDP through an enolization reaction initiated by MgO (basic erosion inhibitor added to the polymer matrix). Enolization depends on both the acidity of the α-hydrogen and the pKa of the base.[13] Studies with PDP-d_0 indicated that no exchange of the α-hydrogen had occurred after a 5-day equilibration in the solvent mixture spiked with MgO (10 mg/sample). The coefficient of variance (CV) of repeated injections of the same sample (standards or polymer derived) was < 0.05% (n = 10). When multiple solutions (n = 10) were prepared with known percentages (n = 5) of the standards (PDP-d_0 and PDP-d_2; Table I) the CV of A_{57} was < 0.1%. When multiple (n = 10) polymer samples were analyzed the CV of A_{57} was < 0.5% provided the polymers were > 90% cured. To define the sensitivity of the method, a Bonferroni's method[14] (t-test for multiple comparison) was performed on the data presented in Table I. All calculated t-values (Table II) were higher than the critical value (2.7745) for the 95% confidence interval suggesting that an 0.25% difference in the extent of polymerization (i.e., %cure = $(1 - \alpha) \cdot 100$) determined by this method was significant.

The method was further validated by a parallel ^{1}H-NMR study. The model reaction was the polymerization of DETOSU and TTEG. ^{1}H-NMR spectra at various stages of the reaction are shown in Figure 4. As the reaction proceeded the methyl groups adjacent to the ketene acetal groups (doublets δ1.49) decreased in size while the methyl groups of the orthopropionate (triplets at δ0.94) increased. The relative amount of free ketene acetals was cal-

culated from the integrated peak areas. The ^{1}H-NMR data were plotted versus the results with the deuterium labeling method (Fig. 5). The highly correlated data (slope = 1.05 ± 0.01; r^2 = 0.998) indicated the deuterium labeling method was accurate.

Cure Kinetics

The polymerization of a reaction mixture composed of ivermectin, DETOSU, HD, HT, and MgO (60°C) was followed by the deuterium labeling method. The deuterium labeling data (Fig. 6) indicated the polymerization reaction was > 99% completed after ca. 30 h, with complete cure observed after ca. 50 h. The cure kinetic profile obtained with the deuterium labeling method agreed well with that determined by DSC (Fig. 6). The deuterium method was more precise than the DSC method (compare the data at t = 48 h). A parallel experiment was also performed to measure the increase in branch sites[15] as the polymer cured. The kinetics of formation of HT branch/crosslink sites (presented as the percent of the device weight; W_{HT}) coincided well with the current method (Fig. 7).

CONCLUSION

Deuterium labeling of unreacted ketene acetal groups is a facile, accurate, and precise method for monitoring the polymerization of poly(ortho ester)s.

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