Quantitative Analysis of Branching in Poly(ortho ester) Networks

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SYNOPSIS

A direct method to quantify the branching attributable to fully reacted hexane-1,2,6-triol (HT) in poly(ortho ester) networks is reported. The method was based on the presence of free hydroxyl groups on incompletely reacted HT in the poly(ortho ester) matrix that were "tagged" prior to matrix hydrolysis. HT molecules that were completely reacted, i.e., acting as cross-linker, within the polymer matrix would have no free hydroxyl groups available for "tagging" and posthydrolysis would be present as free HT. Experimentally, the matrix was swollen in *p*-dioxane, then phenyl isocyanate (PhCNO) was added to "tag" the hydroxyl groups in the presence of a stannous octoate catalyst. After removal of excess PhCNO, the matrix was hydrolyzed under mild acidic conditions. Any "untagged" HT was subsequently derivatized into trimethylsilyl ethers and analyzed by gas chromatography (GC). The level of branching determined by this direct chemical method correlated well with conventional swell ratio measurements. Furthermore, the extent of hydrolysis of the polymer backbone covalent bonds were also measured by this method since the resulting hydroxyl groups were "tagged" by PhCNO. All polyols were analyzed simultaneously by GC. Application of this method in characterization of the solid-state hydrolysis of a poly(ortho ester) network was demonstrated. The degradation profiles of the branch sites and other backbone ortho ester bonds showed that the hydrolysis was initially slow, becoming progressively rapid. © 1993 John Wiley & Sons, Inc.

INTRODUCTION

Bioerodible cross-linked poly(ortho ester)s have been demonstrated as drug-delivery platforms[1,2] where the drug-release kinetics can be affected by the degree of branching. Conventionally, cross-link density has been determined by equilibrium swelling studies.[3] However, this method can be tedious[4] and unreliable,[5] and requires knowledge of the polymer–solvent interaction parameter.[3,5] This parameter is a function of the chemical composition of the matrix and is not available for poly(ortho ester)s. Cross-link density may also be indirectly determined through tensile (stress–strain) experiments.[6] However, this method is influenced by the presence of undissolved solids (e.g., drugs) and physical matrix defects (e.g., entrapped air bubbles) that might be present in a drug-delivery formulation. Therefore, a direct measure of branching was desired. An ideal method must be specific for the branch sites and independent of the chemical composition of the matrix. It must also be free of interference from additives (drugs, antioxidants, stabilizers/accelerators, fillers, plasticizer, etc.) to the polymer matrix and operable in the presence of biological residues to permit *in vivo* assessment of polymer degradation. NMR is one potential method that meets these requirements. It has been reported that solution ^{13}C-NMR can differentiate the carbon atoms at "Y" branches from those at "H" cross-links.[7] However, broad spectral line widths prohibit similar analyses of polymers in the solid state.[8] This article presents a chemical derivatization method that provides a direct measure of branching in poly(ortho ester) matrices.

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EXPERIMENTAL

Chemicals

Phenyl isocyanate (PhCNO), heptane-1,7-diol, triethylamine, trifluoroacetic acid, pyridine, chloromethyldimethylsilane, and 1,1,1,3,3,3-hexamethyldisilazane were received from Aldrich Chemical Co. (Milwaukee, WI). Hexane-1,2,6-triol (HT) was obtained from Sigma Chemical Co. (St. Louis, MO). 3,9-Bis(ethylidene)-2,4,8,10-tetraoxaspiro[5,5]-undecane (DETOSU) was prepared by Merck Research Laboratories (Rahway, NJ). Hexane-1,6-diol (HD) was obtained from BASF (Williamsburg, VA). Stannous octoate was obtained from Pfaltz and Bauer (Waterbury, CT). These reagents were used as received. *p*-Dioxane was obtained from Burdick and Jackson Co. (Muskegon, MI) and was dried over sodium metal and distilled within 1 h of use. Ivermectin was prepared and supplied by Merck Research Laboratories (Rahway, NJ). It was sieved (80 mesh) before use.

Synthesis of Poly(ortho ester)

HD (10.00 g) was placed in a flame-dried two-neck round-bottom flask (250 mL). The flask was equipped with a mechanical stirrer and a rubber septum. DETOSU (11.67 g) was added via an oven-dried syringe. The reaction mixture was allowed to stir (ca. 60 rpm) under nitrogen for 2 h. An aliquot (5.5 g) of the mixture was mixed with HT (0.55 g), ivermectin (0.63 g), and additional DETOSU (1.305 g) to give a final stoichiometric ratio, defined as ketene acetal : hydroxyl, of 0.98 (excluding hydroxyls from ivermectin). The ingredients were mixed vigorously at < 35°C by kneading on a cold steel block. The mixture was transferred into an extruder and filled into FEP tubing (Cole-Parmer; Chicago, IL) to form rod-shaped samples (0.78 mm diameter) that were cured at 60°C for 24 h.

PhCNO Derivatization ("Tagging")

Samples of the cured poly(ortho ester) rods (approx. 10 mm in length) were accurately weighed (approx. 6 mg) and placed in 2 dram vials. Triethylamine (TEA; 50 μL), *p*-dioxane (2 mL), PhCNO (100 μL) and stannous octoate (neat; 20 μL) were added and the vials were capped. The vials were stored without agitation at room temperature (22°C) for ca. 2 h to complete the reaction. Longer reaction times and higher reagent concentrations did not change the observed results. Unreacted PhCNO was quenched by water (50 μL; 3.3-fold excess; 4 h; 22°C). The ortho ester linkages of the polymer matrix were then hydrolyzed by addition of trifluoroacetic acid (60 μL) followed by storage at room temperature (30 min). After the addition of the internal standard (IS; 250 mg/L of heptane-1,7-diol in dioxane), the solution was neutralized by addition of TEA (50 μL). An aliquot (0.5 mL) was transferred into a separate vial and the silating reagent (1 mL; 22°C; 30 min) added. The silating reagent was composed of pyridine, chloromethyldimethylsilane, and 1,1,1,3,3,3-hexamethyldisilazane in a volume ratio of 2 : 1 : 2.[9] The silated samples were analyzed by gas chromatography (GC). The GC (Hewlett-Packard 5880A) was equipped with a split/splitless injector port (280°C) set at a split ratio of 25 : 1. The oven temperature was programmed to increase from 110 to 250°C at a rate of 10°C/min. The postrun conditions were 250°C/5 min. Separation was achieved on a capillary column (Restek Rtx-5, 15 m, 0.25 mm internal diameter with 0.25 μ film thickness). Peaks were detected and quantified by a flame ionization (280°C). Peak area ratios were determined and the amount of free HT was calculated from a linear (r^2 > 0.999) standard calibration curve. The weight percent of HT fully bonded in the polymer matrix (assayed as free HT) relative to the total device weight was calculated ($W_{HT}^{\%}$).

Percent Recovery of HT from the Poly(ortho ester) Matrix

HT (ca. 20 mg) was accurately weighed and dissolved in *p*-dioxane (25 mL). Fifteen 1 mL aliquots of the solution were transferred into 10 mL volumetric flasks. The flasks were divided into groups A, B, and C (n = 5 each). DETOSU (110 mg) and toluenesulfonic acid solution (0.1% in *p*-dioxane; 40 μL; reaction catalyst) were added to groups A and B). These reaction mixtures were refluxed briefly (ca. 10 s) and cooled to room temperature (22°C/30 min). TEA (50 μL), stannous octoate (neat; 20 μL), and PhCNO (100 μL) were added to group B. After standing at 22°C for 2 h, water (50 μL) was added to both group A and B samples and allowed to stand for an additional 4 h. To all samples of groups A, B, and C were added trifluoroacetic acid (70 μL; 30 min), IS (1 mL), and q.s. to volume (10 mL) with *p*-dioxane. Aliquots (0.5 mL) were withdrawn, silated, and analyzed by GC as described

previously. The % recovery of HT was calculated as follows:

$$\% \text{ Recovery} = \text{area}_A/\text{area}_C \times 100\% \quad (1)$$

The conversion of HT to trisubstituted ortho ester was calculated as follows:

$$\% \text{ Conversion} = \text{area}_B/\text{area}_C \times 100\% \quad (2)$$

Area_A, area_B, and area_C are the area ratios (HT/IS) assayed from the samples in groups A, B, and C, respectively.

Kinetics of PhCNO Derivatization

Accurately weighed polymer sections (1 cm; $n = 4$) were placed in 2 dram vials. *p*-Dioxane (2 mL) and TEA (50 μL) were added. The vials were capped and the polymer allowed to swell completely (2 h). PhCNO (100 μL) and stannous octoate (20 μL) were then added. Samples were withdrawn periodically and the reaction quenched by addition of water (50 μL). After all samples were collected, trifluoroacetic acid (70 μL; 30 min) was added and the samples were silated and analyzed by the method described above. The logarithm of the weight percent of "untagged" HT (W_{HT}) minus the value at infinity (W_{HT}^{∞}) was plotted against time and a rate constant (k_{obs}) was obtained from the slope.

Extension Ratio

Poly(ortho ester) samples (2 cm) were allowed to swell in *p*-dioxane (20 mL). Two hours were required to reach swelling equilibrium. The length of the swollen polymer rod was measured with a caliper. The extension ratio was calculated by dividing the swollen length by the length of the original device.

Degradation Studies

Cross-linked poly(ortho ester) samples (1 cm) were accurately weighed and immersed in dissolution test bottles containing isotonic phosphate buffer [0.07*M*; pH 7.4; 75 mL; tonicity adjusted to equal 0.9% NaCl using NaCl(s)]. The bottles were sealed and mounted on a rotating shaft (20 rpm) of a sustained release apparatus (VanKel, Edison, NJ). The water bath was maintained at 37°C. Periodically, samples were withdrawn ($n = 4$) and the devices retrieved. The retrieved devices were lyophilized (FTS Systems, Stone Ridge, NY) for 2 days to remove water and derivatized with PhCNO as described previously.

RESULTS AND DISCUSSION

Poly(ortho ester) matrices were prepared by a thermosetting polymerization reaction between polyols (e.g., HD and HT) and DETOSU according to Scheme 1. The hydrolysis of these matrices was acid-catalyzed to form diol, triol, and pentaerythritol dipropionate (PDP)[10] as decomposition products (Scheme 1).

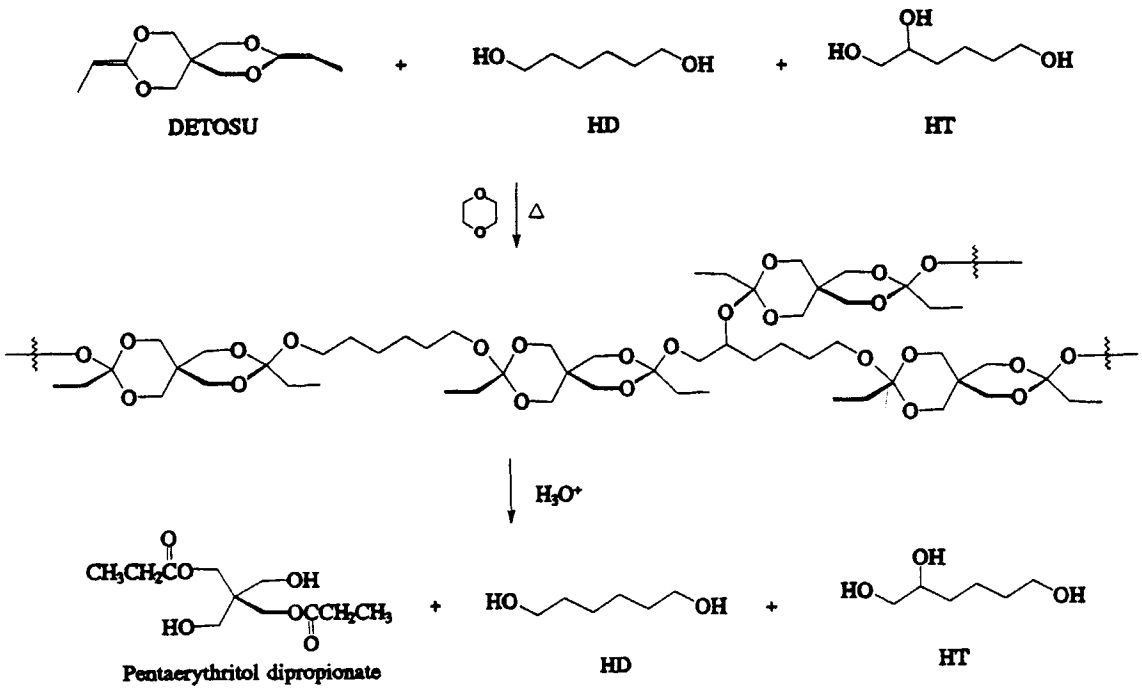
When branching has been effectively achieved, all three hydroxyl groups of the HT branching agent are reacted with DETOSU. However, due to stoichiometry imbalance and steric effects, some HT hydroxyl groups remain unreacted. Such unreacted hydroxyl groups were tagged with PhCNO prior to complete matrix hydrolysis. The "untagged" or free HT cross-linker recovered after matrix hydrolysis must necessarily have been liberated from branch sites (Scheme 2).

The reagent chosen for tagging the unreacted hydroxyl groups was PhCNO. PhCNO has been used to determine the hydroxyl number of polyester and polyether prepolymers used in the synthesis of polyurethanes.[11] The reaction is facile and quantitative. The "un-tagged" hydroxyls were silated[9] before gas chromatographic analysis.

The GC chromatogram (Fig. 1) showed that the silated derivatives of HT, IS, HD, and PDP derived from the backbone hydrolysis were well resolved. Peak assignments were made against reference standards of the respective compound.

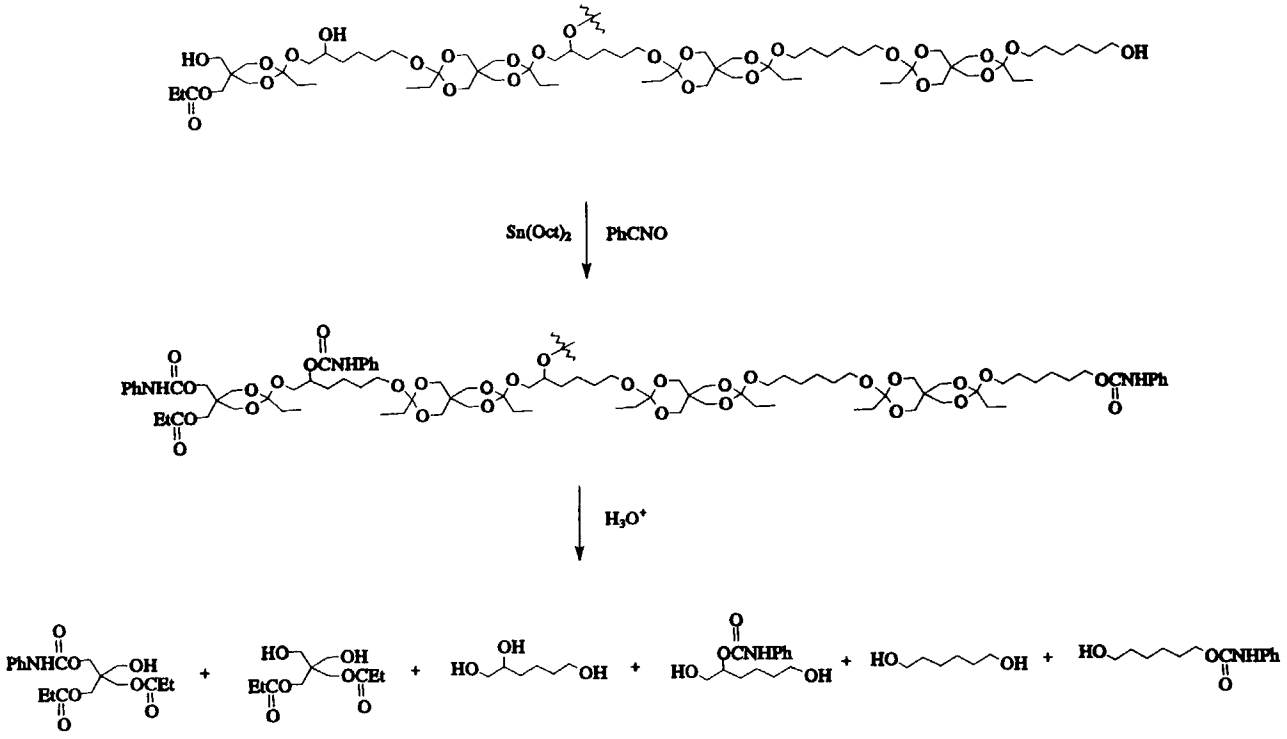
Analytical Method

Owing to the nature of analysis, accuracy of this method relies on at least three parameters: (1) the completion (yield) of the urethane formation reaction; (2) the stability of ortho ester bonds during derivatization; and (3) the recovery of HT from the branch sites. Complete conversion of the HT hydroxyl groups to PhCNO derived urethanes can be accomplished by reaction with excess PhCNO. The kinetics of derivatization as a function of PhCNO concentration are shown in Figure 2. W_{HT}, the weight percent of "untagged" HT, decreased in a *pseudo* first-order manner and eventually leveled off at a minimum (W_{HT}^{∞}). The minimum values for three PhCNO concentrations (Fig. 2) were almost identical (2.53 ± 0.07; $n = 12$), suggesting that the hydroxyl groups in the matrix had been quantitatively converted to urethane. This minimum value remained constant over > 4 h (Fig. 2), suggesting that the PhCNO treatment did not induce polymer hydrolysis.



Scheme 1

To assess the recovery of HT from its branch sites, fully ortho esterified HT was prepared to simulate these sites. HT was allowed to react with a 50-fold excess DETOSU (catalyzed by trace amount of toluene sulfonic acid) in an attempt to convert all the hydroxyl groups into ortho esters without pro-



Scheme 2

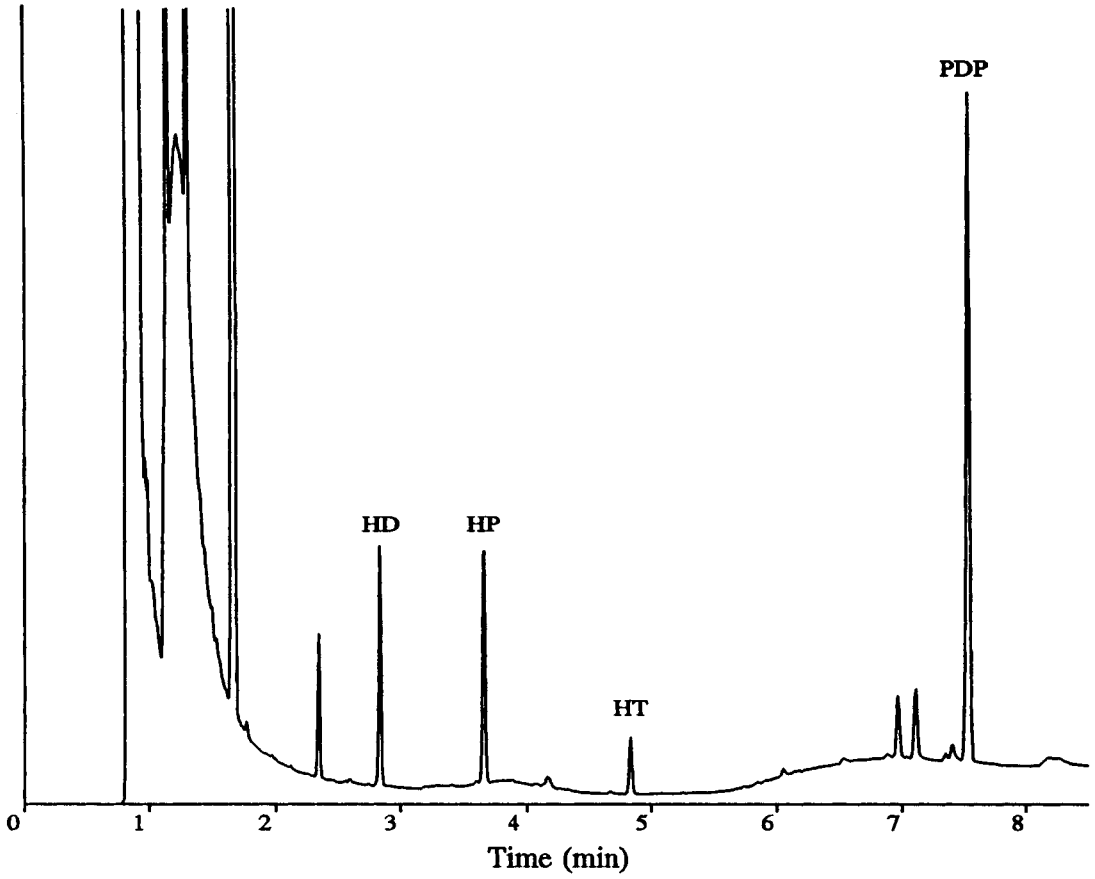


Figure 1 A chromatogram of a typical sample. HD: hexane-1,6-diol, HP: heptane-1,7-diol (IS), HT: hexane-1,2-6-triol, PDP: pentaerythritol dipropionate.

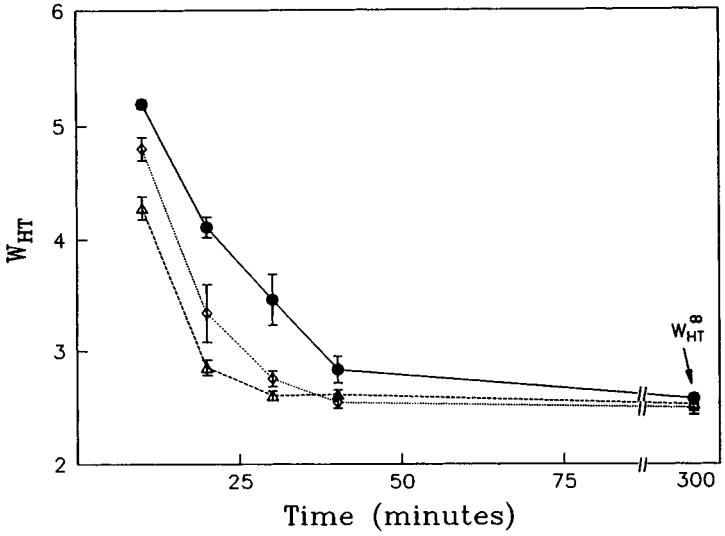


Figure 2 Kinetics of derivatization as a function of PhCNO concentration at room temperature (22°C): 50 (— ● —), 100 (· · · ◇ · · ·), and 200 μL (– – △ – –) of PhCNO. W_{HT} is the "untagged" cross-linker at any time.

ducing an infinite network. The resulting ortho ester was subsequently hydrolyzed and the recovery was calculated using eq. (1). In two independent measurements (each $n = 5$), the recoveries were 99.0 ± 1.5 and 98.5 ± 3.3. Since the 2-hydroxyl group of HT is a vicinal alcohol, it may be sterically hindered. Therefore, it was necessary to confirm that the HT molecules were indeed recovered from fully reacted HT. Thus, the ortho ester bonds in the mixture were stabilized by TEA and subjected to a second derivatization by PhCNO to "tag" any residual hydroxyl groups as urethanes. After the completion of the second derivatization and the subsequent hydrolysis, the "untagged" or free HT was previously fully esterified. Thus, the conversion of HT into its trisubstituted ortho ester can be calculated. The percents of conversion calculated by eq. (2) were 101.5 ± 3.9% and 103.7 ± 2.3%, respectively, indicating that HT was recovered from trisubstituted ortho ester sites and the recovery was quantitative.

The rate of PhCNO derivatization was dependent on the degree of branching. The *pseudo* first-order rate constants (k_{obs}) initially decreased as the W^{∞}_{HT} increased, eventually plateauing at values near 0.1 min^{-1} (Fig. 3). Thus, 2 h reaction time, which represents more than 15 half-lives for the least reactive, highest cross-linked samples, was sufficient. Moreover, the W^{∞}_{HT} values were not affected by the presence of drug, plasticizer, antioxidant, and degradation stabilizer if PhCNO was maintained in large excess. In one study, samples of 1 cm devices (7.77 ± 0.14 mg/cm) were externally added 1.05 mg of ivermectin, 0.78 mg of dibutyl sebacate, 1.36 mg of 2,6-di-*t*-butyl-4-methylphenol (BHT), and 1.99 ± .44 mg ($n = 4$) of magnesium oxide prior to the PhCNO treatment. The W^{∞}_{HT} value obtained in the presence of these additives was 2.66 ± 0.05% ($n = 4$).

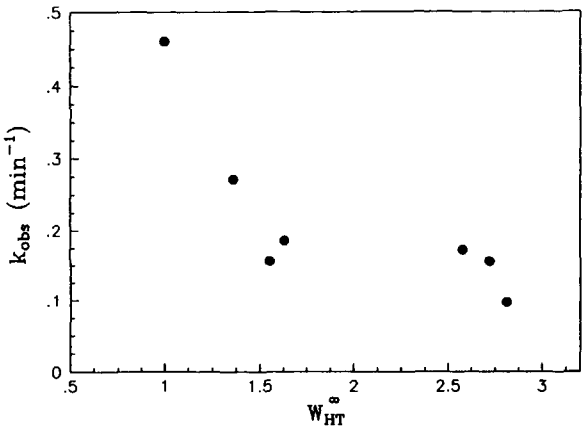


Figure 3 Dependence of the *pseudo* first-order rate constant, k_{obs}, on W^{∞}_{HT}.

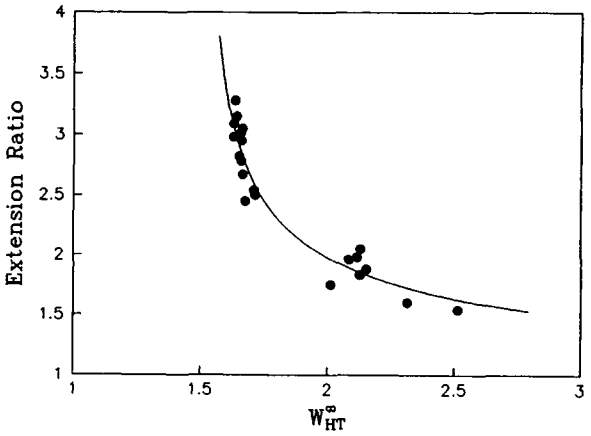


Figure 4 Correlation between extension ratio and W^{∞}_{HT}.

This value was not statistically different from the W^{∞}_{HT} value (2.69 ± 0.04; $n = 4$) of the device. The GC response for HT was linear with $r^2 > 0.999$. The amount of fully bonded HT was also linearly related to the sample size ($r^2 \geq 0.999$). Reproducibility was good with three independent determinations yielding W^{∞}_{HT} values of 2.00 ± 0.10 ($n = 4$), 1.93 ± 0.03 ($n = 8$), and 1.92 ± 0.01% (w/w) ($n = 4$). The percent relative standard deviations within batches and among batches were < 5%.

Correlation with Extension Ratio

One way of assessing the validity of the new chemical method is to compare the results with the extension ratio (swelling behavior) of the matrix. Since the swelling of the matrix was isotropic, the one-dimensional swell ratio, the extension ratio (*ER*), was used. An inverse relationship was observed between the *ER* and the W^{∞}_{HT} values obtained from 22 batches of devices (Fig. 4). It was noted that only a portion of the initial HT (6.05% w/w initially) was incorporated in the polymer matrix with all three hydroxyls esterified to act as a branching agent. The leveling of the *ER* when the W^{∞}_{HT} value was higher than 2% suggested that the PhCNO derivatization method was a more sensitive measure of cross-link density than was a swelling method and was readily applicable to analysis of polymers with low cross-link density where swelling measurements were extremely difficult to perform due to sample fragility or fragmentation.

Application in Kinetic Studies

The degradation profiles of ortho ester bonds in a drug-containing device in pH 7.4 isotonic phosphate

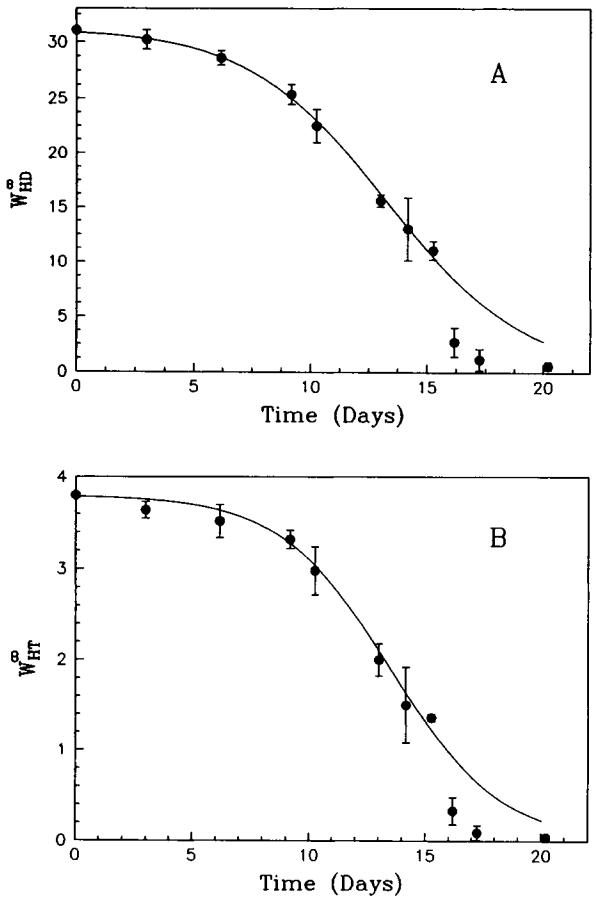


Figure 5 Degradation profiles of (A) fully bonded hexane-1,6-diol (W^{∞}_{HD}), and (B) hexane-1,2,6-triol at branch sites (W^{∞}_{HT}) in a cross-linked poly(ortho ester) matrix in pH 7.4 buffer solution at 37°C.

buffer solution (37°C) are shown in Figure 5. The loss of branch sites (W^{∞}_{HT}) and backbone ortho ester bonds derived from HD (W^{∞}_{HD}; weight percent of "untagged" HD relative to device weight at completion) were initially slow, becoming progressively rapid as hydrolysis proceeded. It was noted that the polymer matrices fragmented and partially dissolved in *p*-dioxane when ca. 10% of the bonds had been hydrolyzed, which placed a practical limit on swelling methods of only the initial 10% of the degradation. With the current method, the complete degradation profiles simultaneously were obtained for all ortho ester bonds in the network. A report of a detailed kinetic model of the poly(ortho ester) hydrolysis and supporting data generated with the current analytical method is being prepared.

CONCLUSION

Using the PhCNO chemical derivatization method, the branch sites and backbone covalent bonds in poly(ortho ester) networks were reproducibly quantified. The degradation profiles of the HT cross-linker as well as various ortho ester linkages in the network were successfully determined from a single GC procedure. This method should prove useful in characterizing the polymerization and gelation of poly(ortho ester) networks in addition to the present hydrolysis studies.

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