A Chemical Means To Study the *In Vitro* Hydrolytic Degradation of Poly(glycolic Acid)

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Synopsis

A chemical means was developed to examine the *in vitro* hydrolytic degradation of both γ-irradiated and nonirradiated poly(glycolic acid) (PGA)-absorbable polymers for the purpose of obtaining information how irradiation affected PGA degradation and how the results related to the previously observed mechanical and morphological data. The method was based on the chemical reaction between the degradation product of the polymer, glycolic acid, and chromotropic acid, and the subsequent measurement of the absorbance of the reaction products by a UV/visible spectrophotometer. It was found that the unirradiated PGA specimens exhibited a two-stage hydrolytic degradation mechanism. This observation supported the previously hypothesized hydrolytic degradation mechanism on the basis of the level of crystallinity data. As the dosage of irradiation increases, the characteristic two-stage degradation mechanism becomes less profound and eventually disappears at 20 Mrads. A monotonic degradation profile was then observed at this dosage level. As reported in the literature, the widespread use of mechanical properties to evaluate the degradation phenomena of this class of polymer does not, however, provide the details of the degradation mechanism as revealed by the present study. The interrelationship between tensile strength, level of crystallinity, glycolic acid concentration, and pH levels of the medium, and their changes as hydrolytic degradation proceeds, are discussed for the purpose of elucidating the mechanism in more detail.

INTRODUCTION

One of the major concerns of using polymeric materials in surgery is their stability, or inertness, in the presence of a hostile biological environment. The degree of degradation is not only a function of time and site of implantation, but also a function of the structure of the polymers and the mechanical force the implant is subject to.

An important application of biodegradable polymers is in wound closure, specifically sutures, which are designed to degrade within the body to minimize foreign-body reaction and subsequent infection. Since the function of wound closure polymeric materials is to support the strength of the wound, it is important that absorbable polymers should maintain adequate strength during the critical period of wound healing. Clinical failure of a synthetic absorbable polymer to close joint capsules has been attributed to the premature degradation of the polymer.[1] Thus, it is important to obtain a better understanding of the degradation phenomena of absorbable polymers.

All existing methods used to study the degradation phenomena of synthetic absorbable sutures are largely based on the loss of mechanical strength of the suture as a function of time.[2-10] The problem with this

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approach, however, is that data during the very early stage of degradation and beyond the period of measurable strength cannot be obtained, making an overall picture of the degradation profile difficult to obtain.

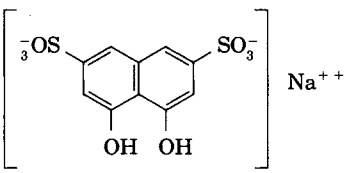
This study reports an alternative means to evaluate the *in vitro* degradation phenomena of synthetic absorbable polymers. The method is based on monitoring the appearance and intensity of color resulting from the chemical reaction of glycolic acid, the degradation product of poly(glycolic acid), with chromotropic acid. It is believed that this new method will make available more complete degradation data to assist polymer scientists to understand better the degradation mechanism of this class of polymers.

EXPERIMENTAL

Materials

A synthetic absorbable polymer, poly(glycolic acid) (PGA), was originally synthesized and commercialized by American Cyanamid. PGA is the simplest linear aliphatic polyester, and, when it is used in wound closure materials, it is melt-spun into multifilaments and subsequently braided into final suture form. It is marketed under the trade name Dexon from Davis and Geck. USP Size 2-0 Dexon with a diameter ranging from 0.25 to 0.29 mm was used in this study. Unopened packets of PGA sutures, obtained from the manufacturer, were subjected to cobalt-60 γ-irradiation at a constant rate of 2.5 Mrad/2 h of exposure. Dosages were 0, 5, 10, and 20 Mrads. The rationale for irradiating PGA specimens up to 20 Mrads was based on the previous study in which the most significant effect of γ-irradiation on the morphological change of PGA fibers was found at 20 Mrads.[11] Oxidative degradation of PGA sutures during the radiation was kept at a minimal and insignificant level by the double-sealed packaging of the suture material. Both the plastic film and the aluminum foil reduce diffusion of oxygen into the suture specimens. All samples were stored in a desiccator filled with Drierite before immersion and testing.

Analytic grade chromotropic acid disodium salt (4,5-dihydroxy-2, 7-naphthalene disulfonic acid disodium salt) was obtained from Fisher Scientific. Its chemical structure is shown below:



Because of its high sensitivity, purple color formation, and quantitative reaction,[12] this chemical has been extensively used to detect and estimate formaldehyde, ethylene glycol, and serum triglycerides, particularly in biological tissues. To prepare chromotropic acid solutions, 1.0 g of the acid disodium salt was dissolved in 250 mL concentrated sulfuric acid.

Pure glycolic acid (70% by weight) was obtained from Polysciences, Inc., and was used to construct a calibration curve.

Preparation of a Calibration Curve

One milliliter of each of the 10 different concentrations of pure glycolic acid (2.05×10^{-1}, 1.03×10^{-1}, 5.13×10^{-2}, 4.10×10^{-2}, 2.05×10^{-2}, 1.03×10^{-2}, 5.0×10^{-3}, 1.0×10^{-3}, 5.00×10^{-4}, $1.00 \times 10^{-4} M$) was mixed with 3 mL of analytical-grade chromotropic acid solution. The resulting absorbance of the most concentrated solution ($2 \times 10^{-1} M$) was determined by scanning the solution through the wavelength ranging from 200 to 900 nm in a Cray 219 UV/visible spectrophotometer. The wavelength at which the maximum absorbance was observed was taken for the determination of the subsequent absorbance of the rest of the sample solutions. The calibration curve was constructed by plotting the observed maximal absorbance vs. the corresponding glycolic acid solution concentration.

Application to PGA Degradation

A fixed amount ($0.08089 \pm 1.548 \times 10^{-4}$ g) of PGA suture materials was immersed in 5 mL of phosphate buffer, pH = 7.4. The solution was kept at 37°C for a predetermined period of time, ranging from as little as 1 h to 120 days. One milliliter of each of the immersion solutions was withdrawn after the predetermined immersion period and immediately mixed with 3 mL of the analytic-grade chromotropic acid solution. One milliliter of the corresponding buffer solution, mixed with 3 mL of the analytic-grade chromotropic acid solution, served as the control. The absorbance maximum of each solution was determined in the manner described above. The corresponding glycolic acid concentration at the absorbance maximum was determined by the use of the previously constructed calibration curve.

RESULTS

When the glycolic acid solution reacted with the chromotropic acid, colors ranging from pink to violet developed, depending on the concentration of glycolic acid. The wavelength of maximum absorbance was 568 nm.

The calibration curve in Figure 1 exhibited a reproducible straight line correlation between glycolic acid concentration and absorbance, with the slope equal to 48.1. Glycolic acid concentration as low as $2.0 \times 10^{-5} M$ could be detected. However, the absorbance of glycolic acid with concentrations greater than $6 \times 10^{-2} M$ exhibited very little increase with increasing concentration, and consequently shows deviation from the linear relationship between absorbance and concentration. This can be explained by the inherent limitation of the Beer–Lambert law.

The degradation profiles of Dexon sutures in terms of the change of glycolic acid concentration with time are shown in Figure 2. The data clearly indicate the advantages of the proposed method over the conventional tensile strength method for evaluating the detailed degradation mechanism of PGA, particularly during the very early and late stages of degradation. Irrespective of the dosage of γ-irradiation, there was no absorbance detected by the instrument as early as the first hour after immersion.

Different degradation profiles were also observed as a result of γ-irradiation and depended on the dosage of γ-irradiation. However, they had one general characteristic: a sharp increase in the concentration of glycolic

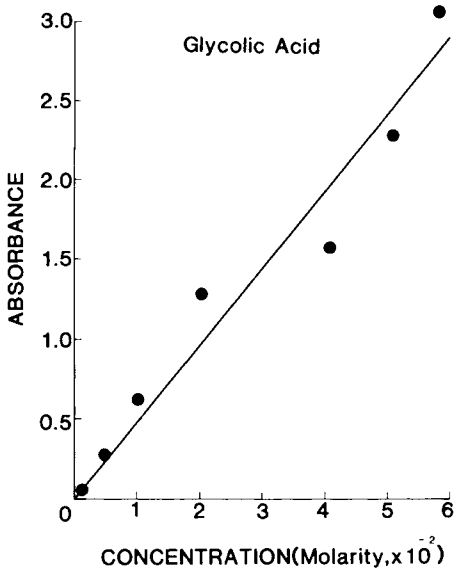


Fig. 1. Calibration curve of glycolic acid determined by plotting absorbance vs. concentration.

acid at an early stage of immersion, and a slow gradual increase in concentration thereafter.

The unirradiated specimens exhibited two distinctive phases of degradation. The first phase encompasses the time frame of immersion to about 20 days. This phase is characterized by both a sharp increase in glycolic acid concentration, and a period of time of relatively constant acid concentration. The second phase begins after 20 days immersion and is characterized by another sharp (but not as sharp as the first phase) increase in glycolic acid concentration.

These two distinctive phases observed in the unirradiated PGA specimens, however, were not profound in the irradiated specimens, particularly the highly irradiated (20 Mrad) specimens. The 20-Mrad specimens exhibited only one monotonic, continuous, and sharp increase of glycolic acid concentration during the first 10–15 days of immersion. Thereafter, the specimens exhibited a gradual, but slight increase in acid concentration. The degradation profiles of the 5 and 10 Mrad specimens were similar and the shape of their degradation profiles falls between those of 0 and 20 Mrad specimens. The two phases observed in the 0 Mrad specimens are not as profound in the 5 and 10 Mrads specimens.

Irrespective of the dosage levels, the tail portions of the degradation profiles of all specimens joined together and reached a common, constant glycolic acid concentration. The difference among the specimens was the time needed to reach such a concentration level, increasing irradiation levels shortening the required time. However, the relationship between irradiation and acid concentration is not limited to the tail portion of the degradation curves. Unirradiated specimens always have a lower glycolic acid concentration than irradiated specimens when compared at the same time interval of immersion.

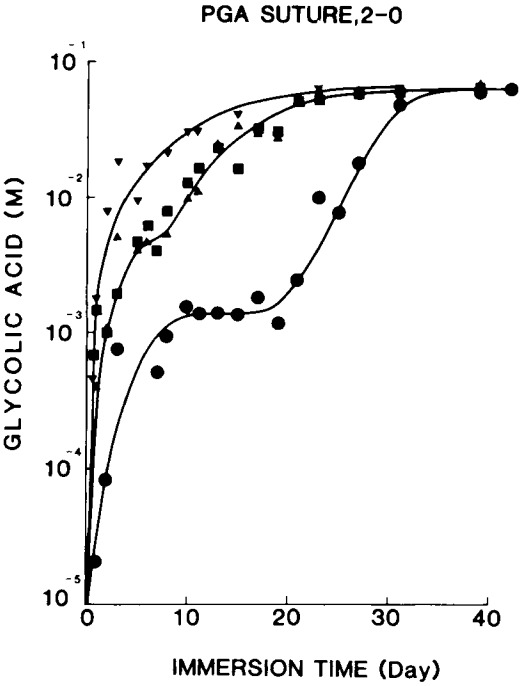


Fig. 2. Hydrolytic degradation profiles of poly(glycolic acid)-braided fibers expressed in terms of the change of glycolic acid concentration as a function of immersion time: (●) 0 Mrad; (▲) 5 Mrad; (■) 10 Mrad; (▼) 20 Mrad.

DISCUSSION

The concentration–time profiles obtained in this study clearly indicate that irradiated specimens degrade differently from unirradiated ones, and that the degradation mechanism of unirradiated PGA fibers involves two stages. This two-stage process is consistent with a recently proposed degradation mechanism of PGA fibers.[7]

In that study a two-stage mechanism was proposed on the basis of crystallinity data.[7] The degradation of this class of polymer was believed to proceed through two main stages, with the first stage in the amorphous region and the second stage in the crystalline region.First stage degradation is predominant before 20 days of immersion, and is characterized by a fast loss of tensile strength due to the scission of tie-chain segments. This results in an increase in the level of crystallinity by achieving better chain mobility and therefore inducing further crystallization. The second stage of degradation begins about the 20th day of immersion and occurs mainly in the crystalline region. It is characterized by a slow destruction of the crystalline region, evidenced by the decrease in the level of crystallinity.

An examination of the times needed to complete the first phase and to initiate the second phase of degradation (20 days) indicates that the time sequence in this study coincides with the previously proposed mechanism.[7]

This study shows that, in the first phase of degradation, glycolic acid concentration increases very sharply and then maintains a relatively con-

stant value until about 20 days post-immersion, before initiating the second phase of degradation. This is exactly what the previously proposed degradation mechanism predicted. Therefore, the glycolic acid released during the first phase degradation comes from the amorphous regions as tie-chain segments, free chain ends, and chain folds in these regions degrade. Only when all or most of the amorphous regions have been removed by hydrolysis, can the water molecules begin to attack the crystalline regions. Because the crystalline regions are less accessible than the amorphous region, hydrolysis can not occur there readily. The observed relatively constant glycolic acid concentration region shown in Figure 2 is evidence for this mechanism. The slope of the second phase degradation is not as steep as the first phase, because the destruction of the crystalline region by hydrolysis is more difficult and slower than in the amorphous regions of the first phase.

Previous studies indicated that the loss of tensile strength of PGA fibers occurred mainly during the first 20 days of hydrolysis and that the materials exhibited no measurable strength after 28 days.[3,4] This loss of strength was attributed mainly to the hydrolytic breakage of tie-chain segments located in the amorphous regions. The sharp increase in glycolic acid concentration in phase one reported in this study is evidence for the proposed mechanism. Figure 3 combines the concentration–time profile of unirradiated PGA specimens obtained in this study with tensile strength–time, pH–time, and crystallinity–time profiles obtained from other studies.[6,7,13] Such a composite figure clearly demonstrates the interrelationships between tensile

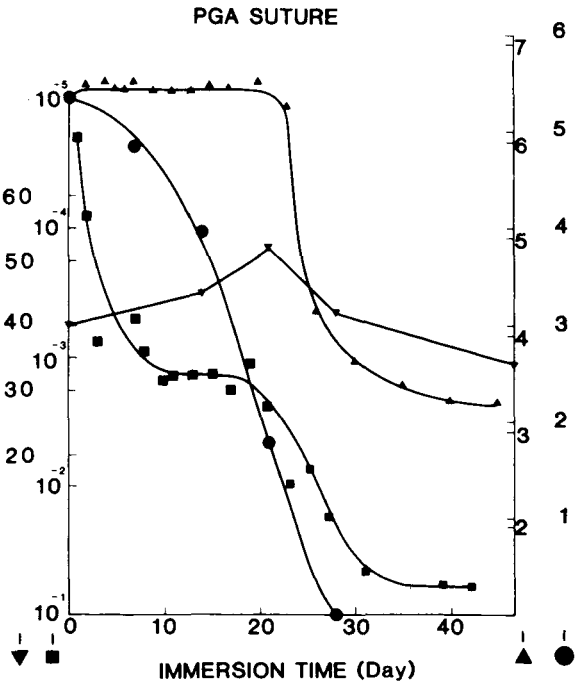


Fig. 3. Interrelationship among glycolic acid concentration (■), tensile strength (●), level of crystallinity (▼), and pH level of the medium (▲), and their changes as a function of time.

strength, level of crystallinity, glycolic acid concentration, and pH levels of the medium, and their change with time.

In addition to the relationship between tensile strength and glycolic acid concentration described above, Figure 3 also shows that, in the concentration-time profiles, the time for the onset of the second (crystalline regions) phase of degradation, about 20 days, coincides with a sharp decrease in pH levels of the medium. This pH decrease indicates the beginning of the hydrolysis of glycolic ester linkages located in the crystalline regions, and results in the release of glycolic acids as shown in the concentration–time profile. This release of glycolic acid from the crystalline regions is sufficient to cause the drastic decrease in the pH level of the medium. The observed rapid increase in the glycolic acid concentration during the first phase of degradation (before 20 days), however, does not cause a change of pH level of the medium during this period of time. It was suggested by Chu that the relaxation of crystal blocks following the hydrolytic scission of tie-chain segments, and the inherent more acids per unit volume in the crystalline regions that could be released through hydrolysis, contributed to the drastic decrease in the pH level during the second phase of degradation.[13] As reported in this study, the observed sharp increase in glycolic acid concentration in the second phase of degradation further supports the pH–time profile previously reported.

The value of using the present method is also demonstrated in Figure 3. Both the mechanical property evaluation and pH level measurement do not provide complete details of the degradation mechanism for this class of polymers. The former merely reveals a continuous loss of tensile strength as degradation proceeds, and does not distinguish the amorphous degradation (first phase) from the crystalline degradation (second phase). The latter only reveals the second half, or second phase, of the total degradation mechanism, but is not sensitive enough to identify the first phase of degradation. The present method, however, is able to reveal and identify both the first and second phases of degradation.

This two-stage degradation mechanism, as reflected in the concentration–time profiles, was not pronounced in the irradiated PGA specimens, and gradually disappeared with an increase in the radiation dosage. It appears that an effect of irradiation is to narrow the difference in susceptibility to hydrolytic degradation in the morphological structure of the amorphous and crystalline regions. In a recently report study, Chu and Williams examined the effect of irradiation on this polymer.[6] On the basis of the reduction in mechanical properties, it was found that the predominant effect of γ-irradiation on PGA fibers is chain scission across the dosage range studied (0–20 Mrads). Consequently, γ-irradiation will accelerate the hydrolytic degradation of PGA fibers. This acceleration effect is also demonstrated in the present study. The amount of glycolic acids released at any time interval before 30 days indicates that the irradiated specimens have higher acid concentrations than the unirradiated and that the concentration increases with dosage.

This predominant chain scission effect of γ-irradiation may be responsible for the less pronounced two-stage degradation mechanism found in irradiated specimens. Before irradiation treatment, the crystalline regions are

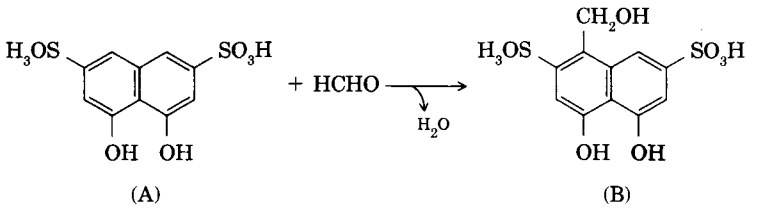
not as equally susceptible to the attack of hydrolytic species as the amorphous regions due to the differences in the morphological structure of the polymers. However, the extensive chain scissions in both the amorphous and the crystalline regions resulting from the irradiation treatment may make the crystalline regions more sensitive to hydrolytic degradation (phase two) immediately after the removal of all of the amorphous regions (phase one). This results in the gradual loss of the characteristic of a two-stage hydrolytic mechanism, and eventually monotonic degradation profiles are observed, as evident in PGA specimens irradiated at 20 Mrads. The fast and early increase in glycolic acid concentration in the highly irradiated specimens (e.g., 20 Mrads) is also consistent with their early appearance of circumferential cracks observed in Chu and Campbell's previous study.[11] Although it is recognized that radiation sterilization of some suture materials is done at 2 Mrads, it is important to examine how radiation, particularly at the high dosage level, could alter PGA chemical structure and subsequently influence its degradation behavior. This influence as evident in the present and previous studies has indeed demonstrated the unique combination effect of radiation and hydrolysis on PGA fibers and its usefulness in elucidating the *in vitro* degradation mechanism.

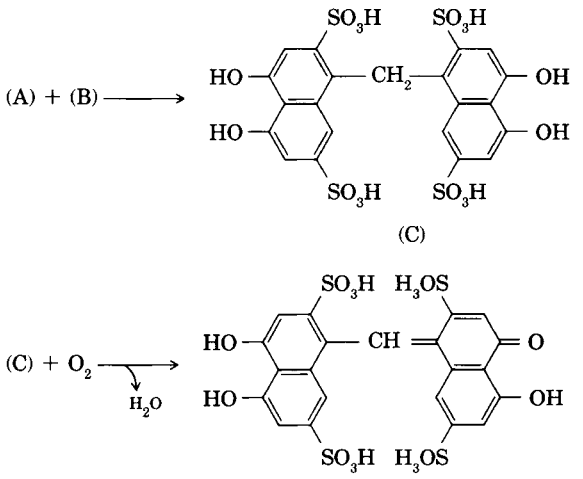
Recently, it was also found that PGA degrades not only by pure hydrolysis but also by enzymes.[14,15] The present study focused only on pure hydrolysis for the purpose of minimizing the complication introduced by enzymes and other biological components. Therefore, it is premature to extend the *in vitro* findings here to *in vivo* degradation of this class of polymer.

The mechanism of the formation of a violet to violet-red color after the chemical reaction between glycolic acid and chromotropic acid in concentrated sulfuric acid is not fully understood, but is believed to occur through a series of oxidation, condensation, and dehydration reactions.[16] By the action of the concentrated sulfuric acid, the glycolic acid is converted to formaldehyde[17]:

$$HO-CH_2-COOH \xrightarrow{H_2SO_4} HCHO + CO_2 + H_2O$$

The resulting formaldehyde then reacts with chromotropic acid through a condensation followed by oxidation, and forms a *p*-quinoidal compound as shown below:





The conjugated double bonds in *p*-quinoidal compound are responsible for the formation of color.

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