Biodegradable polymers for use in surgery — poly(glycolic)/poly(lactic acid) homo and copolymers: 2. *In vitro* degradation

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The degradation mechanism of a series of polyglycolic/polylactic acid, (PGA/PLA), homo and copolymers synthesized as in Part 1[1], has been studied *in vitro*. An *in vitro* test model similar to that described in a previous study[9], was used. The effects of time, temperature and pH on the rate and mechanism of degradation were elucidated. The degree of degradation was monitored molecularly by gel permeation chromatography (g.p.c.), tensile strength determination and mass loss measurements. The mechanism of degradation is shown to be by hydrolysis. The copolymers of PGA and PLA are shown to have a wide range of degradation rates governed by the hydrophilic/hydrophobic balance and crystallinity of the respective copolymer. The effect of the glass transition temperature (T_g) of PGA on its sensitivity to degradation is also demonstrated.

INTRODUCTION

Part I of this series[1] described the synthesis and structural characterization of a range of homo and copolymers of polyglycolic (PGA) and polylactic (PLA) acid. These polymers are known to undergo degradation in the physiological environment[2-7].

This paper describes a study to elucidate the mechanism and kinetics of degradation of the various members of this family of materials[8]. This type of study can best be done *in vitro*. Although PGA has been commercially available for several years as Dexon^R surgical sutures[2] there is little basic information in the literature. The need for the development of meaningful *in vitro* models for testing candidate biomaterials has been discussed elsewhere[9].

The data presented in Part I[1] (see *Figure 1*) show that PGA (Dexon^R) and P-l-LA (as an annealed film) have crystallinities of 50 and 37% respectively by X-ray diffraction (XRD) and differential scanning calorimetry (d.s.c.) techniques. Crystallinity decreases with the increase of either comonomer, and copolymers in the range of compositions between 25 and 70% GA are amorphous. This structural variation would be expected to produce an interesting range of properties, since the two homopolymers represent hydrophilic (PGA) and hydrophobic (PLA) crystalline matrices each containing ester bonds susceptible to hydrolysis. The amorphous range of compositions represents a series of copolymers of varying hydrophilicity. If dl-lactic acid is used instead of l-lactic acid, then the amorphous range is extended from 0–70 m% GA, since P-dl-LA is amorphous.

The *in vitro* test model employed was similar to that described for the PEO/PET series of copolymers[9]. In this case the polymers under investigation were incubated for predetermined periods (0–26 weeks) in buffer solutions of varying pH (pH 5–9), at several temperatures (25°–50°C). On removal from the incubation medium the polymers were taken and the following parameters determined as a function of degradation time and composition:

(1) Loss of tensile strength (in the case of PGA, Dexon^R sutures).
(2) Mass loss.
(3) Loss in molecular weight (*MW*) and changes in molecular weight distribution (*MWD*).

Change in mechanical properties of PGA (Dexon^R) sutures are the key parameters from a practical point of view. Mass loss is significant from the point of view of absorption and removal of the polymers from the body. However the most sensitive parameters for studying degradation effects are *MW* and *MWD*. A significant percentage of polymer chains can be cleaved before there are changes in mechanical properties. Mass loss was expected to be slower than the loss in *MW* and tensile properties, as it is necessary to reduce both *MW* and mechanical strength to a considerable degree before any material will fragment and/or dissolve.

The effect of pH on the above three parameters was studied over the range pH 5–9, for selected materials. Similarly the effect of temperature between 25°–50°C was also investigated.

The effect of enzymes on PGA sutures has been studied simultaneously and independently by Williams *et al.*[10] and these experiments were not repeated in the present study. A series of *in vivo* experiments were undertaken in rats, the results from which will be compared with the *in vitro* data in Part 3[11,8] along with the histological and toxicity findings.

EXPERIMENTAL

On the basis of data presented in Part 1[1] three compositions were chosen for thorough study:

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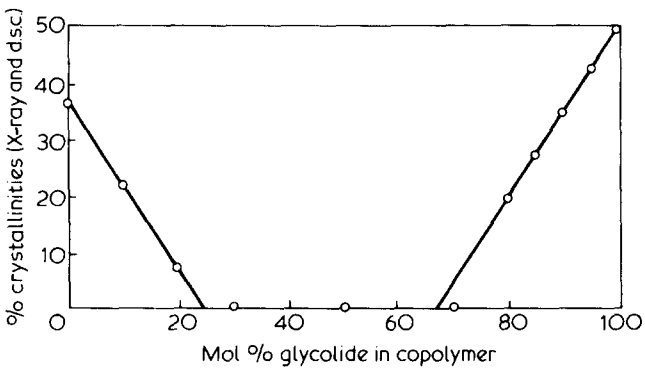

Figure 1 % Crystallinities for GA/LA copolymers as a function of composition (determined by X-ray and d.s.c. measurements)

(1) PGA; in the form of Dexon^R 0, 0'S', 3–0 and 3–0 '*S*' sutures.

(2) P-l-LA; in the form of 0.5 mm thick films cast from $CHCl_3$ [9].

(3) 50/50 m% GA/LA copolymer: in the form of 0.5 mm thick film, cast from $CHCl_3$ [9].

In shorter studies, to evaluate specific effects, P-dl-LA and the 70/30 m% GA/LA compositions were studied.

Polyglycolic acid (PGA) Dexon^R suture

Approximately 50 cm lengths of PGA (Dexon^R) suture of each suture gauge (0 or 3–0) and each filament diameter (6 and 2 denier. '*S*' denotes the finer filament) were used as test pieces.

Each test piece was weighed and placed in a McCartney screw capped phial containing 15 ml of buffer. The pH was monitored as a function of time and remained constant indicating that the buffer capacity was not exceeded. Degradation was carried out in a thermostatically controlled water bath (Grant SB10, Cambridge).

The initial study was carried out over a period of 26 weeks in 0.2 M citrate–phosphate buffer of pH 7 at 37 $\pm$1°C. Citrate–phosphate buffer was used as it allowed the pH to be varied over a wide range (pH 2.6–7.0) without the potential complication of changing to different buffer systems.

Glycolic acid/lactic acid, (GA/LA), copolymers and poly-l-lactic acid, (P-l-LA), polymers

1 cm diameter discs of P-l-LA, 50/50 m% GA/LA and 70/30 m% GA/LA copolymers were used for degradation studies. These discs were cut from films cast from $CHCl_3$ onto glass plates.

As above, each test piece was weighed and placed in a McCartney screw capped phial containing 15 ml buffer. The pH was monitored as a function of time and remained constant indicating that the buffer capacity was not exceeded. Degradation was carried out as described for PGA (Dexon^R) sutures over similar time periods at pH 7 and 37 $\pm$1°C.

On removal from the incubation buffer all samples were washed with distilled water to remove residual buffer and dried over P_2O_5 under vacuum.

In the case of the PGA sutures, tensile measurements were made on the samples. Between 5 and 10 replicates were used for each measurement. The tensile measurements were made using a 2 cm gauge length and a 100% /min strain rate on a Howden Tensiometer.

Mass loss was determined for each sample by comparing the dry weight (m_d) remaining at a specific time with the initial weight (m_0), where:

$$\% \text{ mass loss} = \frac{m_0 - m_d}{m_0} \times 100 \quad (1)$$

The fragments of samples remaining from the above mechanical and mass loss determinations were reused for molecular characterization by g.p.c. Molecular characterization by g.p.c. was carried out using chloroform at 25°C as the eluting solvent for the polymers containing up to 70 m % GA. Above this level of GA, PGA type crystallinity (see *Figure 1*) is present and hexafluoroisopropanol was required as the g.p.c. solvent[8]. Two 1 m columns containing 10^5 and 10^3 Å Styragel^R were used for this work with a flow rate of 1 ml min^{-1}. Although comparative *MW*s and *MWD*s have been used in this study, methods of calibrating the g.p.c. for use with these combinations of solvents and copolymers are discussed at length elsewhere[12].

The effect of pH on the degradation kinetics of some of the materials was determined by incubation in buffers of pH 5, 7 and 9 at 37 $\pm$1 °C as a function of time. Tensile and mass loss profiles were determined as previously described.

In the case of PGA (Dexon^R) sutures we examined the dependence of the kinetics of degradation on temperature.

The rate of degradation was followed by the deterioration of tensile properties and mass loss. Temperatures above and below the glass transition temperature (T_g), i.e. 36°C for Dexon^R sutures, (measured by d.s.c.), were used. The experiments were carried out at pH 7 over a 12 week period.

RESULTS AND DISCUSSION

Polyglycolic acid (PGA) Dexon^R suture

The effect of degradation in 0.2 M citrate–phosphate buffer of pH 7 at 37°C on the change in tensile properties is shown as a function of time in *Figure 2*. The data show no effect of suture gauge (i.e. 0 vs 3–0) or filament denier in the 2–6 denier range. In each case ~80% of the initial tensile strength is maintained for two weeks. From the practical viewpoint this is important as a wound has little strength above that of coagulated fibrin up to 1 week, but is rapidly reinforced by collagen synthesis between 1 and 3 weeks. Most of the suture's strength is lost over the 2–4 week period.

Figure 3 shows the change in stress/strain curve during this time. Elongation decreases simultaneously with tensile strength indicating that the material is becoming more brittle as degradation proceeds.

Mass loss, as a function of time, is shown for two of the Dexon^R configurations (0 and 0'S') in *Figure 4*. Again there is no effect of filament denier size. Mass loss begins in the fourth week, and is complete by 10–12 weeks providing the test medium is not agitated. Agitation will cause total mass loss within 8 weeks.

The observed asyncronization of tensile and mass loss profiles indicates that the degradation process may be complex, and the molecular loss profiles for 'O' Dexon^R shown in *Figure 5* provide the answer. The *MW* loss profiles are essentially identical for both suture gauges and both filament sizes studied. The *MWD* for 0, 1, 2 and 3 week degradation periods show a steady decrease in the *MW* of the peak of the distribution, with the high *MW* tail

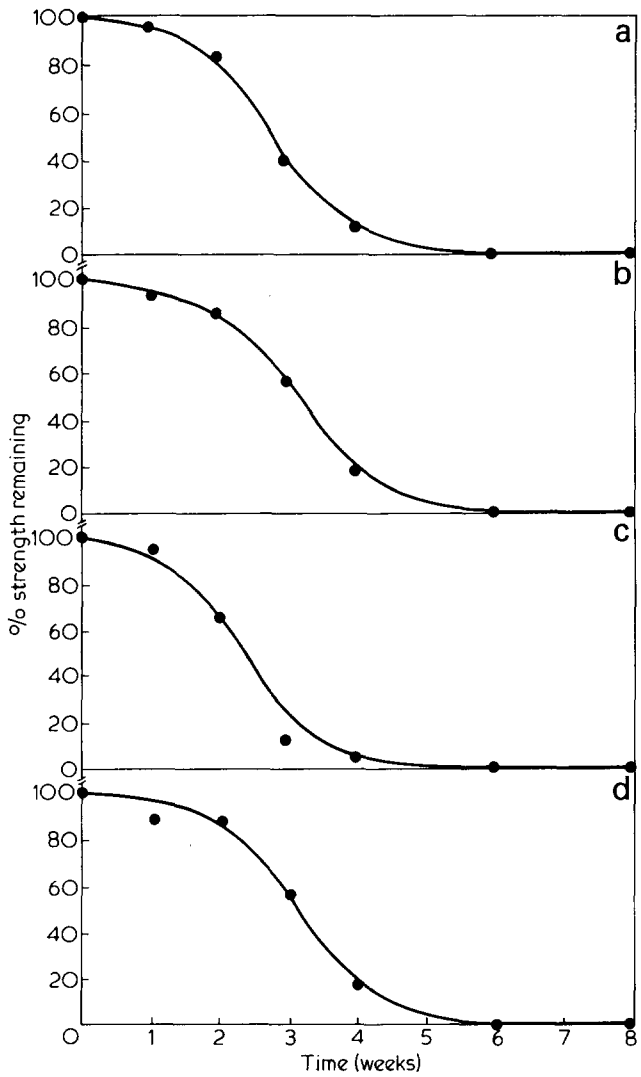

Figure 2 *In-vitro* tensile data, Dexon^R sutures, (PGA) (Degraded at pH 7 and 37°C). (a), '0' Dexon^R; (b), '0' Dexon^R 's'; (c), '30' Dexon^R; (d), '30' Dexon^R 's'

of the distribution being reduced. The low MW tail of the MWD increases up to 3 weeks, and begins to decrease from that point, indicating that mass loss is taking place. The material remains highly crystalline over the initial 6 week period indicating, as expected, that degradation is taking place in the amorphous regions and the remaining mass is contained in small crystallites. At 56 days (*Figure 5*) the majority of the Dexon^R is present as oligomers.

50/50 mol % glycolic acid/lactic acid (GA/LA) copolymer

This copolymer is amorphous and more hydrophobic than the PGA. The mass and MW loss profiles are shown in *Figure 6* and are found to be virtually superimposable, with the major loss in each occurring over the 2–4 week period. This arises due to the amorphous nature of the matrix, and confirms, as was suggested in Part 1[1], that this composition should be an excellent sustained drug release matrix, as it should disappear from the body within 3 months after the drug has been delivered. As the material is inherently hydrophilic[1], i.e. the equilibrium water level is ~25%, it will probably be useful for the more hydrophobic drugs.

Poly-l-lactic acid (P-l-LA)

P-l-LA represents a more complex material than the 50/50 PGA/PLA copolymer as this is a 37% crystalline hydrophobic matrix. The mass and molecular loss profiles are shown in *Figure 7*. The results show that over a 16 week period, only 10–15% of the mass of the 0.5 mm thick disc is lost, whereas $\bar{M}_w$ is reduced to ~50% of its initial value. Although tensile properties were not measured, gross observations indicated relatively small changes in mechanical properties over a 26 week period. Over this period of time there is no change in crystallinity (as

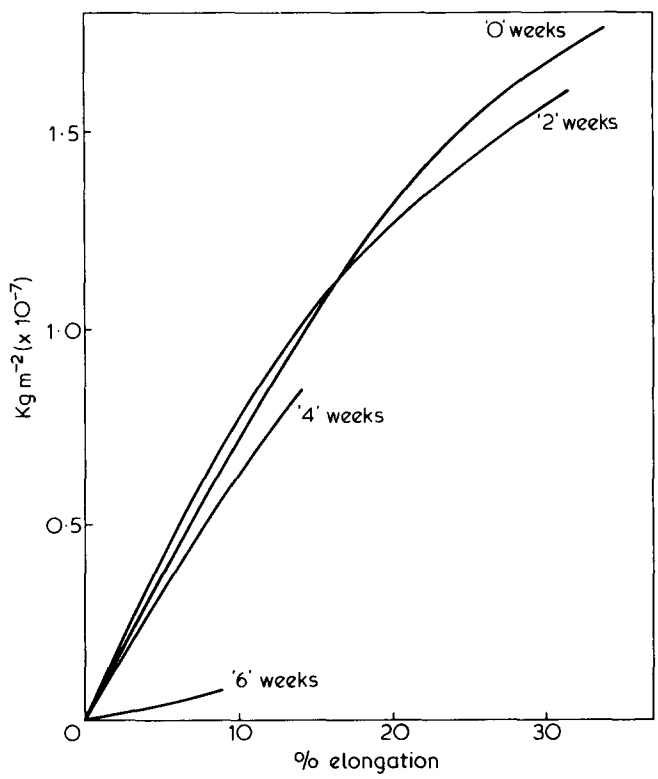

Figure 3 Stress/strain curves for degraded Dexon^R, (PGA), sutures ('0' Dexon^R) (Degraded at 37°C at pH7)

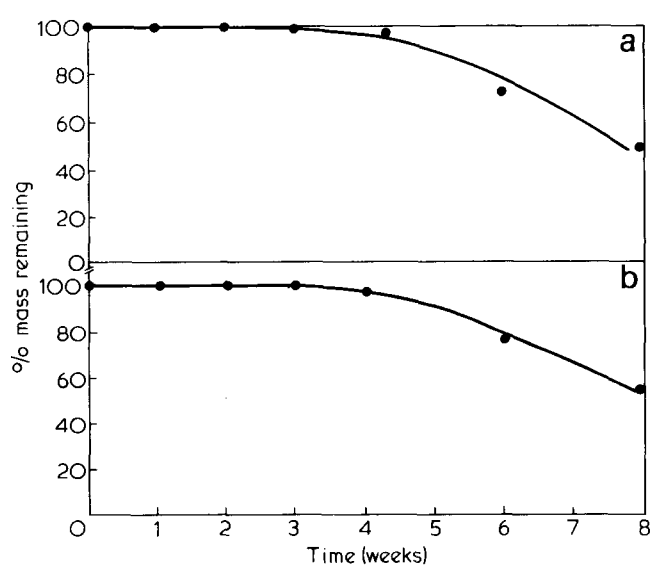

Figure 4 *In vitro* mass loss data, Dexon^R sutures (Degraded at pH 7 and 37°C). (a) '0' Dexon^R; (b), '0' Dexon^R 's'

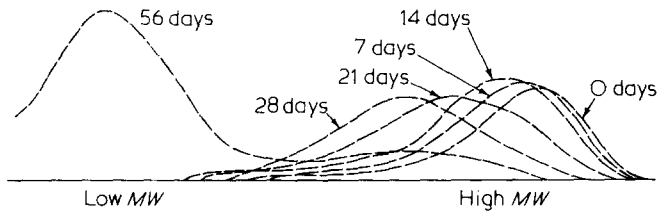

Figure 5 *In vitro* molecular weight distributions for PGA ('0' Dexon^R) sutures, (expressed as a function of degradation time.) Degradation at pH 7 and 37°C. Degradation time expressed in days

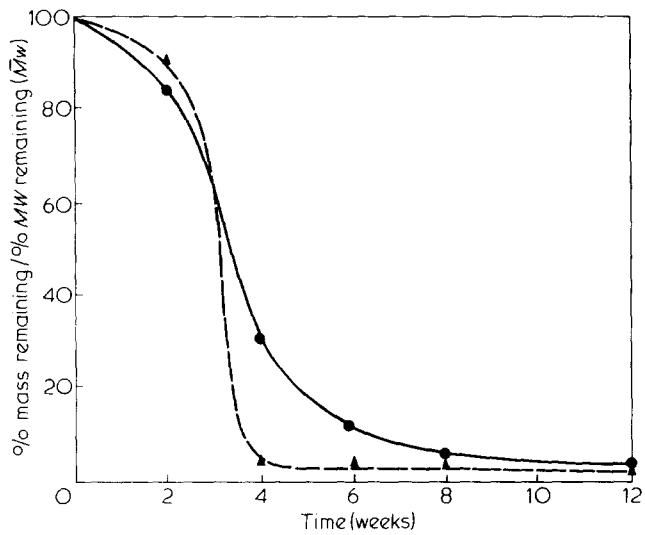

Figure 6 *In vitro* mass and molecular weight loss for 50/50 GA/LA copolymer (Degraded at pH 7 and 37°C). (—●—) % mass remaining, (— —▲— —) % *MW* remaining *in vitro*

measured by d.s.c.) indicating that the mechanical properties are controlled by the crystalline phase.

Effect of pH on degradation kinetics

In vitro degradation was carried out in citrate–phosphate buffers at pH 5 and pH 7 and boric acid–borax buffer at pH 9 (ref 8). In each case there was no significant change in degradation kinetics with change in pH[8]. This was surprising as polyesters are known to be base sensitive. We considered that the reason accelerated degradation was not observed at pH 9 may have been due to the combination of hydrophobicity and crystallinity. The 70/30 GA/LA copolymer has the most hydrophilic composition of the amorphous structures, and it was expected to show greater sensitivity to pH than any other in the family. A limited study showed the 70/30 GA/LA copolymer beginning to fragment at 12 days, and undergo 100% mass loss within 4 weeks at pH 9 (ref 8). This confirms that any sensitivity of these copolymers to pH is dominated by the combined effects of crystallinity and hydrophobicity, and is relatively unimportant, which is converse to the PEO/PET system described previously[9].

Effect of temperature on degradation kinetics

It was shown by d.s.c. in Part I that the glass transition temperature (T_g) for PGA is 36°C. As temperature coefficients of diffusion and hydrolysis rate constants would be expected to show a discontinuity at T_g, an experiment was designed to investigate this phenomenon. Six degradations similar to those described previously for PGA at pH 7 and 37°C were set up at 25°, 30°, 35°, 40°, 45° and 50°C. Three suture sizes, '0', '4–0)' and '5–0', 6 denier/filament Dexon^R were used.

The tensile and mass loss profiles over 12 weeks are shown for '0' Dexon^R as a function of temperature in *Figures 8a* and *b* respectively. As expected, temperature

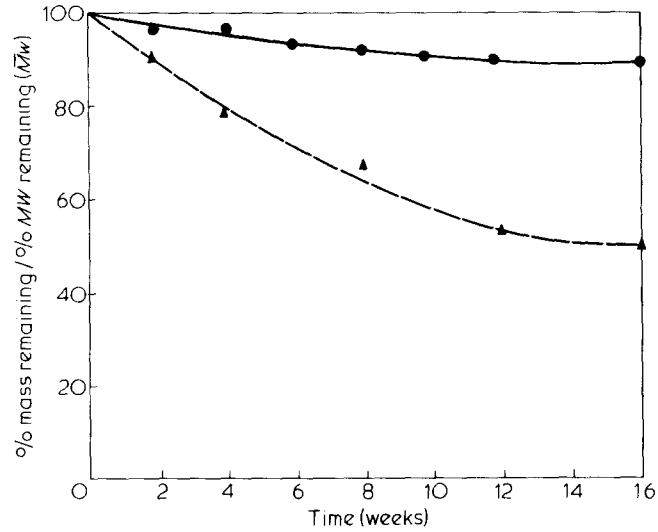

Figure 7 *In vitro* mass and molecular weight loss for P—1—LA polymer (Degraded at pH 7 and 37°C), (—●—) % mass remaining; (— —▲— —) % *MW* remaining *in vitro*

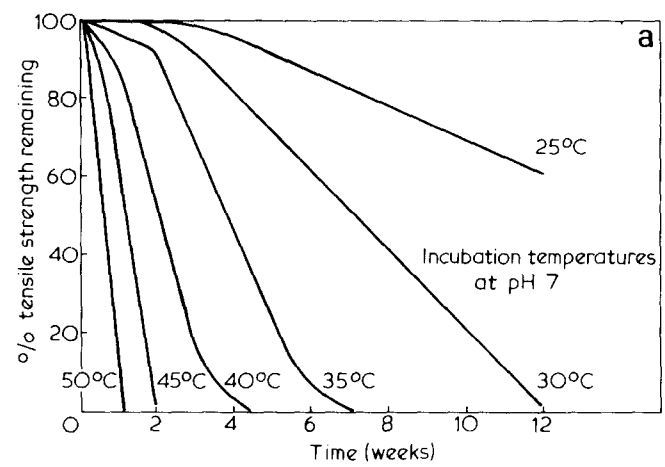

Figure 8a Tensile loss profiles for PGA sutures ('0' Dexon^R) as a function of time

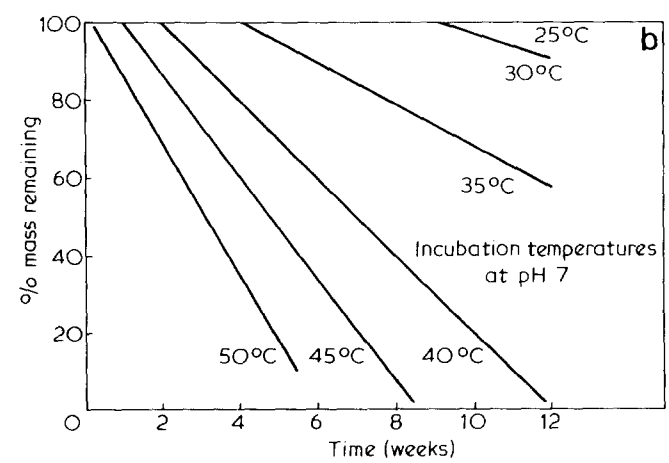

Figure 8b Mass loss profiles for PGA sutures ('0' Dexon^R) as a function of time

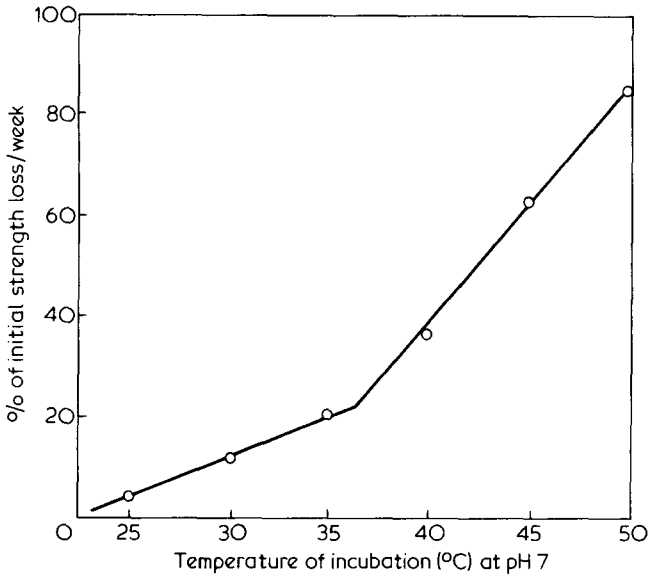

Figure 9 Slopes of the linear portions of the tensile loss profiles plotted as a function of temperature PGA suture: '0' Dexon^R

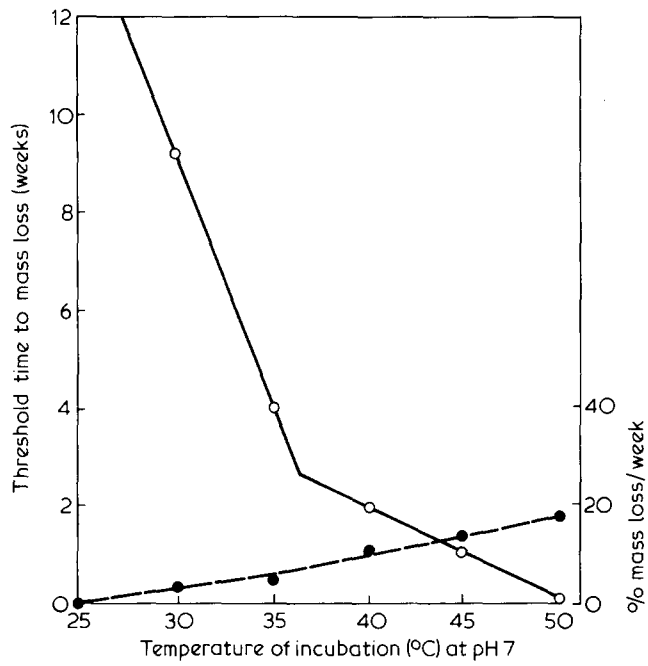

Figure 10 Threshold time to mass loss (—○—) and rate of mass loss (– —●— —) plotted as a function of temperature PGA suture: '0' Dexon^R

has a dramatic effect on the rate of loss of tensile strength and mass.

The slopes of the linear portions of the tensile loss profiles are plotted as a function of temperature in *Figure 9*. The rates are expressed as % initial strength loss per week and show the classical degradation behaviour of a constant temperature coefficient above and below T_g with a clear discontinuity at T_g. The temperature coefficient above T_g is ~7 times greater than that below. This indicates that the glassy state protects PGA from hydrolysis as all short term chain motions are frozen. Above T_g, water diffusion, and therefore hydrolysis, is more facile.

The mass loss profiles in *Figure 8b* shows that not only is the rate of mass loss dependent on temperature, but also the threshold time before its onset. *Figure 10* shows threshold time prior to mass loss, and rate of mass loss, as a function of temperature. Clear discontinuities are seen at 36.5°C for each of the functions indicating the position of T_g. The differences in slope represent differences in the diffusion constants of the degradation products in the glassy and rubbery states.

CONCLUSIONS

This study has shown that the glycolic acid/lactic acid family of homo and copolymers has a wide range of degradation rates that are governed by both hydrophobic/hydrophilic balance and crystallinity. The mechanical properties and property loss profiles during degradation of both homopolymers are governed by the crystalline phase.

The degradation mechanism is one of simple hydrolysis, which is insensitive to pH for most compositions.

The effect of the T_g of PGA on its sensitivity to degradation has been demonstrated.

These simple *in vitro* modelling experiments will be compared with *in vivo* behaviour in Part 3 (ref 11).

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