Biodegradable polymers for use in surgery — poly(ethylene oxide)/poly(ethylene terephthalate) (PEO/PET) copolymers: 2. *In vitro* degradation

A. M. Reed* and D. K. Gilding*
Bioengineering Unit, University of Liverpool, PO Box 147, Liverpool L69 3BX, UK
(*Received* 25 *January* 1980; *revised* 17 *June* 1980)

The degradation mechanism of a series of poly(ethylene oxide)/poly(ethylene terephthalate) (PEO/PET) copolymers, synthesized as described in Part 1[1], has been studied *in vitro*. The need for the development of *in vitro* test methods for candidate biomaterials is set down. The effect of time, temperature, pH and selected enzymes on the rate and mechanism of degradation is elucidated. The degradation products are identified. The degree of degradation was monitored molecularly by gel permeation chromatography (g.p.c.) and end-group titration techniques. The composition of the copolymers was obtained using infra-red (i.r.) and nuclear magnetic resonance (n.m.r.) spectroscopy. Mass loss and water uptake data are also given. The mechanism of degradation is shown to be by hydrolysis. The effect of ethylene oxide (EO) and ^{60}Co γ-irradiation sterilization on the copolymers was investigated.

INTRODUCTION

Part 1 of this series[1] described the synthesis and characterization of a series of copolymers based on poly(ethylene oxide)/poly(ethylene terephthalate) (PEO/PET). These copolymers had been observed[2] to degrade when implanted into biological tissue, and this study was initiated to investigate the mechanism of degradation and its degree of reproducibility[3]. If the degradation profiles, i.e. loss of *MW*, strength and mass, were predictable, and the degradation products non-toxic, then these materials might have potential uses as temporary scaffolds or barriers in surgery.

This paper describes the development of *in vitro* test methods designed to elucidate the degradation mechanisms of the PEO/PET family of copolymers. Subsequently these results were compared with results obtained from *in vivo* experiments where these materials were implanted subcutaneously in rats and intramuscularly in rabbits. In this case the effect of degradation products on adjacent tissues was evaluated simultaneously. These results will be published in Part 3[4].

IN VITRO MODELLING OF THE PHYSIOLOGICAL ENVIRONMENT

The systematic development of *in vitro* models of the physiological environment is of utmost importance in the understanding of the basic mechanisms occurring *in vivo*. *In vitro* modelling, in principle, allows the physiological environment to be simplified such that the effects of single parameters such as pH, ionic strength, temperature and specific enzymes on the test system can be evaluated. The degree of complexity can be varied at will and interplay between multiple parameters can be obtained until the total effect of the physiological environment is simulated. Degradation products can be isolated and obtained in relatively large quantities to enable complete characterization. This information, as a function of time, is the key to the elucidation of degradation mechanisms and kinetics.

To date, *in vitro* modelling has played a minor role in the development of biomaterials[5-7]. However, from the commercial point of view the approach is crucial to the introduction of new products. Relative to animal testing, *in vitro* modelling is low cost, and allows larger statistical studies to be developed at a fraction of the cost of similar studies *in vivo*, which means that smaller companies with lower resources may also contemplate advanced biomedical products. *In vitro* tests can never be a total replacement for animal testing; the animal must always be the primary reference. However, when a battery of *in vitro* tests have been conclusively shown to be equivalent to the *in vivo* situation, it allows itself to be applied more extensively to probing, production and storage problems than could ever be contemplated with the animal model both on humane and economic grounds. Hence accelerated testing is sometimes possible using elevated temperature and/or pH changes, providing the basic correlations with standard conditions have been ascertained. Optimum (and adequate) conditions of packaging and storage can also be evaluated at relatively low cost.

In the work to be described here we have used three PEO/PET copolymer compositions (50, 60, and 70 wt % PEO) based on PEO 1500. These materials were cast into films from which test samples were cut. *In vitro* incubation in buffer at varying pH (pH 5–9) and at temperatures in the range 25°–50°C for predetermined periods of time was used as our initial *in vitro* model. On removal of the

* Present address: Millipore Corporation, Ashby Road, Bedford, MA 01730, USA.

0032-3861/81/040499-05$02.00

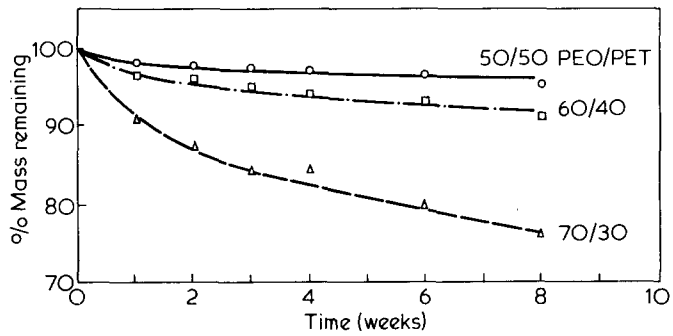

Figure 1 Mass loss data for PEO/PET copolymers at pH 7 and 37°C

polymer samples at the predetermined time periods the following parameters were evaluated:

(1) water uptake;
(2) mass loss;
(3) loss of molecular weight (*MW*) and change in molecular weight distribution (*MWD*);
(4) carboxyl end-group determination;
(5) compositional analysis by ^{13}C nuclear magnetic resonance spectroscopy (n.m.r.) and infra-red spectroscopy (i.r.).

Subsequent experiments examined the effect of selected enzymes on the kinetics of degradation at pH 7 and 37°C. The enzymes investigated were leucine amino peptidase, esterase and α-amylase.

EXPERIMENTAL

In the complete study[3] PEO/PET copolymers based on PEO 600 and 1500[1] molecular weight were synthesized over the composition range 20–80 wt % PEO. The degradation studies were limited to the PEO 1500 based materials with compositions of 50, 60 and 70 wt % PEO. The PEO/PET 60/40 composition has previously been shown to have the optimum elastomeric properties[1].

Preparation of test samples

Film samples of approximately 250 μm thickness were fabricated by casting 20% solutions of the copolymers in chloroform onto deactivated glass plates†. Solvent evaporation was allowed to take place in a refrigerator for 24 h, and residues were removed by heating the films under vacuum to 60°C for 24 h.

The films were cut into 10 mm discs or 'dog-bone' specimens for testing. Each disc was weighed prior to testing to obtain initial weights (m_0), which were ~0.3 g. *In vitro* degradation was carried out in 25 ml of 0.2 M citrate–phosphate buffer at pH 7, contained in 28 ml McCartney screw-cap phials and stored for predetermined periods at 37°C in a thermostatically-controlled water bath (Grant SB10, Cambridge). 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.

On removal, the samples were rinsed thoroughly in distilled water to remove any buffer remaining on the surface, and blotted with Whatman's filter paper to remove surplus surface water. Individual samples were weighed to obtain wet weights m_h. The samples were dried under vacuum over P_2O_5 for 48 h, and reweighed to obtain dry weights m_d.

Water uptake was calculated from equation (1):

$$\% \ H_2O = \frac{m_h - m_d}{m_d} \times 100 \qquad (1)$$

where equilibrium water is expressed as a percentage of dry weight.

Mass loss

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

$$\% \ \text{Mass loss} = \frac{m_0 - m_d}{m_0} \times 100 \qquad (2)$$

In cases at extended time periods, where the sample fragmented, the PEO/PET was filtered on a weighed filter paper, washed with distilled water, dried and reweighed on the filter.

Molecular characterization

The determination of changes in *MW* and *MWD* is particularly important in studies of degradation kinetics, as bond scission is the primary process, and occurs before changes in mechanical properties and mass can be detected. Gel permeation chromatography (g.p.c.) was used to determine *MWD*. Two Styragel columns of 10^5 and 10^3 Å were used with chloroform as the eluting solvent at a flow rate of 1 ml min^{-1}. Analyses were carried out on samples taken from the mass loss studies. The g.p.c. was initially calibrated using narrow *MWD* polystyrene standards[8] and corrected for use with PEO/PET compositions by using samples of PEO/PET copolymers of known *MW*. *MW* was obtained for these samples by vapour phase osmometry (v.p.o.) (see *Figure 2*). A detailed description of this method is given elsewhere[9].

Carboxyl end-group determinations

Carboxyl end-group titrations were undertaken as an increase in carboxylic acid concentration could only occur by ester group scission, the hydrolysis of an ether, producing two hydroxyl groups. The method used was a modified version of that described by Conix[10]. Samples taken from the mass loss studies were dissolved in ~20 ml of a 1:1 benzyl alcohol/chloroform solution and titrated against 0.1 M NaOH in benzyl alcohol, in order to obtain the total number of carboxyl groups.

Compositional analysis

Degraded polymer samples were analysed by ^{13}C n.m.r. spectroscopy in $CDDl_3$. The average compositions and block lengths of the PEO and PET segments were calculated as described in Part 1[1]. Infra-red (i.r.) spectroscopy was also used to obtain average compositions[1], in cases where there were limited quantities of samples available, as in the case of g.p.c. fractions[3].

The composition of PEO/PET copolymers was obtained by i.r. from the ratio of infra-red absorbences at 730 and 2880 cm^{-1} using the calibration curve constructed from n.m.r. compositional data published in Part 1[1].

† Deactivation of $\frac{1}{4}$" glass plates was achieved by steeping the glass for 15–30 min in 10% NaOH solution. This removed grease and provided a light etch. This alkali surface was neutralized with 10% HCl solution for 15 min followed by immediate reaction of the water wet surface with a 10% solution of trimethyl chlorosilane in toluene.

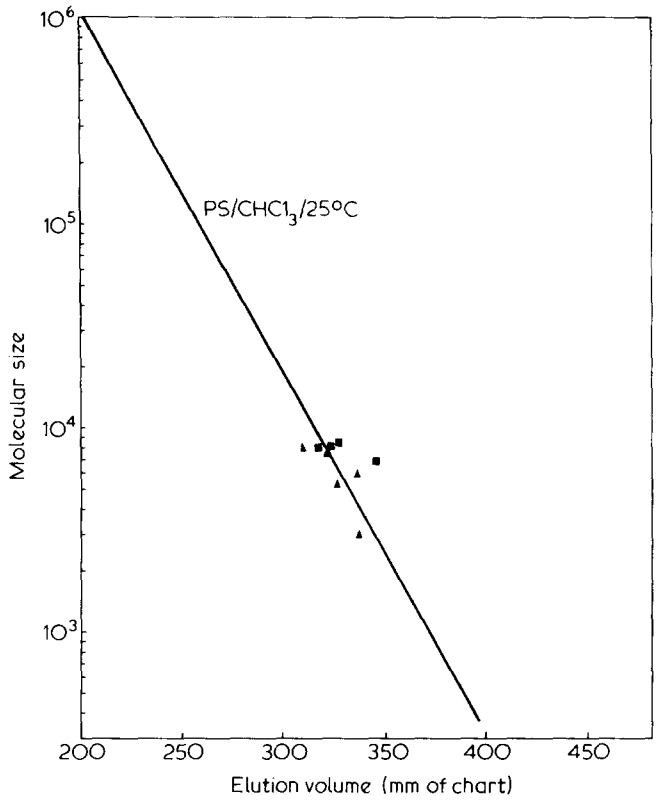

Figure 2 Comparison of $\bar{M}_n$ values for PEO/PET copolymers obtained by gel permeation chromatography and vapour phase osmometry ■, M_n (v.p.o.), 50/50 wt % PEO PET; ▲, M_n (v.p.o.) 70/30 wt % PEO/PET

Table 1 % Water uptake data for PEO/PET copolymers (at pH7 and 37°C). Results expressed as a percentage of dehydrated weight

Time (weeks)	0	1	2	3	4	6	8
50/50 wt. % PEO/PET	62	72	69	74	74	74	78
60/40 wt. % PEO/PET	86	103	101	103	106	103	108
70/30 wt. % PEO/PET	132	163	170	176	169	177	198

RESULTS AND DISCUSSION

In vitro *degradation at pH 7 and 37° ± 1°C*

Water uptake data for the PEO/PET copolymers as a function of incubation time are shown in *Table 1*. The results show a gradual increase in water content with time which may be indicative of chain scission and disruption of the hydrophobic crystalline domains which allow the matrix to swell.

Mass loss profiles are shown in *Figure 1*. The data show that mass loss begins immediately for all compositions, but is more dramatic for the 70/30 composition than the other two. This is consistent with PEO being the continuous phase[1,3]. By 12 weeks all compositions have fragmented to particles of <2 mm across.

The changes in *MWD* as a function of time are shown in *Figure 3*. Initially the *MWD* is narrowed by solubilization of the low *MW* tail of the *MWD* which corresponds to the early mass loss, and by the simultaneous random chain scission which is characterized by a reduction in the high *MW* tail in the *MWD*. The peak of the *MWD* is shifted to lower values due to the degradation of the highest *MW* species producing species of medium *MW*. As degradation continues a low *MW* peak with a high aromatic content (see later) is produced, and the total *MWD* is continuously shifted to lower *MW*s. The final distribution becomes that of an oligomer.

In order to plot the behaviour of an average *MW* with time $\bar{M}_w$ is most applicable because of the sensitivity of $\bar{M}_w$ to low *MW* tails and oligomers. Weight-average molecular weight ($\bar{M}_w$) as a function of time is shown in *Figure 4*. The data show an essentially linear decrease in $\bar{M}_w$ for the first four weeks, with only 60, 40 and 35% of the original $\bar{M}_w$ remaining after 8 weeks for the 50:50, 60:40 and 70:30 compositions, respectively. At 8 weeks, $\bar{M}_w$ in *Figure 4* corresponds to $15–20 \times 10^3$ daltons[1].

Chain cleavage and a simple hydrolytic mechanism has been demonstrated by the measurements described above; it was important to identify which bond was being cleaved, i.e. the ester bond of the PET segment, the ester bond between the PEO and PET segments, or the ether

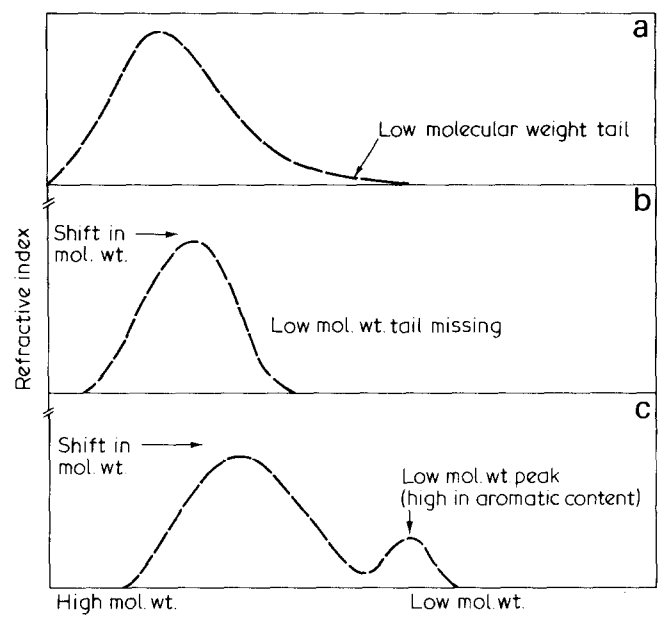

Figure 3 Typical g.p.c. chromatograms for a 50/50 wt % PEO/PET copolymer as a function of time. (a) Initial material (i.e. undegraded), (b) material at 2–4 weeks degradation; (c) material at 4–12 weeks degradation

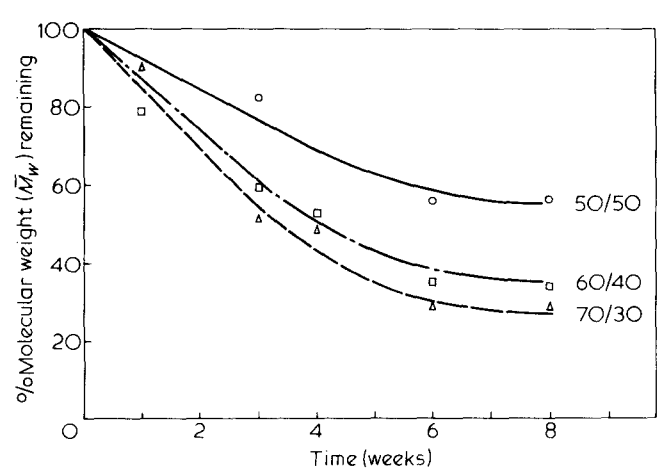

Figure 4 Molecular weight loss data for PEO/PET copolymers at pH 7 and 37°C

Table 2 Carboxyl end-group analysis of degraded PEO/PET copolymers

	Time (weeks)	0	1	2	3	4	8
(a)	50/50 wt % PEO/PET	43 ±2	31 ±7	16 ±2	21 ±3	41 ±3	28 ±2
(b)	70/30 wt % PEO/PET	49 ±8	40 ±7	30 ±2	38 ±2	31 ±2	60 ±9

Results given as g equiv. COOH/10^6g of polymer

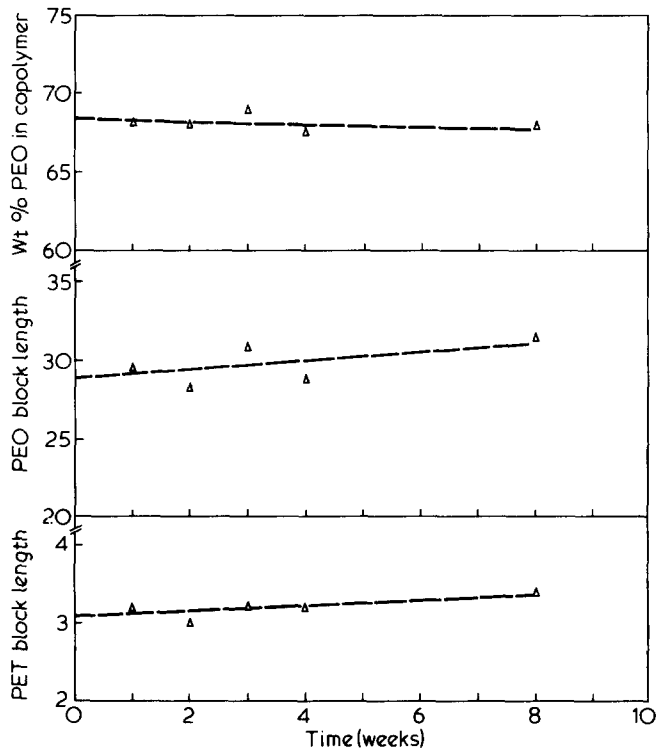

Figure 5 Compositional data obtained from n.m.r. for PEO/PET copolymers at pH 7 and 37°C. 70/30 wt % PEO/PET copolymer

bond of the PEO segment. The second was expected to be preferred as esters are more susceptible to hydrolysis than ethers, and the linkage between the PEO and PET segments represents the ester bond in the most hydrophilic location.

The results of the end-group analysis are shown in *Table 2*. They show a significant fall in the number of –COOH groups over the first 2 weeks, which is simultaneous with the loss of mass. Since there is a fall rather than an increase in –COOH content of the polymer remaining, this suggests that the species containing carboxyl groups were present in the buffer-soluble fraction during the initial 2–3 week period. This was confirmed. The periodic increase in –COOH content between 3 and 8 weeks confirms the continuing ester degradation and the solubilization of degradation products as suggested by the molecular characterization data.

Average compositions as a function of degradation time for the 70/30 composition are shown in *Figure 5*. The data shown are typical for those obtained for each composition, and represent a 65% fall in $\bar{M}_w$ (*Figure 4*) and a 20% loss in mass over the 8 week period (*Figure 1*).

The data show that average composition remains constant over the 1–8 week degradation period, although the PEO concentration is ~2–4 wt % lower than the initial concentration. The constancy of the PEO and PET block lengths indicates that the compositions of the copolymers are narrow.

This conclusion was further verified by i.r. analysis[1] of three fractions cut from a g.p.c. separation, which showed that composition was homogeneous as a function of *MW*. The constancy of the PEO and PET block lengths (especially, as was stated before, 8 weeks is equivalent to a 65% reduction in $\bar{M}_w$), suggest that primary cleavage is at the ester linkages between the PEO and the PET segments as was expected.

The soluble polymer fractions were subjected to molecular compositional characterization. The soluble polymer fractions were obtained by extraction from the incubation medium with chloroform. These polymer fractions were then allowed to evaporate to dryness on NaCl plates under an infra-red lamp. The infra-red spectrum was obtained for each sample and their respective compositions evaluated using the ratios of the peaks at 730 and 2880 cm^{-1} and the i.r./n.m.r. composition calibration previously described[1]. Also 2–5 mg samples of these polymer fractions were used for g.p.c. analysis[3].

The results in *Figure 6* show that the average compositions of the 70/30 solubles contain 86–92 wt % PEO. The *MWD* obtained for these soluble fractions covered a molecular weight range of 1200 to 4000 (relative to the g.p.c./v.p.o. calibration curve[9]) with the majority of the distribution lying between 2000 and 3000. Assuming the most susceptible and probable bond to cleave is the ester linkage between the PET and PEO segments we can calculate the likely structures of the oligomers (using the respective block lengths of PEO and PET gained from n.m.r. as described in Part 1[1]). Below are listed the most probable structures for oligomers which obey the conditions of high % PEO content relative to % PET content and fall in the molecular weight range 1200 to 4000 for the 70/30 wt % PEO/PET composition.

	Structure	Mol. wt	Wt % PEO in soluble oligomers
70/30 Wt % PEO/PET	PEO.PET	2000	69%
	PEO.PET.PEO	3500	79%

Analysis of the insoluble polymer fraction formed late in the degradation process (*Figure 3c*) shows high aromatic content when examined by infra-red and ultra-violet spectroscopy, indicative that low *MW* PET is hydrophobic enough to remain in the insoluble fraction[3].

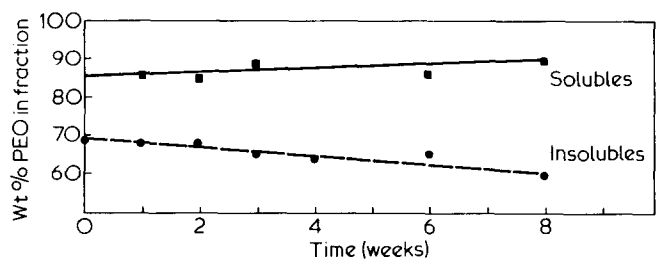

Figure 6 Effect of *in vitro* degradation on the composition of PEO/PET copolymers at pH 7 and 37°C. 70/30 wt % PEO/PET copolymer

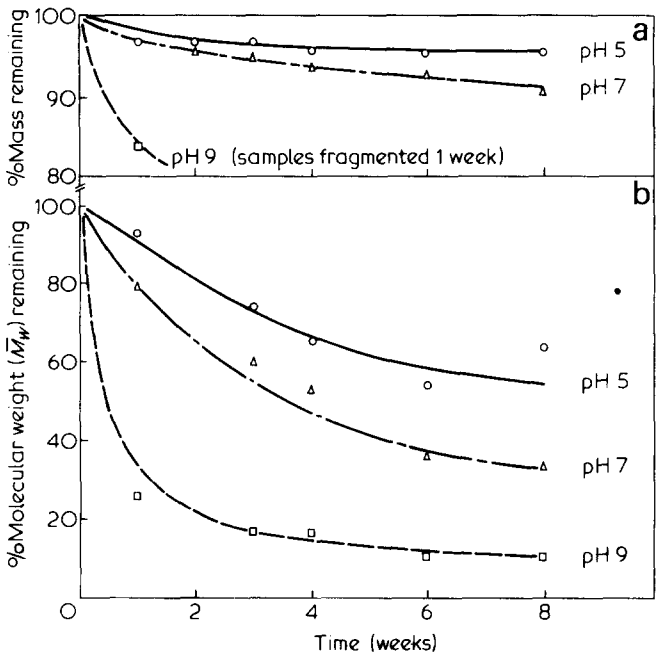

Figure 7 Effect of pH on *in vitro* degradation of PEO/PET copolymers. 60/40 wt % PEO/PET copolymer at pH 7 and 37°C: (a) mass loss; (b) molecular weight loss

The rate of loss of strength depends on two factors expressed in *Table 1* and *Figure 4*, i.e., the degree of plasticization by water, and the loss of *MW*. The 70/30 copolymer had lost all strength within 2 weeks, the 60/40 within 8 weeks, whereas the 50/50 required 12 weeks to fragment, at pH 7 and 37°C in 0.2 M citrate–phosphate buffer.

In addition to the simple simulation of physiological conditions, the effects of pH, temperature and selected enzymes on the degradation were studied.

Effect of pH

Similar experiments to those described above were carried out using 0.2 M, pH 5 citrate–phosphate, and 0.2 M, pH 9 boric acid–borax buffers, borax buffers being substituted for the pH 7 citrate–phosphate buffer used previously.

The results for the 60/40 copolymer are shown in *Figure 7a* for mass loss, and *7b*, *MW* loss. The data show predictable base sensitivity to ester bond cleavage. Samples at pH 5 showed enhanced mechanical properties relative to those at pH 7, whereas the pH 9 medium caused catastrophic failure within 1 week (as compared with 8 weeks at pH 7).

This sensitivity to pH may be put to practical use, as low concentrations of citric acid or sodium bicarbonate may be used as low level fillers for PEO/PET copolymers in order to control micro-pH within the matrix, even though the external environment (as in biological tissues) may be at pH 7. Such localized control of pH allows either acceleration or retardation of the degradation process.

Effect of enzymes

Three enzymes were selected to evaluate the effect of this type of biological catalyst on the degradation mechanism. Leucine amino peptidase (hogs kidney) and esterase, a carboxylic ester hydrolase (hogs liver) were selected as examples of extracellular peptidases, known to be present in connective tissue and to have ester cleavage activity. The third enzyme α-amylase (hogs pancreas) was selected as it was initially planned to use PEO/PET copolymers in dental applications and they would therefore be exposed to high concentrations of amylase. All three enyzmes were supplied by Sigma Chemicals Ltd.

One milligramme of enzyme per 0.4 g of copolymer were used in these experiments (far in excess of physiological concentrations). Experiments were again carried out in 0.2 M citrate–phosphate buffer at pH 7 and 37 C.

Figures 8a and *8b* show the effect of enzymes on the 50/50 and 60/40 copolymers compared with the *MW* loss profiles for simple hydrolysis. The data show that all three enzymes have similar powerful effects on the degradation of the PEO/PET copolymers. The 50/50 composition is more susceptible to cleavage catalysed by the enzymes because of its increased concentration of ester bonds. This is an interesting feature as pH and temperature have greater effects on the higher PEO content copolymers, whereas the three enzymes have the opposite effect. No effort was made to study these enzymes under conditions of maximum activity[11], this would have been unrealistic since the aim was to use the PEO/PET system in connective tissue.

Effect of temperature

The effect of temperature on *PEO/PET* copolymers incubated in 0.2 M, pH 7 citrate–phosphate buffer was studied at 25°, 37° and 50°C. The effect, for example on the 70/30 copolymer in *Figure 9*, shows that degradation at 25°C is insignificant, whereas at 50°C the rate is increased three-fold from that at 37°C.

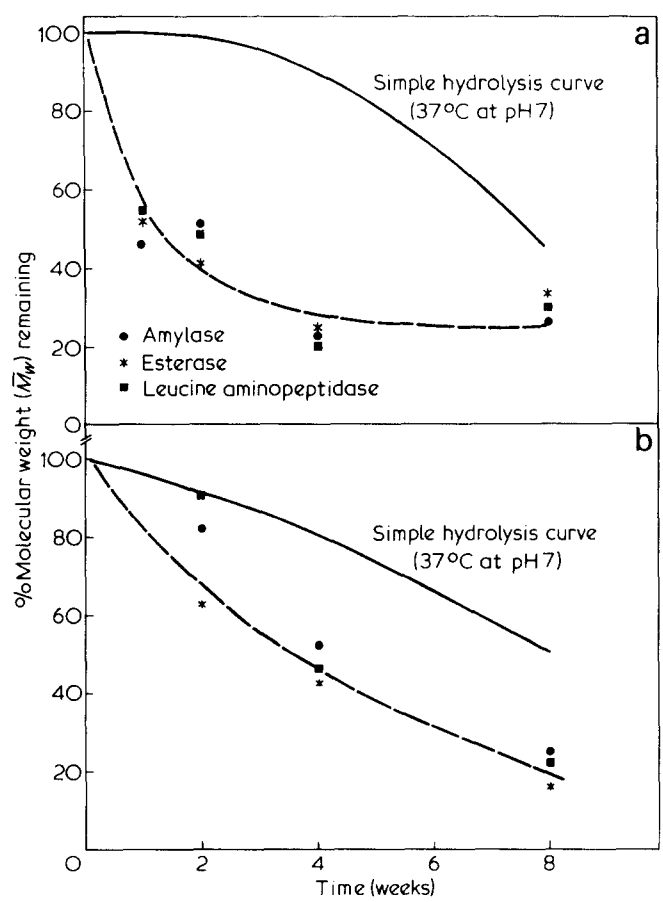

Figure 8 Effect of selected enzymes on the degradation of PEO/PET. (a) 50/50 wt % PEO/PET copolymer, at pH 7 and 37°C; (b) 60/40 wt % PEO/PET copolymer at pH 7 and 37°C

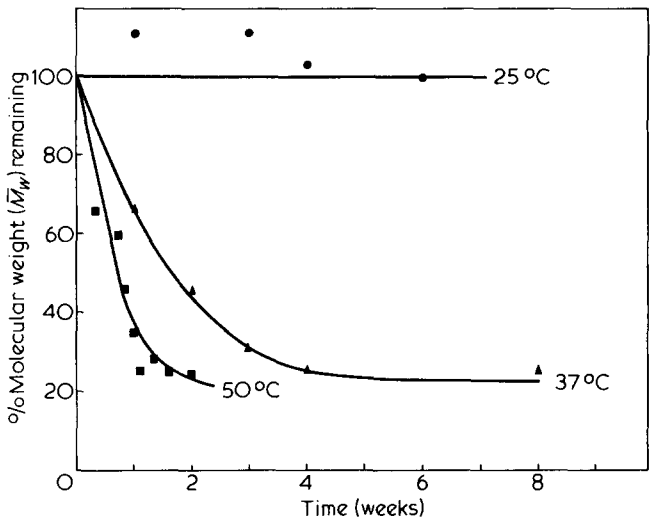

Figure 9 Effect of temperature on degradation of 70/30 wt % PEO/PET copolymer at pH 7

This study has practical significance in that these materials will not tolerate a combination of heat and moisture, whereas should they be exposed to moisture at ambient conditions they may suffer little adverse effect.

Additional studies of the effect of dry heat, 100°C under vacuum for 16 h showed no effect on *MW*. Ethylene oxide sterilization using the Sterivit process at 55°C, caused no deterioration over the period of 2 h within the sterilizer. The EO residues immediately after sterilization were ~500 ppm, which fell to ~150 ppm after 3 days. ^{60}Co γ-irradiation at 2.5 Mrads dosage caused a ~50% reduction in molecular weight ($\bar{M}_w$).

CONCLUSIONS

The systematic development of *in vitro* models for biodegradation *in vivo* has allowed the mechanisms and properties of PEO/PET copolymers during degradation to be understood.

PEO/PET copolymers undergo degradation by a simple hydrolytic mechanism. Primary cleavage is at the ester bond linking the ester and hydrophilic ether segments. The reaction is base-catalysed and sensitive to temperature above 37°C.

Three enzymes with known ester cleavage ability have shown enhanced effects on the simple hydrolytic system.

The samples have been shown to withstand sterilization by dry heat and EO without significant changes in structure and properties. The materials show promise as biodegradable elastomers, especially the 60/40 composition. Their *in vivo* properties will be described in Part 3[3].

ACKNOWLEDGEMENT

We are grateful to the Science Research Council and ICI Corporate Laboratory for the CASE award to AMR during the course of this work.

REFERENCES

1 Gilding, D. K. and Reed, A. M. *Polymer* 1979, **20**, 1454
2 Annis, D. and Jones, M. E. B. Surgical Research Society, London, November 1972
3 Reed, A. M. *Ph.D. Thesis*, University of Liverpool (1978)
4 Wilson, J. G., Gilding, D. K. and Reed, A. M. 1980, to be published
5 Homsey, C. A. *et al.* in 'Biomedical Polymers' (ed. Rembaum, A. and Shen, M.) Marcel Dekker, NY, 1971
6 Bell, J. P., Huang, S. J. and Know, J. R. *NTIS Report No. AD-A009*, 577 (1974)
7 Wade, C. W. R., Hegyeli, A. F. and Kulkarni, J. *Testing & Evaluation* 1977, **5**, (5) 397
8 Gilding, D. K. *Nester/Faust Liquid Chromatography Bulletin* (1970)
9 Gilding, D. K., Reed, A. M. and Askill, I. N. *Polymer* 1981, to be published
10 Conix, A. *Makromol. Chem.* 1958, **26**, 226
11 Williams, D. F. and Mort, E. *J. Bioeng.* 1977, **1**, 231