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In vitro degradation of a polyhydroxybutyrate/polyhydroxyvalerate copolymer

J. C. KNOWLES
Biomedical Engineering Unit, Medical Institute, Hartshill Road, Stoke-on-Trent ST4 7NY, UK
G. W. HASTINGS
Department of Materials, Queen Mary and Westfield College, Mile End Road, London E1 4NS, UK

Injection-moulded PHB/7% PHV (molecular mass 580 000) samples were placed in buffered saline at varying pHs for long-term *in vitro* studies. Anomalies between sample mass loss and Young's modulus were found for the different pHs. The Young's modulus anomaly was explained by examination of the molecular mass distribution (MMD) changes with time. The molecular mass measurements also showed that the MMD moved to a lower molecular mass, and also showed an increase in peak height, irrespective of pH. From the *Mn* data, curves for $(Mn(0)/Mn(t)-1)$ were plotted and the linearity showed that the solution pH was a rate-controlling factor.

1. Introduction

The use of degradable polymers in the medical field is becoming more commonplace [1, 2]. Currently available polymers include polyglycolic acid (PGA) and polylactic acid (PLA), both of which have found considerable use as sutures. Current interest is focusing on degradable polymers for fracture fixation [3–5], but PGA and PLLA do not possess the long-term degradation profile required [6, 7] for a load-bearing fracture fixation device. Polyhydroxybutyrate (PHB) and its copolymers with polyhydroxyvalerate (PHV) offer a range of thermally processable polyesters which exhibit the necessary long-term degradation profile required for a degradable fracture fixation device.

2. Materials and methods

Technical grade PHB/7% PHV (molecular mass 580 000) was obtained from Marlborough Biopolymers, Stockton-on-Tees, UK. The powder was melt processed by using a Fox and Offord (Birmingham, UK) injection moulder to form standard tensile test specimens, conforming to ASTM D638M-81. Buffered aqueous solutions of pH 3, 6, 7, 9, 10 and 11 were prepared using Hydrion buffer powders (Aldrich Chemical Co., Dorset, UK). The required pH was obtained by using a combination of tartaric and phosphoric acid and potassium acid phthalate for pH 2–4; pH 5–8 contained a combination of sodium and potassium phosphate; pH 9–11 contained a combination of sodium carbonate and sodium borate. The buffer powders were dissolved in deionised water. Injection-moulded samples were placed in the solutions, maintained at 37 °C in a water bath. The pH solutions were not sterile, but mould inhibitor was added to reduce any growth. The samples were periodically removed from solution and tested to determine the required parameter. For each test, specific samples were allocated to ensure reproducibility of the testing.

2.1. Wet and dry weight measurements.

Specific samples were used for wet and dry measurements. Following removal from the buffered saline solution, each sample was washed with deionized water and surface wiped by using a filter paper. The specimen was then weighed. After drying for 24 h at 60 °C the specimen was weighed dry. To calculate the percentage weight loss, the following equations were used:

Wet weight:

$$\text{change in wet weight} = \frac{\text{wet weight at time } T - \text{initial dry weight}}{\text{initial dry weight}}$$

Dry weight:

$$\text{change in dry weight} = \frac{\text{dry weight at time } T - \text{initial dry weight}}{\text{initial dry weight}}$$

2.2. Goniophotometry

Goniophotometry is the measurement of reflected light as a function of viewing angle. Measurement of surface roughness or "gloss factor" may be made by analysing the reflectance pattern from an incident

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beam of light. A graphical output of reflectance intensity against angle of reflectance gives a "scattering envelope". Measurement of the scattering envelope and calculation of parameters from this graph are detailed in Knowles and Hastings [8]. The goniophotometer had a specially constructed sample holder attached to enable reproducible sample testing to be performed.

2.3. Mechanical testing

Standard dumbbell-shaped specimens for mechanical testing were air dried and then mounted in the tensile chucks of an ESH hydraulic testing machine. The jaws were set 100 mm apart and loading was applied at a strain rate 5 mm min^{-1}. From the graphical load–displacement output, the Young's modulus was calculated.

2.4. Molecular mass measurements

Molecular mass distribution (MMD) and number average molecular mass (*Mn*) determinations were performed by using gel permeation chromatography (GPC) by RAPRA Technology Ltd, Shawbury, UK. Samples were prepared by dissolving 40 mg of polymer in approximately 10 ml of chloroform (Analar grade). The solutions were allowed to stand overnight and then filtered through a 0.2 µm polyamide mesh. At a flow rate of 1 ml min^{-1}, the polymer solution was passed through a PL gel column and the separated fractions were detected by a refractive index detector.

2.5. Microscopic examination

Specimens were examined under reflected light on a Zeiss Photomicroscope, Pol II. Images were recorded on Pan F black and white film.

3. Results

Fig. 1 shows the percentage change in gloss factor (surface roughness) with time. Initially, the change in gloss factor is erratic, but after approximately 20 days, the effect of differing pH becomes more apparent. The neutral solution produces the least change in surface roughness and the greatest change is produced by the alkaline solution.

Fig. 2 shows the wet weight measurements. All specimens show an initial increase in weight as water is absorbed into the polymer. Specimens at pH 9, 10 and 11 then show a progressive decrease in weight, with a faster weight loss occurring the more alkaline the solution. After 542 days a weight loss of approximately 3% has occurred for the specimens in alkaline solution. This same trend may also be seen in the dry weight measurements (Fig. 3), but this gives a true weight loss of approximately 5%.

Fig. 4 shows the change in Young's modulus with time for samples immersed in solutions of different pH. Up to 300 days, the changes appear erratic, but eventually, the effect of different solution pH can be seen. pH 3 and 11 as expected have the greatest effect on the tensile modulus, but are unexpectedly of the same order of magnitude.

Fig. 5 shows the change in *Mn* and this shows a steady decrease with time and is clearly linked with the change in Young's modulus. The curves do not easily show any differentiation due to solution pH, but do show the general trend for the change in *Mn*.

Fig. 6a–c shows the change in molecular mass distribution for samples in pH 3, 7 and 11.

The polymer shows a low polydispersity (2–3) and there are two major features to the curves. The first, as expected, is the MMD, which exhibits a downward shift with time. The second point to note is that all curves, irrespective of pH, show an increase in peak height with time.

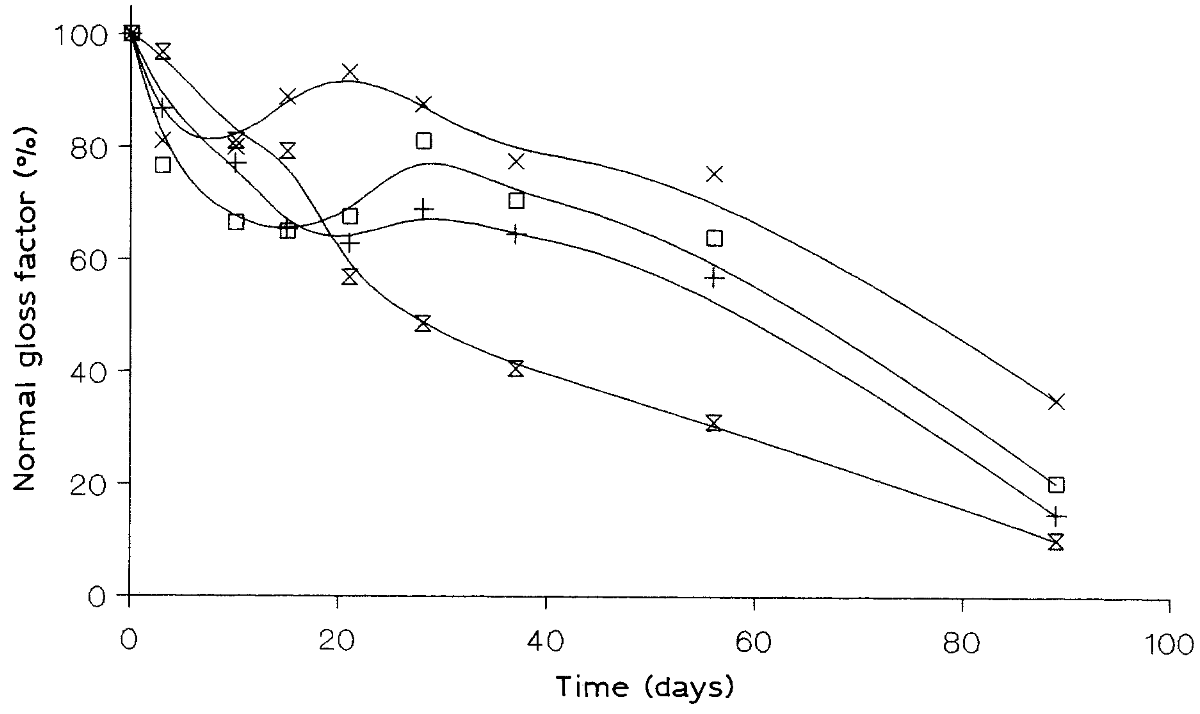


Figure 1 Percentage change in gloss factor for samples in different pH solutions: (+) pH 3, (-⊟-) pH 6, (-×-) pH 7, (-⧖-) pH 11.

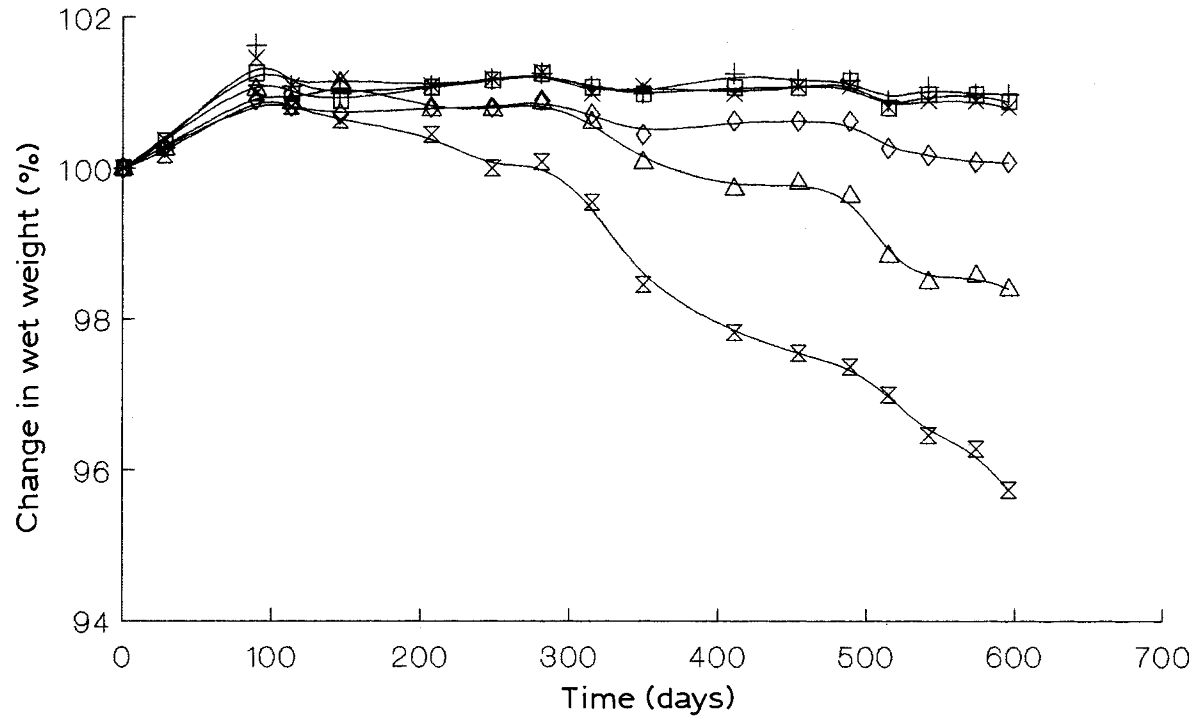


Figure 2 Percentage change in wet weight for samples in different pH solutions. (+) pH 3, (-⊟-) pH 6, (-✱-) pH 7, (-◇-) pH 9, (-△-) pH 10, (-⧗-) pH 11.

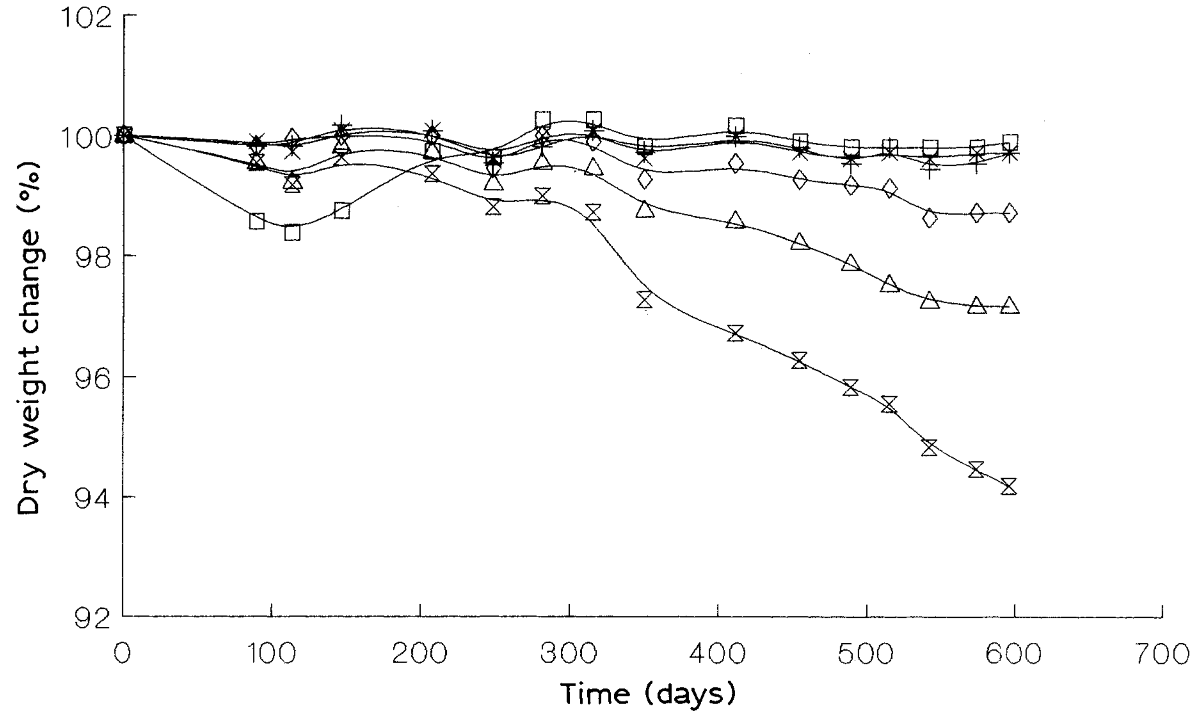


Figure 3 Percentage change in dry weight for samples in different pH solutions. (+) pH 3, (-⊟-) pH 6, (-✱-) pH 7, (-◇-) pH 9, (-△-) pH 10, (-⧗-) pH 11.

Plotting ($Mn(0)/Mn(t)$-1) against time will determine whether the chain scission is a random mechanism [9] and as Fig. 7 shows, the results appear linear. The significance of this will be discussed later.

From microscopic analysis of the polymer surface, differences between the appearance of the surfaces for exposure to pH 3 and 11 solutions may be detected. For pH 3, at 342 and 653 days (Fig. 8a, b), the surface appears rough, but with very fine surface pitting. For pH 11, the degradation is more apparent (Fig. 9a, b). The surface shows very specific areas of degradation, producing large flaws in the surface.

4. Discussion

Polyester degradation is a well-known phenomenon and the difference in the chemical route by which it occurs is influenced by pH. For an acidic/neutral solution, the reaction proceeds by a protonation process, followed by addition of water and cleavage of the

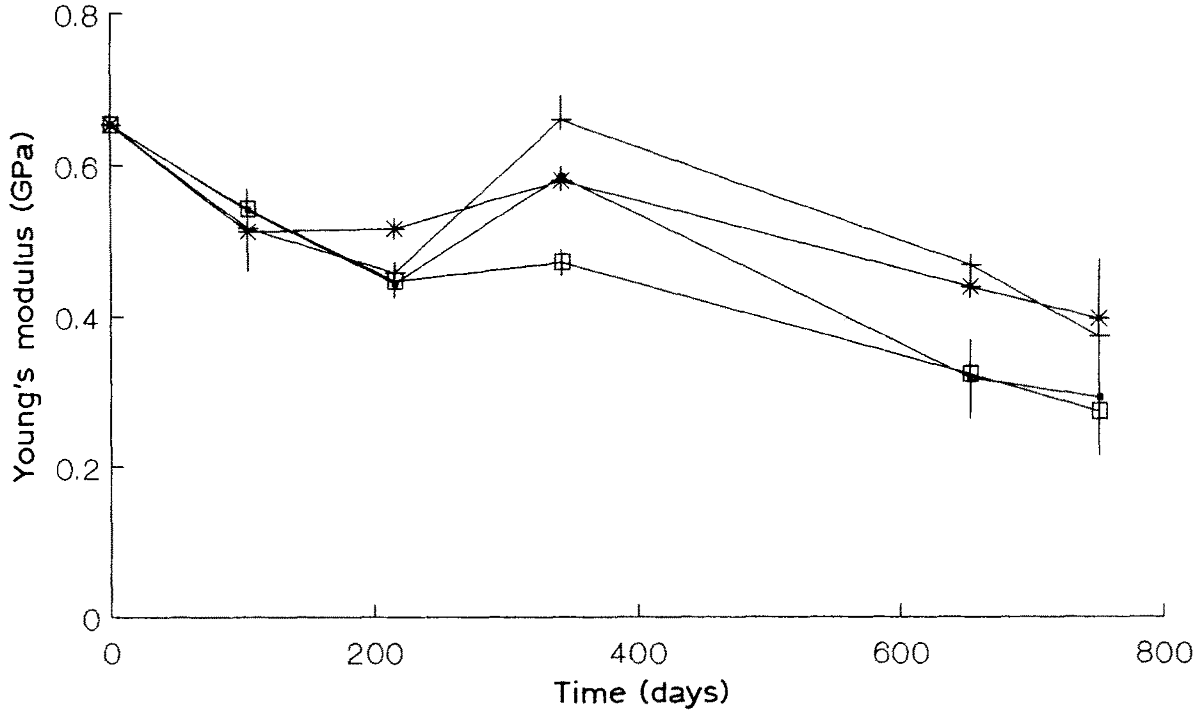


Figure 4 Change in Young's modulus for samples in different pH solutions. (-■-) pH 3, (+) pH 6, (✳) pH 7, (-⊞-) pH 11.

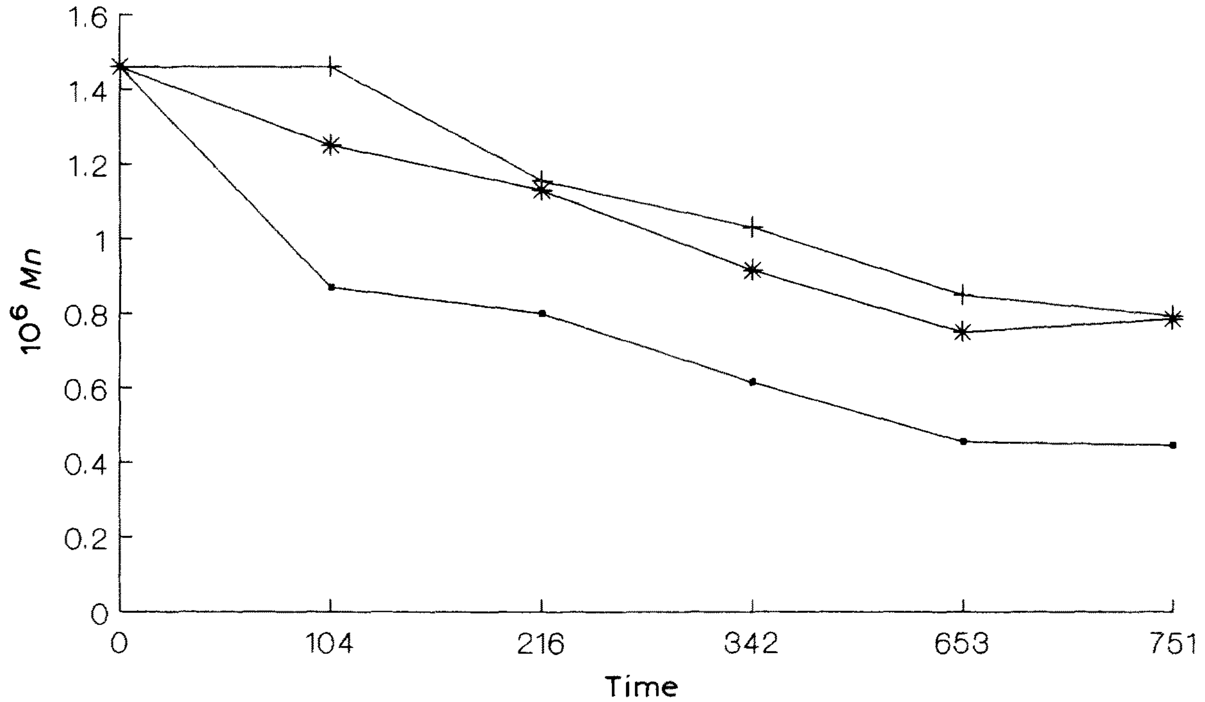
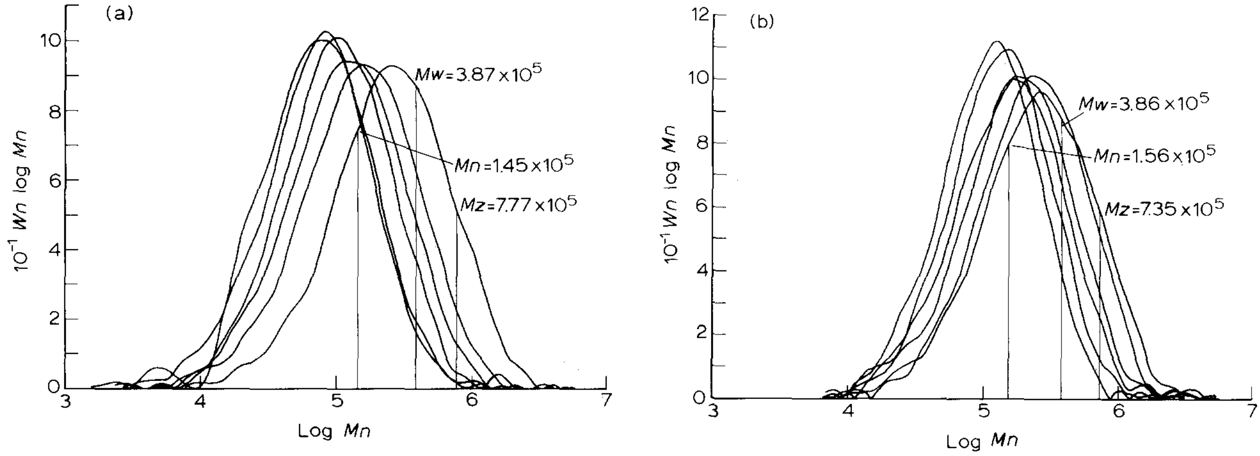


Figure 5 Change in molecular mass number (*Mn*) for samples in different pH solutions. (-■-) pH 3, (+) pH 7, (✳) pH 11.

ester linkage. For polyesters in an alkaline medium, the reaction is slightly different. Hydroxyl ions are attached to the carbonyl carbons and then subsequently, the bonds are ruptured. This major difference in the reaction will result in a difference in the degradation rate of the polymer. From the $(Mn(0)/Mn(t) - 1)$ plot, the linearity shows that the degradation is occurring randomly and it also shows that because the plots for each pH have different slopes, then the pH is a rate-controlling mechanism. For PHB/7% PHV, the effect of the different reaction pathways may clearly be seen in the gravimetric weight change. This same effect is also confirmed by the surface photomicrographs. The explanation of the degradation becomes more complex when looking at pH effects on the mechanical properties. The alkaline and acidic results appear to be similar, which conflicts to some extent with the mass changes. Determination of the molecular mass distribution serves to explain the anomaly found, as the mechanical properties are closely linked to the molecular mass characteristics. Of interest in the MMDs was the change in shape of the curves with time. As the degradation proceeds, the polymer tends towards a narrower distribution. If the



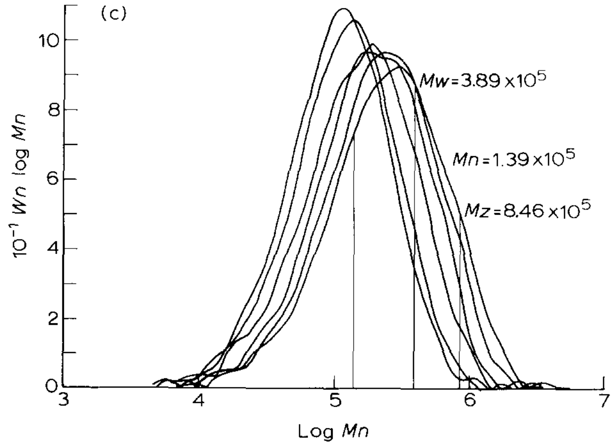


Figure 6 Change in molecular mass distribution for samples in different pH solutions: (a) solution of pH3; (b) solution of pH7; (c) solution of pH11.

polymer was completely degraded, then the MMD would be very narrow with a high count and the increase in peak height showed that this was happening.

5. Conclusion

Considerable work has been performed to analyse the degradation pattern of PHB and its PHV copolymers [10–12]. Early results [13] concluded that the polymer does degrade, but only if subjected to irradiation. This irradiation will have produced a drop in molecular mass and thus enhanced the degradation. When PHB and its copolymers are of a sufficiently high molecular mass, then the degradation changes are more subtle and difficult to detect. The work presented in this paper shows that the polymer does degrade, but easily detectable bulk changes can only be discerned after an extended period of degradation. This slow degradation pattern indicates that the material will find more use than similar materials such as PLA and PGA because they do not degrade sufficiently slowly [14, 15] for the orthopaedic applications envisaged. Because of the rapid degradation of these materials, it has been found in clinical trials [16] that the implant, because of the rapid breakdown of the polymer, forms

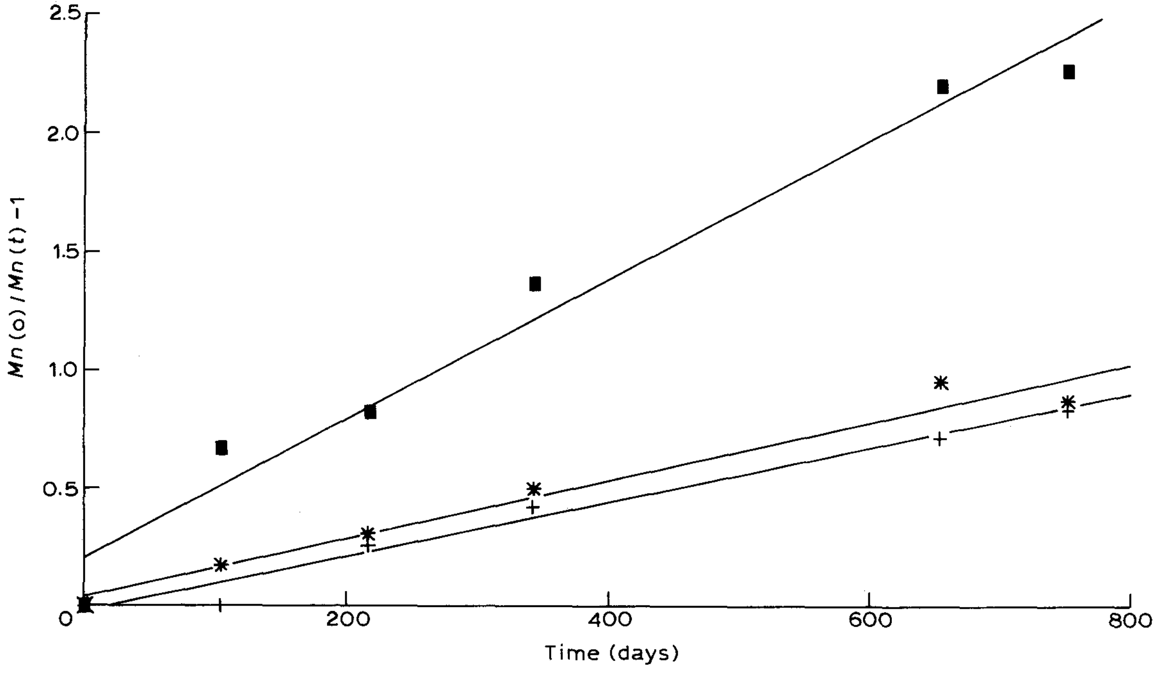


Figure 7 Plot of $(Mn(0)/Mn(t) - 1)$ against time for different pH solutions. (-■-) pH 3, (+) pH 7, (✱) pH 11.

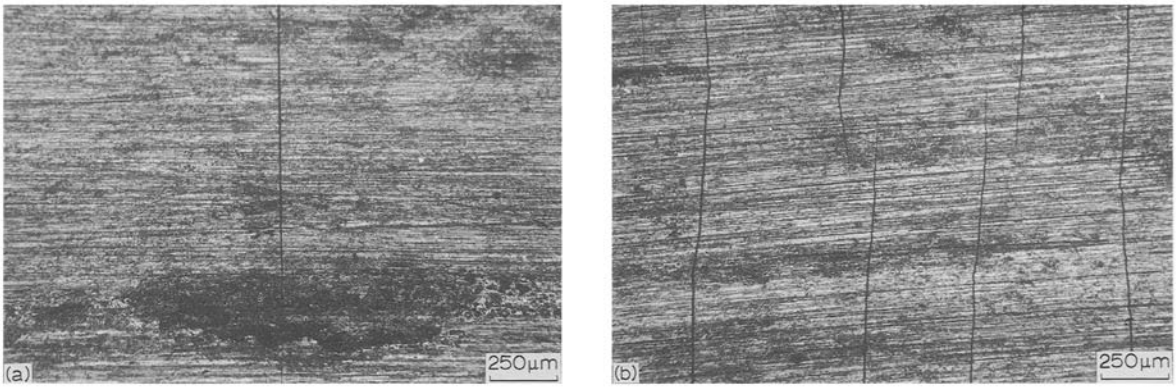


Figure 8 Reflected light micrograph of the surface of injection moulded PHB/7% PHV after *in vitro* degradation in solution pH 3: (a) after 342 days (× 80 magnification); (b) after 653 days (× 40 magnification).

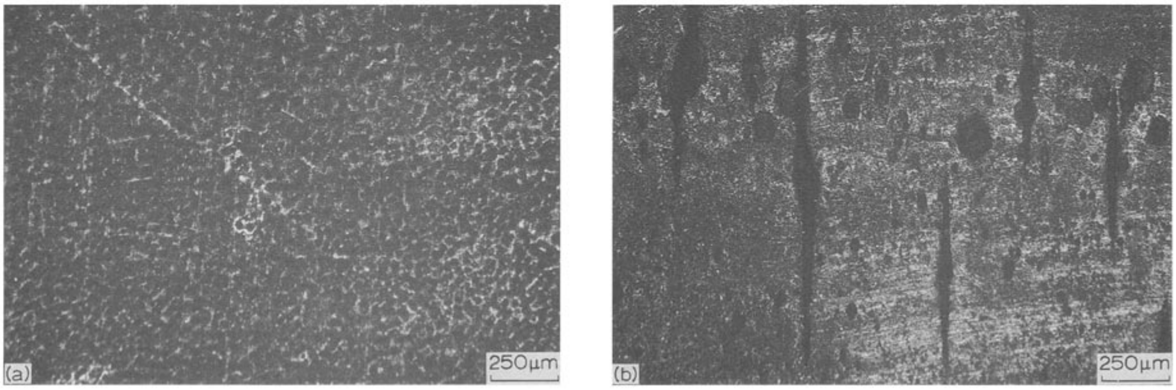


Figure 9 Reflected light micrograph of the surface of injection moulded PHB/7%PHV after *in vitro* degradation in solution pH11: (a) after 342 days (× 80 magnification); (b) after 653 days (× 40 magnification).

an exudate, which elicits a foreign-body reaction, causing swelling.

Miller and Williams concluded, that prediction of the degradation pattern of PHB is difficult, particularly *in vivo*, a view concurrent with ours and the incorporation of PHV to form a copolymer complicates the degradation pattern. The degradation pattern is easily influenced by the pH of the surrounding medium which is important to consider when the material is to be used in a biological environment. Hence, due to the known occurrence of localized pH effects *in vivo* [10], the correlation between *in vitro* and *in vivo* will be complex.

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