

## Ageing phenomena in bacterial poly[(*R*)-3-hydroxybutyrate]

### 1. A study on the mobility in poly[(*R*)-3-hydroxybutyrate] powders by monitoring the radical decay with temperature after $\gamma$ -radiolysis at 77 K

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Poly[(*R*)-3-hydroxybutyrate] (PHB), a bacterial polyester, is subject to a detrimental ageing process, which hampers its application possibilities. This ageing phenomenon is usually studied using moulded specimens, and was found to be related to a loss in segmental mobility. The presence of additives and orientation in moulded PHB samples might influence this ageing process. As a reference for our ageing studies, we therefore conducted a study on the ageing effects in virgin PHB powder. For this purpose a method is required to measure the mobility in a powder without the employment of heat or mechanical strain, since these are known to induce deageing. This paper describes a suitable technique based on the principle that the variation of the radical concentration with temperature after  $\gamma$ -irradiation at 77 K provides a measure of the changes in the segmental mobility of the polymer chains.

(Keywords: poly[(*R*)-3-hydroxybutyrate]; PHB; physical ageing;  $\gamma$ -irradiation; e.s.r.; segmental mobility)

#### Introduction

Poly[(*R*)-3-hydroxybutyrate] (PHB) is a bacterial storage polyester<sup>1</sup>, currently receiving much attention because of its possible application as a biodegradable and biocompatible plastic<sup>2</sup>. Due to its natural origin, PHB has an exceptional stereochemical regularity which enables the polymer to crystallize<sup>3</sup>. For example, injection-moulded samples possess a crystallinity typically around 60%. As-moulded PHB shows a glass transition temperature ( $T_g$ ) at  $\sim 7^\circ\text{C}$  and a crystalline melting point ( $T_m$ ) around  $175^\circ\text{C}$ , as measured by calorimetric analysis<sup>4</sup>.

Based on these data, the material may be expected to be tough at room temperature. Indeed, as-moulded PHB does show ductile behaviour, but subsequently a detrimental ageing process seriously embrittles the material and hampers its applicability<sup>5</sup>. Within several weeks of storage at room temperature, the tensile modulus doubles, and the elongation at break drops below 10%. A typical feature of the ageing process is that it can be partly reversed by the employment of heat or mechanical strain. Using mild 'deageing' treatments, the improvement in toughness is only small and temporary. Interestingly however, annealing the material above  $100^\circ\text{C}$  restores the original toughness and prevents or at least retards ageing to a large extent. All the observed phenomena could be attributed to processes which affect the segmental mobility in the amorphous phase and which will be described extensively in a separate study<sup>6</sup>. In this study, dynamic mechanical analysis was used to monitor the variations in the segmental mobility. However, all the samples used were manufactured via injection-moulding, making them likely to contain oriented regions. Indeed, X-ray

scattering results indicated some orientation to be present. Moreover, the PHB investigated was slightly contaminated with impurities from the industrial recovery process, and a nucleating agent was added to enhance the crystallization. Finally, since PHB is a polyester, the samples are likely to take up some water during storage.

To eliminate any influences of these factors on the process of ageing, a search was made for a method to study the ageing phenomenon in the virgin PHB powder as a reference for further studies. Analogous to dynamic mechanical analysis, this method should produce a measure of the mobility in the material as a function of temperature. The usual techniques are not suitable for this purpose, because they require the polymer to be mechanically strained, which might induce a deageing effect. N.m.r.-based techniques involve rapid rotation of the sample and i.r. spectroscopy as well as dielectric measurements lack signal intensity, unless the powder is pressed into a pellet.

The method, which is the subject of the present paper, involves exposure to high-energy irradiation at low temperatures that ensure that the generated radicals are trapped. Increasing the temperature elevates the mobility of the polymer segments, which enables the radicals to react. Such annealing experiments have been established for a long time as a means of examining the reactivity of radicals generated at low temperatures<sup>7-9</sup>. However, the use of this method to examine variations in the segmental mobility of a polymer has not been reported previously.

#### Experimental

The PHB investigated was a commercial Biopol®

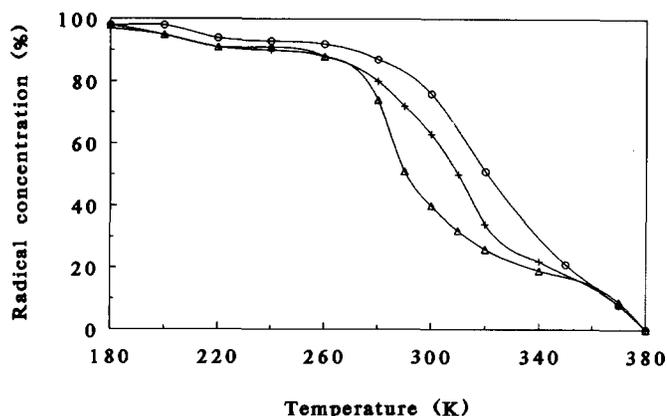


Figure 1 Variation in the relative radical concentration with temperature following  $\gamma$ -radiolysis of PHB in the solid state at 77 K: (O) aged for 4 months; (+) mechanically deaged and subsequently aged for 4 days; ( $\Delta$ ) thermally deaged at 125°C

sample ( $M_w = 539 \text{ kg mol}^{-1}$ ,  $M_w/M_n = 3.5$ ), provided by ICI Bioproducts & Fine Chemicals (Billingham, UK). The off-white powder was further purified by dissolution in chloroform, filtration of the warm solution and precipitation from a chloroform/methanol (70/30) mixture. Subsequently, the powder was dried at 40°C and  $10^3 \text{ Pa}$  for 48 h and allowed to age for 4 months in a desiccator at 25°C. It was packed into three Spectrosil® high purity quartz tubes for  $\gamma$ -radiolysis and subsequent e.s.r. measurements. In one case, the powder was ground before packing in order to mechanically deage the material. The tubes were evacuated at 25°C and  $10^3 \text{ Pa}$  for 30 h and sealed. After 3 days of storage at ambient temperature, one sample was thermally deaged by holding the tube at 125°C for 5 min. Subsequently, the tubes were quenched and stored in liquid nitrogen. Irradiations were carried out in a  $^{60}\text{Co}$ -Gammacell 220 unit at  $0.45 \text{ kGy h}^{-1}$  at 77 K to a total dose of 1.7 kGy. The dose was determined by means of Fricke dosimetry using an acetone/water (2:1) mixture to simulate liquid nitrogen<sup>10</sup>. After irradiation, the samples were kept in liquid nitrogen to avoid any radical loss or premature ageing. E.s.r. spectra were recorded using a Bruker ER 200D X band spectrometer at temperatures ranging from 140 to 400 K at a low microwave power of 0.02 mW (40 dB) to avoid saturation. Radical concentrations were obtained at 140 K by comparison of the integrated e.s.r. signal with a Varian pitch standard reference. Annealing experiments to study the radical decay were conducted as follows. The sample was heated from 140 K to the desired temperature in the variable temperature cavity of the e.s.r. spectrometer and allowed to equilibrate until no evidence for further reaction could be observed through changes in the radical spectrum. The sample was then quenched to 140 K to determine the radical concentration. This allowed a direct comparison of the results and avoided the necessity to employ the Boltzmann correction, which would be necessary if the spectra had been obtained at different temperatures.

As a reference experiment, injection-moulded PHB samples with a corresponding ageing history were studied by dynamic mechanical analysis. For this purpose, the off-white powder was mixed with 1 wt% boron nitride in a Hobart mixer for ~10 min. Boron nitride acts as a nucleating agent<sup>11</sup> to enhance the otherwise slow crystallization process. The powder mixture was extruded

into a single 4 mm diameter strand using a Betol 2520 (diameter 25 mm) operated at a maximum of 180°C and at a screw speed of  $100 \text{ rev min}^{-1}$ . The strand was crystallized in a 60°C water bath and granulated. After drying at 40°C for 20 h, the granulate was injection-moulded using a Boy 15S injection-moulding machine. Processing specifications: maximum barrel temperature 180°C; injection time 15 s; screw speed  $220 \text{ rev min}^{-1}$ ; injection pressure 5 MPa; mould temperature 60°C; cooling time 15 s. The resulting specimens were allowed to age for 4 months at ambient temperature. Subsequent thermal deageing was established by holding the sample at 125°C for 10 min, and cold-rolling was employed in order to establish mechanical deageing. Dynamic mechanical measurements were performed with a Polymer Laboratories dynamic mechanical analyser, operated in the single cantilever bending mode. The specimens ( $2 \times 5 \times 12 \text{ mm}$ ) were investigated at a measuring frequency of 3 Hz and a heating rate of  $2 \text{ K min}^{-1}$ .

### Results and discussion

Upon irradiation of polymeric materials using  $\gamma$ - or electron beam irradiation, various reactive intermediates are generated, including radicals. Below  $T_g$  these radicals are trapped, but upon heating above  $T_g$  various reactions can take place, such as crosslinking, chain scission, etc. Therefore, the radical concentration will change markedly as the polymer passes through the glass transition. The radical decay can be observed by means of e.s.r., and provides a measure of the increase in segmental mobility. An earlier study concerning the radiation chemistry of PHB<sup>12</sup> showed that the radicals generated at 77 K merely decay in the vicinity of  $T_g$  due to chain scission. Therefore, the effects of ageing on the glass transition of PHB should be conveniently identified through monitoring of the radical reactivity.

Figure 1 presents the results for the aged and deaged PHB powders obtained by this method. Due to variations in the packing densities, the samples showed slightly different initial radical concentrations. For the sake of comparison, relative radical concentrations are shown.

Figure 2 shows the corresponding results of the reference experiment. The storage modulus  $G'$  of the moulded PHB samples is plotted as a function of

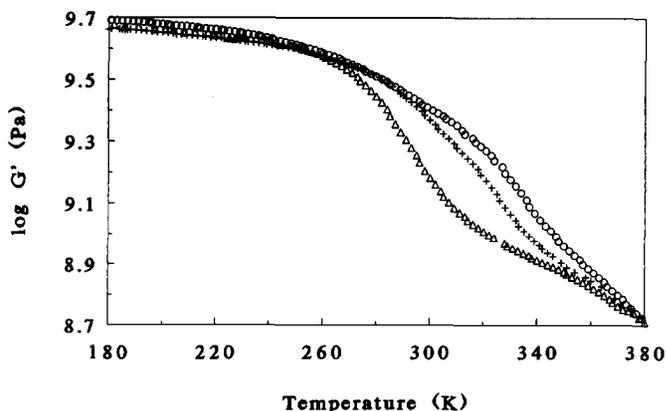


Figure 2 Dynamic storage modulus of PHB in the glass transition range (measuring frequency: 3 Hz): (O) aged for 4 months; (+) mechanically deaged and subsequently aged for 4 days; ( $\Delta$ ) thermally deaged at 125°C

temperature. Obviously, an ageing process has restricted the mobility in the temperature range from  $T_g$  (276 K) up to  $\sim 360$  K. Notably, the largest change in mobility is found around room temperature, and is responsible for the observed embrittlement of PHB upon storage at ambient temperature.

#### Conclusions

Figures 1 and 2, although obtained via completely different techniques, show identical curves. From this striking resemblance, it must be concluded that the ageing process in the virgin PHB powder is analogous to that in the moulded material. Apparently, the ageing phenomenon is an intrinsic property of the polymer and is not related to the presence of extraneous compounds or orientation.

The presented method, the use of e.s.r. in combination with  $\gamma$ -irradiation, for examining the segmental mobility in a polymer powder has proved to be successful in the

case of PHB, and may find applications in studies of the mobility in other polymer materials.

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