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Crystallization phenomena in bacterial poly[(*R*)-3-hydroxybutyrate]:

2. Embrittlement and rejuvenation

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Poly[(*R*)-3-hydroxybutyrate] (PHB) is a bacterial storage polymer, currently receiving much attention because of its potential as a biodegradable plastic. A major drawback of PHB is its intrinsic brittleness. Although as-moulded PHB shows ductile behaviour, upon storage at ambient temperature a detrimental ageing process seriously embrittles the material and restricts its application possibilities. This remarkable embrittlement is delineated in the present study and could be attributed to progressive crystallization. Strikingly, we found that by using a simple annealing treatment PHB can be rejuvenated while subsequent ageing is prevented to a large extent. This observation might considerably enlarge the applicability of PHB.

(Keywords: poly[(*R*)-3-hydroxybutyrate]; biopolymers; ageing)

INTRODUCTION

Poly[(*R*)-3-hydroxybutyrate] (PHB, *Figure 1*) is a microbial storage polymer, accumulated by a variety of bacteria as a reserve of carbon and energy¹⁻³. Owing to its natural origin, PHB is a biodegradable material, i.e. it can be completely degraded and assimilated by a vast range of micro-organisms into harmless, naturally occurring molecules²⁻⁴. Moreover, PHB is a thermoplastic polyester which can be extruded, moulded and spun on conventional processing equipment². These features make PHB an ideal substance for the production of biodegradable packaging materials and other disposables⁵. In 1982, Imperial Chemical Industries (ICI) developed a process for the industrial production of PHB, using the bacterium *Alcaligenes eutrophus*⁶ grown on glucose. Under controlled fermentation conditions, this bacterium can be stimulated to accumulate PHB up to 90% of its total dry mass². At present, PHB is commercially available under the trademark Biopol® (ICI).

Owing to its natural origin, PHB has an exceptional stereochemical regularity. The chains are completely linear and the chiral centres possess only the *R*-stereochemical configuration, which implies that this polymer is completely isotactic and capable of crystallizing. The mass fraction crystallinity of a moulded PHB sample is typically around 60%. As-moulded PHB possesses a glass transition temperature (T_g) at approximately 5°C and a crystalline melting point (T_m) around 175°C^{2,7,8}. Based on these data, the material may be expected to be tough at room temperature. Indeed, as-moulded PHB does show ductile behaviour, but upon storage at ambient temperature a detrimental ageing process seriously embrittles the material.

For the application of PHB as an environmentally

benign plastic, it is of major importance to overcome its brittleness. Surprisingly, however, this problem has hardly been addressed in literature. Grassie *et al.*⁹⁻¹¹ found PHB to be susceptible to a random chain scission, which suggests degradation as a possible explanation for the embrittlement. Alternatively, Barham and Keller¹² related the brittleness to cracks which are present within the large spherulites. When the material is strained, the cracks grow and combine resulting in brittle failure. Moreover, they observed that cold-rolling of PHB sheets improved the ductility as a result of the cracks being 'healed'. However, it must be noted that commercial PHB products possess a very fine spherulitic texture due to the presence of nucleating agents. Finally, Scandola *et al.*¹³ conducted a preliminary study on the ageing phenomenon by means of dynamic mechanical analysis. They concluded that, because of the proximity of T_g to room temperature, PHB is prone to physical ageing, as was described earlier for other semicrystalline polymers by Struik¹⁴⁻¹⁶.

In a preceding paper¹⁷ we have demonstrated that the ageing phenomenon of PHB is an intrinsic property of this polymer and is not related to the presence of extraneous compounds or orientation. The present paper reports a detailed study, which demonstrates that the

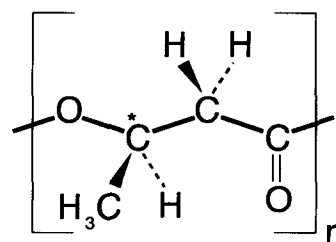


Figure 1 Structural formula of poly[(*R*)-3-hydroxybutyrate]

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embrittlement of PHB should be attributed to progressive crystallization. Moreover, we found that by using a simple annealing treatment the embrittlement of PHB can to a large extent be prevented.

EXPERIMENTAL

Materials

The PHB polymer investigated was a commercial Biopol[®] sample ($M_w = 539 \text{ kg mol}^{-1}$, $M_w/M_n = 3.5$, $T_m = 174^\circ\text{C}$), provided by ICI Bioproducts & Fine Chemicals as a fine white powder. The powder was mixed with 1 wt% boron nitride in a Hobart mixer for approximately 10 min. Boron nitride acts as a nucleating agent¹⁸ to enhance the otherwise slow crystallization process.

Sample preparation

The powder mixture was extruded into a single strand of diameter 4 mm using a Betol 2520 (diameter 25 mm; filters 60 and 100 mesh) operated at a maximum of 180°C and at a screw speed of 100 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 rev min^{-1} ; injection pressure 5 MPa; mould temperature 60°C ; cooling time 15 s. Melt-processing reduced the PHB molar mass (M_w) from 539 to 370 kg mol^{-1} ($M_w/M_n = 2.9$). Ageing of the resulting specimens was established by storage at ambient conditions for a specified time. After 150 days of storage some samples were annealed in a Heraeus hot-air oven. Fully amorphous samples were obtained by quenching from the melt in liquid nitrogen.

Characterization techniques

Tensile testing. Tensile testing was performed at temperatures ranging from ambient to 60°C using an Instron 1122 fitted with a Nene data analysis system. A clamp separation of 50 mm and a crosshead speed of 20 mm min^{-1} were used. The injection-moulded specimens were dumbbell-shaped according to ISO R 537/2, their prismatic part measuring $40 \times 5 \times 2 \text{ mm}$.

Impact testing. The Izod impact strength was determined using a Zwick pendulum impact testing machine on injection-moulded specimens with dimensions of $5 \times 13 \times 50 \text{ mm}$, which were provided with a V-notch possessing a tip radius of 1 mm. All tests were conducted under ambient conditions.

Molar mass measurements

Gel permeation chromatography (g.p.c.) was used to study variations in the molecular weight distribution. The chromatograms were obtained at 30°C in chloroform using a DuPont Instruments apparatus equipped with a set of two $10 \mu\text{m}$ mixed gel columns (Polymer Laboratories, $300 \times 7.5 \text{ mm}$) and a Wilks Miran infra-red detector. The system was calibrated using monodisperse polystyrene standards. True PHB molecular weights were obtained by applying the Mark-Houwink relationship. For polystyrene in chloroform $K = 0.49 \times 10^{-4}$ and $\alpha = 0.794$,

while the equivalents for PHB are $K = 1.18 \times 10^{-4}$ and $\alpha = 0.787$.

Dynamic mechanical thermal analysis (d.m.t.a.)

Dynamic mechanical measurements were performed with a Polymer Laboratories Dynamic Mechanical Analyser, operated in the single cantilever bending mode. Injection-moulded specimens ($2 \times 5 \times 12 \text{ mm}$) were investigated at a measuring frequency of 10 Hz and a heating rate of 2 K min^{-1} .

Dilatometry

Dilatometry was conducted in a Hg-dilatometer¹⁹. The dilatometer was packed with the PHB granulate and mercury was added under vacuum. The sealed dilatometer was subjected to the temperature profile of the processing cycle and, subsequently, positioned in a thermostatted water bath. The density of the sample was monitored over a period of 1 month. The measured densities were converted into sample densities at 20°C using a thermal expansion coefficient of $25 \times 10^{-5} \text{ K}^{-1}$ (estimated from an annealed sample, which was monitored in the range $78\text{--}25^\circ\text{C}$). The density of PHB, ρ_{PHB} , was obtained by correcting the sample density for the presence of 1 wt% boron nitride:

$$\rho_{\text{PHB}} = 0.99 \rho_{\text{sample}} \left(1 - 0.01 \frac{\rho_{\text{sample}}}{\rho_{\text{BN}}} \right)$$

where $\rho_{\text{BN}} = 2.25 \text{ g cm}^{-3}$.

Densities could be translated into mass fraction crystallinities, X_m , using:

$$X_m = \frac{\rho_{\text{PHB}} - \rho_a}{\rho_{\text{cr}} - \rho_a} \frac{\rho_{\text{cr}}}{\rho_{\text{PHB}}}$$

with density of amorphous phase⁷, $\rho_a = 1.179 \text{ g cm}^{-3}$ and density of crystalline phase⁷, $\rho_{\text{cr}} = 1.279 \text{ g cm}^{-3}$.

WAXS and SAXS

To determine the lattice parameters, wide-angle X-ray scattering (WAXS) patterns of injection-moulded samples (thickness: 2 mm) were obtained using a Philips PW 1729 X-ray Generator (Ni-filtered Cu $K\alpha$) and a Statton camera. Small-angle X-ray scattering (SAXS) patterns were recorded using a Rigaku apparatus equipped with a rotating anode (RU-200B) and a Kratky camera provided with a one-dimensional counter (Braun OED-SOM).

Optical microscopy

The spherulitic texture of a slice of a PHB pellet was monitored using a Zeiss optical microscope equipped with a polarizing filter.

Differential scanning calorimetry (d.s.c.)

Melting endotherms were recorded using a Perkin-Elmer DSC-7 differential scanning calorimeter at a heating rate of $20^\circ\text{C min}^{-1}$. Indium was used for temperature and heat of fusion calibration. Mass fraction crystallinities could be calculated from the melting peak area, assuming that the heat of fusion of 100% crystalline PHB⁷ amounts to 146 J g^{-1} .

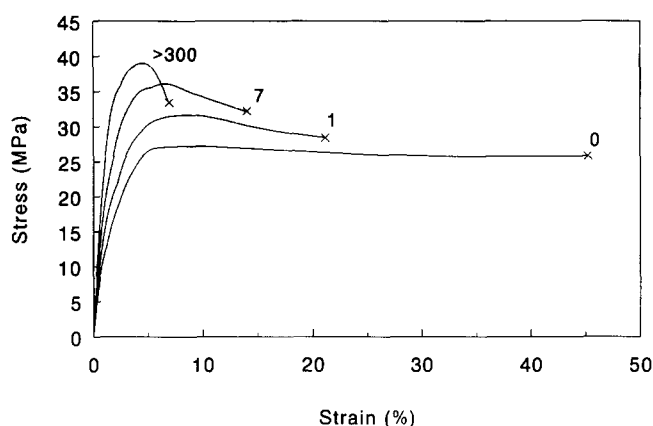


Figure 2 Stress-strain curves at ambient conditions for moulded PHB samples after different periods of storage. The storage times, in days, are indicated in the curves

RESULTS AND DISCUSSION

Effects of storage on mechanical properties

Mechanical testing of PHB revealed the pronounced influence of storage on the mechanical properties. *Figure 2* presents the stress-strain behaviour after different periods of storage at ambient conditions. Initially, the material showed a ductile behaviour with a maximum elongation of approximately 40%. However after 2 weeks storage, the maximum elongation had dropped below 10%, reflecting the embrittlement of the material. *Figure 3* shows the corresponding changes in tensile modulus and Izod impact strength. *Figure 4* presents the dynamic mechanical characteristics as a function of storage time. Notably, the dynamic modulus at room temperature increased by a factor of 2.6 and the dynamic loss peak showed a drastic reduction.

Physical versus chemical deterioration

PHB is known to be susceptible to a random chain scission, producing a decrease in molar mass⁹⁻¹¹. The rate of this degradation process increases with temperature, causing a low melt stability at processing conditions². Considering this, the embrittlement could possibly be related to a drop in molar mass. However, g.p.c. analysis did not show any significant changes in the molecular weight distribution with storage time, indicating that at ambient temperature the occurrence of the scission reaction should be neglected. The reversible character of the deterioration, which will be addressed below, is another indication that the embrittlement of PHB is a physical rather than a chemical process.

Vitrification upon crystallization

A common characteristic of polymer crystallization is that the glass transition is extended to the high-temperature side, reflecting the constraints imposed on the amorphous chains by the crystals. For PHB this broadening of the glass transition could be clearly observed from the changes in the dynamic mechanical spectra (*Figure 4*). Crystallization reduced the height of the dynamic loss peak by a factor of 35, although only about 60% of the material crystallized. The apparent reduction of the ability of the amorphous phase to dissipate energy should therefore be attributed to the constraining action of the crystals. Moreover, while the

amorphous sample (curve (a)) showed a narrow loss peak, crystallization extended the peak basis to approximately 60°C. Importantly, this implies that the amorphous phase will partly vitrify at ambient temperature.

In view of this vitrification, two processes could be involved in the embrittlement of PHB. First, PHB might be prone to 'physical ageing', as was claimed before by Scandola and colleagues¹³. Secondly, moulding for 15 s at 60°C may not be sufficient to complete the crystallization process, so that progressive crystallization will slowly occur upon subsequent storage at room temperature. The observed trends in modulus, impact

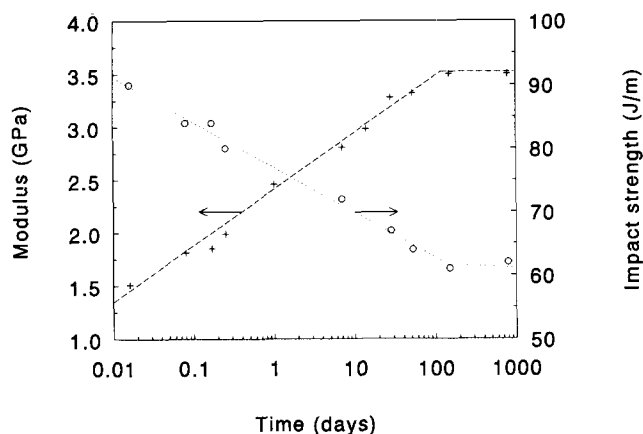


Figure 3 Tensile modulus (+) and Izod notched impact strength (O) of moulded PHB versus storage time

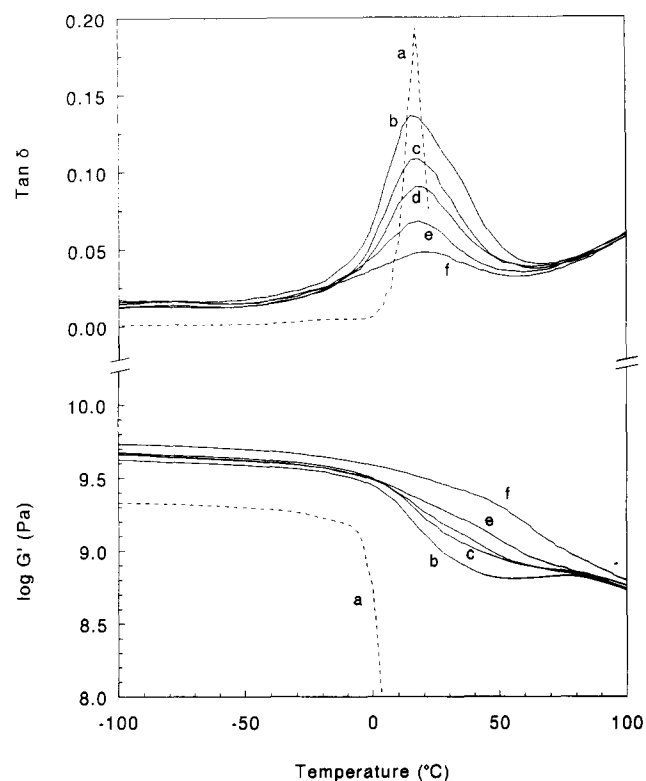


Figure 4 Dynamic mechanical spectra of PHB in the glass transition region. Curve (a) represents a fully amorphous sample. The other samples are moulded and subsequently stored at room temperature for (b) 0 h, (c) 2 h, (d) 1 day, (e) 8 days, (f) 150 days. The storage modulus and the tangent dynamic mechanical loss angle are presented (curve (a) is not on true scale since its peak possessed a maximum at $\tan \delta = 3.5$)

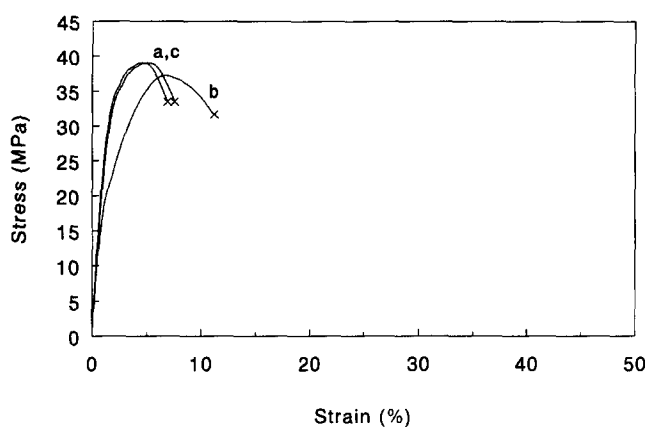


Figure 5 Stress-strain curves at ambient conditions for moulded PHB samples which were (a) stored for 150 days, (b) subsequently annealed for 5 min at 70°C, and (c) stored for another 5 days

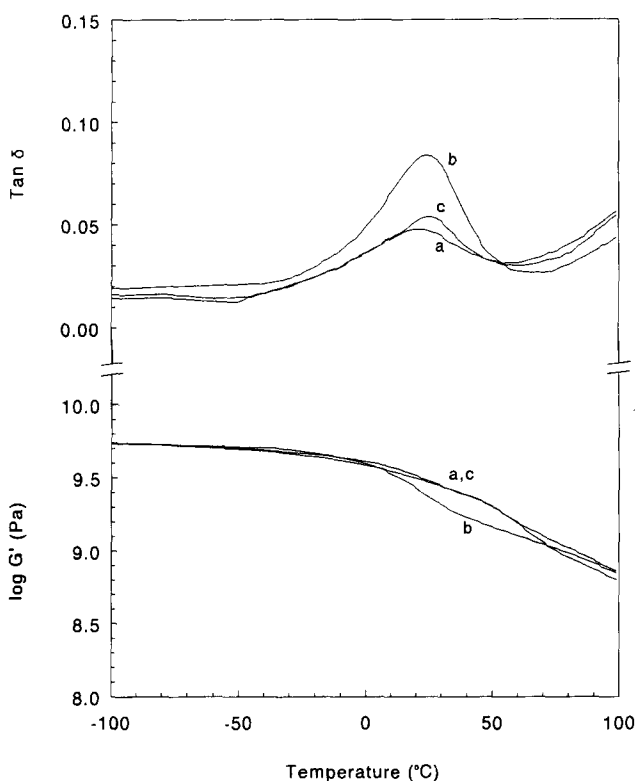


Figure 6 Dynamic mechanical characteristics versus temperature for moulded PHB samples which were (a) stored for 150 days, (b) subsequently annealed for 5 min at 70°C, and (c) stored for another 5 days

behaviour and dynamic mechanical characteristics are consistent with either mechanism.

Physical ageing

The presence of vitrified regions in PHB at room temperature establishes the conditions required for 'physical ageing', which is a phenomenon characteristic of all glassy materials²⁰. Owing to the non-equilibrium character of the glassy state, the residual mobility produces molecular rearrangements that drive the free volume closer to its equilibrium value. The resulting volume relaxation restricts the mobility, which affects all related properties. This process can be completely reversed by regenerating free volume, which can be

achieved by heating the material above T_g or by mechanical strain. Since these treatments do not affect the crystallinity, its reversible character enables physical ageing to be distinguished from progressive crystallization.

Figures 5 and 6 present the effects of heating the aged material at 70°C, which is well above T_g , for a short period of time. The mechanical properties slightly improved and the loss peak increased, reflecting the regained mobility. Upon subsequent storage the material aged again. Obviously, PHB is prone to some physical ageing at room temperature. However, the observed effects are negligible in comparison with the drastic changes accompanying the embrittlement of as-moulded PHB (Figures 2-4). Moreover, tensile testing demonstrated that elevating the temperature to 60°C had no effect at all on the maximum elongation (Figure 7). Since this temperature is well above T_g , this observation eliminates vitrification and physical ageing as possible causes of the embrittlement of PHB.

Progressive crystallization

Apparently, physical ageing does not contribute to the embrittlement of PHB, which suggests that the embrittlement should be merely attributed to progressive crystallization. Dilatometry (Figure 8) showed that the storage of PHB is accompanied by a significant increase in density, which indeed indicates the occurrence of a

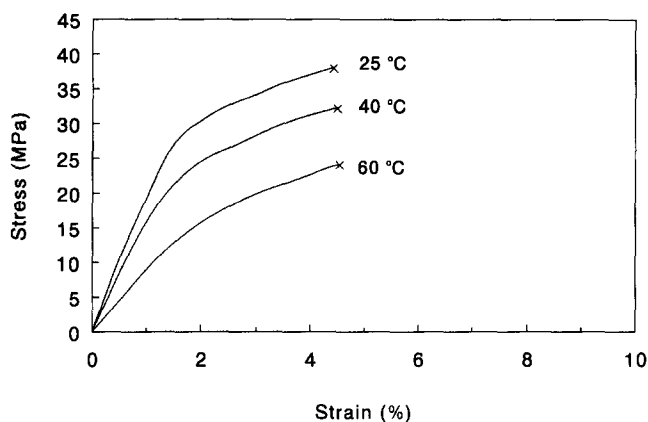


Figure 7 Stress-strain curves at different temperatures for moulded PHB samples which were stored for 400 days

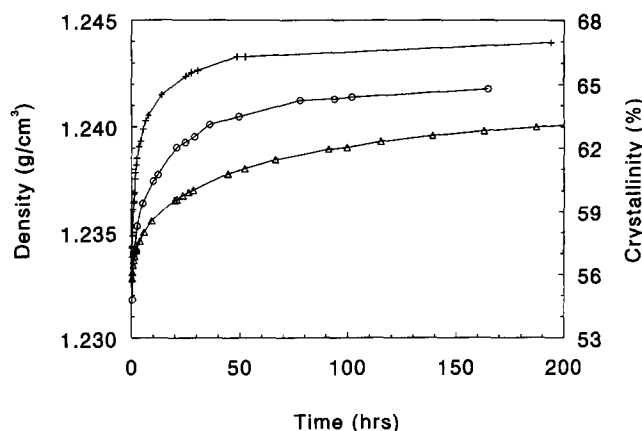


Figure 8 Density changes in as-moulded PHB as observed by dilatometry at 25°C (Δ), 43°C (O), and 60°C (+)

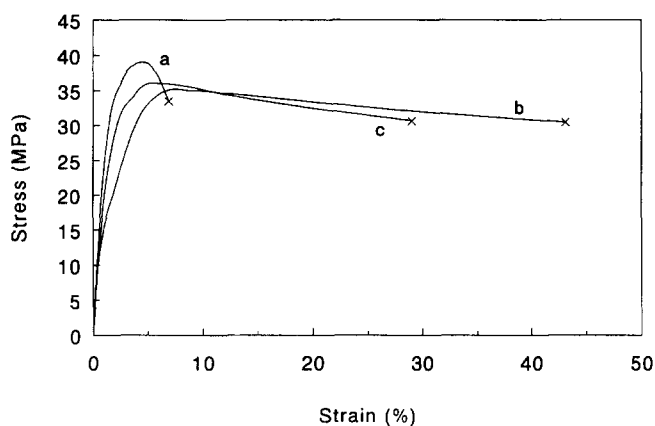


Figure 9 Stress-strain curves at ambient conditions for moulded PHB samples which were (a) stored for 150 days, (b) subsequently annealed for 10 h at 110°C, and (c) stored for another 150 days

crystallization process. Notably, the magnitude of this density effect is excessively high in comparison with the physical ageing of amorphous polymers²⁰. Moreover, storage at elevated temperatures resulted in a higher ultimate density, which conflicts with physical ageing but is consistent with the concept of crystallization. Converting densities into crystallinities showed that the initial 56% crystallinity in as-moulded PHB increased by an additional 7% upon 200 h storage at 25°C. Similarly, from the d.s.c. melting endotherm it was calculated that the crystallinity increased from 55 to 61%. After a period of 1 month, dilatometry revealed a crystallinity of 65%, indicating that crystallization progressed over a considerable length of time parallel to the mechanical changes.

Using WAXS and optical microscopy, no significant variations were observed as a function of time in lattice parameters and spherulitic texture, respectively. In addition, SAXS measurements could not furnish any evidence of lamellar thickening over a period of 3 months. These observations show that the gain in crystallinity indeed results from progressive crystallization and not from some crystal rearrangement.

The comparatively small loss of amorphous fraction upon progressive crystallization is attended by drastic mechanical effects, i.e. complete embrittlement. The concomitant drastic reduction of the dynamic loss peak (Figure 4) reflects a disablement of the amorphous chains to dissipate energy by viscous flow. Moreover, the drop in storage modulus at the glass transition fades out, indicative of a loss of the relaxation strength of the amorphous phase. Apparently, progressive crystallization seriously constrains the amorphous phase, and hence promotes the embrittlement of PHB.

Toughening effects established by annealing

In the previous section it was demonstrated that the properties hardly improved upon annealing at 70°C. Surprisingly, however, annealing at higher temperatures appeared to toughen the material considerably. The original properties were restored and, moreover, subsequent ageing was prevented to a large extent. For example, Figure 9 shows that annealing a sample at 110°C restored the original toughness of the polymer and subsequent storage over a period of 5 months hardly deteriorated the material.

The explanation for this interesting phenomenon must be a physical one, since g.p.c. analysis did not show any significant changes in the molecular weight distribution upon annealing. SAXS measurements revealed that annealing increased the periodicity, indicative of a change in texture. Possibly, a texture change relieves the constraints imposed on the amorphous chains resulting in an increase in relaxation strength, as can be observed from Figure 10.

Like the original material, the annealed PHB is likely to be prone to physical ageing at ambient temperature. This might explain the observed minor deterioration. Consistent with the concept of physical ageing, heating the annealed material at 95°C indeed restored the favourable maximum elongation of over 40%. Dilatometry of the annealed material showed that storage at 25°C elevated the density slightly according to a constant logarithmic rate (Figure 11). This density change corresponds with a 0.1% volume relaxation of the amorphous phase per decade, which is a usual value for physical ageing of amorphous polymers²⁰. However, annealed PHB stored at 40°C exhibited similar ageing, indicating that some crystallization is involved again.

CONCLUSIONS

An explanation for the remarkable embrittlement of PHB at room temperature was presented. In contrast to earlier claims¹³, it was demonstrated that physical ageing does not contribute to this phenomenon and that the embrittlement should be merely attributed to progressive crystallization. During moulding of PHB, crystallization cannot be completed within the processing cycle, so that progressive crystallization will occur upon subsequent storage. At ambient temperature, the rate of this process

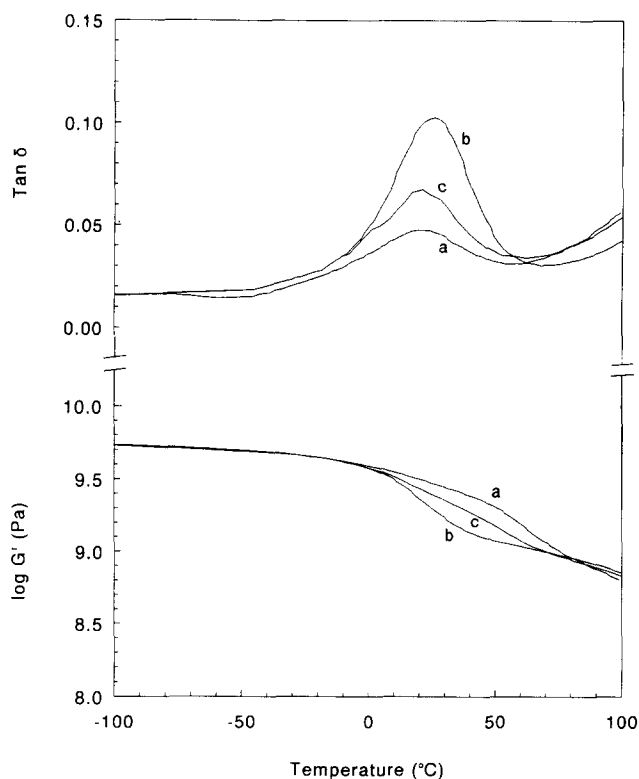


Figure 10 Dynamic mechanical characteristics versus temperature for moulded PHB samples which were (a) stored for 150 days, (b) subsequently annealed for 10 h at 110°C, and (c) stored for another 150 days

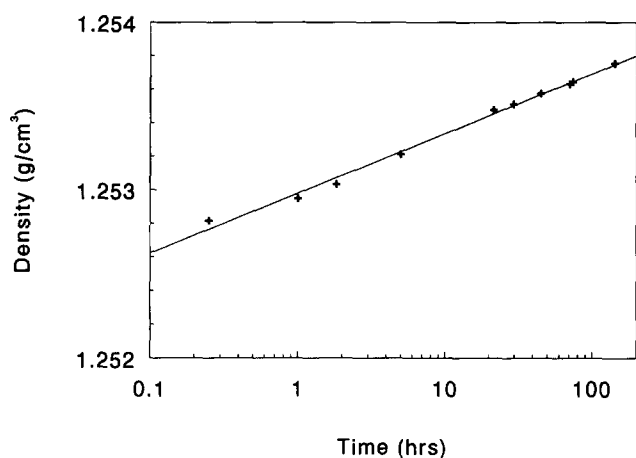


Figure 11 Density changes at 25°C as observed by dilatometry in a sample which was annealed at 110°C for 10 h

is limited because of the proximity of T_g which, in addition, is extended to even higher temperatures as a result of crystallization. Progressive crystallization tightly constrains the amorphous chains between the crystals and, as a consequence, the material embrittles.

Up to now, most attempts to improve the mechanical properties of PHB have focused on incorporating comonomers, such as 3-hydroxyvalerate^{5,21,22} and 4-hydroxybutyrate²³⁻²⁵, by using specific additives in the growth medium of the bacteria. However, the mechanical improvements^{5,26} are at the expense of both production and processing costs. The additives are expensive and, owing to their toxicity in the culture, production yields are considerably lower than for PHB homopolymer. Moreover, the presence of comonomer affects the crystallization kinetics, giving rise to long processing cycle times. Even so, the copolymers constitute the present commercial production of Biopol[®] because of their superior mechanical behaviour.

Strikingly, we have shown that by using a simple annealing treatment PHB homopolymer can be toughened and ageing can be prevented to a large extent. This considerably enlarges the applicability of PHB homopolymer, so that the copolymers might well become obsolete. The simple annealing treatment presented in this paper demonstrates that, contrary to the general opinion, annealing can be beneficial to the properties of semicrystalline polymers. Possibly, the beneficial action consists in relieving the constraints which are imposed on the amorphous chains by the crystals. In the near future, a comprehensive study²⁷ will be presented including the verification of this hypothesis as well as

results concerning the kinetics of the annealing process in order to investigate the practical use of annealing to prevent the embrittlement of PHB.

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