Crystallinity of poly(β-hydroxybutyrate) using the Rietveld technique

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Provided the structure is known, a method has been developed using the Rietveld technique on X-ray powder data collected on a commercial diffractometer to determine the amount of crystallinity in a polymer film such as poly(β-hydroxybutyrate). This film was found to be 46% crystalline for a fresh sample and 60% when degraded.

(Keywords: bacterial polymer; X-ray diffraction; Rietveld technique)

INTRODUCTION

Poly(β-hydroxybutyrate), Biopol™ (ICI), a biodegradable polymer, is a bacterial energy store. The structure, first deduced by conformational analysis, was used[1] to interpret fibre X-ray photographs. Independent work[2] found a space group $P2_12_12_1$, $a=5.76$, $b=13.20$ and $c=5.96$ Å, with c corresponding to the fibre period, $R=0.13$. The two models are similar.

Polyhydroxybutyrate[2] crystallizes as 2_1 chains aligned in groups within an amorphous mass. It consists of well defined polymer chains stacked neatly in rows giving two-dimensional order, but is ill defined along the third c axis. Consequently ($hk0$) reflections are intense with the non-zero l ones weak.

Possible sources of errors in the X-ray data are poorly defined background, extraneous air scattering, scattering by sample holders and beam guides, and surface roughness. These may be accounted for by a polynomial function. The amorphous fraction may be modelled from the crystal structure with particle-size line broadening, and some disorder considerations. For a first approximation, the radial distribution function of the randomly oriented polymer represents the amorphous scattering.

Because poly(β-hydroxybutyrate) (PHB) biodegrades, it may be considered an environmentally friendly material. Typically PHB, precipitated from $CHCl_3$ solution, has about 64% crystallinity[3]. This current work using X-ray powder diffraction measures the crystallinity content of the polymer.

EXPERIMENTAL

In order to carry out this measurement, a solution of commercial polyhydroxybutyrate powder (ICI Biopol™) in $CHCl_3$ was evaporated to dryness on a glass plate, leaving a flat, cast film approximately 100 μm thick. The polymer film was mounted in a Philips PW1050 diffractometer, and a diffraction pattern (Cu Kα, graphite monochromator) was recorded from 2θ 5° to 60° in 0.1° steps. A sample of this film was aged by leaving it exposed for 3 months when it became brown and brittle. The observed powder diffraction patterns are shown in *Figure 1*.

Using the reported structure[2], Rietveld refinements on fresh and degraded polymer data were carried out taking into account strain and crystal thickness (*Table 1*). In the Rietveld refinement, peaks broaden out with increasing 2θ when R_p is reduced to about 0.10, an effect labelled[4] 'anomalous peak broadening'. The sharpness of ($hk0$) and ($0k0$) compared with ($h0l$) and ($0kl$), especially evident in the 10–20° 2θ range, suggested some broadening attributed to particle size and c axis strain. The program DBW3.2[5] was modified to apply platelet size and c axis strain terms to the peak widths.

STRAIN ANALYSIS

This c axis distortion was determined using a peak width formula of Caglioti *et al.*[6] modified by Elcombe and Howard's[4] strain broadening term. The peak width formula was further modified for anisotropic thickness broadening using the Greaves relationship[7]. The pattern displays both effects as the (020), (110) and (040) peaks stand out very sharply, whereas the general trend of all other peaks is to broaden at a greater rate as 2θ increases. The sharpness depends on crystallite size, but strain gives peaks whose width is a function of 2θ.

Other anisotropic size/strain formulae do exist[8] but because the polymers studied have low crystallinity, these corrections were considered unnecessary although a good fit may be obtained by adding sufficient terms.

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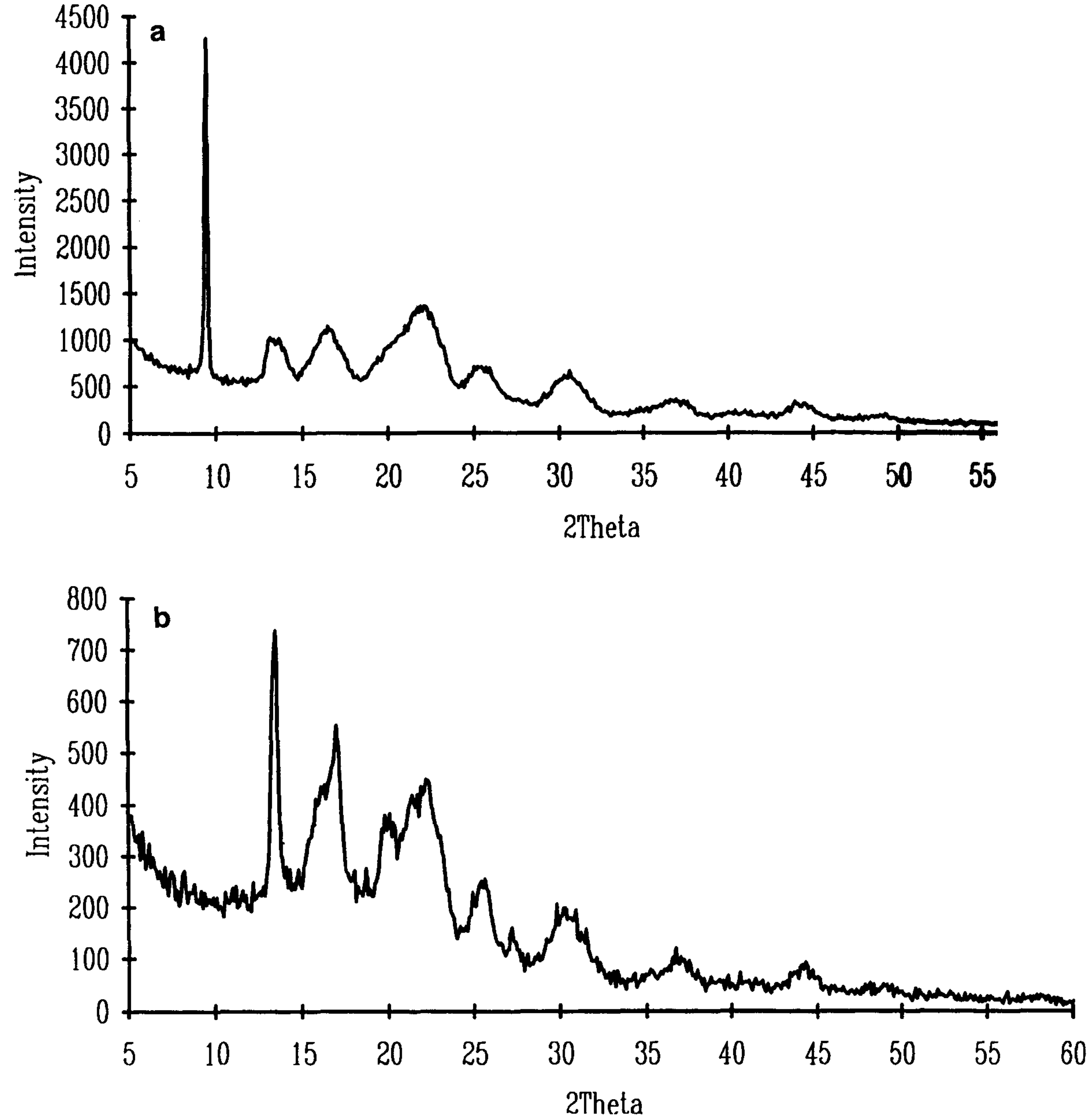

Figure 1 X-ray traces of (a) fresh polyhydroxybutyrate film as cast, (b) degraded polyhydroxybutyrate film

Crystallinity, X, is given by

$$X\% = \Sigma I_x \times 100/(\Sigma I_x + \Sigma I_a)$$

where: ΣI_x = the scattered intensity from the crystalline phase, and ΣI_a = the scattered intensity from the amorphous phase is simplified from ref. 10.

Cell occupancy, volume, molecular weight and mass absorption coefficient are common to both the crystalline and the amorphous materials. This analysis determines the fraction crystallinity in a single phase, in which the formula of Taylor and Matulis[10] reduces to a ratio of the determined Rietveld scale factors. These scale factors relate to the number of unit cells of each phase in the sampled volume.

A 64% crystallinity has been reported[3] for polyhydroxybutyrate, which increases with degradation since the amorphous material reacts preferentially. The Rietveld technique is consistent with previous findings and in addition offers an error analysis (*Table 1*). The large observed estimated standard deviations, ESDs, are probably due to the low count rates, and the shortcomings of the existing profile shape functions (PSFs). The latter problem may be overcome with two PSFs, one to describe the high 2θ and the other for low 2θ sides of the diffuse scatter/diffraction peaks[11].

The Rietveld refinements indicated expansion of a and contraction of b and c, probably due to a rotation of the chains about their axes. As no suitable standard was available for calibration of the diffractometer, values for size and stress/strain analysis are only indicators. However, the degraded sample has lower a-axis and c-axis strain, and smaller crystallites. Particle size derived from the peak width parameters U, W is larger for the degraded sample.

Static solid-state ^{1}H nuclear magnetic resonance (^{1}H n.m.r.) can also indicate[12] increased crystallinity in the degraded polyhydroxybutyrate film. The static solid-state ^{1}H n.m.r. signal consists of two absorptions. The amorphous fraction of the polymer has a broad absorption band. The crystalline signal is sharp due to a limited

Table 1 Rietveld refinement for fresh and degraded films

Parameter	Crystalline fraction	Amorphous fraction
Rietveld refinement for fresh polyhydroxybutyrate film		
Summed peak intensities	24 177(113) $X\% = 46.5(3)\%$	27 780(57)
Preferred orientation, P	0.68	0.81
Overall isotropic thermal, B	−4.9	27.4
c-axis strain, Δc	0.48	0.01
a-axis strain, Δa	0.29	0.01
Crystallite thickness, t	5×10^{14}	75×10^3
Gaussian peak-width quadratic, U	57.79	
Gaussian peak-width constant, W	−0.67	36.52
Lorentzian peak-width component, g	−0.08	
Peak asymmetry factor, A	−0.04	
R_{Bragg}	2.96	1.91
$a = 6.03$ $b = 12.70$ $c = 5.94$ $R_p = 9.60$ Goodness of fit = 23.5		
Rietveld refinement for degraded polyhydroxybutyrate film		
Summed peak intensities	86 627(214) $X\% = 60.3(2)\%$	57 077(65)
Preferred orientation, P	0.76	0.81
Overall isotropic thermal, B	5.1	15.9
c-axis strain, Δc	0.21	0.01
a-axis strain, Δa	−0.12	0.01
Crystallite thickness, t	-5×10^{11}	75×10^3
Gaussian peak-width quadratic, U	28.32	
Gaussian peak-width constant, W	0.98	75.90
Lorentzian peak-width component, g	0.02	
Peak asymmetry factor, A	−0.49	
R_{Bragg}	4.67	2.33
$a = 6.16$ $b = 12.62$ $c = 5.81$ $R_p = 7.58$ Goodness of fit = 37.2		

range of conformations available to the polymer chain. In order to determine the areas of the signals from the amorphous and crystalline fractions, the spectra were curve-fitted with two Voigt functions. In the fresh film, the broader amorphous peak had an area of 1.9×10^8 units, while the area of the crystalline peak was 1.6×10^7 units. This gives an amorphous:crystalline ratio of 11.9:1. The degraded polyhydroxybutyrate ^{1}H n.m.r. spectrum similarly yielded an amorphous:crystalline ratio of only 8.6:1.

Despite higher X-ray crystallinity of the fresh film, the crystallites possess greater strain, leading to a greater range of conformations of the polymer chains, and to n.m.r. peak broadening. The smaller particle size observed in the degraded sample gives an indication of the loss of amorphous matter. The larger 'preferred orientation correction' and 'overall isotropic thermal parameter' are indicators of crystallographic disorder observed in the degraded sample and differ with the n.m.r. These correction factors measure lost long-range order, while the shorter-range order seen in n.m.r. increases with the greater freedom from strain of the system.

Gel-permeation chromatography molecular weight determinations were carried out with $CHCl_3$ solutions of the polymers, and standardized against polystyrene[13]. Polyhydroxybutyrate fresh film was found to have $M_w = 505$ kDa, $M_w/M_n = 4.44$, in accordance with Carswell[14], and the degraded sample had $M_w = 641$ kDa, $M_w/M_n = 6.32$.

Differential scanning calorimetry (d.s.c.) on both samples gave melting points of 161°C for fresh film and 159°C for the degraded film. On repeated heating/cooling cycles from 30 to 250°C at 60°C min^{-1}, with 5 min recycle times, the melting point dropped consistently by 7°C steps between cycles, after the second interval. A slightly faster rate of 10°C per cycle was observed for the degraded film. The endotherm was seen to split into two peaks during the repeats. The separate endotherms have been attributed to other crystalline phases of the material, possibly a β-form proposed by Orts *et al.*[15]. Temperature changes the cell dimensions[16]. The shift in temperature is due to the loss of 'memory' of the material through repeated melts. The apparent faster decrease of the degraded sample's melting point is facilitated by its greater molecular weight dispersion with smaller fragments drifting away from the crystalline matrix in the melt.

CONCLUSIONS

Provided the structure is known, this method may be applied to other commercially important polymers, e.g. polyethylene films and polypropylene casts. These structures may either be determined experimentally by interpreting fibre diagrams or by calculations using molecular mechanics.

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