

# Synchrotron Radiation Laue Diffraction for the Time-Resolved Study of a Transformation in Crystals of $P_4N_4Cl_8$

Graham M. T. Cheetham,\* Paul D. Carr,† Ian M. Dodd, Benson M. Kariuki‡ and Marjorie M. Harding

Chemistry Department, Liverpool University, PO Box 147, Liverpool L69 3BX, UK

(Received 11 August 1995; accepted 12 September 1995)

Experiments are described to show some of the potential of the synchrotron radiation Laue method for the study of structural change within single crystals. In the metastable tetragonal crystals of  $P_4N_4Cl_8$  the eight-membered  $P_4N_4$  ring is in a boat conformation, with symmetry  $\bar{4}$ . On heating to *ca* 340 K the crystals transform, slowly, to a second tetragonal form in which the ring conformation is a chair, its symmetry  $\bar{1}$ . Both structures are known [Hazekamp, Migchelsen & Vos (1962). *Acta Cryst.* **15**, 539–543; Wagner & Vos (1968). *Acta Cryst.* **B24**, 707–713]. In the transformation the molecular packing, unit-cell dimensions and crystal quality remain almost unchanged. To study this transformation, series of Laue diffraction patterns were recorded at 2–3 min intervals over a period of 30–40 min, while the temperature was raised to 373 K. For two series, reflection intensities were measured and they allowed determination and refinement of the fraction of boat and chair molecules present in a mixed boat/chair model of the structure. No significant change in the crystal occurs below *ca* 340 K; at or above 340 K, 40–50% of the molecules are converted from boat to chair conformations within 5 min, but the remainder of the conversion is much slower, even when the temperature is raised towards 370 K.

**Keywords:** Laue diffraction; phase transformations; time-resolved studies.

## 1. Introduction

With the advent of synchrotron radiation came a renewed interest in the Laue method for the recording of X-ray diffraction patterns for the measurement of intensities. The method is able to provide a very large number of intensity measurements in a very short time; outstanding examples include 60 000 reflection intensities from a virus crystal from a single short exposure (Shrive, Clifton, Hajdu & Greenhough, 1990) and very recently, in the work of Moffat and colleagues (Ursby *et al.*, 1995), data from a myoglobin crystal with a 60 ps exposure time at the European Synchrotron Radiation Facility.

Intensity measurements of good quality can be made from Laue diffraction patterns, although in some cases there is a problem with the completeness of the data set. The method should therefore have considerable potential for time-resolved studies of processes involving changes within single crystals. This has proved to be a more elusive goal; it requires high crystal quality throughout the experiment, and detailed consideration and control of the kinetics of the process as it occurs through the whole extent of the crystal. Despite these stringent requirements, a few groups have

succeeded in obtaining electron-density maps showing short-lived reaction intermediates in protein crystals (Singer, Smalås, Carty, Mangel & Sweet, 1993; John *et al.*, 1993; Duke, Wakatsuki, Hadfield & Johnson, 1994). For simpler crystals the requirement of good crystal quality throughout the process of change is difficult to achieve, because most chemical or conformational changes involve some change in cell dimensions. Euler, Gilles & Will (1994) are involved in a study of cation sites in olivine at elevated temperatures. Some qualitative observations have been made on DL-norleucine crystals (Harding, Kariuki, Williams & Anwar, 1995), and the Laue method with time-of-flight neutron diffraction has been proposed as suitable for a study of phase transitions (Wilson, 1995).

Our objective here was to record single-crystal Laue diffraction data at short time intervals over a quite long period of time in order to investigate structural change in crystals of a phosphonitrilic halide,  $P_4N_4Cl_8$ . The crystals were very kindly provided by Professor K. Venkatesan (Bangalore), who suggested their suitability for study. This compound occurs in two crystal forms (see Table 1 and Fig. 1), described by Murthy, Guru Row & Venkatesan (1987), Wagner & Vos (1968) and Hazekamp, Migchelsen & Vos (1962). In the form that we shall call the *B* form (boat, also known as *K*), the eight-membered  $P_4N_4$  ring adopts a boat conformation and the molecular symmetry is  $\bar{4}$ . In the other *C* form (chair, also known as *T*), it has a chair conformation, molecular symmetry  $\bar{1}$ . The *C* form is stable at room

\* Current address: MRC Laboratory for Molecular Biology, Hills Road, Cambridge CB2 2QH, UK.

† Current address: Research School of Chemistry, Australian National University, Canberra, ACT 0200, Australia.

‡ Current address: School of Chemistry, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK.

**Table 1**

Unit-cell data at 298 K for  $P_4N_4Cl_8$  (from Murthy, Guru Row & Venkatesan, 1987).

	$a$ (Å)	$c$ (Å)	$Z$	Space group
<i>C</i> form (or <i>T</i> )	15.320 (2)	5.992 (1)	4	$P4_2/n$
<i>B</i> form (or <i>K</i> )	10.845 (2)	5.965 (1)	2	$P4_2/n$
Unit-cell transformation of the <i>B</i> form gives	15.337 (3)	5.965 (2)	4	$C4_2/a$

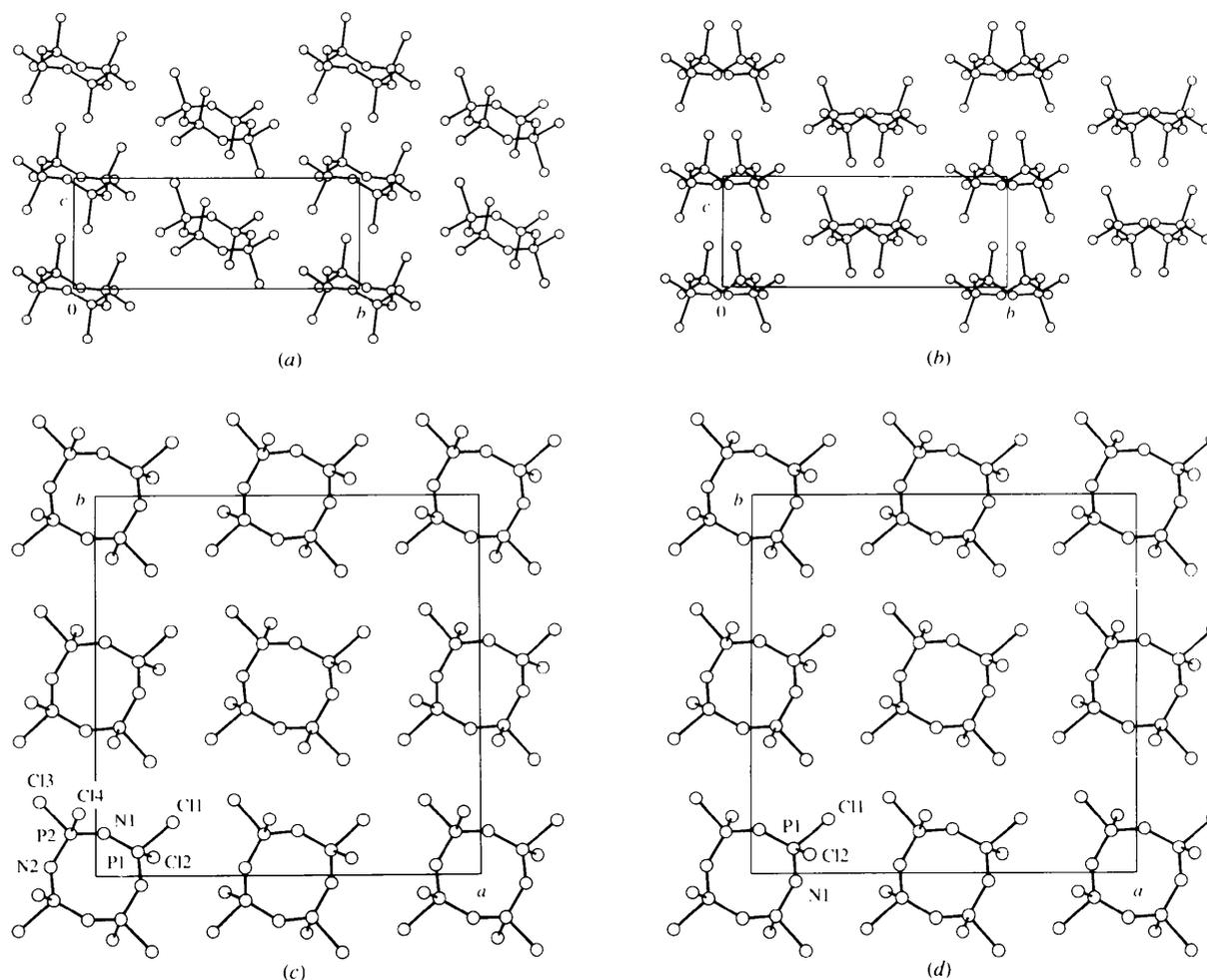
temperature, the *B* form metastable; when the *B* form is heated to temperatures at or above *ca* 337 K an irreversible transition takes place giving the *C* form. Positional and vibrational parameters for both forms have been refined with diffractometer data at several temperatures (Venkatesan, 1989; Bürgi & De Ridder, 1994), and these data were made available to us.

Carr, Cheetham, Harding & Rule (1992) describe the preliminary experiments in which a TV detector and video recorder were used to give a continuous record of the diffraction pattern of a crystal, initially in the *B* form, while the temperature was raised from 293 to 343 K in a period of

10 min. This clearly showed the appearance of additional spots in the diffraction pattern at *ca* 337 K, and gradual changes in the relative intensities of existing spots. To follow the process of the change in more detail it was necessary to record film packs in order that indexed reflection intensities might be obtained.

## 2. Data-collection strategy

The Laue method allows a large number of reflections to be recorded in a single short exposure. The fraction of the unique reflections recorded depends on the crystal orientation, symmetry,  $d_{\min} [= (\lambda/2 \sin \theta)_{\min}]$ , the detector size and distance from the crystal, and the useful wavelength range available. It can be estimated with the program *LCHK* (Elder, 1986) in the *Daresbury Laue Software Suite* (Campbell, 1993). Some aspects of optimizing these fractions have been summarized by Harding (1995) and others discussed by Clifton, Elder & Hajdu (1991). For  $P_4N_4Cl_8$ , with diffraction symmetry  $4/m$ , some possibilities are shown in Table 2. They were calculated using the standard detector size (film, exposed area of radius 60 mm),

**Figure 1**

(a) *a*-axis view of chair-form crystals, (*C*), (b) *a*-axis view of boat-form crystals, (*B*), (c) *c*-axis view of chair-form crystals, (*C*), (d) *c*-axis view of boat-form crystals, (*B*).

**Table 2**

Numbers of unique reflection intensities which could in theory be recorded on one Laue diffraction pattern for a crystal of  $P_4N_4Cl_8$  in the *C* form (chair).

(a)  $\lambda_{\min} = 0.45 \text{ \AA}$  (as on SRS station 9.5)

$\varphi_x$ (°)	$\varphi_y$ (°)	$\varphi_z$ (°)	$d_{\min} = 1.0 \text{ \AA}$		$d_{\min} = 0.75 \text{ \AA}$	
			Number	Fraction of total	Number	Fraction of total
0	0	0	321	0.389	595	0.316
25	25	0	507	0.614	940	0.499
43.6	0.6	-5.7 (crystal G2)	408	0.494	792	0.421
135.4	-0.7	-17.7 (crystal G4)	503	0.609	958	0.509

(b)  $\lambda_{\min} = 0.25 \text{ \AA}$  (as on SRS station 9.7)

$\varphi_x$ (°)	$\varphi_y$ (°)	$\varphi_z$ (°)	$d_{\min} = 1.0 \text{ \AA}$		$d_{\min} = 0.75 \text{ \AA}$	
			Number	Fraction of total	Number	Fraction of total
0	0	0	421	0.510	894	0.475
25	25	0	615	0.745	1343	0.714

$\varphi_x$ ,  $\varphi_y$  and  $\varphi_z$  are the rotations around *x*, *y* and *z* for a crystal initially set with *a* parallel to *x* and the X-ray beam, *c* parallel to *z* and the spindle. Crystal-to-film distance, 55 mm; radius of exposed area on film 60 mm; maximum wavelength 2.0 Å; calculation made with the program *LCHK* (Elder, 1986; Campbell, 1993).

the closest practicable crystal-to-film distance (*ca* 55 mm) and the wavelength ranges available on the two SRS workstations that can be used for Laue experiments. In practice, the fraction quoted is difficult to achieve. Some reflections (up to 17%) are present as components of multiples; it is possible to separate many of these (Campbell & Hao, 1993; Hao, Harding & Campbell, 1995), but the resulting intensity values are not as accurate as those of the singles. A few reflections may be lost as spatial overlaps.  $d_{\min}$ , found with Laue data, is often a little higher (*e.g.* by 0.1 or 0.2 Å) than the best achieved in monochromatic experiments. Nevertheless, for these crystals optimum choice of orientation should give 50–75% of the unique diffraction data. The design of the furnace used for crystal heating (Bhat, Clark, el Korashy & Roberts, 1990) restricted the range of crystal orientations accessible, so that the optimum was never achieved. The use of a nitrogen gas stream heater with a crystal mounted normally on a goniometer head would almost certainly be more satisfactory, but was not available to us at the time.

### 3. Experimental

#### 3.1. Laue data collection

Crystals with dimensions *ca* 1 × 0.5 × 0.5 mm were held between two kaptan sheets in a small electrically heated furnace (Bhat, Clark, el Korashy & Roberts, 1990). In some cases one end of the crystal was glued to the kaptan sheet with epoxy adhesive in order to prevent it moving and fix it in a useful orientation. On SRS workstation 9.7 the furnace could be mounted on a goniometer head on the Laue camera; on workstation 9.5 it was simply placed on a support at the desired position. The temperature, measured by a thermocouple inside the furnace and *ca* 2 mm from the crystal, was slowly raised, and controlled by adjusting the heating current in the furnace. In addition to two series of

observations made with a TV detector and video recorder (see Carr, Cheetham, Harding & Rule, 1992), more than seven series of film packs were recorded for different crystals, heated at different rates to or through the transition temperature region. Of these, crystals *G2* and *G4* gave the results quoted in detail below. They were taken on workstation 9.5, with the SRS running at *ca* 200 mA, exposure time 30 ms for *G4* (1.5 s for *G2*, because of a problem in beamline optics), 0.2 mm collimator, crystal-to-film distance 56 mm and 0.2 mm Al attenuator in the incident beam to reduce the intensity of longer wavelength radiation (helpful for processing and for reducing radiation damage). The crystals were heated at approximately 2 K min<sup>-1</sup>.

#### 3.2. Laue data processing

All the films were digitized, and processed with the *Daresbury Laue Software Suite* (Campbell, 1993), using *GENLAUE* to find and refine the orientation, *INTLAUE* to integrate the reflection intensities, and *AFSCALE* to scale the films of a pack together. Wavelength normalization was carried out separately for each film pack by *LAUESCAL*, using calculated *F*'s for either *B* or *C* form as the standard data. The processing is summarized in Table 3.

#### 3.3. Monochromatic data

Monochromatic data sets were used in the interpretation of the mixed-crystal Laue data sets. They were recorded for three crystals on a Rigaku AFC6S diffractometer at Liverpool University. One crystal, *monoC*, was heated at 373 K for 3 min, allowed to cool and mounted on the diffractometer; a second, *monoBC*, was heated at 345 K for 3 min, cooled and mounted on the diffractometer; the third, *monoB*, was not heated at all. For all these data collections the larger unit cell was used, *i.e.* *a* = *ca* 15.3 Å. Details are given in Table 4.

**Table 3**

Details of Laue data processing.

	Crystal <i>G4</i>	Crystal <i>G2</i>
Number of film packs	11	14
$d_{\min}$ (Å)	0.75–0.77	0.79–0.80
$\lambda_{\min}$ (Å)	0.44	0.44
$\lambda_{\max}$ (Å)	2.0	2.0
<i>GENLAUE</i> mean r.m.s.* (mm)	0.07	0.06
$\varphi_x$ (°)	135.4	43.6
$\varphi_y$ (°)	–0.7	0.6
$\varphi_z$ (°)	–17.7	–5.7
<i>AFSCALE</i> mean <i>R</i> factor	0.047	0.053
<i>LAUESCAL</i> $\lambda$ ranges (Å)	0.44–0.475	0.44–0.475
	0.505–0.905	0.505–0.905
$R_{\text{scale}}^\dagger$	0.13–0.49	0.19–0.41
Number of single-reflection intensity measurements with $I > 3\sigma(I)$ per film pack (a) below 338 K	255–270	185–205
(b) above 338 K	325–360	280–320
Number unique (a) below 338 K	131–138	71–80
(b) above 338 K	162–180	117–134

\*Root-mean-square displacement of observed from predicted spot centres. †Good agreement of observed and calculated  $F$ 's is only to be expected at the ends of the series where the crystal corresponds to a pure chair or boat; at intermediate crystal compositions the wavelength normalization curve should nevertheless be approximately correct.

### 3.4. Interpretation of Laue diffraction data

In both series of Laue intensity measurements the number of observed reflections rose sharply at *ca* 340 K, and there were gradual changes in reflections from there to the ends of the series (Fig. 2). At the end, the intensities correspond approximately to those of the chair crystal. The number of intensity measurements at any one temperature was not sufficient for refinement of all structural parameters,  $xyz$  and  $U_{ij}$ , for either crystal form alone, let alone a mixed crystal. A model was set up which treated the crystal as a random mixture of boat and chair forms, with geometry and  $U_{ij}$  based on those of the separate crystals, *monoB* and *monoC*; the proportion of boat (or chair) form, together with the overall scale factor could be refined.

In the separate boat or chair crystals there are stacks of molecules parallel to  $c$  (Fig. 1). Inspection of packing diagrams and contact distances shows that it is not possible to have a stack in which boat and chair conformation molecules are randomly mixed, but it should be easy to form a crystal containing a random mixture of boat and chair stacks. The positions of the molecular centres are approximately the same, whether the molecules are in the boat or chair conformation, with only a small displacement along  $z$  allowed. In the mixed boat/chair model the crystal symmetry was treated as  $P4_2$ , with  $4_2$  screw axes along 0.25, 0.75,  $z$  and 0.75, 0.25,  $z$ , since this symmetry element is common to both *B* and *C* forms. The coordinates of the boat molecule were taken from those of the pure boat form (refinement of monochromatic data), constrained as a rigid body, and with the  $\bar{4}$  axis parallel to  $z$  and at the coordinate origin. The coordinates of the chair molecule were taken

**Table 4**

Experimental details of monochromatic data collections.

For <i>monoB</i> , <i>monoC</i> and <i>monoBC</i>			
Radiation, wavelength (Å)	Mo $K\alpha$ , 0.71069		
Diffractometer	Rigaku AFC6S		
Temperature (K)	295		
Unit cell from	25 reflections, $\theta = 25$ –30°		
Absorption coefficient (mm <sup>–1</sup> )	2.10		
Data collection, $\theta_{\max}$ (°)	$\omega/2\theta$ scans, 30		
Range of $h, k, l$	0–22, 0–22, 0–8		
Standard reflections	3, at intervals of 150		
	<i>monoB</i>	<i>monoC</i>	<i>monoBC</i>
Crystal dimensions (mm)	0.5 × 0.18 × 0.18	0.5 × 0.3 × 0.3	0.45 × 0.45 × 0.25
Unit-cell dimensions (Å) <i>a</i>	15.343 (3)	15.328 (1)	15.334 (1)
(tetragonal) <i>c</i>	5.965 (5)	5.990 (2)	5.974 (5)
Absorption correction, $\psi$ scans			
$T_{\min}$ – $T_{\max}$	0.844–1.000	0.861–1.000	0.812–1.000
Variation of standard reflections (%)	<2	<1	8
(decay correction applied)			

from those of the pure chair form (refinement of monochromatic data), constrained as a rigid body, with the inversion centre on 00 $z$ . The  $U_{ij}$  values for each molecule, derived from the refinement with monochromatic data, were multiplied by 1.135 to make them more appropriate for temperatures in the region of 343 K; the factor was estimated by comparison of the room temperature and 323 K results of Bürgi & De Ridder (1994) for the *B* form. Obviously, this is only an approximate treatment, but it appeared to be adequate for the purpose.

The three monochromatic data collections described above were helpful in the setting up of this model, described as the mixed boat/chair model. The coordinates and  $U_{ij}$  values used were derived from the data sets *monoB* and *monoC*, by conventional refinement of each with the proper space-group symmetry; the coordinates did not differ significantly from those of Venkatesan (1989) or Bürgi & De Ridder (1994). The results of these refinements are included in Table 5.

The data set *monoBC* apparently corresponded to a mixed crystal, as shown by the refinement of the occupancies of boat and chair (sum = 1.0), together with  $z_{\text{chair}}$ , the displacement of the chair molecule parallel to  $z$ ; initially, the inversion centre of the chair molecule was placed at the origin defined by  $\bar{4}$  for the boat molecule. Further, there are four symmetry-equivalent ways in which a chair molecule of symmetry  $\bar{1}$  may result from a boat molecule of symmetry  $\bar{4}$ . Two of these, related by a mirror plane perpendicular to  $c$ , are plausible on packing grounds, and were both included in the model to be refined. The other two are much less plausible on packing grounds, and structure-factor calculation and attempted refinement showed that they are not helpful in the description of the mixed crystal. Thus, the final mixed boat/chair model included the parameters of one boat and two chair molecules, present in the fractions  $b$ ,  $c_1$  and  $c_2$ . The coordinates are listed in Table 6, and the results of the refinement with the *monoBC*

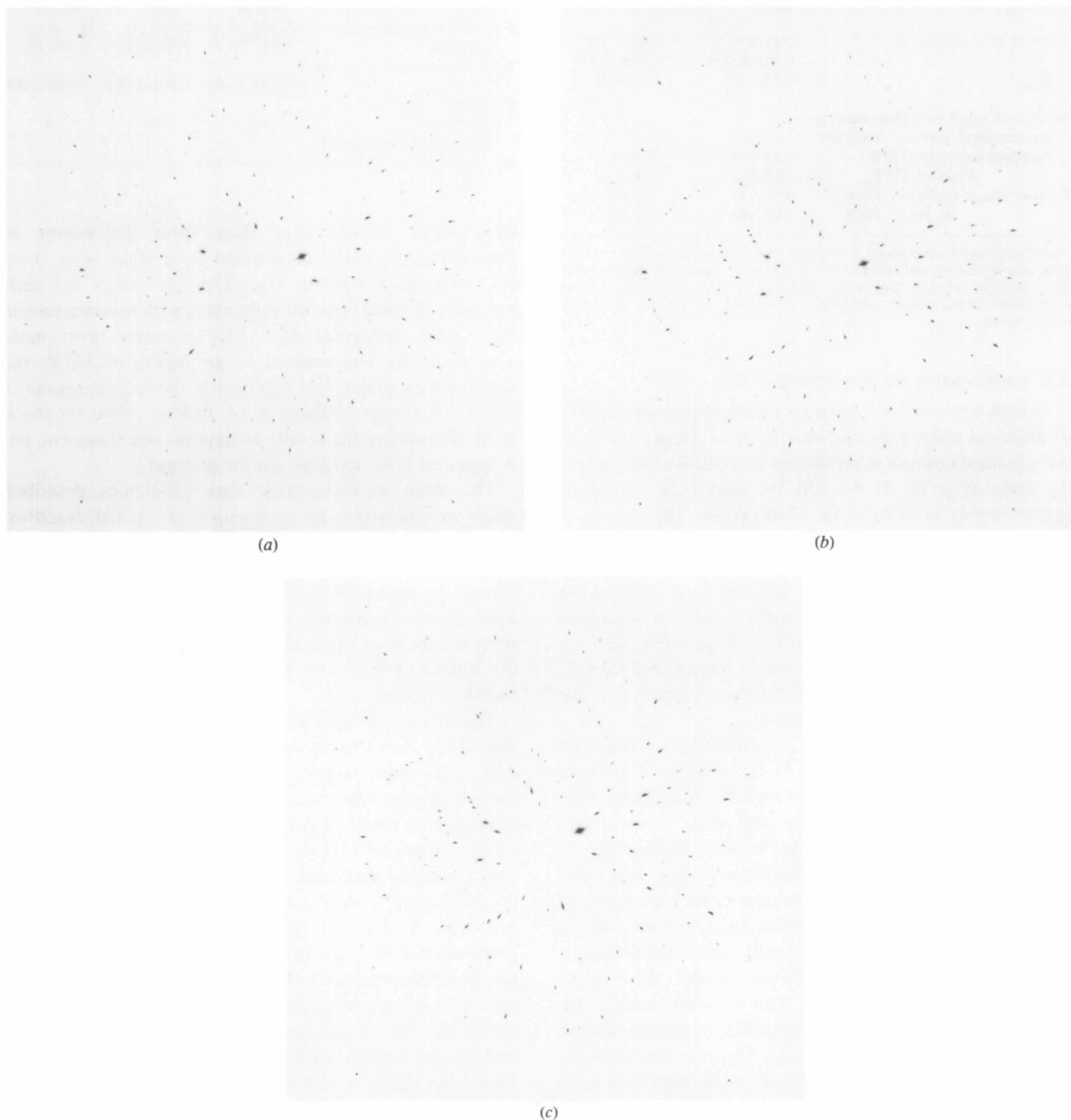
data in Table 5. Since the results of the refinement were not as good as those of the equivalent pure boat and chair forms, ways of improving the model were explored extensively. Electron-density difference maps were examined. A simple program to derive scale,  $k$ , and site occupancy of boat,  $p$ , to fit the observed data as

$$F_{\text{obs}}^2 = (1/k)[pF_{\text{boat}}^2 + (1-p)F_{\text{chair}}^2]$$

was tried. This should be appropriate if the crystal consists of large domains of boat and large domains of chair form.

None of these gave significantly better agreement than the mixed boat/chair model described first, and so that was accepted.

The effect of refinement of the occupancies  $b$ ,  $c_1$  and  $c_2$  using the monochromatic data sets for nominally pure boat and chair forms was checked; the results are shown in Table 5. Table 5 shows first that the mixed boat/chair crystal model gives acceptable agreement ( $R = 0.07, 0.09$ ) with the monoB and monoC data, both when scale only is refined (ii), and when scale and the occupancies  $b$ ,  $c_1$  and  $c_2$  are



**Figure 2** Laue diffraction patterns of crystal G2 at (a) 335, (b) 350 and (c) 370 K. For experimental details see text.

**Table 5**

Results of refinement of different crystal models with the monochromatic data sets.

Data set	Number of reflections Number of unique reflections Number with $F > 4\sigma(F)$	Model, parameters refined (in addition to overall scale factor)	$R$ [for $F > 4\sigma(F)$ ]	$wR_2$ (all)	Occupancies found
monoB	2385	(i) Boat molecule in $C4_2/a$ ; refine $x, y, z, U_{ij}$ (four atoms)	0.038	0.199*	
	2232	(ii) Mixed boat/chair crystal in $P4_2$ (no variable parameters)	0.072	0.326*	$b = 1.0$ , fixed $c_1 = c_2 = 0$ , fixed)
	816*	(iii) Mixed boat/chair crystal in $P4_2$ ; refine $b, c_1, c_2$	0.073	0.368*	$b = 0.955$ (3) $c_1 = 0.037$ (2) $c_2 = 0.008$ (3)
monoC	2019	(i) Chair molecule in $P4_2/n$ ; refine $x, y, z, U_{ij}$ (eight atoms)	0.047	0.127	
	1827	(ii) Mixed boat/chair crystal in $P4_2$ (no variable parameters)	0.090	0.237	$b = 0, c_2 = 0$ , fixed $c_1 = 1.0$ , fixed)
	1459	(iii) Mixed boat/chair crystal in $P4_2$ ; refine $b, c_1, c_2$	0.091	0.240	$b = 0.076$ (4) $c_1 = 0.923$ (6) $c_2 = 0.001$ (4)
monoBC	2034	(i) Mixed boat/chair crystal in $P4_2$ ; refine $b, c_1, c_2$	0.133	0.323	$b = 0.528$ (7) $c_1 = 0.468$ (6) $c_2 = 0.004$ (4)
	1910				
	1460				

$wR_2 = \{\sum[w(F_o^2 - F_c^2)^2] / \sum[w(F_o^2)^2]\}^{1/2}$ . \*This data set contains many reflections with intensities near zero because intensities have been measured at the reciprocal lattice points which should be absent by  $C$ -face-centring. The effect on these numbers should be noted.

refined (iii); the agreement is not as good as in the full refinement because of the approximation made with the  $U_{ij}$  values. Secondly, it can be seen that when this model is used with the monoBC data it is tolerably successful and indicates a mixed crystal. The agreement ( $R = 0.13$ ) is not as good as that with monoB or monoC, and the implication is that in some respect the model is not entirely satisfactory. However, since no better model could be found it was used for this preliminary interpretation of the Laue data series.

Using each Laue data set in turn and these model parameters, the overall scale and the occupancies  $b, c_1$  and  $c_2$  were then refined. Another problem emerged at this stage. While the crystal  $G2$  corresponded to pure boat form at the start of the experiment,  $G4$  appears to have corresponded to ca 25% chair form at the start, and the other crystals used, to between 60 and 100% chair form, at the start of the experiment. Results are therefore presented (Table 7) for crystals  $G2$  and  $G4$  only.

The atom coordinates and atomic displacement parameters derived from refinement with the monochromatic data sets monoB, monoC, and the tables of observed and calculated  $F$ 's resulting from refinement with all three monochromatic data sets, together with the two series of Laue data sets  $G2$  and  $G4$  have been deposited.\* Early trials were performed with *SHELX76* (Sheldrick, 1976), but the final refinements were carried out with *SHELXL* (Sheldrick,

1993), refining on  $F^2$ , and using the atomic scattering factors therein.

#### 4. Results and discussion

The results for crystals  $G2$  and  $G4$  are given in Table 7 and Fig. 3. Crystal quality and data quality are maintained throughout both series, and there is no streaking along reciprocal lattice rows. For both crystals there is no significant change below 338 K, but just above this temperature there is a sudden increase in the number of reflections of significant magnitude with  $h+k$  odd, characteristic of the chair form. In  $G2$ , ca 40% of the part of the crystal under study is then converted to chair form within ca 4 min, and at a temperature not higher than 345 K. In crystal  $G4$  ca 25% chair form appears to have been present initially, but again in the vicinity of the transition temperature, a further 30% is quickly converted. In both cases the remaining conversion is slow and is not quite complete even when the temperature has reached 373 K, 12–20 min later. Combined with the observations made on two further crystals, using TV detector and video recorder (Carr, Cheetham, Harding & Rule, 1992), it is clear that the transformation from boat to chair crystals occurs continuously, with the maintenance of good crystal quality throughout. The data recorded here also confirm that the orientation of the chair crystal, relative to the boat crystal from which it is formed, is that which would be guessed from the packing diagrams (Fig. 1).

There are not yet sufficient data to give a detailed picture of the mechanism of the transformation. Although the

\*Lists of structure factors, anisotropic displacement parameters and complete geometry have been deposited with the IUCr (Reference: HE0138). Copies may be obtained through The Managing Editor, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

**Table 6**

Atom coordinates ( $\times 10^4$ ) and equivalent isotropic displacement parameters ( $\text{\AA}^2 \times 10^3$ ) in final mixed boat/chair model.

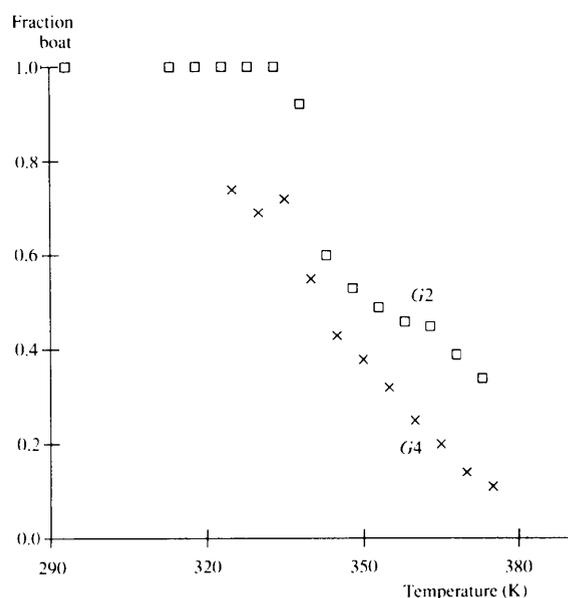
	<i>x</i>	<i>y</i>	<i>z</i>	$U_{eq}$
<b>Boat molecule 1</b>				
P(1)	1100	651	599	30
Cl(1)	2003	1426	-726	54
Cl(2)	1513	490	3728	53
N(1)	1157	-216	-783	41
P(11)	-1100	-651	599	30
Cl(11)	-2003	-1426	-726	54
Cl(12)	-1513	-490	3728	53
N(11)	-1157	216	-783	41
P(21)	651	-1100	-599	30
Cl(21)	1426	-2003	726	54
Cl(22)	490	-1513	-3728	53
N(21)	-216	-1157	783	41
P(31)	-651	1100	-599	30
Cl(32)	-1426	2003	726	54
Cl(33)	-490	1513	-3728	53
N(31)	216	1157	783	41
<b>Chair molecule 1</b>				
P(1)	1086	622	-2234	30
Cl(1)	1991	1405	-950	56
Cl(2)	1482	477	-5375	59
P(2)	-645	1119	-957	27
Cl(3)	-1447	1962	-2422	49
Cl(4)	-432	1657	2025	58
N(1)	206	1130	-2342	45
N(2)	-1167	253	-662	40
P(11)	-1086	-622	606	30
Cl(11)	-1991	-1405	-678	56
Cl(12)	-1482	-477	3747	59
P(12)	645	-1119	-671	27
Cl(13)	1447	-1962	794	49
Cl(14)	432	-1657	-3653	58
N(11)	-206	-1130	714	45
N(12)	1167	-253	-966	40
<b>Chair molecule 2</b>				
P(1)	1086	622	2627	30
Cl(1)	1991	1405	1343	56
Cl(2)	1482	477	5768	59
P(2)	-645	1119	1350	27
Cl(3)	-1447	1962	2815	49
Cl(4)	-432	1657	-1632	58
N(1)	206	1130	2735	45
N(2)	-1167	253	1055	40
P(11)	-1086	-622	-213	30
Cl(11)	-1991	-1405	1071	56
Cl(12)	-1482	-477	-3354	59
P(12)	645	-1119	1064	27
Cl(13)	1447	-1962	-401	49
Cl(14)	432	-1657	4046	58
N(11)	-206	-1130	-321	45
N(12)	1167	-253	1359	40

The coordinates for the boat molecule are those resulting from refinement of mono*B* data, four independent atoms repeated by 4 symmetry. For the molecule chair1, the coordinates are derived from mono*C* data, eight independent atoms repeated by 1 symmetry, and then translated by  $\delta z$ ; the translation  $\delta z$  was derived from refinement with mono*BC* data which gave  $\delta z = -0.0807$  (15). The molecule chair2 is related to chair1 by a minor plane close to  $z = 0$ .  $U_{eq}$  is defined as one third of the trace of the orthogonalized  $U_{ij}$  tensor.

parameter  $c_2$  was refined, its value was always found to be not significantly different from zero. This and the absence of streaks along reciprocal lattice rows, suggest that once initiated within a grain or mosaic block, the transformation may be quickly completed within that block. (Random

transformation of single stacks of molecules should lead to  $c_1 = c_2$ ; if the regions which are ordered as *B* or *C* are very small, streaks should be seen along some reciprocal lattice row lines.) Thus, it seems probable that the transformation is comparatively slow because of a low rate of initiation in at least some of the grains.

These experiments show that it is practicable to record 14 or more single-crystal intensity data sets at 3 min intervals, to follow changes occurring in a crystal on this time scale, and draw conclusions about them. It should be possible to determine the rate of the change at different temperatures, and ascertain whether there is variation from crystal to crystal (several of our other experiment series were intended for this purpose, and it was only when the data were processed that it became clear that prior transformation had occurred). Many improvements can be envisaged in the future. The interval of 3 min is the time required to change the film cassette and complete the SRS safety procedures. A device is available which can record four successive film exposures at intervals of *ca* 30 s, but for more than four such exposures or for shorter time intervals a charge-coupled device detector should be used. In the experiments reported here the number of unique reflections measured with  $F > 4\sigma(F)$  is rather disappointing compared with the theoretical values (compare Tables 2 and 7). The loss was mainly due to the furnace design, which severely limited the accessible  $2\theta$  values; a gas stream heating device without such limitations could be a great advantage. A variety of additions and improvements have already been made to the Laue software since these data were processed (in 1990–1991), so it should now be possible to measure more reflection intensities with somewhat greater accuracy from each Laue exposure. But most important (and difficult) of

**Figure 3**

Fraction of molecules found to be in the boat form in crystals G2 (squares) and G4 (crosses), as a function of temperature. For crystal G2, 20 min elapsed while the crystal was heated from 333 to 373 K, for crystal G4, 27 min.

**Table 7**

Results of refinement of the mixed boat/chair crystal model with each of the Laue intensity data sets in turn.

Temperature (K)	Time (min)	Number of unique reflections with $F > 4\sigma(F)$	$R_1$	$wR_2$	$b$ (boat)	Occupancies	
						$c_1$ (chair 1)	$c_2$ (chair 2)
Crystal G2							
293	0	77 [2]*	0.20	0.47	(1.0)		
313	8	76 [3]	0.20	0.48	(1.0)		
318	10.5	73 [0]	0.17	0.40	(1.0)		
323	13	71 [0]	0.19	0.45	(1.0)		
328	15.5	75 [0]	0.22	0.46	(1.0)		
333	18	77 [0]	0.18	0.41	(1.0)		
338	20.5	80 [5]	0.21	0.48	0.92 (3)	0.08 (3)	0.00 (3)
343	23	123 [46]	0.20	0.48	0.60 (2)	0.34 (2)	0.06 (2)
348	25.5	132	0.19	0.43	0.53 (2)	0.42 (2)	0.05 (2)
353	28	134	0.17	0.40	0.49 (2)	0.47 (2)	0.04 (2)
358	30.5	133	0.19	0.44	0.46 (2)	0.48 (2)	0.06 (2)
363	33	122	0.19	0.42	0.45 (2)	0.50 (2)	0.06 (2)
368	35.5	113	0.18	0.43	0.39 (2)	0.54 (3)	0.06 (2)
373	38	117	0.18	0.43	0.34 (2)	0.61 (3)	0.06 (2)
Crystal G4							
325	0	140 [54]*	0.23	0.50	0.74 (2)	0.25 (1)	0.01 (1)
330	2	136 [51]	0.25	0.58	0.69 (2)	0.26 (2)	0.05 (2)
335	5	134 [50]	0.24	0.56	0.72 (2)	0.25 (2)	0.04 (2)
340	7	162 [76]	0.18	0.42	0.55 (2)	0.42 (2)	0.03 (1)
345	9	180 [87]	0.16	0.41	0.43 (2)	0.53 (2)	0.04 (2)
350	12	182	0.17	0.43	0.38 (2)	0.59 (2)	0.03 (2)
355	19	180	0.15	0.40	0.32 (2)	0.68 (2)	0.01 (2)
360	22	178	0.17	0.43	0.25 (2)	0.73 (3)	0.02 (2)
365	26	164	0.15	0.41	0.20 (2)	0.77 (3)	0.02 (2)
370	29	166	0.17	0.42	0.14 (3)	0.85 (4)	0.01 (2)
375	32	166	0.15	0.37	0.11 (2)	0.86 (3)	0.03 (2)

For crystal G2, at temperatures  $\leq 333$  K, the occupancies were set at  $b = 1.0$  (boat),  $c_1 = c_2 = 0$  (chair) and only the overall scale factor refined. For crystal G2 at temperatures  $> 333$  K, and for crystal G4 at all temperatures, the occupancies  $b$ ,  $c_1$  and  $c_2$  were refined as well as the overall scale factor.  $R_1 = \sum |F_o - F_c| / \sum (F_o)$ .  $wR_2 = \{ \sum [w(F_o^2 - F_c^2)^2] / \sum [w(F_o^2)^2] \}^{1/2}$ . \*Numbers in square brackets are the numbers of reflections with  $h + k = 2n + 1$  [and  $F > 4\sigma(F)$ ]; these reflections should be absent in the pure boat crystal.

all is the choice of crystals and control of the change taking place in them.

We are grateful to SERC (now EPSRC) for studentships (to GMTC and IMD) and financial support, and the provision of synchrotron radiation facilities, to many staff at Daresbury Laboratory for their assistance, to Professor K. Venkatesan for suggesting the study and supplying the crystals, and to him and Dr De Ridder for providing unpublished crystallographic data.

## References

- Bhat, H. L., Clark, S. M., el Korashy, A. & Roberts, K. (1990). *J. Appl. Cryst.* **23**, 545–549.
- Bürgi, H. B. & De Ridder, D. J. A. (1994). Personal communication.
- Campbell, J. W. (1993). *The Daresbury Laue Software Suite*. SERC Daresbury Laboratory, Warrington, UK.
- Campbell, J. W. & Hao, Q. (1993). *Acta Cryst.* **A49**, 889–893.
- Carr, P. D., Cheetham, G. M. T., Harding, M. M. & Rule, R. J. (1992). *Phase Transit.* **39**, 33–43.
- Clifton, I. J., Elder, M. & Hajdu, J. (1991). *J. Appl. Cryst.* **24**, 267–277.
- Duke, E. M. H., Wakatsuki, S., Hadfield, A. & Johnson, L. J. (1994). *Protein Sci.* **3**, 1178–1196.
- Elder, M. (1986). *Information Quarterly for Protein Crystallography*, No. 19. SERC Daresbury Laboratory, Warrington, UK.
- Euler, H., Gilles, R. & Will, G. (1994). *J. Appl. Cryst.* **27**, 190–192.
- Hao, Q., Harding, M. M. & Campbell, J. W. (1995). *J. Synchrotron Rad.* **2**, 27–30.
- Harding, M. M. (1995). In preparation.
- Harding, M. M., Kariuki, B. M., Williams, L. & Anwar, J. (1995). *Acta Cryst.* **B51**. In the press.
- Hazekamp, R., Migchelsen, T. & Vos, A. (1962). *Acta Cryst.* **15**, 539–543.
- John, H., Rensland, H., Schlichting, I., Vetter, I., Borasio, G. D., Goody, R. S. & Wittinghoffer, A. (1993). *J. Biol. Chem.* **268**, 923–929.
- Murthy, G. S., Guru Row, T. N. & Venkatesan, K. (1987). *Acta Cryst.* **A48**, C72–73.
- Sheldrick, G. M. (1976). *SHELX76. Program for Crystal Structure Determination*. Univ. of Cambridge, UK.
- Sheldrick, G. M. (1993). *SHELXL. Program for Crystal Structure Refinement*. Univ. of Göttingen, Germany.
- Shrive, A. K., Clifton, I. J., Hajdu, J. & Greenhough, T. J. (1990). *J. Appl. Cryst.* **23**, 169–174.
- Singer, P. T., Smalås, A., Carty, R. P., Mangel, W. F. & Sweet, R. M. (1993). *Science*, **259**, 669–673.
- Ursby, T., Bourgeois, D., Wulff, M., Pradervand, C., Srajer, V., Legrand, A., Schildkamp, W., Laboure, S., Rubin, C., Teng, T. Y., Roth, M. & Moffat, K. (1995). *Sixteenth European Crystallography Meeting (ECM16), Lund, Sweden*. Abstracts, p. 43.
- Venkatesan, K. (1989). Personal communication.
- Wagner, A. J. & Vos, A. (1968). *Acta Cryst.* **B24**, 707–713.
- Wilson, C. C. (1995). *J. Appl. Cryst.* **28**, 7–13.

## Microfocus X-ray Diffraction of Spherulites of Poly-3-hydroxybutyrate

A. Mahendrasingam,<sup>a</sup> C. Martin,<sup>a</sup> W. Fuller,<sup>a</sup> D. J. Blundell,<sup>b</sup> D. MacKerron,<sup>b</sup> R. J. Rule,<sup>c</sup> R. J. Oldman,<sup>c</sup> J. Liggat,<sup>d</sup> C. Riekkel<sup>e</sup> and P. Engström<sup>e</sup>

<sup>a</sup>Department of Physics, Keele University, Keele, Staffordshire ST5 5BG, UK, <sup>b</sup>ICI Films, PO Box No. 90, Wilton Centre, Middlesbrough, Cleveland TS6 8JE, UK, <sup>c</sup>ICI Chemicals and Polymers Ltd, PO Box No. 8, The Heath, Runcorn, Cheshire WA7 4QD, UK, <sup>d</sup>Zeneca Bio Products, PO Box No. 2, Billingham, Cleveland TS23 1YN, UK, and <sup>e</sup>ESRF, BP 220, F-38043 Grenoble CEDEX, France

(Received 9 June 1995; accepted 18 July 1995)

The microfocus X-ray beamline at the European Synchrotron Radiation Facility has been used to investigate the variation in molecular orientation and crystallinity in spherulites of the organic polymer poly-3-hydroxybutyrate (PHB). This is the first report of the correlation of optical and X-ray measurements on spherulitic polymer films where X-ray diffraction patterns have been recorded and displayed continuously in real time while the specimen was tracked in steps of 10  $\mu\text{m}$  across an incident X-ray beam with a diameter as small as 10  $\mu\text{m}$ .

**Keywords:** spherulites; X-ray diffraction; microfocus; polymers; PHB.

### 1. Introduction

X-ray diffraction provides one of the most powerful techniques for the investigation of polymer conformation and organization within partially ordered materials. From optical microscopy it is clear that the direction of molecular orientation within many polymer materials varies over dimensions much smaller than the  $\sim 100 \mu\text{m}$  beam diameter available from conventional rotating-anode X-ray sources and even from second-generation synchrotron radiation sources. The studies described here demonstrate that by exploiting the high degree of collimation and beam stability of the European Synchrotron Radiation Source (ESRF), together with microfocusing optics, it is possible to characterize the variation in molecular orientation and crystallinity within polymer materials with a spatial resolution as small as 10  $\mu\text{m}$ . The dramatic gains offered by the ESRF in conjunction with a CCD detector system and a high-precision computer-controlled X/Z stage are well illustrated by comparing the observations recorded in the experiments described here with those recorded in studies of crystallite orientation in spherulites using a conventional X-ray source and photographic recording (Barham, Keller, Otun & Holmes, 1984; Fujiwara, 1960).

The homopolymer poly-D(-)-3-hydroxybutyrate (PHB) used for this study is one of the series of optically active thermoplastic polymers made under the commercial name 'Biopol' by Zeneca Bio Products. It is produced by a wide variety of micro-organisms, being used in these organisms for energy and carbon storage. The biological rather than the petroleum origins of 'Biopol', its biodegradability, and its ability to form films with the properties of conventional

thermoplastics give it technological importance as well as fundamental interest (Holmes, 1988). The purity of this polymer leads to an absence of heterogeneous nuclei and gives it the capacity to form large spherulites of high crystallinity.

### 2. Experimental techniques

The microfocus instrumentation on beamline ID13 at the ESRF can provide a highly monochromatic X-ray beam with a wavelength of 0.92  $\text{\AA}$  and a photon flux of  $\sim 10^{11}$  photons  $\text{s}^{-1}$  in a beam diameter at the specimen of  $\sim 7 \mu\text{m}$  FWHM (Engström, Fiedler & Riekkel, 1995). Crucial to the experiments described here was the availability of a computer-controlled X/Y stage which allowed the specimen to be tracked in two dimensions perpendicular to the X-ray beam in steps as small as 1  $\mu\text{m}$ . The backlash in this device was negligibly small so that a specimen could be returned to its original position with respect to the X-ray beam with an accuracy of *ca* 0.1  $\mu\text{m}$ . X-ray diffraction data were recorded using a Photonics Science CCD detector with a sensitive area of 92  $\times$  69 mm and an effective pixel area of 120  $\times$  120  $\mu\text{m}$ . The specimen-detector distance could be as small as 6 cm. At this distance diffraction data could be recorded for *d* spacings from 15 to 1.5  $\text{\AA}$ .

Diffraction patterns were recorded with exposure times of 40 ms. Over this period, the pattern was integrated within the detector in 8-bit pixels before being captured by a Synoptic i860 framegrabber. In order to improve the statistics of the diffraction data, the framegrabber allowed further integration of successive diffraction patterns. In the