Segmental motion of poly(β-hydroxybutyrate-*co*-β-hydroxyvalerate) in chloroform solution studied by ^{13}C n.m.r. relaxation

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^{13}C spin-lattice relaxation times and NOE factors were measured as a function of temperature in two magnetic fields for the protonated backbone carbons of poly(β-hydroxybutyrate-*co*-β-hydroxyvalerate) (PHB/V) copolymer in chloroform-*d*. Analysis of the relaxation data by employing the Dejean–Lauprêtre–Monnerie model reveals the following motional characteristics of the copolymer chain: (1) the local dynamics of a comonomer unit, e.g. HB, is independent of the presence of a nearby HV unit and *vice versa*; (2) different local dynamics prevail not only between the various comonomers, but also within a given comonomer unit in the random copolymer chain; (3) chain segmental motion of the copolymer described by co-operative conformational transitions is similar to that observed for the PHB homopolymer in a previous study.

(Keywords: spin-lattice relaxation; copolymer; comonomer)

INTRODUCTION

Poly(β-hydroxyalkanoate)s are high molecular weight chiral polymers produced by bacteria intracellularly as a carbon and energy reserve material[1]. Studies of these biodegradable polymers have concentrated on the homopolymer poly(β-hydroxybutyrate) (PHB) and poly(β-hydroxybutyrate-*co*-β-hydroxyvalerate) (PHB/V) copolymer which are highly crystalline and thermoplastic materials whose melting point over the 0–30% HV composition range decreases regularly from 175 to ~100°C with the occurrence of cocrystallization.

The microstructure of bacterial and synthetic PHB/V has been investigated by n.m.r. spectroscopy[2–4] and fast atom bombardment (f.a.b.) mass spectrometry[5,6]. In previous papers, the local segmental mobility of PHB has been described by ^{13}C magnetic relaxation in 1,1,2,2-tetrachloroethane[7,8] and chloroform[9] solutions. The purpose of this paper is to extend relaxation studies to the copolyester and compare local dynamics with that observed for PHB homopolymer in the same solvent.

EXPERIMENTAL

A high molecular weight ($M_w = 930000$) PHB/V sample containing 27 mol% HV was obtained from ICI Agricultural Division, Billingham, UK. The composition of the copolyester was measured by ^{1}H n.m.r. spectroscopy, as the ratio of the peak areas due to methyl resonance and the sum of the HB and HV methyl resonances[10]. After completion of relaxation measurements, it was discovered by h.p.l.c. and f.a.b. mass spectrometry[6], that the sample used was not a pure random copolymer, but a mixture containing 4% PHB and 96% (PHB/33% V). However, the very low concentration of pure PHB in the solution used for relaxation experiments (sample concentration 6% w/v) is unlikely to affect the basic conclusions of the present study.

The M_w of the PHB/V sample was determined by the relation[11]:

$$[\eta] = 7.7 \times 10^{-5} M_w^{0.82} \qquad (1)$$

where $[\eta]$ is the intrinsic viscosity in chloroform at 30°C. The intrinsic viscosity and Huggins constant (k') in equation (2) were found to be 2.87 dl g^{-1} and 0.518, respectively.

$$\eta_{sp}/c = [\eta] + k'[\eta]^2 c \qquad (2)$$

^{13}C n.m.r. experiments were conducted on a Varian XL-300, and Bruker WH-400 spectrometers operating at 75.4 and 100.5 MHz for the carbon nucleus, respectively. ^{13}C relaxation times were measured by the standard inversion recovery technique, whereas NOE experiments were carried out by 'gated decoupling'. Experimental details have been given previously[7–9]. Values of spin-lattice relaxation times (T_1s) were determined by a

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Table 1 Experimental and simulated ^{13}C spin-lattice relaxation times (T_1 in ms) and NOE factors[a] of the backbone protonated carbons of (PHB/27% V) copolymer in chloroform as a function of temperature and magnetic field

	75.4 MHz								100.5 MHz							
	CH (HB)		CH_2 (HB)		CH (HV)		CH_2 (HV)		CH (HB)		CH_2 (HB)		CH (HV)		CH_2 (HV)	
Temp. (°C)	Exp.	Calc.	Exp.	Calc.	Exp.	Calc.	Exp.	Calc.	Exp.	Calc.	Exp.	Calc.	Exp.	Calc.	Exp.	Calc.
−30	220	233	135	138	193	208	114	121	293	277	168	164	281	256	156	148
	(2.28)	(2.18)	(2.20)	(2.20)	(2.03)	(2.06)	(2.02)	(2.06)	(2.03)	(2.03)	(2.04)	(2.05)	(2.06)	(1.95)	(2.04)	(1.95)
−20	283	289	157	172	245	255	145	148	339	332	196	197	308	301	177	173
	(2.40)	(2.40)	(2.38)	(2.42)	(2.33)	(2.22)	(2.35)	(2.26)	(2.27)	(2.26)	(2.20)	(2.27)	(2.20)	(2.14)	(2.18)	(2.14)
−10	344	323	193	193	306	305	170	176	346	366	234	218	346	347	221	201
	(2.54)	(2.49)	(2.56)	(2.51)	(2.48)	(2.39)	(2.43)	(2.39)	(2.29)	(2.35)	(2.24)	(2.37)	(2.17)	(2.27)	(2.29)	(2.27)
0	420	414	237	247	364	367	207	213	454	457	262	272	407	412	245	238
	(2.69)	(2.66)	(2.69)	(2.67)	(2.57)	(2.52)	(2.53)	(2.53)	(2.45)	(2.53)	(2.44)	(2.55)	(2.45)	(2.40)	(2.40)	(2.40)
10	476	474	269	283	415	424	241	245	517	516	304	307	467	469	269	272
	(2.76)	(2.73)	(2.81)	(2.74)	(2.65)	(2.61)	(2.65)	(2.61)	(2.53)	(2.62)	(2.54)	(2.63)	(2.56)	(2.49)	(2.50)	(2.49)
20	581	588	336	351	510	513	306	280	638	627	370	373	548	559	329	323
	(2.88)	(2.83)	(2.88)	(2.83)	(2.73)	(2.71)	(2.69)	(2.71)	(2.62)	(2.73)	(2.74)	(2.74)	(2.65)	(2.60)	(2.62)	(2.60)
30	701	698	408	417	615	615	356	355	731	734	434	438	649	660	385	382
	(2.92)	(2.87)	(2.91)	(2.87)	(2.89)	(2.79)	(2.81)	(2.79)	(2.73)	(2.80)	(2.79)	(2.80)	(2.75)	(2.69)	(2.77)	(2.70)
40	850	849	499	496	747	748	439	437	877	880	523	515	781	790	452	437
		(2.91)		(2.91)		(2.85)		(2.89)	(2.87)	(2.85)	(2.84)	(2.85)	(2.83)	(2.78)	(2.88)	(2.77)

[a] Values in parentheses; full NOE is considered when parentheses are omitted

three-parameter non-linear procedure with a root-mean-square error of ±5%. The experiments were repeated until the standard error of the data was determined to be ±5%. Numerical calculations in dynamic modelling have been described in detail in a previous paper[8].

RESULTS AND DISCUSSION

Temperature and field-dependent relaxation parameters

Spin-lattice relaxation times and NOE factors for the backbone protonated carbons of the copolymer over the temperature range −30 to +40°C in two magnetic fields are summarized in *Table 1*. These data represent average values of the relaxation parameters measured for the various components of each peak in the ^{13}C spectrum[2–4]. For instance, the T_1 and NOE parameters of the CH_2 carbon for the HB comonomer is the average of the corresponding values for the BB and BV dyads resolved in the spectrum, which are nearly similar, within experimental error of the measurements. This indicates that the local dynamics of a comonomer unit, e.g. HB unit, is independent of a nearby HV unit and *vice versa*. From the compilation of the data in *Table 1*, it is apparent that the T_1 values of the backbone carbons change monotonically in both fields over the whole temperature range measured and they increase with increasing magnetic field. No minimum in the T_1 *versus* $1/T$ (K) curves is observed for the present data (*Table 1*). In a recent publication, Doi *et al.*[12] reported ^{13}C T_1 relaxation values (at 25 MHz) for PHB/V in chloroform at 27°C and concluded that the chain is rather flexible. Nevertheless, the NOE factors for the backbone carbons in *Table 1* are invariably below the extreme narrowing limit in almost the whole temperature range measured, indicating that a single exponential correlation function, i.e. isotropic motion, is not sufficient to account for the relaxation data in this temperature range.

Another interesting observation is the different relaxation times for the backbone carbons in the HB and HV units, which are reproducible and well outside the experimental error. This observation is in agreement with the relaxation data of Doi *et al.*[12] obtained at 25 MHz. The ratio of T_1 values, $T_1(CH)/T_1(CH_2)$, within each comonomer unit is fairly constant and rather different from the value of 2 expected from the number of directly bonded protons. For instance, the values of this ratio for the HB and HV units are 1.71 ± 0.02 and 1.72 ± 0.05, respectively, at 75 MHz. This discrepancy, which has been observed earlier[7–9] for PHB in solution, indicates that the local motions experienced by the CH vectors at the CH and CH_2 sites within a given sequence are not identical. A general qualitative conclusion that may arise from these observations is that different local dynamics exist not only among the various comonomer sequences, but also within a given sequence.

Although the relaxation parameters (T_1, NOE) of the side chain methyl and ethyl carbons in the HB and HV units, respectively, have been measured, they are not included in the present study, since modelling the dynamics of these groups would not add to our understanding of the segmental motion in the copolymer chain.

Modelling the PBH/V motion in solution

Modelling the dynamics of the PHB/V chain, two types of motion should be considered: the overall rotary diffusion and the segmental motion. Each of these motions is considered as an independent source of motional modulation of the dipole–dipole interactions, so that the composite correlation function is a product of the correlation functions associated with each motion:

$$G(t) = G_0(t)G_s(t) \tag{3}$$

where $G_0(t)$ and $G_s(t)$ are the correlation functions for the overall and segmental modes of reorientation, respectively. For isotropic overall molecular motion of high molecular weight polymers, the corresponding correlation function is a slowly decaying exponential function:

$$G_0(t) \sim e^{-t/\tau_R} \tag{4}$$

with a time constant, τ_R, the correlation time for the overall motion. τ_R can be calculated by using the following hydrodynamic equation[13]:

$$\tau_R = \frac{2M_w[\eta]\eta_0}{3RT} \tag{5}$$

as a function of M_w and $[\eta]$ of the polymer solution in a given solvent of viscosity η_0 and found to be 1.1×10^{-5} s in chloroform at 30°C. This long correlation time compared to the faster segmental motions (correlation time 10^{-9}–10^{-10} s) guarantees the preponderance of segmental motion as the major relaxation source for the backbone carbons of the copolymer, and renders the factorization in equation (3) a good approximation for the total correlation function.

Several correlation functions have been used in recent years to describe the dynamics of synthetic polymers in solution[14]. Among these, the most successful were the three-bond jump model of Jones and Stockmayer (JS)[15], the conformational jump model of Hall, Weber and Helfand (HWH)[16], and a modification of the latter model introduced by Dejean, Lauprêtre and Monnerie (DLM)[17]. All three models have been employed by us to describe the dynamics of PHB in solution[7–9], and the DLM model was favoured over the JS and HWH models in reproducing the relaxation data of the backbone carbons. The same model will be used to simulate the relaxation parameters in the present study.

The DLM model[17] combines the HWH model with a fast anisotropic librational motion to account for the discrepancy observed for the $T_1(CH)/T_1(CH_2)$ ratio. This additional motion, which is assumed to be independent of segmental motion, is a type of wobbling-in-a-cone diffusive motion of the C–H vectors described by Howarth[18]. The composite spectral density combining the HWH segmental description and the fast anisotropic C–H bond libration is given as[17]:

$$J(\omega) = (1 - A)/(\alpha + i\beta)^{1/2} + A\tau_2/(1 + \omega^2\tau_2^2) \tag{6}$$

$$\alpha = \tau_0^{-2} + 2\tau_0^{-1}\tau_1^{-1} - \omega^2 \tag{7}$$

$$\beta = -2\omega(\tau_0^{-1} + \tau_1^{-1}) \tag{8}$$

$$A = 1 - [(\cos\theta - \cos^3\theta)/2(1 - \cos\theta)]^2 \tag{9}$$

Here, τ_1 is the correlation time for the co-operative or correlated jumps responsible for orientation diffusion along the chain according to the HWH model and τ_0 corresponds to damping, either by non-propagative specific motions, or by distortions of the chain with respect to its most stable local conformation. τ_2 is the correlation time associated with the librational motion of the C–H vector inside a cone of half-angle θ, the axis of which coincides with the rest position of the C–H bond.

The simulation parameters of the DLM model, which reproduce the experimental data in *Table 1* are listed in *Table 2*. The calculated relaxation parameters (T_1 and NOE) are shown in *Table 1*. The calculations caused no problem and the discrepancy between experimental and theoretical relaxation parameters was within experimental error. The simulation parameters for the HB unit were $\tau_0/\tau_1 = 3$, $\tau_1/\tau_2 = 200$, and those for the HV unit $\tau_0/\tau_1 = 5$, $\tau_1/\tau_2 = 200$. It should be noted that the τ_1/τ_2 ratios cannot be accurately determined and that good agreement between theory and experiment is obtained in so far as $200 \leqslant \tau_1/\tau_2 \leqslant 800$.

Table 2 Simulation parameters for (PHB/27% V) copolymer using the DLM model

Temp. (°C)	τ_1 (HB) ($\times 10^{-10}$ s)[a]	τ_1 (HV) ($\times 10^{-10}$ s)[b]
−30	4.59	4.65
−20	2.98	2.86
−10	2.46	2.07
0	1.67	1.51
10	1.39	1.21
20	1.05	0.92
30	0.86	0.72
40	0.69	0.57
E_a (kJ mol^{-1})	17	18
τ_∞ ($\times 10^{13}$ s)	1.1	0.5
Corr. coeff.	0.998	0.998

[a] For the HB unit: $\tau_0/\tau_1 = 3, \tau_1/\tau_2 = 200, \theta(CH) = 25°, \theta(CH_2) = 31°$
[b] For the HV unit: $\tau_0/\tau_1 = 5, \tau_1/\tau_2 = 200, \theta(CH) = 21°, \theta(CH_2) = 29°$

The simulated values for the angle θ defined in equation (9) are 25° for the CH carbon and 31° for the CH_2 carbon in the HB unit. These values are similar to the θ values of the corresponding carbons, 26 and 31°, respectively, of the pure PHB in chloroform[9], indicating that the local dynamics in PHB and in HB units of the PHB/V copolymer are identical. This observation supports the earlier conclusion that the local dynamics of the HB unit in the PHB/V copolymer does not depend on the presence of the HV unit(s) in the sequence. The θ values for the CH and CH_2 carbons in the HV units are 21 and 29°, respectively. The different values of θ observed within a given sequence explain the fact that the $T_1(CH)/T_2(CH_2)$ ratio is not approximately equal to 2, and lend support to the second important conclusion of this study, that the internuclear vectors at the two carbon sites within a comonomer sequence do not experience the same local dynamics. The smaller θ values for the CH carbons relative to those for the unsubstituted backbone carbon have been rationalized[8,9,17] on the basis of the greater steric hindrance imposed by the substituent to the librational motion of the corresponding CH vector, and hence restricts the amplitude of the local libration. The greater θ value for the CH carbon in the HB comonomer reflects a lesser steric hindrance to the librational motion of the corresponding CH vector as compared to that for the CH carbon in the HV unit bearing the bulkier ethyl group. This observation gives direct support to the third conclusion of this analysis, that the local dynamics in the comonomer HB and HV units in the copolymer chain are not identical.

Despite differences in the local dynamics for the two comonomers mentioned above, the correlation times, τ_1, derived from fitting the experimental data with the DLM motional model are nearly identical for both HB and HV units (*Table 2*). These values plotted as a function of $1/T$ (K) show linear correlations ($r = 0.998$), and result in similar activation energies (17–18 kJ mol^{-1}) which are not different from that (17 kJ mol^{-1}) obtained for PHB in the same solvent[9]. This very important finding indicates that co-operative transitions occurring in the

PHB/V copolymer chain are of the same nature as those in the PHB chain, namely the type 2 motion according to Helfand's terminology[19].

In summary, the dynamics of PHB/V copolymer in chloroform have been studied through ^{13}C relaxation measurements employing the DLM model for describing chain segmental motion. Although co-operative conformational transitions in the copolymer chain are similar to those observed in the homopolymer PHB chain, discernible differences were observed in the local dynamics for these polymers in chloroform solutions.

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REFERENCES

1 Dawes, E. A. 'Microbial Energetics', Blackie, Glasgow, 1986

2 Doi, Y., Kunioka, M., Nakamura, Y. and Soga, K. *Macromolecules* 1986, **19**, 2860; Kamiya, N., Yamamoto, Y., Inoue, Y. and Chûjo, R. *Macromolecules* 1989, **22**, 3800

3 Bluhm, T. L., Hamer, G. K., Marchessault, R. H., Fyfe, C. A. and Veregin, R. P. *Macromolecules* 1986, **19**, 2871

4 Bloembergen, S., Holden, D. A., Bluhm, T. L., Hamer, G. K. and Marchessault, R. H. *Macromolecules* 1989, **22**, 1663

5 Ballistreri, A., Montaudo, G., Garozzo, D., Giuffrida, M. and Montaudo, M. S. *Macromolecules* 1991, **24**, 1231

6 Nedea, M. E., Morin, F. G. and Marchessault, R. H. *Polym. Bull.* 1991, **26**, 549

7 Dais, P., Nedea, M. E., Morin, F. G. and Marchessault, R. H. *Macromolecules* 1989, **22**, 4208

8 Dais, P., Nedea, M. E., Morin, F. G. and Marchessault, R. H. *Macromolecules* 1990, **23**, 3387

9 Nedea, M. E., Marchessault, R. H. and Dais, P. *Polymer* 1992, **33**, 1831

10 Bloembergen, S., Holden, D. A., Hamer, G. K. and Marchessault, R. H. *Macromolecules* 1986, **19**, 2865

11 Marchessault, R. H., Okamura, K. and Su, C. J. *Macromolecules* 1970, **3**, 735

12 Doi, Y., Kunioka, M., Tamaki, A., Nakamura, Y. and Soga, K. *Makromol. Chem.* 1988, **189**, 1077

13 Riseman, J. and Kirkwood, J. G. *J. Chem. Phys.* 1949, **17**, 442

14 Heatley, F. *Ann. Rep. NMR Spectrosc.* 1986, **17**, 179

15 Jones, A. A. and Stockmayer, W. H. *J. Polym. Sci., Polym. Phys. Edn* 1977, **15**, 847

16 Hall, C. K. and Helfand, E. *J. Chem. Phys.* 1982, **77**, 3275; Weber, T. A. and Helfand, E. *J. Phys. Chem.* 1983, **87**, 2881

17 Dejean de la Batie, R., Lauprêtre, F. and Monnerie, L. *Macromolecules* 1988, **21**, 2045

18 Howarth, O. W. *J. Chem. Soc. Faraday Trans.* 1979, **75**, 863; 1980, **76**, 1219

19 Helfand, E. *J. Chem. Phys.* 1971, **54**, 4651