# $^{13}$C nuclear magnetic relaxation and dynamic behaviour of poly($\beta$-hydroxybutyrate) in chloroform solution

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$^{13}$C spin–lattice relaxation times and nuclear Overhauser effect factors were measured as a function of temperature in two magnetic fields for poly($\beta$-hydroxybutyrate) (PHB) in chloroform-d. The relaxation data were interpreted in terms of chain segmental motion and methyl internal rotation by using various dynamic models. Among these, the Dejean–Lauprêtre–Monnerie model offered the best description of the segmental motion along the PHB chain. However, all models used in this study resulted in similar activation energy (17 kJ mol$^{-1}$) for cooperative segmental motion. An activation energy of 11–12 kJ mol$^{-1}$ was found for methyl internal rotation. Further relaxation experiments on PHB in chloroform-d as a function of concentration and molecular weights were carried out as well.

**(Keywords: nuclear magnetic resonance; relaxation; poly($\beta$-hydroxybutyrate); dynamic models)**

## INTRODUCTION

A detailed analysis of carbon-13 magnetic relaxation of poly($\beta$-hydroxybutyrate) (PHB) in 1,1,2,2-tetrachloroethane (TCE) solvent was reported in the first two papers in this series[1,2]. The chain local motions of PHB were described successfully by using a number of established autocorrelation functions[3–5] relevant to describing local motions in polymer chains.

It seems of interest to examine whether the previously used autocorrelation functions can also be used to interpret the relaxation data of the protonated carbons of PHB in a good solvent, such as chloroform, in which the PHB chain assumes a more extended configuration than in the poor TCE solvent. Extensive studies[6] by means of viscometry, light scattering and optical rotatory dispersion have suggested that an interrupted helical conformation similar to that of PHB in the crystal structure[7,8] may be retained in chloroform solution. Therefore, comparison of the results obtained in TCE solution with those in chloroform would allow a better understanding of the influence of the solvent on the PHB dynamics in solution.

In addition to determining the timescale of local motions of PHB in chloroform, $^{13}$C relaxation experiments have been conducted as a function of concentration to probe the effect, if any, of intermolecular interactions on chain local motions.

Although multiple-field relaxation experiments have shifted the emphasis from measuring relaxation parameters as a function of the molecular weight, such measurements will be carried out in an attempt to investigate the contribution of the overall rotatory diffusion in modulating the dipole–dipole interactions.

## EXPERIMENTAL

The PHB samples used in this study were obtained from ICI Agricultural Division, Billingham, UK. The weight-average molecular weights of the PHB samples ($M_w$ = $6.7 \times 10^3, 1 \times 10^5, 4 \times 10^5, 9 \times 10^5$) were determined by the relation[6]:

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

where $[\eta]$ is the intrinsic viscosity in chloroform at 30°C. Model calculations and concentration-dependent measurements were performed on the same sample ($M_w = 4 \times 10^5$) used in the previous study[1,2]. The intrinsic viscosity and Huggins constant $k'$ in equation (2) were found to be 3.32 dl g$^{-1}$ and 0.478 respectively:

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

$^{13}$C nuclear magnetic relaxation experiments were conducted on Varian XL-300 and Bruker WH-400 spectrometers operating at 75.4 and 100 MHz for the carbon nucleus respectively. $^{13}$C relaxation times were measured by the standard inversion recovery technique, whereas nuclear Overhauser effect (NOE) experiments were carried out by 'gated decoupling'. Further experimental details can be found elsewhere[1]. Values of $T_1$ were determined by a three-parameter non-linear procedure with a r.m.s. error of ±5 or better. The experiments were repeated until reproducibility of the data was better than

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**Table 1** Carbon-13 spin–lattice relaxation times $T_1$ (ms) and NOE factors[a] of protonated carbons in PHB in chloroform-d as a function of temperature, concentration and magnetic field

| Temp. (°C) | 300 MHz, 6% w/v | | | 400 MHz, 6% w/v | | | 300 MHz, 12% w/v | | |
|---|---|---|---|---|---|---|---|---|---|
| | CH | $CH_2$ | $CH_3$ | CH | $CH_2$ | $CH_3$ | CH | $CH_2$ | $CH_3$ |
| −30 | 239 | 137 | 231 | 301 | 176 | 271 | 235 | 133 | 231 |
| | (2.21) | (2.26) | (2.67) | (2.13) | (2.17) | (2.65) | (2.15) | (2.13) | (2.71) |
| −20 | 287 | 162 | 294 | 342 | 199 | 326 | 287 | 164 | 291 |
| | (2.37) | (2.32) | (2.78) | (2.29) | (2.34) | (2.72) | (2.38) | (2.38) | (2.74) |
| −10 | 360 | 209 | 373 | 375 | 234 | 455 | 351 | 200 | 371 |
| | (2.61) | (2.59) | | (2.36) | (2.43) | (2.80) | (2.53) | (2.61) | |
| 0 | 432 | 248 | 458 | 477 | 275 | 491 | 437 | 255 | 461 |
| | (2.65) | (2.71) | | (2.54) | (2.56) | (2.82) | (2.63) | (2.78) | |
| 10 | 512 | 298 | 556 | 566 | 332 | 594 | 474 | 275 | 516 |
| | (2.68) | (2.78) | | (2.62) | (2.65) | (2.83) | (2.70) | (2.90) | |
| 20 | 621 | 362 | 709 | 672 | 397 | 706 | 596 | 347 | 709 |
| | (2.80) | (2.85) | | (2.70) | (2.74) | (2.88) | (2.74) | | |
| 30 | 759 | 450 | 827 | 800 | 467 | 844 | 742 | 436 | 812 |
| | (2.85) | (2.89) | | (2.71) | (2.81) | (2.89) | (2.79) | | |
| 40 | 924 | 540 | 995 | 934 | 555 | 988 | 917 | 537 | 1012 |
| | (2.90) | | | (2.75) | (2.85) | (2.89) | (2.82) | | |

[a] Values in parentheses; full NOE was obtained when parentheses are omitted

**Table 2** Carbon-13 spin–lattice relaxation times $T_1$ (ms) and NOE factors of PHB 6% solution in chloroform-d as a function of molecular weight and temperature at 300 MHz

| Temp. (°C) | Molecular weight | CH | $CH_2$ | $CH_3$ |
|---|---|---|---|---|
| −30 | $6.7 \times 10^3$ | 237 (2.13) | 133 (2.26) | 210 (2.67) |
| | $1 \times 10^5$ | 233 (2.16) | 133 (2.05) | 212 (2.55) |
| | $4 \times 10^5$ | 239 (2.21) | 137 (2.26) | 231 (2.67) |
| | $9 \times 10^5$ | 240 (2.18) | 134 (2.23) | 214 |
| 30 | $6.7 \times 10^3$ | 745 (2.65) | 440 (2.93) | 807 (2.88) |
| | $1 \times 10^5$ | 744 (2.81) | 435 (2.91) | 794 |
| | $4 \times 10^5$ | 759 (2.79) | 450 (2.88) | 827 |
| | $9 \times 10^5$ | 735 (2.61) | 429 (2.90) | 799 |
| 60 | $6.7 \times 10^3$ | 1255 | 767 | 1327 |
| | $1 \times 10^5$ | 1269 | 766 | 1276 |
| | $4 \times 10^5$ | 1378 | 832 | 1424 |
| | $9 \times 10^5$ | 1239 | 742 | 1266 |

±5%. Samples of PHB in $CDCl_3$ were degassed by bubbling with nitrogen gas before use.

Numerical calculations were performed by using the powerful MOLDYN program[9], modified to include the spectral density functions of the motional models used in this study. The description of the program and the procedure used to obtain simulation parameters of the various models and calculated relaxation parameters have been described elsewhere[1,2,10].

## RESULTS AND DISCUSSION

*Table 1* summarizes the $^{13}C$ $T_1$ values and NOE factors for the protonated carbons of PHB as a function of temperature, concentration and magnetic field. *Table 2* contains the molecular-weight-dependent relaxation data at three temperatures. The concentration dependence of the relaxation parameters in *Table 1* show but a minor change going from 6% to 12% w/v solutions. This observation indicates that chain segmental motions and methyl internal rotation are not affected by doubling the concentration, and they may be considered as independent local motions in this concentration range. Unfortunately, higher concentrations cannot be obtained owing to the limited solubility of PHB in chloroform. Therefore, a thorough quantitative interpretation of concentration effects is postponed until a larger concentration range can be studied in other solvents.

Inspection of the data in *Table 2* reveals that, for a given temperature, no discernible trend in any $T_1$ and NOE values as a function of the molecular weight is observed over the range studied. A possible explanation for the molecular-weight independence of local dynamics under good solvent conditions is in terms of excluded-volume interactions, which tend to keep segments far apart. Hence, the local segment concentration is essentially independent of molecular weight in a good solvent such as chloroform.

As can be seen in *Figures 1* and *2*, the $T_1$ values of the backbone carbons change monotonically in both fields over the whole temperature range studied. The NOE factors, which are invariably below the extreme narrowing limit (*Table 1*), indicate that a single-exponential function, i.e. isotropic motion, is inadequate to account for these relaxation data. Another interesting feature of the data in *Table 1* is that the ratio of $T_1$ values, $T_1(CH)/T_1(CH_2)$, is fairly constant at $1.73 \pm 0.03$ at 300 MHz and $1.69 \pm 0.04$ at 400 MHz, rather different from the value of 2 expected from the number of directly bonded protons. This discrepancy, which has been observed[1,2] earlier in TCE solution, indicates that the local motions experienced by the C–H vectors at the CH and $CH_2$ carbon sites are not identical.

### *Modelling the motions of PHB in chloroform solution*

Modelling the dynamics of PHB in chloroform, three general types of motions are considered: (1) the overall

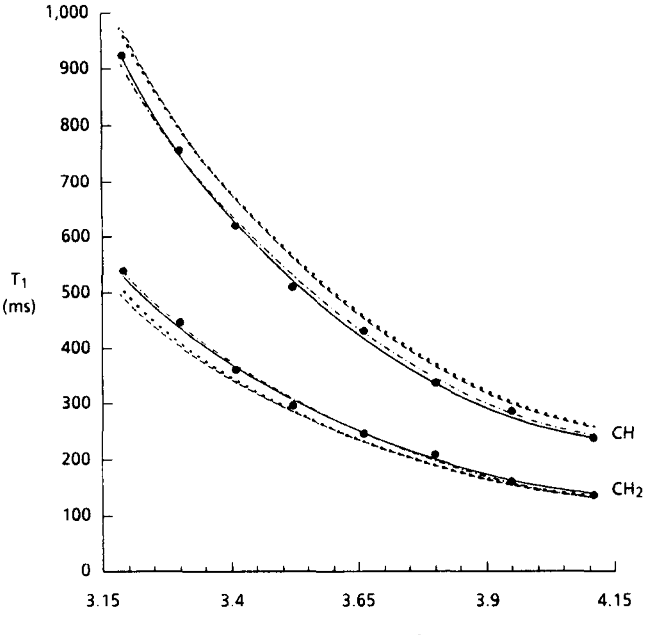

**Figure 1** Plot of $^{13}C$ spin–lattice relaxation times $T_1$ (ms) *versus* reciprocal temperature for PHB in $CDCl_3$ at 300 MHz. Full curves and data points are experimental values; (----) HWH model; (·······) JS model; (—·—·—) DLM model

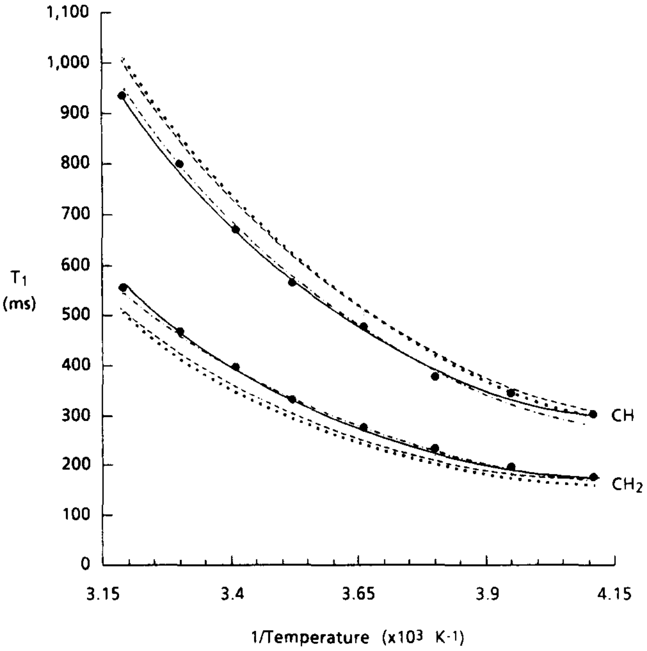

**Figure 2** Plot of $^{13}C$ spin–lattice relaxation times $T_1$ (ms) *versus* reciprocal temperature for PHB in $CDCl_3$ at 400 MHz. Full curves and data points are experimental values; (----) HWH model; (·······) JS model; (—·—·—) DLM model

rotatory diffusion, (2) segmental or backbone rearrangement, and (3) methyl internal rotation. Each of these motions is considered independent, so that the composite autocorrelation function can be written as a product of the autocorrelation functions associated with each motion.

For sufficiently high-molecular-weight polymers, the overall rotatory diffusion is much slower than the chain local motions, and thus makes a negligible contribution to the relaxation of the backbone carbons[11]. This conclusion is supported by the long correlation time $\tau_R$ estimated at infinite dilution as a function of the molecular weight $\bar{M}_w$ and the intrinsic viscosity $[\eta]$ of the polymer solution in a given solvent of viscosity $\eta_0$ through the hydrodynamic equation:

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

which was found to be $1.8 \times 10^{-5}$ s in chloroform at 30°C. This value is relatively independent of concentration and the effect of molecular-weight distribution[1,10].

The next motions considered are local motions and these are segmental motion and methyl internal rotation. Segmental motion will be described by three motional models. The first description is derived from the occurrence of a three-bond jump on a tetrahedral lattice[3], originally proposed by Valeur and collaborators[12]. In the three-bond jump model for segmental motion developed by Jones and Stockmayer (JS)[3], there are two parameters: the harmonic mean correlation time $\tau_h$, which sets the timescale of the backbone motion, and the number of coupled bonds $m$ determining the effective distribution of correlation times. The latter parameter is also given as $2m - 1$, which stands for the chain segment expressed in bonds that are coupled to the central bonds. In the second model developed by Hall, Weber and Helfand (HWH)[4] from consideration of computer simulation of backbone conformational transitions in polymethylene chains, the segmental motion is characterized in terms of a correlation time $\tau_0$ for single conformational transitions, and a correlation time $\tau_1$ for cooperative conformational transitions. The third model as presented by Dejean, Lauprêtre and Monnerie (DLM)[5] describes the backbone reorientation in terms of two independent kinds of motions: (i) a diffusional process along the chain, which occurs via conformational transitions as in the HWH model, and (ii) bond librations, i.e. wobbling in a cone motion of the backbone internuclear CH vectors as described by Howarth[13]. The librational motion is described by a correlation time $\tau_2$ whereas the extent of the libration about the rest position of the CH bond (the axis of the cone) is determined by the cone half-angle $\theta$. Autocorrelation functions of the aforementioned models and their Fourier transforms, the spectral densities, are given in the references cited[3–5,14].

Methyl internal rotation can be described either as jumps between three minima or stochastic diffusion[15] superimposed on segmental motion described by the JS and/or HWH model. Composite spectral densities, involving the correlation time $\tau_{ir}$ for internal motion, can be found in ref. 1.

### *Comparison of the simulation parameters*

The simulation parameters of the three models that reproduce the experimental data of PHB at the two fields are listed in *Table 3*. The best fit of the $T_1$ data is plotted in *Figures 1* and *2* for the CH and $CH_2$ carbons at both fields. Among the three motional models, the best fit ($\leqslant \pm 5\%$) was obtained by using the DLM model. The reproducibility of the data using the JS and HWH models was between ±2 and ±10%. This observation reinforces the earlier statement that the internuclear vectors of the two carbon sites of the chain do not experience exactly the same local dynamics, as the JS and HWH models predict. This is reflected in the simulated values of $\theta$

**Table 3** Simulation parameters for a 6% solution of PHB in chloroform-d using three models

| Temp. (°C) | JS | | | HWH | | | DLM[a] |
|---|---|---|---|---|---|---|---|
| | $2m-1$ | $10^{-9}\tau_h$ (s) | $10^{-10}\tau_{ir}$ (s) | $10^{-9}\tau_0$ (s) | $10^{-9}\tau_1$ (s) | $10^{-10}\tau_{ir}$ (s) | $10^{-9}\tau_1$ (s) |
| −30 | 9 | 0.109 | 0.185 | 2.00 | 0.134 | 0.764 | 0.426 |
| −20 | 9 | 0.079 | 0.145 | 1.20 | 0.103 | 0.607 | 0.304 |
| −10 | 9 | 0.056 | 0.103 | 0.60 | 0.088 | 0.421 | 0.226 |
| 0 | 9 | 0.041 | 0.094 | 0.54 | 0.060 | 0.406 | 0.163 |
| 10 | 9 | 0.032 | 0.077 | 0.46 | 0.044 | 0.345 | 0.128 |
| 20 | 9 | 0.025 | 0.062 | 0.35 | 0.035 | 0.280 | 0.100 |
| 30 | 9 | 0.020 | 0.054 | 0.29 | 0.026 | 0.253 | 0.080 |
| 40 | 9 | 0.016 | 0.046 | 0.23 | 0.022 | 0.220 | 0.065 |
| $E_a$ (kJ mol$^{-1}$) | | 17.5 | 12.5 | 22 | 17 | 11 | 17 |
| $\tau_\infty \times 10^{14}$ (s) | | 2 | 4 | 54 | 3 | 32 | 9 |
| Corr. coeff. | | 0.999 | 0.996 | 0.977 | 0.994 | 0.990 | 0.999 |

[a] $\tau_0/\tau_1 = 3$; $\tau_1/\tau_2 = 200$; $\theta(\mathrm{CH}) = 26°$, $\theta(\mathrm{CH_2}) = 31°$

**Table 4** Comparison of simulation parameters of PHB in TCE[a] and chloroform solutions

(a) Segmental motion

| | Apparent activation energy (kJ mol$^{-1}$) | | | | Arrhenius prefactor ($\tau_\infty \times 10^{14}$ s) | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Solvent | $\tau_h$ | $\tau_1$(HWH) | $\tau_0$(HWH) | $\tau_1$(DLM) | $\tau_h$ | $\tau_1$(HWH) | $\tau_0$(HWH) | $\tau_1$(DLM) | $2m-1$ |
| TCE | 23 | 22 | 40 | 19.5 | 2 | 1 | 79 | 8.5 | 9 |
| CDCl$_3$ | 17.5 | 17 | 22 | 17 | 2 | 3 | 54 | 9 | 9 |

(b) Methyl internal motion

| | Apparent activation energy (kJ mol$^{-1}$) | | Arrhenius prefactor ($\tau_\infty \times 10^{14}$ s) | |
|---|---|---|---|---|
| Solvent | $\tau_{ir}$ (JS) | $\tau_{ir}$ (HWH) | $\tau_{ir}$ (JS) | $\tau_{ir}$ (HWH) |
| TCE | 14 | 12 | 5 | 24 |
| CDCl$_3$ | 12.5 | 11 | 4 | 32 |

[a] From ref. 1

(*Table 3*), which are 26° for the CH carbon, and 31° for the CH$_2$ carbon. The lower value for methine carbons has been observed in many polymers and has been attributed to greater steric hindrance at the methine carbon[2,5,13,16].

The fitting parameters for the DLM model were $\tau_0/\tau_1 = 3$ and $\tau_1/\tau_2 = 200$. However, variation of the latter ratio in the interval $200 \leqslant \tau_1/\tau_2 \leqslant 800$ makes little difference to the calculated relaxation data. In fact, this insensitivity of the correlation time of the fast libration has been noted by Howarth[13]. The ratio $\tau_0/\tau_1$ for the HWH model ranges from 7 to 15 (*Table 3*). However, both sets of $\tau_1$ values of the two models give essentially the same activation energy (17 kJ mol$^{-1}$) and prefactor in an Arrhenius plot (*Table 3*). A rather similar Arrhenius summary is also afforded when segmental motions are described by the Jones and Stockmayer model. Moreover, the $\tau_h$ and $\tau_1$ correlation times in the JS and HWH models are similar. In fact, $\tau_1$ values are longer than $\tau_h$ by a factor of 1.3–1.6. This similarity in $\tau_h$ and $\tau_1$ values, also observed in TCE solutions[1], has been rationalized[17] on the basis that the autocorrelation functions of these models describe cooperative local motions in terms of conformational diffusion, although they were developed from quite different starting points.

The time constant $\tau_0$ for the HWH model is more than an order of magnitude greater than $\tau_1$ (*Table 3*), indicating that single-bond conformational transitions play a lesser role than cooperative transitions for PHB in solution. However, the calculated activation energy for $\tau_0$ (22 kJ mol$^{-1}$) is quite similar to that for $\tau_1$ (17 kJ mol$^{-1}$) within experimental error (±5 kJ mol$^{-1}$), although the pre-exponential constants differ by more than an order (54 for $\tau_0$ vs. 3 for $\tau_1$). This observation is in agreement with the simplifying assumption made by Dejean *et al.*[5] in deriving their model, that is the ratio $\tau_0/\tau_1$ remains constant, in effect giving $\tau_0$ and $\tau_1$ the same activation energy but different prefactors.

Another interesting observation is that the correlation time $\tau_{ir}$ for methyl internal motion is 4–5 times longer for the HWH model than for the JS model over the whole temperature range studied, although both models offer essentially similar activation energy (11–13 kJ mol$^{-1}$) for the internal motion. It appears that the application of the JS and HWH models to methyl internal motion occurring in PHB results in a different timescale as indicated by the different prefactor values of the Arrhenius summary in *Table 3*, but coincide regarding activation energy.

### *Comparison of the dynamics of PHB in two solvents*

Segmental motion and methyl internal rotation in the PHB chain in chloroform can be compared to the local motions in TCE[1]. This comparison is shown in *Table 4*, which displays the Arrhenius parameters for both PHB solutions. The higher apparent activation energy of segmental motion in TCE solvent reflects the influence of solvent viscosity on this type of local motion, whereas this effect appears to be minor for the methyl internal motion. This is not surprising, since the methyl group involves only a minor part of the repeat unit and, if it is an independent motion, it will not be affected by the medium. On the other hand, segmental motion probably involves one to several repeat units and is expected to be affected by the medium, since it sweeps out a significant volume as it occurs.

There is an activation energy difference between $\tau_0$ and $\tau_1$ in TCE, but not in chloroform, indicating that the damping process in the PHB chain as described by $\tau_0$ in the HWH model is more important in chloroform than in TCE solution. However, subtracting the activation energy for the solvent viscosity, $\Delta H_\eta$ (7.48 kJ mol$^{-1}$ for chloroform[18] and 12 kJ mol$^{-1}$ for TCE[1]), from the activation energy obtained from relaxation data (*Table 4*), we obtain similar values (9–11 kJ mol$^{-1}$) for the potential barrier of the segmental motions. This indicates that the same type of segmental motion, namely type 2 motion according to Helfand's notation, prevails in both solvents.

## SUMMARY

In summary, we have studied the dynamics of PHB in chloroform solution through $^{13}$C relaxation measurements employing various dynamic models for describing chain local motions. It appears that the DLM model is favoured over the JS and HWH models in describing segmental motions, but a clear-cut distinction between models cannot be made as long as no minimum in the $T_1$ vs. $1/T$ plot can be reached with the present solvent. No discernible differences in local dynamics were observed for the PHB chain in chloroform and TCE solvents, except perhaps the effect of solvent viscosity.

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