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Phase transitions of poly(octyl isocyanate)

Simulation of the e.p.r. spectra of a dry spin-labelled polymer

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The basic ideas of a novel way to simulate electron paramagnetic resonance (e.p.r.) spectra of spin-labelled polymers are described. The simulation method has been applied in the analysis of the e.p.r. spectra of dry spin-labelled poly(octyl isocyanate) (POIC). POIC is a semiflexible, helical polymer capable of forming a thermotropic liquid crystalline phase. The spectra of a fractionated POIC sample have been analysed in order to find out how the mobility and flexibility of the chain change with increasing temperature, when the polymer turns from the semicrystalline into the liquid crystalline state. The results imply that the sample contains both helical and random chain sequences through the whole temperature range studied. However, the proportion of the helical sequences is dependent on temperature. © 1998 Elsevier Science Ltd. All rights reserved.

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INTRODUCTION

When studying a solution of a polymer which carries a nitroxide radical label as a side group, the interpretation of an electron paramagnetic resonance (e.p.r.) spectrum of the polymer can be based on an assumption of two dynamic processes occurring simultaneously[1–3]. These processes, where also the nitroxide labels are involved are, firstly, the slow isotropic (Brownian) diffusion of the polymer or its segment and, secondly, the fast anisotropic motion of the side groups.

The powder spectra obtainable from dry polymers are interesting because, usually, the former process does not exist in the e.p.r. time scale, whereas the latter exists and affects the shape of the spectra. Thus, in general, the e.p.r. spectra from dry labelled polymers reflect the motion of the side groups, as well as the motional restriction caused by the surroundings of the reporter group. If the nitroxide labels are located in various phases of a heterogeneous polymer system, the complex experimental e.p.r. spectrum will be composed of several spectra which all may be described by a varying degree of averaging of the nitroxide magnetic tensors **A** and **g**, see Ref. [2].

The aim of this paper is to analyse in detail the e.p.r. spectra of spin-labelled poly(octyl isocyanate) (POIC). The present method of spectra simulation has already been applied in the analysis of the e.p.r. spectra of solutions and thermoreversible gels of POIC in non-polar solvents[1]. In the present work, dry polymer has been studied at various temperatures in order to find out how the mobility and flexibility of the polymer chain changes when the sample is heated and it turns from the semicrystalline into the liquid crystalline state.

Poly(alkyl isocyanates) are semiflexible polymers, some of which form thermotropic liquid crystalline phases. Aharoni[4,5] has studied several polyisocyanates, and has shown POIC to be a mesogenic polymer.

Recently, Olayo *et al.*[6] synthesized and investigated an end-labelled poly(butyl isocyanate). These authors observed that, in a dry polymer, the motion of the label is strongly restricted at room temperature; with increasing temperature the polymer softens and a narrow three-line e.p.r. spectrum can be registered even at 60°C. The spectral change was attributed to the increasing mobility of the alkyl side chains in this temperature range. In the present case, the label groups are randomly distributed along the polymer chain. The mobility of the nitroxide covalently attached to the nitrogen in the main chain has been shown[7] to be restricted by the bulky carbonyl groups surrounding the main chain nitrogen, especially when the chain is held in its energetically favourable helical conformation. Owing to this restriction, the e.p.r. spectrum of the label very sensitively reflects changes in the mobility and flexibility of the polymer main chain.

In this paper, the analysis of the e.p.r. spectra registered from carefully fractionated samples of labelled POIC will be described and discussed.

THEORETICAL BACKGROUND

Fast anisotropic motion of a spin label bound to a macromolecule as a side group has been described in detail previously[1–3]. Only the main ideas of this description, and some basic equations used in the calculation, will be presented.

As is known, fast isotropic rotation of a nitroxide radical averages the anisotropy of the **A** and **g** tensors. The result of this averaging is a symmetric triplet e.p.r. spectrum where the distance of the outer lines from the central one is equal to $a_0 = (A_x + A_y + A_z)/3$, and the position of the central line defines $g_0 = (g_x + g_y + g_z)/3$. a_0 is the isotropic coupling constant; A_x, A_y, A_z, and g_x, g_y, g_z are the diagonal components of the **A** and **g** tensors respectively.

In the case of anisotropic rotation of the label radical, the anisotropies of the magnetic tensors **A** and **g** are only partially averaged.

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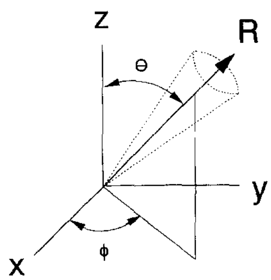

Figure 1 Definition of the oscillation axis R of the label in the nitroxide coordinates. The oscillations occur around the R axis, within an angle $\pm\alpha$. The dashed cone describes the distribution of the possible directions of R

The fast reorientation of the radical may be described by various models, where the equations of the partially averaged tensor components have different forms. In the present case, the spin-labelled polymer is a semiflexible POIC and the label is a pyrrolidine nitroxide, bound with one covalent bond to the polymer as a side group. To describe the fast motion of this side group in a solid or liquid crystalline polymer, an oscillational model has been chosen. In this model, the radical is assumed to oscillate relative to an axis R within an angle $\pm\alpha$. The position of the R axis is defined in the nitroxide radical coordinate system by two angles, θ and ϕ, see *Figure 1*. Alternatively, the position of the R axis may be defined by parameters S and κ which depend on the angles θ and ϕ:

$$S = \frac{3(\cos^2\theta - 1)}{2} \tag{1}$$

$$\kappa = \tan(45^\circ - \phi) \tag{2}$$

We prefer the use of the order parameters S and κ, instead of θ and ϕ, and they serve as comfortable input parameters when describing the oscillational motion of the label.

In the oscillational model, the partially averaged components of the **A** tensor may be written[2]

$$\bar{A}_i = K_1A_i + \tfrac{1}{3}[2K_2T_iA_i + K_3\bar{A}T_i + K_4(T_{(i+2)}A_{(i+1)} + T_{(i+1)}A_{(i+2)})] \tag{3}$$

where A_i is a diagonal component of the tensor **A**. x, y, and z components are permutated cyclically so that e.g. $A_{i+1}|i=z| = A_x$, and $A_{i+2}|i=z| = A_y$.

In equation (3), $\bar{A}$ is defined as

$$\bar{A} = (a_0 + \tfrac{2}{3}\Delta\bar{A}) \tag{4}$$

Constants K_1–K_4 depend on the angle α:

$$K_1 = \frac{1}{2}\left(1 + \frac{\sin 2\alpha}{2\alpha}\right) \tag{5}$$

$$K_2 = \frac{\sin\alpha}{\alpha} - K_1 \tag{6}$$

$$K_3 = 1 - 2\,\frac{\sin\alpha}{\alpha} + K_1 \tag{7}$$

$$K_4 = \frac{1}{2}\left(1 + \frac{\sin 2\alpha}{2\alpha}\right) \tag{8}$$

T_x, T_y, and T_z are functions of S and κ:

$$T_x = (1 - S)(1 - \kappa) \tag{9}$$

$$T_y = (1 - S)(1 + \kappa) \tag{10}$$

$$T_z = 2S + 1 \tag{11}$$

In equation (3) one finally needs to define

$$\Delta\bar{A} = \Delta A S - \delta A(1 - S)\kappa \tag{12}$$

where ΔA and δA are written as

$$\Delta A = A_z - \frac{A_x + A_y}{2} \tag{13}$$

$$\delta A = \frac{A_x - A_y}{2} \tag{14}$$

In general, nitroxide radicals attached to a macromolecule may be expected to be involved in various types of rotational and/or oscillational reorientation. The situation can be visualized as a group (or groups) of nitroxides gathered around a preferential axis (or axes) of rotation, and/or a group of radicals oscillating around a certain axis in the molecular coordinate system. These groups are, in this text, called clusters.

The number of the molecules n in a cluster is proportional to the probability ρ of the corresponding state of the nitroxide in the stationary process leading to the averaging of the magnetic tensors. If N is the total number of molecules in the sample, the probability is defined as $\rho = n/N$.

Each cluster is expected to produce its unique e.p.r. spectrum and thus the experimental spectrum may turn out to be a superposition of several spectra. Because the number of molecules in each cluster most probably varies, the spectra typical to various clusters need to be summed with different weights during the simulation of the experimental spectrum.

From the reasoning above, it follows that the simulation of an experimental e.p.r. spectrum is conveniently started by choosing the model of the averaging of the components of **A** and **g** tensors in the clusters. Then, one needs to estimate the density probability distribution of the clusters in the sample, i.e. to find out the weighted contribution of each spectrum of a corresponding cluster. The latter is defined by

$$\rho_{ij} \approx \exp\left[-\left(\frac{S_i - S_0}{\sigma_S\sqrt{2}}\right)^2\right]\exp\left[-\left(\frac{\kappa_j - \kappa_0}{\sigma_\kappa\sqrt{2}}\right)^2\right] \tag{15}$$

where ρ_{ij} is the weight of the spectrum of the cluster ij. ρ_{ij} is called a spectral mode; consequently, the experimental spectrum is a product of one or several modes, depending on the complexity of the sample. S_0 and κ_0 define the preferential position of the nitroxide z axis in the space of order parameters, and σ_S and σ_κ give the dispersion of S and κ. S and κ are suggested to have a Gaussian distribution.

Now it is possible to calculate a spectrum (or spectra) representing one or several clusters with the axial model of averaging, as well as a spectrum (spectra) resulting from oscillational averaging. By combining these spectra with varying weights, the experimental spectrum should be satisfactorily simulated.

EXPERIMENTAL

The spin-labelled polymer was synthesized by anionic polymerization from n-octyl isocyanate and 1-oxyl-2,2,5,5-tetramethylpyrrolidine-3-isocyanate. The synthesis is described in detail elsewhere[7]. The M_w determined by light scattering was 90 000. By comparing the e.p.r. spectra of the product polymer in solution with known reference solutions, a single chain was concluded to contain two or three nitroxide units.

The polymer was studied by g.p.c. which was calibrated with polystyrene standards. M_w/M_n for the labelled polymer was 4; as a comparison, the same value for a typical non-labelled homopolymer was ≤ 2.

The polymer was fractionated in a gel column (Sephacryl S-400 HR, Pharmacia) using either toluene or tetrahydrofuran as an eluent. In both cases, the polymer was fractionated into 25 fractions. The differences between the spectra of various fractions were only minor. The choice of the eluent did not affect the spectra of the fractions.

E.p.r. spectra of the fractionated polymer were measured as a function of increasing temperature, starting from room temperature, with a Varian E-4 instrument. The spectra measured at temperatures close to the melting point of the polymer were indicative of thermal degradation of the label.

RESULTS AND DISCUSSION

The mesogenic character of POIC is well documented in the reports by Aharoni[4,5], who observed dry polymer to form a liquid crystalline phase at temperatures below the melting transition. Three thermal transitions (denoted as T_{c1}, T_{c2} and T_m in Aharoni[4]) may be observed by d.s.c. for POIC above room temperature, at 60, 114 and 159°C. The highest temperature transition is melting, which is accompanied by thermal degradation. The transition around 60°C is due to the increased mobility of the alkyl side chains; this conclusion is supported by the spin-labelling study of Olayo *et al.*[6], as well as by Aharoni's microscopic observations[5]. According to the latter, the thermal transitions occur at 85, 125 and 184°C.

In the present study the thermal transitions measured by d.s.c. were the same as reported earlier, and it may be concluded that the polymer turns from a semicrystalline solid into liquid crystalline state at temperatures between 114 and 125°C, the exact transition temperature depending on the heating rate and the measuring technique.

The e.p.r. spectra recorded from the bulk polymer showed a clear multicomponent character. Instead of one outer extremum on either side of the central line, two of them were observed in the spectra of solid samples at and above room temperature. This may be explained by the coexistence of at least two different populations of nitroxides; for example, the labels positioned close to the chain ends are usually more mobile than the others. The polymer most probably not only contains helical sequences, but also those with random conformation. These factors, in addition to the known semicrystallinity of the polymer, may affect the shape of the spectra. Because the labelled polymer had a fairly broad molar mass distribution, it was fractionated in a gel column using either toluene or tetrahydrofuran as an eluent. The fractionation did not, however, have any major effect on the shape of the spectra. If the multicomponent nature of the spectra arose from the varying distance of the nitroxide groups from the chain ends, the shape of the spectra should be strongly dependent on the size of the polymer; this kind of effect could not be detected. In our previous investigation on the solutions and gels of POIC, small sharp lines were observed in some of the spectra; these were concluded to have originated from the labels close to the chain ends[1]. The number of the mobile labels at the chain ends is, however, too low to explain the multicomponent character of the spectra of dry polymer. The motional freedom of the nitroxide group is known in this case to be very strongly affected by the conformation and flexibility of the polymer main chain and, thus, these are the factors which need to be considered when analysing the spectra.

The spectra discussed in this report are those obtained from the fraction with the highest molar mass, the fraction number one, when the polymer was eluted through a GPC column using toluene as an eluent. Some representative spectra, together with their simulated counterparts will be shown later in the text. To describe the temperature-dependent changes in the spectra, the separation of the various extrema ($2\bar{A}$) is shown against temperature in *Figure 2*. In this figure, the transition temperatures from Aharoni's calorimetric and microscopic studies are also shown.

The thermal stability of the polymer itself, as well as that of the nitroxide radical, set a limit for the range of temperatures that may be studied using the spin label method. Owing to the thermal degradation of the label even below the T_m of the polymer, the measurements could not be conducted in the isotropic melt state. However, the data in *Figure 2* show that, in general, the changes in the shape of the e.p.r. spectra correlate with the calorimetrically detectable transition temperatures.

The outer extrema, the separations of which are shown in *Figure 2*, move closer to the centre of the spectrum with increasing temperature. The phase transition temperatures are observed as changes in the slopes of the straight lines. In addition, a motionally narrowed three-line spectrum appears when the polymer melts into the liquid crystalline state (the width of the narrow spectrum is shown as curve 3 in *Figure 2*). All the studied polymer fractions with varying molar masses behave identically; the size of the polymer seems to affect only slightly the relative intensities of the various components in the spectra, and even less the transition temperatures.

The simulation of the spectra has been conducted in most cases by combining three different types of theoretical spectra, all produced using the oscillational model of averaging. The oscillational model turned out to be superior over the axially symmetric (rotational) one in reproducing the shapes of the experimental spectra, this indicating the

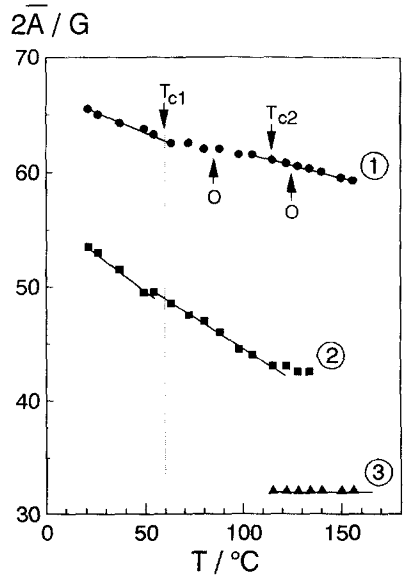

Figure 2 Temperature dependence of the separation of the outer lines ($2\bar{A}$, in gauss) of the three components of the e.p.r. spectra. Transition temperatures measured by d.s.c. are indicated as T_{c1} and T_{c2}. Transitions observed using optical microscopy (from Aharoni[5]) are indicated with O

high barrier for rotational motion of the nitroxide side group in the bulk polymer. The assumption of three types of nitroxide cluster is justified by the simultaneous existence of three spectra with varying widths (see *Figure 2*). In each type of cluster, the angle α within which the label oscillates is supposed to have a certain most probable value. The angle α increases with increasing temperature. In each cluster the values of S and κ, which define the position of the axis of the oscillational motion, have a certain dispersion. The dispersion is strongly dependent on temperature.

The applicability of the present approach is illustrated in *Figures 3–7*, which show the experimental and calculated spectra at room temperature, at 49°C (i.e. below T_{c1}), at 80°C (above T_{c1}), as well as at 128 and 156°C (above T_{c2}, where the polymer is in the liquid crystalline state). In these figures the various modes are also shown, of which the final simulated spectra have been obtained by summation. The parameters used to simulate the spectra are collected in *Table 1*. From *Table 1* it may be seen that, in most cases, the order parameter $S = 0$, which means that the axis of the oscillations is in the magic angle relative to the nitroxide z axis.

It was already stated above that the composite nature of the spectra cannot be sufficiently described by assuming it to be a result of the varying distance of the labels from the mobile chain ends. It should also be noted that the semicrystallinity of the polymer does not seem to explain this feature of the spectra; the spectra also retain their multicomponent shape at temperatures where the crystalline structures have already melted. More probably, the three distinct clusters of nitroxides exist owing to the differences in the chain conformation. The nitroxides producing the broadest of the three spectral components (I mode, see curve 1 in *Figure 2*) are suggested to be those located in the helical chain sequences, where the barrier of the label motion is known to be high[7] and the angle α has its lowest value. The liquid-like component observable at elevated temperatures where the polymer is liquid crystalline (III

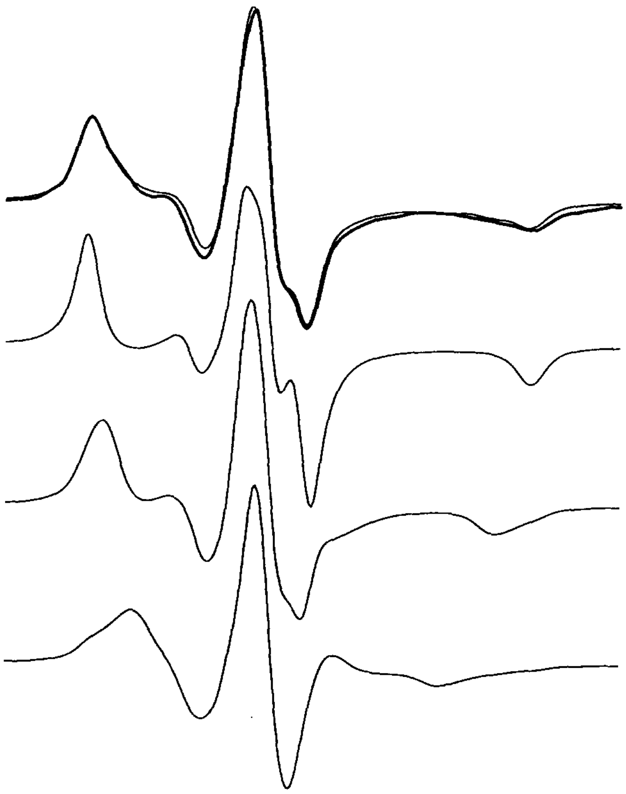

Figure 3 Experimental (bold line) and calculated (thin line) spectra of POIC at 21°C; top row. Below are shown three component spectra from which the final simulated spectrum was obtained. The width of each spectrum is 95.6 G

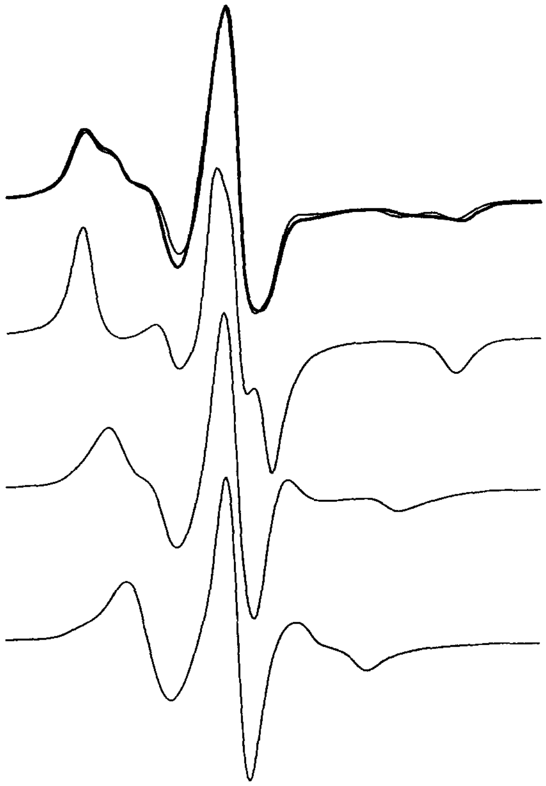

Figure 4 Experimental (bold line) and calculated (thin line) spectra of POIC at 49°C. Three spectral modes are shown below, as in *Figure 3*. The width of the spectra is 95.6 G

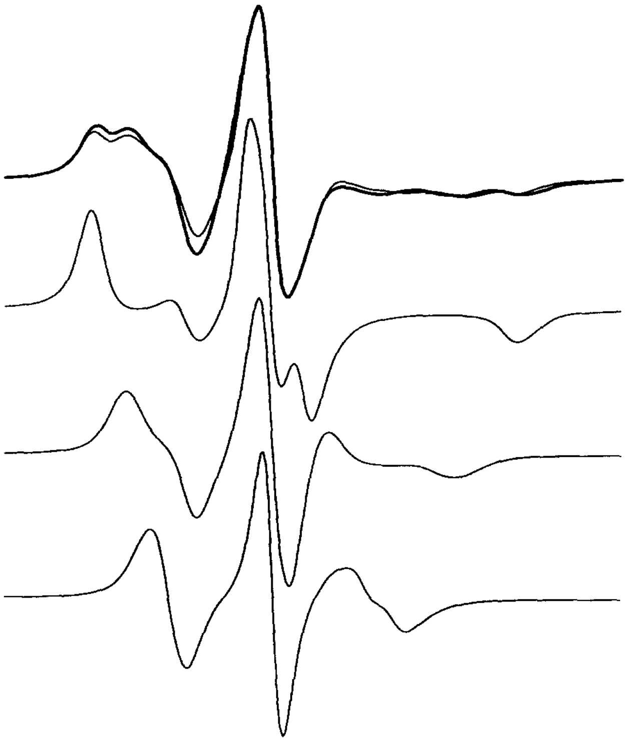

Figure 5 Experimental and calculated spectra of POIC at 80°C, as in *Figure 3*

Table 1 The parameters used in the simulation of the experimental spectra. The final simulated spectra are combinations of three modes summed with weights denoted as W. An exception is the spectrum measured at 156°C, which was simulated using four spectral modes

T (°C)	I mode				II mode				III mode			
	α (deg), δ (G)	S (σ_S)	κ (σ_κ)	W	α (deg), δ (G)	S (σ_S)	κ (σ_κ)	W	α (deg), δG	S (σ_S)	κ (σ_κ)	W
21	31, 2.2	0.0 (0.51)	0.5 (0.60)	0.51	55, 2.1	0.0 (0.69)	0.0 (0.91)	0.33	83, 2.0	0.0 (0.94)	0.0 (0.71)	0.16
49	38, 2.1	0.0 (0.46)	0.0 (0.55)	0.50	74, 2.0	0.0 (0.55)	1.0 (0.64)	0.48	95, 1.9	0.0 (0.72)	0.0 (0.59)	0.02
80	41, 2.1	0.0 (0.53)	1.0 (0.37)	0.52	81, 2.0	0.0 (0.26)	0.0 (0.71)	0.42	111, 1.8	0.0 (0.34)	0.0 (0.50)	0.06
128	47, 2.0	0.0 (0.50)	0.0 (0.57)	0.46	88, 2.0	−0.25 (0.35)	0.40 (0.40)	0.39	136, 1.7	0.05 (0.33)	0.0 (0.18)	0.15
156	52, 2.0	0.0 (0.40)	0.2 (0.47)	0.41	96, 2.0	0.0 (0.32)	0.0 (0.53)	0.26	150, 1.7 158, 1.5	−0.2 (0.18) 0.0 (0.17)	−1.0 (0.67) 0.0 (0.25)	0.25 0.08

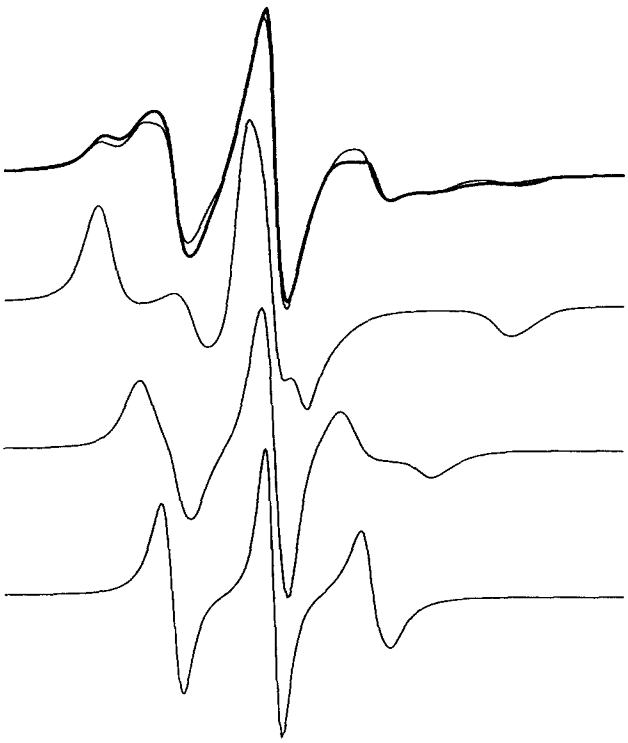

Figure 6 Experimental and calculated spectra at 128°C, as in *Figure 3*

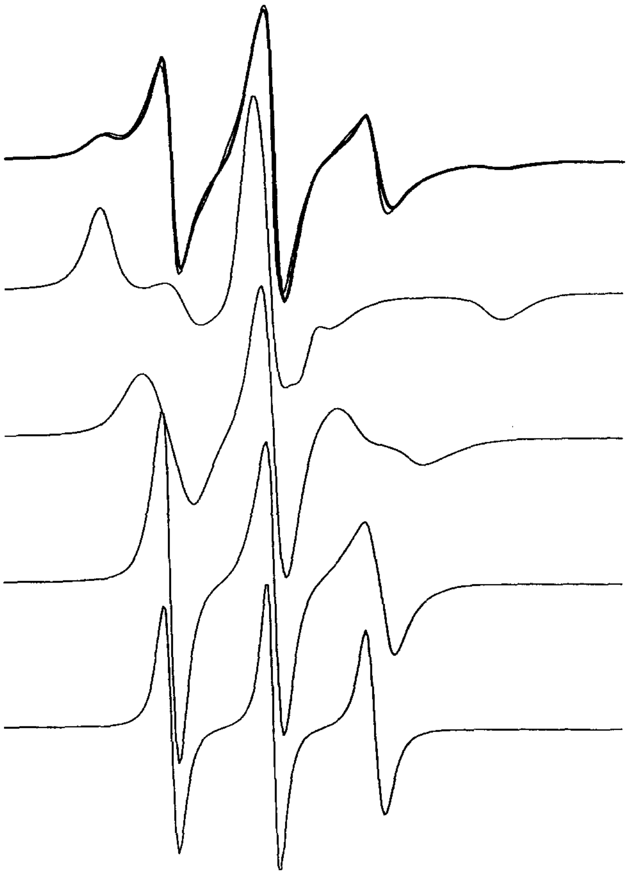

Figure 7 Experimental (bold) and calculated (thin) spectra at 156°C. In this case, the final simulated spectrum is a sum of four components, shown below the top row. The width of the spectra is 95.6 G

mode, curve 3 in *Figure 2*) must be due to the unwinding of the helix, i.e. due to a helix–coil transition occurring at temperatures close to 114°C. This interpretation is in

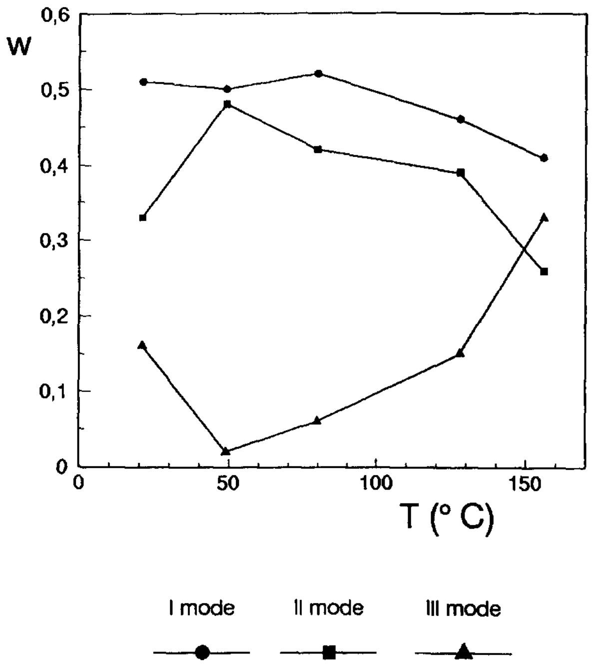

Figure 8 Temperature dependence of the weight of various spectral modes used to simulate the experimental spectra

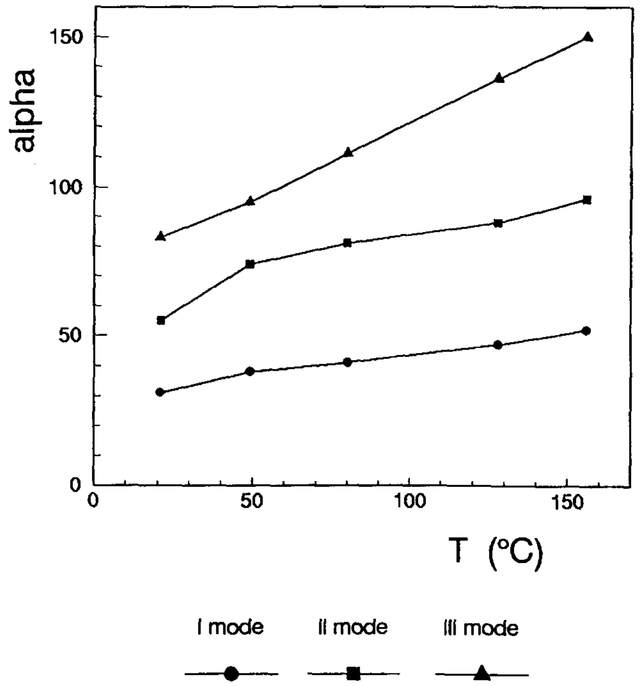

Figure 9 Oscillational angle α against temperature for the various spectral modes

accordance with X-ray diffraction patterns of POIC[4] which show significant changes to occur in the chain packing during the formation of the liquid crystalline phase. The origin of the component having the 'medium' separation of the outer extrema (II mode) is not clear. Whether it originates from the radicals located close to the chain reversals known to occur along the chain[8], or from the thermal fluctuation of the bond angles in the main chain (flexing of the chain), may not be definitely stated.

The spectrum measured at 156°C was best simulated by combining four different spectra. The fourth mode which was added in this case describes a fraction of very mobile nitroxides, these being most probably the radicals at, or close to, the chain ends. The weight of this mode is fairly low, 0.08 (see *Table 1*). The situation is analogous to that observed with solutions and gels of POIC, where the narrow spectrum with sharp lines could be observed only in some of the samples[1].

The important finding, however, is the coexistence of various spectral modes over the whole temperature range studied. This indicates that the polymer has a distribution of chain sequences with varying conformations not only in the solid, but also in the liquid crystalline state. The relative weights of the spectral modes change with temperature; this is illustrated in *Figure 8*. The proportion of the helical chain sequences decreases with increasing temperature, whereas the proportion of the irregular structures increases. The changes of the weights of the spectral modes correlate with the observed phase transitions. On the other hand, the oscillational angle α increases smoothly with temperature, see *Figure 9*. The flexibility of the chain is sensitive to temperature through the whole range of temperatures.

CONCLUSIONS

The e.p.r. spectra of dry spin-labelled POIC measured at temperatures ranging from room temperature to 156°C turned out to be superpositions of several spectra. The complex e.p.r. spectra were simulated by combining three (and at high temperatures, four) spectral modes, all of these representing the oscillational motion of the labelled side group. In each mode, the position of the principal axis of the label oscillation (R) was defined using two order parameters, S and κ. A certain dispersion of the values of the order parameters was allowed. Then, the oscillational angle α was determined.

Most of the experimental spectra were theoretically reproduced using $S = 0$, which implies that the preferential axis of the label oscillation is held close to the magic angle relative to the nitroxide z axis.

The different spectral modes are assumed to reflect differences in the chain conformation and, thus, in the flexibility of the chain. The oscillational angle α increases with increasing temperature in each mode, but the rate of increase is different in various modes. Of the components of the composite spectra, the one with the largest separation of the outer extrema ($2\bar{A}$) is suggested to be produced by the labels in the helical chain sequences. On the other hand, the motionally narrowed component is evidently due to the chain sequences with random conformation.

The results show that helical and random (coiled) chain sequences coexist through the whole temperature range studied. With increasing temperature the semicrystalline polymer melts into the liquid crystalline state; simultaneously, the fraction of polymers with random chain conformation increases.

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