E.p.r. spectroscopy of spin-labelled macromolecules as a tool for determining chain conformations in amorphous solid polymers

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(Received 21 January 1992; accepted 25 March 1992)

The feasibility of using e.p.r. spectrscopy in studying conformations of spin-labelled macromolecules in the amorphous solid state is examined. The algorithm for e.p.r. spectra computation is developed. It was assumed that molecular weights of labelled linear chains are high and their solid solution is diluted. It is shown that the scaling exponent which determines the dependence of mean-square end-to-end distance on molecular weight and stiffness parameter (mean-square length of monomer unit) may be extracted from the spectra. Computed spectra are compared with experimental ones, measured at 77 K, of diluted solutions of spin-labelled poly(4-vinyl pyridine) [P(4-VP)] of different molecular weights in methanol and non-labelled P(4-VP). The conformational state (Gaussian coil), parameter of stiffness, and radius of gyration of spin-labelled P(4-VP) macromolecule in frozen solutions are determined. A conclusion is drawn that e.p.r. spectroscopy may become a new tool for studying chain conformatin in solid polymers.

(Keywords: e.p.r. spectroscopy; polymer conformations in solid state; spin labels; monomer pair distribution function)

INTRODUCTION

Up to now neutron scattering has been almost the single source of information on polymer conformations in the solid state[1]. This technique demands special facilities and observes macromolecules in a unique way. Thus, an increase in the number of methods for probing polymer conformations is urgently required. For this reason a method based on measuring the rate of electronic excitation transport among chromophores attached to polymer chains has recently been developed[2,3].

We present here an approach based on the analysis of e.p.r. spectra of spin-labelled polymer molecules dispersed in amorphous solids. The spin-label method is widely used to study molecular dynamics and the structure of liquids, polymers and biological systems. In polymer science, spin labels (stable nitroxide radicals attached to the macromolecules) are employed in the research of the dynamics of individual molecules, interactions of molecular coils in concentrated solutions, phase equilibrium in polymer solutions, measurements of the local segment concentrations in solid polymers, etc. These findings are summarized by Wasserman and Kovarsky[4].

It was found, in particular, that the shape of e.p.r. spectra depends on the number of spin labels attached to a macromolecule. An example of such a transformation is shown in *Figure 1*. The e.p.r. spectra of solid solutions of free radicals (spin probes) also transform with an increase in their concentration[5,6]. This transformation is mainly due to the dipole–dipole interactions of radicals which strongly depend on the distances between them. The transformation of nitroxide radical spectra are characterized most conveniently by the parameter

$$\Delta = d_1/d - (d_1/d)_0 \qquad (1)$$

where d_1/d is the ratio of the combined intensity of extreme lines to the intensity of the central line (see *Figure 1*) at a given concentration of radical and $(d_1/d)_0$ is the value of d_1/d at such low concentrations that radical interactions may be disregarded. The virtue of this parameter is its insensitivity to the magnetic parameters of nitroxide radicals[5]. The local segment concentration was determined as the concentration of such a spin probe solution for which the Δ parameter of the e.p.r. spectrum is equal to that of the spin-labelled macromolecules under study[4,7]. However, local concentrations only qualitatively characterize polymer conformations.

We shall demonstrate here that the two parameters, ν and a, which appear in a well-known relationship between the mean-square end-to-end distance and the number of monomers, N, composing the chain[8]:

$$\langle r_N^2 \rangle = N^{2\nu} a^2 \qquad (2)$$

may be extracted from e.p.r. spectra of the dilute frozen solution of spin-labelled macromolecules if N is large enough. Here a is the monomer mean-square length[8]. This parameter characterizes the rigidity of the polymer chain. Clearly conformations of labelled polymer may differ from those of non-labelled polymer. However, conformations of random copolymers, which labelled polymers are, pose an independent problem. Moreover, an extrapolation to zero label content cannot be

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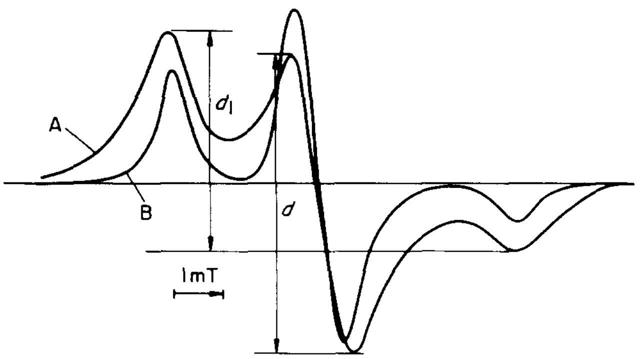


Figure 1 The first derivative of unnormalized absorption e.p.r. spectra of spin-labelled P(4-VP) (number of monomer units $N = 500$) in methanol at 77 K. The number of labels attached to the macromolecule is $m = 85$ (A) and $m = 3$ (B)

excluded. Here, we will investigate the solid solution of spin-labelled macromolecules diluted to such an extent that the interaction between labels attached to different macromolecules can be neglected. A preliminary communication has been published elsewhere[9].

THEORETICAL

In terms of the magnetic resonance theory, solid solutions of spin-labelled macromolecules are magnetically diluted solids with peculiar spatial correlations. The theory of the magnetic resonance line shape in such solids was recently developed[10,11]. This theory allows the calculation of the shape of the absorbtion spectrum, $\mathbf{f}(H)$, provided that the pair distribution function of the paramagnetic centres, $C_s(\mathbf{r})$, and the spectral shape at infinite dilution, $\mathbf{f}_0(H)$, are known. The $C_s(\mathbf{r})$ is equal to the concentration (number density) of paramagnetic centres in the neighbourhood of the point with the radius-vector $\mathbf{r}$ provided the origin of co-ordinates is occupied by a certain paramagnetic centre.

The theory[10,11] is based on the following assumptions.

I. The system is uniform in that it is unimportant which paramagnetic centre occupies the origin. This assumption means that the numbers of monomers composing a polymer molecule, N, and spin labels attached to it, m, are high enough for the contribution of labels located near the ends of the chain to be ignored.

II. The concentration of paramagnetic centres has to be so low that the contributions of multiparticle (triple, quarternary, etc.) distribution functions can be neglected.

III. The effect of forbidden transitions on the e.p.r. spectra can be disregarded and for each radical there are $(2I + 1)$ definite resonance fields, where I is the spin of the nucleus coupled to the electron spin by hyperfine interaction. It is also supposed that the resonance fields are composed of static and fluctuating components. As a result the spectrum at infinite dilution can be presented as a superposition of M Lorentzians of equal width R:

$$\mathbf{f}_0(H) = \frac{R}{\pi}\sum_{p=1}^{M}\frac{W_p}{R^2 + (H - H_p)^2} \tag{3}$$

where $H_p = (p - 1)h + H_{min}$ and $h = (H_{max} - H_{min})/(M - 1)$, where H_{max} and H_{min} being the spectrum bounds. The weight, W_p, should be evaluated as the fraction of radicals with resonance fields belonging to the interval from $(H_p - h/2)$ to $(H_p + h/2)$. If the magnetic parameters of radicals (principal components of g-tensor and hyperfine coupling matrix A) are known, then W_p may be calculated from known formulae for resonance fields[12]. After that M and R should be determined from the requirement of the best fitting of $\mathbf{f}_0(H)$ to experimental spectrum at such low radical concentrations that the spectrum is independent of the concentration. Equation (3) is suitable for numerical computation although it is not unique.

IV. Only the dipole–dipole and exchange interactions are significant for nitroxide radicals[13]. Exchange interactions rapidly decrease with distance, enabling the introduction of the radius of the interactions[14]. This radius is comparable or less than the thickness of the macromolecules. Because of this it was assumed that the exchange interactions can be disregarded.

According to the above theory, the absorption spectrum can be calculated by:

$$\mathbf{f}(H) = \frac{1}{\pi} Re \sum_{n=1}^{M} W_n[i(H_n - H) + R + G(H_n - H - iR, H_n)]^{-1} \tag{4}$$

$$G(Z, H_n) = \sum_{p=1}^{M} W_p F(Z, H_p - H_n) \tag{5}$$

$$F(Z, U) = -4\pi i Z \int_0^1 \mathrm{d}x\, E^2(x) I(x, Z, U) \tag{6}$$

$$E(x) = 3/4 g\mu_B(1 - 3x^2)$$

$$I(x, Z, U) = \int_0^\infty \mathrm{d}r\, r^2 C_s(r) \times \frac{\phi(Z, U)r^6 - E^2(x)}{[V_+ r^6 - E^2(x)][V_- r^6 - E^2(x)]} \tag{7}$$

$$\Phi(Z, U) = 9Z^2 + 11ZU + 4U^2$$

$$V_{\pm}(Z, U) = 5Z^2 + 5ZU + 2U^2 \pm 2(2Z + U)P(Z, U) \tag{8}$$

$$P(Z, U) = (Z^2 + ZU + U^2)^{1/2}$$

where g is the isotropic g-factor, μ_B is the Bohr magneton. It is taken into account here that $C_s(\mathbf{r}) = C_s(r)$ for isotropic media.

Comparison of equations (3) and (4) reveals the function G takes into account the radical interactions effects.

The cofactor of the pair distribution function on the right-hand side of equation (7) behaves as r^2 at small distances, r, between radicals and r^{-4} at large distances.

From this it follows that e.p.r. spectra are sensitive to the form $C_s(r)$ over limited range of r.

The true range of applicability of assumptions II and IV can be determined only through comparison with experimental results. To test the theory, spectra of nitroxide radicals in glassy solution of known concentration C_s were recorded[15]. In this case $C_s(\mathbf{r}) = C_s$ and transformation of spectra due to increase in C_s can be computed by equations (4)–(8) without fitting parameters. It was found that the theory[10,11] provides a quantitative description of e.p.r. spectra transformation if the Δ [see equation (1)] does not exceed 0.35.

If the labels are uniformly or randomly distributed along the linear chain, the number of monomers in the chain, N, and the number of labels, m, are large enough (see assumption I), and the labelling degree $\beta = m/N$ is finite, then

$$C_s(\mathbf{r}) = \beta C(\mathbf{r}) \tag{9}$$

where $C(\mathbf{r})$ is the monomer pair distribution function which is immediately connected with the density–density autocorrelation function.

Here, we will investigate the solid solution of spin-labelled macromolecules diluted to such an extent that the interaction between labels attached to different macromolecules can be neglected.

For an infinitely long linear chain the pair distribution function of monomers can be written as:

$$C(\mathbf{r}) = \int_{-\infty}^{\infty} \mathrm{d}K\, W_{\mathrm{K}}(\mathbf{r}) \tag{10}$$

where $W_{\mathrm{K}}(\mathbf{r})$ is the probability distribution for the vector $\mathbf{r}$ connecting the ends of a chain segment of K monomer units. In a solid, the configuration of each chain is frozen, hence the ensemble necessary for the application of the probability theory is formed by all labelled macromolecules in the sample. Measurements of large-angle light scattering[16–18] and numerical experiments[19] were analysed using distributions of the following type:

$$W_{\mathrm{K}}(\mathbf{r}) = C_{\mathrm{K}} r^{\lambda} \exp[-(r/\sigma_{\mathrm{K}})^{t}] \tag{11}$$

where λ and t are parameters, and the normalizing constant is

$$C_{\mathrm{K}} = \mathrm{t}(4\pi\Gamma[(\lambda+3)/t]\sigma_{\mathrm{K}}^{\lambda+3})^{-1} \tag{12}$$

Γ is the gamma function, and the parameter σ_{K} is proportional to the mean-square end-to-end length of segment of K units:

$$\sigma_{\mathrm{K}}^2 = \frac{\Gamma[(\lambda+3)/t]}{\Gamma[(\lambda+5)/t]}\langle \mathbf{r}_{\mathrm{K}}^2 \rangle \tag{13}$$

A relationship similar to the equation for the end-to-end separation of the entire chain is assumed to be held for a segment:

$$\langle \mathbf{r}_{\mathrm{K}}^2 \rangle = \mathrm{K}^{2v} a^2 \tag{14}$$

It is assumed after Fisher[20] that

$$t = (1-v)^{-1} \tag{15}$$

The distribution (11) contains three parameters, λ, t (or v) and a, and embraces many special cases. Hence, at $t = 2$ ($v = 1/2$) and $\lambda = 0$ we obtain a Gaussian chain. Monte-Carlo simulations by Baumgärtner[19] showed that the distribution of end-to-end lengths of segments within a chain with strong volume interactions, is described with high accuracy by equation (11). However, Lam and Family have shown recently[21] that using equation (11) for the estimation of the coefficients in the expansion of the structure function at small momentum transfers leads to disagreement with Monte-Carlo results for self-avoiding chains in two and three dimensions. It should be noted that large separations between monomers contribute significantly to the higher moments of the density–density correlation function, but for our purposes medium range separations are important. Besides, the most simple form of $C(r)$ is needed in the first stage of the technique development. Therefore, we shall use equation (11) to calculate the pair-distribution function. Substitution of equation (11) into equation (10) with account of equations (12)–(15) gives:

$$C(\mathbf{r}) = \frac{q(v,\lambda)}{a^{\mathrm{D}} r^{3-\mathrm{D}}} \tag{16}$$

where $D = 1/v$ is the fractal dimension of the coil and

$$q(v,\lambda) = \frac{\Gamma[(1-v)(\lambda+3-D)]}{2\pi v\Gamma[(1-v)(\lambda+3)]} \times \left\{\frac{\Gamma[(1-v)(\lambda+5)]}{\Gamma[(1-v)(\lambda+3)]}\right\}^{D/2} \tag{17}$$

Equation (16) coincides with the known scaling law[8].

For the Gaussian chain we get the known result[8]:

$$q(1/2, 0) = 3/\pi \tag{18}$$

It can be assumed with good accuracy[8] for self-avoiding chains that $v = 3/5$. This correlates with the $t = 2.48 \pm 0.05$ obtained by Baumgärtner[19] for the distances between momomer units located far away from the chain ends.

The exponent λ determines the behaviour of distribution $W_{\mathrm{K}}(\mathbf{r})$ at $r \ll \sigma_{\mathrm{K}}$. Using the renormalization group theory to the second order of ε-expansion des Cloizeaux[22] found that for inner segments with a strong volume interaction $\lambda = 0.71$. Therefore, we assume for a highly swollen chain $v = 3/5$ and $\lambda = 0.71$. According to equation (17):

$$q(0.6, 0.71) = 0.43 \tag{19}$$

Nominally, equation (16) also reproduces the globular state at which $D = 3$. Then, the q/a^3 should be equal to the concentration of monomer units in the globule.

Obviously, equation (16) is valid only in the $r^* < r < r^{**}$ interval. The lower limit must be greater than the radius of non-covalent interactions between monomer units (chain 'thickness'). It follows from equation (10) that r^{**} must be of the order of coil size (radius of gyration). The use of equation (16) is possible, first, under the condition:

$$4\pi\beta q v (r^*/a)^{\mathrm{D}} \ll 1 \tag{20}$$

i.e. for small enough degrees of labelling β [see equation (9)], and, second, for such large degrees of polymerization N at which the dipole–dipole interaction of labels separated by r^{**} can be disregarded. It should be mentioned, that the shape of the e.p.r. spectra of solid solutions of nitroxide radicals at concentrations $C_s < 7 \times 10^{-3}$ mol l^{-1} is independent of C_s (ref. 23). This implies that at radical separation $> \sim 60$ Å, their interactions can be neglected. Substituting (16) into (7) and using calculus of residues gives

$$F(Z,U) = \frac{\pi^2}{3}\beta q J(D) H_c^{\mathrm{D}/3} Z\left[1 + i\,\mathrm{ctn}\left(\frac{\pi D}{6}\right)\right] \times B(Z,U,D/6) \tag{21}$$

where

$$B(Z,U,\mu) = \left[1 - \frac{Z+U}{P(Z,U)}\right]\mathbf{V}_{+}^{-\mu}(Z,U) + \left[1 + \frac{Z+U}{P(Z,U)}\right]\mathbf{V}_{-}^{-\mu}(Z,U) \tag{22}$$

$$(0 \leqslant \arg(\mathbf{V}_{\pm}^{-\mu}) < 2\pi\mu)$$

$$H_c = 3g\mu_B/4a^3 \tag{23}$$

$$J(D) = \int_0^1 \mathrm{d}x|1 - 3x^2|^{D/3} \tag{24}$$

In particular, for the Gaussian and swollen coils, respectively, we obtain:

$$J(2) = 0.8016 \quad \text{and} \quad J(5/3) = 0.8192 \tag{25a}$$

and for the globule:

$$J(3) = 4/3\sqrt{3} \tag{25b}$$

It is quite simple to find the analytical asymptotic expression for the far wings of the spectrum. If $|H|$ greatly exceeds the spectrum width, then we can neglect differences $|H_n - H_m|$ and R in comparison with $|H|$ in equations (4) and (5). As a result, we come up with a line shape that coincides with that found previously[10]. This means that at $|H| \gg H_c$ the line shape is:

$$\mathrm{f}(H) = 2\pi\beta q J(D) H_c^{D/3}/3|H|^{1+D/3} \tag{26}$$

Consequently, the less the fractal dimension of the coil, the slower the intensity at the wings. At uniform distribution (in the globule) we get the Lorentzian wings, and for the Gaussian and swollen coil, the so called 'superLorentzian' wings.

Specific calculations were performed for the following radical magnetic parameters:

I $g_x = 2.00917$, $g_y = 2.00619$, $g_z = 2.00224$
$A_x = 0.58$ mT, $A_y = 0.77$ mT, $A_z = 3.64$ mT

II $g_x = 2.00938$, $g_y = 2.00619$, $g_z = 2.00224$
$A_x = 0.55$ mT, $A_y = 0.74$ mT, $A_z = 3.5$ mT

III $g_x = 2.00878$, $g_y = 2.00616$, $g_z = 2.00270$
$A_x = A_y = 0.675$ mT, $A_z = 3.178$ mT

Calculations with magnetic parameters of radicals I and II were performed using $R = 0.4$ mT and with parameters of radical III, $R = 0.325$ mT.

Figure 2 shows examples of the first derivative of absorption spectra, computed using radical I parameters.

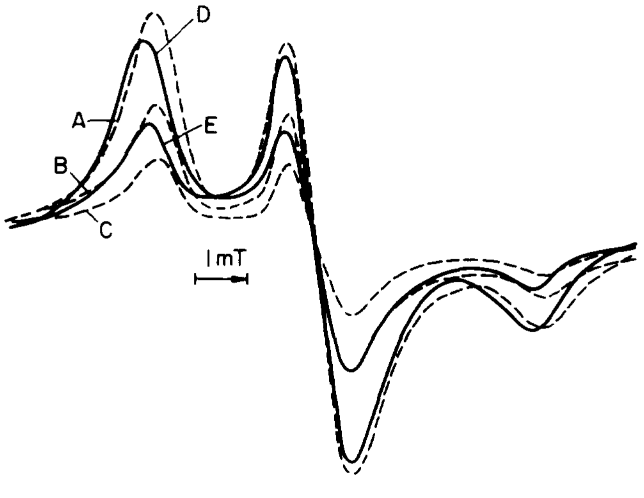


Figure 2 Comparison of calculated and experimental spectra of spin-labelled macromolecules. Broken lines depict e.p.r. spectra calculated using magnetic parameters of radical I for spin probes (A), spin-labelled Gaussian chains with $\beta = 0.17$ and $a = 10$ Å (B), self-avoiding chains with $\beta = 0.17$ and $a = 4.4$ Å (C). Solid lines are normalized experimental spectra of spin probes at concentration 0.11 mol l^{-1} (D) and PVP-1 with $\beta = 0.17$ (E). Parameter $d_1/d = 0.73$ for all spectra

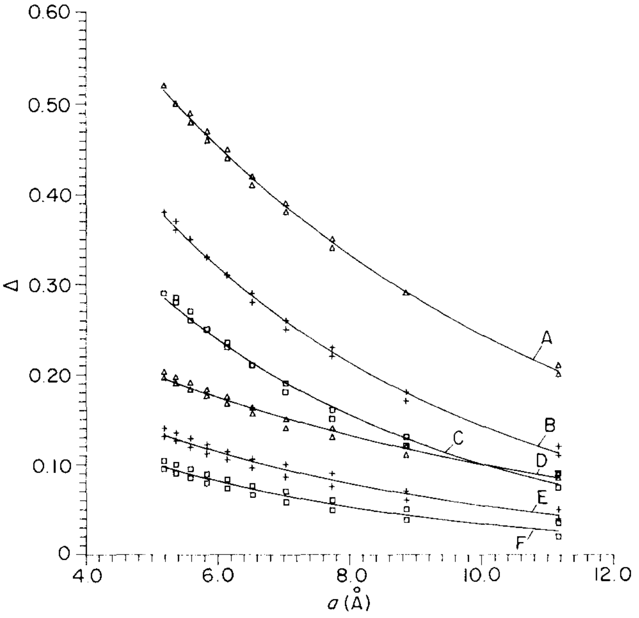


Figure 3 Dependences of parameter Δ on mean-square length of monomer unit (a) computed for Gaussian (A–C) and self-avoiding (D–F) chains. Calculations were done using magnetic parameters of radicals I (upper points) and II, for $\beta = 0.17$ (curves A and D), $\beta = 0.09$ (curves B and E), $\beta = 0.06$ (curves C and F)

Appreciable differences in the line intensities are evident, although all spectra have the same normalizing:

$$\int \mathrm{d}H\mathrm{f}(H) = 1 \tag{27}$$

Probably, the differences in intensities are due to variations in the decay rate at the far spectrum wings (not shown in the figure). These differences can be seen most clearly from equation (26).

Figure 3 presents the dependences of parameter Δ, defined by equation (1) on the mean-square length of the monomer unit (a) for Gaussian and swollen coils. These dependences were obtained from spectra computed for various degrees of labelling. In the figure, each point corresponds to a calculated spectrum.

The dependences of Δ on a are practically insensitive to the choice of radical magnetic parameters, analogous to the dependence of Δ on radical concentration in solid solutions of spin probes[5].

The dependence of Δ on a is explained by the fact that with increasing chain stiffness the local concentration of spin labels drops. It also drops due to coil swelling. Hence, if parameters ν and λ are known in advance, then chain stiffness can be obtained from experimentally observable Δ values.

It follows from spectra presented in *Figure 2* that, on the basis of line intensity analysis we can try to select between the different states of coils. *Figure 4* shows the dependences of central line intensity in spectra normalized by equation (27) on the Δ parameter, estimated for three states of a macromolecule: self-avoiding chain, Gaussian chain and globule. The most remarkable thing about these dependences is that points corresponding to different β values fall onto one and the same curve. This implies that the dependences presented in *Figure 4* are determined only by the conformational state of the macromolecule (the parameter ν). According to equation (21) variation of factor q

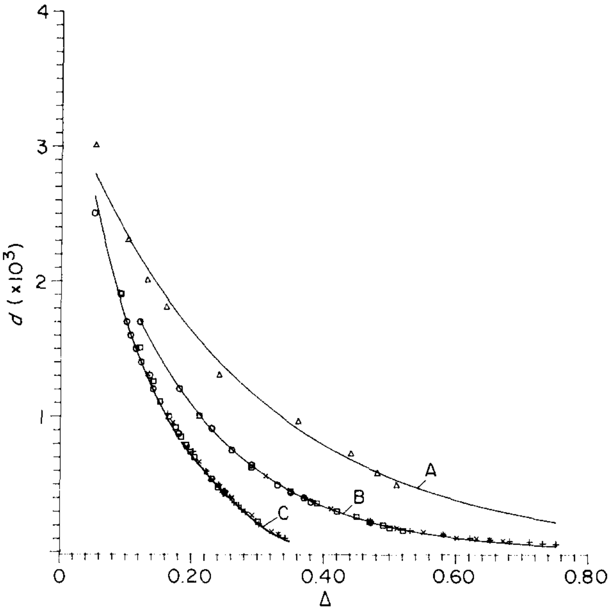


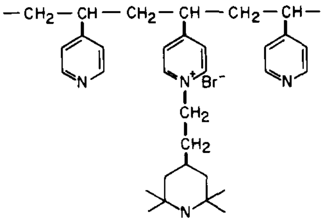
Figure 4 Dependences of central line intensities of normalized spectra [see equation (27)] on parameter Δ, calculated for three conformational states: globule (A), Gaussian chain (B), self-avoiding chain (C) and for labelling degrees $\beta = 0.09$ (○), $\beta = 0.17$ (□), $\beta = 0.30$ (×) and $\beta = 0.40$ (+)

produces the same effect on the line shape as variation of βq. Therefore, points corresponding to various values of λ have to fall onto one curve, since as evident from equation (17) only parameter q depends on λ.

The analysis given above shows that the conformational state of a macromolecule can be determined if the parameter Δ together with the intensity of normalized e.p.r. spectra are accurately measured.

EXPERIMENTAL

Measurements were performed using spin-labelled poly(4-vinyl pyridine) [P(4-VP)]; this polymer was previously repeatedly used to determine the local density of monomer units in a polymer chain[4,7]. The spin-labelled polymer was obtained by the reaction of P(4-VP) and 4-bromomethyl-2,2,6,6-tetramethyl-1-piperidine-1-oxyl in nitromethane[7]:



The content of spin labels on the polymer chain $\beta = m/N$ (m is the number of labelled monomer units) was determined by double integration of e.p.r. spectra. 4-Bromomethyl-2,2,6,6-tetramethyl-1-piperidine-1-oxyl was used as a standard. The spectra of solid polymers and 0.5 wt% polymer solution in methanol at 25°C were integrated; the results of integration differed insignificantly. The following spin-labelled polymers were prepared: PVP-1 ($\beta = 0.17 \pm 0.02$); PVP-2 ($\beta = 0.09 \pm 0.01$); PVP-3 ($\beta = 0.06 \pm 0.01$); PVP-4 ($\beta = 0.005$, $M_w = 5 \times 10^4$) and PVP-5 ($\beta = 0.1 \pm 0.01$, $M_w = 1 \times 10^6$). The molecular weights were determined by light scattering. The labels were assumed to be randomly distributed along the macromolecule; this supposition is based on the fact that the quaternization reaction of P(4-VP), as a rule, leads to statistical distribution of quaternized monomer units along the polymer chain[24].

E.p.r. spectra were obtained using a Radiopan Sex-2544 X-band radiospectrometer at 77 K. Recording conditions (modulation amplitude, power level of radio-frequency field) were such that effects of power saturation and line broadening were excluded.

Spectra of 0.5 wt% solutions of spin-labelled polymers in methanol, as well as in non-labelled P(4-VP) were registered. To prepare a solution of labelled polymer in non-labelled polymer, both polymers were dissolved in a 1:200 ratio in methanol, and then the solvent was slowly evaporated.

For solid solutions of spin-labelled polymer in methanol the following values for d_1/d and Δ parameters were obtained:

PVP-1 $d_1/d = 0.73$, $\Delta = 0.24$

PVP-2 $d_1/d = 0.63$, $\Delta = 0.14$

PVP-3 $d_1/d = 0.52$, $\Delta = 0.03$

PVP-4 $d_1/d = 0.49$

PVP-5 $d_1/d = 0.62$, $\Delta = 0.13$

For spin-labelled polymer in a non-labelled polymer:

PVP-1 $d_1/d = 0.77$, $\Delta = 0.20$

PVP-2 $d_1/d = 0.65$, $\Delta = 0.08$

PVP-3 $d_1/d = 0.58$, $\Delta = 0.01$

PVP-4 $d_1/d = 0.57$

PVP-5 $d_1/d = 0.73$, $\Delta = 0.16$

These Δ values were defined as the difference in d_1/d values of polymer under consideration and PVP-4; the spin-label content in PVP-4 was so small that no dipole–dipole interaction was evident in its spectra.

Magnetic parameters of spin-labels have been determined by 2-mm e.p.r. spectroscopy[25]. These parameters depend on the solvent, hence their values were separately estimated for 0.5 wt% solutions of spin-labelled PVP-4 in methanol and in non-labelled polymer at 77 K. The aforementioned parameters of radical I (see Theoretical section) are those determined for a solution of spin-labelled polymer in methanol, those of radical II have been determined for PVP-4 in non-labelled polymer. Magnetic parameters of radical III are known[26] as the parameters of di-tret-butylnitroxide radical.

RESULTS AND DISCUSSION

Theoretical analysis has shown that to determine the conformational state of a spin-labelled macromolecule (value of ν) it is necessary to measure the absolute intensities of e.p.r. spectra lines (*Figure 4*). However, it is more convenient to register relative intensities of spectra, reduced to a given concentration of paramagnetic centres, rather than absolute intensities. Therefore, the e.p.r. spectra of solid solutions of spin-labelled macromolecules and spin probe with known concentration of radicals should be compared.

The macromolecule conformational state can be

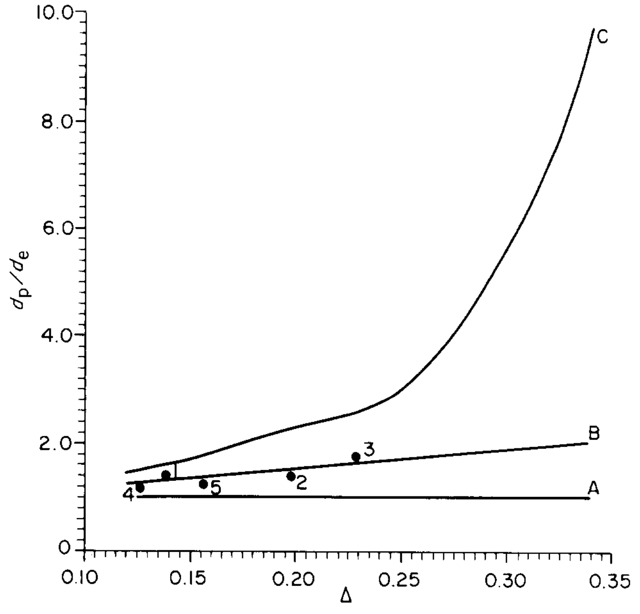


Figure 5 Dependences of the ratio of central line intensities in normalized spectra of probe, d_p, and label, d_l, on parameter Δ: (A) globule; (B) Gaussian chain; (C) self-avoiding chains. Experimental values of this ratio are shown for PVP-2 in methanol (1), PVP-1 in P(4-VP) (2), PVP-1 in methanol (3), PVP-5 in methanol (4), PVP-5 in P(4-VP) (5)

determined using dependences presented in *Figure 5*. This figure depicts the dependences of ratios of normalized [see equation (27)] e.p.r. spectra intensities of probe solution and spin-labelled macromolecules on the Δ parameter, computed for three polymer chain states: globule, Gaussian chain and self-avoiding chain. The spatial distribution of labels in globules of high molecular weight have an identical form to those of probes in solution, and this is why the ratio is equal to unity. It is readily seen that these ratios differ significantly, especially in the $0.02 < \Delta < 0.35$ interval.

Experimental values of the ratios of intensities of normalized (reduced to a given concentration) label and probe spectra are also plotted in *Figure 5*. The 4-bromomethyl-2,2,6,6-tetramethyl-1-piperidine-1-oxyl was used as a probe and the glassy solution of this radical in methanol and/or in non-labelled polymer was used for reducing intensities of spectra labels. The concentrations of probe solutions were chosen from the condition of coincidence, the Δ parameter, of the label spectrum with those of the probe spectrum. For solutions of PVP-1, PVP-2 and PVP-5 in methanol, and for a solution of PVP-1 and PVP-5 in non-labelled P(4-VP) the experimental ratios of intensities of normalized label and probe spectra agree well with the theoretical estimate for the Gaussian coil. Unfortunately, for solutions of PVP-3 in methanol and in non-labelled P(4-VP), as well as for solutions of PVP-2 in non-labelled P(4-VP), no decisive conclusion on chain conformations can be drawn, since in these cases experimental values of Δ are < 0.1; they correspond to the interval in which ratios of intensities for three polymer chain states differ insignificantly.

At first notice, the reason for the spin-labelled macromolecule of P(4-VP) in solid methanol to have the conformation of a Gaussian chain [methanol is known to be a good solvent for P(4-VP) at room temperature[27]] is quite obscure. However, it should be kept in mind that we determined the conformation of P(4-VP) modified by spin labels. Moreover, the quality of the solvent depends on temperature, and it cannot be excluded that the methanol glass transition temperature is close to the θ temperature of P(4-VP) solution in methanol.

The fact that the conformation of spin-labelled P(4-VP) macromolecule in solid P(4-VP) is that of a Gaussian coil corresponds to the Flory theorem on the conformation of macromolecules in non-dilute amorphous polymers[8] and to a number of experimental data[1,3].

The mean-square length of monomer unit was obtained from curves $\Delta = f(a)$ for the Gaussian chain, shown in *Figure 3*, and from experimental values of the parameter Δ (see Experimental section). Data are summarized in *Table 1*. Using these values the spectra of solid solutions of spin-labelled P(4-VP) were calculated and compared with experimental values (*Figure 2*). It is clearly seen that the experimental spectrum corresponds well to the theoretical one.

Table 1 also contains data on the macromolecule root mean-square radius of gyration, $(\bar{R}_G^2)^{1/2}$, computed using[8]:

$$\bar{R}_G^2 = \frac{1}{6}\langle r_N^2 \rangle \qquad (28)$$

and equation (2) with $\nu = 1/2$ (Gaussian chain).

It is interesting to note that for P(4-VP) in a θ solvent (butanone–isopropanol mixture at room temperature) light scattering experiments[27] give $a = 7.5$ Å. The mean-square length of the monomer unit of spin-labelled P(4-VP) is greater than that of the non-labelled polymer. This is not surprising since chain stiffness and the dimensions of the polymer coil might increase due to the attachment of spin labels. Moreover, the conformations determined here have to be compared with the conformations at the glass transition temperature of solvent.

The radii of gyration values listed in *Table 1* are interesting to compare with the mean separation of centres of polymer coils. Knowing the polymer concentration in solution (0.5 wt%) and the degree of polymerization N, it is quite simple to find that the mean coil separation for polymer with $M_w = 5 \times 10^4$ is ~260 Å, i.e. greater than the macromolecule radius of gyration (~100 Å). This comparison reveals that the polymer coils do not overlap and, indeed, they can be regarded as isolated molecules. Polymer coils with $M_w = 1 \times 10^6$ at the same concentration (0.5 wt%) are separated by ~700 Å, and the values of the radius of gyration are ~400 Å. This suggests there is insignificant overlapping of the coils and thus they can also be regarded as isolated molecules.

Table 1 Mean-square length of monomer unit (a) and radius of gyration $[(\bar{R}_G^2)^{1/2}]$ of spin-labelled P(4-VP) at 77 K

Polymer	Solvent	a (Å)	$(\bar{R}_G^2)^{1/2}$ (Å)
PVP-1	Methanol	10	90
PVP-2	Methanol	10	90
PVP-1	P(4-VP)	11	100
PVP-5[a]	Methanol	11	440
PVP-5[a]	P(4-VP)	10	400

[a] Linear interpolation was used to obtain values of a for PVP-5

It is important to note that values of a for labelled polymers of substantially different molecular weights (5×10^4 and 1×10^6) coincide to within the error of determination ($\sim 10\%$). This fact substantiates the assumption that a $M_w = 5 \times 10^4$ of spin-labelled PVP is sufficiently high for the validity of the algorithm presented in the Theoretical section.

CONCLUSIONS

The results of the present work indicate that e.p.r. spectroscopy of spin-labelled macromolecules can be used for the determination of the conformation of inner parts of high molecular weight polymer chains in the amorphous solid state. In treating this problem, it is necessary to compare e.p.r. spectra of spin-labelled macromolecules and spin probe reduced to a known concentration and using curves presented in *Figure 5*, to determine the state of polymer chain (Gaussian coil, swollen coil or globule). Further, with the aid of curves analogous to those shown in *Figure 3* the mean-square length of the monomer unit (in the case of a globule, the density of monomer units) and, using equations (2) and (28), the mean-square end-to-end distances and the radius of gyration of the macromolecule can be found.

There is no doubt that the information provided by the e.p.r. method can considerably amplify data obtained by other methods, in particular, by neutron scattering. However, it should be kept in mind that the conformation of a spin-labelled macromolecule, i.e. a polymer chain modified by spin labels, is studied. The proposed method is close to the method for the determination of polymer chain conformation by measuring the rate of electronic excitation transport among chromophores[2,3], which is also carried out via the dipole–dipole mechanism.

Here, we put forward a method especially designed for studying variations of polymer chain conformation in different physico-chemical processes: phase separation of polymer blends, macromolecule deformations, adsorptions on solid surfaces, gel formation, etc.

ACKNOWLEDGEMENTS

The authors express their thanks to O. G. Poluektov for determining the magnetic parameters of radicals via 2-mm e.p.r. spectroscopy, and to A. A. Lundin for valuable discussions.

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