Pure & Appl. Chem., Vol.54, No.2, pp.507-520, 1982.
Printed in Great Britain.
0033-4545/82/020507-14$03.00/0
Pergamon Press Ltd

THE STRUCTURE AND DYNAMICS OF MACROMOLECULES IN SOLUTIONS AS STUDIED BY ESR AND NMR TECHNIQUES

A.L. Buchachenko and A.M. Wasserman

Institute of Chemical Physics, the USSR Academy of Sciences, 117334 Moscow, USSR

Abstract - New methods of investigation of the structure and dynamics of macromolecules and their interactions in solutions based on the ESR and NMR of spin labelled molecules are considered. It is shown that such important characteristics of macromolecules as the local monomer unit density in a macromolecular coil, segment size, characteristic rotational times of segments, local translational diffusion coefficients of segments, interpenetrability of macromolecular coils, can be determined by these methods.

In the last decade new ideas have been advanced and new techniques based on using spin labels and spin probes developed for characterization of macromolecules. The physical fundamentals underlying these methods reside in the electron-nucleus and electron-electron interactions modulated by both rotational and translational molecular dynamics that affect the linewidth of ESR and NMR spectra of spin labelled macromolecules. Nitroxyl radicals are currently the most commonly used spin labels and probes.
These ideas and methods have since received considerable acclaim and were discussed, in particular, in Midland, USA at the 1978 conference initiated and organized by Prof. R. Boyer (Ref. 1).
In this paper new results obtained in Institute of Chemical Physics by ESR and NMR of spin labelled macromolecules will be reported. It is shown below what kind of information about the characteristics of macromolecules may be expected from these methods.

CHARACTERISTICS	INFORMATION
Physical structure	the local density of monomer units in macromolecular coil; the size of segments;
Molecular dynamics	characteristic rotational times of segments; local translational diffusion coefficients of segments in a macromolecular coil;
Macromolecular interactions	interpenetrability of macromolecular coils; local monomer unit densities of the guest and host macromolecules

THE ESR OF SPIN LABELLED MACROMOLECULES: ROTATIONAL DYNAMICS AND SIZE OF SEGMENTS

Molecular rotation of the spin labels and probes modulates the magnetic electron-nucleus interaction and Zeeman interaction anisotropy; therefore, the linewidths of the ESR spectra of spin labels and probes are extremely sensitive to the rotation frequency. This phenomenon is the physical foundation on which the spin label and probe techniques are based. However, spin labels as a rule are joined to the polymer chain via several chemical bonds with free internal rotation rather far distant and isolated from the polymer segment. For this reason the rotational motion of the spin label is not immediately related with the rotational or re-orientational motion of the macromolecular segment to which the spin label is attached.

To be able to measure the dynamics of segment rotation it is necessary to find out the relation between label rotational frequency and segment reorientation frequency. The first approach to this problem was formulated by Hubbell and McConnell (Ref. 2) and their findings were later developed by Timofeev et al. (Ref. 3) and applied to the investigation of biomolecular dynamics. We too have used their approach for the characterization of synthetic polymer macromolecules (Ref. 4).
The underlying physical considerations are as follows. A spin label takes part in two types of motion: fast anisotropic rotation with respect to macromolecular segment with a period $\tau_L \lesssim 1 \times 10^{-9}$ sec and a slow rotation or reorientation together with the segment with the characteristic time $\tau \gg \tau_L$ (Fig. 1). The fast rotation of spin label inside a cone with a precession

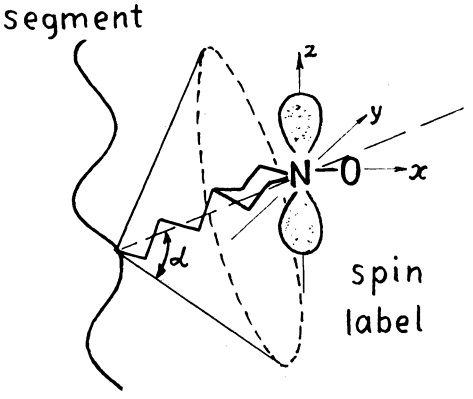


Fig. 1. Schematic representation of the spin label precession relative to a macromolecular segment.

angle α with respect to the macromolecular segment leads to a partial averaging of the dipolar electron-nucleus interaction and anisotropy of the Zeeman interaction that may be quantitatively represented by the parameter

$$S = \overline{\Delta A} / \Delta A \qquad (1)$$

where $\Delta A = A_{zz} - 1/2(A_{xx} + A_{yy})$; $\overline{\Delta A} = \overline{A}_{\parallel} - \overline{A}_{\perp}$;

A_{xx}, A_{yy}, A_{zz} are the principal values of the dipolar electron-nucleus interaction tensor.
$\overline{A}_{\parallel}$ and $\overline{A}_{\perp}$ are partially averaged components of dipolar interaction given by the formulas:

$$\overline{A}_{\parallel} = a_{iso} + b/3(\cos^2\alpha + \cos\alpha) \qquad (2)$$

$$\overline{A}_{\perp} = a_{iso} + b/6(\cos^2\alpha + \cos\alpha) \qquad (3)$$

where $a_{iso} = 1/3(A_{xx} + A_{yy} + A_{zz})$; $b = A_{zz} - (A_{xx} + A_{yy})/2$.

The physical meaning of parameter S is that it defines what portion of the dipolar electron-nucleus interaction is averaged due to label rotation and what portion of it is averaged by rotational or reorientational motion of segment itself. It characterizes the steric hindrances limiting the label mobility and is related with the precession angle by the following relationship:

$$S = 1/2\,(\cos^2\alpha + \cos\alpha) \qquad (4)$$

For a spin label rigidly fixed to macromolecule, S = 1. The smaller S corresponds to a larger precession angle α and at S = 0 the label rotates freely and isotropically, its motion being practically independent from the segment motion and totally averaging the dipolar electron-nucleus interaction.

The characteristic segment rotation times can be found in the following manner. The effect of viscosity on the parameter $2A'_{\parallel}$ of the ESR spectrum (Fig. 2) is investigated and plotted as a function of (T/η). As an example,

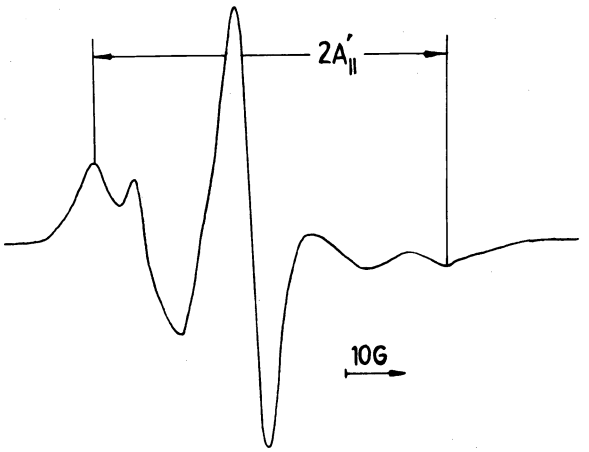


Fig. 2. A typical ESR spectrum of a spin label with a partially retarded rotation.

Fig. 3 illustrates such a relationship for a spin labelled polyvinyl pyridine solutions in ethanol-glycerin mixtures. The fact that the $2A'_{\parallel}$ versus $(T/\eta)^{0.84}$ relationship is linear means that the theoretical description of

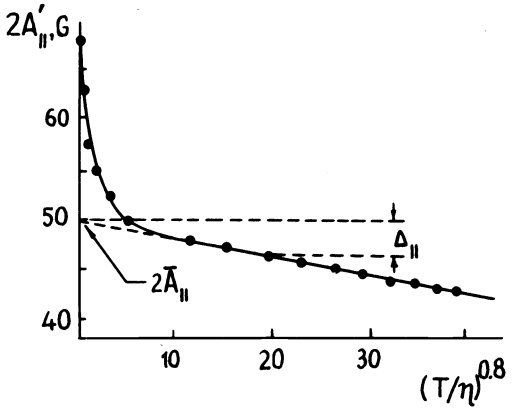


Fig. 3. Viscosity effect on parameter $2A'_{\parallel}$ for spin labels with partially retarded rotation.

the spin label motion is adequate to real situation. A deviation from the model at higher viscosities may occur as a result of violation of the theoretical model conditions, viz. the label rotation times begin to exceed 1×10^{-9}s. Extrapolating the $2A'_{\parallel}$ values for $T/\eta \to 0$ we find the averaged value of $2\bar{A}_{\parallel}$ and using Eq. (1) may calculate S. Then, for every point of the curve in Fig. 3 we determine $\Delta_{\parallel} = 2A'_{\parallel} - 2\bar{A}'_{\parallel}$ and using the theoretically calculated $\Delta_{\parallel}$ versus τ relationship we find the characteristic segment rotation time τ. Such theoretical relationships are presented in

Fig. 4 for different S.

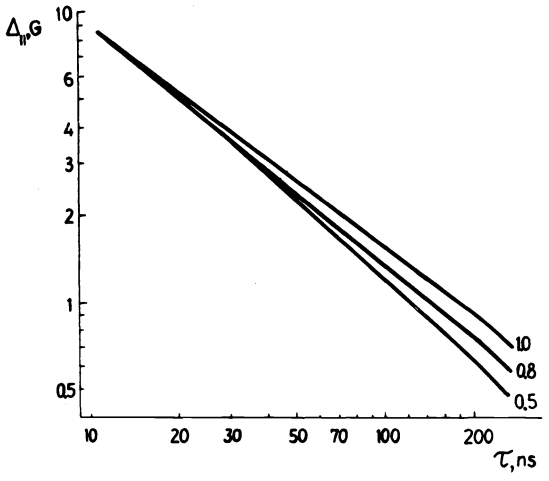
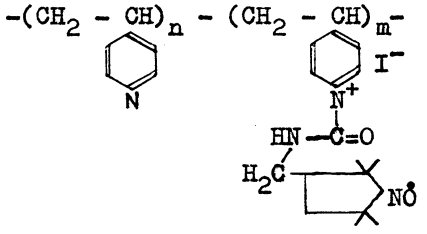


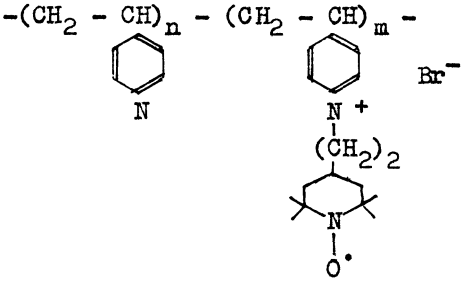
Fig.4. Relationship between parameter Δ_{II} and characteristic rotation time of segment τ.

We have used this procedure for characterization of the segment motion in the following spin labelled macromolecules:

poly-4-vinyl-pyridine (PVP) I and II

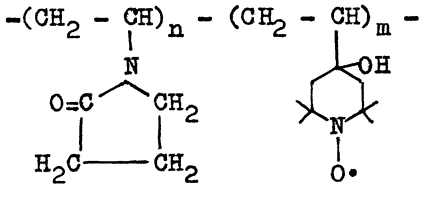


I

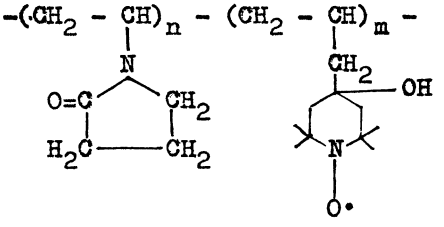


II

polyvinyl pyrrolidone (PVPyr) III and IV

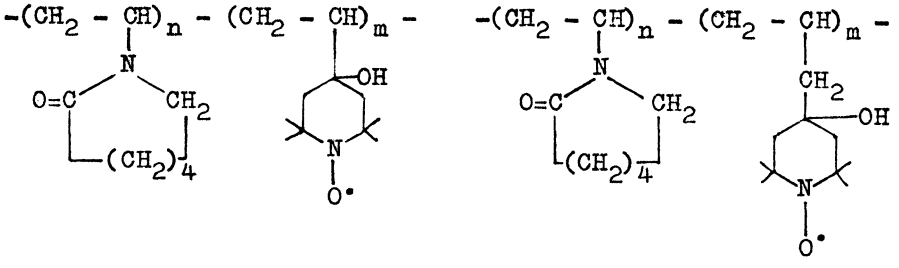


III



IV

polyvinyl caprolactam (PVC) V and VI



V VI

labelled with different nitroxyl radicals. The concentration of spin labels is small and does not exceed one per 100–200 monomer units.
In Table 1 are listed the characteristic times of the rotational motion of segments, τ, and parameters S characterizing the freedom of rotation of the spin label itself.

TABLE 1. Segmental mobility and size of segment in spin labelled macromolecules (ethanol, 25°C)

Macromolecules	Mx10^3	S	τ,ns	r, Å	v, Å^3
PVP-I	250	0.56	3.3	14	11.5x10^3
PVP-II	250	0.20	3.3	14	11.5x10^3
PVPyr-III	70	0.81	4.4	15.5	15.6x10^4
PVPyr-IV	60	0.74	4.4	15.5	15.6x10^3
PVC-V	25	0.81	8.0	18.0	24.5x10^3
PVC-VI	30	0.76	8.0	18.0	24.5x10^3

It is important to note that τ does not depend on the spin label, i.e. the times listed characterize the motion of the macromolecular segments themselves rather than that of a spin label. Parameter S does depend on the spin label: the more rigidly the label is fixed to the polymer segment the more limited is the label motion and the closer is S to unity.
The effect of solvent viscosity on the segmental mobility (in ethanol-glycerine mixtures) obeys the Stokes-Einstein formula, whence one can determine the hydrodynamic segment radii r and the corresponding segment volumes v (see Table 1).
In PVP the activation energy of segment rotation is 4.4 kcal/mol and the pre-exponential factor has a "normal" value of $\tau_0 = 2\text{x}10^{-13}$s.
Parameter S does not depend on the viscosity when temperature is constant but greatly varies with temperature (Table 2).

TABLE 2. Temperature effect on parameter S and precession angle α in PVP

T°C	S	α
-20	0.78	30°
0	0.68	40°
25	0.56	48°
40	0.49	50°
60	0.44	56°

The fact that S and α increase indicates that the amplitude of spin label motion increases with temperature and the motion itself becomes more independent from that of the macromolecular segment.
The physical structure and reorientation dynamics of segments are, besides,

sensitive to the solvent quality. For example, water is a bad solvent for PVC and the macromolecular coil is more densely packed in water. This means that the spin label motion is more restricted and the parameter S increases. In good solvents (ethanol, butanol) the monomer units in macromolecular coil are less close-packed and, therefore, the spin label has a greater freedom of motion and S decreases (Table 3).

TABLE 3. Parameter S and characteristic time τ in PVC (25°C)

Solvent	S	τ, ns
Water	0.87	5
Ethanol	0.81	8
Butanol	0.81	17

In a similar manner we have investigated the macromolecule behavior near the point of phase separation and have shown that macromolecular coils tend to contract close by that temperature.

THE ESR OF SPIN LABELLED MACROMOLECULES: LOCAL DENSITY OF MONOMER UNITS, TRANSLATIONAL DIFFUSION OF SEGMENTS AND INTERACTIONS OF MACROMOLECULES

The information about these characteristics is derived from line broadening in the ESR spectra of spin labels induced by dipolar and exchange interactions of unpaired electrons. Dipolar interactions are efficient in systems with low molecular mobility and yield information about the local concentration and static distribution of paramagnetic particles. Exchange interactions modulated by collisions (or, better to say, encounters) of the radicals - spin labels or probes - allow to measure intramolecular translational diffusion coefficients.
Both these contributions to the line broadening are proportional to the paramagnetic particle concentration and the problem is only to separate tnem and measure individually. As regards nitroxyl radicals we have succeeded in solving this problem experimentally having measured the ESR line broadening as a function of the translational diffusion coefficient (Fig.5).

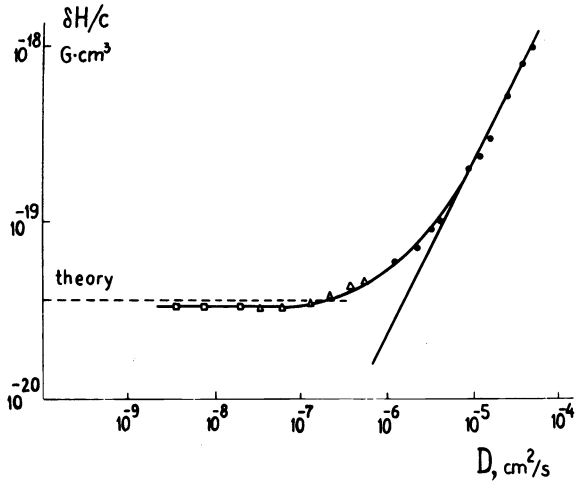


Fig. 5. Effect of diffusion coefficient on reduced ESR line broadening $\delta H/C$. Dotted line indicates the theoretical limit of dipolar contribution.

In systems with highly mobile molecules ($D \gtrsim 5 \times 10^{-6}$ cm^2/s) the reduced broadening $\delta H/C$ is a linear function of diffusion coefficient. In this

region the main contribution to the broadening effect comes from exchange interactions:

$$\delta H = 2\pi 3^{-1/2} \gamma^{-1} k_{ex} C \qquad (5)$$

where δH is the line broadening, γ is electron gyromagnetic ratio; C is radical concentration, k_{ex} is spin exchange rate constant given by

$$k_{ex} = pk_o = 16\pi\, prD \qquad (6)$$

where p is probability of spin relaxation at the radical encounter, D is diffusion coefficient.
The theoretical curve in Fig. 5 coincides with the experimental one for pr = 1 Å. From exchange broadening data it is possible using Eqs (5) and (6) to determine the translational diffusion coefficient D.
In systems with low molecular mobility ($D \leq 1 \cdot 10^{-7}$ cm^2/s) the reduced line broadening does not depend on the diffusion coefficients and is totally controlled by the dipole interactions. For a statistic distribution of spin labels or probes,

$$\delta H/C = (3.6 \pm 0.5) \times 10^{-20} \text{ Gauss/cm}^3 \qquad (7)$$

and all deviations from this value are due to nonrandom distribution. What they show is how much the local spin concentrations differ from the mean values.
The local concentration of spin labels in a macromolecular coil corresponds to their concentration within a small element of volume near a given spin label (Fig. 6). The radius R of this local spherical domain is about 30 Å and is also the distance over which the dipolar contribution to the line broadening of the central label from the spin labels located inside of

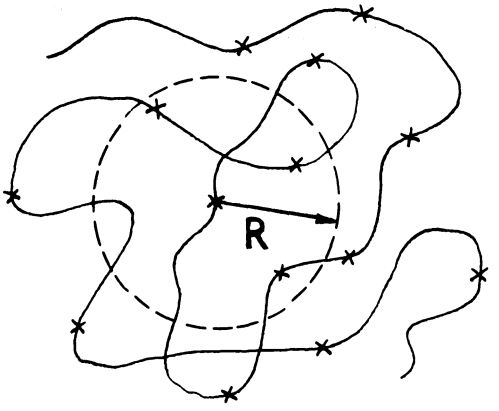


Fig. 6. Schematic representation of a sphere with local spin label concentration (labels are shown as crosses).

sphere is comparable with the total linewidth, i.e. is about 1 Gauss. The volume of this domain is normally much smaller than that of a macromolecule.
The method of separation of the dipolar and exchange contributions to the line broadening has been used to investigate the physical structure and molecular dynamics of polyvinyl pyridine (PVP).
The local densities of spin labels were measured in spin-labelled PVP in which the molar percentage of the spin labels $\beta = m/(m+n)$ was varied in the range from $1 \cdot 10^{-2}$ to $20 \cdot 10^{-2}$. Local concentration of monomer unit ρ_{loc} is easily calculated from the spin label concentration C_{loc} by the formula:

$$C_{loc} = \beta \rho_{loc}$$

(provided, of course, the spin labels are distributed on the macromolecule in a random, statistical manner).
The averaged monomer unit density in a macromolecular coil is given by the formula:

$$\langle \rho \rangle = N/V \simeq 0.42\,[\eta]\,M\varphi'$$

where N is the number of monomer units in the macromolecule, $[\eta]$ is characteristic viscosity, $\varphi' = 3.1 \cdot 10^{24}$.
The averaged and local densities of monomer units in PVP are compared in Table 4.

TABLE 4. Averaged and local monomer unit densities in PVP (ethanol, 20°, $\beta = 0.20$, 1% solution)

$M \cdot 10^4$	$\langle \rho \rangle$, M	ρ_{loc}, M	ρ_{loc}, M(−196°)
25	0.05	0.30	0.40
5	0.20	0.28	0.36

These results prompt some conclusions.
First, the local concentrations of monomer units are only slightly dependent upon the molecular mass of the polymer.
Second, in large macromolecules the local densities of monomer units may be 5 to 6 times greater than the average values (in accordance with the Gaussian model of a polymer coil).
Third, local concentrations are almost independent of temperature, that is, both the monomer units distribution in a coil and coil size are little affected by temperature. Only at very low temperature, in frozen solutions, do these variations become substantial.
At higher (above 20°) temperatures in dilute solutions the main contribution to ESR line broadening is provided by the intramolecular exchange interactions which can be used to determine the spin label encounter rate constants and local intramolecular segment diffusion coefficients. The diffusion coefficients of segments and spin probes are compared in Fig. 7.

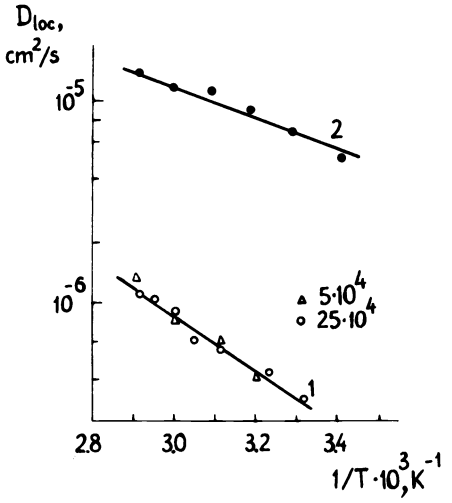


Fig.7. Temperature effect on local diffusion coefficient:
1 - spin label, 2 - spin probe.

Segments diffuse at a rate at least an order of magnitude slower than spin probes. Segment diffusion coefficients are practically independent from the mass of macromolecule. The activation energy of segment diffusion (8.4 kcal/mole) exceeds considerably the activation energy of probe diffusion (3.3 kcal/mole).
Another important problem is behavior of polymer coils when they interact in concentrated solutions. It is important to know what is the interpenetrabi-

lity of coils, how the monomer unit densities vary in both the host and guest macromolecules, how the molecular dynamics vary in combined macromolecular coil.
To answer these questions we have investigated the dipolar and exchange broadening of the ESR lines in concentrated solutions of spin labelled macromolecules and concentrated solutions of unlabelled macromolecules in the presence of a minor quantity of labelled macromolecules (Ref. 5). In the first case the broadening effect is due both to the intra- and intermolecular interaction of spin labels. In the second case it is only the intramolecular interaction which induces the line broadening. Separating the dipolar and exchange contributions to the intra- and intermolecular broadening one can determine the local densities of monomer units in the host and quest macromolecules. The results are shown in Fig. 8.

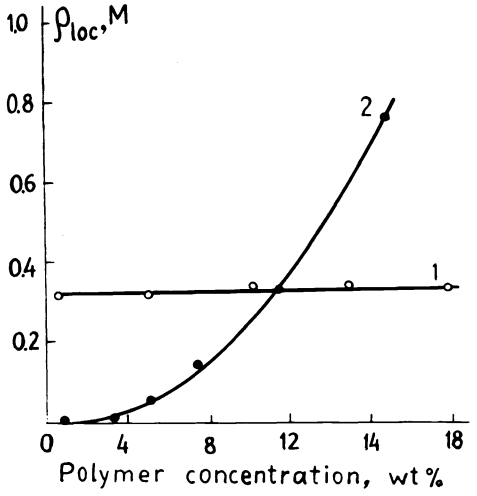


Fig. 8. Local concentrations of the monomer units of host macromolecule (1) and guest macromolecules (2) in macromolecular coil as function of total macromolecule concentration in solution (PVP, 20°, ethanol).

As in an isolated macromolecule, the local density of monomer units in a host molecule is normally 5 to 6 times greater than the mean density in a coil. When the polymer concentration in solution is increased the local monomer unit density of the host macromolecule remains almost unchanged, which means that the size and density of a macromolecular coil are almost insensitive to the presence of any other macromolecule. The local monomer unit density of the guest macromolecules increases monotonously with polymer concentration in solution. When the concentration is about 10 wt.% the local monomer unit densities of quest macromolecules and host macromolecules are comparable, i.e., at this concentration the macromolecular coils interpenetrate and substantially overlap each other. At higher concentrations the local density of monomer units of guest molecules exceeds that of the host molecule. In other words, the entire volume of the host macromolecule is filled with the monomer units of the guest macromolecules.
If one knows the exchange contribution to the ESR line broadening and local spin label concentrations it is easy to find the coefficients of segment translational diffusion in polymer coils as well as local diffusion activation energy. Their relationships with polymer concentration are presented in Table 5 and Fig. 9.

TABLE 5. Activation energies E_D and preexponential factors D_o for the local translational diffusion coefficients

Polymer concentration, wt.%	E_D, kcal/mole	D_o, cm^2/s
1.0	8.7	0.5
5.5	8.0	0.3
11.3	8.6	0.5
25.6	8.9	0.8
32.5	9.6	0.8

Local diffusion coefficients of segments decrease in proportion with an increase of the local monomer unit density in macromolecular coil. So long as the local density is constant the diffusion coefficient is constant too (Fig. 9). At a concentration between 10 and 15 wt.% when the macromolecular coils overlap the total local density of monomer units in a polymer coil increases and the local diffusion coefficient decreases. The decrease of

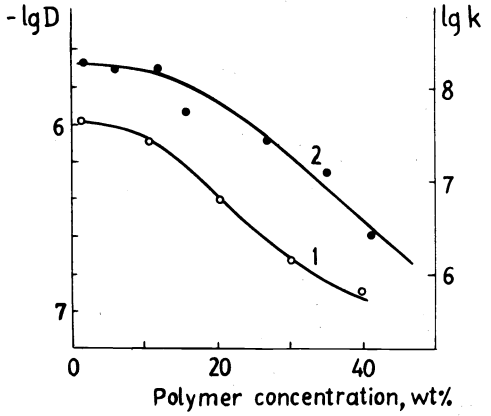


Fig. 9. Local translational diffusion coefficients of segments (1) and kinetic chain termination rate constants (2) as functions of macromolecule concentration in solution: 1 - PVP, 20°, ethanol; 2 - polymethylmethacrylate in methylmethacrylate, 22.5°C.

the local diffusion (or microdiffusion) coefficient of segments occurs simultaneously with the decrease of chain termination rate constant in the radical polymerization process (Fig. 9). This analogy suggests that macroradical recombination is controlled by the local segment diffusion in overlapping macromolecular coils. The rate of polymer coil entanglement (or disentanglement) is also controlled by the local segment mobility in a coil.

NMR OF SPIN LABELLED MACROMOLECULES

High resolution NMR spectroscopy of spin labelled macromolecules can also be a good means of investigating the macromolecular structure, dynamics and interactions. Spin labels and probes induce the paramagnetic line broadening in NMR spectra of macromolecules the main reason for which is the dipolar interaction between unpaired electron and protons (or some other nuclei) modulated by translational diffusion of macromolecular segments.
A theory of paramagnetic line broadening in NMR spectra in a liquid where paramagnetic particles are present was advanced by Hubbard (Ref. 6). We have laid his theory at the basis of our new method of investigation of spin labeled macromolecules (Ref. 7).
Protons of a macromolecule may interact either with the spin labels of the same macromolecule (host) or with those of other (guest) macromolecules. Therefore the observed paramagnetic line broadening is caused by two factors: intermolecular due to the interaction between protons and labels of other molecules, and intramolecular induced by the host molecule labels. Collisions between protons and the nearest spin labels occurs much oftener than with remote ones, and therefore, the line broadening in the NMR spectra of protons is controlled by the local spin label concentration, that is, label concentration in the vicinity of a given proton within a volume element with a radius $r \leqslant 30$ Å (Fig. 10). This volume may be much smaller than the volume of macromolecule itself and the local spin label concentration (and, consequently, local density of monomer units) may differ widely from the average label concentration within a polymer coil volume.

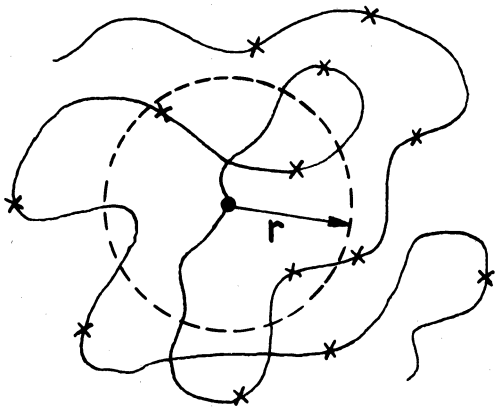


Fig. 10. Schematic representation of a sphere containing the spin labels (shown as crosses) that induce dipolar line broadening in NMR spectra of proton (in the center of the sphere).

The intramolecular contribution into the line broadening is dictated by the local concentration of spin labels of the host macromolecule, whereas the intermolecular contribution is determined by that of the guest macromolecules. The relative importance of these two contributions depends on the extent to which a given volume element of macromolecular coil is filled with the monomer units of host or guest macromolecules. The contributions to linewidths are given by the following formulae:

$$(1/T_2)_{intra} = 2\pi \Delta\nu_{intra} = \frac{3.6\pi \gamma_I^2 \gamma_S^2 \hbar^2 N_{host}}{50d \cdot 1/2(D + D_L)} \qquad (8)$$

$$(1/T_2)_{inter} = 2\pi \Delta\nu_{inter} = \frac{3.6\pi \gamma_I^2 \gamma_S^2 \hbar^2 N_{guest}}{50d \cdot 1/2(D + D_L)} \alpha \qquad (9)$$

Here, N_{host} is intramolecular local concentration of labels in the host macromolecule; N_{guest} is local concentration of labels belonging to the guest macromolecules; D_L and D are translational diffusion coefficients of labels and segments, respectively, and may be assumed to be equal since the labels are attached to macromolecular segments; d is the limiting approach distance between a proton and spin label (d = 3.6 Å, Ref. 8); $\Delta\nu$ is experimentally measured paramagnetic line broadening expressed in Hz. The factor $0 \leqslant \alpha \leqslant 1$ accounts for the fact that the access to monomer units of host macromolecule for the labels of guest macromolecules may be harder than for its intrinsic labels and therefore the line broadening can be less efficient. The spin probe induced line broadening is defined by a similar equation:

$$(1/T_2)_{probe} = 2\pi \Delta\nu_{probe} = \frac{4.8\pi \gamma_I^2 \gamma_S^2 \hbar^2 N_{probe}}{50d \cdot 1/2(D_{probe} + D)} \qquad (10)$$

where D_{probe} is translational diffusion coefficient; for small radicals it may be assumed that $D \ll D_{probe}$, i.e., that the translational mobility of a spin probe is much higher than that of a spin label.
Now one can rewrite Eqs (8)-(10) in the following form:

$$(1/T_2)_{inter} = \frac{3.6\pi \gamma_I^2 \gamma_S^2 \hbar^2 N_{guest} \alpha}{50dD_L} \qquad (11)$$

$$(1/T_2)_{intra} = \frac{3.6\pi \gamma_I^2 \gamma_S^2 \hbar^2 N_{host}}{50dD_L} \qquad (12)$$

$$(1/T_2)_{probe} = \frac{4.8\pi\gamma_I^2\gamma_S^2\hbar^2 N_{probe}}{25dD_{probe}} \qquad (13)$$

and, therefore, one can now measure both the local density of monomer units and local diffusion coefficients of segments in macromolecular coil.
The method will now be illustrated for spin-labelled poly-4-vinyl-pyridine which had initially been studied by the ESR technique (see the previous section of this paper).
The problem therefore is to separate the intramolecular and intermolecular contributions to the paramagnetic line broadening. It can be solved by measuring the paramagnetic NMR line broadening of labelled macromolecules as well as of various mixtures of labelled and unlabelled macromolecules. For example, in a weakly labelled polymer (1:10) the paramagnetic line broadening of the α-protons of the pyridine ring is $\Delta\nu_{intra} + \Delta\nu_{inter}$ (10% solution in CD_3OD, Fig. 11). In a 10% solution of a mixture of strongly la-

Fig. 11. NMR spectra of α-protons of pyridine ring in PVP (1), spin-labelled PVP (2) and a mixture of labelled and unlabelled PVP in the 1:3 ratio (3); 60 MHz, 20°, deuterated methanol.

belled (1:5) and unlabelled polymers presented in the 1:3 ratio, the total spin label concentration is one half of that in the previous case, whereas the label concentration in a spin labelled macromolecule is twice as high. In a mixture of labelled and unlabelled macromolecules the line broadening measured for the protons of an unlabelled macromolecule is equal to $(1/2)\Delta\nu_{inter}$ and for those of a labelled macromolecule it is $2\Delta\nu_{intra} + (1/2)\Delta\nu_{inter}$. In NMR spectrum of a mixture of macromolecules the signal of the α-protons of the pyridine ring is in fact a superposition of the signals from a labelled and unlabelled macromolecules (Fig. 11, Spectrum 3). The first signal amounts to 1/4 of the total intensity and is strongly broadened, therefore its contribution to the linewidth may be ignored and the intermolecular line broadening for unlabelled macromolecules can be straightly found. After that it is possible to calculate the two contributions, $\Delta\nu_{inter}$ and $\Delta\nu_{intra}$, separately.
It is very simple to measure the paramagnetic NMR line broadening of unlabelled macromolecules in the presence of a known concentration of spin probes, $\Delta\nu_{probe}$. Having then measured all contributions to the line broadening individually it is possible to find ratios between them:

$$\frac{\Delta\nu_{intra}}{\Delta\nu_{probe}} = 0.375\left(\frac{N_{host}}{N_{probe}}\right)\left(\frac{D_{probe}}{D_L}\right)\alpha \qquad (14)$$

$$\frac{\Delta\nu_{inter}}{\Delta\nu_{probe}} = 0.375 \left(\frac{N_{guest}}{N_{probe}}\right)\left(\frac{D_{probe}}{D_L}\right)\alpha \qquad (15)$$

whence local spin label concentrations may be easily determined (provided the ratio of local diffusion coefficients is known) or, conversely, knowing local label concentration it is easy to determine the local diffusion coefficients. However, the first way is preferrable since the ratios of spin label and probe diffusion coefficients can be readily found from the ESR spectra of spin labelled macromolecules and spin probes under identical conditions. It is easy then, once the local spin label concentrations have been calculated, to convert them into local concentrations of monomer units in a macromolecular coil.
Pertinent data are given in Table 6.

TABLE 6. Local monomer unit densities of host and guest macromolecules (PVP, methanol, 20°C)

Polymer concentration, wt.%	ρ_{host}, M	ρ_{guest}, M
2	0.24	0
10	0.26	0.35
20	0.27	0.60
30	-	1.0

The local densities of monomer units are seen to be the same as those measured by a different and independent method - from the dipolar line broadening of ESR spectra (cf. the preceding section). The local monomer unit densities in a host macromolecule are 5 to 6 times as high as the averaged densities and are almost independent from the concentration of macromolecules in solution, that is, a macromolecular coil is insensitive to the presence of foreign macromolecules in solution. The local density of monomer units of guest macromolecules increases monotonously with the total concentration of macromolecules indicating that there is interpenetration of coils. The local concentrations listed in Table 6 have been calculated under the assumption that = 1 and the fact that they are in a good agreement with the results of ESR measurements indicates that the coils can penetrate through each other without hindrance and that the macromolecule protons are almost equally accessible for the spin labels of both the host and guest macromolecules, i.e. is close to unity.
Knowing the local concentration of spin labels and spin probe diffusion coefficient (calculated from the measured exchange broadening of the ESR lines) it is not difficult to determine the local diffusion coefficient of segments D_L. At 20°C it is $4.4 \cdot 10^{-7}$, $4.1 \cdot 10^{-7}$ and $3.4 \cdot 10^{-7}$ cm^2/s in solutions containing 10, 20 and 30% polymer by weight, respectively (which is in good agreement with the diffusion coefficients measured by the ESR technique). The activation energy of the local translational diffusion of macromolecular segments in methanol solution is 6.4 kcal/mole.
The new method described herein which is based on an analysis of the NMR linewidths of spin-labelled macromolecules has some advantages over the ESR technique discussed in the first section of this paper. In the ESR method the local densities of spin labels (in other words, local densities of monomer units) in a macromolecular coil are found from the dipolar line broadening under conditions of limited molecular mobility so that the dipolar interaction of unpaired electrons is not averaged by the molecular motion. On the contrary, local diffusion coefficients are determined from the exchange line broadening under condition that the molecular mobility is high and the dipolar interaction is averaged.
These limitations do not exist in the NMR method: both the local monomer unit concentrations in a coil and local diffusion coefficients in macromolecule may be measured under the same conditions. However, this method is not independent. To measure local monomer unit concentration or local diffusion coefficient information about one of these must be at hand. In this sense the two methods are essentially complementary.

CONCLUSION

The methods of studying macromolecular structure and dynamics we have dis-

cussed in this paper require labelling of the macromolecules with spin labels. But the chemistry of nitroxyl radicals is now at such an advanced stage of development that, in principle, it is no trouble to implant a spin label into any macromolecule or functional group. What is really a problem is that labelling may modify the macromolecule (especially when the number of spin labels per molecule is large). However, in most cases it is not necessary to introduce too many labels into a macromolecule to enable macromolecular dynamics studies. In any case the combination of the ESR and NMR techniques allows to establish the general features of the structural and dynamic behavior of polymer coils under different conditions.

REFERENCES

1. Molecular Motion in Polymers by ESR, ed. by R.F. Boyer and S.E. Keinath, Hardwood Academic Publishers (1980).
2. W.L. Hubbard, H.M. McConnell, J. Amer. Chem. Soc., 93, 314-322 (1971).
3. I.V. Dudich, V.P. Timofeev, M.V. Volkenstein and A.Yu. Misharin, Molekulyarnaya Biol., 11, 685-691 (1977); V.P. Timofeev, I.V. Dudich, Yu.K. Sokulev and R.S. Neslin, Febs. Lett., 89, 191-193 (1978).
4. A.M. Wasserman, T.A. Alexandrova, I.V. Dudich, V.P. Timofeev, Europ. Polym. J. (in press); T.A. Alexandrova, T.M. Karaputadze, A.B. Shapiro, A.M. Wasserman, Vysokomol. Soed. (in press).
5. A.L. Buchachenko, A.M. Wasserman, T.A. Alexandrova and A.L. Kovarski, see Ref. 1, p. 33.
6. P.S. Hubbard, Proc. Roy. Soc., A291, 537-545 (1966).
7. N.A. Sysoeva, N.N. Maslovich, A.M. Wasserman and A.L. Buchachenko, Vysokomol. Soed., A 22, 2065-2070 (1980).
8. K. Endo, I. Morishimo, T. Yonezawa, J. Chem. Phys., 67, 4760-4766 (1977).