

ration between the donor and acceptor.

Intuitively, a structural dependence is expected, yet these results suggest that a dependence will be found only when the structural difference between two quenchers is sufficient to change the distance of separation between the donor and the acceptor.

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## Electron Spin Resonance Study of Anisotropic Rotational Reorientation of Spin Labels Attached to the Side Chains of Soluble Poly(methacrylamide)-Type Copolymers

J. Pilař, J. Labský, J. Kálal,

*Institute of Macromolecular Chemistry, Czechoslovak Academy of Sciences, 162 06 Prague 6, Czechoslovakia*

and J. H. Freed\*

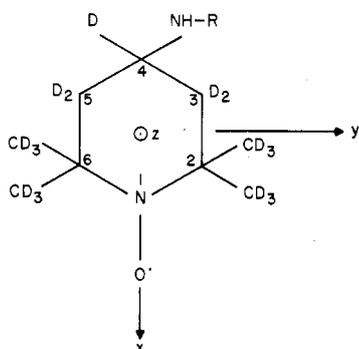
*Department of Chemistry, Cornell University, Ithaca, New York 14853 (Received December 7, 1978)*

Soluble copolymers of *N*-(2-hydroxypropyl)methacrylamide (HPMA) with *N*-methacryloylated  $\omega$ -amino acids, which form side chains with 4-aminoperdeuterio-2,2,6,6-tetramethylpiperidinyl-1-oxy (PD-Temp-NH<sub>2</sub>) attached to their end, were investigated. The EPR spectra of methanolic solution of these copolymers recorded at 313–173 K were analyzed by using a model of anisotropic but axially symmetric rotational reorientation of the spin label. An analysis of these spectra in the motional narrowing region showed that the symmetry axis  $z'$  of the rotational-diffusion tensor describing this rotational reorientation was an axis close to that of the N–O bond, and that, depending on the type of side chain, the rotational reorientation about this axis is four to six times faster than about the remaining two axes. The correlation time,  $\tau_R$ , characterizing the rate of rotational reorientation of the spin label decreases monotonically with increasing side chain length. Linear log  $\tau_R$  vs.  $1/T$  dependences were obtained and were characterized by a low activation energy,  $E = 15 \pm 2$  kJ/mol, that was the same within the limits of experimental error for all the copolymers. Slow motional spectra were given a preliminary interpretation with a model of very anisotropic rotational reorientation.

### I. Introduction

The EPR method is able to provide information on the rotational reorientation of radicals of the nitroxide type, whose characteristic feature consists of anisotropy in their  $g$  tensor and hyperfine interaction tensor  $\mathbf{A}^N$  of the nitroxide group nitrogen atom.<sup>1a,b</sup> An investigation of the EPR spectra of stable nitroxide radicals bound at the ends of side chains of polymers allows one to obtain information

on the way in which the rotational reorientation of a nitroxide (spin label), characterized by a temperature-dependent correlation time, is affected by the polymer, side chain, and solvent.<sup>2-6</sup> Copolymers of *N*-(2-hydroxypropyl)methacrylamide (HPMA) with 3 mol % of 4-nitrophenol esters of *N*-methacryloylated  $\omega$ -amino acids were chosen for the investigation. These amino acids form side chains of copolymers at the end of which 4-amino-

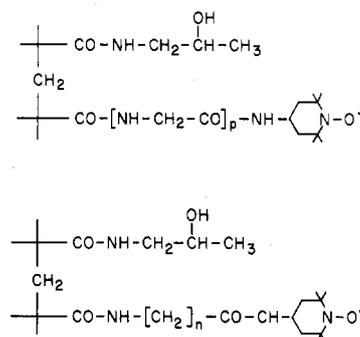


**Figure 1.** The structure of spin-label derivatives used: R = H, 4-aminoperdeuterio-2,2,6,6-tetramethylpiperidinyloxy (PD-Temp-NH<sub>2</sub>); R = CH<sub>3</sub>CO, 4-acetylaminoperdeuterio-2,2,6,6-tetramethylpiperidinyloxy; R = COCH<sub>2</sub>NH<sub>2</sub>, 4-glycylaminoperdeuterio-2,2,6,6-tetramethylpiperidinyloxy; R = COCH<sub>2</sub>NHCOCH<sub>2</sub>NH<sub>2</sub>, 4-glycylglycylaminoperdeuterio-2,2,6,6-tetramethylpiperidinyloxy.

2,2,6,6-tetramethylpiperidinyloxy (Temp-NH<sub>2</sub>) was attached by a polymer analogous reaction.<sup>7,8</sup>

EPR spectra of nitroxides in the motional narrowing region ( $\tau_R = 1 \times 10^{-11}$ – $1 \times 10^{-9}$  s) have the character of three components of nitrogen splitting with different line widths, and consequently also with different amplitudes. Intrinsic peak-to-peak first derivative line widths of these three components may be fitted to the expression  $\Delta H(M) = A + BM + CM^2$  ( $M$  is the spectral index number;  $M = +1, 0, 1$ , for low-field, central, and high-field lines, respectively).<sup>1a,b,9,10</sup> The basic problem arising in the analysis of nitroxide spectra in this region is the determination of variations among the three intrinsic line widths of the components of nitrogen splitting, i.e., the determination of  $A$ ,  $B$ , and  $C$ . Using the inverse proportionality of the ratio of line widths to the square root of their first derivative peak-to-peak heights, we can determine  $A$ ,  $B$ , and  $C$  with a higher accuracy from the measured peak-to-peak heights and the line width of one, usually central, line. However, in the majority of nitroxides used as spin labels, the three observed components of nitrogen splitting form an envelope of another, usually unresolved, hyperfine structure arising from an unpaired electron interaction with the hydrogen or deuterium nuclei of the spin-label molecule. In these cases it is necessary to simulate spectral lines on the basis of known splitting constants of these nuclei for various intrinsic line widths; the observed line widths and peak-to-peak height ratios are then transformed into intrinsic line widths by employing a calibration procedure analogous to that employed previously.<sup>3,10</sup> Since deuterium splittings are much reduced from proton splittings, the use of perdeuterated analogues of spin labels significantly reduce the corrections required for obtaining the intrinsic line widths.<sup>10</sup>

Results of the extensive theory of EPR spectra of nitroxides, which allows one to calculate theoretical line shapes for EPR spectra within a broad range of correlation times, have been summarized by Freed.<sup>1b</sup> This theory allows one to treat various models of anisotropic rotational reorientation. In the simplest case, the rotational reorientation of nitroxides is characterized by an axially symmetric rotational-diffusion tensor. The degree of anisotropy of the rotational reorientation,  $N$ , is determined by the ratio of the rotational-diffusion component of this tensor,  $R_3$ , about its symmetry axis,  $z'$ , to the rotational-diffusion component,  $R_1$ , about the remaining principal axes  $x'$  and  $y'$ .<sup>9,10</sup> It is assumed in the first approximation that the symmetry axis of the rotational-diffusion tensor  $z'$  is identical with one of the molecule-fixed axes of the nitroxide molecule (Figure 1). The correlation time,  $\tau_R$ ,



**Figure 2.** The structure of copolymers. The subscript denotes the number of atoms in the backbone of the side chain (used as a measure of length of the side chain  $d$ ). A: HPMA-I<sub>2</sub> ( $p = 0$ ); HPMA-IV<sub>5</sub> ( $p = 1$ ); HPMA-IV<sub>8</sub> ( $p = 2$ ); HPMA-IV<sub>11</sub> ( $p = 3$ ); HPMA-IV<sub>14</sub> ( $p = 4$ ). B: HPMA-II<sub>6</sub> ( $n = 2$ ); HPMA-II<sub>8</sub> ( $n = 4$ ); HPMA-II<sub>9</sub> ( $n = 5$ ); HPMA-II<sub>10</sub> ( $n = 6$ ); HPMA-II<sub>11</sub> ( $n = 7$ ); HPMA-II<sub>15</sub> ( $n = 11$ ).

is then defined by using this tensor (for the model of Brownian rotational diffusion by  $\tau_R = [6(R_1R_3)^{1/2}]^{-1}$  and for an approximate model of free rotational diffusion by  $\tau_R = (7)^{1/2}[6(R_1R_3)^{1/2}]^{-1}$ ).<sup>9</sup> Equation 5 of ref 9 allows one in addition to calculate  $A$ ,  $B$ , and  $C$  in the motional narrowing region as a function of magnetic parameters of the nitroxide in the given solvent, and of the correlation time, degree of anisotropy, and symmetry axis of the rotational-diffusion tensor characterizing rotational reorientation. By comparing the experimentally determined  $C$  vs.  $B$  temperature dependence and the calculated  $C$  vs.  $B$  dependences on the correlation time  $\tau_R$ , it is possible to determine the character of the rotational reorientation and the respective correlation times. In the motional narrowing region the theoretical line shapes of EPR spectra are model independent. In the slow motional region ( $\tau_R > 10^{-9}$  s) the EPR spectra of the nitroxides lose the simple character of the three components of nitrogen splitting and their analysis must be carried out by means of simulation of their theoretical line shapes. In calculating the theoretical line shapes in this region, the model of rotational reorientation (e.g., Brownian diffusion, free diffusion, jump diffusion) must also be specified.<sup>1,9</sup>

In our earlier papers<sup>7,8</sup> we reported results obtained in the study of methanolic solutions of the above-noted spin-labeled copolymers in the temperature range 313–213 K, assuming that the rotational reorientation was isotropic. Later investigations showed that such an assumption was too simplified. In this paper we report the results obtained for the investigation of the same copolymers within the range 313–113 K, using the deuterated spin-label PD-Temp-NH<sub>2</sub> (Figure 1), with an analysis carried out in terms of the theory of Freed et al.<sup>1b,9,10</sup> and compare these results with our earlier ones.

## II. Experimental Section

The preparation of copolymers of HPMA with ca. 3 mol % 4-nitrophenol esters of *N*-methacryloylated  $\omega$ -amino acids has been described earlier.<sup>7,8</sup> 4-Aminoperdeuterio-2,2,6,6-tetramethylpiperidinyloxy (PD-Temp-NH<sub>2</sub>) (Figure 1) was bound at the ends of amino acid side chains via the polymer analogous reaction with 4-nitrophenol esters. All the operations involving spin-labeled copolymers of HPMA-I, HPMA-II, and HPMA-IV type thus prepared (Figure 2) were carried out in a manner similar to the case where the proton derivative Temp-NH<sub>2</sub> was used as the spin label.<sup>7,8</sup> The spin-labeled copolymers HPMA-IV<sub>11</sub> and HPMA-IV<sub>14</sub> were prepared by a polymer analogous reaction of 4-nitrophenol esters of the copolymer HPMA-IV<sub>8</sub> with 4-glycylamino- and 4-glycylglycyl-

TABLE I: Characteristics of Copolymers of 2-Hydroxypropyl-*N*-methacrylamide<sup>a</sup>

copolymer	nitrophenol ester content mol %	mol weight ( $M_w$ )	spin-label concn <sup>b</sup>
HPMA-I <sub>2</sub>			$4.6 \times 10^{19}$
HPMA-II <sub>6</sub>	3.19	37 000	$5.9 \times 10^{19}$
HPMA-II <sub>8</sub>	2.95	34 000	$7.1 \times 10^{19}$
HPMA-II <sub>9</sub>	3.2	37 000	$1.4 \times 10^{20}$
HPMA-II <sub>10</sub>	3.1	41 000	$7.0 \times 10^{19}$
HPMA-II <sub>11</sub>	3.02	42 000	$6.0 \times 10^{19}$
HPMA-II <sub>15</sub>	2.9	37 000	$1.3 \times 10^{20}$
HPMA-IV <sub>5</sub>	3.45	42 000	$3.7 \times 10^{19}$
HPMA-IV <sub>8</sub>	3.0	27 000	$5.2 \times 10^{19}$
HPMA-IV <sub>11</sub>	3.0	27 000	$1.1 \times 10^{19}$
HPMA-IV <sub>14</sub>	3.0	27 000	$1.3 \times 10^{19}$

<sup>a</sup> See Figure 2 for structure of the copolymers. <sup>b</sup> The spin label used is 4-aminoperdeuterio-2,2,6,6-tetramethylpiperidyl-1-oxy.

aminoperdeuterio-2,2,6,6-tetramethylpiperidyl-1-oxy (Figure 1). The subscript in the abbreviation for the copolymers indicates the number of atoms in the backbone of the side chain.

The synthesis of 4-aminoperdeuterio-2,2,6,6-tetramethylpiperidyl-1-oxy has been described elsewhere.<sup>11</sup> Its derivatives, namely, 4-acetyl-amino-, 4-glycyl-amino-, 4-glycylglycylaminoperdeuterio-2,2,6,6-tetramethylpiperidyl-1-oxy, and 4-methacryloylamino-1-hydroxyperdeuterio-2,2,6,6-tetramethylpiperidine hydrochloride, were prepared in a manner similar to the respective proton derivatives described in earlier papers.<sup>7,8</sup> The molecular weights of copolymers (Table I) were determined by light scattering with a Photo Gonio diffusometer, Sofica 42 000. The content of 4-nitrophenol esters (Table I) was determined spectroscopically.<sup>7</sup>

**Measurements of EPR Spectra.** EPR spectra were recorded with a JEOL-PE-3X spectrometer provided with an EC-100 computer. The measurements were performed at a microwave output of 1 mW and with a 100-kHz magnetic modulation. The cavity temperature was stabilized with a JES-VT-3A temperature controller to  $\pm 0.5$  K and measured with a platinum resistance thermometer. The line widths were determined by means of a JES-FC3 NMR marker. The spin-label concentrations in the samples (Table I) were determined by comparing the second integral of the spectra of the sample with the second integral of the spectrum of the standard (benzene solution of DPPH). The data show that the yield of the polymer analogous reaction with PD-Temp-NH<sub>2</sub> lay within 60–80%, with the exception of the copolymers HPMA-IV<sub>5</sub>, HPMA-IV<sub>11</sub>, and HPMA-IV<sub>14</sub>, where it was lower. However, the latter two copolymers were prepared by polymer analogous reaction with 4-glycyl-amino- or 4-glycylglycylaminoperdeuterio-2,2,6,6-tetramethylpiperidyl-1-oxy.

The copolymers were measured in methanolic solutions deoxygenated by bubbling with nitrogen, which were filled under nitrogen into JES-LC-02 quartz aqueous solution sample tube, active volume 0.05 mL. The basic copolymer concentrations in solution, 0.3 wt %, used in the HPMA-II type copolymers, had to be raised owing to the lower spin-label concentration (Table I) to 0.6 wt % for the HPMA-IV type copolymers and to 1.2 wt % for the copolymer concentration in this range do not affect the results (the results obtained by analyzing spectra of 1.2% solutions of several copolymers of the HPMA-II series were the same as with 0.3% solutions).

**Analysis of EPR Spectra** The first derivative peak-to-peak line widths and amplitudes of envelopes of the

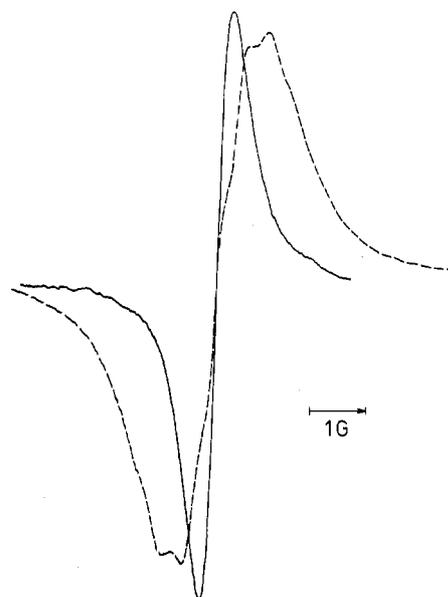
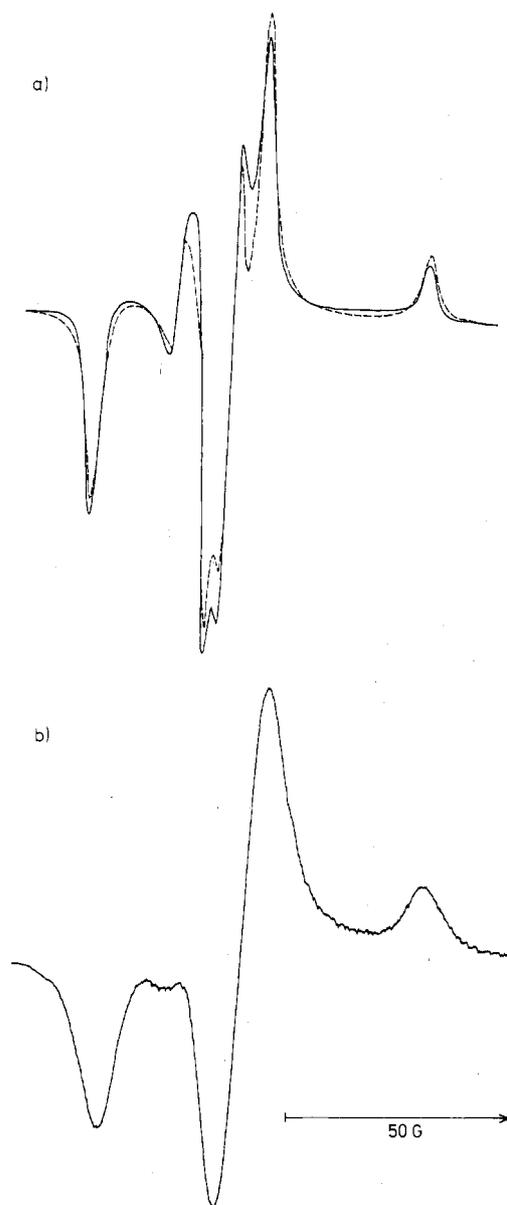


Figure 3. Central line ( $M = 0$ ) of the nitrogen splitting of copolymer HPMA-II<sub>15</sub> spin-labeled by Temp-NH<sub>2</sub> (---) and PD-Temp-NH<sub>2</sub> (—) in methanol at 313 K.

hyperfine structure forming three components of the nitrogen splitting were read by means of an EC-100 computer. Their ratios were then transformed into values corresponding to the intrinsic line width by using calibration curves. These curves were constructed from data obtained by the simulation of spectral lines as envelopes of an unresolved hyperfine structure arising by an interaction of the unpaired electron with protons of the piperidine ring of the spin label. In the case of Temp-NH<sub>2</sub>, the simulation was carried out by using splitting constants determined by the NMR method<sup>11</sup> ( $a_{2,6}^{\text{CH}_3} = 0.46$  G,  $a_{2,6}^{\text{CH}_2} = 0.05$  G,  $a_{3,5}^{\text{H}} = 0.30$  G,  $a_{3,5}^{\text{D}} = 0.49$  G,  $a_4^{\text{H}} = 0.09$  G; the axial and equatorial methyl groups and protons in positions 2,6 and 3,5 are characterized by different splitting constants). In the case of PD-Temp-NH<sub>2</sub>, the deuterium splitting constants were estimated by using the known ratio of the proton and deuterium splitting ( $a^{\text{H}}/a^{\text{D}} \approx 6.5$ ). In Figure 3 one can see the advantage offered by the use of the deuterated spin label, consisting in an essential narrowing of spectral lines, and in the ensuing decrease in the error introduced into the analysis of spectra by the calibration procedure described above. A, B, and C were obtained in the motional narrowing region as the final result of analysis of each spectrum.

Experimental spectra were analyzed in the slow motional region by comparing them with simulated line shapes. In calculations of the simulated spectra with an IBM 370 computer we used the program published by Freed.<sup>1b</sup>

The magnetic parameters of the spin label used in methanol, i.e., the hyperfine interaction tensor  $\mathbf{A}^{\text{N}}$  and the  $\mathbf{g}$  tensor, were determined by an analysis of the rigid-limit spectrum of 4-acetylaminoperdeuterio-2,2,6,6-tetramethylpiperidyl-1-oxy recorded at 113 K in CD<sub>3</sub>OD.<sup>11</sup> The latter derivative can be prepared as a pure crystalline compound and the structure of its substituent in position 4 resembles that of the spin label bound onto a side chain of a copolymer (Figure 2) more than the amino group of PD-Temp-NH<sub>2</sub>. This spectrum was analyzed by employing the simulation procedure<sup>9</sup> in which the Lorentzian line shape and the orientation-dependent line width  $X = \alpha + \beta \cos^2 \theta$  were used, where  $\theta$  is the polar angle between the spin-label molecular  $z$  axis and the direction of the external magnetic field. The best fit with the experimental spectrum (Figure 4) was reached for the magnetic pa-



**Figure 4.** Rigid-limit spectra (— experimental and ---- simulated) of 4-acetylamino-perdeuterio-2,2,6,6-tetramethylpiperidiny-1-oxyl in  $\text{CD}_3\text{OD}$  (a) and 4-acetylamino-2,2,6,6-tetramethylpiperidiny-1-oxyl in  $\text{CH}_3\text{OH}$  (b) at 113 K.

parameter values listed in Table II and for  $\alpha = 1.55$  G and  $\beta = 0.11$  G.

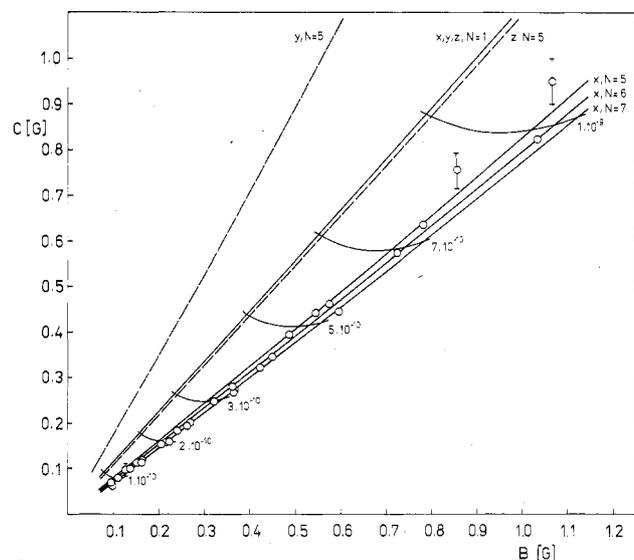
### III. Results and Discussion

*a. Motional Narrowing Region.* The  $C$  vs.  $B$  dependences determined from spectra of all the HPMA-II series copolymers spin labeled by PD-Temp- $\text{NH}_2$  (at 313, 293, 273, and 253 K, and for the majority of copolymers at 233 K) are given in Figure 5 (for spin-labeled copolymers HPMA-II<sub>6</sub> and HPMA-II<sub>8</sub>, values for 233 K are not given, because the respective spectra already lie at the boundary of the slow-motional region; for copolymer HPMA-II<sub>15</sub>, on the other hand, the value for 213 K is also given). The same figure also shows the  $C$  vs.  $B$  dependences calculated from eq 5<sup>9</sup> for various degrees of the anisotropy of rotational reorientation,  $N$ , assuming that the  $z'$  symmetry axis of the rotational diffusion tensor becomes in turn the molecular fixed axes of the spin-label  $x$ ,  $y$ , and  $z$  (Figure 1). It can be seen from the Figure 5 that within the temperature range studied the experimentally determined  $C$  vs.  $B$  dependence is almost linear and agrees well with

**TABLE II: Magnetic Parameters of 4-Acetylamino-perdeuterio-2,2,6,6-tetramethylpiperidiny-1-oxyl<sup>a</sup>**

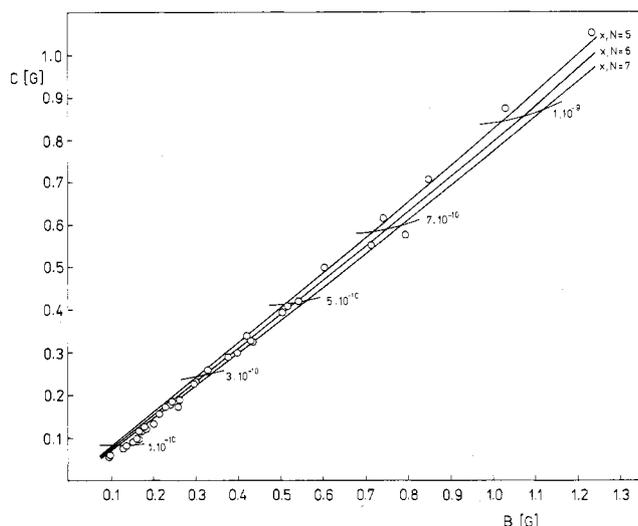
	$z' \equiv x$	$z' \equiv y$	$z' \equiv z$
$A_{x'}$ , G	5.2	35.9	7.0
$A_{y'}$ , G	35.9	7.0	5.2
$A_{z'}$ , G	7.0	5.2	35.9
$g_{x'}$	2.0061	2.0022	2.0090
$g_{y'}$	2.0022	2.0090	2.0061
$g_{z'}$	2.0090	2.0061	2.0022
$\xi_{\text{ND}}^{(0)b}$ , MHz	-15.5	-18.6	34.1
$\xi_{\text{ND}}^{(2)c}$ , MHz	-21.5	20.2	1.26
$g^{(0)d}$	$3.96 \times 10^{-3}$	$4.08 \times 10^{-4}$	$-4.37 \times 10^{-3}$
$g^{(2)e}$	$1.95 \times 10^{-3}$	$-3.40 \times 10^{-3}$	$1.45 \times 10^{-3}$

<sup>a</sup> Magnetic parameters  $A_x$ ,  $A_y$ ,  $A_z$ ,  $g_x$ ,  $g_y$ , and  $g_z$  were determined by an analysis of the rigid-limit spectrum measured in  $\text{CD}_3\text{OD}$  at 113 K (Figure 4). All the values of magnetic parameters given in the table are expressed in the coordinate system of the rotational-diffusion tensor  $x'$ ,  $y'$ ,  $z'$ , where  $z'$  is the symmetry axis of the tensor. The table gives values for cases where the  $z'$  axis is identical with the molecular fixed axes of PD-Temp- $\text{NH}_2$  (Figure 1).  
<sup>b</sup>  $\xi_{\text{ND}}^{(0)} = (1/\gamma_e)(1/2\pi)[2(6^{1/2})]^{-1}[2A_{z'} - (A_{x'} + A_{y'})]$ .  
<sup>c</sup>  $\xi_{\text{ND}}^{(2)} = 1/4(1/\gamma_e)(1/2\pi)(A_{x'} - A_{y'})$ .  
<sup>d</sup>  $g^{(0)} = (6)^{-1/2}[2g_{z'} - (g_{x'} + g_{y'})]$ .  
<sup>e</sup>  $g^{(2)} = 1/2(g_{x'} - g_{y'})$ .



**Figure 5.** Comparison of experimental and calculated  $C$  vs.  $B$  dependences. Experimental values were obtained by analysis of the spectra of the HPMA-II type copolymers labeled with PD-Temp- $\text{NH}_2$  (cf. text). Theoretical values calculated by using eq 5 of ref 9 for the indicated degrees of anisotropy of rotational reorientation,  $N$ , and symmetry axes of the rotational-diffusion tensor,  $z'$ . For cases in which  $z' \equiv x$  (solid lines), correlation times  $\tau_R$ , also determined from eq 5, are shown in the figure as parameter.

that calculated for  $z' \equiv x$ , and  $N = 6 \pm 1$ . One can also see that experimental data cannot be interpreted by assuming that  $z' \equiv y$  or  $z' \equiv z$  (with  $z'$  being the fast axis) because in both these cases the calculated  $C/B$  reaches much higher values than the experimental ones. Hence, the spin label bound at the ends of side chains of copolymers of the HPMA-II type in methanolic solutions undergoes anisotropic rotational reorientation; rotation about the symmetry axis of the rotational-diffusion tensor represented by the molecular fixed axis  $x$  of the spin label is about six times faster than rotation about the remaining two axes. Such a conclusion is in agreement with the finding that roughly in the direction of the  $x$  axis, the spin-label molecule is bound on the side chain of the copolymer, and one could scarcely expect a faster rotation



**Figure 6.** Comparison of experimental and calculated  $C$  vs.  $B$  dependences. Experimental values were obtained by analysis of the spectra of the HPMA-II type copolymers labeled with Temp-NH<sub>2</sub> (cf. text). Further cf. Figure 5.

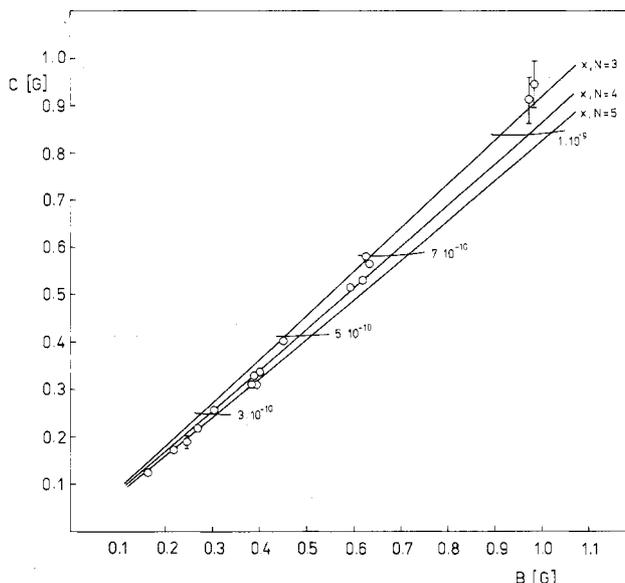
about the axis perpendicular to this bond.

Figure 6 shows the  $C$  vs.  $B$  dependence determined by an analysis of the experimental spectra of the HPMA-II type copolymers spin-labeled by Temp-NH<sub>2</sub>.<sup>7</sup> This dependence leads to a conclusion qualitatively identical with that reached in the case of the deuterated spin label. On the other hand, the figure also shows the higher inaccuracy of experimentally determined  $B$  and  $C$ , which is most likely due to errors introduced into the analysis of spectra by the calibration procedure described above. In this case, experimentally determined  $C/B$  values in the region of short correlation times cannot be interpreted at all within the range of reasonable degrees of anisotropy.

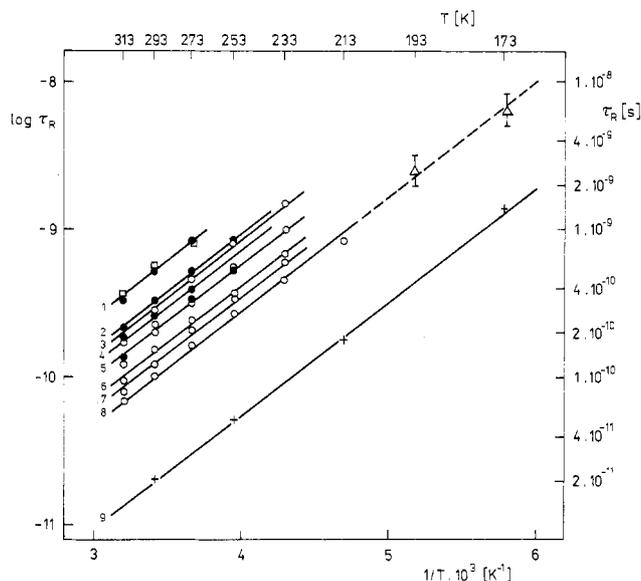
Results obtained for the second group of copolymers spin labeled by PD-Temp-NH<sub>2</sub>, for copolymers of the HPMA-IV type, and for copolymer HPMA-I, at 313, 293, 273 and (with the exception of HPMA-I and HPMA-IV<sub>5</sub>) 253 K are given in Figure 7. The experimentally determined  $C$  vs.  $B$  dependence is again almost linear and may be interpreted by assuming that the symmetry axis of the rotational diffusion tensor  $z'$  is again the spin-label molecular-fixed  $x$  axis, but with a lower degree of anisotropy of the rotational reorientation,  $N = 4 \pm 1$ . Such a conclusion is in agreement with an earlier finding, namely, that at the same length the side chains of the glycine type of the HPMA-IV type copolymers are more rigid with respect to the mobility of the spin label than side chains of the methylene type of the HPMA-II type copolymers.<sup>8</sup> The reason probably consists of the presence of a higher number of CO-NH bonds, which can be regarded as rigid with respect to rotation about its axis.

Attempts were made during the calculations to improve the agreement between the experimentally determined and calculated  $C/B$  dependences in the region of longer correlation times by introducing an experimental adjustment parameter  $\epsilon'$  into expressions for pseudosecular spectral density,<sup>10</sup> but it was found to be unnecessary for most of the range of correlation times studied. Therefore we did not use this experimental parameter in our final analyses, although there may well be theoretical justification for it.<sup>10</sup> The experimental error in the determination of  $B$  and  $C$  was estimated as  $\pm 5\%$ .

We have not in this analysis of the motional-narrowing line widths considered the possibility of a tilt of the fast axis  $z'$  relative to the  $x, y, z$  coordinate system, because



**Figure 7.** Comparison of experimental and calculated  $C$  vs.  $B$  dependences. Experimental values were obtained by analysis of the spectra of the HPMA-I<sub>2</sub> and HPMA-IV type copolymers spin labeled by PD-Temp-NH<sub>2</sub> (cf. text). Further cf. Figure 5.

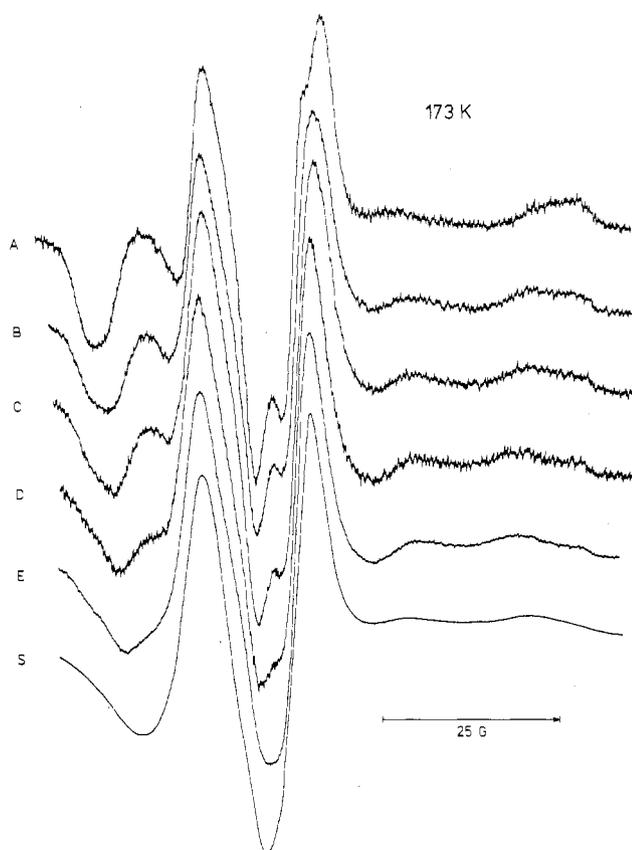


**Figure 8.** Motional narrowing  $\log \tau_R$  vs.  $1/T$  dependences (—) for copolymers HPMA-I ( $\square$ ), HPMA-II ( $\circ$ ), HPMA-IV ( $\bullet$ ) spin labeled by PD-Temp-NH<sub>2</sub> and for free 4-acetylamino-*p*-deuterio-2,2,6,6-tetramethylpiperidyl-1-oxy in methanol, and extrapolation to the slow motional region (---) for copolymer HPMA-II<sub>15</sub> ( $\Delta$ ). To make the results more graphic, values for copolymer HPMA-II<sub>11</sub> situated between those for copolymers HPMA-II<sub>10</sub> and HPMA-II<sub>15</sub> were omitted. (1, HPMA-I<sub>2</sub> and HPMA-IV<sub>5</sub>; 2, HPMA-IV<sub>6</sub>; 3, HPMA-II<sub>6</sub>; 4, HPMA-IV<sub>11</sub>; 5, HPMA-II<sub>8</sub> and HPMA-IV<sub>14</sub>; 6, HPMA-II<sub>9</sub>; 7, HPMA-II<sub>10</sub>; 8, HPMA-II<sub>15</sub>; 9, free spin label.)

with only the  $B$  and  $C$  terms it would be impossible to obtain such information unambiguously. This is discussed further below.

Using this analysis of the  $C$  vs.  $B$  dependences, we can now assign the correlation time  $\tau_R$  to each pair of experimentally determined  $B$  and  $C$  values, i.e., each measured EPR spectrum. The  $\log \tau_R$  vs.  $1/T$  dependences for copolymers spin labeled by PD-Temp-NH<sub>2</sub> are given in Figure 8.

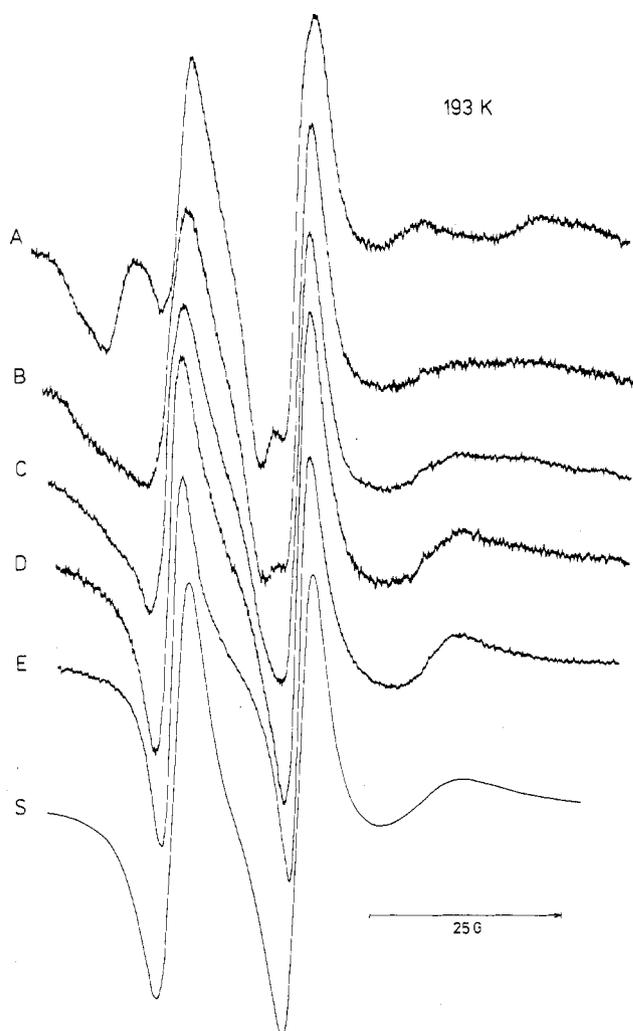
*b. Slow Motional Region.* Simulated spectra in this region of correlation times ( $\tau_R > 10^{-9}$ s) were calculated by using magnetic parameters of the spin label (Table II), the anisotropy of rotational reorientation,  $N$ , and the sym-



**Figure 9.** Slow-motional spectra of copolymers HPMA-IV<sub>5</sub> (A), HPMA-II<sub>6</sub> (B), HPMA-II<sub>8</sub> (C), HPMA-II<sub>10</sub> (D), and HPMA-II<sub>15</sub> (E) recorded in methanol at 173 K and the simulated spectrum (S) calculated by using the parameters  $\tau_R = 7 \times 10^{-9}$  s, rotational independent line width  $X = 0.3$  G,  $N = 6$ ,  $z' = x$ , free rotational diffusion, magnetic parameters, cf. Table II.

metry axis of the rotational-diffusion tensor  $z' \equiv x$  as determined from the motional narrowing region. Certain computational limitations involved in the calculation of simulated spectra hindered us from calculating line shapes for  $\tau_R > 2 \times 10^{-8}$  s. Based upon conclusions reached by Freed et al.,<sup>9,10</sup> the simulated spectra were calculated by using models for Brownian rotational diffusion and approximate free rotational diffusion [the molecule rotates freely owing to inertia for  $\tau$  ( $\tau(R_1 R_2)^{1/2} = 1$ ) and after that passes jumpwise into a new orientation]. Freed et al.<sup>9,10</sup> note that spectra calculated for free diffusion and for moderate jump diffusion are quite similar, although the latter one is probably more realistic; (the former, however, converges more readily in the computer program). In this way, it was possible to simulate quite satisfactorily the spectra of copolymers HPMA-II<sub>15</sub> at 173 and 193 K (Figures 9 and 10). The correlation times determined for HPMA-II<sub>15</sub> by employing the simulation procedure are also given in Figure 8 and correspond fairly well to linear extrapolation of the motional narrowing data to the slow motional region. However, in calculations of simulated spectra on these assumptions, we were not able to reproduce changes which occur in the HPMA-II type copolymers spectra if the side chain is shortened (typical examples are given in Figures 9 and 10). These changes are markedly reflected in the splitting of their low-field and central parts. The spectra of copolymer HPMA-I<sub>2</sub> (Figure 11) in which these trends are strongest could not be simulated at all.

The described low temperature spectra of copolymers with short side chains strikingly resemble the shapes of the spectra obtained by Wee and Miller<sup>4</sup> in their study of



**Figure 10.** Slow-motional spectra of copolymers (spectra denoted as in Figure 9) recorded in methanol at 193 K, and the simulated spectrum calculated by using the parameters  $\tau_R = 3.5 \times 10^{-9}$  s,  $X = 0.1$  G, the other cf. Figure 9.

poly- $\gamma$ -benzyl- $\alpha$ ,L-glutamate, end spin labeled by Temp-NH<sub>2</sub>. These spectra were successfully interpreted by Mason et al.,<sup>12</sup> assuming a very anisotropic axially symmetric rotational reorientation of the spin label, in which the symmetry axis of the rotational-diffusion tensor  $z'$  lies in the  $x, z$  plane with a tilt angle  $\theta$  between  $z'$  and  $z$  axes.

It is believed, therefore, that in the case of copolymers with short side chains, the character of rotational reorientation about the  $z'$  axis is determined by the properties of the side chain, while the rotation about the  $x'$  and  $y'$  axes is determined mainly by rotational reorientation of the whole polymer chain. With a decrease in the temperature of the sample, the rotational reorientation of the polymer chain may be slowed down more quickly than the rotational reorientation of the side chain, and this would lead to an increase in the degree of anisotropy ( $N > 6$ ). Moreover, as follows from Figure 12, in the case of long side chains, the symmetry axis of the rotational-diffusion tensor is represented by an axis very close to the molecular  $x$  axis of Temp-NH<sub>2</sub>. On the basis of data on similar types of nitroxides,<sup>1c</sup> we assume that Temp-NH<sub>2</sub> occupies the chair form with the mirror plane, and that the N<sub>1</sub>-O bond forms an angle of 15–20° with the N<sub>1</sub>C<sub>2</sub>C<sub>6</sub> plane. Shortening of the side chain of the HPMA-II type copolymers leads to a decrease in the number of the CH<sub>2</sub>-CH<sub>2</sub> bonds about which the chain can rotate, and

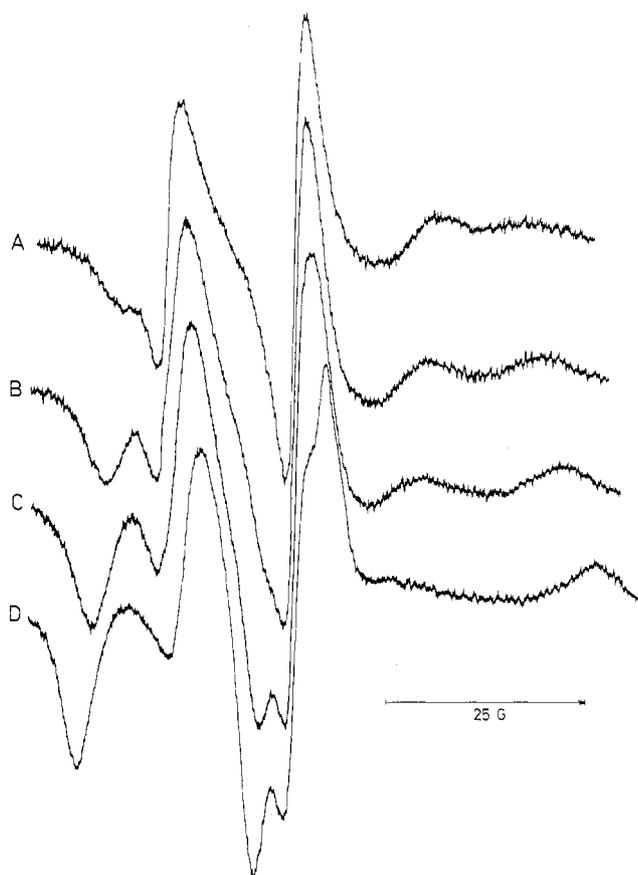


Figure 11. Slow-motional spectra of copolymer HPMA-I<sub>2</sub> recorded in methanol at 233 K (A), 213 K (B), 193 K (C), and 173 K (D).

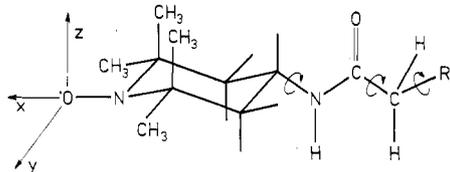


Figure 12. The assumed conformation of spin label bounded at the end of the side chain of the copolymer.

rotation about the C<sub>4</sub>-NH bond becomes increasingly predominant. This bond lies (given our assumptions) in the *x, z* plane and forms a tilt angle of 40–45° with the *z* axis. Such a conformation is virtually identical with that for which Mason et al.<sup>12</sup> calculated the simulated spectra already referred to. In the case of copolymers with a long and flexible side chain (HPMA-II<sub>15</sub>), flexibility of the side chain also contributes to some extent to the rotational reorientation about the *x'* and *y'* axes, and therefore there is no essential rise in the degree of anisotropy with decreasing temperature. On the contrary, the higher rigidity of side chains of the HPMA-IV type copolymers in the motional narrowing region causes a decrease in the degree of anisotropy described above, because it slows down the rotational reorientation about the symmetry axis of the rotational-diffusion tensor. For this type of copolymer, shortening of the side chain reflects a similar effect as on the HPMA-II type copolymers. A detailed analysis of the slow-motional spectra discussed here will be presented in one of the forthcoming papers, but we do wish to note here that we still have to investigate the possibility that for these short-chain copolymers, a single consistent model with *N* > 6 but with *z'* tilted in the *x, z* plane can explain both the slow-motional and fast-motional spectra, even though the fast motional results would not in themselves

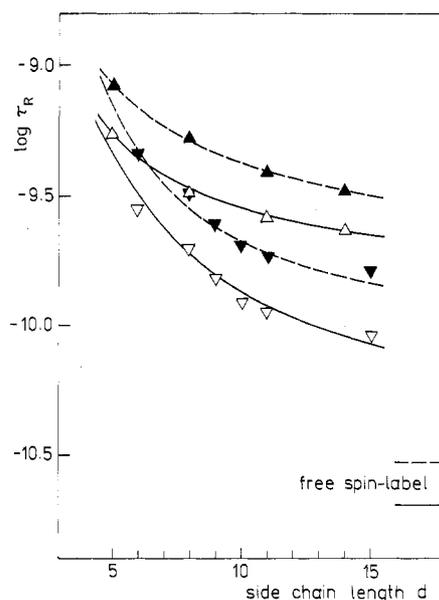


Figure 13. Dependence of the correlation time on the length of the side chain (expressed through the number of atoms in the backbone of the side chain *d*) for the HPMA-II (▽) and HPMA-IV (Δ) type copolymers spin labeled by PD-Temp-NH<sub>2</sub> at 293 K (white symbols and solid lines) and at 273 K (black symbols and dotted lines) in methanol. Bottom right: limiting values for the free spin label at given temperatures.

be unambiguous. Spectra recorded at 113 K in methanol are, for all copolymers, practically identical with the rigid-limit spectrum of Temp-NH<sub>2</sub> (Figure 4b).

*c. Temperature Dependence of Correlation Times.* It follows from Figure 8 that just as in the investigation of copolymers spin-labeled by Temp-NH<sub>2</sub>,<sup>7,8</sup> the log τ<sub>R</sub> vs. 1/*T* dependences can be regarded within the limit of experimental error as linear for all the copolymers under study. The activation energy of rotational reorientation of PD-Temp-NH<sub>2</sub> determined from these dependences is the same within the limits of experimental error (*E* = 15 ± 2 kJ/mol), irrespective of the type of the side chain to which it is attached. The same activation energy characterizes also the temperature dependence of the correlation time of the free spin label in methanol, also shown in Figure 8. A virtually identical value of the activation energy also follows from the results obtained by Hwang et al.<sup>10</sup> in their investigation of PD-Tempone in ethanol. If one assumes, in accordance with Bullock and Cameron,<sup>2</sup> that the determined activation energy *E* is the sum of the activation energy of rotational reorientation of the spin label, *E*<sup>\*</sup>, and of the activation energy for viscous flow of the solvent, *E*<sub>η</sub>, one obtains a value of *E*<sup>\*</sup> = 4.5 kJ/mol, since for methanol *E*<sub>η</sub> = 10.5 kJ/mol.

These facts lead to a conclusion that in this case the decisive role in the thermal activation of rotational reorientation of the spin label in methanol is played by a strong interaction between solvent and the molecule of PD-Temp-NH<sub>2</sub>, irrespective of the fact whether the spin label is free or bound on the side chain of the copolymer. We believe that this interaction is mediated by a hydrogen-bonded complex formed between PD-Temp-NH<sub>2</sub> and solvent. For this reason, the rate of rotational reorientation of the spin label characterized by the correlation time τ<sub>R</sub> varies with temperature slowly. The various side chains by means of which the spin label is bonded to the polymer act only as a hampering factor which increases the correlation time τ<sub>R</sub> depending on the length and type of the side chain.

The dependence of the correlation time τ<sub>R</sub> on the length and type of the side chain can be seen in Figure 13. Unlike

linear dependences obtained in the study of copolymers spin labeled by Temp-NH<sub>2</sub>,<sup>8</sup> the correlation time decreases with lengthening side chain asymptotically to a value characterizing the free spin label at a given temperature. The slower decrease in the correlation time in the case of the HPMA-IV type copolymers compared with the HPMA-II type copolymers is connected with the different structure of side chains of these two types. The structural unit of the side chain of the HPMA-IV type copolymers is the glycine group NH-CH<sub>2</sub>-CO. The side chain is then formed from the CH<sub>2</sub>-CO, NH-CH<sub>2</sub>, and CO-NH bonds, where especially the latter is regarded as rigid with respect to rotation about its axis. Such a side chain is much more rigid and represents a stronger hindering factor than the side chain of the HPMA-II type copolymers formed mainly by the CH<sub>2</sub>-CH<sub>2</sub> bonds. A similar conclusion was reached in the discussion of the lower degree of anisotropy of rotational reorientation in the case of the HPMA-II type copolymers.

Results given in Figure 8 indicate that within the limits of experimental error the rotational reorientation of the spin-label bound on copolymer HPMA-I<sub>2</sub> and HPMA-IV<sub>5</sub> is characterized by the same correlation times. On the other hand, a lengthening of the side chain by one glycine group considerably affects the correlation time; this effect can be seen from differences between copolymers HPMA-IV<sub>3</sub>, HPMA-IV<sub>11</sub>, and HPMA-IV<sub>14</sub> (Figure 13). However, the side chains of HPMA-I<sub>2</sub> and HPMA-IV<sub>5</sub> copolymers are respectively shorter or comparable with the side chains formed by the 2-hydroxypropylmethacrylamide group ( $d = 5$ ).

Since these groups form ca. 97% monomeric units, they obviously form a region of intensive steric interactions along the copolymer backbone. Inside this region the rotational reorientation of the spin label is obviously hampered by steric hindrance and is approximately constant irrespective of side chain length. Changes in the correlation time can be observed only starting from longer side chains, which allow the spin label to move outside this region.

#### IV. Conclusion

The analysis of the EPR spectra of spin-labeled copolymers carried out by using a model of axially symmetric rotational reorientation allowed, at least in first approximation, the interpretation of experimental spectra in the slow motional region, and also the interpretation of spectra in the motional narrowing region, including the experimentally determined  $C$  vs.  $B$  dependences, on the basis of a concrete physical model. A necessary condition for this

analysis was to obtain magnetic parameters of Temp-NH<sub>2</sub> in methanol, which was the main reason for the synthesis of PD-Temp-NH<sub>2</sub>. The use of PD-Temp-NH<sub>2</sub> in the spin labeling of copolymers enabled a higher accuracy to be achieved in the analysis of spectra.

The results obtained confirm the main features concerning the linearity of the  $\log \tau_R$  vs.  $1/T$  dependences characterized by a low activation energy, and the monotonic dependence of the correlation time on the length of the side chain of a given type, as they were summarized in earlier papers<sup>7,8</sup> (numerical differences in the correlation times are not essential in this comparison). Higher accuracy of the analysis of spectra allowed us to obtain a more realistic dependence of the correlation time on the length of the side chain. In our further studies we intend to concentrate on the same or similar copolymers in media which do not have such a strong interaction between solvent and the spin-label molecule, and to examine slow motional spectra of copolymers in which the spin label is undergoing very anisotropic rotational reorientation.

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