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# **Dynamic processes in polymer systems studied by electron spin resonance spin label technique**

Komise pro obhajoby doktorských disertací  
v oboru makromolekulární chemie

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## **Introduction**

Study of local segmental dynamics of polymers plays a significant role in understanding structure-property relationships shown by different polymers. The relationships represent an important subject in polymer research. It is useful to study local chain motions of an isolated chain in dilute solutions before chain-chain interactions are taken into account. Conformation of a polymer chain in dilute solution resulting, among others, from polymer-solvent interactions depends strongly on the dynamics on the length scale of a few monomer units. Understanding of diffusion processes in swollen polymer networks (polymer gels) and in concentrated polymer solutions has a direct impact on numerous practical applications such as drug delivery systems and transport across membranes.

**The thesis was aimed at** the development of special techniques of electron spin resonance (ESR) spectroscopy to get a powerful tool for studying local dynamics at molecular level and diffusion processes in polymer systems together with description and interpretation of the results obtained.

## **Results**

### **Local dynamics in polymer systems**

Interaction of the magnetic moment of an unpaired electron with the nuclear magnetic moment of nitrogen nucleus in nitroxide free radicals characterized by the tensors of hyperfine interaction,  $A$ , and g-factor,  $g$ , is highly anisotropic. The anisotropy can be completely averaged out when the nitroxide is subjected to sufficiently fast thermal Brownian rotational diffusion in very low viscous media. Regarding that nuclear spin of nitrogen nucleus is equal to unity and assuming that no other nuclei interact with unpaired electron in the nitroxide, three equidistant lines of equal intensities and widths should be observed in ESR spectra of nitroxides in such media. In fact, even in media of very low

viscosity, the averaging is not complete and anisotropy affects the shape of the nitroxide spectrum.

In real media nitroxides change their orientations with respect to the external magnetic field created by the spectrometer due to Brownian thermal rotational mobility characterized usually by the rotational correlation time  $\tau_R$ . Anisotropic hyperfine interaction between unpaired electron and nitrogen nucleus is modified by these changes with a frequency dependent on  $\tau_R$ . In such way frequency-dependent perturbation is generated which modifies energy levels and transition probabilities in the system. As a result, the line shape of ESR spectra of nitroxides (and generally of all free radicals in which anisotropic magnetic interactions occur) depends on the correlation time  $\tau_R$ . The line shape of the ESR spectrum can be calculated using spin Hamiltonian  $\hat{H}_S(t)$  for the nitroxide radical. The time dependence of the spin Hamiltonian describes orientation-dependent part  $\hat{H}_I(t)$  which contains terms characterizing anisotropy of magnetic interactions in the free radical (components of the tensors of hyperfine interaction,  $\mathbf{A}$ , and g-factor,  $\mathbf{g}$ ) and terms characterizing rotational reorientation of the free radical as a classic stochastic process using Wiegner rotation matrix elements. The “ESR working window” in which line shapes of the spectra are sensitive to rotational reorientation depends on the anisotropy of the  $\mathbf{A}$ - and  $\mathbf{g}$ -tensors characterizing a free radical and for nitroxides covers the range  $\tau_R \in (10^{-12}-10^{-5} \text{ s})$ , which means that the technique is applicable to polymer solutions, polymer gels and solid polymers at temperatures close to or above  $T_g$ . At the X-band ESR frequency (9 GHz), rotational dynamics in most liquids is usually sufficiently fast, i.e.,  $\tau_R \Delta\omega \ll 1$  (where  $\Delta\omega$  is a measure of the magnitude of the orientation-dependent part of the spin Hamiltonian  $\hat{H}_I(t)$ ), and such motions ( $\tau_R < 10^{-8} \text{ s}$ ) fall within the motional narrowing regime. In these cases, the fast-motional nitroxide ESR spectrum is a simple superposition of three Lorentzian lines of different widths and, subsequently, with different amplitudes when recorded as first derivative of the absorption, which is common in ESR. For slower motions in more viscous media where  $\tau_R \Delta\omega \geq 1$ ,

the ESR spectrum depends more dramatically on the combined influence of molecular motion and magnetic interactions. Thus, the slow-motional ESR line shapes, in principle, provide a more detailed picture of rotational dynamics when compared with fast-motional line shapes.

A comprehensive theory for interpretation of ESR spectra of nitroxides based on numerical solution of the stochastic Liouville equation has been step-by-step developed by Professor J. H. Freed and his coworkers at Cornell University as described in contributions to two monographs<sup>(1,2)</sup> and papers quoted therein resulting in computer programs<sup>(2,3)</sup> suitable for calculation of theoretical line shapes of ESR spectra experimentally accessible not only in continuous wave experiments but in various pulse experiments as well.

**Poly(N-(2-hydroxypropyl)methacrylamide) - fast-motional spectra.** After solving basic problems concerning synthesis of spin-labeled polymers, the research was started by testing sensitivity of the ESR spin label technique to local reorientational processes occurring inside polymer chain in solution. Soluble copolymers of *N*-(2-hydroxypropyl)methacrylamide (HPMA) and 4-nitrophenyl esters of *N*-methacryloylated  $\omega$ -amino acids forming side chains were prepared. The nitroxide spin label was attached to the copolymer by the reaction of 4-amino-2,2,6,6-tetra([<sup>2</sup>H<sub>3</sub>]methyl)-3,3,5,5-[<sup>2</sup>H<sub>4</sub>]piperidin-1-yloxy with the 4-nitrophenyl ester. Both fast and slow-motional ESR spectra of dilute methanolic solutions of these copolymers were measured in suitable temperature range.<sup>[20,22,26]\*</sup> Regarding theoretical tools available that time we focused on the analysis of fast-motional spectra. The results clearly showed decrease of correlation time for the nitroxide reorientation  $\tau_R$  with increasing temperature and with increasing length of the side chain. Several important conclusions resulted from these studies. Deuterated spin labels significantly simplify analysis of both fast and slow-motional spectra and should be used in further research. Contribution of unresolved hyperfine interaction of unpaired electron with deuterium nucleus to the width of lines in nitroxide spectra is

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\* references given in square brackets refer to relevant author's papers

significantly smaller than contribution of its interaction with proton due to significantly smaller nuclear magnetic moment of deuterium nucleus when compared with proton. Even in the case when simple axially symmetric rotational diffusion with symmetry axis oriented along one of the axes of the nitroxide axis system, analysis of fast-motional spectra is not able to characterize the rotation fully. It follows that attention should be focused on the slow-motional spectra.

Studies based on qualitative characterization of rotational reorientation of nitroxides and on determination of the proportion of nitroxides subjected to slower and faster rotational diffusion in selected samples were also performed. The amount of spin-labeled water-soluble model polymers immobilized on macroporous spherical carrier formed by glycidyl methacrylate – ethylene dimethacrylate copolymer and on epoxy-activated Sepharose was determined by ESR.<sup>[25]</sup> It was confirmed that the free nitroxide is cleaved off by chymotrypsin from 4-[(glycyl-L-phenylalanyl)amino]-2,2,6,6-tetramethylpiperidin-1-yloxy attached as a substrate with paramagnetic leaving group to the end of side chains of water-soluble HPMA copolymers.<sup>[27]</sup> The character, amount and accessibility of oxirane groups modified in various glycidyl methacrylate – ethylene dimethacrylate copolymer particles by reaction with 4-amino-2,2,6,6-tetra([<sup>2</sup>H<sub>3</sub>]methyl)-3,3,5,5-[<sup>2</sup>H<sub>4</sub>]piperidin-1-yloxy were determined by ESR as well.<sup>[38,42,44,48]</sup>

**Segmental mobility.** At the next step our research benefited from the progress reached in theoretical description of the effects of dynamics on line shape of nitroxide ESR spectra and in resulting software. That time we were able to analyze slow-motional spectra of nitroxides subjected to anisotropic rotational diffusion with rotation symmetry axis oriented quite arbitrary with respect to the nitroxide axis system using Schneider-Freed<sup>(2)</sup> set of programs. When aiming at characterization of rotational mobility of segments of polymer chain, a way had to be found to extract this information from the parameters characterizing rotational diffusion of the nitroxide. On the basis of theoretical



CO-NH, and NH-SL, where SL stands for 2,2,6,6-tetramethylpiperidin-1-ylloxy). Given that the second bond, which is a peptide bond can be considered to be fixed regarding the time window of the relevant motions, and that there are steric constraints around the first bond, the only bond through which conformational changes of the tether are expected to occur is the NH-SL bond.

Using this model and the Schneider-Freed<sup>(2)</sup> set of programs, we studied local segmental dynamics in poly(methyl methacrylate) (PMMA), poly(methacrylic acid) (PMA), poly(acrylic acid) (PAA), polystyrene (PS) and poly(2-hydroxyethyl methacrylate) (PHEMA). All these polymers were spin-labeled by incorporating spin-labeled chain unit (Chart 1) in polymer chain at randomly distributed sites using various synthetic techniques (polymer reaction, copolymerization with a suitable monomer carrying an amine precursor of the spin label and subsequent oxidation). Weight-average ( $M_w$ ) of the spin-labeled polymers exceeded in all cases 20 000 which is considered as a rough limit above which the contribution of the rotational dynamics of polymer coil as a whole in solution to the rotational dynamics of the spin label can be neglected. Molar concentration of the spin-labeled chain units ranged between 1 and 3 mol %.

**Poly(methyl methacrylate).** Randomly spin-labeled PMMA carrying the same spin label units as given in Chart 1 and end spin-labeled PMMA were prepared. Highly consistent data characterizing segmental dynamics of the randomly labeled PMMA were obtained by analysis of the experimental spectra using a simulation approach. All the best fits were obtained for  $\beta \sim 51^\circ$  which is the value very close to that determined for the orientation of the above mentioned NH-SL bond calculated for the chair form of piperidine nitroxides from crystallographic data determined by diffraction methods on nitroxide single crystals.<sup>(51)</sup> The Arrhenius plot of the values determined for the parameter characterizing PMMA segmental rotational diffusion ( $R_s$ ) in ethyl acetate was divided into two parts by a break at room temperature while the low-temperature part of the plot was characterized by a higher activation energy than

the high-temperature part. The sudden decrease in the activation energy at ca. 300 K was explained by the conformational transition of PMMA chain. Such conformational transition of PMMA (and some other polymethacrylates) chains above the transition temperature characterized by an increase in flexibility and a decrease in unperturbed dimensions of the polymer coil  $K_0$  by 8 - 25 % was observed by Katime et al.<sup>(52)</sup> in the studies of molecular weight and temperature dependences of intrinsic viscosity of polymethacrylates in non-polar organic solvents. For PMMA in ethyl acetate they found the transition temperature range 318–328 K. No such break was observed when plotting data for PMMA in dibutyl phthalate, probably because the transition temperature range in this solvent, if exists, is shifted outside the temperature range studied. Analysis of the end-labeled PMMA spectra in both solvents reveals much faster rotational diffusion of the nitroxide attached to the end of PMMA chain compared with the randomly labeled chain, where most spin labels are expected to be attached to the inner chain segments (for statistical reasons). The higher value of  $\beta \sim 65^\circ$  observed for end-labeled PMMA in ethyl acetate solvent indicates participation of more bonds in the internal rotation of the spin label attached to the end of the chain which probably shifts the  $\beta$  towards average value for all-trans chain ( $\sim 90^\circ$ ).

**Polyelectrolytes** offer an excellent opportunity to study the effect of polymer chain conformation on the character of local segmental motions. A polyelectrolyte molecule in solution may greatly expand by electrostatic repulsion between polar groups, and this effect manifests itself in a very high intrinsic viscosity. The size of the polyelectrolyte random coil is, among other things, a function of the degree of neutralization  $\alpha$ . The reduced viscosity of a typical polyelectrolyte in aqueous solution, which exhibits the so-called “normal polyelectrolyte behavior” (e.g. PAA), increases with  $\alpha$  increasing up to 0.4 and then slowly decreases due to the saturation effect.<sup>(53)</sup> PMA deviates from the normal polyelectrolyte behavior and is known to undergo a conformational transition in an aqueous solution upon ionization. It exists in a very compact



conformation stabilized by short-range interactions at low  $\alpha$  values, whereas at  $\alpha > 0.3$ , long-range electrostatic interactions become predominant and its compact structure is converted to an extended one, which then shows a normal polyelectrolyte behavior upon further increase of  $\alpha$ . This conformational transition, which can be characterized by the fact<sup>(54)</sup> that at 293 K 50 % of the very compact conformation disappears at about  $\alpha = 0.17$ , is responsible for the steep increase in reduced viscosity at  $\alpha = 0.15$ .<sup>(51)</sup>

Randomly spin-labeled PMA<sup>[41]</sup> and PAA<sup>[47]</sup> carrying the same spin label units as given in Chart 1 were prepared. Dilute solutions of both polyacids neutralized to the required degrees were prepared by mixing a polyacid stock solution ( $\alpha = 0.0$ ) in quartz-bidistilled water with an appropriate amount of a polysalt stock solution ( $\alpha = 1.0$ ) or of a 0.4 M NaOH. Extreme care was taken to prevent accidental neutralization of the polyacids during preparation. The degree of neutralization  $\alpha$  achieved was checked by titration. ESR spectra of the solutions were measured in the temperature range 273 – 353 K.

For PMA much slower segmental rotational mobility with much lower activation energy for the very compact conformation, which prevails at low degrees of neutralization  $\alpha$ , than the values characterizing extended conformation, which prevails at higher  $\alpha$  was found<sup>[41]</sup>. Both conformations were found to exist simultaneously in the  $\alpha$  range 0.0-0.2 at higher temperatures in agreement with spectrophotometric data.<sup>(52)</sup> The segmental rotational mobility increases with coil expansion in the  $\alpha$  range 0.2-0.5, which may be explained on the basis of results published by Bahar et al.<sup>(55)</sup> by increasing number of segments in the moving sequence of the PMA chain. Our results are in good agreement with the published data resulting from NMR studies.<sup>(56)</sup>

In contrast, quite different results were obtained for PAA<sup>[47]</sup>, which is expected to exhibit normal polyelectrolyte behavior. The PAA segmental dynamics nearly independent of the degree of neutralization  $\alpha$  in the range 0.0-1.0 was found at temperatures from 273 to 353 K. The absence of methyl groups is probably responsible for a higher segmental rotational mobility at all

degrees of neutralization and in the whole temperature range studied when compared with PMA under the same conditions.

**Polystyrene.** A styrene copolymer with methacrylic acid containing less than 5 mol % of spin-labeled methacrylic acid units distributed randomly along the main chain was synthesized. ESR spectra of spin-labeled polystyrene were measured at X-band (9 GHz) in dilute solutions (ca. 1 wt %) over a broad temperature range in four solvents differing in thermodynamic quality, viscosity, and activation energy of viscous flow.<sup>[60]</sup> The temperature dependence of parameter  $R_s$ , characterizing the local dynamics in polystyrene chains, and of the other parameters characterizing dynamics in the copolymer studied were determined by fitting experimental ESR spectra to the MOMD model using NLSL program.<sup>(3)</sup> The MOMD (microscopic orders with macroscopic disorder) model allows for constraints in the rotational diffusion. More precisely, the polymer chain segmental motion sensed by the tethered nitroxide, i.e., the wobbling motion of the effective axis of internal rotation, is considered as constrained by an orienting potential, typically given by

$$-U(\theta, \varphi)/kT = \sum_{L=2,4} \{ c_0^L D_{00}^L(\theta, \varphi) + c_2^L [ D_{02}^L(\theta, \varphi) + D_{0-2}^L(\theta, \varphi) ] \}$$

where the  $c_0^L$  terms refer to the strength and shape of the restricting potential for the wobbling motion and the  $c_2^L$  to its asymmetry, and  $D_{0K}^L(\theta, \varphi)$  are Wigner rotation matrix elements. The angles  $\theta$  and  $\varphi$  are polar and azimuthal angles, respectively, which characterize orientation of the effective axis of internal rotation relative to the director expressed in the principal axes of nitroxide rotational diffusion tensor. The MOMD model, when applied to the system of nitroxide spin labels attached via short side chains (tethers) to the polystyrene main chain in solution, implies a preferred orientation for the axis of internal rotation of each attached nitroxide label with respect to the polymer main chain, but there is an isotropic distribution of these preferred orientations in the macroscopic sample due to the random distribution of orientations of polymer coils. The nature of the preferred orientational distribution within each polymer

coil is determined by the shape of the orienting potential. The best-fit shape can be found during the fitting process by varying potential parameters  $c_0^2$ ,  $c_2^2$ ,  $c_0^4$ , etc., which become additional parameters of the fit when MOMD model is used and can be optimized during the fitting process. The first three mentioned parameters were found to be sufficient in the present study. The best-fit values of the fitting parameters again demonstrate general consistency of the model used. The angle  $\beta$  was found to be solvent-dependent; it increases with increasing solvent viscosity in the range 55 °C - 70 °C and slightly decreases or keeps practically a constant value with increasing temperature in each of the solvents. The parameter  $c_0^2$  characterizes the component of the orienting potential symmetric in the plane perpendicular to the direction of the preferred rotational tensor symmetry axis orientation specified by angle  $\beta$ . This parameter increases with increasing temperature in all solvents used up to a maximum around 1.5 reached at intermediate temperatures at which a fast-motional character of the spectra begins to prevail and it sharply decreases towards zero at higher temperatures. The parameter  $c_0^4$ , which affects the shape of orienting potential significantly, behaves in a similar way being negative at low temperatures. The parameter  $c_2^2$  characterizing rhombic distortion of the orienting potential keeps a relatively high value (above 1.0) at temperatures at which the slow-motional character of the spectra prevails; at intermediate and higher temperatures, it sharply decreases towards zero. For the nitroxide spin label chemically attached to polymer chains in solution, the conclusions presented above generally confirm the importance of microscopic ordering for the dynamics resulting in slow-motional ESR spectra and its decreasing effect on the dynamics in the motional narrowing region. A significantly higher quality of the fits obtained is visible at first sight when comparing with fits presented in previous papers.

Unlike the authors of papers published so far (see references given in the relevant paper [60]), we have concluded that polystyrene in the solvents

exhibits non-Kramers' behavior described by the power law relationship

$$\tau_c = K [\eta(T)]^\alpha \exp(E_a/RT) = K' \exp(E_{\text{exp}}/RT)$$

$$E_{\text{exp}} = E_a + \alpha E_\eta, \quad \eta(T) = \eta_0 \exp(E_\eta/RT)$$

between  $\tau_c$  and the solvent viscosity  $\eta$  suggested<sup>(12)</sup> on the basis of papers published by Fleming and co-workers.<sup>(13)</sup> The pre-factor  $K$  in this equation is independent of temperature and viscosity,  $E_{\text{exp}}$  is the activation energy determined from the Arrhenius plot of a parameter characterizing local segmental mobility (e.g.  $\tau_c$ ), and  $E_\eta$  is the activation energy of viscosity of the solvent.  $K'$  is independent of temperature,  $E_a$  is the height of the potential barrier for local segmental motions,  $T$  is the absolute temperature, and  $R$  is the gas constant. The exponent  $\alpha$  is assumed to depend on the moment of inertia and the size of the isomerizing unit and on the curvature at the top of the potential barrier. A larger size, a larger moment of inertia, or a lower barrier prolong the time required to get across the barrier and thereby lead to a larger value of  $\alpha$ . When this time period is sufficiently long, then the high-frequency behavior of friction is less significant, and behavior close to Kramers' high-friction limit should be observed. The value of the exponent  $\alpha$  is expected to vary from polymer to polymer. The height of the intramolecular potential barrier  $E_a$  should not depend on the solvent viscosity but it may vary with conformation of the polymer in solution. It follows that it should be solvent-independent for all good nonpolar solvents for a particular polymer but may change in bad solvents and in  $\Theta$  solutions.<sup>(12)</sup>

We found that local dynamics of polystyrene in all the solvents used -  $\Theta$  solvent dioctyl phthalate, marginal solvent dibutyl phthalate and good solvents toluene and dimethylformamide - regardless of their thermodynamic quality, can be characterized by parameter  $\alpha = 0.73 \pm 0.02$  and by the height of the potential barrier for local conformational transitions  $E_a = 10.5 \pm 0.6$  kJ/mol. The value agrees with the value published by Waldow et al.<sup>(18)</sup> for good solvents ( $E_a = 11 \pm 3$  kJ/mol) within the given error limits. The difference between the

value of parameter  $\alpha$  determined in the present paper and the value determined by Waldow et al.<sup>(18)</sup> using the fluorescence depolarization technique and anthracene chromophore bonded in the polystyrene chain ( $\alpha = 0.9$ ) is minor. Nevertheless, a higher  $\alpha$  value has been found by the fluorescence depolarization technique in the presence of bulky chromophore label than by ESR in the presence of the nitroxide spin label affecting the chain geometry to a small extent. A similar case was observed with polyisoprene when comparing values determined by the fluorescence depolarization technique<sup>(11)</sup> and no-label NMR<sup>(12)</sup> (0.75 and 0.41, respectively).

During stays in Professor Jack Freed's laboratory at Cornell University I completed study of spin-labeled polystyrene (using a slightly different copolymer of styrene with spin-labeled acrylic acid unit) with measurements on the then unique far-infrared (FIR) ESR spectrometer and with comparison of the results obtained for this polymer by X-band (9 GHz) and FIR (250 GHz) ESR. Although a reasonable agreement between the results obtained by NLSL analysis at 9 GHz and 250 GHz has been found<sup>[55]</sup>, there were systematic discrepancies such that the orienting potentials obtained from the 250 GHz spectra were about twice (or more) higher than those from the 9 GHz spectra, and the rotational diffusion tensor components from the 250 GHz spectra were at least twice higher than those from the 9 GHz spectra. This implies a greater sensitivity of the 9 GHz spectra to slower tumbling motions. For the faster "time-scale" of the 250 GHz spectra, such motions are likely "frozen-out", consistently with the MOMD model. Nevertheless, the results at both frequencies yielded a common activation energy,  $E_{\text{exp}} = 20.7 \pm 1.5$  kJ/mol, which, when corrected for the viscous flow contribution according to classic Kramers' theory, yielded  $E_a = 11.9 \pm 1.5$  kJ/mol.<sup>[55]</sup>

**Poly(2-hydroxyethyl methacrylate).** Recently our attention has been focused on hydrogels based on 2-hydroxyethyl methacrylate (HEMA). As the next step in the study of HEMA-based polymer systems, an investigation of local segmental dynamics of spin-labeled PHEMA in methanolic solution (linear

PHEMA is unfortunately insoluble in water) over a wide concentration range was performed.<sup>[61]</sup> Measurements characterizing local polymer dynamics of PHEMA have not been reported so far, and data on local dynamics in nondilute solutions can only scarcely be found in literature.

A copolymer of HEMA with spin-labeled methacrylic acid units distributed randomly along the main chain at concentration  $\sim 3$  mol % was synthesized. ESR spectra of methanolic solutions of the copolymer at concentrations between 2 wt% and 50 wt% were measured at X-band (9 GHz) over a broad temperature range. The temperature dependence of the  $R_S$  parameter characterizing the local segmental dynamics in PHEMA and of the other parameters characterizing local dynamics and conformation of the copolymer were determined by fitting experimental ESR spectra to the theoretical spectra calculated using the MOMD model and NLSL program.<sup>(3)</sup> The best-fit values of the fitting parameters demonstrate general consistency of the model used. At all the concentrations investigated, the angle  $\beta$  first increases with increasing temperature in the range 55 °C - 70 °C, reaching maximum at ca. 250 K, and then decreases. The isotropic inhomogeneous Lorentzian broadening  $w^L$  reaches minimum at temperatures close to 230 K and then increases with increasing temperature. The best-fit values of the three potential parameters employed characterize the shape of the ordering potential at a particular temperature and concentration. The parameters  $c_0^2$  and  $c_0^4$  characterize the component of the ordering potential independent of the  $\theta$  coordinate, and the parameter  $c_2^2$  characterizes its rhombic distortion. Best-fit values of parameters  $c_0^2$  and  $c_2^2$  are positive and decrease with increasing temperature at all concentrations studied. Best-fit values of parameter  $c_0^4$  reach negative minimum values at temperatures close to 250 K and then increase toward zero with increasing temperature. Typical shapes of the orienting potential  $U(\theta, \varphi)$  in the coordinates  $\theta$  and  $\varphi$  ( $\theta = 0^\circ$  for the directions in the symmetry plane of the piperidine moiety – in the xz plane of the nitroxide axis system defined in Chart 1 and  $\varphi = 0^\circ$  for the

direction along the director) show the probability distribution of effective orientation of the nitroxide rotational tensor symmetry axis with respect to the director. The appearance of two clearly visible maxima of the distribution separated by an angle of  $2\varphi_{\max}$  increasing from  $60^\circ$  to  $100^\circ$  ( $\varphi_{\max} \div 30^\circ - 50^\circ$ ) with temperature in this temperature range is typical of negative values of parameter  $c_0^4$  and positive values of  $c_0^2$ . The ordering potential keeps the plane of symmetry of the piperidine moiety and the maxima appear at  $\theta = 0^\circ$  and at  $\theta = 180^\circ$  as follows from the positive values of the parameter  $c_2^2$ .

Arrhenius-type plots of rotational parameters  $R_s$  and  $R_l$ , as determined by the fitting process of experimental spectra of spin-labeled PHEMA in methanol at all the concentrations studied, differ at first sight by their nonlinearity for both rotational parameters from the published plots characterizing segmental dynamics of a number of polymers in dilute solution, in particular from the recently published plots characterizing local dynamics of polystyrene in dilute solution, which are linear for both rotational parameters regardless of the thermodynamic quality of the solvent.<sup>[60]</sup> The plots exhibit an atypical nonlinear behavior characterized by two breaks at specific temperatures in the  $R_s$  plot. The difference between the lower break temperature ( $\sim 250$  K for all the concentrations studied) and the upper break temperature was found to increase with increasing concentration of PHEMA in methanol. Local dynamics of the polymer was observed to be practically independent of temperature in the temperature range between both breaks. On the basis of our previous results and literature data<sup>(52)</sup>, we concluded that, similarly to the behavior of PMMA and some other polymethacrylates in a number of non-polar solvents, PHEMA, which is more hydrophobic due to the presence of the hydroxyethyl groups, undergoes in methanol above the lower break temperature a conformational transition from a less compact to more compact conformation of the polymer chain. The decrease in the polymer globule radius observed in 2% methanolic solution of PHEMA with temperature increasing in the range in which the

saddle part for this PHEMA concentration exists, as determined by dynamic light scattering (DLS) and small ange X-ray scattering (SAXS) experiments, supports this conclusion. Intramolecular interactions in a dilute solution (2 wt%) in the range between both break temperatures, when PHEMA exists in a compact conformation, hinder local polymer dynamics. The contribution of the intermolecular interactions in entangled nondilute solutions (at polymer concentrations higher than ca. 20 wt%) is probably responsible for the same effect in more concentrated solutions and for the observed increase in the upper break temperature with increasing PHEMA concentration and simultaneously decreasing  $\zeta_H$ . Analysis of the shape of the orienting potential, which constrains the preferred orientation of the effective axis of internal rotation of the tethered nitroxide, revealed the presence of two different conformations of the nitroxide moiety. The UB3LYP/6-31+G\* quantum chemical calculations provided two conformational minima of the model system (nitroxide moiety plus tether) in a good agreement with the geometry required by the shape of the ordering potentials. However, the presence of the conformations revealed by these calculations can be accepted as an explanation of the obtained experimental data only providing that the effects omitted in the calculations, in particular the neglect of solvent effects and of interactions mediated by the polymer chain(s), would increase the probability of occurrence of the more tightly packed nitroxide conformation.

**Comparison with another techniques.** When comparing various mentioned techniques used for characterization of local segmental dynamics in polymers, only NMR technique does not require the presence of any label affecting the chain dynamics; on the other hand, its applicability requires appearance of suitable lines in the NMR spectrum of the polymer ( $^{13}\text{C}$  enrichment is frequently needed). The presence of bulky chromophore label bonded in the main chain, required when applying fluorescence technique, affects both main chain conformation and dynamics in the vicinity of the “signal” group significantly. ESR technique requires the presence of a spin label, which is usually attached to



a short side chain; the volume and structure of the spin-label-bearing chain unit is usually very similar to the volume and structure of the main chain units. Information on the local main chain dynamics is extracted from the parameters characterizing anisotropic rotational diffusion of the label. The ESR spin label technique is applicable to polymer systems (copolymers, gels) regardless of their complicated chemical structure providing that a suitable method of spin label attachment to them is found.

### **Macroscopic translational diffusion in polymer systems**

Electron spin resonance imaging (ESRI) is able to provide information on the distribution of paramagnetic molecules in the sample along the direction of magnetic field gradient (created by special coils) applied to the sample in addition to the external magnetic field created by the spectrometer magnet. The ESR spectrum measured in the presence of the magnetic field gradient (“projection”) is a superposition of the spectra of all individual paramagnetic molecules in various positions inside the sample (i.e. at various effective external magnetic fields) weighted by the spectrometer sensitivity at particular position. In principle, two main modifications of the technique can be used. Spectral-spatial two-dimensional ESRI (2D ESRI) requires measurement of a large number of projections (usually more than 50) taken in the presence of different but specified magnetic field gradients. After application of a suitable reconstruction procedure, three-dimensional image in spectral-spatial-intensity coordinates can be obtained.<sup>(26-28)</sup> Such images can show or exclude possible dependence of line shapes in ESR spectra of paramagnetic molecules on the spatial coordinate, which is the only significant advantage of this time-consuming technique. Concentration profiles of paramagnetic molecules along the magnetic field gradient can be constructed by integration of ESR spectra at selected spatial coordinates. Application of the 2D ESRI, requiring for collection of experimental data in the most favorable cases a time period of about 60 min during which the profile should not change significantly, to the

study of macroscopic diffusion is limited to very slow processes.

Providing that there is no line shape variation in the ESR spectrum of the paramagnetic molecules throughout the sample, the projection could be mathematically expressed as a convolution of the spectrum measured at the gradient-off with the concentration profile of the paramagnetic molecules inside the sample multiplied by the spatial sensitivity function of the spectrometer. In order to get the concentration profile, a reverse deconvolution process must be performed, i.e. the spectrum at the gradient-off must be deconvoluted out of the projection measured at the gradient-on.<sup>(29,30)</sup> This technique called one-dimensional ESRI (1D ESRI) is relatively fast. In principle, it enables determination of diffusion coefficient for macroscopic translational diffusion of a paramagnetic tracer (e.g. added onto the surface of the sample) by measuring spectrum at the gradient-off and the projection at the gradient-on once at a particular time after the start of the diffusion proceeding from the surface inside the sample provided that the magnetic field gradient is applied in the direction of the diffusion (perpendicular to the sample surface). By fitting a theoretical concentration profile of the tracer in the sample, expressed as a solution of Fick's equation for proper sample configuration<sup>(31)</sup>, to the experimental profile, the diffusion coefficient as one of the parameters of the fit can be deduced. Measurements performed at various times after start of the diffusion make it possible to determine the time dependence of the diffusion coefficient.

**2D ESRI studies.** During stays in U.S.A. at University of Detroit Mercy in Detroit, I was given an opportunity to participate in assembling one of the first X-band ESRI spectrometer in the world. After completed assembling and testing procedures, application of this technique to measurement of macroscopic translational diffusion of paramagnetic tracers in polymer systems became the main theme for cooperation between the Institute and Detroit laboratories. Study of diffusion of low-molecular-weight nitroxide spin probes and spin-labeled poly(ethylene oxide) (PEO) oligomers as the tracers in HEMA-based hydrogels as the matrix by 2D ESRI was selected to start with. Hydrogels are

polymer materials of soft and rubbery-like consistency, which have the ability to retain substantial amounts of water.<sup>(61)</sup> Hydrogels based on the HEMA monomer exhibit excellent biocompatibility and physical properties similar to those of living tissue.<sup>(62,63)</sup> For these reasons they have found large-scale applications in medical practice.<sup>(64)</sup> In particular, such hydrogels have been used for manufacturing soft contact and intraocular lenses.<sup>(65)</sup>

First hydrogel matrices in which diffusion of the selected tracers ranged in measurable limits and suitable experimental arrangement considering the time needed for taking all necessary projections for 2D ESRI (this requirement excluded very fast diffusions) and stability of the hydrogel samples (very slow diffusions excluded) had to be found. Samples for measurement were prepared by adding a small amount of tracer solution on the surface of cylindrical hydrogel sample in glass capillary with diameter smaller than 1 mm. Care was taken to prevent solvent evaporation from the sample by sealing the capillary after adding the tracer. Only hydrogels swollen with the solvent to equilibrium were selected as matrices to exclude contribution of swelling process to the diffusion. After sealing, the sample was immediately placed inside the microwave cavity of spectrometer and sets of ESRI projections were taken after suitable periods of diffusion with magnetic field gradient oriented along the long axis of the cylindrical sample i.e. in the direction of macroscopic diffusion.

In the first pioneering paper published on this subject<sup>[50]</sup> diffusion coefficients for three different tracers in three hydrogels prepared using different ratios of HEMA and 2-(2-hydroxyethoxy)ethyl methacrylate (DEGMA) monomers, which, when swollen to equilibrium, contained various amounts of water, were presented. The diffusion coefficients were found to depend primarily on the molecular weight of the tracer. Their dependence on the weight fraction of the gel in the samples roughly followed the free volume model. I have also participated in the study<sup>[52]</sup> devoted to a comparison of diffusion coefficients for a simple low-molecular-weight spin probe in concentrated toluene or dimethylformamide solutions of polystyrene with diffusion coefficients of the

same tracer in polystyrene gels crosslinked to various degrees and swollen with the same solvents. It was concluded that the dependence of diffusion coefficients on the weight fraction of the polymer or gel in the samples is consistent with the free volume theory and that the temperature dependence of diffusion coefficients fits well the Arrhenius behavior. A significant dependence of the diffusion coefficients for the spin probe on the degree of crosslinking of polystyrene gel forming the matrix is the most important finding in this study. It was also concluded that the extremely time-consuming and complicated 2D ESRI technique used so far should be used only for checking the independence of spectral line shapes on the spatial coordinate, which follows from all so far performed experiments. When the independence is confirmed, the much faster and much more effective 1D ESRI technique should be used.

**1D ESRI studies.** Considering all findings of the first stage of the studies devoted to diffusion processes, new software for treatment of both 1D and 2D ESRI data based on much more stable and accurate deconvolution and back projection processes was first compiled.<sup>[57]</sup> Then the project continued by studies of diffusion of paramagnetic tracers in cross-linked poly(1-vinylpyrrolidone) (PVP) hydrogel swollen to equilibrium with water and in concentrated aqueous solutions of PVP using 1D ESRI technique. Practically the same diffusion coefficients for low-molecular-weight tracers in PVP gels and solutions containing the same concentration of linear PVP were found.<sup>[54]</sup> Also, practically the same hydrodynamic correlation length  $\zeta_H$  was measured in the gels and solutions by DLS. On the other hand, a significantly lower diffusion coefficient in PVP gel when comparing with a PVP solution containing the same concentration of linear PVP was found for a bulky paramagnetic tracer (spin-labeled PEO oligomer). It was concluded that even small concentration of permanent cross-links in the lightly cross-linked PVP gels are able to reduce diffusion coefficients of a bulky paramagnetic tracer to the value corresponding to a two-fold increase in concentration of linear PVP in

the solution containing entangled polymer chains. In the last paper for the time being concerning diffusion processes, information on the effect of permanent crosslinks in gels on the diffusion coefficients were given. Diffusion of paramagnetic tracers in HEMA-based gels and in concentrated solutions of linear PHEMA was compared. The insolubility of linear PHEMA in water precludes a comparison of the diffusion in crosslinked HEMA gels and in aqueous PHEMA solutions and therefore methanol was selected as solvent. Hydrodynamic correlation length  $\zeta_H$  in both the HEMA gels cross-linked to various degrees and equilibrium-swollen with methanol and in concentrated methanolic solutions of linear PHEMA was measured by DLS. It was found<sup>[57]</sup> that diffusion coefficients for four tracers (both low-molecular-weight and oligomers) decrease with increasing molecular weight of the tracer in all the matrices studied. The dependence of the diffusion coefficients on the concentration of linear PHEMA in methanolic solutions were well fitted within the frame of both Phillies'<sup>(44)</sup> and Petit's<sup>(43)</sup> models on approximately the same level of accuracy. The best-fit values of characteristic parameters for each of the models were compared with theoretical predictions and experimentally determined values. Lower values of diffusion coefficients of all four tracers were found in HEMA gels compared with a polymer solution containing the same concentration of the polymer. The data found indicate that slowing down the diffusion of the tracers in gels depends not only on the presence of additional permanent crosslinks demonstrated by shortening hydrodynamic screening length of the matrix, but also on the character of barriers to the diffusion (temporary entanglements vs. permanent crosslinks) as well. The results of the described research were also summarized in two review papers.<sup>[56,67]</sup> All the ESRI measurements described were performed in Detroit using a laboratory-assembled ESRI spectrometer during the stays. The experiments were significantly complicated by inevitable transportation of the samples prepared in Prague to Detroit as far as HEMA and PVP gels are concerned. At present the research in this field continues in Prague using a commercial Bruker

ELEXSYS E-540 ESRI spectrometer.

## **Conclusions**

Application of special techniques of electron spin resonance spectroscopy to studies of local dynamics at molecular/segmental level and diffusion processes in polymer systems has been continuously developed on the top levels of the understanding and description of theoretical basis of such processes using available experimental techniques. Using the ESR spin label technique at various stages of development, local dynamics in some polymer systems has been described. Both data complementary to some other experimental techniques and data characterizing some newly observed effects have been obtained. Interpretation of experimental data based on the currently available theoretical models has been found. Experimental technique for measurements of diffusion coefficients of paramagnetic tracers in polymer solutions and gels based on ESRI was introduced and applied also to studies of systems important for medical applications. At present, laboratory in this Institute belongs to a very limited number of workplaces in the world where experiments in the described fields can be successfully performed, evaluated and interpreted in accord with the present state of knowledge.

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## Summary

Special techniques of electron spin resonance (ESR) spectroscopy of spin labels, based on the theory developed by Professor J. H. Freed and his coworkers at Cornell University, have been applied to studies of local dynamics in polymer systems at molecular/segmental level. Local segmental dynamics in various polymers (poly(methyl methacrylate), polystyrene, poly(methacrylic acid), poly(acrylic acid) and poly(2-hydroxyethyl methacrylate)) with spin-labeled chain unit incorporated in polymer chain at randomly distributed sites has been studied. Both data complementary to some other experimental techniques and data characterizing some newly observed effects have been obtained and interpreted using available theoretical models.

An experimental technique for measurement of coefficients characterizing macroscopic translational diffusion of paramagnetic tracers, based on electron spin resonance imaging (ESRI), was introduced and applied to studies of diffusion processes in polymer solutions and gels. The effect of permanent crosslinks in gels on the diffusion coefficients was studied in particular. Diffusion of paramagnetic tracers in gels based on 2-hydroxyethyl methacrylate was compared with diffusion in concentrated solutions of linear poly(2-hydroxyethyl methacrylate). It was found that diffusion coefficients of oligomer tracers decrease with increasing molecular weight. The dependence of the diffusion coefficients on the concentration of linear polymer in solution was well fitted within the frame of theoretical models. Lower values of diffusion coefficients of oligomer tracers were found in gels compared with solutions containing the same concentration of the polymer. The data indicate that slowing down the diffusion of the tracers in gels depends not only on the presence of additional permanent crosslinks but also on the character of barriers to diffusion (temporary entanglements, permanent crosslinks).