Dynamic Nuclear Polarization Studies of TANOL/water-glycerol Solutions

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Dynamic Nuclear Polarization (DNP) studies of TANOL/water-glycerol solutions were performed at 1.53 mT. The samples have either various water-glycerol ratios at constant concentration or various concentrations at a constant water-glycerol ratio. The low field EPR spectra were recorded, the DNP parameters(enhancement factors, coupling constant, leakage factor) were determined, electronic relaxation times were measured. Depending on the values obtained, the interactions between nuclei and electrons are discussed.

Key words: DNP; EPR; Relaxation; Nitroxides.

Introduction

In recent years low field Dynamic Nuclear Polarization (DNP) studies have attained importance in the investigation of biological samples [1 - 4]. Nitroxides are widely used stable free radicals in EPR applications because of their spectral sensitivity to the environment [5, 6]. In recent studies DNP measurements of biological fluids were performed at 6.85 mT using various nitroxides [7]. The earlier studies on DNP were reviewed by Potenza [8] and Bates [9]. As to DNP studies of liquids at low fields, there are many studies on the molecular dynamics of nitroxide systems. Of especial interest for us are nitroxide radical-water and nitroxide radical-viscous liquid systems. An extensive study on aqueous solutions of piperidine nitroxides at 0 - 100 °C was performed by Meise et. al [10]. NMR relaxation of nitroxide radicals in water and various alcohols was also studied [11 - 13]. Although there are many studies on NMR relaxation and its frequency-, temperature- and concentration-dependence, studies on electronic relaxation of nitroxide radicals are limited [14 - 16], and viscosity studies are generally done for various temperatures only. In the presented study, DNP and electronic relaxation time measurements were carried out for a series of samples having different glycerol-water ratios .The aim of this study was to obtain the changes in the observed enhancement and electronic relaxation times depending on the viscosity and concentration at 1.53 mT for the further studies on biological fluids.

Theory

It is well known that the DNP experiments are based on the enhanced number of transitions between the nuclear spin states as a result of relaxation processes between nuclei (**I**) and electrons (**S**). The observed nuclear enhancement in DNP experiments is given by

$$G(P) = \frac{P_z}{P_o} - 1 = -\rho f s \frac{\gamma_S}{\gamma_I}. \qquad (1)$$

Here P_z and P_o are the nuclear polarizations in the presence and absence of EPR power, respectively. ρ is the nucleus electron coupling parameter. Its value is 0.5 for dipolar interaction and –1 for scalar interaction [17, 18].

The leakage factor f determines the efficiency of the electrons in the relaxation processes of nuclear spins. It may be written as

$$f = 1 - \frac{T_1}{T_{10}} = \frac{kCT_{10}}{1 + kCT_{10}}, \qquad (2)$$

where T_1 is the free radical induced solvent nuclear relaxation and T_{10} is the pure solvent nuclear relaxation [19]. On the right hand side of (2), k is the

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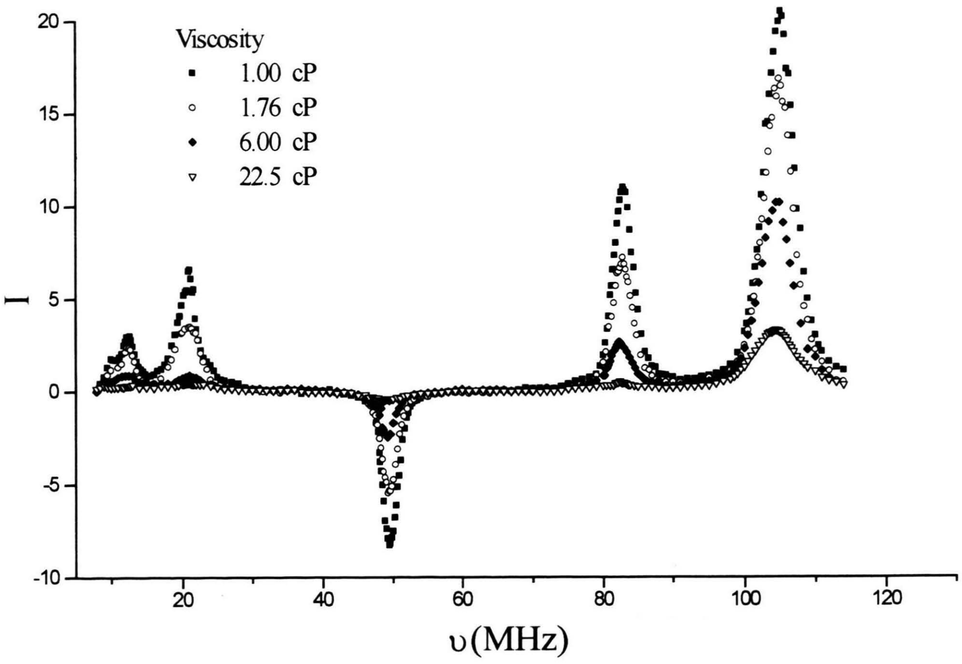

Fig. 1. The EPR spectra of the samples having various glycerol ratios.

relaxivity of the free radical and C its concentration. For high concentrations of free radicals f is close to 1.

The saturation factor s determines the amount of saturation for each resonance line and it is given by

$$\begin{aligned} s &= \sum_i s_i = \sum \frac{\sigma_i g(\omega-\omega_i)}{1+\sigma_i g(\omega-\omega_i)} h(\omega_i), \\ \sigma_i &= \pi\gamma_S^2 B_{1e}^2 (T_{1e}^i)^2. \end{aligned} \tag{3}$$

Here $g(\omega-\omega_i)$ is the shape function and $h(\omega_i)$ the weight factor for the i^{th} resonance line [20, 21]. B_{1e} is the applied EPR field and T_{1e} is the electronic spin lattice relaxation time.

The saturation factor for each resonance transition can be rewritten as

$$s_i = \frac{\alpha P}{1+\alpha P} h(\omega_i), \tag{4}$$

where P is the EPR power and α a constant which includes the circuit parameters. Using (2) and (4) in (1), the following relation for the observed enhancement is obtained:

$$G(P)^{-1} = \frac{\gamma_S}{\gamma_I}\left(1+\frac{1}{kCT_{10}}\right)\left(1+\frac{1}{\alpha P}\right)\frac{1}{\rho h(\omega_i)}. \tag{5}$$

An inspection of (5) indicates the proportionality of the observed enhancement to the applied power and free radical concentration [22]. The graph of $G(P)^{-1}$ against P^{-1} is a straight line, and from extrapolation to infinite power the extrapolated enhancement factor can be obtained. The extrapolated enhancement factor for infinite power is proportional to the free radical concentration. Therefore the graph of $F(\omega_i)^{-1}$ against C^{-1} gives a straight line also. From the extrapolation of the line to infinite concentration the nucleus-electron coupling constant ρ can be calculated.

The electronic relaxation times are given by

$$T_{1e}^i = \frac{|(P_z - P_o)/P_o|_{\text{lowpower}}}{F(\omega_i)} \frac{1}{\pi\gamma_S^2 B_{1e}^2 g(\omega-\omega_i) h(\omega_i)} \tag{6}$$

and calculated from the saturation measurements at each resonance line.

Experimental

The samples were prepared using various water-glycerol ratios. The concentration of TANOL (4-hydroxy-TEMPO) was $2\cdot 10^{-3}$ mM and the glycerol ratio covered a range from 20% to 70%. Except for

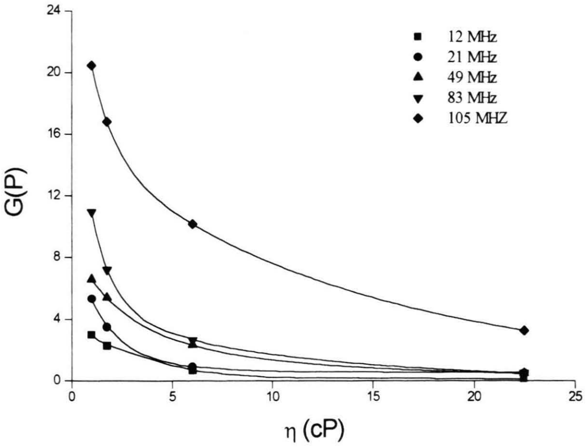

Fig. 2. The change of the observed enhancements depending on viscosity values.

the investigation of the concentration dependence of the polarization, the 20% glycerol-80% water samples were prepared for ten different concentrations. The concentration ranged from 0.2 to 10 mM. Both, undegassed and degassed samples were studied. In the studies, 10 ml samples were used in 18 mm o.d. sample tubes.

The EPR spectra and DNP measurements were recorded at 1.53 mT using a home made double resonance spectrometer. The proton resonance frequency was fixed to 65 kHz and the EPR frequency scans ranged from 5 to 120 MHz. In obtaining the EPR spectra, the amplitude of the rf field was kept constant and the frequency varied in desired steps [23, 20].

Results and Discussion

The low field spectra of samples having various glycerol ratios but the same concentration are given in Figure 1 . Inspection of these spectra shows that an increase in the viscosity results in a restriction of the translational and rotational motion of the molecules and an increase in the related correlation times τ_r and τ_t. This in turn causes a decrease in the nucleus-electron coupling parameter ρ and also in the observed polarization [15, 24 - 26]. The decay curves of the observed polarization against viscosity exhibit an exponential behaviour (Figure 2):

$$G(P) = G_0(P)\exp(-A\eta). \qquad (7)$$

The $G_0(P)$ and A values are listed in Table 1 for each resonance transition. Interestingly, the decay of the line at 105 MHz is slower than that of the other lines. From the concentration dependence studies the weighted coupling constant for 105 MHz ($\rho_i h(\omega_i)$) was found to be much higher than the other resonance transitions. Although this study is performed at a single viscosity value it supports the slow decay obtained for this transition.

Table 1. The observed polarizations $G_0(P)$ ($\eta = 0$) and decay constants A for the samples having various viscosities.

ν (MHz)	12	21	49	83	105
$G_0(P)$	3.94	7.66	7.90	14.14	21.60
A	0.299	0.403	0.205	0.317	0.113

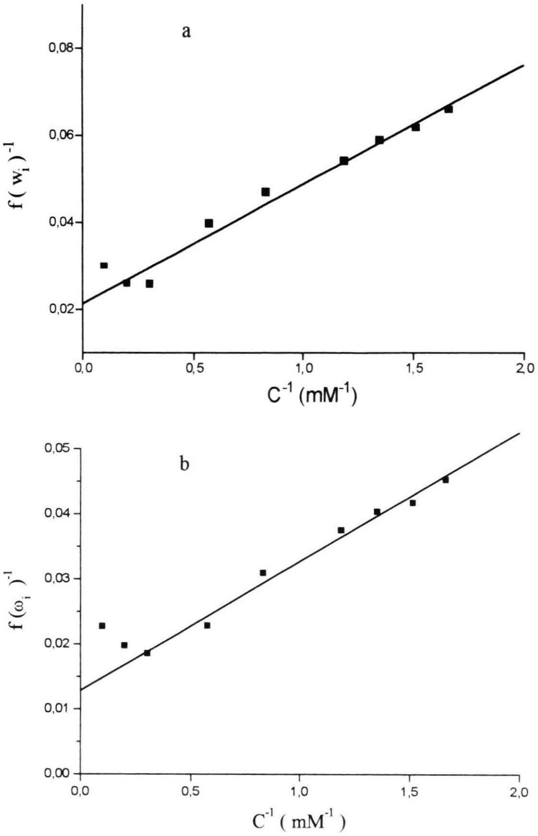

Fig. 3. For TANOL / 20% glycerol + 80% water, the change of the extrapolated enhancements depending on concentration C. a) 21 MHz b) 105 MHz.

In the concentration dependent studies, the observed enhancements were measured both for undegassed and degassed samples. The observed enhancements for degassed samples were approximately 30% higher than those for undegassed ones.

As mentioned in [10, 27, 28], the low field spectra of nitroxides are complex because of exchange

Table 2. Weighted coupling constans for TANOL / (20% glycerol + 80% water).

ν (MHz)	10	12	21	49	83	105
$\rho h(\omega_i)$	0.035	0.063	0.052	0.063	0.045	0.173

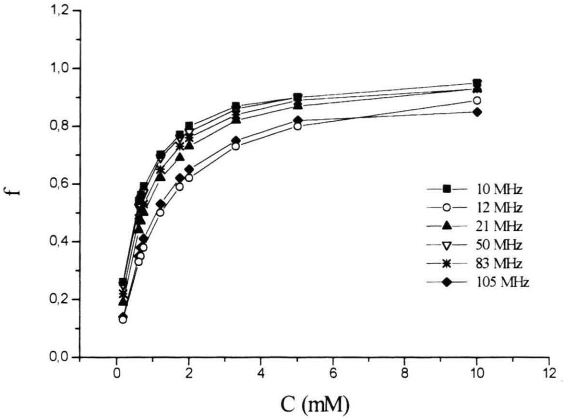

Fig. 4. The calculated leakage factors for TANOL / 20% glycerol + 80% water.

phenomena. For lower fields, where the number of permitted resonance transitions increase as a result of the Breit-Rabi effect [29], the situation is more complex. Therefore, unlike the three line spectra, the transition probabilities are also different and each resonance transition could only be saturated depending on its weighting factor [21]. However, for low field studies, rather than the absolute values of the parameters, relative ones are important.

For all samples, saturation experiments were performed and extrapolated enhancements were obtained. The graphs of extrapolated enhancements against concentration exhibit a linear behaviour for low concentrations (Figure 3). With increasing radical concentration, as a result of exchange and dipolar interactions a deflection from the linear behaviour is observed. These results were obtained for all hyperfine lines, and they are consistent with recent studies at higher magnetic fields [26, 30]. From the slopes of these graphs, the f leakage factors were determined for various concentrations (Figure 4). From an extrapolation of the $F(\omega_i)^{-1}$ - C^{-1} lines to infinite concentration weighted coupling constants were determined (Table 2). There is no ordering of the weighted coupling constants depending on the frequency, as mentioned in a recent study. Since $\Sigma h(\omega_i) = 1$,

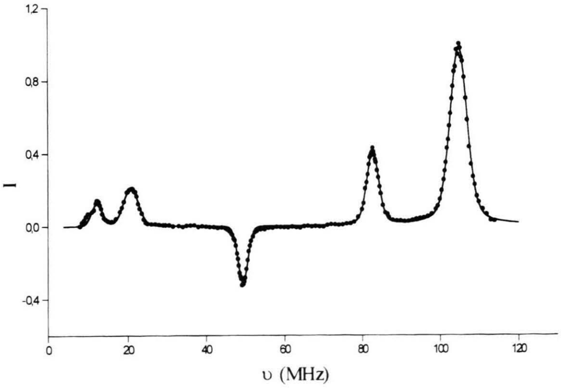

Fig. 5. Calculated (—) and experimental (···) spectrum for TANOL / 20% glycerol + 80% water.

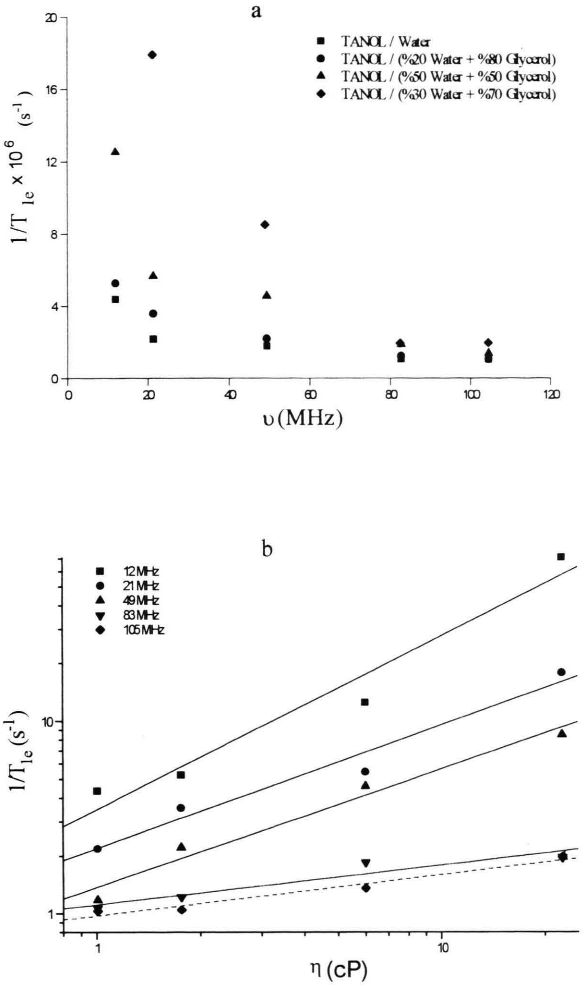

Fig. 6. a) The relaxation rates of the samples having various viscosities. b) The change of relaxation rates against viscosity.

$\Sigma \rho h(\omega_i) = \rho = 0.43$ was obtained. As expected, this value is close to 0.5.

The electronic relaxation times of the samples were measured using cw saturation technique. In the calculation of relaxation times, the spectra were fitted using the Gaussian+Lorentzian line width approximation, and a good consistency between the experimental and calculated spectra was obtained (Figure 5). The changes of relaxation rates against frequency and viscosity are given in Figs. 6a) and b), respectively. In Fig. 6a) the relaxation rates decrease as the peak frequency increases, and these results are consistent with our previous studies [31].

The relaxation rates depend linearly on the viscosity (Figure 6b)). This is consistent with earlier studies, in which this result was reached in organic solutions of free radicals using temperature dependent viscosity changes [14, 15]. This type of variation is expected from the translational motion of the molecules. The translational correlation time is proportional to the viscosity and inversely proportional to temperature. At a constant temperature, the slope of the lines in the logarithmic graph of Fig. 6b) should be equal to unity but, as it can be followed from the figure, the value of the slope decreases with increasing frequency. In other words, the increase in the relaxation rates with increasing viscosity dominates for the low frequency peaks.

Acknowledgements

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