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Overhauser Dynamic Nuclear Polarization Parameters of a Nitroxide Radical in Liquid

Handan ENGİN KIRIMLI[1*]

ABSTRACT: Solution state Overhauser-effect-type dynamic nuclear polarization (ODNP) has been studied in the last years. The basis of ODNP is a polarization mechanism that presents nuclei with electron spin polarization. ODNP enhancements studies are presented 4-fluorobenzyl alcohol (4FBA) with the stable nitroxide radical as the polarizing agent for 15, 30 and 60 mM concentrations at 1.53 mT. Major ODNP parameters were obtained. The significant parameter is the coupling parameter, since it determines the maximum NMR signal enhancements and the interactions between the nuclear spin and the electron spin. The coupling parameter can vary from -0.152 to 0.001. The obtained ODNP parameters show that both scalar and dipolar interactions occur. The results show that nitroxide radical can be taken as polarizing agent for ODNP studies of 4-fluorobenzyl alcohol solvent. The effect of dissolved molecular oxygen on the enhancement factor for the sample with the smallest concentration was experimentally investigated.

Keywords: Dynamic nuclear polarization, Overhauser effect, free nitroxide radical, 4-fluorobenzyl alcohol (4FBA), the polarizing agent

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INTRODUCTION

Dynamic nuclear polarization (DNP) has recently received great attention for its ability to increase signal densities by several times of magnitude in nuclear magnetic resonance (NMR) experiments. DNP, a technique well known in paramagnetic solutions and suspensions, depends on the interaction between polarisation agent and objective nuclei adjusted by molecular move (Yalciner, 1981; Griffin et al., 2019; Levien, 2020). This technique is becoming more and more popular as it magnifies nuclear spin polarization. The magnetic interactions between the nucleus and the free electron by the DNP technique are very strong. As a result of the diffusion and rolling of solvent with radical molecules, the nucleus and the free electron are in action relative to each other. These movements create dipolar interactions, while molecular collisions create scalar interactions. Molecular collisions are caused by direct contact between the nucleus and the unpaired electron or by the temporal overlap of localized molecular orbitals during the diffusion operation (Kirimli and Peksoz, 2011).

Overhauser DNP is DNP technique in liquids at room temperature (Overhauser, 1953; Hausser and Stehlik, 1968). It is a pair of magnetic resonance technique in which nuclear magnetic resonance is observed at the moment when the electron resonance of a paramagnetic substance is occured. In solution-state, nuclear polarization increases by the methods of Overhauser DNP based on the effect of transfer the polarization from electronic spins to a system of nuclear spins. In our study, the nuclear spins are proton spins, which are the nuclei of the hydrogen atom in the solvent molecule. The electron spins belong to the unpaired electron delocated on the radical (Kirimli, 2017). While the first studies of the Overhauser DNP technique used paramagnetic metals, modern works included fixed free radicals such as nitroxide (Ardenkjaer-Larsen et al., 2019). Nitroxide radicals, which are paramagnetic, are a good polarization target. These free radicals predominantly have the unpaired electron in the N-O bond. However these electrons are of low energy due to the four methyl groups shielding the free electrons (Bunyatova, 2004).

Today, the ODNP technique is a very active research area and the theory and practice of the ODNP method are constantly evolving. Therefore ODNP technique may enable new applications to physics, chemistry, biology and medicine (Doll et al., 2012; Biller et al., 2018; Franck and Han, 2019; Jaudzems et al., 2019; Keller and Maly, 2021). Especially it can be used in clinical imaging (Nelson et al., 2008), hyperpolarization (Johansson et al., 2004; Ardenkjaer-Larsen et al., 2019) and particle physics (Goertz et al., 2002; Bunyatova, 2004).

The goal of this study is to examine the ODNP parameters by ODNP technique for nitroxide/4-fluorobenzyl alcohol (4FBA) samples at 1.53 mT. In this technique, intermolecular spin spin interactions occur between the nuclear spins in the diffused solvent molecules and the unpaired electron spins in free radical. Nitroxide radical as 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO) used. The DNP experiments indicated that nitroxide as the polarizing agent with 4FBA exhibit both scalar and dipolar interactions were seen. According to these results the dominant is scalar and the rest is dipolar.

Experimental application of ODNP and its theory can be provided in literature (Potenza, 1972; Müller-Warmuth and Meise-Gresch, 1983; Guiberteau and Grucker, 1998; Franck et al., 2013; Bentum et al., 2016). Including the observed nuclear polarizations, P_z, the thermal equilibrium values, P_0; the Overhauser DNP enhancement factor is given by

$$E = \frac{P_Z}{P_0} = 1 - \rho f s \left|\frac{\gamma_e}{\gamma_n}\right| \tag{1}$$

where, γ_e and γ_n, are the gyromagnetic ratios of the electron and nuclear spins (for ^{1}H; $|\gamma_e/\gamma_n| = 658$), respectively. The leakage factor *f*, the saturation factor *s* when the Electron Spin Resonance (ESR) is at full saturation, it becomes equal to 1. The nuclear-electron coupling parameter ρ, in the white spectrum approach, is +0.5 for pure dipolar interaction and -1 for pure scalar coupling. The ρ values obtained vary between the limit values of +0.5 and -1.

When being complete the Electron Paramagnetic Resonance (EPR) saturation (s=1), the inverse of E factor is extrapolated for the infinite ESR power is derived as

$$\left(\frac{P_z - P_0}{P_0}\right)^{-1}_{s\to 1} = -\left(\rho f \left|\frac{\gamma_e}{\gamma_n}\right|\right)^{-1} = E_\infty^{-1} \ . \tag{2}$$

ρ determines the nuclear-electron interaction and given as

$$\rho = -\frac{E_\infty}{658 \cdot f} \ . \tag{3}$$

The importance on dependent scalar coupling is K parameter. Since ρ is an experimentally obtainable, the parameter K can be easily calculated.

$$K = \frac{1 - 2\rho}{1 + \rho} \tag{4}$$

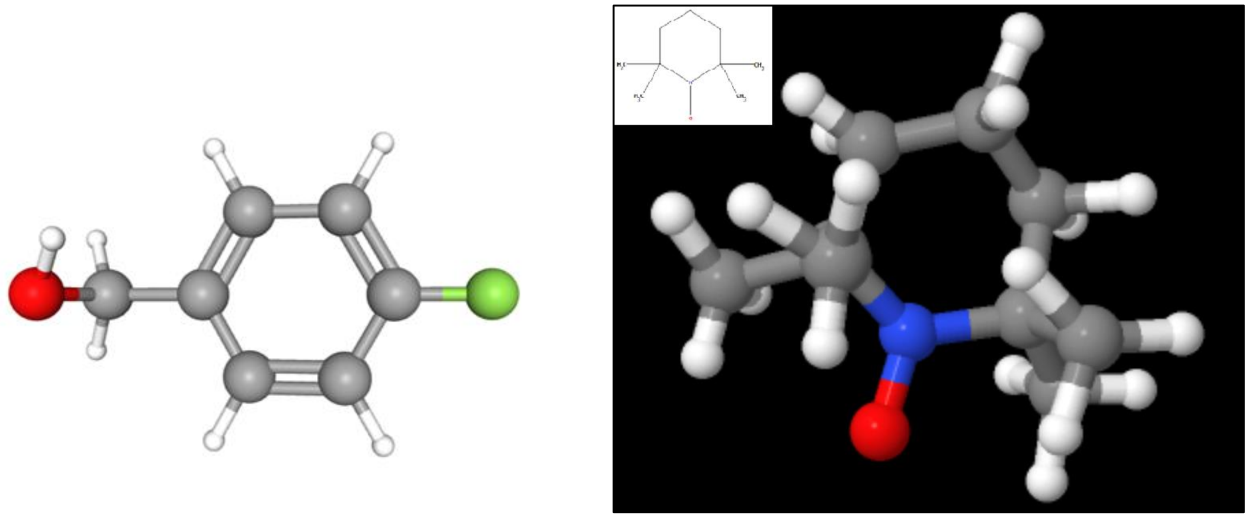
MATERIALS AND METHODS

Materials

Stable nitroxide radical such as 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO) were used. The stable free radical TEMPO and solvent were purchased from Aldrich Chemical Co. (USA). The solvent was 4-fluorobenzyl alcohol (4FBA). The main properties of 4FBA are given in Table 1 and of structural view in Figure 1 (a). The molecular structure of the selected free radical is shown in Figure 1 (b).

Table 1. NMR sensitivity of 4-fluorobenzyl alcohol and basic constants.

Solvent	Molecular Weight (g mol^{-1})	Melting Point (°C)	Boiling Point (°C)	Density (g cm^{-3})	NMR Sensitivity (x10^{22} spin/cm^3)
4-fluorobenzyl alcohol (4FBA) (C_7H_7FO)	126.13	23	210	1.15	5.21



(a) (b)

Figure 1. (a) Structural View of 4-fluorobenzyl alcohol (4FBA) and **(b)** Nitroxide radical TEMPO (red color "oxygen; green color "fluorine"; white color "hydrogen";blue color "nitrogen" and grey color "carbon")

Nitroxides radicals are soluble in water, benzene and heptane. For the majority of the ODNP studies that address the polarization of the solvent by free radicals nitroxides radicals are used (Sezer, 2013). Therefore, to investigate experimentally the effect of ODNP in liquids, we chose 4FBA as the solvent and nitroxide radical TEMPO as the DNP agent dissolved in the solvent. This radical is suitable for extensive DNP enhancements (Kryukovet al., 2010).

Experimental procedure

The solutions were prepared in three different concentrations as 15, 30 and 60 mM. For each concentrations, TEMPO radical was first weighted and dissolved in 4FBA. The sample volumes were 6.5 cm^3. Then the prepared samples were placed in Pyrex tubes of 18 mm diameter.

The presence of oxygen affects the enhancement factor. Degassing treatment should be performed in order to minimize the interactions with air molecules, to saturate the EPR line easily and to ensure the long life of the sample. Degassing process was performed in Leybold-Heraeus vacuum system at 7.5×10^{-4} Pa pressure value. Since the melting temperature is 23 ^{o}C, the sample melted quite late in degaze process.

DNP measurements were done at 1.53 mT fixed magnetic field NMR spectrometer. The spectrometer is hand-made and has a resonance frequency of 61.2 kHz for protons and 43.8 MHz for electrons. The spectrometer was explained in the previous articles (Akay and Yalciner, 1995; Kirimli, 2017).

RESULTS AND DISCUSSION

DNP parameters

For each sample, E_∞ values were determined by extrapolating from the optimal fit lines drawn by obtaining the inverse of the enhancement factors as a function of the inverse of the square of the high frequency voltage, which is proportional to the inverse of the ESR power. The E_∞^{-1} values correspond to the intersection points of the extrapolated optimal fit lines passing through the experimentally obtained points with the line $V_{eff}^{-2}=0$ (Figure 2).

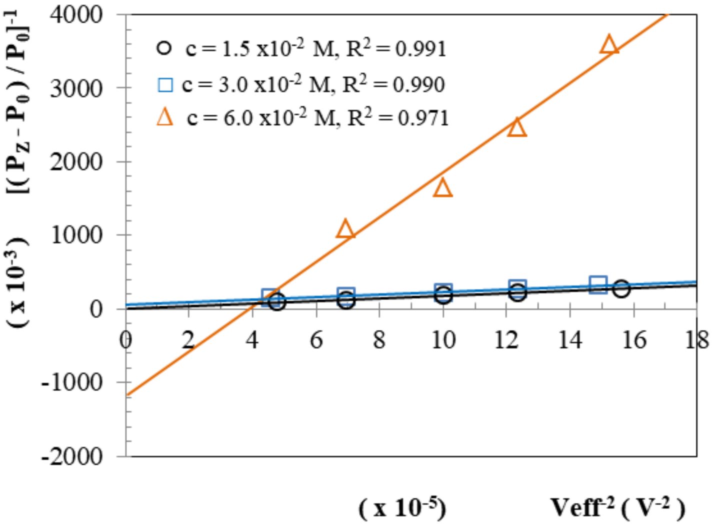


Figure 2. Obtaining E_∞ for TEMPO/4FBA sample. The R^2 values indicate the regression of the experiment points

The leakage factor f was taken as 0.90 ± 0.05 for all the samples based on the literature values (Müller-Warmuth et al., 1970; McCarney and Han, 2008; Kirimli and Peksoz, 2011). After ρ value calculated from equation (3), K value can be found by equation (4). The DNP parameters obtained for the TEMPO/4FBA sample, E_∞, E_{end}, ρ, s (E_{end}/E_∞), K are presented in Table 2. Dipolar coupling between electron spin and nuclear spin causes negative sign enhancements while scalar coupling causes positive sign enhancements. E_∞ can therefore be positive or negative, depending on which process is dominant. As seen in Table 2, the E_∞ values are negative and positive, these values vary between 90.09 and -0.84.

Table 2. Summary of ODNP parameters (E_∞, E_{end}, ρ, s, K) (E_{end}, the enhancement factor for maximum available the EPR power)

	c (mM)	E_∞	E_{end}	ρ	***s***	***K***
TEMPO/	15	90.09	9.82	-0.152	0.109	1.538
4-fluorobenzyl alcohol	30	15.01	6.68	-0.025	0.445	1.077
(4FBA)	60	-0.84	0.91	0.001	1.083	0.997

Strong dissolution takes place in solutions with nitroxide radicals in solvents containing hydroxyl. This results in DNP looking quite different (Müller-Warmuth et al., 1970). Kryukov et al. (2010) observed that the proton-spin coupling dipolar interaction dominates in solutions with nitroxide radicals in toluen. A similar study, Enkin et al. (2015) reported that measured DNP enhancements for nitroxide derivatives at 0.35 T in toluene are the negative sign. This indicates that the polarization transfer mechanism is dipolar dominant.

Samples with 15 and 30 mM concentrations from the studied samples show scalar interaction, while sample with 60 mM concentration shows dipolar interaction. This result shows that the dipolar interaction occurs with the increase of free radical concentration, i.e. the increase of S spin population. K is the relative importance of the scalar and dipolar interaction and the values of 1.538, 1.077 and 0.997 also support these results on interactions.

It was reported that DNP studies on a number of aliphatic and aromatic nitroxide radicals with hexafluorobenzene show scalar interactions. The collision of the radical oxygen to the center of the hexafluorobenzene ring in aliphatic nitroxides and the plane collision in aromatic nitroxides contribute to the scalar interaction (Glazer and Poindexter, 1971).

Müller-Warmut et al. (1970) reported that the spin density can be temporarily transferred from the radical to the molecular orbital of the solvent by hydrogen bridge. They found certain effects occuring in solutions of nitroxide radicals in solvents containing hydroxyl by the formation of hydrogen bridges between = NO radical and - OH of the solvent. Thus for investigated solutions dipolar and little scalar contact interactions between solvent protons and radical electrons have been observed. It can be seen that the DNP data obtained by working with the same solutions here support the results given by Müller-Warmut et al. (1970).

Dissolved oxygen effect on DNP signal enhancement

The solution of TEMPO radical in 4FBA solvent with 15 mM concentration was degassed at 7.5 x 10^{-4} Pa. In this work, the effect of degassed oxygen molecules on DNP signal enhancement was investigated. Firstly, DNP enhancements of the degassed sample were carry out. Then, the top of the Pyrex tube including the sample was cut to reach atmospheric pressure, and closed by a cork stopper. Experiments were completed with this prepared sample to determine the effect of dissolved oxygen on DNP enhancement signals over time. All these experiments were done at V_{eff} = 100 V EPR power. It was found that the DNP signal enhancements decrease exponentially with time as seen in Figure 3.

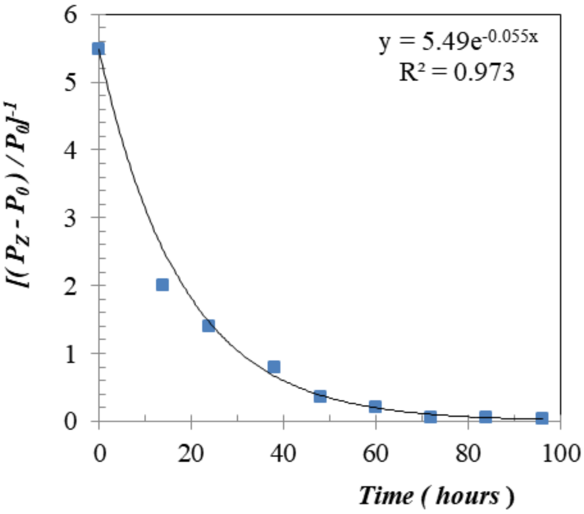


Figure 3. Time dependent change of DNP signal enhancement

EPR oximetry is a technique with the necessary features for clinical use. This technique is based on the ability of molecular oxygen to interact with paramagnetic materials such as nitroxide and influencing the EPR spectra to be proportional to the amount of oxygen (Khan et al., 2005; Ardenkjær-Larsen et al., 1998). In the presence of oxygen the line-width of the EPR lines is broadened to an extent depending on the local oxygen concentration as molecular oxygen is paramagnetic (Krzic et al., 2001). It is known that in samples with dissolved oxygen molecules, the EPR line width increases and signal enhancement decreases (Clarkson et al., 1998). Especially, DNP studises in the samples existing dissolved oxygen molecules can be useful in EPR oximetry working. In our study, the effect of dissolved molecular oxygen on the signal enhancement has been clearly demonstrated with the DNP method, which is known as a very susceptible and credible method.

CONCLUSION

The ρ value ranges from -1 to +0.5, depending on the type of interaction. That is, it varies from -1 in the pure scalar state to +0.5 in the pure dipolar state. The results of our studies and the calculated DNP parameters between the nuclear spin and the electron spin, indicate that both scalar and dipolar interactions occur for TEMPO/4FBA samples. Especially the coupling parameter ρ gives information about interactions and depends on the dynamics of the electron–nuclear spin system and supports the investigation of the effects of molecular dynamics on the DNP enhancement. This conclusion is also supported by the parameter K. Selected nitroxide radical TEMPO has the spin density settled on the NO group (Levien et al., 2020). The effect of the spin density on radical concentration and thus on DNP enhancement was observed. It is noticed that TEMPO radical is a good polarizing target for DNP experiments.

The effect of dissolved molecular oxygen on signal enhancements was determined. In general, it was observed that paramagnetic oxygen molecules greatly affect NMR signal enhancements. The experimental data obtained are in accordance with the results in the literature. Especially, the results given in this study may be useful for biological studies with nitroxide radicals in an oxygen present environment.

Finally, the results of our study may add to investigated of ODNP on 4-fluorobenzyl alcohol solvent for a nitroxide radical TEMPO and may thus provide useful information for applications of ODNP.

Conflict of Interest

The article author declares that there is no conflict of interest.

Author's Contributions

I hereby declare that the planning, execution and writing of the article was done by me as the sole author of the article.

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