

Studies of Dynamic Nuclear Polarization with Nitroxides in Aqueous Solution

P. Höfer¹, P. Carl¹, G. Guthausen¹, T. Prisner², M. Reese³, T. Carlomagno³,
C. Griesinger³, and M. Bennati³

¹EPR Division, Bruker Biospin, Rheinstetten, Germany

²Institute for Physical and Theoretical Chemistry, Center for Biomolecular Magnetic Resonance,
University of Frankfurt, Frankfurt, Germany

³Max Planck Institute for Biophysical Chemistry, Göttingen, Germany

Received 30 October 2007; revised 20 February 2008

© Springer-Verlag 2008

Abstract. The dynamic nuclear polarization effect between the nitroxide radical TEMPO and the ¹H protons of water solution was investigated at an electron pumping frequency of 9.7 GHz for different experimental conditions. In particular, we compared ¹⁴N-4-hydroxy-TEMPO (TEMPOL) with 4-oxo-TEMPO and evaluated the effect of ²H and ¹⁵N isotope labelling. Furthermore, we tested the effect of concomitant irradiation on both electron paramagnetic resonance (EPR) lines with the ¹⁵N-labelled compound. Our results show that the reduction in the EPR line width given by the ²H and ¹⁵N labelling and the collapse in the number of hyperfine lines due to ¹⁵N substitution leads to the highest enhancement of $\varepsilon = -140$ ever reported in the literature. Concomitant pumping on two hyperfine lines does not give higher enhancements at our experimental conditions.

1 Introduction

Dynamic nuclear polarization (DNP) of nuclei in liquid solutions containing paramagnetic centers has been studied since the early years of magnetic resonance to achieve information about molecular motion and electron–nuclear spin relaxation [1, 2]. In the past few years, this technique has attracted a lot of attention again since it could provide a means to overcome the sensitivity limits in solution and solid-state nuclear magnetic resonance (NMR) towards studying macromolecular complexes. Large enhancements of NMR signals at high fields (up to 10 T) have been reported in the solid state using TEMPO, trityl or biradical systems [3–6]. These enhancements are due to thermal mixing or cross effects within weakly coupled electron and nuclear spins. In contrast, DNP in liquids is much more challenging due to technical problems related with microwave penetration, losses and heating of the sample, as the Overhauser effect dominating the DNP mecha-

nism in liquids depends linearly on the saturation factor of the electron paramagnetic resonance (EPR) line [7]. Furthermore, the Overhauser effect loses efficiency with increasing magnetic field. Several studies have been reported about DNP on solvent molecules at low magnetic fields ($B \leq 1$ T) using organic radicals as polarizers [2]. Considerable enhancements of different nuclei, such as ^{31}P , ^{15}N and ^{13}C (but not ^1H), were reported at 5 T in nonaqueous solutions [8]. We have recently reported ^1H DNP enhancements up to -100 at 0.34 T and -20 at 3.4 T in nitroxide aqueous solution [9]. The results compared with the trityl-water system under similar experimental conditions indicated that nitroxide radicals are the most attractive candidates for DNP experiments in combination with solution NMR of biomolecules at various magnetic fields. In this paper we report a more systematic study on the experimental conditions for DNP with nitroxides as polarizers and pumping at 9.7 GHz microwave frequency. Although similar experiments had been reported in the literature by several groups [10–13], the enhancements observed here using state-of-the-art EPR technology are the highest ever reported and give new insights into the experimental conditions determining the DNP mechanism under study. The present data are consistent with our previous predictions that all enhancements reported so far with nitroxides are not limited by the intrinsic Overhauser coupling factor but rather by the difficulties associated with saturating the EPR line in aqueous solution.

2 Experimental

The experiments were performed on a commercial Bruker EPR spectrometer (e-scan) operating at 9.7 GHz and a dielectric electron–nuclear double resonance (ENDOR) resonator (Bruker EN 4118X-MD4) that achieves a B_1 field of 3 MHz with a 5 W microwave amplifier (Bruker AmpX). To allow for NMR detection, the ENDOR probehead was connected to a Bruker Minispec ($\nu(^1\text{H}) \approx 2\text{--}65$ MHz) and the ENDOR circuit was externally matched to the NMR receiver. The spectrometer fundamental frequency was on resonance with the low-field hyperfine line for the ^{15}N -labelled sample or with the central hyperfine line for the ^{14}N sample and tuned to the resonator dip. Dual irradiation was accomplished using a second tuneable microwave source. The second variable frequency was set 62 MHz apart (for the ^{15}N sample) on the other EPR transition. In the dual-frequency setup, two separate microwave amplifiers were used for the two frequencies resulting in power levels at the resonator input of 1.5 and 0.5 W at the fundamental frequency (MW1) and the second frequency (MW2), respectively. Due to technical reasons the power levels at the resonator input were different for the two frequencies. The polarizer samples 4-hydroxy-TEMPO (TEMPOL), 4-oxo-TEMPO (TEMPONE), and the $^2\text{H}/^{15}\text{N}$ uniformly labelled TEMPONE were purchased from Sigma-Aldrich and were dissolved in water at concentrations of 2.5, 5, 10, and 25 mM. The solutions were degassed and loaded into quartz capillary tubes with an inner diameter of 0.9 mm. The samples were continuously irradiated with microwaves at room temperature for 2 s and subsequently the NMR free induction decay (FID) was recorded.

3 Results

In Fig. 1, we report ^1H -DNP enhancements of the water protons at 9.7 GHz under various sample conditions. First, we compare the effect of the substituent (hydroxyl- versus oxo-groups) at the nitroxide ring in the unlabelled samples.

We observe no significant difference between the enhancements of the two compounds and we reach a maximum enhancement of about -100 for the 5 mM concentrated samples at the maximum available power. The enhancements decrease with increasing concentration to about -60 in both compounds at 25 mM and maximum power. This latter effect is correlated with the increasing line width at higher concentration as indicated in Fig. 1, due to Heisenberg spin exchange effects. A shortening of the relaxation time T_2 causes a decrease of the saturation factor and, therefore, of the resulting enhancement as will be discussed below. Isotope labelling with ^2H and ^{15}N leads to the line narrowing and reduction in the number of the hyperfine lines. With this sample we report the highest enhancement of -140 ever observed with nitroxides at a concentration of 25 mM. Figure 1 shows that at the concentration of 5 mM an enhancement close to saturation can be achieved for all three samples with the available microwave power.

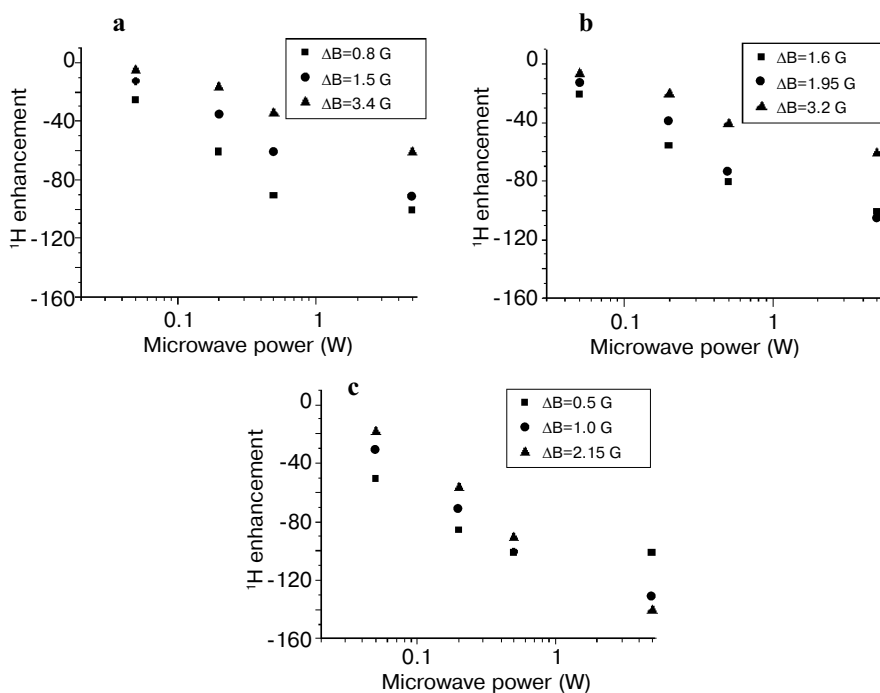


Fig. 1. Concentration and power dependence of ^1H -DNP enhancements of the water protons in solutions with different nitroxide polarizers. The EPR pumping frequency was 9.7 GHz and resonant with the low-field EPR hyperfine line. Comparison of unlabelled ^{14}N -TEMPONE (a) and ^{14}N -TEMPOL (b). c $^2\text{H}/^{15}\text{N}$ -labelled TEMPONE. The respective EPR line widths are indicated. The concentrations were 5 mM (squares), 10 mM (circles) and 25 mM (triangles).

The fact that in this case all samples show a maximum enhancement in the range from -80 to -100 indicates that here the maximum enhancement is concentration-limited. Conversely, saturation is not observed for the $^2\text{H}/^{15}\text{N}$ -labelled sample at higher concentration. This indicates that for this case the enhancement is power-limited and higher enhancements might be achieved with higher microwave power.

Since our previous [9] and current results seemed indicative that the observed enhancements can be limited by the feasibility of saturating the EPR line under the given experimental conditions, we tested the possibility of increasing the effective saturation factor by irradiating concomitantly on both hyperfine lines in the ^{15}N -labelled sample with a concentration of 2.5 mM. To our knowledge, such a DNP experiment with dual irradiation has never been reported previously in the literature. The results are illustrated in Fig. 2.

The DNP enhancements were measured first by irradiating with a single frequency on the low- and high-field lines, respectively. With MW1 tuned to the resonator dip and resonant with the high-field line, a maximum enhancement of -60 was achieved. The low sample concentration was chosen so that the saturation point would be easily achieved under the current experimental conditions. As can be seen in Fig. 2, the power available with MW1 was sufficient to drive the enhancement into a saturation level. To evaluate the efficiency of the second microwave channel, MW2 was adjusted with a frequency offset of 62 MHz to the low-field line while MW1 was switched off. As the available power for MW2 was only 0.5 W and the resonator efficiency was reduced outside of the dip, the maximum enhancement was just about -20 . The apparent levelling off of the enhancement is due to a saturation of the microwave amplifier in the MW2 channel, indicating that the power of 0.5 W was actually not reached in this experiment. In the dual-frequency experiment, MW2 was set to the maximum available power, i.e., enhancement of about -20 , and then the power of MW1 was successively increased. With increasing MW1 power the enhancement increases;

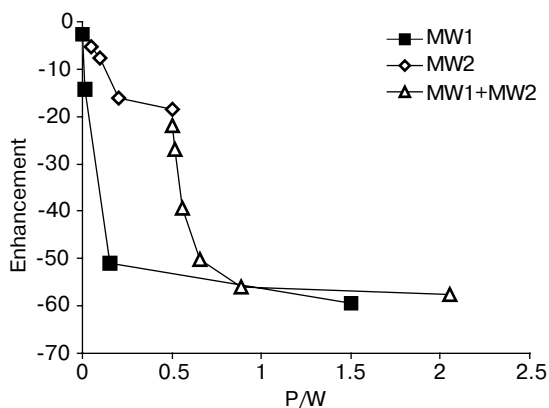


Fig. 2. ^1H -DNP enhancements of the water protons in a solution doped with $^2\text{H}/^{15}\text{N}$ TEMPONE at a 2.5 mM concentration. MW1 with power P_1 was tuned at the resonator dip (9.76 GHz) and was on resonance with the high-field hyperfine line. MW2 with power P_2 was applied at the low-field line.

however, the maximum value did not exceed the value achieved with MW1 irradiation only.

4 Discussion

DNP in liquid solution is governed by the Overhauser effect [7], by which saturation of the electronic transitions in an electron–nuclear coupled spin system leads to the transfer of electron spin polarization first to the nearby and subsequently to distant nuclei. The maximum achievable enhancement ε was given by [1]

$$\varepsilon = \frac{\langle I_z \rangle}{I_0} = 1 - \xi s f \frac{|\gamma_s|}{\gamma_I}, \quad (1)$$

where I_z and I_0 are the dynamic and the Boltzmann nuclear polarizations, respectively, γ_s and γ_I are the magnetogyric ratios of the electron and the nuclei, f is the leakage, s is the saturation and ξ is the coupling factor. The coupling factor ξ depends on the involved relaxation mechanisms and can achieve values in the range from -1 (pure scalar relaxation) up to $+0.5$ (pure dipolar). In ^1H liquid DNP with water, the enhancements are usually dominated by dipolar relaxation, leading to negative enhancements with a maximum possible value $|\varepsilon|$ of about 330. Using nuclear magnetic resonance dispersion (NMRD) experiments, we recently assessed that the relaxation mechanism for the water protons in solutions with nitroxides is pure dipolar and we employed the $1/T_1$ nuclear relaxation data to estimate the coupling factor independently from the DNP experiments. We obtained $\xi \sim 0.4$ for 5 to 10 mM TEMPOL in water solutions at 0.34 T [9]. Using Eq. (1) and the achieved enhancement of -100 with the unlabelled compounds, we arrived at an effective saturation factor of about 0.5 for our DNP experiments at 9.7 GHz. This saturation factor is higher than the expected one when saturating only one of the three hyperfine lines ($s = 1/3$). In agreement with the proposal of other authors [13] (although our reported ξ are substantially different), the large saturation factors can be interpreted in terms of an electron–electron double resonance effect which is caused either by the very fast nuclear spin–lattice relaxation of the strongly coupled nitrogen or by intermolecular Heisenberg spin exchange. Both mechanisms compete with the internal electron T_1 relaxation and cause indirect saturation of the adjacent hyperfine lines [14, 15]. Using Eq. (1), a leakage factor of 0.83 and a coupling factor ξ of about 0.4 [9], our present enhancement of $\varepsilon = -140$ with the 25 mM ^{15}N -labelled TEMPONE leads to an effective saturation factor of about 0.64.

5 Conclusions

Our data permit to draw some conclusions about optimal experimental conditions for DNP in liquid solution with nitroxide radicals as polarizers. First, the effective saturation factor can be substantially increased by using ^2H and ^{15}N isotope-

labelled samples. Second, if by single-frequency irradiation the enhancement can be driven into saturation, it cannot be further increased by dual-frequency irradiation. However, if saturation cannot be achieved at high polarizer concentration by single-frequency irradiation, it should be possible to drive the enhancement to higher levels by dual-frequency irradiation. Alternatively, improved microwave resonance structures with higher conversion factors could lead to saturation factors close to 1 without requirement of higher microwave power. We are currently using the present nitroxide-water solution polarizer system to test and optimize DNP with a new shuttle spectrometer operating at 9 GHz EPR pumping frequency and at 600 MHz ^1H NMR frequency [16].

Acknowledgments

We gratefully acknowledge technical support by A. Tavernier and T. Marquardsen. We thank D. Lennartz for preparing the nitroxide solutions in initial DNP experiments. The work has been supported by the European Design Study BIO-DNP as well as the Max Planck Society and the Fonds der Chemischen Industrie.

References

1. Hausser, K.H., Stehlik, D.: *Adv. Magn. Res.* **3**, 79–139 (1968)
2. Müller-Warmuth, W., Meise-Gresch, K.: *Adv. Magn. Res.* **11**, 1–45 (1983)
3. Maly, T., Debelouchina, G.T., Bajaj, V.S., Hu, K., Joo, C., Mak-Jurkaskas, M.L., Sirigiri, J.R., van der Wals, P.C.A., Herzfeld, J., Temkin, R.J., Griffin, R.G.: *J. Chem. Phys.* **128**, 052211 (2008)
4. Hall, D., Maus, D.C., Gerfen, G.J., Inati, S., Becerra, L.R., Dahlquist, F.W., Griffin, R.G.: *Science* **276**, 930–931 (1997)
5. Bajaj, V.S., Farrar, C.T., Hornstein, M.K., Mastovsky, I., Bryant, J., Kreisler, K.E., Elena, B., Viereg, J., Temkin, R.J., Griffin, R.G.: *J. Magn. Reson.* **160**, 85–90 (2003)
6. Ardenjaer-Larsen, J.H., Fridlund, B., Gram, A., Hansson, G., Hansson, L., Lerche, M.H., Servin, R., Thaning, M., Golman, K.: *Proc. Natl. Acad. Sci. USA* **100**, 10435–10439 (2003)
7. Abragam, A.: *Principles of Nuclear Magnetism*. Clarendon, Oxford (1961)
8. Loening, N., Rosay, M., Weis, V., Griffin, R.G.: *J. Am. Chem. Soc.* **124**, 8808–8809 (2002)
9. Höfer, P., Parigi, G., Luchinat, C., Carl, P., Guthausen, G., Reese, M., Carlomagno, T., Griesinger, C., Bennati, M.: *J. Am. Chem. Soc.* **130**, 3254–3255 (2008)
10. Meise, D., Müller-Warmuth, W., Nientiedt, H.-W.: *Ber. Bunsenges. Phys. Chem.* **80**, 584–590 (1976)
11. Grucker, D., Guiberteau, T., Eclancher, B., Chambron, J., Chiarelli, R., Rassat, A., Subra, G., Gallez, B.: *J. Magn. Reson.* **106**, 101–109 (1995)
12. Bates, R.D., Drozdowski, W.S.: *J. Chem. Phys.* **67**, 4038–4044 (1977)
13. Armstrong, B.D., Han, S.J.: *Chem. Phys.* **127**, 104508 (2007)
14. Hyde, J.S., Chien, J.C.W., Freed, J.H.: *J. Chem. Phys.* **48**, 4211–4226 (1968)
15. Robinson, B.H., Haas, D.A., Mailer, C.: *Science* **263**, 490–493 (1994)
16. Reese, M., Lennartz, D., Marquardsen, T., Höfer, P., Tavernier, A., Carl, P., Schippmann, T., Bennati, M., Carlomagno, T., Engelke, F., Griesinger, C.: *Appl. Magn. Reson.* **34**, 301–311 (2008)

Authors' address: Peter Höfer, EPR Division, Bruker Biospin, 76287 Rheinstetten, Germany
E-mail: peter.hoefler@bruker-biospin.de