Hyperpolarization quenching in ^{13}C nuclei bound to fast relaxing quadrupolar ^{14}N mediated by scalar coupling relaxation in amide groups exposed to Earth's magnetic field

Enrico Chiavazza[1,2], Eugen Kubala[2,3], Concetta V. Gringeri[2,4], Stephan Düwel[2,5], Markus Durst[2,5], Rolf F. Schulte[2], and Marion I. Menzel[2]

[1]*Chemistry, University of Turin, Turin, Italy,* [2]*GE Global Research, Munich, Germany,* [3]*Johannes Kepler University, Linz, Austria,* [4]*Klinik im Klinikum Rechts der Isar, Technische Universität München, Munich, Germany,* [5]*Institute of Medical Engineering, Technische Universität München, Munich, Germany*

Purpose: The possibility of using hyperpolarized agents in either MR spectroscopy or in MR imaging is strictly dependent on their relaxation time. Glutamine is an important metabolite, its utilization is greatly enhanced and linked to the energetic metabolism in tissues where a proliferative state is activated (e.g injuries, tumor)[1]. Working with [5-^{13}C] glutamine for this purpose, a rapid polarization loss was observed after completing the dissolution process, yielding an almost zero signal in the resulting NMR spectrum (Figure 1). The same behavior has been observed in [^{13}C] urea. Despite a number of articles published on these substrates[2-5], to the best of our knowledge no-one has described and explained similar transient fast relaxation phenomena. Polarization levels have been measured on samples kept at 0.2T during the polarizer/scanner transfer and using ^{15}N labeled isotopomers.

Method: 20 μL [^{13}C]urea ($^{14}N/^{15}N_2$ – 8M) (Euriso-top, Saint-Aubin, France), 25 mM OXO63 radical (GE Healthcare, Milwaukee, WI, USA), 2.5mM Dotarem (Guerbet, Roissy, France);

100 μL [5-^{13}C]glutamine (5-$^{14}N/^{15}N$ – 0.6M) (Euriso-top,Saint-Aubin, France), 45 mM OXO63 radical (GE Healthcare, Milwaukee, WI, USA), 5mM Dotarem (Guerbet, Roissy, France); both the mixtures were dissolved in glycerol and hyperpolarized with an Hypersense 3.35T polarizer (Oxford Instrument, Abingdon, UK) for 1h at 94.115 and 94.105 GHz, respectively. The solid samples were rapidly dissolved with 5ml Tris (30mM) buffered D_2O. The final concentration of hyperpolarized substrates was 32 and 12 mM for [^{13}C]urea and [5-^{13}C]glutamine, respectively. The dissolved samples were transferred to the MR scanner within 16 - 18 sec.

The polarization was measured in a 3T GE Signa HDx scanner (GE Healthcare, Milwaukee, WI, USA) set up with a purpose-built solenoid ^{13}C coil. The acquisition sequence used was a series of small flip angle pulses (5° for the [^{13}C]/[^{13}C,^{15}N] urea and 10° for the [5-^{13}C]/[^{13}C,^{15}N] glutamine samples). Thermal polarization was obtained from a 2048 scan averaged measurement after adding 4%v/v Dotarem to the dissolved sample (flip angle 90°,TR 1s). Liquid polarization was calculated from the integrated hyperpolarized and thermal spectrum assuming a typical Boltzmann spin distribution for the thermal measurement. The polarization values were not corrected for the T_1 decay since the T_1 at low field was supposed to be markedly different from that measured at 3T.

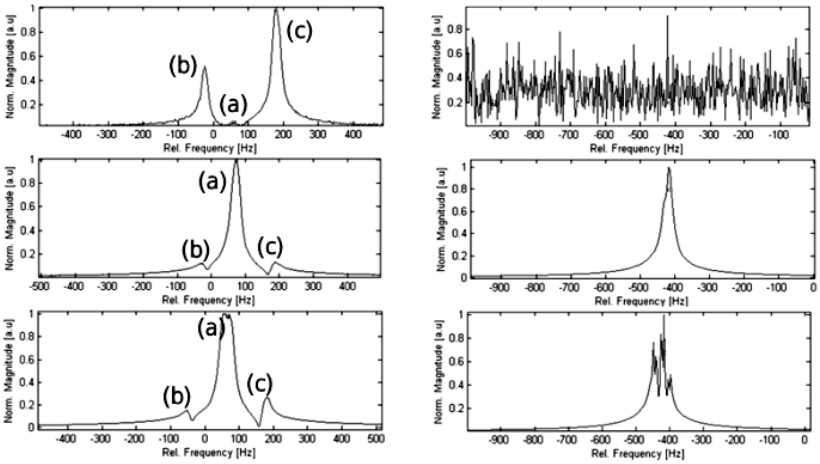

Figure 1. Hyperpolarized spectra of [5-^{13}C]glutamine (left) and [^{13}C]urea (right): first row, samples transferred at low field magnetic field (<1mT); second row, samples transferred with a 0.2T auxiliary magnetic field; third row ^{15}N labeled samples transferred at low magnetic field (<1mT). Glutamine signal is indicated as (a); (b) and (c) are assigned to [5-^{13}C] glutamate and [5-^{13}C] pyroglutamate, respectively, which are formed during the dissolution process due to the presence of a base in the glycerol glassy mixture.

Equation 1

$$R_{1sc} = \frac{8\pi^2 J^2}{3} I_X (I_x + 1) \frac{\tau_{SC}}{1 + (\omega_{13C} - \omega_X)^2 \tau_{SC}^2}$$

Table 1. Polarization values and relaxation times of [^{13}C]urea and [5-^{13}C]glutamine, measured at 3T.

	T_1(s) at 3T	Transport in earth's magnetic field	Sample attached to 0.2T permanent magnet during transport	Transport in earth's magnetic field, ascorbate added as radical scavenger
		Liquid Pol (%)	Liquid Pol (%)	Liquid Pol (%)
[^{13}C,$^{14}N_2$]urea	78±4	$3\cdot10^{-3}\pm1\cdot10^{-3}$	13±1	$3\cdot10^{-3}\pm1\cdot10^{-3}$
[^{13}C,$^{15}N_2$]urea	85±7	30±2	25±1	- - -
[5-^{13}C, ^{14}N]glutamine	8.0±0.1	$0.02\pm5\cdot10^{-3}$	0.7±0.1	$0.05\pm5\cdot10^{-3}$
[5-^{13}C, ^{15}N]glutamine	7.7±0.4	0.7±0.2	0.8±0.1	- - -

Results: The hyperpolarized signal is strongly enhanced by the presence of an auxiliary magnetic field during the transfer, as well as by the use of ^{15}N labeled amides (Figure 1). No polarization preserving effect was observed when a radical scavenger (sodium ascorbate 5mM) was added to the dissolution agent. The polarization values in all conditions are reported in table 1.

Discussion: The observed low field relaxation behavior for ^{14}N-^{13}C amides suggested that, in such conditions (relatively strong J coupling, short ^{14}N nucleus T_1 and weak magnetic field), a new relaxation mechanism becomes dominant. Scalar coupling (II° kind) is known to be an efficient relaxation mechanism in closely resonant nuclei (^{79}Br-^{13}C). Its contribution to relaxation has been theoretically estimated[6] using equation 1 and has been found to be equivalent to an averaged R_1 of 1.5±0.1 s^{-1} (measured $J_{^{14}N-^{13}C}$=11.3±0.1Hz, $T_{1,^{14}N}=\tau_{sc}$=1±0.1ms and B_0 magnetic field along the transfer path, where $\omega_x=\gamma_x \cdot B_0$ and I_x=1). This polarization quenching has been succesfully overcome by keeping the hyperpolarized sample close to a permanent magnet (0,2T) but an even lower field is sufficient to avoid this relaxation (10mT). Alternatively, ^{15}N labeled substrates appeared to be effective and may be a safer solution. The low polarization obtained for both [5-^{13}C] glutamine isotopomers is due to a relatively long transfer time if compared to its short T_1. The T_1 itself appeared to be shorter than the one reported in the literature (16s in H_2O, 310K, 9.4T[7]) most likely because of the high concentration of Gd complex used in these experiments. The difference between the polarization obtained value in [^{13}C,$^{14}N_2$] urea and [^{13}C,$^{15}N_2$] urea can be related to the fact that the samples were always exposed to a low magnetic field for a few second after the dissolution, when the solution was still in the upper part of the polarizer.

Conclusion: We have shown that nuclei, such as ^{14}N and ^{13}C, with different γ ratios under unfavorable conditions, such as J coupling, short ^{14}N T_1 and weak magnetic field can engage in mutual relaxation. The polarization loss that occurrs during the transfer of hyperpolarized amides to the MR scanner is shown to be due to fast scalar coupling relaxation at the low field which is present between the polarizer and the scanner itself. Thus, the use of some physiologically important metabolites as hyperpolarizable DNP probes might therefore be eventually impaired. This phenomenon, although it has never been reported in literature, should be taken into account during the design of a DNP-MRI laboratory, either by locating the polarizer in the stray field of the MR scanner or by connecting it to the MR scanner with a suitable sustained magnetic field transfer system.

References: [1]. Lehninger AL, Nelson DL, Cox MM, *Principles of Biochemistry* 1993**,** Worth Publishers; [2]. Ardenkjaer-Larsen JH, Fridlund B, Gram A, et al. *Proc Natl Acad Sci USA.* 2003;100(18); [3]. Golman K, Ardenaer-Larsen JH, Petersson JS et al*. Proc Natl Acad Sci USA.* 2003;100(18); [4]. von Morze C, Bok RA, Sands JM et al. *Amer J Physiol-Renal Physiol.* 2012;302(12); [5]. Wilson DM, Keshari KR, Larson PEZ et al. *J Magn Res.* 2010;205(1); [6] Becker J, Shoup RR, Farrar TC. *Pure App. Chem.* 32 (1972) 51-66; [7]. Gallagher FA, Kettunen MI, Day SE et al. *Magn Res Med.* 2008;60(2). Acknowledgements: co-funding by BMBF grant number 01EZ1114.