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Over 35% liquid-state ^{13}C polarization obtained *via* dissolution dynamic nuclear polarization at 7 T and 1 K using ubiquitous nitroxyl radicals†

Tian Cheng,* Andrea Capozzi, Yuhei Takado, Riccardo Balzan and Arnaud Comment

The most versatile method to increase liquid-state ^{13}C NMR sensitivity is dissolution dynamic nuclear polarization. The use of trityl radicals is usually required to obtain very large ^{13}C polarization *via* this technique. We herein demonstrate that up to 35% liquid-state ^{13}C polarization can be obtained in about 1.5 h using ubiquitous nitroxyl radicals in ^{13}C-labeled sodium salts by partially deuterating the solvents and using a polarizer operating at 1 K and 7 T.

The dissolution dynamic nuclear polarization (DNP) method which combines the low-temperature DNP technique developed in the realm of particle physics with a fast dissolution process was established a decade ago.[1] It allows enhancing the ^{13}C nuclear magnetic resonance (NMR) signal-to-noise ratio (SNR) of substrates several thousand fold and led to the development of a broad range of new applications in NMR spectroscopy and magnetic resonance imaging (MRI). With the advent of dissolution DNP, it became possible to monitor chemical and biochemical transformations in real time *in vitro* and *in vivo* by ^{13}C NMR.[2–4] Most studies published so far were performed using Hypersense™, a commercial dissolution DNP instrument operating at 3.35 T. The highest liquid-state ^{13}C polarization reported at this field is on the order of 30% in the optimized preparation of [1-^{13}C]pyruvic acid doped with trityl radicals and 1–2 mM Gd^{3+} ions.[5–7] Upon increasing the polarizing field to 4.6 T, up to 64% solid-state polarization was obtained.[8] Although the polarization time constants increase with increasing magnetic field, it was shown that it is possible to reach about 53% in 1.5 h ($\tau_{Buildup} = \sim 3000$ s). It must however be noted that the specific self-glassing property of neat pyruvic acid is particularly favorable for DNP and such high polarization levels can usually not be achieved in other compounds, in particular in salts that must be dissolved in a mixture of solvents containing a so-called glassing agent. For instance, the highest polarization reported to date at 3.35 T for preparation based on [1-^{13}C]acetate salts using Ox063 trityl radicals was 18%.[9] The use of choline chloride to form deep eutectic mixtures was recently proposed to obtain liquid-state polarization close to 30% in various substrates.[10] Compared to trityl radicals, stable nitroxyl radicals, namely TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl) and its derivatives, lead to more modest ^{13}C polarization. However, their wide availability, relatively low toxicity,[11,12] and the possibility of efficiently scavenging them make nitroxyl radicals attractive for *in vitro* and *in vivo* applications.[13,14] In samples prepared using nitroxyl radicals, it was observed that solvent deuteration leads to an increase in polarization by a factor of about 2.[15,16] It was also recently shown that upon increasing the polarizing field to 6.7 T and using a low-temperature solid-state ^{1}H to ^{13}C cross-polarization scheme, it is possible to obtain liquid-state ^{13}C polarization of $\sim$35%.[17] However, the solid-state NMR hardware is rather complex since high B_1 fields are required for transferring the polarization from ^{1}H to ^{13}C.[17]

We herein propose a method to obtain room-temperature solutions containing molecules with a ^{13}C polarization of up to 35% within about 1.5 h using the widely available TEMPOL (4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl) nitroxyl radical without requiring any solid-state ^{1}H to ^{13}C polarization transfer sequence. It is based on the use of an in-house designed polarizer adapted to perform at 1 $\pm$ 0.05 K and 7 T, the highest field ever reported for dissolution DNP, and a careful choice of solvent deuteration level.

The polarizer developed for the present study is a modified version of the instrument described in an earlier publication.[18] The magnetic field of the 89 mm room-temperature bore superconducting magnet (Oxford Instruments, Oxford, UK) was set to 7 T. A 197 GHz millimeter wave (mm-wave) source (ELVA-1, St. Petersburg, Russia) with a 0.5 GHz bandwidth and a maximum output of 60 mW was used to irradiate the samples. The challenging part of working at 1 K at such high field comes from the high losses inherent to mm-wave frequencies close to the far infrared region of the electromagnetic spectrum. These losses cannot be compensated by increasing the power because of the large amount of heat dissipating into the system which increases

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the superfluid helium bath temperature and leads to reduced DNP efficiency.[18] To minimize the losses, the mm-waves were transmitted from the output of the source to the sample space through two components only: a 22 mm-long WR-06 rectangular to circular waveguide transition (ELVA-1, St. Petersburg, Russia) connected to a 1.3 m long 6 mm internal diameter gold-plated stainless steel circular waveguide. As compared to the original design used at both 3.35 T and 5 T,[16,18] the inner diameter of the mm-wave cavity was reduced from 30 mm to 23 mm, corresponding to a 41% reduction in volume and thus an increase in mm-wave magnetic field B_1 of 30% for a given mm-wave power. The influence of the cavity size on the mm-wave power requirement was previously studied using a similar DNP polarizer.[19] The inner surface of the cavity was also gold-plated to reduce the mm-wave absorption. Thanks to these modifications, it was possible to reach maximum ^{13}C polarization at 1 K with a source output power of 50 mW (see Fig. 1). This was not the case with the original 30 mm diameter cavity and components that were not gold-coated (see Fig. S1 in the ESI†). The mm-wave power dependence of the NMR signal enhancement has been previously measured using a different DNP polarizer at 4 K and 200 GHz and the maximum polarization could not be reached with the maximum available power, namely 70 mW.[20] A possible reason for this discrepancy is the presence, in the system described herein, of a cavity that confines the mm-waves.

All samples measured in the present study were prepared by dissolving sodium [1-^{13}C]acetate (3 M) and TEMPOL in water–ethanol or water–glycerol mixtures. All chemicals were purchased from Sigma-Aldrich, Buchs, Switzerland. The solutions were prepared in containers that were sealed and placed in a 40 °C water bath for 1 h. Once the solutions returned to room temperature, droplets of 2 ± 0.5 μL were poured in liquid nitrogen to form frozen beads. A total volume of 250 μL of frozen beads was inserted into a 7 T polarizer. The mm-wave frequencies corresponding to the maximum positive and negative ^{13}C polarization

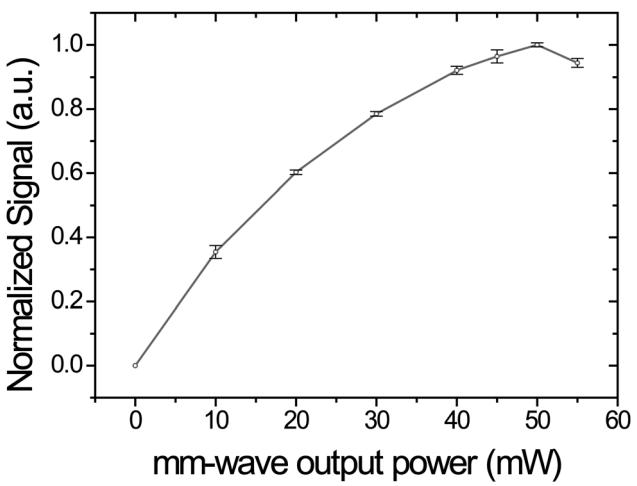

Fig. 1 Maximum ^{13}C NMR signal as a function of the mm-wave source output power. The measurements were performed at 1 K in a sodium [1-^{13}C]acetate sample (sample type GlyHD66; see Table 1) irradiated at 196.85 GHz. The line connecting the data points is drawn to help guide the eye.

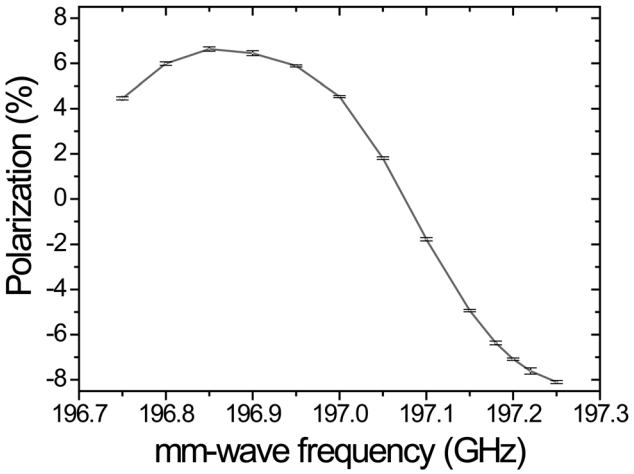

Fig. 2 Maximum ^{13}C polarization as a function of the irradiation mm-wave frequency. The measurements were performed at 4.2 K in a sodium [1-^{13}C]acetate sample (sample type GlyHD66; see Table 1). The source output power was set to 50 mW. The absolute polarization was determined from liquid-state measurements performed following dissolution. The line connecting the data points is drawn to help guide the eye.

were determined at 4.2 K by measuring the ^{13}C solid-state NMR signal for different irradiation frequencies separated by steps of 0.04 GHz over the full range of the source output frequency (Fig. 2). The ^{13}C NMR signals were measured by applying a small flip angle radiofrequency pulse every 5 minutes. The coil implemented in the low-temperature probe used for solid-state NMR measurements was printed on a flexible support (0.1 mm printed circuit boards (FR-4), 35 μm Cu). Following a scheme described in an earlier publication,[21] ceramic chip capacitors (ATC-100B, American Technical Ceramics, USA) were used to pre-tune and -match the coil and an external serial matching circuit was used to properly match (50 ohms) and fine-tune the probe to 75.16 MHz, the ^{13}C resonance frequency at 7 T.

To quantify the liquid-state enhancement, each sample was polarized at 1 ± 0.05 K with 50 mW mm-wave power at 197.25 GHz. Once the maximum ^{13}C polarization was reached, the dissolution was performed with 5 mL of superheated D_2O (12 bar, 450 K). Following dissolution, a constant helium gas pressure of 5 bar was applied for 2 s to transfer the solution inside a 5 m long and 2 mm inner diameter PTFE (polytetrafluoroethylene) tube from the output of the polarizer to an in-house designed infusion pump located inside the bore of an adjacent 9.4 T MR scanner (Agilent, USA).[21] A volume of 2.8 mL was collected inside the main compartment of the pump around which the copper coil of a dedicated NMR probe was wound.[14] All measurements were performed at 17 ± 3 °C, the temperature at which the pump rapidly thermalized the transferred solution.[14] Acquisition of the hyperpolarized ^{13}C signals started 1 s after the transfer and was performed using a calibrated 5° radiofrequency pulse applied every 3 s. The liquid-state enhancement factor was calculated from the ^{13}C thermal equilibrium signal recorded 15 min after dissolution using a calibrated 90° pulse (average of 8 acquisitions). The longitudinal ^{13}C relaxation time was deduced from the signal decay corrected

Table 1 ^{13}C polarization build-up time constants, solid-state ^{13}C relaxation time, and liquid-state ^{13}C polarization for all 3 M sodium [1-^{13}C]acetate samples hyperpolarized at 7 T and 1 ± 0.05 K. The errors represent the observed standard deviation between different dissolution experiments

Sample type	TEMPOL concentration (mM)	Solvents	Build-up time constant (s)	Solid-state ^{13}C relaxation time (s)	Liquid-state ^{13}C polarization (%)
EtDD50	50	D_2O–d_6-EtOD (2 : 1 v/v)	4950 ± 200	>40 000	14.5 ± 1
EtDD66	66	D_2O–d_6-EtOD (2 : 1 v/v)	1600 ± 50	14 000 ± 3000	8 ± 0.5
EtHD66	66	H_2O–d_6-EtOD (2 : 1 v/v)	3800 ± 400	—	5.5 ± 0.5
GlyDD66	66	D_2O–d_8-glycerol (1 : 1 w/w)	2550 ± 100	35 000 ± 4000	16.5 ± 1
GlyHD50	50	H_2O–d_8-glycerol (1 : 1 w/w)	4250 ± 400	21 000 ± 2500	19 ± 1
GlyHD58	58	H_2O–d_8-glycerol (1 : 1 w/w)	2200 ± 200	15 000 ± 2000	35 ± 3
GlyHD66	66	H_2O–d_8-glycerol (1 : 1 w/w)	1400 ± 200	—	25 ± 1
GlyHH66	66	H_2O–glycerol (1 : 1 w/w)	3000 ± 100	—	16 ± 3

for the effect of the 5° pulses. At least two dissolution experiments were performed for each type of sample.

The sample used as reference for testing the adapted DNP polarizer was identical to the one measured at 5 T in a previous study,[16] *i.e.*, a frozen 3 M sodium [1-^{13}C]acetate solution prepared in a D_2O–d_6-EtOD (2 : 1 vol/vol) mixture and doped with 50 mM TEMPOL. The maximum ^{13}C polarization value and the associated build-up time for this reference sample are presented in Table 1 (sample type EtDD50). The observed maximum polarization was larger than the one obtained at 5 T and 1.2 K but the increase was less than about 20%. This is substantially less than what could be expected if the ^{13}C polarization simply scaled with the magnetic field (40% increase in magnetic field) as was previously observed when comparing results obtained at 5 T and 3.35 T.[16] This could be at least partially due to the fact that the bandwidth of the 197 GHz source was not large enough to unequivocally identify the frequency corresponding to the maximum negative ^{13}C polarization. The build-up time constant was more than two times longer than the one measured at 5 T. The working temperature was however lower in the present study (1 K instead of 1.2 K). Although increasing the TEMPOL concentration to 66 mM resulted in, as a consequence, a faster ^{13}C polarization build-up, the maximum ^{13}C polarization significantly decreased in this type of sample (see Table 1). The results of measurements performed with lower TEMPOL concentrations (between 33 and 50 mM) are not reported since the build-up time constants were too long to be accurately determined and the liquid-state ^{13}C polarization levels obtained after 5 h of polarization were not as large as the ones presented in Table 1. The use of deuterated glycerol instead of EtOD as a glassing agent led to increased maximum polarization (sample type GlyDD66 in Table 1). It was also observed that replacing D_2O by H_2O reduced the build-up time and considerably improved the ^{13}C polarization. The optimal TEMPOL concentration for a 3 M [1-^{13}C]acetate sample in H_2O–d_8-glycerol (1 : 1 w/w) was around 58 mM (sample type GlyHD58 in Table 1). The ^{13}C NMR signal enhancement factor obtained at 9.4 T following dissolution of samples of type GlyHD58 was 45 000 ± 2000, corresponding to a liquid-state polarization of 35 ± 3%. The longitudinal ^{13}C relaxation was strongly affected by the presence of TEMPOL and the decay of the liquid-state signal corresponded to a relaxation time constant T_1 = 27 ± 2 s for all sample types presented in Table 1. As proposed in an earlier study,[13] nitroxyl radicals can be scavenged by vitamin C and, following a protocol previously described,[14] 1 M deuterated sodium ascorbate was preloaded into the main compartment of the infusion pump prior to dissolution. The resulting [1-^{13}C]acetate T_1 increased to 57 ± 1 s (see Fig. 3). The slight deviation from the mono-exponential fitting curve is most likely due to the finite reaction time between ascorbate and TEMPOL.[14]

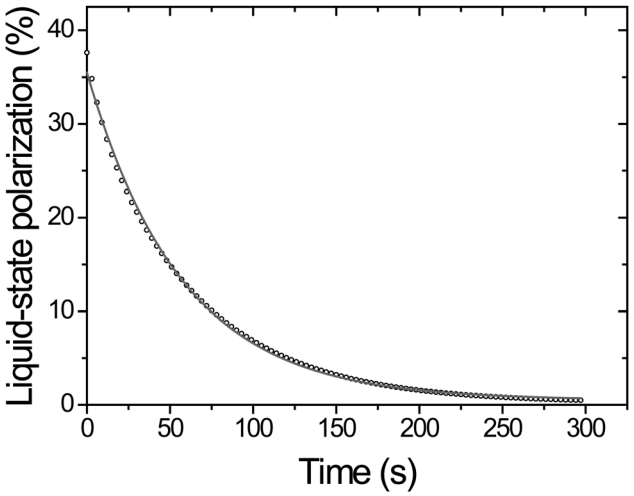

Fig. 3 ^{13}C signal decay measured inside the infusion pump following dissolution and radical scavenging in 1 M deuterated ascorbate solution. The fit is a mono-exponential decay function from which a longitudinal relaxation time constant of 57 ± 1 s was deduced.

The fact that in samples doped with nitroxyl radicals the build-up and nuclear relaxation time constants become shorter when protons are replaced by deuterons has already been observed at 3.35 T.[15] It was also shown that full solvent deuteration leads to substantially larger ^{13}C polarization in both water–ethanol and water–glycerol matrices at 3.35 T as well as at 5 T.[15,16,22] We observed that this is not the case at 7 T, at least in water–glycerol matrices doped with TEMPOL. The most striking difference compared to what has been observed at lower field is that partial deuteration of the water–glycerol solvent mixture can increase the ^{13}C polarization by more than a factor of 2 as compared to fully protonated or fully deuterated matrices. For a given radical concentration, the presence of both protons and deuterons in the solvent mixture also shortens the ^{13}C build-up time constant as compared to the fully deuterated (D_2O–d_8-glycerol) and fully protonated (H_2O–glycerol) samples. Comparable ^{13}C polarization levels (29 ± 3%) were measured in samples prepared in D_2O–glycerol with a similar proton to deuteron ratio. Since the solutions were kept at 40 °C for 1 h

before preparing the frozen samples, the proton to deuteron ratio at the exchangeable sites of the molecules equilibrated to the total proton to deuteron ratio of the solution. We concluded that the exchangeable protons of the solvent molecules are at the origin of the increased polarization efficiency. There is thus no relationship with the effect of the methyl groups observed in a previous study.[23]

The lower ^{13}C polarization measured in samples prepared with H_2O–d_6-EtOD mixtures as compared to samples prepared with a fully deuterated water–ethanol solvent mixture shows that the larger ^{13}C polarization in H_2O–d_8-glycerol mixtures cannot simply be explained using spin thermodynamics arguments related to the heat capacity of the proton and deuteron baths.[15,22] The maximum polarization is rather obtained for samples in which the proton to deuteron ratio allows establishing the most effective dynamic equilibrium. Large liquid-state ^{13}C polarizations could also be obtained in other salts dissolved in the same H_2O–d_8-glycerol solvent mixture containing 66 mM TEMPOL ($\sim$20 $\pm$ 2% in 1.5 h in 3 M sodium [1-^{13}C]pyruvate samples). The optimal radical concentration was however not determined and it is very likely that higher polarization could be obtained with different TEMPOL concentrations.

The first dissolution DNP results obtained using a 7 T polarizer presented herein show that a relatively low power mm-wave source is sufficient to reach optimal ^{13}C polarization at 1 $\pm$ 0.05 K using ubiquitous nitroxyl radicals. Sample preparation was optimized for [1-^{13}C]acetate, a substrate that has already proven to be important for *in vivo* metabolic studies.[24–26] Since large liquid-state ^{13}C polarizations of up to 35 $\pm$ 3% were measured inside an in-house designed infusion pump that can be used to inject the substrate in small animals,[14] the proposed method is directly applicable for real-time *in vivo* metabolic studies.

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