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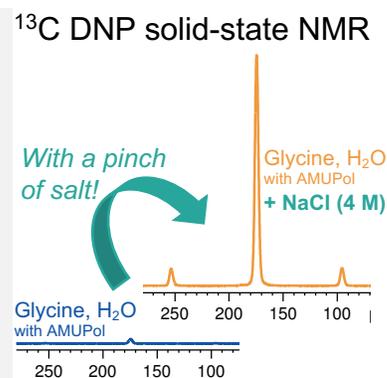
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Polarization Amplification in Dynamic Nuclear Polarization Magic-Angle Spinning Solid-State Nuclear Magnetic Resonance by Solubilizing Traditional Ionic Salts

Florian Ferrer, Marie Juramy, Ribal Jabbour, Samuel Cousin, Fabio Ziarelli, Giulia Mollica, Pierre Thureau,* and Stéphane Viel*

ABSTRACT: Dynamic nuclear polarization can improve the sensitivity of magic-angle spinning solid-state NMR experiments by one to two orders of magnitude. In aqueous media, experiments are usually performed using the so-called DNP juice, a glycerol-*d*₈/D₂O/H₂O mixture (60/30/10, v/v/v) that can form a homogeneous glass at cryogenic temperatures. This acts as a cryoprotectant and prevents phase separation of the paramagnetic polarizing agents (PAs) that are added to the mixture to provide the source of electron spin polarization required for DNP. Here, we show that relatively high ¹H DNP enhancements (~60) can also be obtained in water without glycerol (or other glass forming agents) simply by dissolving high concentrations of electrolytes (such as NaCl or LiCl), which perturb the otherwise unavoidable ice crystallization observed upon cooling, hereby reducing PAs phase separation and restoring DNP efficiency.



Dynamic nuclear polarization has emerged as an efficient method for improving the sensitivity of magic-angle spinning (MAS) solid-state NMR (ssNMR) experiments.¹ Thanks to the pioneering works of R. G. Griffin and coworkers,²⁻⁴ sensitivity enhancements over one to two orders of magnitude can now be readily achieved for a wide range of samples,⁵⁻¹² including at high magnetic fields (9.4 T and above).¹³⁻¹⁴ DNP is based on the transfer of electron spin polarization to nuclei promoted by microwave irradiation of the sample,¹⁵⁻¹⁶ usually at cryogenic temperatures. For diamagnetic samples, the required source of electron spin polarization is provided by specific paramagnetic species (so-called polarizing agents or PAs)¹⁷ that are added to the sample of interest. In aqueous media, as is typically the case for biological samples, one of the preferred strategies consists of mixing water with organic compounds such as glycerol,³ DMSO,¹⁸ or trehalose¹⁹ in predetermined relative volume fractions to form an amorphous glass at cryogenic temperatures (*ca.* 100 K). Indeed, water is known as a bad “glass former”,²⁰ and only cooling rates $>10^6$ K.min⁻¹ have been shown to promote the formation of glassy water at atmospheric pressure (*i.e.* hyperquenching).²¹⁻²² These cooling rates are far superior to those that can be achieved by inserting the sample into the pre-cooled low-temperature MAS ssNMR probehead used for DNP, which can be estimated in the 10^2 – 10^3 K.min⁻¹

range under typical implementation conditions.²³ Therefore, without the use of glassy agents, ice crystallization cannot be prevented.²⁰ This may lead to PA phase separation and self-aggregation (or crystallization), which in turn translate into a loss of DNP efficiency. As such, any DNP MAS ssNMR experiment performed in pure water typically ends up with hardly any sensitivity enhancement at all. In contrast, using glycerol or alternative “glass forming” compounds, good-quality glasses can be obtained in which PAs can be homogeneously dispersed, hereby allowing DNP efficiency to be restored. In addition, in some circumstances, these mixtures allow the analyte of interest (*e.g.* biomolecules) to be structurally preserved at low temperature (cryoprotection). One example of these binary, glass-forming mixtures involves glycerol and water. In particular, a 60/30/10 (v/v/v) glycerol-*d*₈/D₂O/H₂O mixture has found widespread use in the related literature, where it has been referred to as ‘DNP juice’ for some time,²⁴ allowing large DNP sensitivity enhancements (>200 at 9.4 T) to be reached for a range of samples. Decreasing the amount of glycerol usually decreases the glass stability of the sample,²⁵ unless special rapid freezing techniques are used.²⁶

In the framework of our ongoing work related to the investigation of crystallization processes of glycine by DNP MAS ssNMR,²⁷⁻³⁰ we unexpectedly observed in one particular study a substantial ¹H DNP enhancement (x8) for

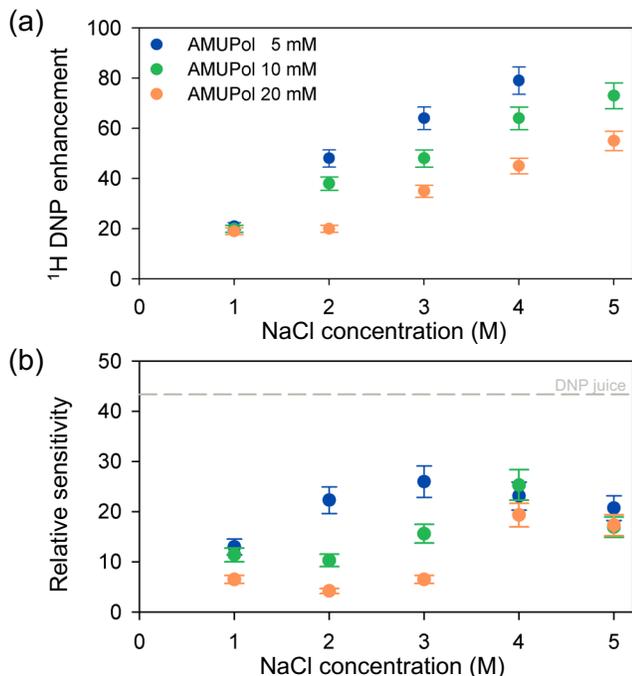


Figure 1. Evolution of the (a) ^1H DNP enhancement and (b) relative sensitivity measured at 9.4 T and *ca.* 100 K on aqueous solutions (100% H_2O) of 0.5 M $[1-^{13}\text{C}]$ glycine with increasing concentration of NaCl for 3 distinct concentrations of AMUPol (5, 10, and 20 mM). In (b), the grey dashed line indicates the relative sensitivity obtained on a comparable glycine solution but in DNP juice (glycerol- $d_8/\text{D}_2\text{O}/\text{H}_2\text{O}$, 60/30/10, v/v/v) with 10 mM of AMUPol (and without NaCl).

glycine in water when NaCl was used for selecting a particular glycine polymorph (whereas no DNP enhancement could be observed in the absence of NaCl).³¹ In the present work, we aim to comprehensively investigate this effect. As such, we first studied the impact of the NaCl concentration (from 0 M to 5 M) by recording $^1\text{H} \rightarrow ^{13}\text{C}$ cross-polarization magic-angle spinning (CP MAS) spectra at 9.4 T and *ca.* 100 K (with and without microwave irradiation) for a series of aqueous solutions (100% H_2O) of $[1-^{13}\text{C}]$ glycine (0.5 M) at 3 different AMUPol concentrations (5, 10 and 20 mM). Further experimental details can be found in SI. We chose AMUPol because it can provide high ^1H DNP enhancements at 9.4 T (>200),³² and its DNP properties (*e.g.* depolarization) have been thoroughly described in the literature.³³ For all 0.5 M $[1-^{13}\text{C}]$ glycine samples, we measured two quantities. First, we measured the ^1H DNP enhancement defined as the intensity ratio of the $^1\text{H} \rightarrow ^{13}\text{C}$ CP MAS resonances recorded with the microwave field *on* and *off* ($I_{\text{on}}/I_{\text{off}}$). Second, because AMUPol is affected by depolarization,³³ a process that artificially improves the amplitude of the DNP enhancement by reducing the spectral intensities measured *without* microwave irradiation,³⁴ we also measured the *relative sensitivity*. The latter was herein expressed as the ratio of the integral of the ^{13}C O resonance of a given glycine sample

analyzed with microwaves to the integral of the same resonance for a reference glycine sample in pure water analyzed without microwaves (and without AMUPol). Moreover, to account for differences in recycle delays, this ratio of integrals was divided by the square root of the respective experimental time constants (see SI for details). Both the ^1H DNP enhancement and the relative sensitivity are valuable in assessing the actual improvement in experimental sensitivity achieved through DNP. Results are reported in Figure 1.

As can be seen in Figure 1, substantial ^1H DNP enhancements were observed (Figure 1a), even for moderately high NaCl concentrations (1 M), for which a ^1H DNP enhancement of 20 could be measured. For comparison, as mentioned above, the ^1H DNP enhancement of glycine in water (without NaCl) using the same range of AMUPol concentrations is 1. This increase in ^1H DNP enhancement also translated into significant improvement in terms of relative sensitivity, as shown in Figure 1b. For instance, the relative sensitivity measured for an aqueous solution (100% H_2O) with NaCl (4 M) and AMUPol (10 mM) is about 58% of that measured on the same glycine sample at the same concentration but with DNP juice (evidenced by the grey dashed line in Figure 1b). Interestingly, a 2 M NaCl solution in water (100% H_2O) with 5 mM of AMUPol performs almost just as well. This increase in sensitivity allows 2D experiments to be readily recorded, as illustrated with a 2D ^{13}C - ^{13}C INADEQUATE experiment³⁵ performed in *ca.* 25 min on a 0.5 M $U-^{13}\text{C}$ glycine sample containing LiCl and AMUPol at 4 M and 15 mM, respectively (Figure S1). Moreover, a similar gain in sensitivity (56% with respect to DNP juice) was obtained for another water-soluble compound, nicotinamide, which is a natural form of vitamin B3 that is widely used to form cocrystals with drugs to modify their properties³⁶ (Figure S2).

Furthermore, note that the DNP juice only contains *ca.* 10% of protons, as it is composed of glycerol- $d_8/\text{D}_2\text{O}/\text{H}_2\text{O}$ (60/30/10, v/v/v).¹² Using the same relative concentration of protons, the relative sensitivity of a 4 M NaCl solution of glycine with 10 mM AMUPol in a $\text{D}_2\text{O}/\text{H}_2\text{O}$ mixture (90/10, v/v) reached 75% of that obtained with the DNP juice (Figure 2). Interestingly, in Figure 2a, the ^{13}C resonances due to the glycerol are clearly visible, which makes it difficult to observe other signals not only in the 50-80 ppm spectral region, but also in other regions due to the presence of multiple side bands of substantial intensity that may also cause spectral interference with signals of lower intensity. This is even more pronounced when considering proline as an example because the glycerol signals overlap almost entirely with the alpha-CH signal due to proline (Figure S3).

In addition, we investigated the effect of using different types of salts (Figure 3). In this case, the salt concentration was maintained constant (4 M) for comparison and we only used plain water (100% H_2O) for all the electrolytic solutions. Figure 3 shows that substantial sensitivity

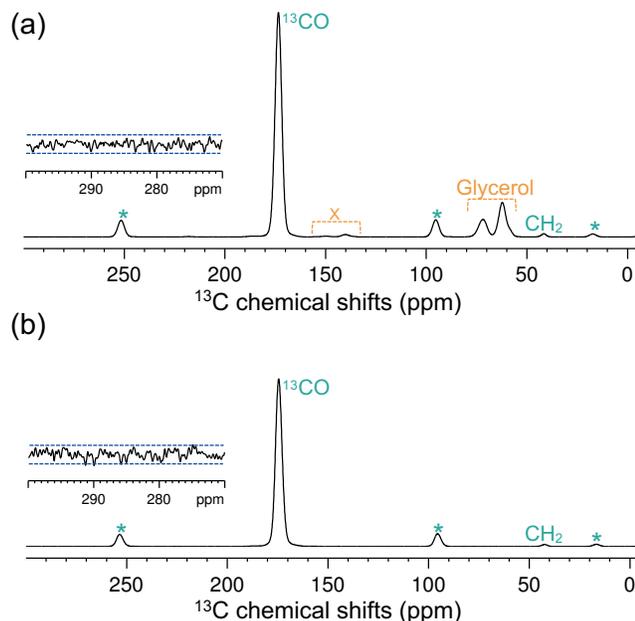


Figure 2. DNP-enhanced $^1\text{H}\rightarrow^{13}\text{C}$ CP MAS spectra of $[1-^{13}\text{C}]$ glycine (0.5 M) with AMUPol (10 mM) recorded at 9.4 T and *ca.* 100 K in (a) DNP juice and (b) a 4 M NaCl solution in $\text{D}_2\text{O}/\text{H}_2\text{O}$ (90/10, v/v). The noise levels of both spectra are illustrated in the corresponding insets. These spectra have been normalized to the square root of their ^1H DNP build-up time constants (3.4 s and 1.6 s, respectively, see SI for details). The * and x symbols indicate spinning side bands due to glycine and glycerol, respectively. The ^{13}CO and CH_2 resonances due to $[1-^{13}\text{C}]$ glycine and the CH and CH_2 resonances due to glycerol are shown with green and orange labels, respectively.

enhancements could be obtained for electrolytic solutions containing Na^+ or Li^+ ions, with a relatively low impact of the respective counterion (Cl^- , Br^- , or I^-), whereas solutions containing K^+ ions only yielded disappointing results. For comparison, recall that the ^1H DNP enhancement and the relative sensitivity of 0.5 M $[1-^{13}\text{C}]$ glycine in pure water (100% H_2O) without any added salt and without AMUPol are both equal to 1.

The fact that we do observe such sizeable DNP sensitivity enhancements suggests that, in these experimental conditions, ice crystallization may be hampered somehow (with a concomitant reduction in PA phase separation). In fact, we observed that ^1H spin-lattice relaxation times of aqueous solutions (100% H_2O) of NaCl without AMUPol increased steadily with NaCl concentration, from $T_1[100\text{ K}] = 1\text{ s}$ without NaCl (*i.e.* a situation where water is likely to be present in the form of ice only) to $T_1[100\text{ K}] = 20\text{ s}$ at 6 M NaCl (near the solubility limit at 298 K). This indicates a possible change in the state of matter of water, although variations in the concentrations of dissolved paramagnetic O_2 in these samples could also be contributing factors.³⁷ More precisely, data in Figure 3 showed that the best results were obtained for NaCl and LiCl, with ^1H DNP enhancements that reached 73 and 128,

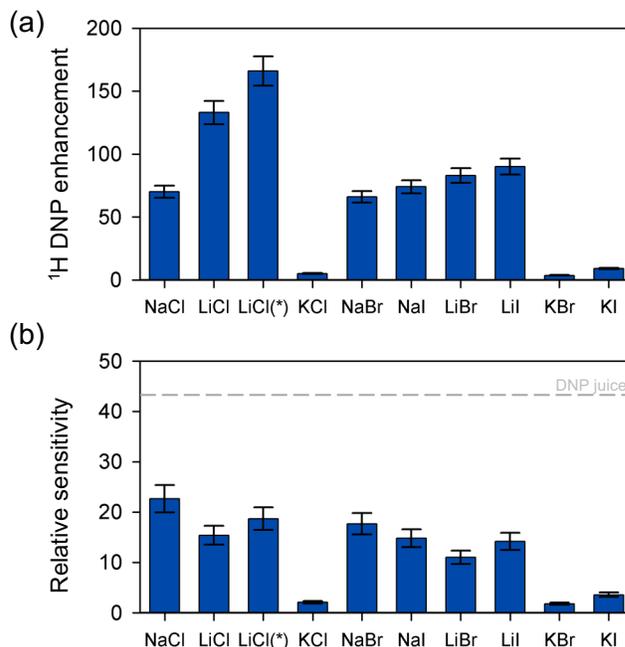


Figure 3. Evolution of (a) the ^1H DNP enhancement and (b) the relative sensitivity measured in aqueous solutions (100% H_2O) of 0.5 M $[1-^{13}\text{C}]$ glycine in different salts (at 9.4 T and *ca.* 100 K). In each case, the AMUPol concentration is 15 mM and the salt concentration is 4 M, except for the 3rd case from the left (LiCl^*) for which the LiCl concentration is 9.5 M.

respectively. Still, the best relative sensitivity was observed for the NaCl solution due to a lower ^1H DNP build-up time constant ($\tau_{^1\text{H,DNP}} = 0.74\text{ s}$ and $\tau_{^1\text{H,DNP}} = 1.1\text{ s}$ for NaCl and LiCl, respectively), and because the integral of the glycine signal in the spectrum recorded on the LiCl solution without microwaves was 40% of that measured for the *off* spectrum of the NaCl solution. This would suggest that signal losses due to depolarization are more pronounced for LiCl solutions than for NaCl solutions.

Overall, the trends observed in Figure 3 are in line with the so-called “glass-forming” properties of these electrolytic solutions. It is indeed well established in the literature that concentrated aqueous solutions of electrolytes containing sodium, lithium, or potassium ions, behave differently upon cooling with respect to pure water,³⁸ allowing for the formation of glasses depending on experimental conditions such as pressure or cooling rates. For instance, for $\text{LiCl}/\text{H}_2\text{O}$ solutions, it has been shown that even low cooling rates such as $10\text{ K}\cdot\text{min}^{-1}$ (amenable to our DNP setup) can lead to solution amorphization at ambient pressure – yielding a rather homogenous glass – as long as the LiCl concentration is close or above the eutectic (7.5 M).²⁰ In this respect, we also analyzed a highly concentrated $\text{LiCl}/\text{H}_2\text{O}$ solution (9 M), well above the eutectic point (Figure 3). A ^1H DNP enhancement of 166 was obtained in this case – the highest value of all – but the corresponding relative sensitivity was comparable to that obtained for LiCl at 4 M, mainly because

of a relatively higher ^1H DNP build-up time constant ($\tau_{^1\text{H,DNP}} = 4.4$ s instead of $\tau_{^1\text{H,DNP}} = 1.1$ s). In contrast, the cooling rates achievable in our DNP conditions should not be sufficiently fast to obtain glassy NaCl/water solutions, even at the eutectic concentration, because cooling rates larger than 10^5 K.min $^{-1}$ are required at ambient pressure,³⁹ unless the aqueous NaCl solutions are confined in nanosized pores (which is not the case here).⁴⁰ Moreover, in that latter study,⁴⁰ it was observed that nano-confinement could vitrify NaCl/H $_2$ O solutions but not KCl/H $_2$ O solutions, indicating that glasses from KCl/H $_2$ O solutions are even more difficult to obtain than those from NaCl/H $_2$ O solutions.⁴¹ In other words, while it is reasonable to assume that our experimental DNP setup could lead to the formation of glasses for the Li $^+$ solutions, this should not be the case for the Na $^+$ solutions (and even more so for the K $^+$ solutions). Clearly, one could argue that the pressure inside the spinning rotor is not exactly equal to the ambient pressure because of the fast-spinning speed of the rotor that exerts an additional centrifugal pressure on its inner wall (which can reach up to *ca.* 2 MPa when spinning at room temperature a 3.2 mm MAS rotor at 8 kHz).⁴² Still, the pressures necessary to suppress crystallization for concentrated NaCl solutions (4.5 M) were shown to be superior to 100 MPa.⁴¹ Remarkably, however, the ^{23}Na MAS ssNMR spectra recorded at *ca.* 100 K on all the aqueous solutions of NaCl only showed a single and broad resonance at *ca.* -6 ppm (with a linewidth at half height of *ca.* 1400 Hz/13 ppm), which indicated that the sodium

ions were not in a well-defined crystalline state but experienced a variation of local chemical environments that is symptomatic of a microscopically disordered state. This suggests that the presence of NaCl, even at low concentrations, affects ice crystallization in our experimental conditions, leading to a diminished or absent PA phase separation typically observed in water. Consequently, the restoration of DNP efficiency becomes feasible and reliable through an approach referred to as PASTIS (Polarization Amplification by Solubilizing Traditional Ionic Salts). We emphasize here that this approach is different from the work by Kubicki *et al.* where they added solid (insoluble) dielectric particles to the solution, resulting in an amplification of the microwave field in the sample and a concurrent increase in the DNP enhancement.⁴³ PASTIS represents a possible alternative to the use of the DNP juice whenever glycerol should be avoided,¹² but it is likely to be more suitable for analyzing water-soluble molecules of small to moderate molecular weights rather than proteins or other large biological assemblies, since the structure of the latter can be altered in the presence of large amounts of electrolytes. Still, using these experimental conditions, it could be of interest to study other water-soluble PAs,¹⁷ including at higher magnetic fields and faster MAS rates. Applications in dissolution DNP could also be explored.⁴⁴ Investigations along these lines will be addressed in our lab.

■ ASSOCIATED CONTENT

SI Supporting Information

Experimental details regarding sample preparation, NMR measurements and data analysis, are available (PDF).

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■ ABBREVIATIONS USED

CP, cross-polarization; DNP, dynamic nuclear polarization; MAS, magic-angle spinning; NMR, nuclear magnetic resonance; PA, polarizing agent.

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