J|A|C|S COMMUNICATIONS

Published on Web 02/04/2010

Optimizing the Polarization Matrix for *ex Situ* Dynamic Nuclear Polarization

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NMR spectroscopy is widely used in analytical sciences from basic chemistry to biomedical applications, although it has always displayed inherently low sensitivity. Despite the drive toward increasing field strengths and the introduction of cryogenically cooled probes, the improvement in sensitivity has been modest. This reality has driven interest in hyperpolarization techniques such as parahydrogen-induced polarization,[1,2] optical pumping,[3] and transfer of polarization from electrons by dynamic nuclear polarization (DNP).[4,5] The most limiting factor of DNP is that the conditions required for optimal polarization are not the same as normally employed to record NMR spectra. This has led to several implementations of DNP where samples are polarized at low temperatures and NMR spectra are subsequently recorded at higher temperature after heating of the samples.[4,5]

Golman and co-workers developed an experimental setup where the polarization is carried out *ex situ* at very low temperatures (1.3−1.5 K), followed by rapid dissolution with hot solvent to record the NMR spectrum in a separate high-field magnet at room temperature.[6] This implementation adds a large temperature factor and affords overall signal enhancement factors in the order of >10 000.[5]

However, such enhancements come at the price of the short lifetime of the polarization, which limits applications to the longitudinal relaxation times (T_1) of the involved nuclei.

This constraint has encouraged the use of ^{13}C observation because the T_1's of ^{13}C nuclei in small molecules are typically much longer than those of protons. In particular, quaternary carbon nuclei can have lifetimes of minutes. This led to *in vivo* applications of DNP where the metabolization of an isotopically labeled metabolic precursor was observed.[6,7]

DNP could also revolutionize NMR applications in structural analysis of small drug-like molecules. This is probably the largest field where routine NMR methods are commonly employed. However, for this, two-dimensional (2D) spectra such as ^{13}C/^{1}H correlation spectra, demanding long acquisition times, are required. This highlights the need for reliable polarizations that enable fast 2D NMR experiments yielding spectra of small molecules within the lifetime of the polarization of NMR nuclei. The principle choice of pulse sequences is between ultrafast NMR and small flip-angle heteronuclear multiple-quantum coherence (HMQC).[8−10]

Here we present an improvement in matrix design, which helps to obtain 2D NMR spectra in sufficiently short time to acquire heteronuclear 2D NMR spectra of small drug-like molecules. This is demonstrated for small molecules with limiting T_1 relaxation times. These improvements are driven by a key methodological improvement, the optimization of the polarization matrix.

In our experiments we addressed these points (i) by using an acetone/DMSO mixture which forms a glass, (ii) by the addition of ^{13}C(2)-acetone as a copolarization agent to optimize the network of nuclei that can participate in spin diffusion, (iii) by using DMSO-d_6 or D_2O to reduce the density of protons which can cause relaxation to ^{13}C nuclei, and (iv) by polarizing at $\omega_e + \omega_N$ rather than $\omega_e - \omega_N$ to achieve constructive interference of quantum rotor and Zeeman polarization.[11]

By combining an optimized gradient-selected HMQC sequence (see Supporting Information) using an incremented small flip-angle, adiabatic pulses to minimize losses, and a novel nonuniform sampling schedule based on the model described by Kazimierczuk et al.[12] (see Supporting Information), we obtained excellent results for many small molecules, here exemplified by 1D and 2D HMQC spectra of thiamine and naproxen.

The thiamine sample without copolarization agent was prepared by dissolution of 67.4 mg of thiamine (Sigma-Aldrich) and 2 mg of the trityl radical Ox63 (GE Healthcare) in 100 μL of a solution of glycerol and D_2O (1:1 v/v). The sample with copolarization was prepared by dissolution of 67.4 mg of thiamine and 2 mg of Ox63 in 100 μL of a solution of glycerol/D_2O/^{13}C(2)-acetone (1:1:1 v/v/v). A naproxen sample without copolarization agent was prepared by dissolution of 46 mg of naproxen (Sigma-Aldrich) and 2 mg of Ox63 in 100 μL of a solution of DMSO-d_6 and methanol (1:1 v/v). For the sample containing copolarization agent, 64 mg of naproxen and 2 mg of Ox63 were dissolved in 100 μL of a solution of DMSO-d_6/methanol/^{13}C(2)-acetone (1:1:1 v/v/v).

All samples were hyperpolarized for 90 min at 1.3 K in a HyperSense system (Oxford Instruments, UK) using a microwave frequency of 94.165 GHz. The hyperpolarized samples were then dissolved with 4 mL of hot (186 °C), pressurized (9 bar) methanol (final concentration in both samples 50 mM) and within 1.6 s transferred to a 500 MHz NMR spectrometer for NMR data acquisition. Small flip-angle excitation 2D-HMQC spectra were recorded, a nonlinear sampling scheme was employed to obtain a larger acquisition time in the indirect time domain, and the spectra were processed using an analytical Fourier transform with subsequent artifact removal[12] in the NMRLab[13] software package. See Supporting Information for a more detailed description of the methods.

Figure 1 shows 1D spectra for thiamine after polarization with and without a copolarization agent. While the NMR spectrum of the sample recorded without a copolarization agent shows only the quaternary carbons, most ^{13}C resonances of thiamine can be observed at a much better signal-to-noise ratio in the presence of the copolarization agent; only the resonances of nuclei C_2, C_6, C_{P6}, and C_{E2} are not observed as a consequence of fast relaxation. A maximum enhancement factor of ∼15 compared to the DNP experiment without the copolarization agent has been obtained for C_{E1} (see Supporting Information for additional details). For naproxen (Figure 2) in the absence of a copolarization agent, very small signals are observed for the aromatic carbon atoms, which become much stronger in the presence of the copolarization agent.

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10.1021/ja909984w

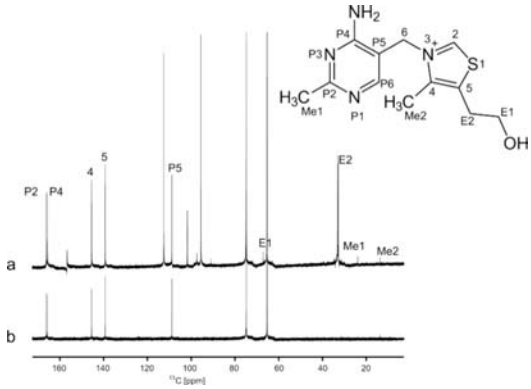

Figure 1. (a) ^{13}C 1D DNP-NMR spectrum of thiamine in the presence of ^{13}C(2)-acetone as a copolarization agent. The ^{13}C 1D spectrum was recorded after 90 min of polarization at 1.3 K. (b) The spectrum of thiamine was recorded after 90 min of polarization without copolarization agent. Artifacts and radical signals are indicated by "*" and "Rad", respectively.

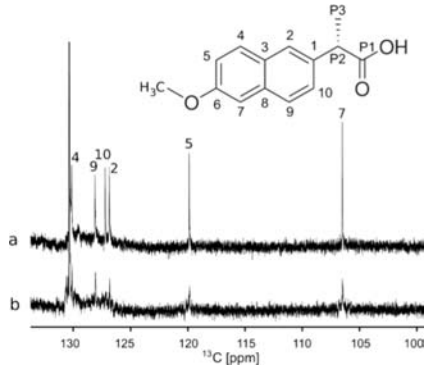

Figure 2. Aromatic region of the ^{13}C 1D DNP-NMR spectrum of naproxen. The compound was polarized at 1.3 K for 90 min either in the presence (a) or in the absence (b) of ^{13}C(2)-acetone as a copolarization agent.

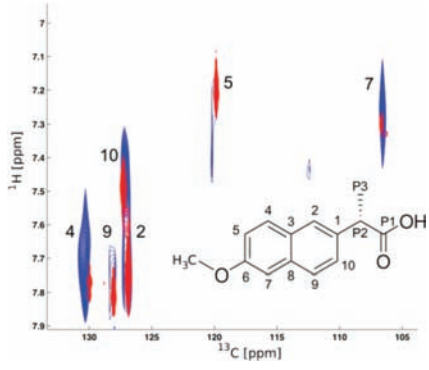

Figure 3. Overlay of the aromatic region of a 2D-HMQC NMR-spectrum of naproxen acquired with 32 data points in the indirect dimension using either conventional (blue) or nonuniform (red) sampling. Nonlinear sampling considerably reduces the line width in the incremented dimension.

For instance, the enhancement factor arising from the copolarization agent is ∼5 for most atoms. This is further illustrated by the 2D spectrum (Figure 3) recorded in the presence of ^{13}C(2)-acetone, which could not be obtained without the addition of the copolarization agent. Overall enhancement factors were up to 500 (see Supporting Information for details).

A copolarization agent must fulfill the following requirements. (i) It needs to form a glass state together with the other components of the polarization matrix.[14] The residual molecular movement possible in a solid glass is vital for the molecules to make optimal contact to the radical. (ii) More importantly, the copolarization agent needs to transfer polarization from initial polarization centers across the polarization matrix to the sample molecules, a process that involves spin diffusion involving mutual spin flips of adjacent spins. Spin diffusion scales proportional to r^{-6}, where r is the distance between individual spins and therefore depends on the availability of carbon spins in close proximity. Adding 50% of ^{13}C(2)-acetone yields a 6.8 M concentration of acetone, a factor of ∼340 higher than the concentration of the sample and a factor of 500 larger than that of the radical. By using a suitable polarization matrix, it is possible to obtain 2D spectra of small molecules with carbon nuclei that have relaxation times in the range of few hundred milliseconds.

Ex situ DNP-NMR should become widely applicable in analytical chemistry, in particular for pharmaceutical and biomedical applications, if a broad range of 2D spectra becomes possible. The acquisition of 2D NMR spectra is now possible in less than 1 min after 1−2 h of polarization. With foreseeable improvements of the technology, in particular reduction of sample dissolution and transfer times, at least another order of magnitude in sensitivity seems feasible.

Acknowledgment. We thank Oxford Instruments for the loan of equipment and supply of materials, the Wellcome Trust for supporting the HWB-NMR facility, the EU for support in the context of the EU-NMR grant (RII3-026145) and for supporting I.M.-M. in the context of the PRISM EU project, and BBSRC for supporting M.G.S. by a CASE studentship.

Supporting Information Available: Detailed pulse sequence, precise description of the nonuniform sampling regime, and additional spectra and parameters. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA909984W