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^{1}H and ^{13}C Dynamic Nuclear Polarization in Aqueous Solution with a Two-Field (0.35 T/14 T) Shuttle DNP Spectrometer

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Dynamic nuclear polarization (DNP) permits increasing the NMR signal of nuclei by pumping the electronic spin transitions of paramagnetic centers nearby.[1] This method is emerging as a powerful tool to increase the inherent sensitivity of NMR in structural biology aiming at detection of macromolecules.[2−4] In liquid solutions, DNP is governed by the Overhauser effect (OE), which depends on various physical parameters but overall loses efficiency with increasing magnetic fields.[5−10] In aqueous solution, additional issues associated with the penetration of microwaves in water and heating effects aggravate the experiment.[7−10] For this reason, to our knowledge, to date no work has been published on solution DNP in combination with high resolution NMR for structural biology. We have recently reported large ^{1}H-DNP enhancements (>100) on water solutions containing 4-hydroxy-amino-TEMPO (TEMPOL) by using state-of-the-art microwave technology at 9.7 GHz pumping frequencies.[8] To examine the feasibility of low-field (9.7 GHz/0.35 T) DNP in high resolution NMR, we have constructed the prototype of a two-field shuttle DNP spectrometer that polarizes nuclei at 9.7 GHz/0.35 T and detects the NMR spectrum at 14 T.[11] The advantage with respect to the method in ref 4 is that the sample is not frozen at any point and the experiment can be repeated unlimitedly, as required for example to acquire multidimensional NMR spectra. In this communication we report our first ^{1}H and ^{13}C DNP enhancements with an improved version of this spectrometer. Enhancements up to 15 were observed for small molecules at 14 T as compared to the Boltzmann signal. The results provide a proof of principle for the feasibility of a shuttle DNP experiment and open up perspectives for the application potential in solution NMR.

The principle of the shuttle DNP experiment is illustrated in Figure 1. In our prototype spectrometer, a 0.35 T EPR magnet is located above the 14 T NMR magnet at a center-to-center distance of 1.5 m (Figure 1a). The NMR signal is polarized via OE-DNP in a Bruker microwave (mw) resonator at 9.7 GHz/0.35 T and subsequently shuttled within 140 ms into the NMR 14 T magnet. Within this concept, the enhancements at the low field (0.35 T) have to overcome the magnetization ratio given by the field ratio 14 T/0.35 T = 40 to provide an effective enhancement at 14 T of $\epsilon_{\mathrm{eff}} = \epsilon(0.35\ \mathrm{T})/40$. Furthermore, in this prototype setup the sample travels through a very low field region (Figure S1) located between the two magnets.[11] Our previous calculations based on ^{1}H spin relaxation as a function of the magnetic field[12,13] let us predict that in this setup ^{1}H in proteins would relax completely during the transfer time and only enhancements on small molecules would be observable. Despite these limitations, the present results provided the essential step to define and optimize the feasibility of this experiment, as illustrated in the following.

The DNP enhancement at the low field was optimized by monitoring the ^{1}H NMR signal of the water protons with a Bruker Minispec spectrometer (5−60 MHz) and a tuned radio frequency (RF) circuit. A CW mw amplifier (Varian) provided up to 20 W of power. TEMPONE-^{2}H,^{15}N was employed as a polarizer at concentrations ranging from 5 to 25 mM. The samples were loaded into 0.9 or 0.46 mm inner diameter (ID) capillaries to a height of 3 (for ^{1}H) and 10 mm (for ^{13}C). The tubes were sealed with UV glue and placed in the shuttle container (Figure S2). At the low field position, the samples were irradiated for variable times (1−20 s) and then transferred to the high field position, where a 90° pulse was applied and the NMR spectrum was recorded (Figure 1b).

In Figure 1c we compare the NMR signal of the water protons with and without shuttle DNP. To avoid fast ^{1}H relaxation during the transfer time, we chose a low polarizer concentration of 5 mM, which gave a low field enhancement of −110 consistent with our previous data.[8] The effective signal enhancement at 14 T resulted in $\epsilon_{\mathrm{eff}} = -2.6$ when measured shortly after shuttle arrival in the high field position (i.e., with a delay time t_{d} of 145 ms).

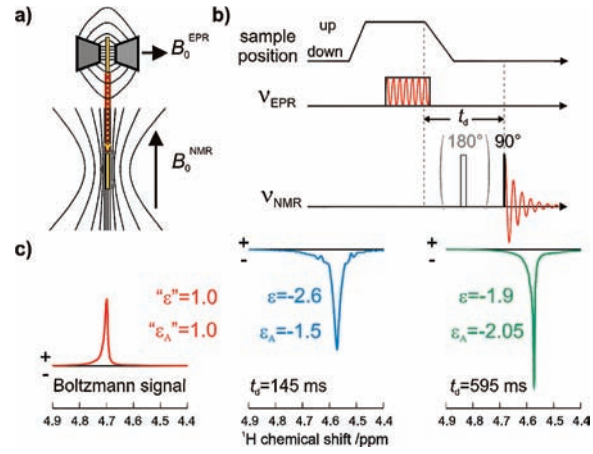

Figure 1. (a) Schematic of the shuttle spectrometer showing the two magnets and the sample pathway. (b) Time sequence of the DNP experiment. The 180° pulse probes the polarization sign via inversion recovery. (c) ^{1}H shuttle DNP enhancement of water doped with 5 mM TEMPONE-^{2}H,^{15}N. Compared are enhancements of the line integrals (ϵ) and the amplitudes (ϵ_{A}). The line widths are (Hz): 8.0 (left), 16.8 (middle), 6.8 (right). The line shape asymmetry is caused by difficult shimming of the small sample. Experimental parameters: 3 s mw irradiation (t_{irr}), $P_{\mathrm{mw}} = 5$ W, 0.9 mm ID sample tube, single scan.

The ratio of the measured low field and effective high field enhancements gives a factor of −110/−2.6 = 42 and indicates that no substantial water magnetization is lost during shuttling. At t_{d} = 595 ms the integral of the line decreases due to relaxation but the

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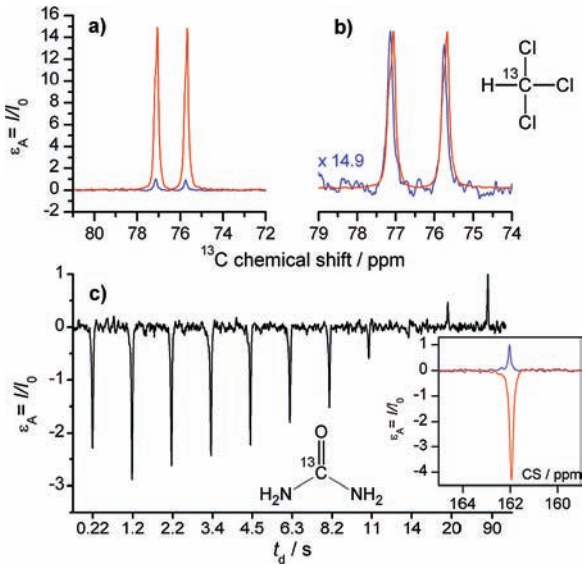

Figure 2. ^{13}C shuttle DNP enhancement of chloroform-^{13}C (a,b) and of urea-^{13}C (c, inset) in water with 25 mM TEMPONE-^{2}H,^{15}N. The enhanced signals (red) are compared with the Boltzmann polarization (blue) at 14 T (t_{irr} = 10 s (urea), 15 s (chloroform), 16 scans, P_{mw} = 20 W). Bottom: signal recovery of urea-^{13}C after shuttle DNP (8 scans/point, t_{irr} = 5 s, P_{mw} = 20 W). The time axis is in a logarithmic scale.

amplitude increases because mechanical ringing attenuates and the line narrows. Additionally, after shuttle DNP a shift in the resonance line of ~ 0.12 ppm is visible, which is attributed to sample heating ($\Delta T = 12°$ at the conditions in Figure 1c).

Figure 2 displays the ^{13}C signal enhancement of 99% ^{13}C labeled chloroform ($CHCl_3$) and of aqueous 4 M urea-^{13}C. Since the low field signal of ^{13}C was not detectable in our setup, the well documented[14] DNP experiment with $CHCl_3$ was used as a test for ^{13}C DNP. The high field enhancement of $CHCl_3$ is ~ +15 (Figure 2a) consistent with large positive enhancements previously observed.[14] A superposition of the spectra before and after DNP with t_d = 220 ms does not show broadening of the ^{13}C lines (Figure 2b), but sometimes the line intensity raises up until t_d is ~1 s (Figure 2c) indicating ringing times up to 1000 ms.

For 4 M urea, we observed negative enhancements with an ϵ_{eff} of -4 ± 1 at polarizer concentrations of 25 mM, Figure 2c. The sign of the enhancement was verified by observing a zero crossing in the recovery of the enhanced signal to Boltzmann equilibrium (Figure 2c). It has been pointed out[15] that the DNP enhancements in ^{13}C nuclei are contributed by a direct Overhauser effect between electrons and ^{13}C, which could be either dipolar (negative) or scalar (positive), but also by the indirect NOE from surrounding protons. To test a possible contribution of the ^{1}H−^{13}C NOE in the enhancements of urea, a series of samples containing an increasing D_2O/H_2O ratio (i.e., 25, 50, 75, 100% D_2O) and 25 mM polarizer were measured. All these samples showed negative enhancements in the range $3 < |\epsilon_{eff}| < 5$ without systematic dependence on the degree of deuteration. We therefore concluded that the ^{1}H−^{13}C NOE played a minor role, which is consistent with previous DNP data on different small molecules at large radical concentrations.[14] To gain additional insight into the observed DNP enhancement, we recorded ^{13}C-T_1 relaxation data of 4 M urea with 23 mM TEMPONE-^{2}H,^{15}N as a function of the external magnetic field (Figure S3). The curves for H_2O and D_2O are similar and show a smooth decrease in the relaxation rates from $1/T_1 \approx 0.85$ s^{-1} at $B = 5 \times 10^{-4}$ T to ~ 0.2 s^{-1} at 9.4 T. The absence of a sharp dispersion and a low field to high field ratio larger than 10/3 suggest that outer-sphere and inner-sphere dipolar and contact contributions should be included in the fit. However, a reliable fit is not possible due to the relatively large errors of the data. A small contact contribution could account for counteracting dipolar and scalar mechanisms. The complex mechanism of hyperfine interaction with ^{13}C nuclei, which strongly depends on the bond polarization,[16] leads to the expectation that both mechanisms contribute to DNP in a way that is difficult to predict because it depends on the detailed chemical structure. The T_1 data were used to estimate the ^{13}C relaxation during the shuttling. A calculation based on the field-path diagram (Figure S1) and t_d = 140 ms indicates a 7% magnetization loss after the low-field DNP process and corresponds to $\epsilon(0.35\ T) \approx -220$ in Figure 2c.[17]

In conclusion, our results demonstrate that DNP in aqueous solution combined with high resolution NMR detection is feasible in a shuttle DNP spectrometer. The limitations were posed by the presented construction, which shuttles the sample between two magnets through a very low field region, and no enhancement on molecules larger than urea could be observed. The sample volumes used so far are an order of magnitude (>10) smaller than those in conventional NMR experiments. While efforts are undertaken to increase the volume and filling factors,[18] the method should be valuable to investigate biomolecules available in very small quantities. The results prompted the construction of a new two center magnet that will transfer the sample from 0.35 T through an increasingly higher field up to 14 T. The much shorter (~ 0.5 m) pathway will reduce the shuttle times and the relaxation losses. Predicted from our considerations on relaxation[12,13] (Figure S1) the new setup should allow for DNP enhancements of biomolecules.

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Supporting Information Available: Magnetic field profile of the setup; Relaxation rates as a function of the sample position and of τ_c; Improved hardware; Urea-T_1 relaxation dispersion in H_2O and D_2O. This material is available free of charge via the Internet at http://pubs.acs.org.

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(17) The negative enhancements are consistent with enhancements of−200 observed directly at X-band (D. Hinderberger, private communication).
(18) The NMR filling factor could be improved by reducing the dimensions of the NMR coil (10 mm), which adjusts a shuttle of 4.1 mm outer diameter.

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