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Producing over 100 ml of highly concentrated hyperpolarized solution by means of dissolution DNP

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Abstract

New low-temperature inserts compatible with an existing hyperpolarizer were developed to dynamically polarize nuclei in large samples. The performance of the system was tested on 8 ml glassy frozen solutions containing ^{13}C-labeled molecules and doped with nitroxyl free radicals. The obtained ^{13}C low-temperature polarization was comparable to the one measured on 20 times smaller sample volume with only 3–4 times higher microwave power. By using a dissolution insert that fits to the new design, it was possible to obtain about 120 ml of room-temperature hyperpolarized solution. The polarization as well as the molecule concentration was comparable to the values obtained in standard size hyperpolarized samples. Such large samples are interesting for future studies on larger animals and possibly for potential clinical applications.

Keywords

Hyperpolarization; Dissolution DNP; Dynamic Nuclear Polarization; 13C; TEMPO

INTRODUCTION

Biomedical applications of the recently developed DNP-based hyperpolarization technique, where DNP stands for Dynamic Nuclear Polarization, seem very promising. The clinical potential of the technique in tumor treatment was recently discussed [1], but it can be of great interest for many in vivo and in vitro MR pre-clinical studies and several applications using hyperpolarized ^{13}C-labeled molecules have been reviewed by Golman and Petersson [2]. The intrinsic limitation of the technique is the finite life time of the hyperpolarized state of the molecule, which is determined by the longitudinal relaxation time of the nuclei of interest. The hyperpolarized state cannot be recovered once the magnetization is destroyed and therefore, although it is very intense, the signal is only available for a limited amount of time. In addition to this time restriction, the delay between two consecutive experiments can hardly be shorter than one hour with the current methods [3;4]. Various in vivo non-proton MR studies would

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benefit from the ability to perform consecutive injections of small quantities of hyperpolarized solution separated by short time intervals. This is particularly true for substances which have limited uptake. Since the nuclear spin-lattice relaxation times in a typical DNP-polarized sample are usually on the order of tens of minutes at liquid helium temperature, a possible scheme to obtain consecutive amounts of hyperpolarized solution is to implement a multiple-dissolution system which allows for the successive extraction of a fraction of a large solid-state polarized sample.

The first aim of the present study was to demonstrate that it is possible to highly polarize a large volume of frozen biologically-compatible substance by slightly modifying an existing DNP prepolarizer and with a modest increase in microwave power. This is a first and required step towards a multiple-dissolution system. The second aim was to develop an apparatus to dissolve the entire large sample at once and hence produce the largest possible volume of hyperpolarized solution while keeping the same molecule concentrations and liquid-state polarizations. So far, the largest quantity of hyperpolarized media obtained via the dissolution-DNP method was reported to be on the order of 19 ml and was used for in vivo pig experiments [5]. Although this corresponds to quite a large volume, the solid-state sample prior to dissolution was relatively small (about 0.4ml) and not all molecules are as highly soluble as pyruvic acid in biologically-compatible solvents. With most molecules, in order to obtain a large volume of highly concentrated hyperpolarized solution, it is therefore necessary to increase the solid-state sample volume. The ability to produce a large volume of hyperpolarized solution would allow in vivo studies in large animals and would open the way for potential clinical use. Additionally, recent studies showed that spin order can be preserved during time intervals that were up to approximately 40 times longer than the spin-lattice relaxation time using long-lived states [6;7]. Methods for creating long-lived states are still under development and certain requirements are imposed on the number of interacting spins in the sample and on the geometry of their spatial distribution [8;9;10]. However, these results indicate that room-temperature liquid-state polarization could be stored for a long time while small fractions are extracted for experiments.

EXPERIMENTAL

DNP hardware components

Hyperpolarizing a sample by means of DNP consists essentially in a two-step process: first the low-temperature nuclear spin polarization is enhanced via DNP, a technique that has been used in particle physics for several decades [11]. This process is most efficient at low temperature when the electron spin polarization is close to 100% and the nuclear spin relaxation times are very long (on the order of 1000 s). This step is performed by placing the molecule embedded in a frozen glassy solution doped with free radicals in a pumped liquid helium bath and by irradiating it with microwaves at a frequency close to the radicals electron spin resonance frequency. The second step consists in dissolving the frozen sample in superheated water and was only recently developed [3]. Between the two steps, the setup has hence to be changed from an irradiation configuration to a dissolution configuration.

The DNP prepolarizer used for this study was described in a previous paper [12], but the DNP inserts were redesigned. A cross-sectional view of the low-temperature inserts in the irradiation configuration is sketched in Fig.1(a). The modified sample holder has a net volume of 13.5 ml instead of 0.6 ml, corresponding to a useful volume of about 8–9 ml for sample beads. It is placed in a 29 mm external diameter glass fiber tube. The microwave cavity of the modified main insert consists in a brass cylinder of 34 mm inner diameter and 64 mm height, open at the top. The coupling horn antenna of the microwave insert located inside the microwave cavity during microwave irradiation has an external diameter of 26.6 mm.

The dissolution insert used in the dissolution configuration was modified to adapt to the large sample holder. The water boiler capacity is 170 ml and 4 identical 2 mm inner diameter capillary tubes connect it to the sample volume. The coupling between the dissolution insert and the sample holder is made through a solid PTFE (polytetrafluoroethylene) piece that is fixed to a 25 mm external diameter carbon fiber tube. It has 4 capillary inlet ports and one outlet port. Two of the 4 capillary inlet ports as well as the 3 mm diameter outlet port are shown on the cross-sectional view sketched in Fig.1(b). Immediately after dissolution, the hyperpolarized solution is pushed out of the prepolarizer through a 3 mm inner diameter capillary tube.

Methods

The low-temperature solid-state NMR measurements were performed with a remotely-tuned NMR coil placed just outside the glass fiber tube (see Fig.1). The coil is connected to tuning capacitors located outside the cryostat via a 1.2 mm diameter coaxial cable (Rosenberger Micro-Coax UT-141-SS). This circuit is rather lossy, but the signal was large enough to evaluate and follow the polarization. Once the polarization has reached a satisfactory level, the setup is changed to dissolution configuration. The most critical point is the insertion of the dissolution insert inside the cryostat and in particular, docking the PTFE piece around the sample holder to form a leak-tight seal to keep the hot water from leaking into the cryostat. This is especially important when operating with such a large amount of water. The delay between the docking time and the dissolution was rather long (on the order of 10 s) because several manual safety checks have to be performed before opening the valve releasing the water from the heater. A complete automation of the procedure could greatly shorten this delay.

About 1 s after dissolution of the beads in 120 ml of superheated deuterated water (~170 °C), a high-pressure (~4 bars) helium gas stream was used to drive the solution out of the polarizer, through a 12 m long capillary, to the isocenter of an actively-shielded 9.4 T animal scanner (Varian, Palo Alto, CA, USA/Magnex Scientific, Abingdon, UK). The sample was collected in a two-stage phantom connected to a gas exhaust to release the pressure and separate the solution from the gas. The labeled molecule and free radical concentrations in the final solution are about 16 times lower than in the initial sample. This number corresponds to what is commonly called the dilution factor. Carbon spectra were acquired through single pulse experiments using a three-loop surface coil of 10mm diameter. A small sphere filled with ^{13}C-labeled formic acid was fixed in the center of the coil in order to generate a reference signal. The coil was placed on the bottom surface of the phantom along with a proton butterfly surface coil used for shimming. Shimming was performed using the FASTESTMAP algorithm [13].

RESULTS AND DISCUSSION

Solid-state nuclear polarization

To test the performances of the system, glassy frozen 3 M 1-^{13}C labeled sodium acetate solutions containing 33 mM of TEMPO (2,2,6,6-tetramethyl-1-piperidinyloxy) were prepared in the form of ~2 mm diameter beads. The solvent was composed of 90% D_2O and 10% d_6-ethanol. This composition leads to the largest ^{13}C polarization on acetate in water/ethanol mixtures doped with TEMPO [14]. The typical sample volume was 8 ml. The nuclear spins were dynamically polarized at a sample temperature of 1.4 K. The working temperature was higher than with the standard size low-temperature inserts (1.1 – 1.2 K) due to the larger size glass fiber tube yielding greater thermal losses. The optimal irradiation frequency was found to be 93.91 GHz by scanning the microwave frequency. A typical ^{13}C polarization curve of 1-^{13}C acetate is presented in Fig.2 for both the standard insert described in [12] and the new large sample insert. Both samples were from the same batch. The polarization time constants were 1800±100 s and 2300±100 s respectively. Note that the microwave power setting was

separately optimized in each case. The polarization values measured on the large samples were about 20% smaller than for the standard size samples due to the fact that the sample space temperature was about 20% higher (1.4 K instead of 1.15 K). The Borghini model indeed predicts a linear dependence of the nuclear spin polarization on the inverse temperature [12; 15], even when the electron spin polarization is near unity. Similar results were obtained on 2.8 M sodium pyruvate solutions (18/82 d_6-ethanol/D_2O volume ratio) doped with 33 mM of TEMPO, although the maximum ^{13}C polarization observed in such samples was slightly lower (7±0.5%).

The microwave power dependence of the maximum polarization is shown in Fig.3. As already observed previously, the largest polarization is obtained with 20–30 mW (at the source output) for standard size samples [12]. The optimum power for large samples was found to be about 100 mW, which is rather low considering the 20-fold increase in sample volume. However, the cavity volume V is only about 2.5 times larger and, if the differences in Q factor and in filling factor η between the two cavities are neglected, it is expected to only need 2.5 times more power P to obtain the same microwave magnetic field B_1 since $B_1 \alpha \sqrt{P\eta Q/V}$ [16]. Finally, it is worth mentioning that the observed polarization time constants for large samples slightly decrease with increasing power (1800±100 s at 300 mW) while the polarization stays essentially constant.

Liquid-state nuclear polarization

A volume of about 120 ml of room-temperature hyperpolarized solution was collected in the phantom. The time evolution of the liquid-state NMR signal was measured by applying an adiabatic 10 degree BIR- 4 pulse every 3 s. A typical set of resulting spectra are plotted in Fig. 4. Only a fraction of the hyperpolarized solution was located within the sensitive volume of the surface coil, but the whole volume is expected to be homogeneous as a consequence of mixing forced by the high pressure helium gas used to transfer the solution into the phantom. A strong signal at the frequency of the carboxyl ^{13}C of sodium acetate was detected 8 s after dissolution. Its integral was about 1800 times larger than the one measured afterwards on the thermal equilibrium signal. The doublet centered at 169.4 ppm corresponds to the ^{13}C-labeled formic acid reference. The observed decay time constant of the hyperpolarized acetate signal was 40 ± 2 s in all experiments. It was determined by fitting the time dependence of the signal integral with an exponential decay curve after having corrected for the lost in signal due to the RF pulses. The T_1 was subsequently determined by performing an inversion recovery relaxation measurement. Its value was found to be identical to the decay time constant (42 ± 2 s). Note that the observed decay time constant is the same as the one measured on standard size samples.

Slight discrepancies were observed in the time evolution of the spectra 16 s after dissolution, time at which the helium gas overpressure was stopped. As can be seen on Fig.4, the amplitude of the spectra recorded 17 s and 20 s after dissolution is indeed slightly lower than the expected value based on the extrapolation of a smooth exponential decay. This is most likely due to turbulences in the phantom, which yielded to a slight modification of the coil loading. The enhancement factor observed after dissolution is about 3 times smaller than the one measured in standard-size samples for the same initial polarization. The reason for this difference comes from the non-optimized dissolution procedure and in particular from the fairly long delay between the docking time and the actual dissolution. During this delay, the sample is still in a solid-state but at a much higher temperature than liquid helium temperature and its relaxation time is therefore expected to be rather short.

In vitro measurements were independently performed in a 400 MHz high–resolution NMR system (Bruker, Germany) in order to determine the dependence of T_1 on the TEMPO

concentration in a 0.1 M 1-^{13}C acetate solution dissolved in D_2O. The results presented in Fig. 5 show that the relaxation rate $1/T_1$ is proportional to the TEMPO concentration, in agreement with the theory of Korringa, Seevers and Torrey [17]. According to the linear least squares fit plotted in Fig.5, the extrapolated TEMPO concentration corresponding to a relaxation time of 42 s is about 2.2 mM. This is precisely the expected concentration calculated from a dilution factor of 16 and an initial concentration of 33 mM. This confirms that the entire frozen solution was dissolved and transferred into the phantom, and that the acetate concentration in the hyperpolarized solution should be 3/16= 0.19 M, as expected.

CONCLUSION

Using new hardware components compatible with an existing DNP prepolarizer, it was demonstrated that it is possible to increase the solid-state sample volume by a factor larger than 20 while essentially keeping the same polarization efficiency as for standard size samples. A multiple-dissolution system is expected to be compatible with this large sample volume design. From a large solid-state sample, it was shown that about 120 ml of highly concentrated hyperpolarized solution can be produced. Part of the low-temperature polarization was kept through the dissolution process although the enhancement factor was smaller than the one observed with standard volumes. This is most likely due to the non-optimized dissolution procedure. After optimization, the main results previously obtained on standard volumes should essentially hold.

These results open the way to new potential hyperpolarized MR studies in large animals and the volume of hyperpolarized media that can be produced would be large enough to consider clinical applications. However, toxicity issues due to the presence of a few mM of free radicals in the hyperpolarized solution will have to be addressed and it is not the purpose of the present paper to discuss the various constraints, such as the maximum injection rate, related to clinical experiments.

Acknowledgments

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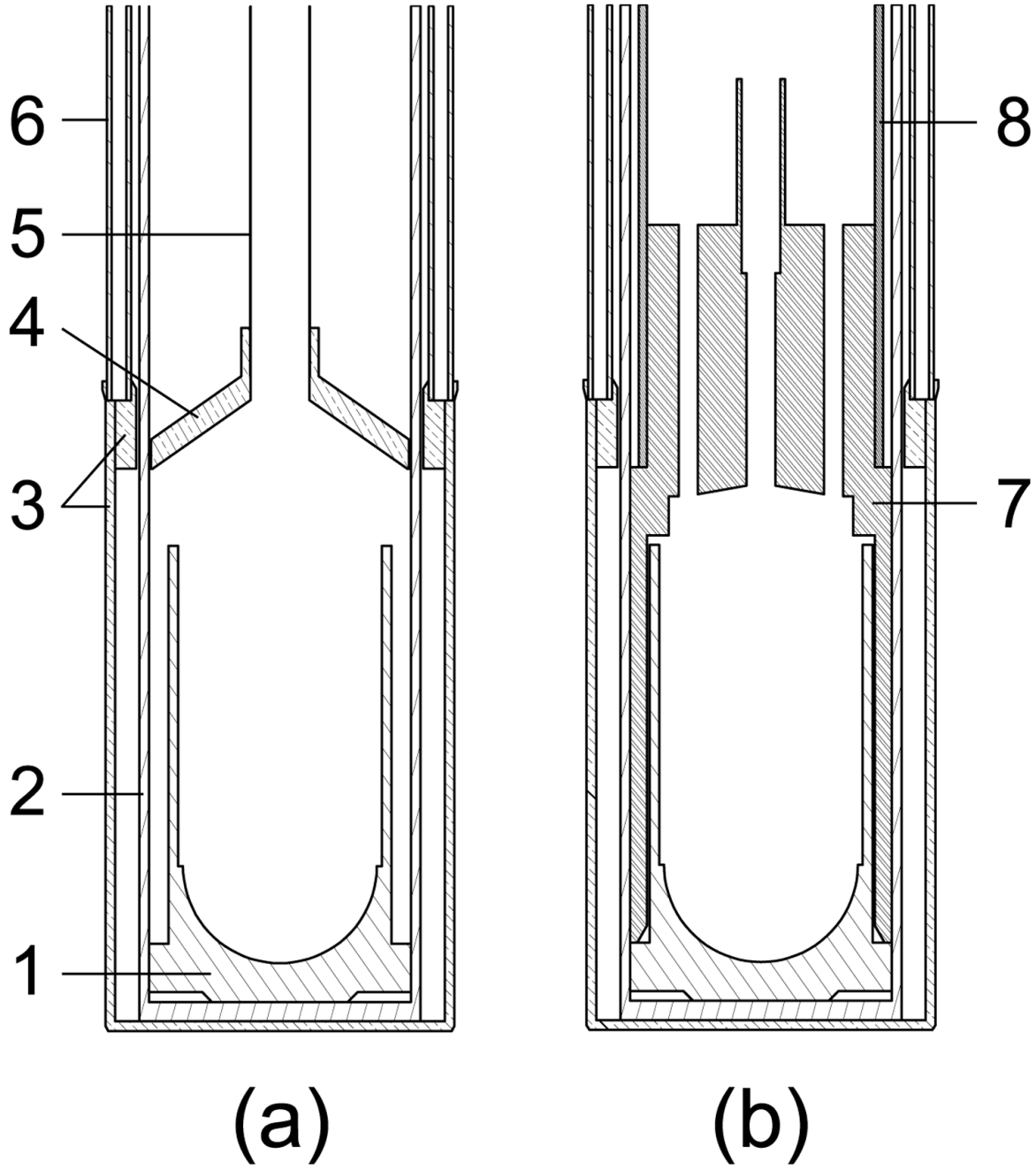

Figure 1.
Sketch of the inserts cross-sectional view: (a) irradiation configuration (b) dissolution configuration. The parts are numbered as follows: (1) sample holder, (2) glass fiber tube, (3) microwave cavity, (4) horn antenna, (5) cylindrical waveguide, (6) supporting thin tubes, (7) PTFE coupling piece, (8) carbon fiber tube.

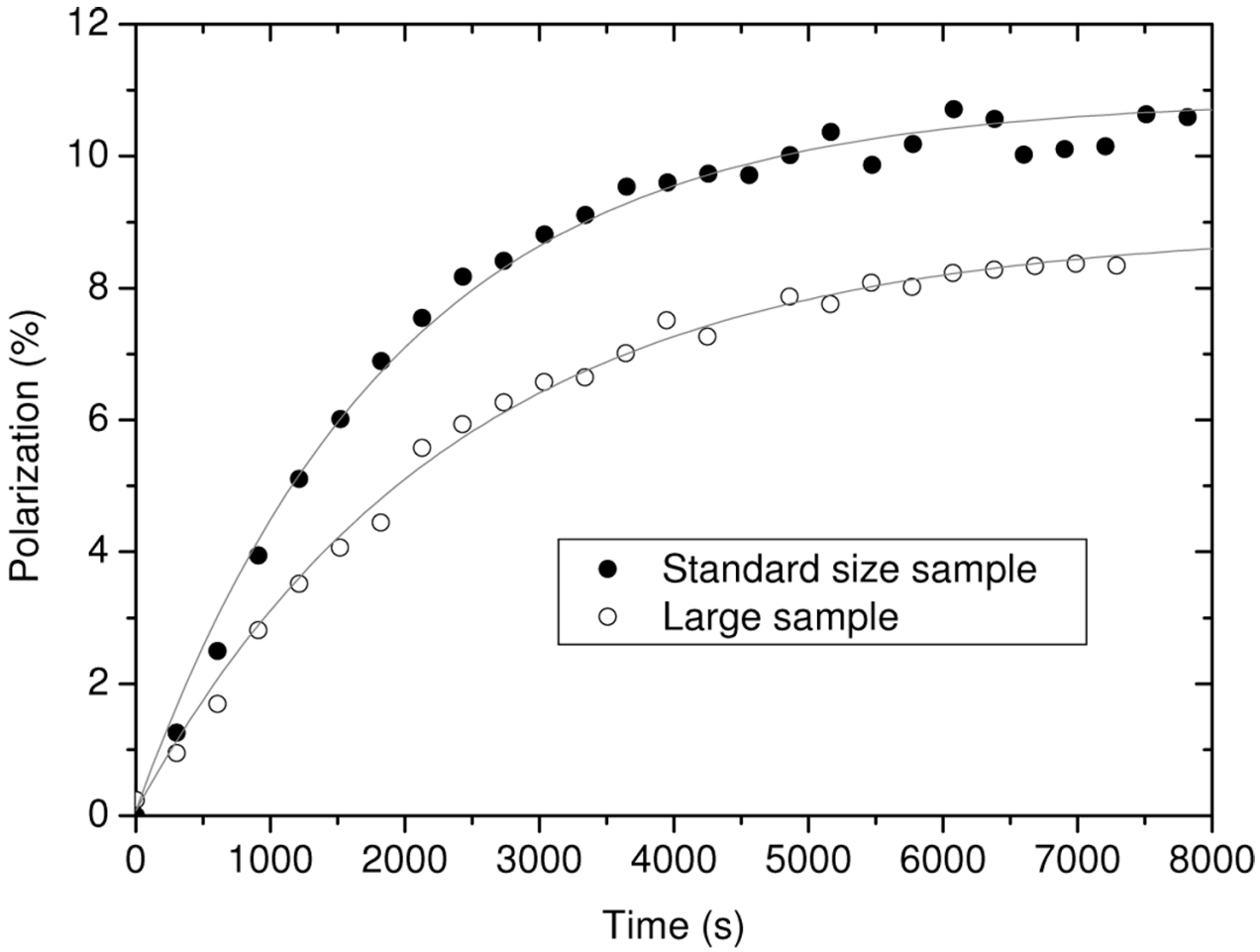

Figure 2.
Polarization curves of ^{13}C nuclei in 1-^{13}C labeled sodium acetate. The microwave power was set to 30 mW for the standard size sample and 100 mW for the large sample. The polarization time constant was found to be about 1800 s for the standard size sample and 2300 s for the large sample.

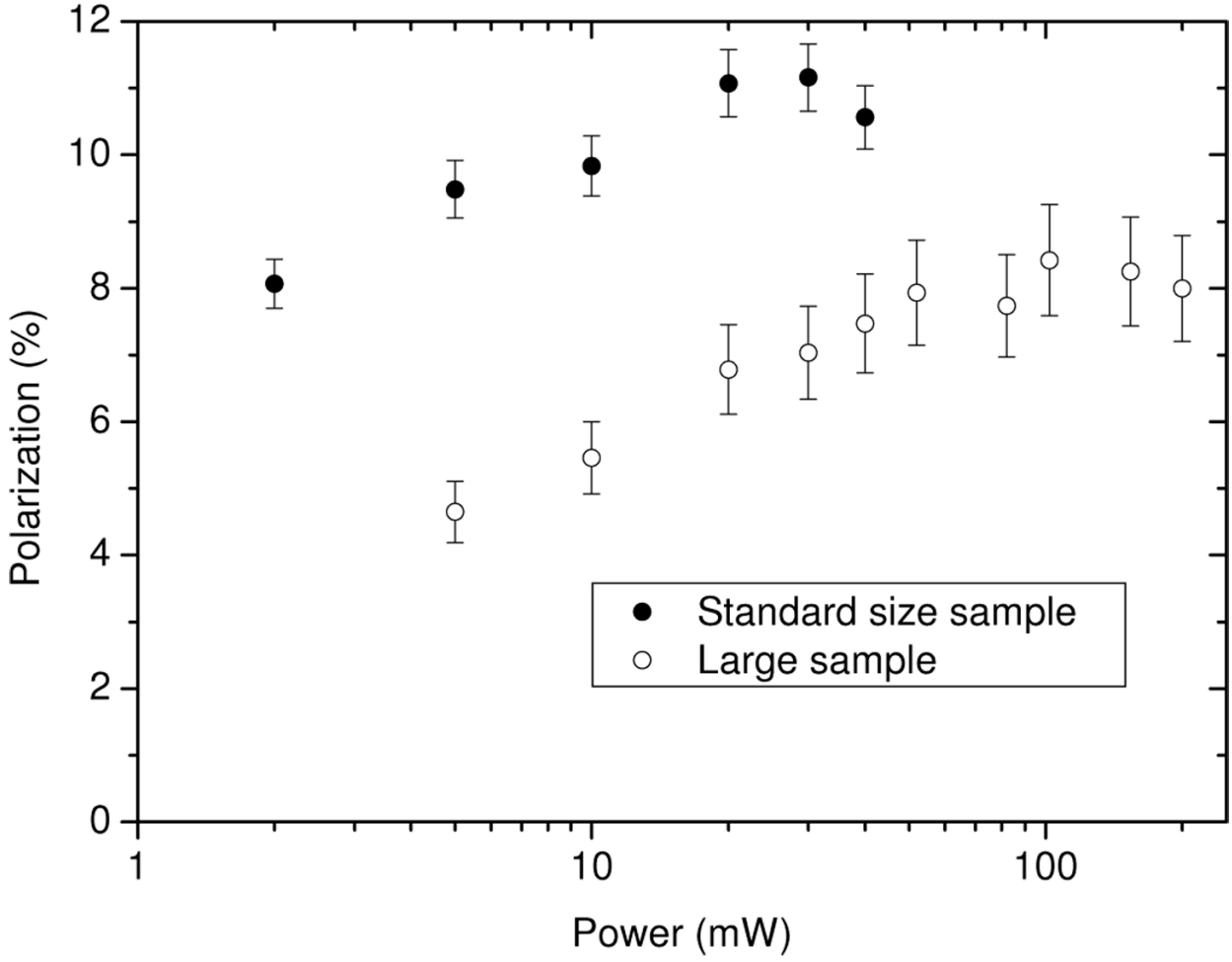

Figure 3.
Maximum ^{13}C polarization as a function of the microwave power at the source output for both the standard size sample and the large sample.

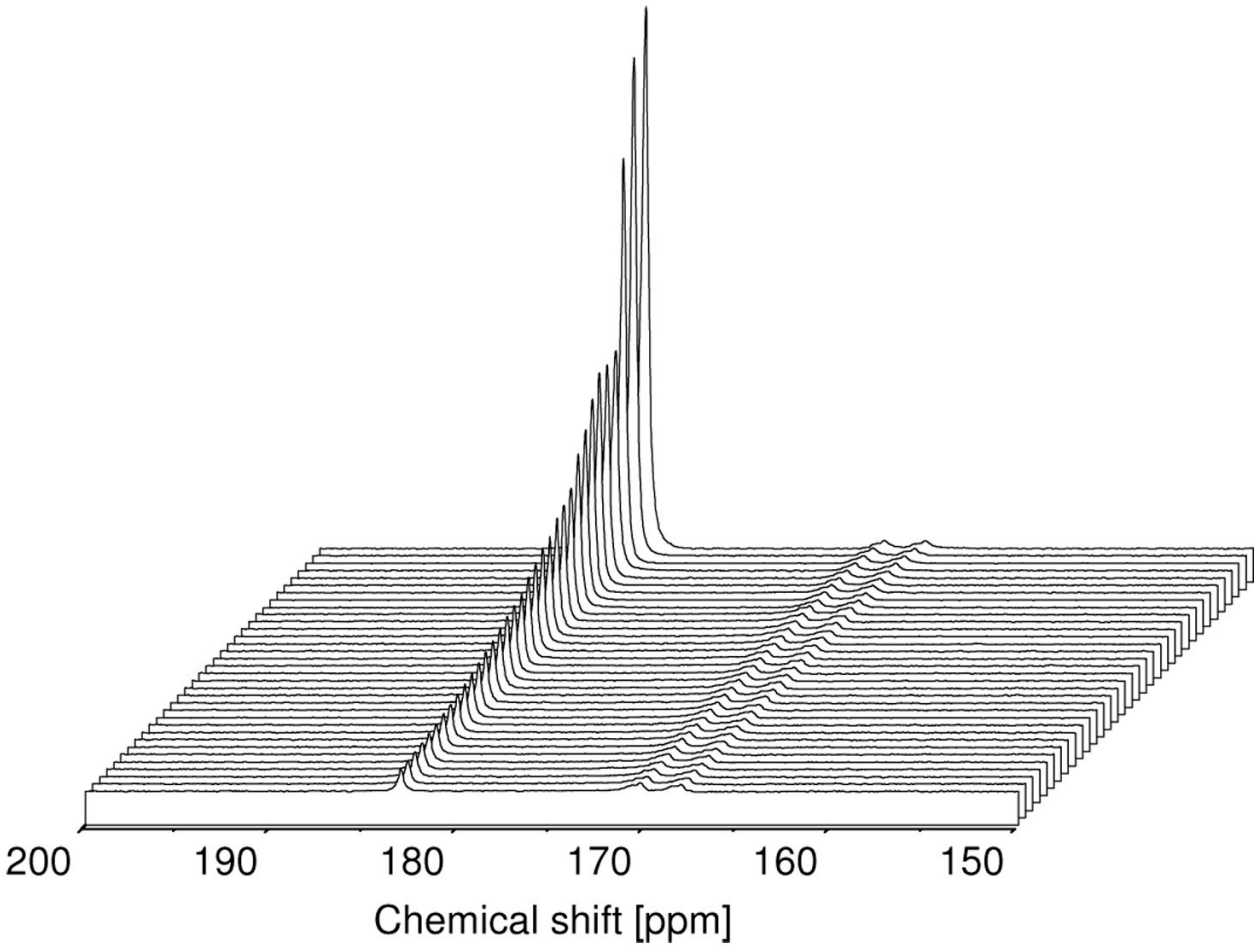

Figure 4.

Time evolution of the ^{13}C signal in the 9.4T scanner after dissolution of the DNP-enhanced ^{13}C-labeled acetate frozen solution. Spectra were recorded at 3 s intervals, starting 8 s after dissolution. The doublet centered at 169.4 ppm corresponds to the formic acid reference.

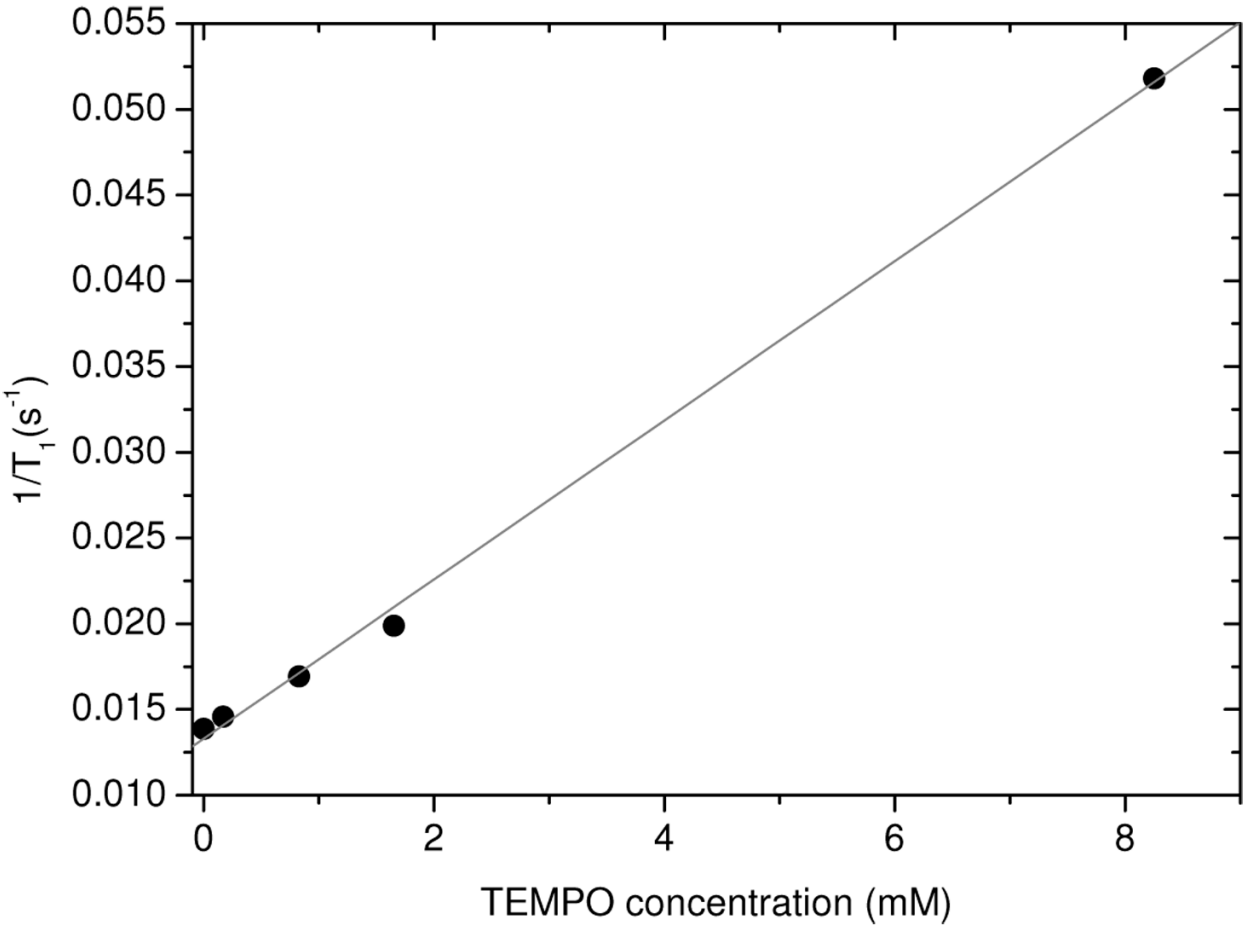

Figure 5.
^{13}C spin-lattice relaxation rate of 1-^{13}C sodium acetate dissolved in deuterated water (0.1 M solutions) as a function of TEMPO concentration. The samples were degassed using a standard freeze-pump-thaw procedure and the measurements were done on a 400 MHz high-resolution system.