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Achieving 1% NMR polarization in water in less than 1 min using SABRE

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Abstract

The development of biocompatible hyperpolarized media is a crucial step towards application of hyperpolarization *in vivo*. This article describes the achievement of 1% hyperpolarization of 3-amino-1,2,4-triazine protons in water using the parahydrogen induced polarization technique based on signal amplification by reversible exchange (SABRE). Polarization was achieved in less than 1 min.

Keywords

SABRE; Parahydrogen; Hyperpolarization

Hyperpolarization can improve NMR sensitivity by producing nuclear spin polarization far beyond that available at thermal equilibrium. Using improved sensitivity generated by hyperpolarization, NMR can now detect less concentrated molecules. Recent progress has shown applicability to the study of the distribution [1] and metabolism [2–4] of hyperpolarized molecules *in vivo*. For instance, the metabolism of hyperpolarized ¹³C-labeled pyruvate to lactate has been used to study cancer malignancy [2–6]. This technique is already being translated to clinical scanners and a first trial is ongoing [7].

Compared to dynamic nuclear polarization (DNP) [8,9], parahydrogen induced polarization (PHIP) [10–15] is a relative cost effective technique to hyperpolarize small molecules. Parahydrogen is a spin isomer of hydrogen with an antisymmetric singlet spin state. Large NMR signal enhancements have been obtained by incorporating this pure spin state into a molecule through a hydrogenation reaction [11,13]. In 2009, Duckett's group developed a parahydrogen polarization technique named signal amplification by reversible exchange

(SABRE), in which chemical modification of the substrate is not necessary [16]. In this approach, when the substrate and the parahydrogen bind to a catalyzing metal complex concurrently, the polarization is transferred to the substrate through the scalar coupling network. Release of the polarized substrate allows new substrate to bind to the metal center for polarization. SABRE has already been applied to detect trace amounts of chemicals [17–20] and can be used in conjunction with zero-field NMR spectroscopy [21].

Preparation of hyperpolarized samples for *in vivo* application requires that the sample is in aqueous solution, generally physiologically buffered saline. In addition, it is important to remove the toxic additives that are used for hyperpolarization and to control for appropriate pH and temperature. For DNP, samples can be prepared in water solution by dissolution of a cold (~1 K) polarized sample with over-heated water buffer. In order to obtain PHIP polarized substrates in water, the unsaturated substrate is reacted with parahydrogen in acetone solution first, followed by fast evaporation and dissolution in water [22–24].

Most SABRE polarization studies, however, have been done using methanol-d₄ as a solvent, which is not suitable for injection *in vivo*. According to the SABRE theory, the signal enhancement level depends heavily on the binding kinetics and spin relaxation [25], which are related to the properties of the solvent. Therefore, it is crucial to choose the appropriate solvent to achieve maximum polarization. In our previous work, we investigated SABRE polarization of two drugs in methanol, ethanol and DMSO [26]. Others measured a slight enhancement in an ethanol and water mixture [27]. Unfortunately, at present, the best IMes based catalyst, IrCl(COD)(IMes) (COD = cyclooctadiene, IMes = 1,3-bis(2,4,6-trimethylphenyl)imidazole-2-ylidene) is insoluble in water. One way to address this is by incorporating some hydrophilic groups [28]. However, modification of the structure of the catalyst decreases the SABRE enhancements. We therefore investigated the possibility of SABRE polarization using IrCl(COD)(IMes) in water, which is ready for *in vivo* applications.

The chemical to be polarized was chosen to be 3-amino-1,2,4-triazine (ATZ). ATZ exhibits various interesting biochemical properties [29,30] and its structure has been studied in detail [31,32]. ATZ is a heterocyclic aromatic organic compound, containing three nitrogen atoms in the aromatic ring, which are able to bind to the iridium atom of the catalyst precursor. Therefore, it is suitable for SABRE polarization.

The polarization magnetic field was optimized using a sample containing 0.40 mM catalyst and 4.0 mM ATZ in methanol solution at room temperature. The best enhancement of ~50 times was achieved at 65 G (see Fig. 1), similar to SABRE polarizations of other molecules when using the same catalyst [33,34].

The sample in methanol was bubbled with hydrogen for at least 2 h after which 3 mL water was added. This process has been described to improve polarization in water-methanol mixtures [35]. The methanol in the mixture was evaporated in vacuum using a 40 °C water bath for 10 min. After filtering, the sample was injected back into the mixing chamber for polarization. The solvent contained 90% of water and 10% of methanol in molar fraction.

In the SABRE scheme, a sample is polarized outside the main magnet by bubbling parahydrogen through it. When the polarization is finished, the sample is transported pneumatically to the pre-shimmed 750 MHz NMR probe in the main magnet using nitrogen pressure. The solvent signals were suppressed using excitation sculpting with gradient dephasing [36]. The pulse sequence was [hard $\pi/2$ -G₁-shaped π -hard π -G₁-G₂-shaped π -hard π -G₂-acquisition]. This dephases the two solvent peaks while refocusing all other signals. Different from other solvents, the water-methanol mixture is prone to form a large amount of foam during hydrogen bubbling. Therefore, it is very tricky to transport the sample in water-methanol mixture. For practical consideration, we therefore minimized the number of transportation cycles. Because only a limited number of polarization cycles could be achieved for each freshly made sample, we did not optimize the polarization conditions in water-methanol mixture. According to our previous work, in most solvents, the best SABRE polarizations were achieved at a magnetic field of 65 G and a temperature of 54.4 °C with a polarization time of 30 s. The sample in 90/10 water/methanol was therefore polarized under these conditions. Compared to the spectrum acquired with thermal polarization, an enhancement of 170 ± 45 times (Fig. 2 left) was achieved, corresponding to an absolute polarization of 1%. The peaks between -10 and -5 ppm in the spectral with catalyst (Fig. 2. top and middle) belong to various forms of iridium hydride. The peaks are broadened due to that the soft shaped pulses do not refocus this spectral range properly. The spectrum of pure substrate in the same solvent is plotted for reference.

It would be very important to polarize compounds such as drugs and metabolites. We therefore polarized some compounds with similar structure as ATZ, such as pyridine, isoniazid and pyrazinamide. However, no SABRE enhancement was observed, even though the enhancement of ATZ in methanol, was similar to that of pyrazinamide and higher than isoniazid and pyridine (Table 1). Further study on the mechanism of SABRE polarization in water is needed in order to polarize more compounds.

In this communication, we presented an easy and cost effective way to achieve 1% of ¹H polarization in water with SABRE in less than 1 min. We expect that the polarization can be further improved by using a higher concentration of catalyst [37]. Compared to DNP (2–8% of ¹H polarization) [38–40] less polarization could be achieved, but DNP polarization takes 30 min and needs a large amount of liquid helium. The recent development of SABRE allows continuous polarization of substrates [41]. We speculate that combination of this approach with polarization in water has the potential for injecting SABRE polarized substrates *in vivo*.

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Fig. 1. Enhancement of the two ATZ protons (red and blue curves) as a function of polarization magnetic field strength in methanol at room temperature. It was not possible to assign the protons. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fig. 2. Enhancement of ATZ protons in 90% water 10% methanol at 54.4 °C (top) compared to thermal polarization (middle). The bottom is the spectrum of the substrate without catalyst (16 averages).

Table 1

Summary of the substrates studied in water/methanol mixture.

Substrate	3-Amino-1,2,4-triazine (ATZ)	Pyridine	Isoniazid	Pyrazinamide
Solvent Polarization	90/10 water/methanol 1%	<i>a</i>	<i>a</i>	<i>a</i>

^a No signal was observed.