High-throughput continuous-flow system for SABRE hyperpolarization

Petr Štepanek a,*, Clara Sanchez-Perez b,1, Ville-Veikko Telkki a, Vladimir V. Zhivonitko a, Anu M. Kantola a,∗

aNMR Research Unit, Faculty of Science, University of Oulu, P.O. Box 3000, FI-90014, Finland
bEnvironmental and Chemical Engineering, Faculty of Technology, University of Oulu, FI-90014, Finland

Corresponding authors.
⇑
1 Present address: Department of Chemistry, University College London, 20 Gordon Street London WC1H 0AJ, United Kingdom.

E-mail addresses: petr.stepanek@oulu.fi (P. Štepanek), clara.sanchez@oulu.fi (C. Sanchez-Perez), ville-veikko.telkki@oulu.fi (V.-V. Telkki), vladimir.zhivonitko@oulu.fi (V.V. Zhivonitko), anu.kantola@oulu.fi (A.M. Kantola).

© 2019 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

1. Introduction

Nuclear magnetic resonance (NMR) is an invaluable method finding applications in many fields ranging from physics through chemistry to biological studies. It is a very versatile technique, offering ways for elucidation of complex molecular structures [1], measuring dynamic molecular processes [2] or investigating porosity of materials [3]. Moreover, it forms a basis of magnetic resonance imaging (MRI), an indispensable tool in contemporary medical diagnosis and a generally versatile method for non-invasive visualization of spatial density of atomic nuclei.

The broad impact of NMR and MRI was achieved despite their rather poor signal strength. The NMR signal acquired during the experiment is directly proportional to the difference in populations of the spin states of the observed nuclei. In a traditionally designed experiment at thermal equilibrium, the spin state populations are governed by Boltzmann distribution, which is a function of temperature and the strength of the magnetic field the sample is subjected to. At ambient temperatures the population differences between spin states achievable in such way are only on the order of 10−5, even when strong magnetic fields of several Tesla are used. Consequently, the development of methods for increasing the spin polarization has received a significant attention.

In order to boost the NMR signal, several methods have been devised. These so-called hyperpolarization (HP) techniques can increase the nuclear spin polarization beyond the thermal equilibrium state, often by several orders of magnitude. The HP process uses an external source of spin order that is transferred to the sample and creates large differences in spin-state populations. The relative population differences as high as tens of percent have been achieved [4–7]. However, unless the spin order is converted to a long lived state [8], the hyperpolarized samples usually rapidly relax towards thermal equilibrium populations, constraining the usable lifetime of the magnetization.

In one HP method, dynamic nuclear polarization (DNP) [5], the nuclei are polarized using unpaired electrons from free radicals.
DNP is a very versatile method, capable of polarizing a wide range of molecules. However, it requires very expensive facilities, has high operational costs and the repeatability of the level of polarization may be difficult to predict.

Another HP method is spin-exchange optical pumping (SEOP) [6], in which polarization is transferred from electrons of vaporized alkali metal atoms to noble gas nuclei. The SEOP process can achieve a very high degree of polarization, up to 90% [9], but it is in practice usable only for polarizing of noble gas atoms, such as helium, xenon or krypton [10].

Lastly, a number of hyperpolarization methods, called para-hydrogen induced polarization (PHIP) [6], use parahydrogen (para\textsubscript{-}H\textsubscript{2}, one of the spin isomers of hydrogen) as the source of the spin order. In some PHIP approaches, para-H\textsubscript{2} can be directly incorporated into the molecule via addition reaction with a suitable unsaturated bond, producing molecules with non-equilibrium spin populations in schemes known as PASADENA [11,12] or ALTADENA [13].

Recently, it has been shown that PHIP can also be used to transfer the magnetization from para-H\textsubscript{2} to the hyperpolarized molecules without actual chemical modification taking place. Such effect can be achieved by a reversible association of the substrate molecule with para-H\textsubscript{2} mediated by a suitable metal catalyst. The technique is known as Signal Amplification By Reversible Exchange (SABRE) [14]. The SABRE polarization mechanism is based on the transfer of spin order via the scalar couplings of the para-H\textsubscript{2} and the substrate molecule, and its efficiency depends on the strength of the magnetic field in which the polarization transfer takes place, so-called polarization transfer field (PTF) [14–18]. Protons are most efficiently polarized in the field of several mT, but in the case of heteronuclei, very low fields on the order of \(\mu T\) are optimal, requiring magnetic shielding from the Earth’s natural magnetic field [19–22]. While SABRE is not as versatile as, e.g., DNP [5,7], it is nevertheless capable of hyperpolarizing a significant range of nuclei and molecular substrates, provided that they can associate with the polarization-transferring Ir catalyst. Moreover, its costs for instrumentation facilities are orders of magnitude smaller than for DNP, making it an affordable hyperpolarization option. In addition, since SABRE does not modify its substrate, it can be used to produce stable, predictable levels of nuclear spin polarization in a continuous fashion, as has been shown [23–37].

Since the first description of SABRE, a number of significant developments have been made. Following the initial report [14], which used [Ir(COD)(py)(PCy\textsubscript{3})][BF\textsubscript{4}] \((\text{COD} = \text{cyclooctadiene, py = pyridine, PCy}_3 = \text{tri-cyclohexyl-phosphine})\), synthetic efforts yielded more potent catalysts allowing more efficient polarization transfer or solvent compatibility [15,17,38–41]. Currently, one of the most efficient SABRE catalysts is of the general form [Ir(IMes\((L)_3(H_2))\) \((\text{IMes} = 1,3\text{-bis}(2,4,6\text{-trimethylphenyl})\text{-imidazolium, L is a ligand molecule, not necessarily identical in all three coordination places})\)]. It is an activated form of an air-stable precursor [Ir(COD)(IMes)Cl], which can be in turn readily prepared from commercial reagents [42,43].

Apart from protons (see, e.g., [14,16,44–46]), other nuclei such as \(^{13}\text{C} [18,24,25,44,47],^{15}\text{N} [20–22,30,41,44,45,48–50],^{31}\text{P} [14,51,52],^{33}\text{P} [19,53],^{29}\text{Si} [54] and ^{119}\text{Sn} [54] have been successfully polarized. The SABRE polarization transfer has also been achieved in high magnetic fields [28,31,55,56] and the suitability of the SABRE for 2D NMR techniques [57–59], low concentration detections [46] and imaging [14,21,25,29,47,51,53,60–62] has been demonstrated as well. In addition to the NMR measurements in high-field instruments, the SABRE has also been successfully used in low-field set-ups [25,47,63], where use of hyperpolarized samples yields even more pronounced results due to the much smaller achievable thermal polarization. The issue of contaminating the sample with the SABRE catalyst can be overcome by immobilization of the catalyst on solid support [49,64,65] or via extraction of the analyte using two-phase system of immiscible liquids [66].

The first experimental approach to SABRE, which is still in use today, is a manual shaking of the sample under propan-\textsubscript{2}-H\textsubscript{2} atmosphere inside a pressurized valve NMR tube to introduce the hydrogen gas into the solution. After thorough mixing the sample is immediately inserted into NMR spectrometer for measurement. Since the relaxation is one of the crucial parameters affecting the efficiency of the technique, an effort has been directed towards instrumentation that would shorten the delivery time of the sample. One such approach is an automated computer-controlled flow system with pre-polarizing magnet and a flow probehead, which allowed for repeatable preparation and transfer of hyperpolarized sample into NMR spectrometer by applying gas pressure [15,24]. Such system allows to polarize the same sample multiple times by cycling it between the NMR probe and the polarizer where it is periodically repolarized. Another option is an automated mechanical shuttling of the whole sample using a motorized system [67,68]. One can also use a continuous in situ polarization without transfer of the sample. The approach has been shown either in low field setup of several mT [25–27,29], simultaneously exploiting the favorable conditions for \(^1\text{H}\) SABRE transfer, or in high fields [30–32]. The experiments can be performed either with stopping of the flow of propan-\textsubscript{2}-H\textsubscript{2} before individual acquisitions [32–34] or with the constant flow of propan-\textsubscript{2}-H\textsubscript{2} at all times, even during measurement time, creating a truly continuous polarization [27,28,35–37]. Yet another method, a continuous flow system using a membrane reactor has been described recently [69] and its features are compared with the setup presented in this paper in the Results section.

Here we present a different approach to sample preparation and transfer based on a continuously flowing liquid. Our system is inherently well-suited for flow imaging and delivers a steady flow of sample with a stable level of nuclear spin-polarization. It also provides a high throughput of the sample on the order of milliliters per second. We describe the overall system, its main components and features and discuss its performance parameters, such as the influence of the pumping speed of liquid, influx of hydrogen gas and polarization transfer field strength and their influence on the signal strength. We focus mainly on the unique features of our system, such as flow rates and efficiency of the hydrogen exchange with the liquid phase. The details of kinetic aspects of the SABRE in solution have been discussed extensively in the literature before (see, e.g., [24] and references cited therein) and it is reasonable to assume they are preserved irrespective of the mode of sample preparation and transportation into the spectrometer.

2. Methods

2.1. Polarizer overview

The overall schematic of the system is presented in Fig. 1. The main parts of the setup are: high pressure hydrogen cylinder (A), custom made para-H\textsubscript{2} converter (B), low-field electromagnet (C), vacuum pump (G), peristaltic pump for sample (I), and main framework with tubing, valves and polarizing vessel. The main body of the polarizer is fixed onto a plexiglass (see Fig. S1 in Supporting Information for full view).

2.2. Principle of operation

The system allows for several modes of operation. After introducing the sample into the degassing compartment (H), a vacuum
pump can be used to degas the sample via the freeze-pump-thaw cycle. The sample is then transferred to the polarizing cell by applying slight overpressure into the degassing vessel via the three-way valve V1. During the polarization operation the hydrogen gas passes through the converter immersed in liquid N2 (B), where it is enriched to 50% para-H\textsubscript{2} by conversion over FeO(OH), and flows through the sample in the polarizing cell (C). The hydrogen is introduced into the sample in polarizing cell via three 1/16 inch thick pieces of tubing. The gas flow is regulated by a needle valve and monitored by a flow-meter. The valve V2 allows to route the connection from vacuum pump to back-pressure regulator and maintain a stable pressure inside the whole system even as new para-H\textsubscript{2} flows constantly in. Pressure of up to 7 bar has been successfully tested.

The liquid sample is pumped independently of the hydrogen flow via the peristaltic pump. The pump is placed in the line after the NMR spectrometer in order to minimize travel time from cell to spectrometer (connected via 2.4 m long tubing, corresponding to less than two seconds travel time at the highest speed) and hence the loss of polarization due to the relaxation. The pumping direction can be chosen freely, but pumping the liquid in downward direction opposite to the flow of the rising hydrogen bubbles provides better mixing of the sample, allows the freshest incoming para-H\textsubscript{2} to be in contact with the sample immediately leaving the polarizing cell and minimizes probability of stray bubbles to enter the sample. Moreover, it also allows using a smaller amount of sample. After passing through the NMR spectrometer, the sample is returned to the cell for refreshing of the polarization.

During the experiment the degassing vessel (H) can be used as a solvent trap by immersing it in cold bath in order to condense escaping solvent vapours, which can be then periodically returned back to maintain a stable sample volume.

2.3. Sample preparation

The sample solutions consisting of methanol (non-deuterated), pyridine and [Ir(Imes)(py)(H\textsubscript{2})\textsubscript{3}] were degassed prior to measurements. Pyridine was chosen as one of the most well-studied molecules in SABRE. The first portion of the sample was prepared by dissolving 180 \mu l (2.25 mmol) of pyridine in 45 ml of methanol and transferred to the polarizing cell. The second portion of the sample was prepared by dissolving 180 mg of Imes catalyst in 20 ml of methanol and subsequent addition of 80 \mu l (1 mmol) of pyridine. The second solution thus had identical concentration 50 mmol/l of pyridine as in the first batch, allowing us to make a titration with the catalyst while maintaining constant concentration of pyridine. The titration was performed in four steps, each time transferring 5 ml of the catalyst solution from the degassing vessel to the polarizing cell and mixing thoroughly. The four samples yielded concentrations 1.4, 2.6, 3.5 and 4.3 mmol/l of catalyst and were labelled c\textsubscript{1} to c\textsubscript{4} in order of increasing concentration, corresponding to ratios of catalyst to pyridine 1:35, 1:20, 1:14 and 1:12.

The total methanol loses during the degassing steps were measured as a decrease in level of the liquid surface in the degassing vessel and are estimated to be under 1 ml and thus not significant, given the total sample volume (varied between 45 and 65 ml).

Unless otherwise stated, the flow rate of hydrogen was set to 0.9 ml/s, flow rate of the sample to 170 ml/min and hydrogen gas pressure to 5 bar.

2.4. NMR measurements

All the NMR and MRI experiments were performed on Bruker AVANCE III 300 spectrometer equipped with micro-imaging unit, using Micro 2.5 probe with a 25 mm birdcage coil tunable for 1H.

The sample was flowing through a straight piece of perfluoroalkane (PFA) tubing (outer diameter 6.35 mm (1/4 inch), inner diameter 3.96 mm (0.156 inch), Swagelok). PFA tubing was fixed concentrically with the coil axis by a 3D printed PET (polyethylene terephthalate) holder. The proton NMR spectra on the straight PFA tubing were recorded with one scan using a 45 deg pulse.

As our interest in the present study was focused on hyperpolarized signals of pyridine and methanol, we left the hydride signals of SABRE catalyst outside the spectral width. Their presence was confirmed in our preparatory SABRE experiments.

2.5. MRI measurements

MR images were recorded either for the identical tubing as was used for NMR experiments or for a phantom flow channel created...
by connecting two pieces of PFA tubing of different inside diameters (3.2 and 3.96 mm) by a nylon compression fitting with inside diameter of 4.8 mm (see Fig. S2 in SI for details). The fitting was fixed in the center of the probe using a padding constructed from extruded polystyrene. Due to the shape of the resulting object, the shimming proved to be rather difficult, and the field inhomogeneity gave rise to broad baseline (Fig. S3 in SI). This prohibited use of selective pulses as excitations targeting the pyridine region would also excite a non-negligible portion of methanol nuclei. For this reason, the images were recorded using hard pulses covering the whole spectral region of interest, including both methanol and pyridine, and the individual component intensities were obtained by subtraction.

The 2D spin density images in the straight PFA tubing were measured using a gradient echo sequence with first order flow compensation. The images were recorded with an echo time, $T_E$, of 12.3 ms and repetition time, $T_R$, of 50 ms. The signal of interest (pyridine or methanol) was excited using a selective Hermitian pulse with an excitation bandwidth of 270 Hz. The slices were oriented in the plane of the tube axis, with thickness 2 mm, the field of view (FOV) 20 × 20 mm, and a resolution of 156 μm/pixel on the 2D plane. For each image 128 averages, $N_{av}$, were collected (experimental time ca. 14 min).

The 2D flow images were recorded using the velocity encoded gradient-echo provided by the Paravision 5.1 software (Bruker BioSpin). Bipolar gradients were used to produce a phase shift proportional to the velocity, thus encoding the velocity distribution of the moving spins into each pixel made by the two spatial dimensions (read and phase directions). In the Fourier flow mapping mode used in this work, two encoding steps were required to velocity encode one main spatial (vertical) direction. The experiments on the phantom constructed from the fitting and tubings were recorded using the following parameters: $T_E = 2.1$ ms, $T_R = 10.6$ ms, $N_{av} = 200$, FOV = 20 × 20 mm (resolution of 156 μm/pixel in the 2D plane), and slice thickness 1.25 mm. The slices were oriented along the axis of the tube. The maximum velocity and the velocity resolution for the flow encoding were 80 cm/s and 10 cm/s, respectively (experimental time ca. 54 min).

3. Results and discussion

All numerical NMR data are reported as signal enhancements of NMR peaks with respect to the signal of fully thermally polarized sample in 300 MHz (7 T) magnet (see SI for details). Specific ranges for integration are given in Table S1 in SI. Note that in the flowing sample the signal of methanol is dramatically reduced due to the short time period spent in the field before being introduced into the probe, significantly reducing the thermal polarization.

3.1. Effect of the flow rate on the signal amplitude

The first set of experiments demonstrates the effect of different flow rates of the sample from polarizing cell to the NMR spectrometer. We have measured NMR spectra in the region of flow rates from 0 to 170 ml/min in steps of 17 ml/min for each sample $c_1$ to $c_4$. The results are plotted in Fig. 2. We note that the flow rate is considerably higher than 10 ml/min of the previously reported continuous flow system [69]. This allows for new applications where a large volume of sample would be required, such as imaging of larger flow channels. It, however, also requires somewhat larger sample volume of 50 ml, compared to 30 ml in Ref. [69], in order to assure sufficient time in the reactor for the polarization transfer to take place.

The first apparent trend is the steadily increasing observed magnitude of the hyperpolarized (HP) signal with the flow rate. This is an interesting contrast to the continuous flow system in [69], where an optimal flow rate was found below its maximum value. The likely cause are different methods of mass transfer of hydrogen into the solution in the two systems, which influence the kinetics of SABRE transfer. An interesting point is that at slower speeds the lowest concentration of catalyst seems to provide the largest enhancement, while samples with increasing catalyst loadings provide progressively smaller enhancement. On the other hand, higher catalyst loadings achieve larger enhancement at higher flow rates (faster delivery speeds). In addition, the maximum achieved enhancement, while not significantly dependent on the catalyst concentration, is the largest for the medium catalyst loadings.

Since faster delivery speeds offer higher HP signal, the trends suggest that proton relaxation, rather than the reaction kinetics associated with the spin-transfer, is the key factor influencing the observed signal intensity. While the SABRE catalyst builds up the sample polarization over time, it also shortens the effective relaxation time of pyridine [24]. At low catalyst concentrations the loss of polarization due to this effect is diminished owing to the lesser probability of association of hyperpolarized pyridine with the scarce catalyst molecules. On the other hand, higher catalyst concentrations offer higher reaction throughput, leading to increased HP signal when the flow rates are high enough to offset the shortened relaxation times. However, at the highest catalyst loading the effect of the fast relaxation seems to start to overcome even the fastest delivery once again (sample $c_4$). A new signal appears at ca. 4.5 ppm, which can be assigned to hyperpolarized dissolved hydrogen gas [55] (see also Fig. S4 in SI). This signal is not apparent in the thermally polarized sample. As documented throughout Figs. 2–6, the signal depends on a number of system parameters and increases in concert with the pyridine protons, underlining its origin due to the SABRE hyperpolarization process.

As another representation, the enhancements of the proton signals are plotted in Fig. 3 for each individual signal, showing rather limited effect of the catalyst concentration and more clearly demonstrating the highest polarization for concentration $c_3$.

The maximum obtained signal enhancement (as a ratio of integrated hyperpolarized signal to the thermal one in 300 MHz magnet) in this set of experiments is 105 for the sample $c_3$, corresponding to the polarization of about 0.25%. Full table of values is given in SI (Table S2).

3.2. Effect of the hydrogen flow rate on the signal amplitude

The second set of experiments consisted of measuring the dependence of signal strength on the hydrogen flow. With increasing flow, one to three independent streams of bubbles form from the three capillaries and ascend through the solution, dissolving $para$-$H_2$ enriched gas into the liquid while also mixing the solution. We performed three separate measurements at flow rates of 0.5, 0.9, or 1.8 ml/s. The results are shown in Fig. 4.

It is apparent that at the lowest catalyst loading, the effect of hydrogen flow rate is quite limited. At such low concentration the catalyst availability is likely the rate determining step and the additional $para$-$H_2$ gas thus has a limited impact. Following the trend of the lowest $para$-$H_2$ flow rate across different concentrations (red *bars*, we see that the SABRE efficiency slightly decreases with higher catalyst loadings. This effect is likely due to the fact that at higher concentrations there is not enough fresh $para$-$H_2$ to bind to the abundant catalyst for new polarization transfer, but
the additional catalyst instead shortens the effective relaxation time.

On the other hand at higher para-H$_2$ influx, more catalyst molecules can bind with the now available para-H$_2$ and increase the polarization, which is reflected as a progressively higher dependence of the HP signal amplitude on the gas influx at the medium flow rates (green). In this case the achieved polarization increases from $c_1$ to $c_2$ and drops off only at $c_4$. At the highest para-H$_2$ flow rate the polarization dependence on the concentration does not decrease significantly even at $c_4$, consistently with our hypothesis. In summary, there appears to be a delicate balance between the para-H$_2$...
H₂ flow rate at higher catalyst concentrations does not fully employ the catalyst is the rate determining factor and the superfluous flow rate and SABRE catalyst concentration. At low concentrations, NMR signals (sample c₄ = 4.3 mmol/l). The six signals correspond to protons in ortho-, para-, meta-position of pyridine, hydroxyl proton of methanol, polarized H₂ and methyl protons of methanol. Note that methanol signals also reflect significant portion of thermally polarized molecules. Moreover, because of very large thermal equilibrium reference signal, their intensities are multiplied by 1000 for visibility. The concentrations of SABRE catalyst increase in the order c₁ = 1.4, c₂ = 2.6, c₃ = 3.5, c₄ = 4.3 mmol/l. 

At first glance different behaviour of the ortho- and para- protons compared to meta- can be seen where the former show negative values. It is well established that the efficiency of the SABRE effect is dependent on the strength of the magnetic field which facilitates the polarization transfer, the so-called polarization transfer field (PTF). We explored this effect in our system by measuring the signal coming from the hyperpolarized hydrogen at 4.5 ppm is similar to the pyridine signals by being rather insensitive to the para-H₂ flow rate at the lowest concentration and increasing only at higher concentrations. Moreover, it can be seen that both proton signals of methanol also respond to the hydrogen flow in a similar way, providing an evidence for slight hyperpolarization of methanol in addition to the thermal contribution coming from transport through the stray fields on the way to the NMR probe. The polarization of solvent by SABRE has been reported before for acetonitrile [18,70], methanol [17,71], ¹³C in neat pyridine [72], and water was also polarized in a similar way [73]. In our case the total contribution from the SABRE process is rather large, likely because the SABRE experiments are usually conducted in deuterated solvents, in contrast to our proton-rich solvent. Thus, our system contains much more available ¹H nuclei as receivers of the polarization, leading to substantial total increase of the signal. Interestingly, such polarization of methanol has not been observed in previous continuous flow SABRE experiment [69], possibly as an effect of different PTF, which is quite strong in the low mT region [17].

Apart from the bubbles raising through the sample, there is a second interface between the gas and the liquid at the point where the sample returns from the spectrometer to the polarizing cell. The liquid here forms a thin film on the inner wall of the cell and at higher flow rates even splashes through the hydrogen atmosphere. In both cases, a significant interface between the liquid and the gas is created, enabling another way for dissolving hydrogen.

In order to gain some insight into the efficiency of this process, we ran an experiment where at time t = 0 the flow of hydrogen was stopped, thus ceasing the creation of the bubbles, but the sample was let to circulate, preserving the gas-liquid interface at the sample-return inlet. An NMR spectrum was taken every 30 s for 10 min with results shown in Fig. 5. As can be clearly seen, an easily discernible hyperpolarization is observed even after several minutes. For comparison, the average time for the whole sample volume to circulate through the system is ca. 23 s. Such long persistence considering short relaxation time of protons leads us to the conclusion that there must be an ongoing process refreshing the polarization of the sample. Since the bubbling can no longer be a contributing factor, the interface at the sample inlet is likely the source of persistent introduction of fresh para-H₂ into the sample. As expected, the overall signal intensity gradually decreases over time due to the depletion of the para-H₂ atmosphere. In addition, a similar loss of polarization can once again be seen for both methanol protons as well as for H₂ signal.

### 3.3. Effect of the polarization transfer field strength

It is well established that the efficiency of the SABRE effect is dependent on the strength of the magnetic field which facilitates the polarization transfer, the so-called polarization transfer field (PTF). We explored this effect in our system by measuring the signal strength at several values of PTF produced by the electromagnet outside of the polarizing cell (Fig. 6).
We can also see that the field dependence of the H₂ signal more closely resembles that of the ortho- and para- hydrogen atoms rather than that of the meta- in the sense that it is quite small at low fields, after which it grows rapidly.

Generally the signal increases with higher PTF. Our setup did not allow to test stronger fields than those reported in Fig. 6. In the future, the setup could be upgraded with the coils allowing stronger PTF and, therefore, potentially higher polarization degree.

3.4. Spin density imaging

As a test of the imaging capabilities of the system, we acquired a set of FLASH images of the sample flowing through the tubing (Fig. 7). The images were recorded using selective excitation pulses (see Methods section) on either the pyridine or methanol signal. As can be seen, under the given conditions no signal can be discerned in the image of thermally polarized pyridine. On the other hand, hyperpolarized pyridine produces a strong signal, clearly showing the sample inside the tubing. The signal is even stronger than that from the thermally polarized methanol, which is in large excess in comparison to the pyridine (concentration of pure methanol is 24.7 mol/l, while the pyridine concentration is 50 mmol/l, a ratio of almost 300 when accounted for number of protons per molecule).

An interesting note is the spin density profile across the tubing diameter. As seen in the lower panel of Fig. 7, methanol produces stronger signal near the edges of the tubing, while the pyridine signal is strongest in the center. This is readily explained by the origin of the spin magnetization - a significant portion of the methanol nuclei is polarized thermally, which means that the longer they stay inside the magnet, the stronger signal they will produce. Since the slowest liquid layers are found near the wall of the tubing, the spins in these volume elements spend longer time in the field and acquire higher thermal polarization than the ones closer to the center of the tube. In the case of pyridine the situation is reversed as practically all signal comes from the hyperpolarized nuclei with negligible signal arising from the thermal polarization due to the low concentration of pyridine. Thus, the slower molecules near the walls lose their polarization via relaxation, giving rise to weaker signal compared to the fast flowing fresh hyperpolarized sample in the middle. It is worth pointing out that Fig. 7 shows image of a thin slice from the center of the tubing and the intensity variation is thus not affected by the cylindrical geometry of the tubing.

3.5. Velocity mapping

As a second set of imaging experiments, we have performed the velocity mapping on the phantom object (see Methods section and Fig. S2) using FLOWMAP. The phantom was chosen in order to demonstrate different velocities of the sample flowing through channel of changing diameter. One set was recorded with hyperpolarization of the sample and the second set without. The contribution of hyperpolarized pyridine was then obtained as a difference between these two images, bearing in mind that a small contribution from hyperpolarized methanol is likely present. The experiment was run at a single average linear flow rate of 12 cm/s through the tubing. It should be noted, however, that the maximum speed in the center of the tubing can reach higher values during flow. Moreover, the used pump exhibits pulsating flow, giving rise to even faster instantaneous velocities. The distribution of different velocities along the vertical direction is shown in Fig. 8.

As the measured intensity values were rather low, the scale of Fig. 8 is truncated to range 0–30% of maximum intensity to provide larger contrast. Full-scale image can be found in the SI (Fig. S5). A difference can be seen between the second row, corresponding to thermally polarized nuclei - thus, effectively, only the methanol - and the third row, showing difference between thermally polarized and hyperpolarized sample. Near the edges of the tube, particularly in the central, widest part of the channel, the methanol signal clearly dominates in the range of velocities up to 20 cm/s. In the region of 20–50 cm/s the contributions get more even, possibly because the flow is too fast to sufficiently thermally polarize the methanol while also being too slow to retain the hyperpolarization before a significant portion of nuclei relax. In the region of 50–
60 cm/s the pyridine nuclei contribute a large portion of the total intensity. At even higher speeds the pyridine is the dominant contributor while methanol is rather weak. It is apparent that the regions of the slowest velocities, corresponding mostly to methanol, are at the edges of the widest, central region of the phantom. This is expected as the incoming liquid pushes more easily straight through the center of the fitting, leaving the fringes relatively undisturbed. The observed behaviour shows that faster moving liquid carries relatively more pyridine polarization compared to slowly moving regions. This is in general agreement with the previous conclusions from the spin density imaging.

These findings clearly point out the possibility to use hyperpolarized samples to study fast flow rates, that could be impossible to image with non-polarized samples due to lack of time to build up thermal polarization.

4. Conclusion

We have constructed and successfully tested a polarization technique for steady production of hyperpolarized samples using the SABRE technique. The system features constantly flowing liquid, which is being continuously mixed with the stream of hydrogen bubbles. A rather high volume of sample required in the current iteration of the instrument can be offset by using a non-deuterated solvent, the signal of which is efficiently suppressed via the insufficient thermal polarization during the flow. Optimization of the delivery tubing to achieve smaller dead volume is also a viable route to improve the issue. The set-up allows a rapid (less than two seconds) introduction of the sample into the NMR instrument for measurements and the tests of the performance show that we are able to obtain over 100 times enhancement of the pyridine signal compared to the fully thermally polarized sample (about 0.29% total polarization). It shall be noted that combined use of a higher polarizing transfer field and more enriched para-H2 gas (up to 3-fold enhancement) and a deuterated solvent could possibly provide up to an order of magnitude higher signal, corresponding to ~3% proton polarization. The increase in signal due to the deuterated solvent would come due to more favourable relaxation and a larger SABRE enhancement of pyridine because of less polarization wasted by transfer to solvent protons.

One of the features of our system is the stability of the delivered polarization, which is not decaying due to the relaxation by virtue of being continuously replaced by fresh sample. In addition, the flow helps to suppress the thermal polarization of the solvent, allowing to perform measurements in normal non-deuterated solvents, signals of which are diminished to about 7% compared to the full thermal polarization. In addition, the bulk methanol is slightly polarized by SABRE, leading to increase of its signal by about 50% as compared to the thermal polarization during the flow. The system design is very suitable not only for NMR spectroscopy and MRI, but also for flow imaging.

Acknowledgement

This project has received funding from the European Union’s Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement NMOXPEC No 654967 (PS) and from Academy of Finland (Grant 316180) (PS). We acknowledge the generous support provided by the European Research Council (ERC) under Horizon 2020 (H2020/2018-2022) ERC grant agreement No. 772110 and the Academy of Finland (Grant Nos. 289649 and 294027). We also thank the Magnus Ehrnrooth Foundation for financial support (PS). The authors acknowledge financial support from the Kvantum institute (University of Oulu). We are grateful to Anne Selent for the visual documentation of the experiments.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jmr.2019.01.003.

References

[1] J. Keeler, Understanding NMR Spectroscopy, John Wiley & Sons, 2010.
[2] P.T. Callaghan, Translational Dynamics and Magnetic Resonance, Oxford University Press, 2011.
[3] J. Kärger, D.M. Ruthven, D.N. Theodorou, Diffusion in Nanoporous Materials, John Wiley & Sons, 2012.
[4] P.J. Rayner, M.J. Burns, A.M. Olaru, P. Norcott, M. Fekete, G.G.R. Green, L.R. Highton, R.E. Mewis, S.B. Duckett, Delivering strong 1H nuclear hyperpolarization levels and long magnetic lifetimes through signal amplification by reversible exchange, Proc. Natl. Acad. Sci. U. S. A. 116 (16) (2017) E3188–E3194, https://doi.org/10.1073/pnas.1620457114.
[5] J.H. Ardenkjær-Larsen, B. Fridlund, A. Gram, H. Hanson, M.H. Lerche, R. Servin, M. Thaning, K. Golman, Increase in signal-to-noise ratio of >10,000 times in liquid-state NMR, Proc. Natl. Acad. Sci. U. S. A. 100 (18) (2003) 10158–10163, https://doi.org/10.1073/pnas.1733835100.
[6] D.A. Barskiy, A.M. Coffey, P. Nikolaou, D.M. Mikhaylov, B.M. Goodson, R.T. Branca, G.J. Lu, M.G. Shapiro, V.-V. Tekkki, V.V. Zhivonitko, I.V. Kopytug, O.G. Salnikov, KV. Kortunov, V.I. Buldzhyanov, M.S. Rosen, M.J. Barlow, S. Safavi, I.P. Hall, L. Schröder, E.Y. Chekmenev, NMR hyperpolarization techniques of gases, Chem. A Eur. J. 23 (2017) 725–751, https://doi.org/10.1002/chem.201603884.
[7] T. Maly, G.T. Debelouchina, V.S. Bajaj, K.-N. Hu, C.-G. Joe, M.L. Mak-Jurkauskas, J. Maly, H. Tracy, P.C.A. van der Wel, J. Herzfeld, R.J. Temkin, R.G. Griffin, Dynamic nuclear polarization at high magnetic fields, J. Chem. Phys. 128 (2008), https://doi.org/10.1063/1.2833582 (art. no 052211).
[8] M.H. Levitt, Singlet nuclear magnetic resonance, Annu. Rev. Phys. Chem. 63 (2012) 1415–1442, https://doi.org/10.1146/annurev-physchem-022511-143724.
[9] P. Nikolaou, A.M. Coffey, L.L. Walkup, B.M. Gust, N. Whiting, H. Newtond, S. Barlow, E.Y. Chekmenev, B.M. Goodson, Near-unity nuclear polarization with an opensource Xe-129 hyperpolarizer for NMR and MRI, PNAS 110 (35) (2013) 14140–14145, https://doi.org/10.1073/pnas.1306586110.
[10] D.M. Lilburn, G.E. Pavlovskaya, T. Meersmans, Perspectives of hyperpolarized noble gas MRI beyond ‘He, J. Magn. Reson. 229. doi: https://doi.org/10.1016/j.jmr.2012.11.014.
[11] C.R. Bowers, D.P. Weitekamp, Parahydrogen and synthesis allow dramatically enhanced nuclear alignment, J. Am. Chem. Soc. 109 (18) (1987) 5451–5452, https://doi.org/10.1021/ja00252a049.
[12] T.C. Eisenach, R.M. Rios, P.P. Deutsch, S.B. Duckett, G.G.R. Green, I.G. Khazal, J. López-Serrano, D.C. Williamson, Reversible interactions with para-hydrogen enhance NMR sensitivity by polarization transfer, Science 323 (2009) 1708–1711, https://doi.org/10.1126/science.1168877.
[13] M.G. Pravica, D.P. Weitekamp, NMR alignment by adiabatic transport of parahydrogen addition products to high magnetic field, Chem. Phys. Lett. 145 4 (1988) 255–258, https://doi.org/10.1016/0009-2614(88)80002-2.
[14] R.W. Adams, J.A. Aguilar, K.D. Atkinson, M.J. Cowley, P.I.P. Elliot, S.B. Duckett, G.G.R. Green, I.G. Khazal, J. López-Serrano, D.C. Williamson, Reversible interactions with para-hydrogen enhance NMR sensitivity by polarization transfer, Science 323 (2009) 1708–1711, https://doi.org/10.1126/science.1168877.
[15] M.J. Cowley, R.W. Adams, K.D. Atkinson, M.C.R. Cockett, S.B. Duckett, G.G.R. Green, J.A.B. Lohman, R. Kerssebaum, Iridium N-heterocyclic carbene complexes as efficient catalysts for magnetization transfer from para-hydrogen, J. Am. Chem. Soc. 133 (2011) 6134–6137, https://doi.org/10.1021/ja202099u.
[16] H. Zeng, J. Xu, J. Gillen, D. Artemov, J.-M. Tyburn, J.A. Lohman, R.E. Mewis, I. Golman, Increase in signal-to-noise ratio of 10,000 times in liquid-state NMR, Proc. Natl. Acad. Sci. U. S. A. 100 (18) (2003) 10158–10163, https://doi.org/10.1073/pnas.1733835100.
[17] V.V. Zhivonitko, I.V. Skopiv, I.V. Kopytug, Strong 13C nuclear spin hyperpolarization produced via reversible chemical interaction with parahydrogen, Chem. Commun. 51 (2015) 2566–2569, https://doi.org/10.1039/c4cc08115c.
[18] T. Theis, M.L. Truong, A.M. Coffey, R.V. Shepchin, K.W. Waddell, F. Shi, B.M. Goodson, W.S. Warren, E.Y. Chekmenev, Microtesla SABRE enables 10X...
16

P. Šepedová et al./Journal of Magnetic Resonance 300 (2019) 8–17

nitrorgen-15 nuclear spin polarization, J. Am. Chem. Soc. 137 (2015) 1404–1407, https://doi.org/10.1021/jacs.5b01242d.

[21] M.L. Truong, T. Theis, A.M. Coffey, R.V. Shchepin, K.W. Waddell, F. Shi, B.M. Goodson, W.S. Warren, Direct hyperpolarization of nitrogen-15 in aqueous media with parahydrogen in reversible exchange using SABRE-SHARE, J. Phys. Chem. C 119 (2015) 8786–8797, https://doi.org/10.1021/jp5107799.

[22] D.A. Barskiy, K.V. Kovtunov, T.L. Sculptor, W.S. Warren, T.S. Weisen, B.M. Goodson, E.Y. Chekmenev, J. Phys. Chem. A 122 (2018) 9107–9114, https://doi.org/10.1021/acs.jpca.8b07163.

[23] J.F. Colell, D.A. Barskiy, K.V. Kovtunov, I.G. Khazal, A.C. Whitwood, S.B. Duckett, G.G.R. Green, R.A. Green, L. Highton, S.M. Kenny, C.S. Wijmenga, M. Tessari, 2D NMR trace analysis by continuous hyperpolarization of a biomolecule in water, J. Phys. Chem. B 120 (25) (2016) 5670–5677, https://doi.org/10.1021/acs.jpcb.6b08280.

[24] A.N. Pravdivtsev, V.A. Bukhtiyarov, H. Zimmermann, M.-H. Vietich, K.L. Ivanov, Transfer of SABRE-derived hyperpolarization to spin-1/2 heteronuclei, RSC Adv. 5 (2015) 6315–6316, https://doi.org/10.1039/c5ra13808f.

[25] A.N. Pravdivtsev, A.V. Yukrovskaya, H.-M. Vietich, K.L. Ivanov, RF-SABRE: a way to continuous spin hyperpolarization at high magnetic fields, J. Phys. Chem. B 119 (43) (2015) 13619–13629, https://doi.org/10.1021/acs.jpcb.5b03032.

[26] K.V. Kozhukhov, V.A. Nesterov, T.S. Revunov, W.S. Warren, Using signal amplification by reversible exchange (SABRE) to improve the hyperpolarization of 15N NMR signals, Angew.
