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Dissolution Dynamic Nuclear Polarization capability study with fluid path

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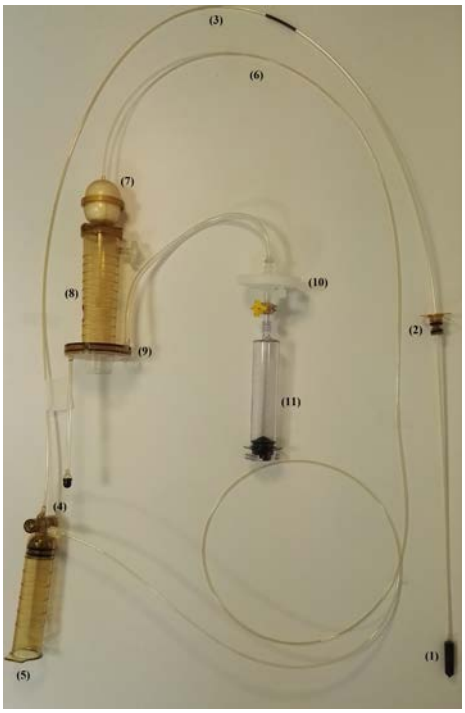
ABSTRACT

Signal enhancement by hyperpolarization is a way of overcoming the low sensitivity in magnetic resonance; MRI in particular. One of the most well-known methods, dissolution Dynamic Nuclear Polarization, has been used clinically in cancer patients. One way of ensuring a low bioburden of the hyperpolarized product is by use of a closed fluid path that constitutes a barrier to contamination. The fluid path can be filled with the pharmaceuticals, i.e. imaging agent and solvents, in a clean room, and then stored or immediately used at the polarizer. In this study, we present a method of filling the fluid path that allows it to be reused. The filling method has been investigated in terms of reproducibility at two extrema, high dose for patient use and low dose for rodent studies, using [1-^{13}C]pyruvate as example. We demonstrate that the filling method allows high reproducibility of six quality control parameters with standard deviations 3-10 times smaller than the acceptance criteria intervals in clinical studies.

GRAPHICAL ABSTRACT



QC output

Quality control (process capability)	
Pyruvate conc. (mM)	246±19
pH	7.71±0.17
EPA conc. (µM)	1.17±0.28
Temperature (°C)	32.0±1.5
Volume (mL)	44.2±1.0
Polarization (%)	34.2±2.9

HIGHLIGHTS

- d-DNP of sample sizes up to 1 mL
- d-DNP with re-useable plastic fluid path
- Reproducibility data for [1-^{13}C]pyruvate for the clinical formulation

INTRODUCTION

Increase of the NMR signal of molecules by hyperpolarization is a technique, which has many applications in the field of NMR spectroscopy and imaging. The most commonly used method is dissolution-Dynamic Nuclear Polarization (d-DNP) [1], in which the hyperpolarized nuclear spins are transferred from the solid state to the liquid state by a quick dissolution while retaining the nuclear spin polarization.

In this study, the d-DNP technique will be demonstrated by hyperpolarization of pyruvate; being a key intersection in various metabolic pathways, [1-^{13}C]pyruvate is by far the most common molecule for studies with d-DNP [2] [3]. It has an advantageously long relaxation time T_1 and high polarization levels can be achieved [4]. Other molecules can be used to probe biological pathways as well; [U-^{13}C;U-^{2}H]glucose [5], [1,4-$^{13}C_2$]fumarate [6], [1-^{13}C]bicarbonate [7] among many other biologically relevant molecules. Similarly, hyperpolarized ^{13}C-labelled biologically inert molecules have been applied for angiography and perfusion studies [8] [9]. Besides the bioprobe, an electron paramagnetic agent (EPA) should be present to enable Dynamic Nuclear Polarization. The commonly used EPAs for hyperpolarization are OXO63 [10], BDPA [11] and TEMPO [12]. In this study, AH111501 is used, a stable trityl radical, which precipitates in low pH and can be removed by filtration in the receiver previous to the neutralization step. In 2013, the first clinical study with hyperpolarized [1-^{13}C]pyruvate was published, demonstrating technical feasibility and showing promising results in the phase I trial on patients with prostate cancer [13].

Studies on the performance of the d-DNP process with the aim of demonstrating efficient dissolution, i.e. high concentration and large volume with minimal loss of polarization have been published [14] [15]. The principle of dissolution in these studies is the use of a solvent that is heated to an elevated temperature to create a vapor pressure that can drive the flow of solvent past the cold, polarized sample. In the case of aqueous dissolutions, a temperature of 180 °C is typically used, creating a vapor pressure of 10 bar. Another principle of dissolution was introduced in 2011 [16], where the concept of a closed fluid path (FP) was presented. The primary objective of the closed FP was to secure a sterile environment throughout the polarization and dissolution process [16]. In terms of dissolution capability, two key principal differences between the FP and previous designs should be noted: 1) the solvent is not driven by the vapor pressure at the given temperature, but by a syringe, 2) the independent inlet and outlet tubes are replaced by two concentric tubes with the inlet as the inner lumen and with return flow in the outer lumen. From the first point follows that solvent

temperature can be controlled independently of solvent flow rate. In [17] it was shown that a syringe and solvent temperature of 130 °C and piston drive pressure of 16 bar was optimal for the dissolution. These conditions are extreme for most plastic materials, but is tolerated by e.g. polyphenylsulfones or polyetheretherketones. The second point is expected to affect dissolution efficiency, but was introduced to facilitate a dynamic seal that allows sample introduction into the cryostat through an air lock without raising the pressure of the 1 K sample space to atmospheric pressure. The FP consists of two major parts: part A, which contains sample and solvent, and allows the hyperpolarized solution to leave the polarizer through an open ended tube, and part B, that consists of a receiver connecting to the exit tube of part A, allowing non-contact quality control of the dissolved product. Part B also includes a filter that removes the EPA in-line with the dissolution process. The EPA precipitates in aqueous solution at acidic pH and can be removed effectively by mechanical filtration.

The objective of this work was to study the dissolution capability of the FP for two different sample sizes of [1-^{13}C]pyruvic acid. (1) A sample of 1.47 g, producing a human dose, dissolved in dissolution medium (DM) and neutralized with Neutralization Medium (NM) to a target concentration of 250 mM [1-^{13}C]pyruvate using the SPINlab polarizer and quality control system (involving FP part A and B). (2) A sample of 40 mg, producing a small animal dose, dissolved in DM to a target concentration of 80 mM, using a home-built polarizer involving only part A of the FP.

MATERIALS AND METHODS

Polarizer

Large samples were polarized on a SPINlab (GE Healthcare), Fig 1, operating at 5 T and 0.85 K. Small samples were polarized on a home built polarizer [1] operating at 6.7 T and 1.1-1.2 K modified to accommodate the fluid path dissolution system.

Sample

[1-^{13}C]pyruvic acid (PA) (GE Healthcare, Norway) containing 15 mM AH111501 (EPA), 94.9% purity (GE Healthcare, USA).

Dissolution Medium (DM)

Large sample: Ultra pure water (CHROMASOLV®Plus HPLC grade, Sigma Aldrich) with 0.1 g/L ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA) (VWR).

Small sample: 0.2 M tris-hydroxymethyl aminomethane (TRIS base) (VWR) with 0.1 g/L EDTA.

Neutralization Medium (NM)

Large samples: 0.72 M NaOH (MERCK), 0.4 M TRIS base, 0.1 g/L EDTA.

Small sample: NaOH (10 M).

Fluid Path (FP)

The FP (type 1293, GE Healthcare, USA) used for this study is shown in Fig 1. Part A of the fluid path consists of a vial which can hold up to 2 mL of sample, the dynamic seal that allows the tubing to move inside the polarizer under vacuum, the co-axial tubing for DM inlet and outlet, the valve that opens the inlet and outlet simultaneously and the dissolution syringe that can hold up to 60 mL of solvent. Fig 2B and 2C show the vial with the position of the inner tube. The inner tube has a nozzle to provide a more efficient dissolution as demonstrated in [15] and [17]. Part B of the FP, Fig 1, consists of the transfer tube, the EPA filter, receiver vessel with quality control (QC) appendage, the sterile assurance filter and a Medrad 65 mL MR syringe (Bayer, Denmark). On first use of the FP, the vial was attached to the outer tube by the use of UV adhesive (Dymax 1161-M). On reuse, the FP part A was cleaned with 40 mL washing buffer (10% NM in distilled water), and then twice with 40 mL distilled water. Initially, the FP was thoroughly examined for any potential defects or irregularities. The FP was placed on a pressure check fixture and briefly flushed with Helium gas to secure unhindered flow. Reused FPs containing visible moisture were flushed for 10 min, or until dry, with compressed air or helium gas. Part B is not reused since it contains a small amount of HPTS (8-hydroxypyrene-1,3,6-trisulfonic acid, trisodium salt) dye used for the optical pH determination.

Filling tube for sample addition

For adding the sample, a syringe with a filling tube (Mikrolab Aarhus A/S, PEEK O.D. 1/32” I.D. 0.5 mm, 1.5 m, VI JRT5620M3) attached through an adapter, a ferrule and a screw (Tefzel P628, Tefzel P200 and Tefzel P215, Mikrolab Aarhus A/S, Denmark) were used. To fit into the adapter, a sleeve (Sealtight, 840 um, F247, Mikrolab Aarhus A/S, Denmark) was glued (UV adhesive) onto the filling tube, Fig 2. The end of the tube was cut in a slanted manner in order to pass the nozzle

and minimize residual sample at the end of the tube. Before first use of the filling tube, it was inserted through the dissolution syringe valve bore and through the inner tube, all the way to the sample vial. When the tube reached the bottom of the empty vial, it was retracted such that the end of the tube just passed the nozzle of the inner tube (Fig 2B). Thus no sample would contaminate the inner tube upon addition. A mark was put on the filling tube by the dissolution syringe valve bore, so the correct position would be easily found upon next use.

Addition of sample, DM and NM

a. Addition of large samples

Approx 0.5 mL of sample was pulled into a 3 mL single use plastic syringe (carefully avoiding air bubbles) after which the filling tube was attached. The sample was then pushed through the filling tube in order to fill the dead volume (approx. 380 µL). An Eppendorf tube with sample was placed on the balance, tared, and sample (1.47 g) was drawn into the syringe. Air (few centimeters) was then drawn into the tube to prevent contamination of the FP inner wall. The syringe and filling tube were weighted before and after addition, to confirm correct addition of sample. The surface of the filling tube was thoroughly cleaned for potential impurities and inserted through the inner tube of the FP until the tube reached the bottom of the vial. The slanted tip made it possible for the filling tube to pass the nozzle of the inner tube. The filling tube was then retracted to the correct position, as indicated by the mark. The sample was carefully added by push of the syringe piston. After addition, air was drawn into the filling tube, and it could then be retracted from the FP without contaminating the inner tube.

38.0 g of DM was added to the dissolution syringe. The outlet of the syringe was plugged and the syringe put on the filling stand purge. The sample vial was carefully submerged into liquid nitrogen (vertical position to avoid splashing) and allowed to freeze (2 min). The FP was then flushed with helium for 2 min after which the system was pressure tested (40 psi for 2 min without losing pressure). When the FP tests were completed, the valve rotor was inserted and put into the closed position. The dissolution syringe was then connected to a part B, Fig 1. Ca. 15.0 g of NM and 26.2 g of DM were added to the receiver of part B, a Medrad power injector syringe was attached and the FP and receiver were ready to be inserted into the polarizer and QC module, respectively.

b. Addition of small samples

The filling tube was cleaned, dried and attached to a 1 mL syringe. Sample (40 µL) was placed in an Eppendorf tube, and drawn into the filling tube as a bolus, followed by ca. 10 cm air (Supplementary data Video 1). The filling tube was carefully inserted into the FP and pushed to the bottom of the vial. It was then retracted 0.5 cm. The syringe was removed, and helium was flushed through the filling tube (10 psi, 2 min) (Supplementary data Video 2). The filling tube was then removed from the FP. 13.3 g of DM and 138 µL NM were then added to the dissolution syringe. The FP was pressure tested as described for large samples. A short exit tube was attached (50 cm) and the FP was ready for being inserted into the polarizer. An empty 50 mL Falcon tube was used as a receiver for small samples.

SPINlab QC (online measurements; large sample only)

The QC module measures six parameters by methods that are non-contact with the product. The syringe valve is open for 10 s during the dissolution. The dissolution takes approx. 6 s. 5 s after the syringe valve closes, a part of the hyperpolarized sample is allowed to flow through a series of cavities by opening a valve in the bottom of the receiver. The QC parameters are measured in approx. 15 s, which means that the product (in the Medrad syringe) is released approx. 30 s after the dissolution is initiated. Pyruvate concentration is determined by absorbance at 360 nm, EPA concentration is determined by absorbance at 470 nm, pH is determined by ratio metric absorbance at 405 and 450 nm for a hydroxypyrene-1,3,6-trisulfonic acid (HPTS) dye in one of the cavities, polarization is measured by NMR and corrected for relaxation, temperature is measured by IR on the receiver and volume is a capacitance threshold sensor on the Medrad syringe. The QC module was calibrated prior to the experiments. Pyruvate concentration, EPA concentration and pH all required measurement of a dark, blank and several calibrants (GE Healthcare, USA) according to manufacturer's instructions. Polarization and flip angle calibration were performed by the use of a ^{13}C reference sample ($^{13}C_3$-glycerol doped to shorten T_1, QC daily check sample, GE Healthcare, USA) according to manufacturer's instructions. Temperature was calibrated by the use of a receiver filled with water of known temperature.

Offline measurements

pH: LAQUA pH/ION meter F-72 (HORIBA) with pH electrode DJ 113 662-1385 (VWR) or Knick Portamess pH-meter 913 with pH electrode Hamilton mini electrode (KLC). Polarization: measured on a 400 MHz NMR (Agilent Technologies, USA). Acquisition parameters: 3° flip angle every 2 s, starting approx. 10 s post dissolution. The thermal signal was acquired by adding Omniscan (15 µL)

to reduce T_1 to <1 s. 2000 averages with repetition delay 0.5 s, 3° flip angle. Software for analysis: MestReNova 10.0 and MATLAB R2015a, by which polarization and T_1 were calculated. PA concentration: For large samples the concentration was measured by quantitative NMR. Sample: sample from receiver (450 µL), [^{13}C]urea (15 µL, 400 mM), D_2O (100 µL) and Omniscan (15 µL). Acquisition parameters: 90° flip angle, repetition delay 1.5 s. Software for analysis: TopSpin 3.2. For small samples the concentration was measured by absorbance on a BioTek EPOCH 2 microplate reader (Holm & Halby, Brøndby, Denmark) at wavelength 360 nm. Temperature was measured by the use of thermometers of type OMEGA HH804U Thermometer or Testo 110, AG Germany. EPA concentration: measured by absorbance at 465 nm (EPOCH plate reader with 10 mm cell). Output volumes were measured by weighing an empty Falcon tube before and after dissolution (Discovery Ohaus balance).

RESULTS

a. Large samples

Eleven experiments were performed using four FP. The samples were polarized for 2.5 h on average to more than 90% of maximum polarization, followed by dissolution. The data from the QC module have been compared to offline measurements of the respective samples, Table 1. To secure a physiological acceptable pH, the NM in the receiver was adjusted accordingly to the amount of PA added (PA/NaOH ratio 1.6), which was in the range of 13.9-14.6 g. The target concentration in the case of large samples was 250 mM, which mimics a human dose.

Table 1 - Results from dissolutions compared to the respective off-line measurements (n=11)

	Average	**Standard deviation**	**Relative STD (%)**
PA/EPA added (g)	1.47	0.02	1.4
DM in syringe (g)	38.0	0.1	0.2
DM in receiver (g)	25.8	0.3	1.0
NM in receiver (g)	14.3	0.2	1.4
PA/NaOH mol ratio	1.6	0.002	0.2
Online results			
Pyruvate conc. (mM)	246	19	7.7
pH	7.71	0.17	2.2
EPA conc. (µM)	1.17	0.28	24

Temperature (°C)	32.0	1.3	4.0
Volume (mL)	>40 mL		
Polarization (%)	34.2	2.9 (n=3)*	8.5
Offline results			
Pyruvate conc. (mM)	247	20	8.3
pH	7.75	0.15	1.9
EPA conc. (µM)	0.68	0.21	31
Volume (mL) (product in Medrad syringe)	44.2	1.0	2.3
Temperature (°C)	31.7	0.5	1.6

* The NMR flip angle was incorrectly calibrated in the first experiments leading to saturation of the receiver and incorrect estimation of the polarization.

b. Small samples

To demonstrate filling of small samples, seven experiments were performed, Table 2, using the same four FP as in the large sample study. The experiments were designed to mimic a target concentration, suitable for small animal dose (80 mM). Polarization measurements were made for four of these.

Table 2 – Results from dissolutions with small sample filling (n=7)

	Average	**Standard deviation**	**Relative STD (%)**
PA/EPA added (mg)*	40		
DM in syringe (g)	13.4	0.6	4.5
NaOH (10M) syringe (g)	0.138		
Pyruvate conc. (mM)	70	7.4	11
pH	7.8	0.1	2
EPA conc. (µM)	70.5	7.5	11
Product in receiver (g)	5.3	0.6	12
Temperature (°C)	54	2.6	5
Polarization (%)	43.7	5.5 (n=4)	13
T_1	53.8	2.0 (n=4)	4
Recovery (%)	82	12	15

* 40 mg was assumed to be added with no loss under the addition process

DISCUSSION

The study shows that the new filling method of the FP can be performed in a reproducible manner, validated by QC parameters from the SPINlab and offline measurements. The method is robust with

small relative standard deviations. We have demonstrated two extreme conditions; filling of large samples mimicking a human clinical dose, and small samples suited for small animal studies.

For the human dose, base is added to the receiver, in order for the radical to precipitate and be filtered off before entering the receiver. The acceptance criterion for residual EPA in the product is less than 3 µM [18]. The measured value is 1.2±0.3 µM, well within specification, and demonstrating >99% removal. The mean value of the EPA concentration is five standard deviations below the acceptance criterion. The pyruvate concentration acceptance criteria are 220-280 mM [18], which is also achieved with three standard deviations margin. The acceptance criteria for pH are 6.7-8.2 [18]. The study was not perfectly centered in the range, which could be achieved by changing the volume of NM in the receiver. However, the range is about ten standard deviations in this study. pH is a critical safety parameter, demonstrating that filling and process performed very well and is robust. Since only approx. 44 mL are transferred to the Medrad syringe, recovery has been disregarded in the large sample case, since some of the sample is used in the QC and thereby trapped in the dead volume of the system. However, the volume transferred to the Medrad syringe is four standard deviations above the acceptance criterion. The FP's have been reused for demonstrational purposes and are not sterile, but we believe the approach could be translated into a low bioburden version by appropriate cleaning of the FP before filling. Sterility assurance can then be achieved by a validated, in-line sterile filter. The filling procedure should be compatible with normal pharmacy clean room environments.

For small samples, base is added to the dissolution syringe, which means that all the PA and EPA is transferred to the receiver upon dissolution, and recovery can thereby be estimated. Recovery is calculated from the product of pyruvate concentration and volume, and assume that the full 40 mg was deposited in the sample vial. Most of the 15-20% loss of sample is likely due to loss in the filling process. The pyruvate concentration in the receiver is highly linked to the performance of the fluid path, since the dead volume vary. The variability of 11% for the pyruvate concentration is acceptable for most animal studies. We have not systematically studied the minimal possible sample amount that can be deposited in the vial by our method, but we have tested volumes down to 10 mg. This could have significance for e.g. cell or perfused organ studies that only require dilute substrate concentrations. More important is the minimal volume that can be applied in the dissolution. We believe that the product volume of app 5 mL achieved in this study, is close to the minimum volume that can be reached without loss of polarization and reduced recovery. This is close to the same performance as e.g. the Hypersense optimized for small sample polarization. It would be reasonable

to expect that the use of two-phase dissolution could reduce the effective dissolution volume even further as previously demonstrated [19].

The lower temperature of the solvent in the dissolution allows the use of all plastic components. Plastic components eliminates leaching of paramagnetic ions from e.g. stainless steel components and potential enhanced relaxation from these ions. The polyphenylsulfone that is used in the fluid path is a plastic with high toughness and impact strength, good long-term hydrolytic stability and chemical resistance. It withstands steam sterilization without any significant loss of properties. For other solvents than water, the chemical resistance at the given temperature and pressure conditions needs to be taken into consideration and tested.

The SPINlab corrects the polarization to the start of dissolution by assuming a T_1 of 65 s. This means that the measured polarization is corrected by a factor of approx. 1.5 in this study. The polarization for low dose is the actual measurement without any correction. However, the delay is only approx. 10 s from start of dissolution to measurement. The difference in polarization for the two situations is partly due to the higher magnetic field strength of the prototype polarizer (6.7 T vs 5.0 T), but also that some polarization loss is known to occur in the QC system.

The reusability of the FP's means reduced cost for research purposes, and no failed FP were observed within the ten times reuse that we arbitrarily defined as the maximum number of reuses on the SPINlab. The filling method has been tested extensively (>100 dissolutions) at Aarhus University Hospital, Skejby on the SPINlab, without failure of the fluid path during dissolution that have led to contamination of the polarizer. Failure in dissolution can happen due to e.g. moisture in the tubing that lead to blockage and prevention of flow. This happens at a low rate depending on operator skills. In our laboratory we use the fluid paths until failure since contamination of the polarizer is easier to clean. However, most failures occur during handling as fatigue of glue joints, typically after 20-50 uses. Wear and tear can also lead to failure in the pressure test and discard of the fluid path. More rarely the FP fails early in use due to defects in the plastic. The failures due to defects in the plastic appear to be unrelated to the filling method, but can lead to a burst inside the polarizer during dissolution depending on location of the defect. With the hundreds of dissolutions that we have performed with this method, there does not seem to be a higher frequency of fatal incidents than by single use.

CONCLUSION

The principle of the fluid path for dissolution-DNP enables independent control of solvent temperature and flow rate. The benefit is efficient dissolution at lower solvent temperature and use of all plastic components. The reproducibility of the dissolution process is high on all parameters relevant for clinical use. The fluid path can be used for any sample size from 10 uL to 1.1 mL, and produces a minimum product volume of approx. 5 mL up to the full volume of 44 mL for a patient.

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REFERENCES

[1] J. H. Ardenkjær-Larsen, B. Frindlund, A. Gram, G. Hansson, L. Hansson, M. H. Lerche, R. Servin, M. Thaning and K. Golman, "Increase in signal-to-noise ratio of >10,000 times in liquid state NMR," *PNAS,* vol. 100, no. 18, pp. 10158-10163, 2003.

[2] S. Kohler, Y. Yen, J. Wolber, A. Chen, M. Albers, R. Bok, V. Zhang, J. Tropp, S. Nelson, D. Vigneron , J. Kurhanewicz and R. Hurd, "In Vivo13Carbon Metabolic Imaging at 3T With Hyperpolarized13C-1-Pyruvate," *Magn. Reson. Med.,* vol. 58, pp. 65-69, 2007.

[3] H. Gutte, A. E. Hansen, H. H. Johannesen, A. E. Clemmensen, J. H. Ardenkjær-Larsen, C. H. Nielsen and K. Andreas, "The use of dynamic nuclear polarization (13)C-pyruvate MRS in cancer," *Am. J. Nuc. Med. Mol. Imaging,* vol. 5, pp. 548-560, 2015.

[4] K. Golman, R. in't Zandt, M. Lerche, R. Pehrson and J. H. Ardenkjaer-Larsen, "Metabolic Imaging by Hyperpolarized 13C Magnetic Resonance Imaging for In vivo Tumor Diagnosis," *Cancer Res.,* vol. 66, no. 22, pp. 10855-10860, 2006.

[5] S. Meier, P. R. Jensen and J. Ø. Duus, "Real-time detection of central carbon metabolism in living Escherichia coli and its response to perturbations," *FEBS Lett.,* vol. 585, pp. 3133-3138, 2011.

[6] F. A. Gallagher, M. I. Kettunen, D.-E. Hu, P. R. Jensen, R. in't Zandt, M. Karlsson, A. Gisselsson, S. K. Nelson, T. H. Witney, S. E. Bohndiek, G. Hansson, T. Peitersen, M. H. Lerche and K. M. Brindle, "Production of hyperpolarized [1,4-13C2]malate from [1,4-13C2]fumarate is a marker of cell necrosis and treatment response in tumors," *PNAS,* vol. 106, no. 47, pp. 19801-19806, 2009.

[7] F. A. Gallagher, M. I. Kettunen and K. M. Brindle, "Imaging pH with hyperpolarized 13C," *NMR BIOMED,* vol. 24, pp. 1006-1015, 2011.

[8] C. von Morze, P. E. Larson, S. Hu, H. A. Yoshihara, R. A. Bok, A. Goga, J. H. Ardenkjær-Larsen and D. B. Vigneron, "Investigating tumor perfusion and metabolism using multiple hyperpolarized 13C compounds: HP001, pyruvate and urea," *Magn. Reson. Imgaing,* vol. 30, pp. 305-311, 2012.

[9] K. W. Lipsø, P. Magnusson and J. H. Ardenkjær-Larsen, "Hyperpolarized 13C MR Angiography," *Curr. Pharm. Design,* vol. 22, pp. 90-95, 2016.

[10] J. Ardenkjaer-Larsen, S. Macholl and H. Jóhanneson, "Dynamic Nuclear Polarization with Trityls at 1.2 K," *Appl. Magn. Reson.,* vol. 34, pp. 509-522, 2008.

[11] L. Lumata, S. J. Ratnakar, A. Jindal, M. Merritt, A. Comment, C. Malloy, A. D. Sherry and Z. Kovacs, "BDPA: An Efficient Polarizing Agent for Fast Dissolution Dynamic Nuclear Polarization NMR Spectroscopy," *Chem. Eur. J.,* vol. 17, pp. 10825-10827, 2011.

[12] B. v. d. Brandt, E. Bunyatova, P. Hautle and J. Konter, "DNP with the free radicals deuterated TEMPO and deuterated oxo-TEMPO," *Nucl. Instrum. Meth. A,* vol. 526, pp. 53-55, 2004.

[13] S. J. Nelson, J. Kurhanewicz, D. B. Vigneron, P. E. Z. Larson, A. L. Harzstark, M. Ferrone, M. van Criekinge, J. W. Chang, R. Bok, I. Park, G. Reed, V. K. Weinberg, J. H. Ardenkjær-Larsen, A. P. Chen, R. E. Hurd, L.-I. Odegardstuen, F. J. Robb, J. Tropp and J. A. Murray, "Metabolic Imaging of Patients with Prostate Cancer Using Hyperpolarized [1-13C]Pyruvate," *Sci. Transl. Med.,* vol. 5, no. 198, pp. 1-11, 2013.

[14] A. Comment, J. Rentsch, F. Kurdzesau, S. Jannin, K. Uffmann, R. van Heeswijk, P. Hautle, J. Konter, B. van den Brandt and J. van der Klink, "Producing over 100 ml of highly concentrated hyperpolarized solution by means of dissolution DNP," *J. Magn. Reson,* vol. 194, pp. 152-155, 2008.

[15] S. Bowen and J. H. Ardenkjaer-Larsen, "Enhanced performance large volume dissolution-DNP," *J. Magn. Reson.,* vol. 240, pp. 90-94, 2014.

[16] J. H. Ardenkjær-Larsen, A. M. Leach, N. Clarke, J. Urbahn, D. Anderson and T. W. Skloss, "Dynamic Nuclear Polarization Polarizer for Sterile Use Intent," *NMR Biomed.,* vol. 24, pp. 927-932, 2011.

[17] J. Jain, S. Dey, L. Muralidharan, A. M. Leach and J. H. Ardenkjær-Larsen, "Jet Impingement Melting With Vaporization: A Numerical Study," in *ASME Heat Transfer Summer Conference*, Jacksonville, Florida, 2008.

[18] "Hyperpolarized [13C] Pyruvate Documentation Page," National Cancer Institute - the National Institute of Health, [Online]. Available: http://imaging.cancer.gov/programsandresources/cancer-tracer-synthesis-resources/hyperpolarized-C13-pyruvate-documentation. [Accessed 28 06 2016].

[19] T. Harris, C. Bretschneider, L. Frydman, Dissolution DNP NMR with solvent mixtures: Substrate concentration and radical extraction, J. Magn. Reson. 211 (2011) 96–100. doi:10.1016/j.jmr.2011.04.001.

FIGURE LEGENDS

Fig 1: (A) SPINlab polarizer. The polarizer operates at 5 T and 0.9 K [16]. The polarizer has four independent channels that allow simultaneous polarization of up to four samples (fluid paths). The QC module (cylindrical black unit) is seen to the right of the photo below the touch screen. (B) Fluid Path (FP). Part A: (1) vial (2) dynamic seal (3) co-axial tube (4) valve (5) dissolution syringe. Part B: (6) transfer tube (7) EPA filter (8) receiver vessel (9) QC appendage (10) sterile assurance filter (11) Medrad 65 mL MR syringe. Part A loads into the polarizer (behind the sliding door). The vial is inserted into one of the four airlock and the dissolution syringe is inserted into the corresponding heater-pressure module. After some pump-flush cycles, the airlock gate-valve will open and the vial can be pushed through the dynamic seal to the to the 0.9 K sample space. Part B is initially loaded into the corresponding warmer module seen to the lower right in (A). Shortly before dissolution part B is moved from the warmer to the QC module.

Fig 2: (A) PEEK tube (a), sleeve (b), adapter (c), ferrule (e) and screw (f) assembled to result in the filling tube used for filling of the FP (B) Filling tube placed in correct position in a demonstrational set-up (C) section of a vial with sample; (1) outer lumen (2) inner tube (3) nozzle (4) sample (pyruvic acid)

FIGURE 1

FIGURE 2