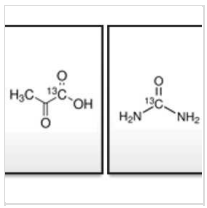

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Mar 22, 2022

Pyruvate and Urea preparation for dissolution DNP V.1

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1

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This document codifies the probe preparation of pyruvate and urea for dissolution dynamic nuclear polarization. Additionally, this also describes the steps recommended for estimation of T1 and polarization and quality assurance to ensure repeatability and robustness of hyperpolarized ^{13}C probe formulation.

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DISCLAIMER:

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Contents

I) General Remarks

A. Glassing and high compound concentration

The highest levels of nuclear spin polarization and MR signal are attained when the ^{13}C concentration is as high as possible.The ideal compound formulations therefore are small molecule liquids prepared neat.If needed, the compounds must be formulated with appropriate (co)solvents and at a concentration such that they reliably form an amorphous glass when cooled rapidly.Glassing is critical to achieve high levels of polarization.

B. Stability of compound formulations

The various compounds are stable to various degrees and the *radicals are light sensitive*.Formulated compounds are best stored frozen and protected from light. Gadolinium(III) chelates, e.g. Dotarem / Gd-

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DOTA, are relatively unstable at low pH, such as when dissolved in pyruvic acid, and will dissociate to free Gd^{3+} ions when in liquid state.This can have a significant negative impact on the T_1 relaxation rate upon dissolution, which results in the hyperpolarized signal decaying faster.To summarize:
Note:

- Protect from light exposure whenever possible
- Do not let compound formulations sit at room temperature for extended periods

II) Trityl Radicals

A. The stabilized trityl radicals used as the source of spin polarization are costly and must be stored and handled properly.They are best stored long term cold, dry and protected from light, e.g. in a jar containing a dessicating agent in the freezer.To prevent the buildup of water condensation, allow them to warm to room temperature before handling.The trityl radicals are typically light, lyophilized solids of various shades of green.To reduce their tendency to cling to surfaces when handling them, it helps to use an antistatic gun to dissipate static electricity on the plasticware and weighing containers.

B. The structures and molecular weights of the radicals:

R =	Name	Formula	Molecular Weight
-CH2CH2OH	OX-63	C52H60Na3O18S12	1426.77
-CH2CH2OCH3	“GE Trityl” / AH-111501	C64H84Na3O18S12	1595.09
-CH3	Finland	C40H36Na3O18S12	1066.46

III) Recipes

(Note: this is for a large batch; scale down as needed)

A. [1-^{13}C] pyruvic acid

Materials

1. **130 mg** GE-Trityl
2. **6.0 g** (~ **4.9 mL**) [1-^{13}C] pyruvic acid (Aldrich: 677175, CIL: CLM-8077) MW: **89.05 g /mol** ; density 1.281
3. **14.4 µL** Dotarem / **500 Milimolar (mM)** Gd-DOTA

Protocol:

1. Set sonicator bath to 40°C.
2. Remove pyruvic acid from freezer and allow to warm to room temperature.
3. Weigh **130 mg** GE-Trityl and transfer to **15 Milimolar (mM)** conical plastic centrifuge tube.
4. Add **6 g** pyruvic acid to tube.
5. Dissolve GE-Trityl with a combination of vortex mixing and bath sonication.
6. Add Gd-DOTA to get net **1.5 Milimolar (mM)**

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7. Aliquot **200 µL** into **1 mL** screw-top cryovials and store in **-80 °C** freezer

B. ^{13}C Urea

Materials

1. **216 mg** OX-63
2. **3100 mg** ^{13}C Urea (Aldrich: 603430) MW: **61.05 g /mol**
3. **7150 mg** Glyerol (Aldrich 56-81-5), MW: 92.09
4. **3.2 µL** of **500 Milimolar (mM)** Gd-DOTA

Protocol:

1. Set sonicator to **60 °C**
2. Static gun zap **15 mL** conical tube
3. Mix **3100 mg** urea with **7150 mg** glycerol in the conical tube. Dissolve by vortexing and sonication.
4. Static gun zap weigh paper and **15 mL** conical tube
5. Weigh **216 mg** OX-63 into the **15 mL** conical tube
6. Cut tip off a pl000 pipette tip
7. Pipette the urea+glycerol mix into the radical tube
8. Shake, vortex, sonicate (**00:00:30**), and centrifuge to dissolve
9. Add **3.2 µL** Gd-Dota (**500 Milimolar (mM)**).
10. Aliquot **450 µL** into **1 mL** screwtop cryovials and store in **-80 °C** freezer

IV) QA Procedure for HP Formulations using Hypersense

A)Microwave sweep, polarization buildup:

1. Load 50-100 µL of HP formulation (pyruvate and urea separately) into HyperSense cup, load into polarizer
2. Perform microwave sweep to obtain polarization frequency
   a. Start with these parameters : 300 min buildup time,100 MHz sweepwidth, 25 mW power
   b. Recommended to do a coarse frequency sweep (5 MHz resolution) to capture full lobe of sweep curve, then do a fine frequency sweep (2--3 MHz resolution) to identify maximum amplitude
   c. If polarizer contains background signal, perform a sweep using same parameters prior to loading sample
   d. Perform routine sweeps every 2--3 months
3. Start polarization at optimal microwave frequency, monitor buildup using RINMR program
4. Note the following parameters, compare with historic values:
   a. Microwave frequency
   b.Buildup curve maximum amplitude (solid state build-up)
   c. Buildup curve time constant (Typical time-constant in HyperSense 957 seconds)

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B) HP dynamic ^{13}C NMR spectroscopy to measure % polarization, T_1:

1. Prepare NMR spectrometer for HP polarization and T_1 measurement:

   a. Insert sample with similar ionic strength, tune coils (^{1}H,^{13}C) and shim

   b. Set up arrayed ^{13}C sequence, verify that it runs properly. Ensure the following parameters are set properly:

      i. Spectral width to cover the signal of interest (Pyruvate at 171 ppm and Urea at 163.5 ppm)
      ii. Transmit + receive frequency
      iii. Pulsewidth + power for a low flip angle pulse (5 - 15°)
      iv. Acquire multiple transients to capture the entire relaxation of the HP signal

2. Prepare empty NMR tube with cap for measurement
3. Prepare polarizer for dissolution, inject dissolution buffer, dissolute. Note the time of start of dissolution
4. Quickly inject HP dissolution into NMR tube, insert into spectrometer, start sequence
5. Use timer to record transfer time from polarizer to spectrometer
6. Save data once acquisition finishes

C) Thermal ^{13}C NMR spectroscopy to measure %polarization, concentration:

1. Setup NMR spectrometer using the following parameters

   a. TR = 5 * T_1 (Pyruvate is ~ 48 s and Urea is ~44 s @ 11.7 T or measured in HP dynamic spectra section IV.D.1)

   b. Alternatively, add 1/100 volume of [M]**500 Milimolar (mM)** Gd-DOTA to reduce required TR

   c. Tip angle = 90°

   d. averages = 8 - 32 (depending on final agent concentration in dissolution)

2. Start acquisition, save once finished

D) Calculation of T_1 and % polarization

1. ForT_1: fit HP dynamic peak integrals to exponential decay

Tip angle correction: multiply each n^{th} transient by $\sec(\alpha)^{n-1}$, where α is the tip angle

2.

For % polarization (P_{HP}): use the peak integrals in (a) the 1st HP spectrum (S_{HP}); and (b) the thermal spectrum (S_T), using the following equation:

$$P_{HP} = P_T \frac{S_{HP}}{S_T} \csc(\alpha)$$

a. $P_T = \tanh\left(\frac{1}{2}\frac{\hbar\gamma B_0}{k_B T}\right)$ is the polarization at the NMR spectrometer field strength

   b. Depending on the NMR spectrometer, S_T may need to be normalized by the number of averages

   c. The "back--calculated" % polarization to the time of dissolution is often reported, assuming constant T1 loss over the transfer time ($t_{transfer}$). This is calculated as

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$$P_{HP,back-calculated} = P_{HP} e^{\frac{t_{transfer}}{T_1}}$$

For each batch of HP probe preparation, the % polarization and T1 are logged (along with polarizer characteristics to enable QA. Typically the % polarization achieved with pyruvate is ~ 14-20% .

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