Published in final edited form as:

RSC Adv. 2016 ; 6(74): 69728–69732. doi:10.1039/C6RA15808K.

Toward Production of Pure ^{13}C Hyperpolarized Metabolites Using Heterogeneous Parahydrogen-Induced Polarization of Ethyl[1- ^{13}C]acetate

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Abstract

Here, we report the production of ^{13}C -hyperpolarized ethyl acetate via heterogeneously catalyzed pairwise addition of parahydrogen to vinyl acetate over TiO_2 -supported rhodium nanoparticles, followed by magnetic field cycling. Importantly, the hyperpolarization is demonstrated even at the natural abundance of ^{13}C isotope (ca. 1.1%) along with the easiest separation of the catalyst from the hyperpolarized liquid.

Graphical abstract

Nuclear Magnetic Resonance (NMR) hyperpolarization is a rapidly expanding area of research, because it provides an opportunity to overcome the low sensitivity problem of conventional NMR spectroscopy and imaging *in vitro* and *in vivo*.^{1–4} One of these

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Electronic Supplementary Information (ESI) available: Catalysts preparation procedure, additional NMR spectra. See DOI: 10.1039/x0xx00000x

approaches is the dynamic nuclear polarization (DNP) method based on polarization transfer from electrons to nuclei via microwave irradiation of a sample containing free radicals.^{5,6} DNP enables hyperpolarization of ^{13}C -labeled substrates such as lactate and pyruvate^{7,8} for metabolic MRI to provide unique information about different pathologies in cancer, heart disease and others.^{9,10} Despite the wide range of molecular targets that can be hyperpolarized by DNP, it is a very expensive hyperpolarization technique, and it requires a relatively long time (~ 1 h) for production of polarization, which significantly limits a broad application of this technique. An alternative approach to achieve hyperpolarized (HP) state in biomolecules is Parahydrogen-Induced Polarization (PHIP),^{11,12} which relies on hydrogenation of some unsaturated precursor with parahydrogen. In PHIP, pairwise hydrogen addition leads to formation of compounds in which the two nascent H atoms from parahydrogen molecule are no longer magnetically equivalent, which is manifested as near unity nuclear spin polarization (P).^{13,14} While fluids hyperpolarized by PHIP can be employed as contrast agents for imaging applications,^{15,16} they typically have relatively short T_1 values, i.e. depolarize within seconds.¹⁷ To increase short lifetime of HP fluids, more sophisticated agents with ^{13}C labeled sites were developed, where these ^{13}C sites are hyperpolarized via polarization transfer from nascent parahydrogen atoms using spin-spin couplings. While this polarization transfer can be accomplished by radio frequency pulse sequences, a less instrumentation demanding alternative is a magnetic field cycling (MFC) approach.^{15,18–20} Angiographic ^{13}C HP agent 2-hydroxyethyl propionate (HEP) produced by PHIP was demonstrated at the very dawn of hyperpolarization era.¹⁵ A few other ^{13}C -labeled PHIP hyperpolarized compounds were developed later for potential biomedical applications: succinate,^{21,22,2} tetrafluoropropionate,²³ phospholactate,^{24–26} and others, despite the chemistry-related challenge of unsaturated precursor design with sophisticated isotope labelling patterns. However, PHIP using side-arm hydrogenation (SAH) that was pioneered by Reineri and co-workers recently,^{27,28} may significantly expand the scope of the PHIP method with regard to the potentially amenable biomolecules. Hyperpolarization of pyruvate and acetate were reported to date,^{27,28} with many more biomolecules likely to follow soon. Furthermore, novel ^{13}C enrichment chemistry to enable simple and low cost incorporation of ^{13}C tags was recently developed for PHIP-SAH method, which can potentially yield hyperpolarized contrast agents with % $P_{^{13}\text{C}}$ of 20–50%.²⁹

However, until recently the PHIP technique primarily relied on homogeneous pairwise hydrogenations with transition metal complexes as catalysts for production of HP contrast agents.^{30,31} The main disadvantage of this approach is the presence of the dissolved homogeneous catalyst, which is expensive and is challenging to separate from the hyperpolarized compounds.^{32,33} Recently, however, PHIP was observed in the heterogeneous reactions in the gas³⁴ and in the liquid phases^{35,36} over immobilized complexes,³⁷ supported metals^{34,38} and ligand-capped nanoparticles.^{39,40} Observation of ^{13}C HP resonance and therefore, production of ^{13}C -hyperpolarized molecules via heterogeneous hydrogenation with parahydrogen was so far shown only for ^{13}C HEP during hydrogenation of 2-hydroxyethyl acrylate over platinum nanoparticles capped with glutathione.⁴⁰ However, the conversion level was extremely low (see Figure S3 in the ESI of reference 41) and may be estimated as *ca* 0.03% for 15 seconds of reaction time. Moreover, along with low degree of substrate conversion the filtration procedure to separate 2 nm

nanoparticles is also a translational challenge, which cannot be mitigated by a straightforward decantation of HP fluids from the solid catalyst during the time frame comparable to ^{13}C T_1 (typically on the order of tens of seconds). Furthermore, HEP has no direct metabolic relevance, and its potential use is limited to angiography.¹⁵

Herein, we report ^{13}C NMR hyperpolarization of a biomolecule via heterogeneous liquid phase hydrogenation of vinyl acetate over solid Rh/TiO₂ catalysts. The ^{13}C nuclear spin polarizations achieved in these experiments are the first reported to date involving heterogeneous reactions over supported metal catalysts.

Titania-supported rhodium catalysts are the best supported metal catalysts for HET-PHIP in both liquid^{36,41} and gas phases.¹⁷ Therefore, two variants of Rh/TiO₂ catalysts corresponding to two different loadings of the metal (1 wt% and 23.2 wt%) were prepared (see ESI for details).

In the first set of experiments, both catalysts were tested in the gas phase hydrogenation of propene to propane with parahydrogen to check their efficiencies in terms of both the percentage of pairwise hydrogen addition level and the overall hydrogenation activity/yield. Both catalysts were active and yielded HP propane (Fig. S1 in the ESI), which is in good agreement with the previous observations.¹⁷ It should be noted that Rh/TiO₂ catalyst with 23.2 wt% rhodium loading resulted in the NMR spectrum with lower intensities of HP lines in comparison to that with 1 wt% rhodium loading despite a higher (*ca.* 100% vs. 75%) chemical yield of the reaction product. Next, both catalysts were used in the liquid phase heterogeneous hydrogenation of vinyl acetate in three organic solvents: benzene-d₆, dimethyl sulfoxide (DMSO)-d₆ and methanol-d₄. A high-pressure setup allowing to carry out hydrogenation reaction at *ca.* 7 atm of gas pressure *in situ* was employed⁴² which allowed us to monitor the reaction inside a 5 mm NMR tube filled with vinyl acetate solution. The schematic presentation of the experimental setup with field cycling procedure is shown in Fig. 1. Utilization of DMSO-d₆ as the solvent was ineffective because no ethyl acetate formation was detected in the case of both catalysts. In contrast, when benzene-d₆ was used as a solvent (Fig. S2 in the SI) and hydrogenation of vinyl acetate with parahydrogen was performed *in situ* in the 9.4 T NMR magnet (corresponding to PASADENA¹¹ conditions), very intense and clearly recognized HP peaks of ethyl acetate were detected at *ca.* 7 atm of hydrogen pressure. A relatively high pressure of H₂ gas along with its high solubility in benzene allowed us to observe weak PHIP effects even several minutes after interrupting hydrogen bubbling (Fig. S2 in the ESI), indicating that hydrogenation of vinyl acetate is relatively slow. The very strong polarized ALTADENA-shaped¹² signatures of HP NMR lines for ethyl acetate were also observed when hydrogenation experiments were conducted in the Earth's magnetic field (Fig. 2) or at ~0.1 μT inside the magnetic shield with the subsequent fast transfer to the high field of the 9.4 T NMR spectrometer (Fig. S3 in the ESI). Importantly, utilization of Rh/TiO₂ catalyst with 23.2 wt% rhodium loading for vinyl acetate hydrogenation in benzene-d₆ solution results in the loss of parahydrogen-derived hyperpolarization of ethyl acetate despite greater chemical reaction yield compared to Rh/TiO₂ with 1 wt% Rh loading. The use of high parahydrogen pressure is very important, and it is clear that the concentration of dissolved H₂ increases the reaction yields. For example, the intensities of HP lines were approximately four-fold

greater when ~7 atm parahydrogen total pressure was used vs. ~1 atm under otherwise the same heterogeneous hydrogenation conditions in methanol-d₄ solvent (Fig. S4 in the ESI). This situation is common, and utilization of relatively high parahydrogen pressures along with the use of efficient rhodium catalyst (23.2 wt% Rh loading) in methanol-d₄ solvent allowed us to observe HP lines in the ALTADENA experiments (Fig. 2b) with ca. 9-fold enhancement e_H corresponding to % $P_H \sim 0.03\%$. The possibility to produce HET-PHIP in the liquid phase opens up new horizons not only for the liquid phase ¹H contrast agents production but also for polarization transfer from HP protons to hetero nuclei via MFC.^{15,27–29,43}

By utilization of the MFC approach and heterogeneous hydrogenation over supported metals we demonstrate hyperpolarization of 1-¹³C resonance of ethyl [1-¹³C]acetate ($e_{13C} \sim 44$, % $P_{13C} \sim 0.035\%$) at 98% ¹³C enrichment (Fig. 3). ¹³C HP signal is also readily detectable at natural abundance (~1.1%) of the ¹³C isotope (Fig. S5 in the ESI). Hydrogenation reaction was carried out in the Earth's magnetic field via parahydrogen bubbling through 80 mM solution of vinyl acetate-1-¹³C in methanol-d₄ for approximately 25 s. Then the bubbling was terminated and the NMR tube with the reaction mixture and the solid catalyst was transferred inside a magnetic shield with a weak (~0.1 μT) magnetic field for a short period of time (~2 s) and then to the 9.4 T NMR spectrometer for ¹³C NMR spectra acquisition (Fig. 3b). This MFC procedure was found to be over three times more efficient (Fig. S6 in the ESI) than carrying out the hydrogenation reaction inside the magnetic shield.²⁹ A plausible explanation is a more efficient proton relaxation at ~0.1 μT vs. that at the Earth's magnetic field. In addition to achieving $e_{13C} \sim 44$ for ethyl [1-¹³C]acetate via HET-PHIP, the chemical conversion was relatively high (ca. 25% conversion of 80 mM vinyl acetate-1-¹³C reagent in benzene-d₆). ¹³C enhancement of ~44 is approximately 50 times lower than that ($e_{13C} \sim 2200$) obtained using similar MFC procedure when homogeneous Rh-based hydrogenation catalyst in methanol-d₄ was utilized. This significant difference can be primarily explained by two factors: (i) longer hydrogenation time (~25 s vs. ~10 s²⁹) resulting in T_1 depolarization of proton hyperpolarization pool at the Earth's magnetic field, and (ii) lower percentage of pairwise parahydrogen addition on supported metal catalysts⁴⁴ vs. that for homogeneous catalysts. Further optimization of HET catalysts for production of solutions of HP contrast agents is required to improve % P_{13C} and the reaction yields. Moreover, the use of near 100% parahydrogen produced with a parahydrogen generator rather than ~50% parahydrogen used here would effectively triple % P_{13C} to ~0.1%. Furthermore, 100% conversion of vinyl acetate to ethyl acetate may provide a four-fold increase in the amount of polarized product, thereby improving the production of the payload (defined as the product of concentration and % P) of HP contrast agent.

To further emphasize that HET-PHIP catalyst should be optimized and matched for a specific task/PHIP precursor system, we utilized the 23.2 wt% Rh/TiO₂ catalyst in PHIP-MFC experiments. It was shown that hydrogenation of 80 mM vinyl [1-¹³C]acetate via parahydrogen bubbling at ca. 7 atm in methanol-d₄ solution yielded very strong ¹³C PHIP polarized resonance of ethyl [1-¹³C]acetate (Fig. S7 in the ESI) with the intensity similar to that observed with the 1 wt% Rh/TiO₂ catalyst in benzene-d₆ (Fig. 3). Moreover, these relatively high ¹³C hyperpolarization levels (along with high chemical conversion) of ethyl [1-¹³C]acetate allows detecting ¹³C PHIP-MFC hyperpolarized resonance even at the natural

abundance of ^{13}C nuclei (Fig. S5 in the ESI). This ^{13}C PHIP polarization produced by using HET hydrogenation with parahydrogen is reported for the first time for an unlabeled compound. The relative simplicity of the presented setup (coupled with convenient production of ~50% parahydrogen using liquid N_2) will enable screening of wide range of molecular PHIP-SAH precursors for their utility to serve as HP contrast agents. In particular, screening of SAH in esters of pyruvate, lactate, amino acids, etc. may enable HET-PHIP of molecules with biological relevance to answer questions about metabolism and function *in vivo*.

In conclusion, we have prepared and successfully used two Rh/TiO₂ HET catalysts for effective pairwise hydrogen addition to produce HP biomolecule in two organic solvents. Optimization of the experimental setup and the use of elevated pressure of hydrogen for heterogeneous hydrogenation experiments allowed us to produce strong ^1H PHIP hyperpolarized patterns for ethyl acetate, which can be potentially a useful molecular probe for cancer and brain metabolism.²⁹ Magnetic field cycling (MFC) allowed us to observe strong hyperpolarized ^{13}C resonance of ethyl [1- ^{13}C]acetate via polarization transfer from protons to slower relaxing 1- ^{13}C site. Because a wide range of biomolecules can be potentially hyperpolarized using PHIP-SAH and MFC, the approach presented here would be useful for preparing pure (i.e., catalyst free) HP contrast agents. The reported results are the first to date demonstration of HP ^{13}C resonance in ^{13}C isotopically labeled and unlabeled (natural abundance of ^{13}C) biomolecule produced over supported metal catalysts in the liquid phase. And presented technique can get 0.1% ^{13}C polarization.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

ITC team acknowledge the grant from the Russian Science Foundation (14-13-00445) for the support of heterogeneous hydrogenation experiments, and FASO Russia project # 0333-2014-0001 for basic funding. VIB and LMK thank RSF grant (# 14-23-00146) for the support of catalysts preparation. The US team thank NIH 1R21EB018014 and 1R21EB020323, NSF CHE-1416268, DOD CDMRP W81XWH-12-1-0159/BC112431, and W81XWH-15-1-0271, and Exxon Mobil Knowledge Build for MFC experiments and setup building.

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Fig. 1. Schematics of the experimental setup for liquid phase heterogeneous hydrogenation over supported metal catalysts with magnetic field cycling (MFC) for effective polarization transfer from ^1H to ^{13}C using PHIP-MFC.

Fig. 2.

(a) Reaction scheme of vinyl acetate hydrogenation with parahydrogen to yield HP ethyl acetate. (b) ¹H NMR spectrum of the solution after heterogeneous hydrogenation of vinyl acetate to ethyl acetate with parahydrogen over 23.2 wt% Rh/TiO₂ catalyst in methanol-d₄ (ALTADENA-type experiment). (c) Corresponding ¹H NMR spectrum of fully relaxed reaction mixture obtained after PHIP hydrogenation experiment shown in (b).

Fig. 3.

(a) Reaction scheme of vinyl acetate-1- ^{13}C heterogeneous hydrogenation by parahydrogen over 1 wt% Rh/TiO_2 catalyst in benzene- d_6 solution with subsequent polarization transfer from protons to ^{13}C (via MFC), (b) ^{13}C NMR spectrum of HP ethyl [1- ^{13}C]acetate, and (c) corresponding ^{13}C spectrum of thermally polarized reaction mixture after hyperpolarization experiments shown in (b).