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Production of Pure Aqueous ^{13}C-Hyperpolarized Acetate Via Heterogeneous Parahydrogen-Induced Polarization

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Abstract

Supported metal catalyst was designed, characterized and tested for aqueous phase heterogeneous hydrogenation of vinyl acetate with parahydrogen to produce ^{13}C-hyperpolarized ethyl acetate for potential biomedical applications. The Rh/TiO_2 catalyst with 23.2 wt% loading produced strong hyperpolarized ^{13}C-enriched ethyl acetate-1-^{13}C detected at 9.4 T. Approximately 14-fold ^{13}C signal enhancement was detected using ~50% parahydrogen gas without taking into account relaxation losses before and after polarization transfer via magnetic field cycling from nascent parahydrogen-derived protons to ^{13}C nuclei. This first observation of ^{13}C PHIP-hyperpolarized products over supported metal catalyst in the aqueous medium opens up new possibilities for production of catalyst-free aqueous solutions of nontoxic hyperpolarized contrast agents for a wide range of biomolecules amenable to Parahydrogen Induced Polarization via Side Arm Hydrogenation (PHIP-SAH) approach.

Graphical Abstract

Supported metal catalyst was designed, characterized and tested for aqueous phase heterogeneous hydrogenation of vinyl acetate with parahydrogen to produce ^{13}C-hyperpolarized ethyl acetate for potential biomedical applications. Approximately 14-fold ^{13}C signal enhancement was detected using ~50% parahydrogen gas at 9.4 T.

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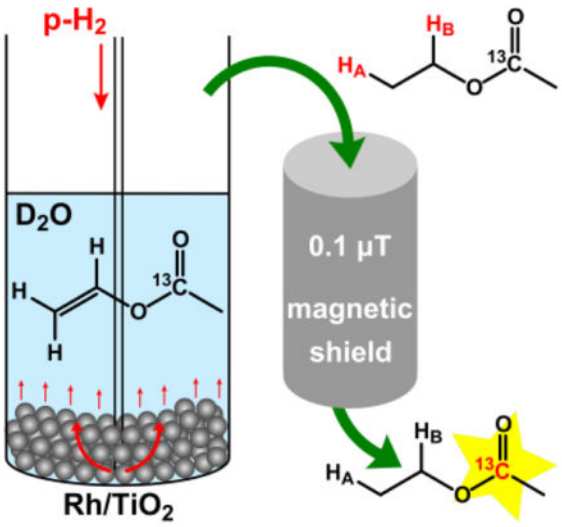

Keywords

parahydrogen; PHIP; ^{13}C NMR; hyperpolarization; polarization transfer

Nowadays, the methods based on magnetic resonance (NMR, MRI) are routinely used as sensitive analytical tools in chemistry and medicine. However, despite their impressive achievements, a potentially wider reach of these techniques is limited by their inherently limited sensitivity associated with the low nuclear spin polarization at thermal equilibrium. The advent of hyperpolarization NMR techniques significantly expands the range of potentially feasible MR applications.[1–9] One of hyperpolarization techniques is the dynamic nuclear polarization (DNP) that allows one to hyperpolarize a wide range of ^{13}C-labeled compounds[10,11] and utilize them for metabolic NMR/MRI.[12–14] However, the main limitations of the DNP technique are (i) a long hyperpolarization time and (ii) high *hyperpolarizer* cost. Parahydrogen-induced polarization (PHIP) is an alternative hyperpolarization technique using singlet spin order of parahydrogen.[15–17] Pairwise addition of parahydrogen (p-H_2), that is addition of the two H atoms from one p-H_2 molecule to magnetically nonequivalent positions of substrate molecule, creates hyperpolarization on the nascent protons in the product molecule. Importantly, the polarization transfer from nascent parahydrogen atoms via spin-spin couplings to heteronuclei (*e.g.* to ^{13}C) results in hyperpolarized (HP) contrast agents with the lifetimes sufficient for their biomedical applications.[18–20]

PHIP allows production of HP liquids that can be directly used for signal enhancement in various NMR/MRI applications.[18,20] Despite the relative synthesis complexity of ^{13}C-labeled PHIP precursor compounds, many successful examples with high polarization levels on ^{13}C nuclei (P_{13C}>10%) in biomolecules have been reported: ^{13}C-succinate,[21] ^{13}C-tetrafluoropropionate,[22] and more recently ^{13}C-phospholactate.[23] While the corresponding pioneering *in vivo* studies are encouraging,[22,24,25] they were performed using injections of liquid HP contrast agent containing homogeneous Rh-based catalyst,[26] which is potentially toxic, and thus it is a definite roadblock for ultimate in-human

translation. Two alternative approaches can potentially address this obstacle: (i) catalyst removal by filtration, or (ii) utilization of heterogeneous catalyst for pairwise p-H_2 addition (HET-PHIP).[9,27–29] The second approach is more advantageous, because it additionally allows catalyst recycling.

Importantly, PHIP medium must be biocompatible, *i.e.* aqueous. The first observation of heterogeneous PHIP effects in aqueous medium was reported in 2010[30] utilizing supported metal catalysts. Recently a similar observation (indicating the possibility of heterogeneous PHIP formation in water) was made using ligand-capped platinum nanoparticles.[31,32] Although the transfer of polarization from nascent parahydrogen protons to ^{13}C nuclei was accomplished at P_{13C} = ~0.013% level,[31] subsequent applications of this system face serious challenges, because (i) separation of HP solution from 2 nm size nanoparticles is required, (ii) very low conversion levels were achieved (*ca.* 0.03% after 15 seconds of chemical reaction),[31] and (iii) the reaction product 2-hydroxyethyl propionate (HEP) has no biomedical relevance beyond angiographic applications.[18]

Recently, an efficient procedure of polarization transfer from nascent parahydrogen protons to ^{13}C using side arm hydrogenation (SAH) approach in combination with adiabatic magnetic field cycling was introduced. It was exemplified with a range of esters bearing an unsaturated alcoholic or carboxylic moiety, including vinyl acetate which yielded HP ethyl acetate-1-^{13}C upon hydrogenation and magnetic field cycling.[33,34] However, these previous studies employed homogeneous catalyst in organic medium, which is incompatible with the long-term goal of in-human translation of PHIP-SAH, which otherwise paves a straightforward and scalable route to efficient ^{13}C hyperpolarization of many biomolecules including ^{13}C-pyruvate.[33–35] Moreover, recent results based on the vinyl acetate heterogeneous hydrogenation in the gas phase with subsequent dissolution in aqueous phases and hydrolysis resulting in hyperpolarized ethanol and non polarized acetic acid mixture production allow to produce pure, biocompatible and catalyst free hyperpolarized fluid.[36]

In this work, we demonstrate that both previous shortcomings of PHIP-SAH can be resolved. Specifically, 2-3 mm beads of TiO_2 with supported rhodium nanoparticles were utilized for the aqueous phase heterogeneous hydrogenation of vinyl acetate-1-^{13}C with p-H_2, followed by polarization transfer to ^{13}C nuclei by means of magnetic field cycling.[33–35]

Recently it was shown that decreasing the size of metal nanoparticles leads to the increase in the pairwise p-H_2 addition level,[9,36] and therefore, the catalyst under investigation should have the smallest possible metal nanoparticles. However, large amounts of metal introduced upon impregnation of a porous support often lead to larger particle sizes, which are disadvantageous in terms of PHIP efficiency. Therefore, in the preparation of the 23.2 wt% Rh/TiO_2 catalyst an aqueous rhodium nitrate solution was used to obtain relatively small metal particles even at the high rhodium loading. Rh was chosen as an active component, because it usually provides the highest PHIP signal enhancements compared to other metals.[37] The catalyst was characterized in detail by transmission electron microscopy (TEM) and X-ray photoelectron spectroscopy (XPS) techniques (Figures 1 and S2). The

average particle size of the 23.2 wt% Rh/TiO_2 catalyst was estimated from TEM data as *ca.* 3 nm, which is favorable for the observation of PHIP effects.[38] Therefore, this catalyst was used for heterogeneous hydrogenation of vinyl acetate in aqueous medium with subsequent polarization transfer from parahydrogen-derived protons to ^{13}C nuclei and ^{13}C NMR spectra detection (Figure 3).

The catalyst's activity for vinyl acetate hydrogenation is expected to be lower in the aqueous medium (vs. that previously shown in organic solvents[39]), and the increase in the total available metal active centers by using 23.2 wt% Rh/TiO_2 catalyst was intended to compensate for this activity loss. Indeed, it was found that 20 seconds of hydrogen bubbling in our setup (~0.5 mL of 0.08 M vinyl acetate-1-^{13}C solution, ~7.1 atm p-H_2 pressure at 150 standard cubic centimeters per minute (sccm) flow rate) was sufficient to reach more than 90% conversion level of vinyl acetate to the reaction products (Figures 3 and S3). ^{13}C signal enhancement of ~14 was observed under these conditions (see Figure 3).

Importantly, the hyperpolarized ^{13}C NMR signal was detected both when hydrogenation was carried out directly inside the magnetic shield and when the NMR tube with the hydrogenation products was briefly placed in the shield after termination of hydrogen bubbling. The latter approach generally resulted in greater ^{13}C polarization values. The size of catalyst beads and the Rh loading can be potentially further optimized to maximize chemical conversion efficiently and the degree of pairwise addition, *i.e.* to maximize the overall yield of ^{13}C hyperpolarization.

Figure 3 also shows that in case of polarization transfer only the ^{13}C NMR signal from hyperpolarized ethyl acetate can be detected. However, in the ^{13}C NMR spectrum of the thermally polarized reaction products (Figure 3c), the equilibrium signals of ^{13}C-labeled unreacted vinyl acetate, ethyl acetate and acetic acid can be seen. Therefore, the formation of acetic acid by hydrogenolysis of ethyl acetate or via direct hydrogenation of vinyl acetate can be easily verified via ^{13}C NMR. Along with the acetic acid the ethanol also is formed as the byproduct of the vinyl acetate heterogeneous hydrogenation with the estimated concentration of approximately 4 mM over supported rhodium catalyst, and the corresponding ^{1}H NMR spectra before and after reaction are shown in Figure S3.

It should also be emphasized that the HET-PHIP ^{13}C signal enhancement (ε_{13C}) of ~14 achieved here is a factor of ~130 lower than that obtained under similar conditions using homogeneous Rh catalyst in methanol-d_4.[35] However, it should be noted that hydrogenation duration was 20 s in this study vs. 10 s in the previous report,[35] and additional T_1 relaxation losses have contributed to lower ε_{13C} observed here (^{13}C T_1 of carboxyl ^{13}C sites is generally on the order of 30-60 seconds *in vitro*[21,22,33–35] and *in vivo*,[10,41] and T_1 of protons at low magnetic fields is generally on the order of 5–10 seconds[42,43]). It is likely that optimization of the catalyst structure and reaction conditions are the potential routes for major improvement for the presented approach.

The stability of the catalyst under reactive conditions is also important, and XPS investigations of the catalyst before and after the reaction were carried out (see SI for details). It was successfully confirmed that the catalyst did not undergo any changes after the

reaction, and Rh nanoparticles are present at the surface of titania only in the metallic state (Figure S2).

Glöggler et al.[31] utilized ~2 nm beads that will likely be challenging to separate fast. In contrast, the 2–3 mm catalytically active beads used here can be easily separated, *e.g.* via simple decantation of hyperpolarized aqueous solution from the catalyst. For instance, Figure S4b shows that even during hydrogenation the beads reside at the bottom of the NMR tube. Alternatively, if the average beads are decreased down to 0.1 mm in diameter, the catalyst suspension (Figure S4a) can be potentially filtered.

The ability to produce hyperpolarized compounds in water using solid catalysts opens up new possibilities for the production of catalyst-free aqueous solutions of nontoxic hyperpolarized contrast agents. Furthermore, polarization transfer from parahydrogen-derived protons to heteronuclei via magnetic field cycling increases the lifetime of hyperpolarization of the produced compounds and minimizes signal background. All these factors are of paramount importance for *in vivo* applications.[18] Therefore, the first observation of ^{13}C hyperpolarized molecules formed via heterogeneous hydrogenation of vinyl acetate with p-H_2 over supported rhodium nanoparticles is a major step forward to biomedical applications of HET-PHIP approach, and to HET-PHIP-SAH in particular, because the latter can be used to hyperpolarize a wide range of biomolecules.[33–35]

In conclusion, we have presented a new approach to produce pure (from catalyst) aqueous solutions of a biomolecule carrying ^{13}C hyperpolarization. It is based on the heterogeneous side arm hydrogenation (SAH) of a suitable precursor molecule with p-H_2 over supported metal catalysts with subsequent or simultaneous polarization transfer from parahydrogen-derived protons to ^{13}C nuclei via magnetic field cycling. The ~90% conversion in the heterogeneous hydrogenation reaction in water achieved here (which is comparable to the conversion achieved by homogenous PHIP hyperpolarization[23] for *in vivo* ^{13}C imaging[24]) can be further increased by increasing p-H_2 pressure and flow rate, and by increasing the amount of catalytically active sites on the catalyst surface. We believe that combination of aqueous phase heterogeneous catalysis by supported metals with HET-PHIP-SAH and polarization transfer approach offers a powerful tool for the production of hyperpolarized biocompatible contrast agents for MRI, because the demonstrated method is scalable and not demanding instrumentationally.

Experimental Section

For the liquid phase heterogeneous hydrogenation 23.2% Rh/TiO_2 solid catalyst was placed at the bottom of a 5 mm high-pressure medium-wall NMR tube which was then filled with the solution of ^{13}C-labeled vinyl acetate in D_2O. After that the NMR tube was pressurized up to ~7.1 atm of p-H_2 pressure and heated to 90 °C, and then p-H_2 was bubbled through the solution for the period of 20 seconds at the flow rate of 150 sccm. Note the samples were not degassed before reaction and hyperpolarization process. As soon as bubbling was stopped, the NMR tube was transferred from the Earth's magnetic field to the very low magnetic field (~0.1 μT) inside the magnetic shield and then adiabatically transferred to the 9.4 T high field of the NMR spectrometer, where ^{13}C NMR spectra were acquired (Figures 2, 3). The

detailed experimental setup is presented in Figure S1. The procedures for catalysts preparation, hydrogenation and XPS experiments, and additional NMR spectra are given in SI.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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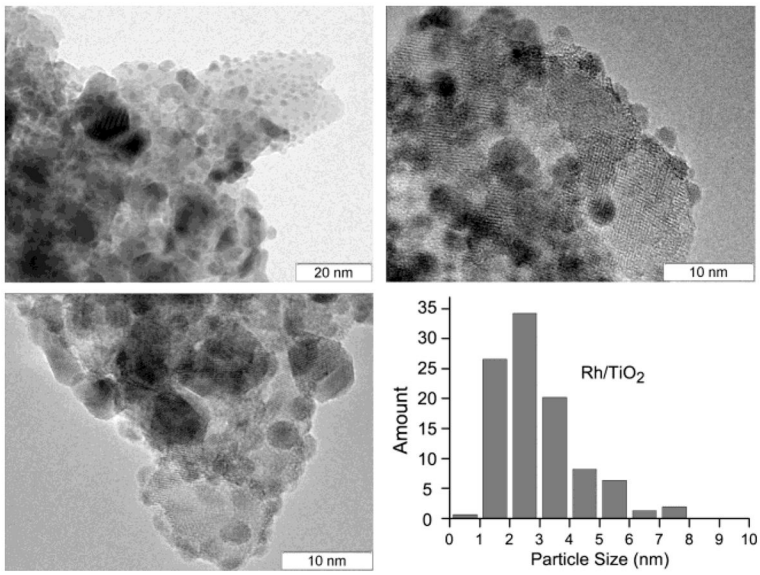

Figure 1.

TEM images and metal particles size distribution for 23.2 wt% Rh/TiO_2 catalyst obtained via wet precipitation from aqueous rhodium nitrate solution. The average size of metal particles is *ca.* 3 nm.

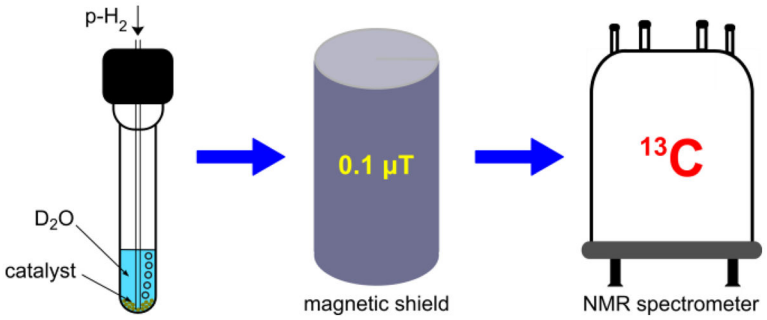

Figure 2.
Experimental procedure for ^{13}C hyperpolarization and NMR spectra detection via PHIP-SAH and magnetic field cycling.

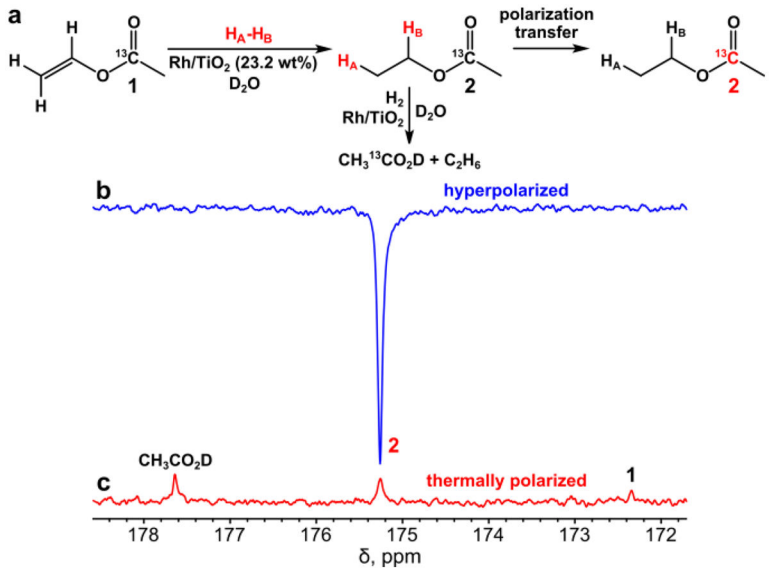

Figure 3.

(a) Reaction scheme of vinyl acetate-1-^{13}C heterogeneous hydrogenation with parahydrogen (p-H_2) over 23.2 wt% Rh/TiO_2 catalyst in D_2O solution with subsequent polarization transfer from protons to ^{13}C nuclei, (b) ^{13}C NMR spectrum of HP ethyl acetate-1-^{13}C, and (c) corresponding ^{13}C spectrum of thermally polarized sample after waiting for a time period longer than $5 \cdot T_1$. All spectra were acquired with 1 signal scan. NMR tube with the hydrogenation products was placed in a magnetic shield after termination of hydrogen bubbling. The ^{13}C signal enhancement (ε_{13C}) is ~14, $\%P_{13C}$ ~ 0.011%.