Electronic Supplementary Material (ESI) for Chemical Communications.

Electronic Supplementary Information for: Combined Homogeneous and Heterogeneous Hydrogenation to Yield Catalyst-Free Solutions of Parahydrogen-Hyperpolarized [1-^{13}C]Succinate

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1 Materials and Methods

All NMR experiments (unless otherwise stated) were performed in a 1.4 T ^{1}H-^{13}C dual resonance SpinSolve benchtop NMR system (Magritek, Aachen, Germany).

Parahydrogen at >95% enrichment was generated by passing hydrogen gas (>99.999% purity) over a hydrated iron oxide catalyst in a cryostat operating at 30 K (Advanced Research Systems, Macungie, U.S.A.).

For magnetic field sweeps for polarization transfer, an MS-2 four-layer mu-metal magnetic shield (Twinleaf LLC, Princeton, U.S.A.) was used to provide a 10^4 shielding factor against external magnetic fields. No static shim fields were required, since the residual field within the shield was on the order of 5 nT. The time-dependent applied magnetic field was generated using the built-in B_y shim coil, with current supplied with an NI-9263 analog output card (National Instruments, Aachen, Germany) with 10 μs time precision. The guiding magnetic field for sample transport in and out of the magnetic shield was provided with a handmade solenoid, with current supplied with a Keysight U8001A current source (Keysight Technologies, Böblingen, Germany).

1.1 Generating and Purifying Hyperpolarized Fumarate

The precursor solution for all fumarate experiments was 125 mM acetylene dicarboxylic acid monopotassium salt, 125 mM NaOD, 125 mM sodium sulphite and 3.5 mM ruthenium catalyst $[RuCp^*(CH_3CN)_3]PF_6$ (product number 667412) in D_2O. All chemicals were purchased from Sigma Aldrich.

For fumarate experiments a stainless steel reactor with an internal volume of 20 mL was used. 1/8 in O.D. PEEK (polyether ether ketone) tubing was used to flow the para-enriched hydrogen gas, and 1/16 in O.D. 0.5 mm I.D. PTFE (polytetrafluoroethylene) capillaries were used for solution flow. These tubes were connected to the reactor via 1/4-28 PEEK fittings, and to the gas flow-control manifold via Swagelok fittings (Swagelok, Frankfurt, Germany). Two heater mats were wrapped around the reactor and surrounded by glass fibre insulation, maintaining a constant 85°C temperature.

To form fumarate, 0.8 mL of precursor solution was loaded into the reactor and given 10 s to reach 85 °C. The reactor was sealed, and parahydrogen was bubbled into the reaction solution at approximately 6 L/min for 60 s at a pressure of 8.5 bar. After bubbling, a two-way microfluidic valve was opened and the sample was ejected through a PTFE capillary into a 10 mm NMR tube in the magnetic shield underneath. To prevent the sample from passing through low-magnetic-field regions that could cause polarization loss during sample transport in/out of the shield, a guiding solenoid was used to provide a 10 μT field. After the sample reached the 10 mm NMR tube, the field was nonadiabatically (rapidly) dropped to 50 nT and then adiabatically ramped to 2 μT in 1 s to transform the proton singlet order into ^{13}C magnetization. The solution was extracted through a 1/16 in PTFE capillary into a syringe, and 200-250 μL of this solution was injected into a 5 mm NMR tube and placed into the 1.4 T benchtop NMR magnet (SpinSolve) for signal acquisition as a control sample (sample A).

The remainder of the sample (sample B) underwent the precipitation purification procedure, and was then further hydrogenated to form succinate. The sample was mixed with 370 ± 20 μL 1 M disodium fumarate in D_2O to raise the overall fumarate concentration, and this was injected into a glass filter funnel containing 1 mL 12 M HCl. The low pH induced immediate precipitation of fumaric acid as a solid powder, and the remaining solution was vacuum filtered away. A Halbach magnet array surrounded this filtration stage, to provide a constant 400 mT magnetic field.[1] The hyperpolarized solid fumaric acid sample was dissolved in (unless otherwise specified) 1 mL of 1 M NaOD in D_2O. This sample was the precursor for the 2nd hydrogenation step to form succinate.

1.2 Generating Hyperpolarized Succinate

During the fumarate purification procedure, the heterogeneous catalyst (Pd/Al_2O_3 powder at 15 wt.% loading unless otherwise specified) was loaded into a PEEK reactor vessel kept at 85°C. The purified hyperpolarized fumarate solution was injected into the reactor, and it was sealed. Hydrogen gas was bubbled in at approximately 6 L/min for 20 s at a pressure of 8.5 bar. After bubbling, a two-way microfluidic valve was opened and the sample was ejected through a 1/8 in. PTFE capillary into a 5 mm NMR tube, and this sample was placed into the 1.4 T benchtop NMR magnet for signal acquisition. Much of the solid catalyst was caught in the 1/8 in. PTFE capillary and removed before the start of the next experiment.

1.3 Heterogeneous Catalyst Preparation and Characterization

Table 1. Pd content obtained from XRF, average particle sizes measured from chemisorption and TEM data, and Pd dispersion measured from CO chemisorption uptakes.

Catalyst designation	Pd content (wt.%)	Average Pd particle size (nm)		BET area (m^2/g)
		Chemisorption	TEM	
Pd/Al_2O_3-15	13	6.7±0.8	n/a	n/a
Pd/Al_2O_3-20	18.4	8.3±0.4	7±2	152

1.3.1 Preparation

The Pd/Al_2O_3 catalysts were prepared by incipient-wetness impregnation[2] of alumina Al_2O_3 (Sasol TKA-423, S_{BET} = 200 m^2/g) with an aqueous solution containing palladium (II) nitrate to obtain ≈20 wt% or 15 wt% Pd metal loading. After impregnation, the sample was dried in the air and then calcined at 400°C. The prepared catalysts demonstrated pellet diameter ranging from 0.2 to 0.3 mm.

The adsorption method was used to prepare palladium catalyst on a carbon support (S_{BET} = 2000 m^2/g). $(NH_4)_2PdCl_4$ salt was used as a Pd source. The prepared powder was then reduced with an aqueous solution of sodium borohydride. Then the sample was thoroughly washed with water and dried at 90°C for 4 h. The Pd content was found to be 1% (measured by XRF). Commercially available Pt/Al_2O_3 catalyst (Engelhard 4759; Pt content 5 wt.%; 2.5 nm Pt NPs average size; S_{BET} = 168 m^2/g) was used as received.

1.3.2 Catalyst characterization

Elemental analysis

The precise Pd metal content in prepared catalysts was determined by the X-ray fluorescence method using ARL PERFORM'X XRF spectrometer (Thermo Scientific, USA) equipped with a rhodium anode as an X-ray source. The results are shown in Table 1.

TEM analysis

Transmission electron microscopy (TEM) images were obtained on a JEOL JEM 2010 microscope (JEOL, Japan) with a spatial resolution of 1.4 Å. The microscope was operated in the transmitted electron detection mode at an accelerating voltage of 200 kV. The ImageJ software package (National Institutes of Health, USA) was used for analysis of collected TEM images. To obtain statistical information, at least 300 particles of the Pd/Al_2O_3-20 sample were processed to determine the size distribution and average size of Pd nanoparticles. A TEM micrograph depicting Pd nanoparticles of Pd/Al_2O_3-20 is shown in Fig. 1. The black dots correspond to Pd nanoparticles. The Pd/Al_2O_3-20 catalyst displays unimodal Pd particle size distribution and the average diameter is 7±2 nm.

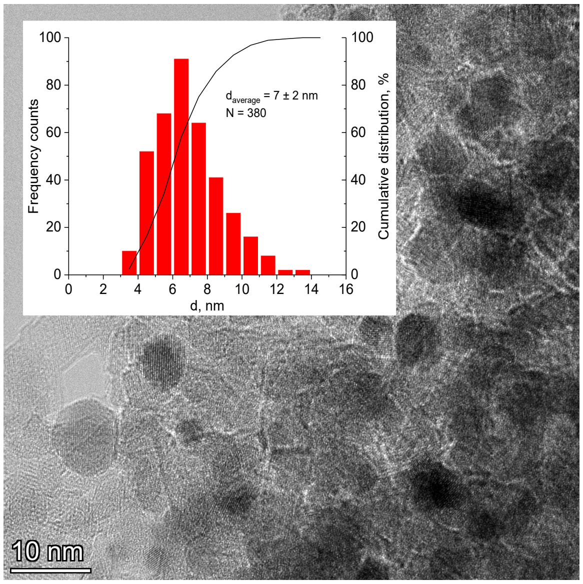

Figure 1. High-resolution TEM image of Pd/Al_2O_3-20 catalyst. The inset shows the distribution of Pd nanoparticles.

N_2 sorption

The surface area and porosity of Pd-20 catalyst were examined by N_2 isoterms using an automatic Micromeritics ASAP 2400 analyzer (Micromeritics Instrument Corp., Norcross, USA). The specific surface area was calculated using the Brunauer-Emmett-Teller (BET) equation at five different relative pressure (P/P_0) values (from 0.06 to 0.20). The pore size distribution was obtained from desorption isotherm using the Barrett-Joyner-Halenda (BJH) method. The catalyst showed a type IV isotherm, which is an intrinsic feature of mesoporous materials.[3] The catalyst showed the H1 hysteresis loop and a unimodal pore size distribution, indicating the existence of uniform pores. The surface area and average pore diameter of the Pd/Al_2O_3-20 catalyst were found to be 152 m^2/g and 10.4 nm.

CO chemisorption

The average Pd particle size on the surface was evaluated using pulse chemisorption of CO at 25°C by assuming a CO-to-surface Pd atom stoichiometry of unity. For each catalyst the measurements were repeated two times with different loadings. The calculated average diameter of Pd NPs in Pd-20 catalyst using CO chemisorption data is 8.3±0.4 nm, which is within the uncertainties range of TEM analysis. Pd-15 catalyst shows smaller NPs, the calculated average size is 6.7±0.8 nm (from CO chemisorption analysis).

1.4 Catalytic tests

For sample preparation, 20 mg of Pd/Al_2O_3 catalyst was placed at the bottom of a medium-wall 5 mm NMR tube (Wilmad) tightly connected with 1/4 in. outer diameter PTFE tube. Next, 0.5 mL of 100 mM sodium fumarate in deuterated water was added. The samples were pressurized up to 6 bar and preheated in a water bath to 100°C for 30 s. The volumetric feed flow rate of hydrogen was regulated using a thermal mass flow controller (Brooks Instruments, Netherlands). Hydrogen was bubbled through the sample positioned inside water bath. Next, the sample was cooled down to room temperature and ^{1}H NMR spectra were acquired for conversion evaluation. The experiments were repeated two times for each catalyst.

1.4.1 Products identification

NMR experiments were carried out on a 7.05 T Bruker AV 300 MHz NMR spectrometer. All NMR spectra of a reaction mixture were acquired in thermal equilibrium using a $\pi/2$ rf pulse. For deuterium NMR acquisition, the lock coil was used as a transceiver. For ^{2}H NMR the sample was prepared as follows: the heterogeneous Pd catalyst was filtered out, deuterated water was evaporated from the sample and distilled water was added.

1.4.2 Catalyst recyclability

The recyclability of the Pd/Al_2O_3 catalysts (both Pd-15 and Pd-20) was studied in fumarate hydrogenations in 5 mm NMR tubes. Each hydrogenation run involved bubbling hydrogen gas (purity >99.999%) through a solution of 100 mM sodium fumarate dibasic (purity >99%), in D_2O (isotopic purity of 99.9%) at a pressure of 6 bar for 30 s. Upon completion of the reaction, the catalyst was filtered from the reaction mixture and washed with distilled water. Then the catalyst was either dried in air at 100°C for 1 h or reduced under H_2 flow (30 sccm) at 100°C for 1 h before the next reaction cycle. It can be seen from Fig. 2 no decrease in catalytic activity was observed over three consecutive runs for reduced Pd-20. Reduced Pd-15 catalyst showed decrease in catalytic performance (from 100 to 81% in conversion) only in the third run. Drying between cycles was found to be less effective for maintaining high catalytic activity.

1.5 ICP-MS Analysis of Pd content in filtered solutions

To assess the content of residual Pd in solution (from leaching from the heterogeneous catalyst) after filtering the succinate solution, ICP-MS (inductively-coupled plasma mass spectrometry) measurements were carried out on two samples prepared in the same way. First, the heterogeneous hydrogenation was carried out in a 5 mm NMR tube using 15 wt.% Pd/Al_2O_3 catalyst in a 500 µL solution of 100 mM sodium fumarate in D_2O. Hydrogen was bubbled through the solution for 30 s at 6 bar pressure, after which the tube was depressurized. The sample was extracted and syringe-filtered in <3 s using a 0.2 µm pore diameter syringe filter (Chromafil Xtra H-PTFE-20/25). This sample was diluted by a factor of 100 for the ICP-MS measurement. The detection limit of the ICP-MS instrument (Agilent 8800) was 5×10^{-7} mass %, corresponding to 5 ng of Pd per mL of solution, or 47 nM. No Pd was observed in the measurement, so the Pd concentration in the measured sample was less than 47 nM. Accounting for the 100× dilution factor, this means the Pd concentration in the undiluted succinate solution is less than 4.7 µM (less than approx. 5 µM).

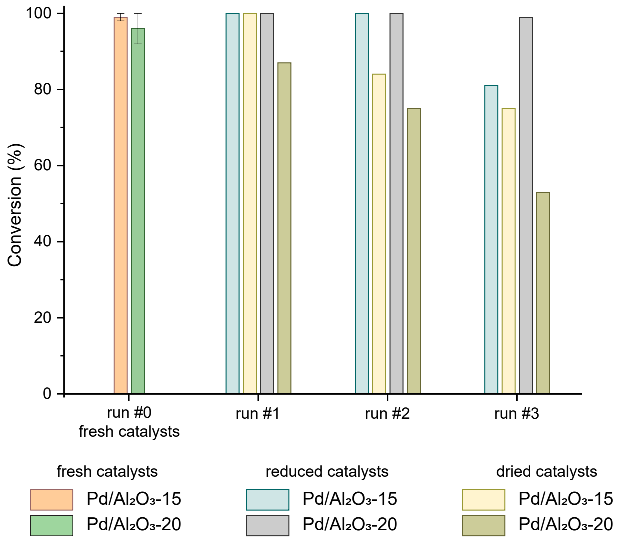

Figure 2. Results of recycling test of Pd-15 and Pd-20 catalysts.

The Al content in the solutions was not determined since we don't expect contamination of the solution with Al_2O_3 given its high stability (it is unlikely to disintegrate in water, whereas Pd may in principle be leached into the water, although we didn't observe this either). Moreover, alumina is used in drinking and wastewater purification as an adsorbent due to its stability in wide range of conditions.

2 Polarization and Error Calculations

Thermal equilibrium ^{1}H NMR spectra were acquired of the fumarate control sample (sample A) and the succinate sample (sample B) to measure the concentration of fumarate in sample A (C_F^A) and the concentration of fumarate and succinate in sample B (C_F^B and C_S^B). These values were corrected for the partial deuteration (see Section 3). Knowing these concentrations, in addition to the volume of sample A (V^A) that was mixed with a volume V^0 of the 1 M unpolarized fumarate solution, allowed us the calculate the polarization of the hyperpolarized fumarate and succinate molecules.

The absolute polarization $P_\mathrm{F}^\mathrm{abs}$ of fumarate in sample B was measured by comparing the hyperpolarized fumarate

^{13}C signal from sample B S_F^B to the thermal equilibrium ^{13}C signal (S^standard) from a standard of known-concentration (C^standard, 0.5 M ^{13}C spins).

$$P_\mathrm{F}^\mathrm{abs} = \frac{1}{\Gamma} \times \frac{1}{\chi} \times \frac{C^\mathrm{standard}}{C_\mathrm{F}^\mathrm{B}} \frac{S_\mathrm{F}^\mathrm{B}}{S^\mathrm{standard}} \frac{\sin\left(\theta^\mathrm{standard}\right)}{\sin\left(\theta^\mathrm{B}\right)}, \qquad (1)$$

where θ is the pulse flip-angle used, Γ is the thermal equilibrium ^{13}C polarization at 1.4 T and room temperature (1.2×10^{-6}) and χ is the fraction of fumarate molecules that contain a ^{13}C spin in the carboxylate position (2.2% at natural abundance).

The dilution factor D which represents the fraction of fumarate molecules in sample B that underwent the hyperpolarization procedure is given by:

$$D = \frac{C_\mathrm{F}^\mathrm{A} \times V^\mathrm{A}}{C_\mathrm{F}^\mathrm{A} \times V^\mathrm{A} + (1\,\mathrm{M}) \times V^0}. \qquad (2)$$

Using this we can calculate the polarization of the hyperpolarized fumarate molecules in sample B:

$$P_\mathrm{F} = P_\mathrm{F}^\mathrm{abs} \times D. \qquad (3)$$

The same process was used to calculate the polarization levels of hyperpolarized succinate.

The error bars reported in Fig. 3 come from error in measuring sample volumes that are used to calculate the dilution factor. To calculate the total error for each measurement we have propagated the error of each individual sample volume measurement.

3 Partial Succinate Deuteration

We observed in high-resolution ^{1}H NMR spectra that the succinate molecules were partially deuterated during the heterogeneous hydrogenation, presumably due to addition of one or two deuterons from D_2O across the fumarate double bond. This is shown in Fig. 3. We did not see deuterated fumarate form in any experiments (in either ^{1}H or ^{13}C NMR spectra).

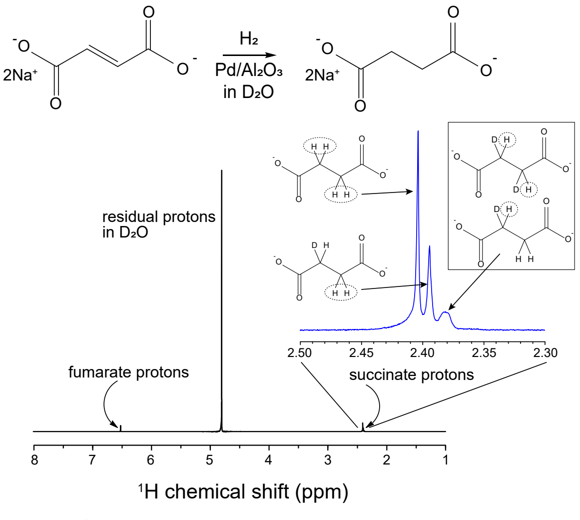

Figure 3. ^{1}H NMR spectrum of a solution following partial hydrogenation of fumarate to succinate. Three peaks are visible which correspond to different proton environments in succinate, succinate-2-d, and succinate-2,3-d_2.

To quantify the degree of deuteration we carried out additional hydrogenation reactions under different conditions. The hydrogenation of 100 mM sodium fumarate in 0.5 mL D_2O was performed in an NMR tube containing 30 mg of 10, 15 or 20 wt% Pd/Al_2O_3 catalyst, kept at 98°C in a water bath. Hydrogen was bubbled through the solution for 30 s at 150 sccm and an absolute pressure of 6 bar. The NMR tube was then depressurized and left to cool to room temperature. ^{1}H NMR spectra of the reaction mixture were acquired to measure the degree of succinate deuteration. We carried out six experiments, two replicates for each of the catalyst types, and observed the following succinate composition on average: $43 \pm 1.3\%$ succinate, $50 \pm 1.0\%$ succinate-2-d_1, $7.7 \pm 1.4\%$ succinate-2,3-d_2. This means we underestimated the succinate concentration in the hyperpolarization solutions by $16 \pm 1.3\%$, and this factor is accounted for in all results shown in the manuscript.

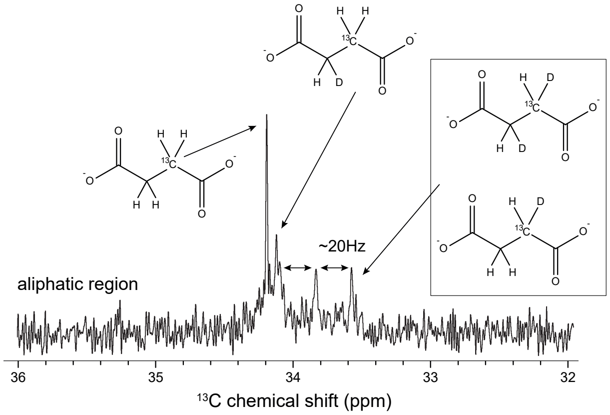

Figure 4. ^{13}C NMR spectrum of a solution following partial hydrogenation of fumarate to succinate.

To confirm that no other succinate isotopomers were forming, we additionally acquired a high-resolution ^{13}C NMR spectrum from one of the reaction mixtures, shown in Fig. 4. There is no quintet peak pattern visible, indicating that succinate-2,2-d_2, succinate-2,2,3-d_3, and succinate-2,2,3,3-d_4 are not formed.

Bibliography

(1) Knecht, S.; Blanchard, J. W.; Barskiy, D.; Cavallari, E.; Dagys, L.; Van Dyke, E.; Tsukanov, M.; Bliemel, B.; Münnemann, K.; Aime, S., et al. Rapid hyperpolarization and purification of the metabolite fumarate in aqueous solution. *Proc. Natl. Acad. Sci. U.S.A.* **2021**, *118*, e2025383118.

(2) Marceau, E.; Carrier, X.; Che, M. *Synthesis of Solid Catalysts*; John Wiley Sons, Ltd, 2009; Chapter 4, pp 59–82.

(3) Sing, K. S. Reporting physisorption data for gas/solid systems with special reference to the determination of surface area and porosity (Recommendations 1984). *Pure and applied chemistry* **1985**, *57*, 603–619.