Heteronuclear Polarization Transfer Using Selective Pulses during Hydrogenation with Parahydrogen

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Received February 21, 1996

The PHIP technique was introduced in 1986 by Bowers and Weitekamp (2, 3) and has since been used as a method for the examination of homogeneous hydrogenation reactions *in situ*. Widespread applications have been found, for example, the detection of reaction intermediates, examination of reaction mechanisms, and kinetic studies (4-9). The PHIP method takes advantage of the selective overpopulation of spin states of the hydrogenated products, due to the addition of parahydrogen to double or triple bonds of organic compounds, or directly to transition metal compounds.

If both hydrogen atoms from the parahydrogen retain their phase correlation during the transfer reaction, then the corresponding spin levels of the product (i.e., the $\alpha\beta$ and $\beta\alpha$ states) become highly overpopulated. Accordingly, the difference in population of individual energy levels can be as much as five orders of magnitude higher than those resulting from the thermal Boltzmann distribution.

In terms of product-operator formalism, the density matrix of parahydrogen can be described as

$$\boldsymbol{\sigma}(t_0) = 0.25 - I_1 I_2.$$
[1]

After the development under the Hamiltonian of the product spin system in which these protons form an AX system, and after averaging over the whole evolution time, the spindensity matrix becomes

$$\boldsymbol{\sigma}(t_{\text{ave}}) = 0.25 - I_z^{\text{A}} I_z^{\text{X}}.$$
 [2]

In short, addition of parahydrogen in the presence of a static magnetic field leads to a product-operator term corresponding to longitudinal two-spin order for the hydrogenation product. This abnormally high population, in the following termed "polarization,"¹ persists for a time on the order of the spin–lattice relaxation time. Up to now, most applications of this method exploit proton polarizations; only a few publications have dealt with the subsequent transfer of polarization to heteronuclei (4, 9, 10).

Two simple pathways for heteronuclear polarization transfer have been found, which can be detected after application of a simple hard 90° pulse to the heteronucleus. The first mechanism is that of the well-known nuclear Overhauser effect, which transfers polarization by cross relaxation from the added hydrogen atoms to the heteronucleus. The first example of using PHIP for this purpose was signal enhancement in a 31 P spectrum (4). In the case of generating a reaction product via hydrogenation whereby the former parahydrogen protons end up in an AA' or AB part of a spin system, even stronger enhancements can be achieved owing to higher-order effects (11). More recently, we were able to detect a signal enhancement of up to 2500 in a ¹³C spectrum as a result of hydrogenation of acetylenedicarboxylic dimethyl ester (10). A second method of transferring polarization to nuclei with low gyromagnetic ratio and low natural abundance is the application of the INEPT+ sequence to the

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¹ "Polarization," strictly speaking, is restricted to a spin system for which a spin temperature can be defined. An alternate, more general expression, would be "alignment."

polarized hydrogenation products, which in the case of an iridium catalyst generates a 160-fold enhancement of the signal strength of a 13 CO group (9).

All these techniques of polarization transfer have some disadvantages with respect to a general *in situ* NMR experiment. The transfer by way of cross relaxation is dependent on the magnitude of the dipolar couplings and may be severely decreased by competing relaxation processes. Cross relaxation and the application of the INEPT+ sequence both lead to anti-phase signals. Consequently, broadband decoupling of the protons cannot be used to increase the signal-to-noise ratio of the signals from the hydrogenation product. Both techniques lead to signals that are anti-phase with respect to long-range couplings, particularly when applied to ¹³C spectroscopy. As these splittings are often on the same order as the experimental linewidths, the intensities of the signals may suffer significantly. Finally, only very few hydrogenation reactions lead to an AA' or an AB spin system.

The recently introduced SEPP technique (12) transforms the longitudinal two-spin order term $I_z^A I_z^X$ into in-phase magnetization on one of the protons originating from the parahydrogen. In this publication, we show that this technique can be used for efficient polarization transfer to other nuclei by first transforming the $I_z^A I_z^X$ magnetization of the PHIP-polarized protons into in-phase magnetization, followed by an INEPT or DEPT pulse sequence. (Note that the heteronuclear polarization-transfer step prepares the proton signals in an anti-phase configuration with respect to the ¹³C-H coupling, not with respect to the H–H coupling.)

The experiments were carried out in a spinning 10 mm NMR tube in a 400 MHz Varian spectrometer (VXR-400). Parahydrogen was enriched to about 50% by passing ordinary hydrogen over charcoal in a U-tube cooled to 77 K in liquid nitrogen. This gas was bubbled into a solution containing 3.1 ml CDCl₃, 20 mg of the catalyst [Rh(norbornadiene)(PPh₃)₂], and 100 μ l of 1-hexyne or phenylacetylene (13). In order to carry out the experiments while spinning the sample tube, a long glass tube with an outer diameter of 5 mm was fixed centrally inside the magnet bore. One end of this glass tube (the gas inlet) extended about 3 cm outside the magnet; the other end reached about 2 cm inside the 10 mm NMR sample tube. Its purpose was to hold in place a long polyethylene tube which carried the parahydrogen gas to a 1 mm glass capillary that dipped into the reaction solu-



FIG. 1. The three proposed pulse sequences; in each case, the delay t_1 is adjusted to $1/(2J_{CH})$. (a) SEPP-INEPT with refocusing, (b) SEPP-INEPT+, and (c) SEPP-DEPT.



FIG. 2. ¹³C-NMR spectra recorded during hydrogenation of phenylacetylene to styrene with parahydrogen, starting with an E-BURP-2 pulse on M (at 5.13 ppm) and generating a signal from the ¹³C nucleus directly bound to A. (a) SEPP-DEPT ($\theta = 54^{\circ}$) with decoupling during acquisition. (b) Proton-coupled SEPP-DEPT ($\theta = 90^{\circ}$). (c) SEPP-INEPT (evolution through 54°) with refocusing and decoupling. (d) Proton-coupled SEPP-INEPT+ (evolution through 45°), starting with a soft pulse on A (6.61 ppm). (e) The same as (d) but decoupled.

tion. This could be retracted just before the radiofrequency pulse sequence so that the field homogeneity was not disturbed by the hydrogen bubbles.

To permit a proper comparison of the performance of the different pulse sequences, the hydrogenation conditions must be the same in order to achieve the same degree of initial polarization. Consequently, we carried out a series of experiments using six different pulse sequences. Any individual experiment in this series involved 35 s of hydrogenation (with the capillary inside the solution), followed by an interval of 3 s after the capillary had been removed, before starting the pulse sequence. Two dummy experiments were carried out before each run. A two-step phase cycle was used to cancel spurious signals.

Consider an AMX spin system in which the parahydrogen is transferred to spins A and M, while X is a ¹³C spin that is directly bonded to A but two bonds away from M:

$$R_{1}^{-13}C = C - R_{2}^{-13} - R_{2}^{-13} - R_{1}^{-13} - R_{1}^{-1$$

In order to transform the $2I_z^A I_z^M$ magnetization of the highly polarized proton spins into I_x^A magnetization, we apply a 90° E-BURP-2 pulse (14) to spin M, generating $2I_z^A I_y^M$. Immediately afterward, a hard 90° proton pulse converts this into $2I_y^A I_z^M$. This avoids having to apply separate selective pulses to excite the outer ¹³C satellites of the A proton. Simulations (15) indicate that the side effects due to evolution during a simple selective pulse (for example, a rectangular pulse) are less serious than those that occur in more sophisticated (and, therefore, longer) shaped pulses. This makes the experiment feasible even if no pulse shape generator is available. During an evolution time of $1/(2J_{AM})$, the $2I_y^A I_z^M$ magnetization converts into $-I_x^A$, if a simultaneous 180_x^{α} proton pulse is applied after an interval $1/(4J_{AM})$ to refocus the ¹³C–H coupling. Once the in-phase magnetization $-I_x^A$ has been created, signal enhancement of the ole-finic carbon sites can be achieved by the well-known techniques of heteronuclear polarization transfer (Fig. 1) (16).

We have successfully applied SEPP-INEPT (with refocusing), SEPP-INEPT+, and SEPP-DEPT (12, 15) to olefinic products of the hydrogenation of phenylacetylene and 1-hexyne. These new techniques have been compared with INEPT+ and a DEPT sequence, modified by the addition of a hard proton 90° pulse after the last refocusing delay. All the results have also been compared with a simple ¹³Cpulse-acquire experiment. The initial selective pulse (E-BURP-2) was applied to one or the other of the newly polarized protons (from parahydrogen) added across the triple bond. The final refocusing interval of the refocused INEPT and the INEPT+ sequences was optimized to achieve spectral editing (16) according to the number of directly bound protons at the ¹³C site, i.e., 45° of relative precession in the case of two protons, 90° precession for one proton, and 54° to achieve the optimum for both sites. For the DEPT sequences, the proton editing pulse was modified in a corresponding manner.

Almost all the selective pulse experiments gave a higher signal-to-noise ratio than the nonselective sequences. The resulting spectra (Figs. 2 and 3) show stronger signals than the INEPT+ sequence or the modified DEPT sequence. Because of the strong cross relaxation between the directly bonded protons, application of a single hard 90° pulse generates two- to threefold stronger signals than the nonselective INEPT+ or modified DEPT sequence. In these cases, the NOE is more effective than the transfer by the nonselective pulse sequences, which do not convert the NOE enhancement into detectable magnetization. However, the application of pulse sequences starting with SEPP leads to signals



FIG. 3. ¹³C-NMR spectra obtained during hydrogenation of 1-hexyne with parahydrogen, starting with a selective pulse on proton A (5.78 ppm) followed by selective DEPT ($\theta = 54^\circ$) with decoupling (a) and coupled (b). Note the eightfold expansion of the frequency scale in (b).

with a comparable signal-to-noise ratio, even if no decoupling is carried out (Figs. 2b, 2d, and 3b). Moreover, these signals are in phase and therefore are easier to interpret in cases where multiple couplings lead to more complicated signals. In addition, phase cycling in the SEPP experiments permits the suppression of artifacts and undesirable conventional signals not generated by the PHIP effect. But the most striking feature of this technique is the possibility of decoupling during acquisition (Figs. 2a, 2c, 2e, and 3a), which leads to spectra with a signal-to-noise ratio as high as 400 after only two scans. This gain is between three and four times higher than that after a hard 90° pulse. The attractiveness of parahydrogen as a tool for the examination of catalytic hydrogenation in situ will benefit from the increased sensitivity, especially in cases where the resulting hydrogenation products exhibit polarization signals that are split by many couplings and where the initial polarization is weak.

ACKNOWLEDGMENTS

H. Sengstschmid thanks the Austrian government as well as the government of the Federal State of Upper Austria for financial support. J. Barkemeyer thanks Professor C. Griesinger and Dr. S. J. Glaser, University of Frankfurt am Main, Germany, for providing their simulation program SIMONE, and the Graduiertenkolleg of the Deutsche Forschungsgemeinschaft Bonn, Germany, for financial support.

REFERENCES

J. C. Eisenschmid, R. U. Kirss, P. P. Deutsch, S. I. Hommeltoft, R. Eisenberg, J. Bargon, R. G. Lawler, and A. L. Balch, *J. Am. Chem. Soc.* 109, 8089 (1987).

- 2. C. R. Bowers and D. P. Weitekemp, *Phys. Rev. Lett.* 57, 2645 (1986).
- 3. C. R. Bowers and D. P. Weitekamp, J. Am. Chem. Soc. 109, 5541 (1987).
- T. C. Eisenschmid, J. McDonald, R. Eisenberg, and R. G. Lawler, J. Am. Chem. Soc. 111, 7267 (1989).
- C. R. Bowers, D. H. Jones, N. D. Kurur, J. A. Labinger, M. G. Pravica, and D. P. Weitekamp, *in* "Advances in Magnetic Resonance" (W. S. Warren, Ed.), Vol. 14, p. 269, Academic Press, San Diego, 1990.
- 6. R. Eisenberg, Acc. Chem. Res. 24, 110 (1991).
- 7. M. S. Chinn and R. Eisenberg, *J. Am. Chem. Soc.* 114, 1908 (1992).
- R. Eisenberg, T. C. Eisenschmid, M. S. Chinn, and R. U. Kirss, *in* "Advances in Chemistry" (W. R. Moser and D. W. Slocum, Eds.), Vol. 230, Washington, DC, 1992.
- S. B. Duckett, C. L. Newell, and R. Eisenberg, J. Am. Chem. Soc. 115, 1156 (1993).
- J. Barkemeyer, M. Haake, and J. Bargon, J. Am. Chem. Soc. 117, 2927 (1995).
- H. J. Bernstein, J. A. Pople, and W. G. Schneider, *Can. J. Chem.* 35, 65 (1957).
- 12. H. Sengstschmid, J. Barkemeyer, R. Freeman, and J. Bargon, J. Magn. Reson., in press.
- 13. J. Bargon, J. Kandels, and K. Woelk, Z. Phys. Chem. 180, 65 (1993).
- 14. H. Geen and R. Freeman, J. Magn. Reson. 93, 93 (1991).
- S. J. Glaser and G. P. Drobny, *in* "Advances in Magnetic Resonance" (W. S. Warren, Ed.), Vol. 14, p. 35, Academic Press, San Diego, 1990.
- 16. O. W. Sørensen and R. R. Ernst, J. Magn. Reson. 51, 477 (1983).