

A SHARP Method for High-Resolution NMR of Heteronuclear Spin Systems in Inhomogeneous Fields

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Received March 14, 1985

We describe experiments which provide sharp chemical-shift spectra, with or without scalar couplings, for heteronuclear spin systems in inhomogeneous fields. This adds to an array of conceptual developments, beginning with the work of Maudsley and Ernst (1), which has broadened the applicability of heteronuclear NMR as a probe of complex molecules and mixtures. Sensitivity has been improved by using coherence transfers with protons (1-27), allowing experiments for which the signal intensity is independent of the gyromagnetic ratio γ_S of the dilute heteronucleus (3, 5, 7, 10-17). Selectivity is improved as well in these methods since the heteronuclear coupling serves as a label to distinguish the magnetizations of different heteronuclear systems from one another and from other spin groupings.

Resolution, however, is the major consideration limiting the application of these heteronuclear techniques to topical and *in vivo* spectroscopy and in general to samples where spatial magnetic-field inhomogeneity prevents accurate chemical-shift measurement. This communication presents sharp chemical-shift spectra of $^{13}\text{CH}_n$ groups in an inhomogeneous static magnetic field, opening up the possibility of working with samples that are very large or that have an inhomogeneous magnetic susceptibility. The sensitivity is that of the protons directly bound to the heteronucleus, while the resolution is determined by the homogeneous linewidths and the heteronuclear shifts. Scalar interactions, both heteronuclear and homonuclear, may be observed or decoupled as desired by variations of the pulse sequences. These features suggest the acronym SHARP: sensitive, homogeneous and resolved peaks.

The key concepts for these two-dimensional (28, 29) experiments can be described using the schematic pulse sequences of Fig. 1. Each begins with longitudinal proton magnetization and prepares a coherence involving the carbon transition operator S_+ . Each incorporates a heteronuclear coherence transfer echo (3, 11, 12) in which linear combinations of the gyromagnetic ratios γ_I and γ_S of the protons and carbons determine the rates of dephasing and rephasing in the inhomogeneous static field. In analogy to earlier experiments designed to measure chemical-shift differences in inhomogeneous fields (12, 30, 31), the echo occurs at the end of the evolution period t_1 , which consists of two or more subintervals whose lengths are incremented proportionally. In each case the evolution is terminated by transfer of the echoed coherence to transverse proton magnetization for detection. The Fourier transform

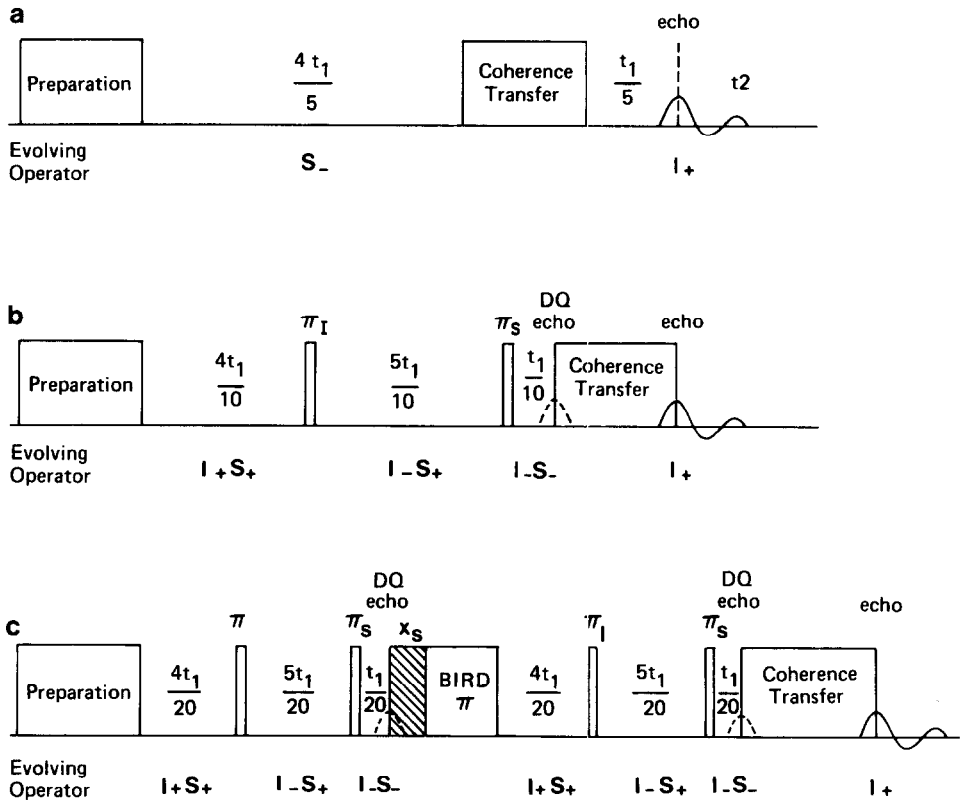


FIG. 1. Schematic pulse sequences for SHARP heteronuclear spectroscopy. In (a) carbon dephasing occurs during the period $4t_1/5$ and is refocused by subsequent proton evolution for a time $t_1/5$. In (b) double-quantum heteronuclear coherence evolves during t_1 with π pulses placed to eliminate inhomogeneous broadening and heteronuclear coupling while retaining chemical shifts and homonuclear coupling. In (c) resolution is further increased by using BIRD to eliminate the homonuclear couplings.

with respect to t_1 gives the homogeneous spectrum. Thus the sequences presented combine the sensitivity advantages of recent heteronuclear double-quantum experiments (13, 14) with the resolution advantage of heteronuclear coherence-transfer echoes (3, 12) and add the options of heteronuclear and homonuclear (20, 21) decoupling.

The scheme of Fig. 1a is the simplest and is exemplified by the detailed sequence of Fig. 2a. For the spin system consisting of a ^{13}C and its directly bound protons, the prepared density operator is $I_x S_y$ (4, 7), a product of the transverse magnetization operators for the two-spin species. Protons not directly bonded to a ^{13}C (nonsatellite protons) experience only the small multiple-bond homonuclear and heteronuclear couplings and so to a good approximation their density operator at time τ is simply refocused transverse magnetization I_y .

The evolution period starts with a period $4t_1/5$ with the protons decoupled (Fig. 2a). The x phase of the decoupling field is chosen so that it spin locks the I_x factor

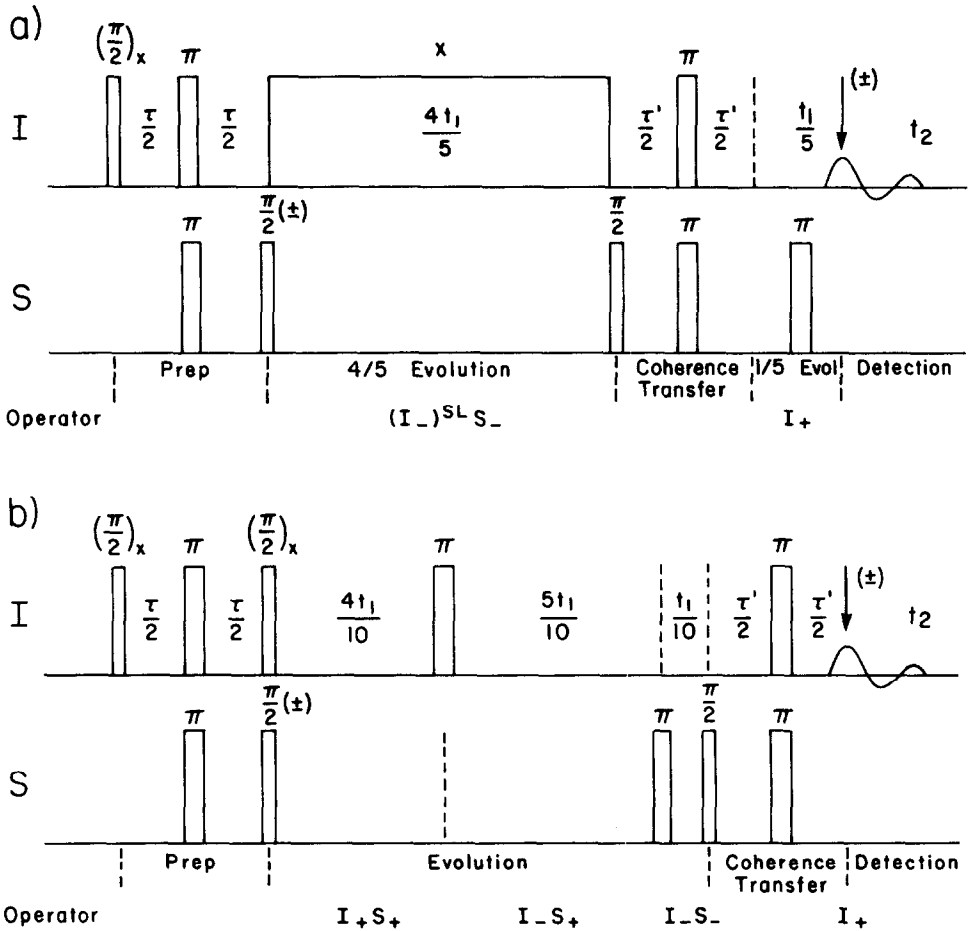


FIG. 2. Details of two SHARP heteronuclear pulse sequences. The sequence in (a) has a period of proton-decoupled S -spin evolution, followed by a proton evolution period one-fourth as long. Proton decoupling was effected experimentally using x decoupling with 180° phase alteration, not shown in detail. The sequence in (b) produces heteronuclear double-quantum echoes (indicated by dashed lines) that are converted into single-quantum proton echoes for measurement. In both cases, a constant purging period (not shown) of unmodulated proton x decoupling is applied for 4 ms just before the evolution period to dephase unwanted nonsatellite magnetization. The symbols $\pi/2(\pm)$ and $t(\pm)$ indicate that two experiments differing by a 180° phase shift on the first S -spin $\pi/2$ are subtracted to further suppress nonsatellite signal.

of the prepared heteronuclear coherence. Thus the only evolution is precession due to the ^{13}C chemical shift and inhomogeneous dephasing at a rate proportional to γ_S . The nonsatellite magnetization starts along y and thus is nutated by the decoupling field. Due to the usual small inhomogeneities of the rf field strength over the sample, this has the effect of dephasing the nonsatellite magnetization and severely reducing its contribution to the eventual spectrum. The evolution period is now interrupted by a $\pi/2$ pulse on the ^{13}C , which starts a mixing period τ' during

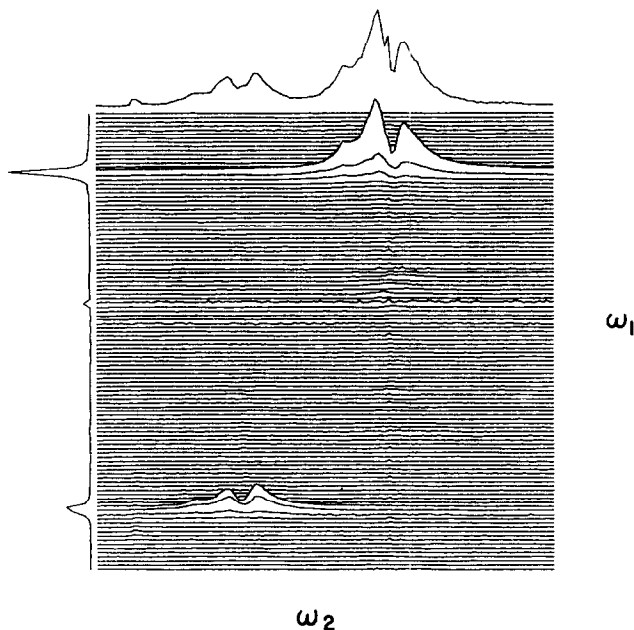


FIG. 3. A two-dimensional SHARP spectrum of ^{13}C -enriched ethanol obtained using sequence 2a. The plot is 256 points in ω_2 and 512 points in ω_1 . Note the relatively sharp (~ 6 Hz) lines of the echo spectrum in ω_1 versus the inhomogeneously broadened ^1H spectrum in ω_2 . The line positions in ω_1 are as expected from Eq. [1] and the increment $\Delta t_1 = 1.25$ ms, which resulted in folding over twice in this dimension.

which observable proton magnetization develops. The evolution period then resumes with rephasing determined by γ_I and is completed in a period $t_1/5$, just $\gamma_S/\gamma_I \cong \frac{1}{4}$ times as long as the dephasing period. The heteronuclear coupling is removed during this period by a ^{13}C π pulse at its center.

The resulting FID acquired in t_2 is thus modulated by the ^{13}C chemical shift acting for $4t_1/5$ and by the proton shift and homonuclear coupling terms acting on transverse proton magnetization for time $t_1/5$. For a sufficiently inhomogeneous field this is pure phase modulation since the "antiecho" contribution is negligible (29, 32). Therefore, this is a quadrature experiment with respect to both t_1 and t_2 and the resulting 2D Fourier transform contains all information on the sign of the frequencies. An example is shown in Fig. 3 for a 50:50 mixture of 1- ^{13}C ethanol and 2- ^{13}C ethanol, each enriched to 45% ^{13}C . The ω_2 projection indicates the poor field homogeneity (~ 1 ppm), while the ω_1 projection gives a singlet from the methyl and from the methylene sites. With t_1 defined as in Figs. 1a and 2a, line positions are given by

$$\nu_1 = -\frac{4}{5}\nu_C + \frac{1}{5}\nu_H \quad [1]$$

where ν_C and ν_H are chemical shifts.

The resolution achieved in this experiment (~ 6 Hz) was inadequate to resolve the homonuclear scalar couplings, which are scaled down by one-fifth. A better

approach is sketched in Fig. 1b and detailed in Fig. 2b. This sequence uses the same preparation period as before. The heteronuclear operator $I_x S_y$ has a component $I_+ S_+$ of double-quantum coherence, which may be separately detected from all other components ($I_- S_-$, $I_+ S_-$, $I_- S_+$) by selecting the fraction of t_1 for which this operator is allowed to dephase and rephase. Since I_+ has four times the dephasing rate of S_+ , $I_+ S_+$ dephases at a rate of 5 relative to a factor -3 for $I_- S_+$ and -5 for $I_- S_-$, respectively, and it alone leads to an $I_- S_-$ echo at t_1 . This is achieved by suitably placed π pulses as in Fig. 2b. At the same time, this pulse timing ensures that heteronuclear coupling is eliminated, while homonuclear coupling is full scale. The ^{13}C and proton chemical shifts are scaled as before but have the opposite sign. A period of coherence transfer involving a $\pi/2$ pulse on the ^{13}C and a τ' period, as before, creates a one-quantum proton echo which is detected. The selection of only one of the original components of heteronuclear double-quantum coherences again

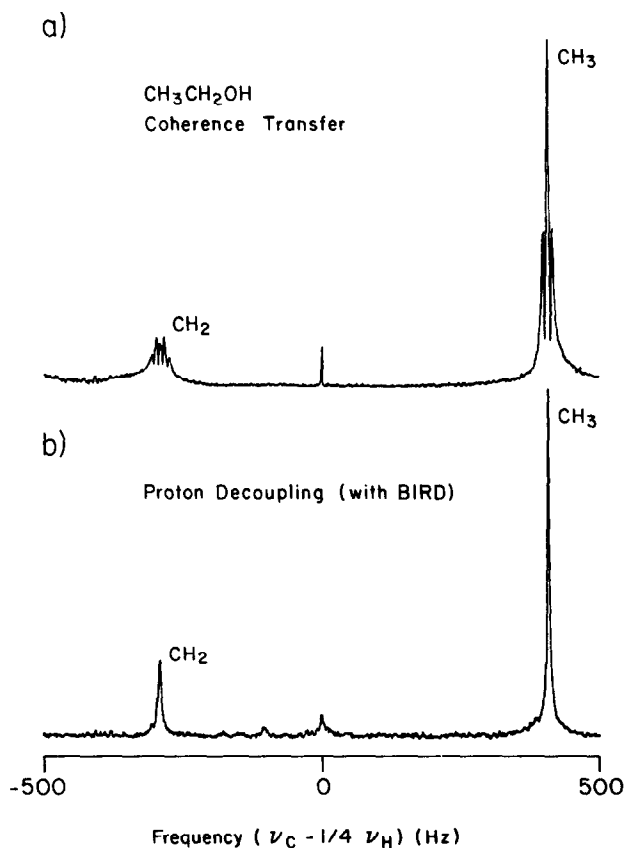


FIG. 4. Heteronuclear double-quantum SHARP spectra. The spectrum in (a) was obtained with sequence 2b measuring only one point at the tip of the echo as a function of t_1 . The two multiplets arise from the methyl and ethyl groups and show resolved proton-proton splittings. A fully decoupled spectrum is shown in the lower trace, obtained by using the SHARP BIRD sequence sketched in Fig. 1c. Both spectra are folded over due to an increment $\Delta t_1 = 1$ ms.

ensures that the experiment is quadrature in t_1 and t_2 and contains all sign information.

Figure 4a shows the spectrum of ethanol produced by the pulse sequence of Fig. 2b. The line centers are given by the expected combination of chemical shifts. The resolved homonuclear multiplet structure is due to the splitting of the heteronuclear methyl resonance by the two methylene protons and of the heteronuclear methylene resonance by the three methyl protons. The heteronuclear multiplet structure may be retained with a scale factor of $\frac{1}{4}$ by using the same sequence as Fig. 2b, except that the proton π in the evolution period occurs at $3t_1/8$ and the $^{13}\text{C}\pi$ is omitted.

In a more complex system it will be desirable to collapse all multiplet structure to improve the resolution of the chemical-shift information. This is accomplished by the incorporation of bilinear rotation decoupling (BIRD) (20, 21), which uses the one-bond scalar interaction to decouple the satellite protons from the nonsatellite protons. The coherence pathway sketched in Fig. 1c is similar to that of Fig. 1b, but now there are two distinct 4:5:1 echoes making up the evolution period with a BIRD π pulse sandwiched in between. The resulting fully decoupled ethanol SHARP spectrum is shown in Fig. 4b.

We have demonstrated the possibility of observing sharp heteronuclear chemical-shift spectra from liquids in an inhomogeneous magnetic field. An obvious application of this work, currently under way, is to topical NMR with high resolution.

ACKNOWLEDGMENTS

This work was supported by the Director, Office of Energy Research, Office of Basic Energy Sciences, Materials Science Division of the U.S. Department of Energy, and by the Director's Program Development Funds of the Lawrence Berkeley Laboratory under Contract DE-AC03-76SF00098.

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