Sensitivity Enhanced Detection of Weak Nuclei Using Heteronuclear Multiple Quantum Coherence

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Abstract: A new type of cross correlation spectra for heteronuclear spin systems is described which has some desirable features for the observation of magnetically weak nuclei. In previous two-dimensional experiments it has been the practice to correlate the single quantum transitions of two species to each other. In the new experiments described in this paper the single quantum transitions of one spin species (I) are correlated to the forbidden heteronuclear multiple quantum transitions (HMT). It turns out that this correlation leads to an indirect detection of the single quantum transitions of the second spin species (S) since these transitions have common energy levels with the HMTs. The sensitivity of these correlation spectra can become completely independent of the gyromagnetic ratio of the nuclei (S) since the HMTs are generated and observed through the nuclear spins I. Thus nuclei with weak magnetic moments can be detected with the full sensitivity of magnetically strong nuclei such as protons or fluorine.

I. Introduction

Two-dimensional Fourier transform NMR spectroscopy has provided techniques for correlating chemical shifts based on spin–spin coupling in the spectra of two coupled nuclear species (e.g., ^{1}H and ^{13}C in an organic compound). In many cases it has been shown[1-5] that such a correlation of the single quantum NMR spectra provides more detailed information than the individual spectra of either of the coupled spins. In general this results from the fact that correlated two-dimensional spectra[7] display peaks which correspond to both the single transitions (dia peaks) and to correlations of different transitions (cross peaks). As an example, in weakly coupled spin systems the various cross peaks derived from the correlation of the individual single quantum spectra may be used to assign the protons and carbons which are directly bonded. In strongly coupled spin systems, on the other hand, detailed information about the composition of the eigenfunctions may be extracted from the phases and intensities of the observed cross peaks.

Cross correlation of various spectra can be achieved in a two-dimensional NMR experiment. The transverse magnetization of the spin species I, generated by an rf pulse, evolves for a certain evolution time t_1 and is partially converted into transverse magnetization of the S spin species which can be detected during the detection period t_2. For a series of equidistant t_1 values a two-dimensional heteronuclear free induction decay $s(t_1,t_2)$ is obtained which, after two-dimensional Fourier transformation with regard to t_1 and t_2, results in the cross correlation spectra. Apart from their rich information content, these spectra are of interest because they permit an indirect detection of rare nuclei with an increased sensitivity.[1] However, the sensitivity of such cross correlation spectra could be enhanced considerably if proton free induction decays were detected and their Zeeman polarization used to generate the indirectly observed nonequilibrium state. The resulting signal $s(t_1,t_2)$ would then be completely independent of the gyromagnetic ratio of the indirectly detected nuclei.

In the following paragraphs such an optimal method will be described which is related to the method invented by Vaughan et al.[6]

II. Heteronuclear Multiple Quantum Transitions

It is not difficult to generate heteronuclear multiple quantum coherence between two different spin species which are coupled to each other. Calculations show[5] that, during each kind of heteronuclear coherence transfer, heteronuclear multiple quantum coherence is involved. This heteronuclear multiple quantum coherence can be indirectly detected by converting it into single quantum coherence (transverse magnetization). As long as the two spin species I and S are weakly coupled to each other no fixed phase relationship between the I and S spin carrier frequencies is necessary. In Figure 1 the heteronuclear multiple quantum spectrum of CH_3I is presented, which was indirectly obtained via the proton decoupled free induction decay of carbon-13 in a two-dimensional type experiment (i.e., the experiment defined in Figure 4 without the 180° pulse in the evolution period).

There are four heteronuclear zero and four double quantum transitions producing the two triplets shown in Figure 1. The zero quantum transition triplet is centered around the frequency

$$\omega_z{}^0 = (\omega_I{}^0 - \omega_I) - (\omega_S{}^0 - \omega_S)$$

while the double quantum transition triplet is centered around the frequency

$$\omega_d{}^0 = (\omega_I{}^0 - \omega_I) + (\omega_S{}^0 - \omega_S)$$

where $\omega_I{}^0 \equiv$ proton Larmor frequency, $\omega_S{}^0 \equiv {}^{13}$C Larmor frequency, $\omega_I \equiv$ proton carrier frequency, and $\omega_S \equiv {}^{13}$C carrier frequency. The HMTs can be converted into a transverse magnetization of any involved spin system.

III. Generation of Heteronuclear Multiple Quantum Coherence

Generation of homonuclear multiple quantum coherence has been accomplished[7] by means of two nonselective 90° pulses separated by an interval τ_p. Analogous heteronuclear higher order coherence can be achieved by the simultaneous application of the same pulse sequence to both spin systems. Because the generation of multiple quantum coherence depends, for a fixed pulse interval τ_p, on both the chemical shifts of the excited spins and the heteronuclear coupling constants, it is very difficult to determine the optimal τ_p for the entire spin system. A considerable simplification can be achieved by the additional application of two echo-180° pulses to both spin systems in the center of the interval τ_p. Then the generation of heteronuclear multiple quantum transitions will only depend on the heteronuclear coupling constants. Since the heteronuclear coupling constants between direct neighbor spins in organic liquids are in most cases dominant (e.g., ^{1}H and ^{13}C, or ^{1}H and ^{15}N) the selection of a sufficiently short preparation

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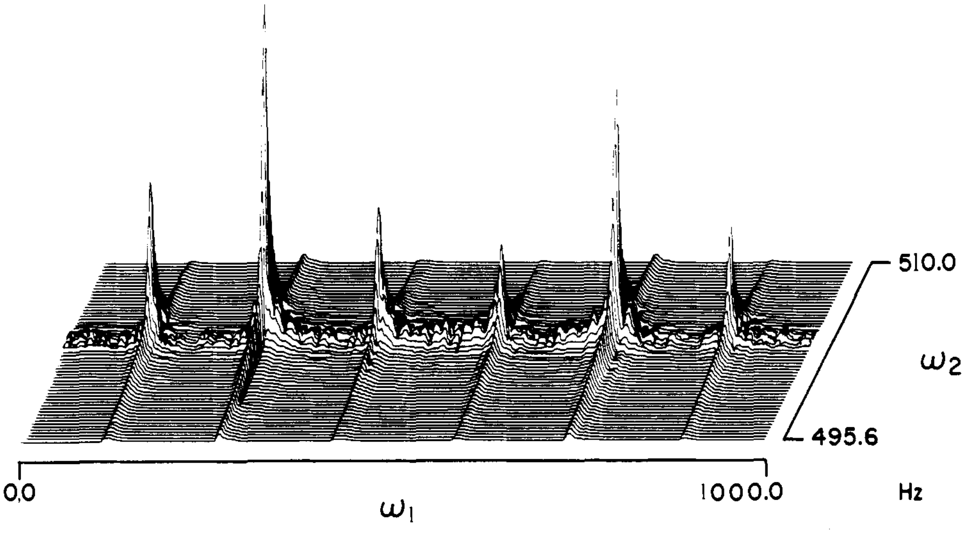

Figure 1. Heteronuclear multiple quantum spectrum of methyl iodide. Observation via carbon-13 FID, pulse sequence of Figure 4 without 180° pulse at $t_{1/2}$. Sample was a 20% solution of 65% carbon-13 enriched CH_3I in acetone-d_6. $s(t_1,t_2)$ were measured at 512 different t_1 values. The more intense triplet to the left represents the zero quantum transitions and the triplet to the right, the double quantum transitions.

interval τ_p permits a selective generation of zero and double quantum transitions between direct neighbor heteronuclear spins (see preparation periods in Figures 2 and 4).

For an isolated I_nS group the spin density operator at the beginning of t_1 (Figures 2 and 4) is

$$\rho(t_1 = 0) = \frac{1}{\mathrm{Tr}(1|)}\left[1| + \beta\left(F_{Iz}\cos\left(\frac{J_{ax}}{2}\tau_p\right) + 2S_yF_{Iy}\sin\left(\frac{J_{ax}}{2}\tau_p\right)\right)\right] \quad (1)$$

where

$$F_{Iz} = \sum_{i=1}^{n} I_{iz}$$

$$\beta = \frac{\hbar\omega_I{}^0}{kT}$$

J_{ax} = heteronuclear coupling constant

assuming that the I spins were in thermal equilibrium at the beginning and S is a spin ½ nucleus. Thus for $(J_{ax}/2)\tau_p = \pi/2$ the Zeeman polarization is completely converted into heteronuclear zero and double quantum coherence.

It is important to note that all pulses applied in the preparation period have the same phase for each frequency. If the last 90° pulse acting on the I spin is phase shifted by 90°, instead of zero and double quantum coherence only odd-order coherence is generated, i.e., for a sufficiently short mixing time τ_p only single quantum coherence. This single quantum coherence transfer has been independently discovered by Morris[8] and exploited for a sensitivity enhancement of rare spins.

IV. Exploitation of Heteronuclear Zero and Double Quantum Coherence

Figure 1 provides an example of the useful information which can be obtained from an indirectly detected heteronuclear multiple quantum transition spectrum. The peak positions may be analyzed to obtain an indirect observation of the spectrum of a second spin species. In addition, the line widths may be used to determine some elements of the Redfield relaxation matrix.[6]

It is important to note the symmetrical relation which exists between heteronuclear transitions and the two spin systems. The transitions can be generated from either spin system and observed by converting to single quantum coherence for any involved spins. It is therefore possible to both generate and observe the transitions with the spins (I) having the larger magnetic moment in order to obtain the full achievable sensitivity (Figure 2).

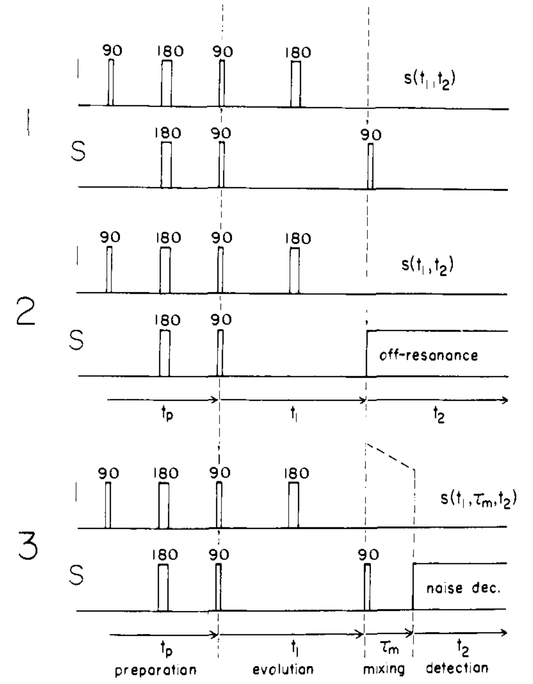

Figure 2. Pulse sequences which can be used in proton observed heteronuclear multiple quantum experiments for the indirect detection of nuclei with weak magnetic moments. All pulses of a given frequency have the same phase.

One can thus indirectly observe the chemical shifts of weak nuclei via strongly magnetic nuclei (e.g., ^1H or ^{19}F) to which they are spin coupled.

Since the analysis of HMT spectra is complicated by splittings due to heteronuclear spin-spin couplings, it is advisable to simplify the spectra by the application of a refocusing nonselective 180° pulse to one of the two spin systems in the center of the evolution period t_1 (Figures 2 and 4). This pulse interchanges zero and double quantum transitions in such a way that it refocuses the chemical shift of the spins to which the 180° pulse is applied as well as all heteronuclear spin-spin

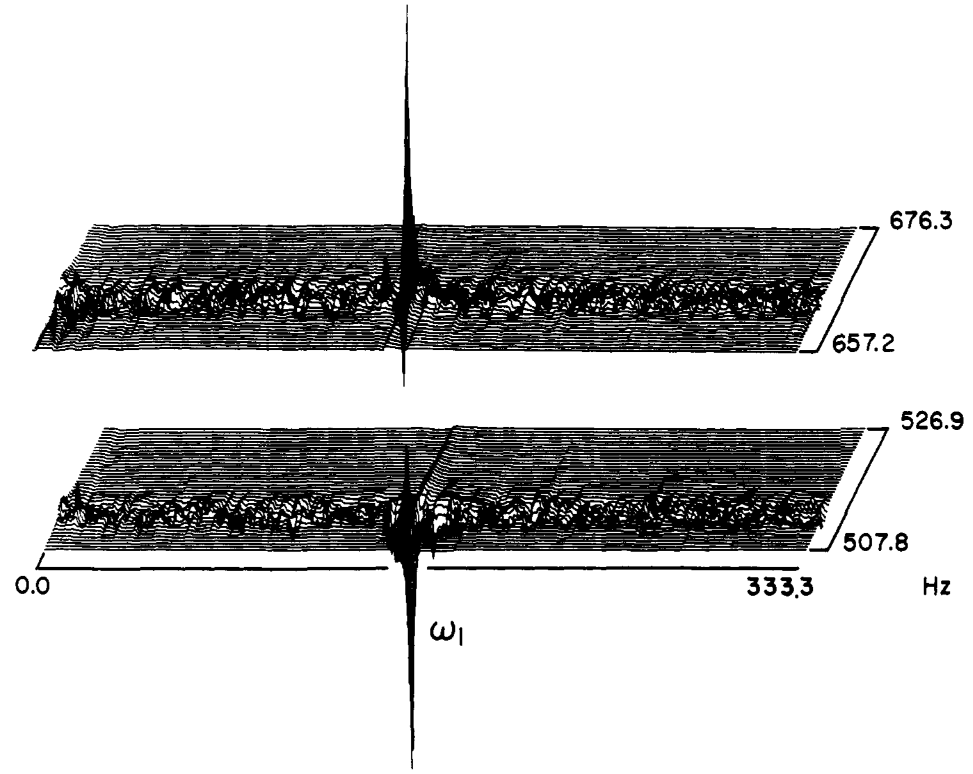

Figure 3. A phase sensitive cross correlation spectrum of methyl iodide (same sample as in Figure 1), the result of method 1 in Figure 2. The doublet splitting in the vertical proton frequency direction is due to the coupling to the carbon-13. A total of 460 t_1 experiments were performed (t_1 max = 0.687 s). The peak positions along ω_1 correspond to the carbon-13 chemical shift.

interactions, provided that all spins are weakly coupled.[9] This echo experiment (Figures 2 and 4) leads to cross correlation spectra in which the HMTs along ω_1 direction (Figure 3) only oscillate with the chemical shift of the indirectly detected spins and the homonuclear spin-spin interactions.

This is demonstrated with a sample of methyl iodide, which shows a cross correlation spectrum (Figure 3) in which all HMTs of the spectrum in Figure 1 are collapsed into a single peak along ω_1 at the position of the carbon chemical shift. Along the vertical proton frequency axis this peak is split into a doublet with opposite signed branches, since the converting 90° pulse applied to the carbon at t_1 does not provide a net coherence transfer between the HMTs and the observed proton single quantum transitions. Applying heteronuclear decoupling during t_2 (Figure 2, method 3) increases the sensitivity by only a factor of 2 for the indirect detection of a rare spin with S = 0.5.

For experiments represented in Figure 2, the full I spin magnetization of a symmetric I_nS group (e.g., CH_3) can be converted into HMT population by an appropriate setting of τ_p. Therefore the spectra of nuclei with weak magnetic moments can be indirectly observed with the optimal sensitivity (see eq 1).

The principal disadvantage is the requirement of a minimum number of experiments with different t_1 values for getting the desired resolution in ω_1. Another difficulty arises if the indirectly detected spin species has low abundance. For perfect pulses the magnetization of protons not coupled to a rare spin is invisible. However, pulse imperfections may produce signals from uncoupled protons which can overwhelm the rare-spin satellites. This problem can be circumvented with a compensation scheme[10] in which the phase of the proton echo pulse in the center of t_1 is varied and a floating point Fourier transform is applied.

Generally homonuclear proton spin-spin interactions are also effective in the frequency domain of the HMTs (ω_1). Thus proton multiplets are expected to lay tilted by 45° in the (ω_1, ω_2) frequency plane of the correlation spectra. The only restriction for the proposed methods is the requirement of resolved heteronuclear spectra for the I spins, which may fail in ^{14}N NMR owing to the efficient quadrupolar relaxation. Nevertheless indirect detection of ^{14}N resonance is possible for selected examples.

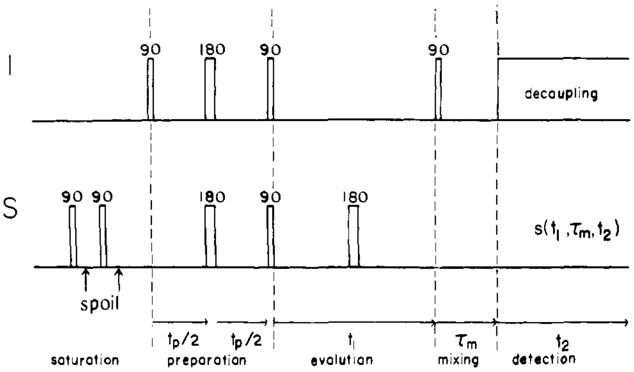

Figure 4. Pulse sequence for the carbon-observe cross correlation spectra. The proton decoupled spectrum at carbon-13 is correlated to the carbon refocused heteronuclear multiple quantum transitions.

V. Alternative to the Conventional Cross Correlation Spectra

It is possible to design a pulse sequence (Figure 4) in which the HMTs are generated from the strong I spins followed by detection of the rare S spins for a series of t_1 experiments in close analogy to the published methods of heteronuclear cross correlation.[1-5] For illustration this pulse scheme was applied to 2,3-dibromothiophene (Figure 5). Because proton decoupled carbon-13 spectra are detected the introduction of a mixing

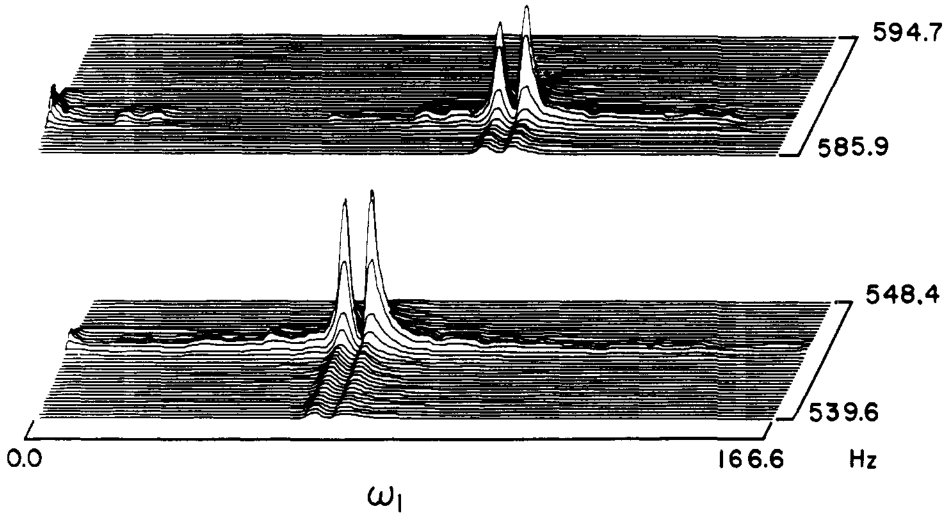

Figure 5. An absolute value plot of the carbon-observe cross correlation spectrum of 2,3-dibromothiophene in acetone-d_6 (application of the method of Figure 4). The projection to the vertical axis gives the proton decoupled carbon-13 spectrum, and frequencies along ω_1 are those of the proton spectrum with average line width of the heteronuclear multiple quantum transitions.

period τ_m was mandatory and thus only absolute value plots are represented.[2]

VI. Conclusion

It has been demonstrated that echoes of heteronuclear zero and double quantum transitions can be used for the indirect detection of magnetically weak nuclei with the full sensitivity of strong nuclei such as ^1H and ^{19}F to which they are coupled. This is possible because these higher order heteronuclear transitions have common energy levels with the indirectly detected transitions.

The proposed excitation of heteronuclear multiple quantum transitions selectively suppresses the generation of disturbing single quantum coherence on both spin systems. This is very useful if the observed spins are much more abundant than the indirectly detected spins. Effects of imperfect pulses can be suppressed by compensation schemes.[10]

Since the full I spin polarization of an I_nS group can be converted in HMTs and they are observed via the I spin FID, S spin nuclei can be indirectly detected with a sensitivity which is completely independent of their magnetic moments.

A second method (Figure 4) in which HMTs are generated from the strong I spin and observed via the S spins leads to results similar to those in ref 1-5. In this case, however, the free induction decay of the strong spin can be detected without any radiation damping.

VII. Experimental Section

All experiments were performed on a Varian FT-80A spectrometer linked to a set of Sykes floppy disks for data storage. All data acquisition and processing were done under computer control with specially developed software. The carbon-observe experiments were performed with the carbon-proton switchable probe. The proton frequency pulses were generated with the proton decoupler, which was externally locked to a Harris PRD 7938 synthesizer to reduce its phase instability.

For the proton-observe experiment the broad-band probe was used with proton FIDs detected with the decoupler coil. The proton pulses were generated through the observe channel where the carbon pulses were obtained from an external rf gate which was switchable by the drive voltage to the proton decoupler. The carbon frequency was generated by a second Harris synthesizer and after the rf gate amplified by an Amplifier Research AR-30h transmitter, which was connected over a 20-MHz band-pass filter to the broad-band circuit. Both synthesizers were phase locked to the FT-80A 1-MHz master clock. The methyl iodide sample was a 20% solution of 65% ^{13}C-enriched CH_3I in acetone-d_6. The acetone was used for the internal deuterium lock. The 2,3-dibromothiophene was mixed with 20% acetone-d_6.

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