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Practical Aspects of Carbon-13 Double Quantum NMR

AD BAX

Department of Chemistry, Colorado State University, Fort Collins, Colorado 80523

AND

T. H. MARECI

Department of Radiology and Department of Physics, University of Florida, Gainesville, Florida 32610

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We want to address an erroneous view that has recently been advanced on ^{13}C double quantum NMR. A recent publication (*1*) introduced a modified version (*1*) of the original double quantum NMR experiments; this new version claims to offer an attractive increase in sensitivity by a factor of three, or a decrease in the size of the required data matrix by a factor of four. To avoid disappointment among spectroscopists we wish to make it clear that this modified version offers no gain in sensitivity whatsoever in comparison with the other double quantum experiments (*2–6*), provided they are properly optimized. It is shown here that the sensitivity of this modified experiment can be even slightly lower. The claim of a reduction of the size of the required data matrix is also shown to be incorrect.

In the original two-dimensional double quantum ^{13}C experiments, the acquisition time t_2 starts immediately after the final "read pulse" (Fig. 1), and the time during which the double quantum coherence evolves is labeled t_1. The detected ^{13}C–^{13}C satellites (*7*) are modulated in amplitude as a function of t_1 with the double quantum frequencies (*2*). In the quadrature-F_1-detection version of this experiment (*3*), results are combined with those of a second experiment which contains a composite 45°_z pulse (*8*) in the evolution period. This allows the sign of the modulation frequency to be determined, which simplifies the interpretation of the results. A simpler and more sensitive alternative to obtain quadrature F_1 detection has been proposed more recently (*6*); an increase of the flip angle of the final "read pulse" to 135° provides directly satellite signals which are modulated in phase as a function of t_1. In either case, data acquisition is started after the final read pulse and the coherence transfer echo (*9*) is detected (*4, 6*). The time domain signal of a carbon A, directly coupled to carbon X, is then given by

$$s_A(t_1, t_2) = C \exp[-i(\Omega_A + \Omega_X)t_1] \exp[i(\Omega_A \pm \pi J_{AX})t_2] \qquad [1]$$

where C is a constant and Ω_A and Ω_X are the angular chemical shift frequencies of carbons A and X. Effects of static magnetic field inhomogeneity and transverse relaxation are neglected in this expression and will be mentioned later.

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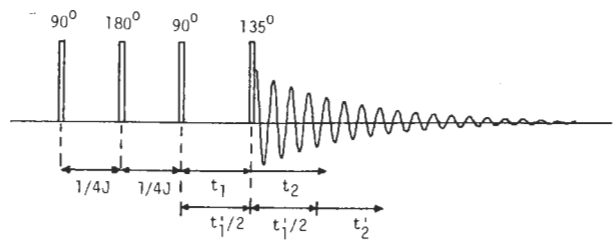


FIG. 1. Pulse scheme of the experiment for correlating ^{13}C shifts via double quantum coherence in natural abundance samples. In the original experiments, the detection period t_2 starts immediately after the read pulse. In Turner's modification, detection t'_2 starts a time t_1 later, and therefore the evolution period t'_1 has a length equal to $2t_1$.

In the recently proposed modification (*1*), the pulse scheme remains unchanged, but the detection period, now labeled t'_2, starts a time t_1 after the read pulse. The new evolution period, labeled t'_1 (Fig. 1), equals $2t_1$. The detected signal is now described by

$$s'_A(t'_1, t'_2) = C \exp[-i(\Omega_A + \Omega_X)t'_1/2] \exp[i(\Omega_A \pm \pi J_{AX})t'_1/2] \exp[i(\Omega_A \pm \pi J_{AX})t'_2]$$
$$= C \exp[-i(\Omega_X \pm \pi J_{AX})t'_1/2] \exp[i(\Omega_A \pm \pi J_{AX})t'_2]. \qquad [2]$$

As can be seen from Eq. [2], the modulation frequency in Turner's version equals $(\Omega_X \pm \pi J_{AX})/2$ rather than the value, $\Omega_A + \Omega_X$, in the original experiments (Eq. [1]). In principle, this allows a sampling frequency in the t_1 dimension which is four times lower than in the original versions, and therefore would require a data matrix a quarter the size. However, in the original experiments the spectral windows in the F_1 and F_2 dimensions can be set to identical values. This introduces folding in the F_1 dimension, as will be shown below, without introducing any ambiguity. Coupled ^{13}C nuclei are identified by the following two criteria: they must show an identical double quantum frequency in the F_1 dimension, and the centers of the bars connecting the double quantum resonances (see, e.g., spectra in Refs. (*3*) and (*5*)) must be on the "diagonal," $F_1 = 2F_2$. If the spectral widths in both frequency dimensions of the original experiments are identical, this diagonal will be folded in a way schematically indicated in Fig. 2, and all folded double quantum resonances will be present in the shaded areas. All nonfolded resonances are present in the unshaded area in Fig. 2. A practical illustration of this kind of folding is found in the spectrum in Ref. (*6*). No ambiguity is caused by this folding, and the required sizes of the data matrices for the original double quantum experiments would be twice that for the modified experiment. However, Eq. [2] shows that in the modified experiment the modulation frequency is halved, and therefore the separation of resonances in the F_1 dimension is halved. If transverse relaxation during the evolution period is neglected, doubling the length of the acquisition time in the t_1 dimension would be necessary to give the same effective resolution; such doubling would lead to identical sizes for the data matrices.

A similar paradox arises if in the original experiment a 180° pulse is applied at, for example, $0.45t_1$. This would mean that the phase of the double quantum coherence would refocus at time $0.9t_1$, and then evolve for a time $0.1t_1$ before it is converted

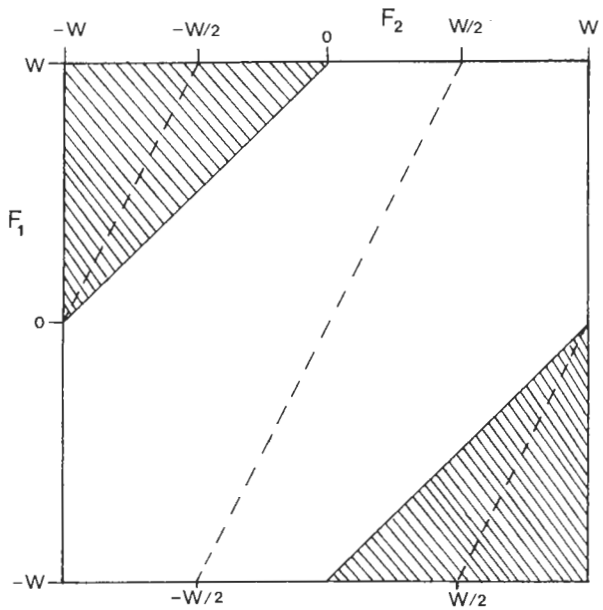
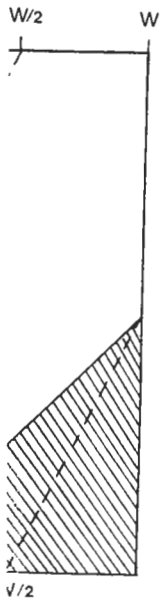


FIG. 2. Schematic representation of a 2D double quantum spectrum where the sampling frequencies i both dimensions are identical. The shaded areas only contain resonances folded in the F_1 dimension, th blank area only contains nonfolded resonances. Double quantum resonances of coupled ^{13}C nuclei ar always symmetrically displaced in the F_2 dimension with respect to the "diagonal" $F_1 = 2F_2$ (broken line) i.e., the center of the bar connecting correlated double quantum frequencies falls on this line.

by the final 90° pulse into observable magnetization. This would induce a modulatio frequency which would be a factor of *ten* lower. However, nothing would be gaine as the sampling time in the t_1 dimension would have to be a factor of ten longer t obtain the same effective resolution (neglecting transverse relaxation). In general, high F_1 modulation frequency should be favored, because this allows short sampling times in the t_1 dimension, and hence minimizes relaxation effects while optimizing sensitivity. The argument about sensitivity that was given in Ref. (*1*) is clearly incorrect even if a lower sampling frequency in the t_1 dimension could be used. The paper b Aue *et al.* (*10*) is misinterpreted when claims are made that a lower sampling frequenc in the t_1 dimension, allowing a longer acquisition time in this dimension, yields highe sensitivity. This is only true for one-dimensional spectroscopy, where audiofrequenc filters avoid high frequency noise from being folded into the spectrum; in two-di mensional spectroscopy the sensitivity is independent of the sampling frequency i the t_1 dimension. This is easily seen by considering, for example, a double quantu modulation frequency equal to zero. In this consideration, decay as a function of t due to relaxation is at first neglected, the same simplification used in reference (*1*) Independent of the t_1 sampling frequency, a number of spectra (t_1 sampling points with equal intensity are obtained and with *uncorrelated* noise. Fourier transformatio with respect to t_1 will thus give an F_1 spectrum with a signal-to-noise ratio independen of the t_1 sampling frequency or sampling time. In practice, the signal decays as function of t_1 and the longer t_1 values offer lower sensitivity. Therefore, the lowe



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sampling frequency in the t_1 dimension will give poorer sensitivity than a high sampling frequency if an identical number of t_1 values are used.

Symmetrization routines (*11, 12*) can be used in the original double quantum experiments, even if the spectral widths in both frequency dimensions are identical, and the folding that is schematically indicated in Fig. 2 occurs. Symmetrization should in this case be used on F_2 traces, about the dashed diagonal $F_1 = 2F_2$, and should be simpler than with two-dimensional symmetrization in the case of a nonsquare data matrix. Of course, signals in the shaded areas in Fig. 2, which contain folded double quantum signals, should be symmetrized about the folded diagonal.

Because of sensitivity considerations, one always wants to use a sampling time in the t_2 *dimension* which is at least of the order of the transverse relaxation time T_2. Because of data storage space limitations, this often restricts the maximum t_1 value to the order of 30 msec or less. However, a 30 msec sampling time in the t_1 dimension usually provides more than sufficient digitization in the F_1 dimension to identify identical double quantum frequencies, i.e., coupled ^{13}C nuclei. Whether the coherence transfer echo or antiecho (*6*) is detected will not be very important for such short t_1 values, unless the magnet has extremely poor homogeneity. Nevertheless, one usually favors detection of the coherence transfer echo, as even the smallest improvement in sensitivity is welcome in this type of experiment.

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