

Deconvolution and Measurement of Spin–Spin Splittings by Modified J Doubling in the Frequency Domain

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A new implementation of J doubling in the frequency domain is proposed. This modified J doubling uses novel sets of delta functions [..., +1, -1, +1, +1, -1, +1, ...] for in-phase multiplets and [..., -1, -1, -1, +1, +1, +1, ...] for antiphase multiplets. The convolution process together with the couplings found by it generates a deconvoluted multiplet that preserves the integral and the position of the original one. If the number of delta functions tends to infinity, the whole operation behaves like a formal deconvolution of the multiplet, which is a linear process. Modified J doubling allows for multistage procedures. This makes it possible to analyze 2D multiplets and to measure coupling constants as small as 0.11 Hz with an accuracy of ± 0.03 Hz. © 2001 Academic Press

Key Words: J doubling; spin–spin coupling constant; convolution; deconvolution.

A plethora of methods have been developed to measure spin–spin coupling constants (J) for cases where the direct method, which consists in locating peak maxima, breaks down. This usually happens when the linewidth at half-height is around the value of J ; passive splittings are underestimated due to peak overlap, while active ones are overestimated due to signal cancellation. Among those methods, the simpler ones are those that need only one NMR experiment and in which J can be measured directly from the multiplet, or from 2D or 3D experiment traces. These procedures can be performed in the time domain (2–5) or in the frequency domain (6–9). Methods in the frequency domain have been shown to be faster since they avoid iterative use of Fourier transformations. In this paper, we focus on a modification of J doubling in the frequency domain which entails the introduction of novel sets of delta functions (δ 's) that provide several advantages over the ones already reported. We test the method by comparison with known experimental results of the spectra obtained from 1D and 2D NMR data.

Classical J doubling has been easily implemented in both domains, time (3, 4) and frequency (8, 9). J doubling in the

frequency domain is based on the convolution. The convolution of two functions f and g is defined as

$$h(\nu') = \int_{-\infty}^{\infty} f(\nu) g(\nu' - \nu) d\nu,$$

where $f(\nu)$ corresponds to the original multiplet, $g(\nu' - \nu)$ to a set of δ 's which are evenly spaced, and $h(\nu')$ to the convoluted multiplet. Since the operation allows for the use of any array of δ 's, the implementation turns out to be quite flexible.

Once a digitized, isolated, and baseline-corrected multiplet is extracted from a spectrum, J doubling in the frequency domain convolutes the signal with several δ 's spaced by a trial J , J^* , which is varied within the interval where the real J is expected to be. The absolute integral is obtained for each J^* . The graph of the absolute integral vs J^* is named the integral function (3). The minimum of this function is the real coupling constant, i.e., J_R . When more than a pair of δ 's is used, local minima are created due to subharmonics coincidences, the number of which depends on the kind (in-phase or antiphase) and number of couplings that are present. All these subharmonics are less intense than J_R and tend to disappear when the linewidth increases. Knowledge of the exact subharmonic positions might be useful for proper interpretation of the integral function, Table 1.

Diverse arrays of δ 's generate similar integral functions but different convoluted multiplets. Figure 1 shows patterns produced when a doublet is convoluted with sets of δ 's spaced by a distance equal to J_R . An antiphase convoluted pattern is generated when an even number of δ 's with alternating signs is used for in-phase multiplets (Fig. 1a). If an odd number of δ 's with C_2 symmetry is employed, an in-phase convoluted signal is produced (Fig. 1b). The C_2 axis is defined in Fig. 1. Each signal at the extremes is, in fact, a deconvoluted multiplet, but it is called a submultiplet because it is not positioned at the center of the original spectral width (sw) and it has only half the integral of the original signal. The separation between the

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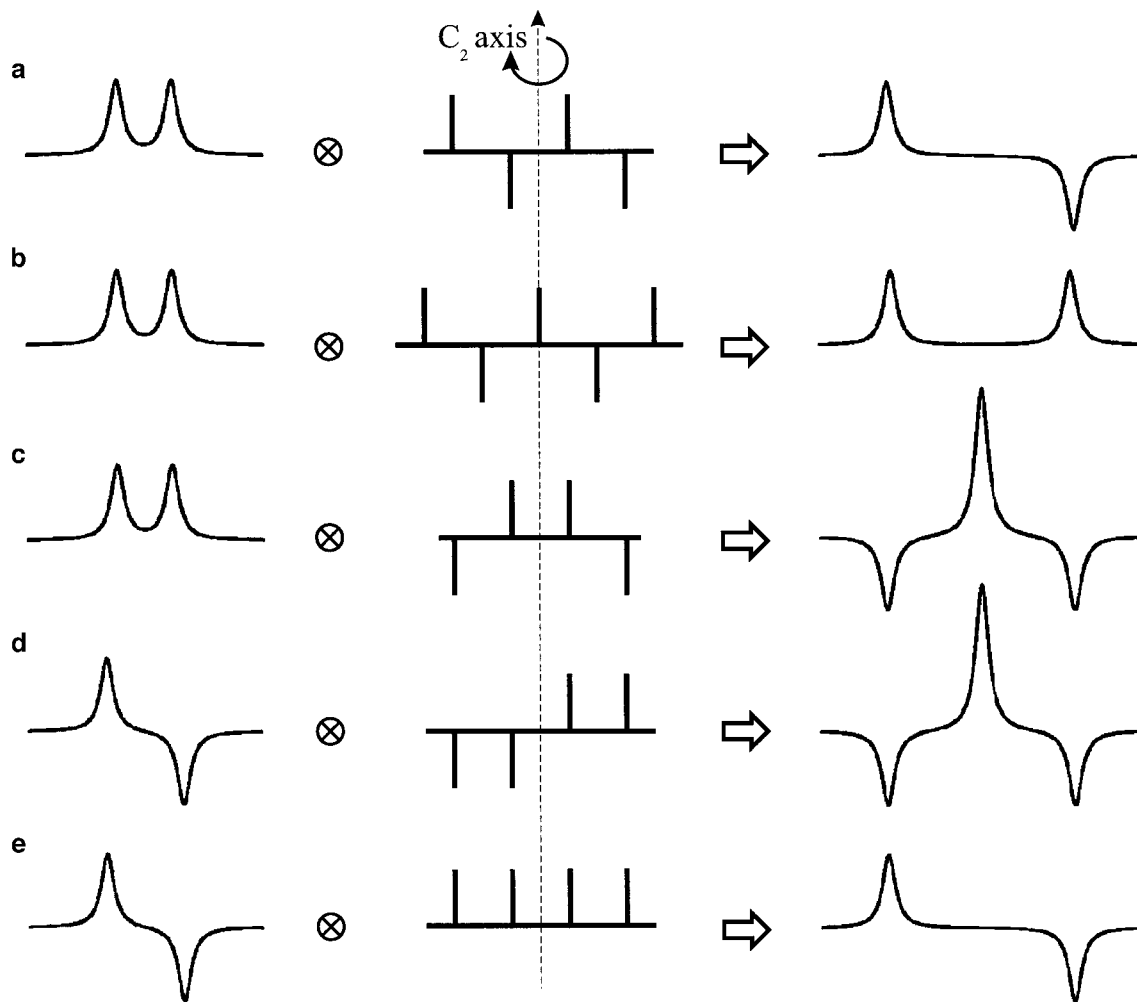


FIG. 1. Convolution of in-phase and antiphase multiplets with different sets of delta functions (δ 's) spaced by a distance equal to the real coupling constant (J_R). (a) Convolution of an in-phase multiplet with an even number of δ 's with alternating signs produces two submultiplets in antiphase. (b) Convolution of an in-phase multiplet with a \mathcal{C}_2 symmetry set of an odd number of δ 's with alternating signs generates two submultiplets in-phase. (c) A \mathcal{C}_2 symmetry set of an even number of δ 's with alternating signs gives rise to two submultiplets at the extremes and an effectively deconvoluted multiplet at the center of the original multiplet. (d) An \mathcal{S}_2 symmetry set of δ 's creates two submultiplets at the extremes and an effectively deconvoluted multiplet at the center of the original multiplet. (e) Convolution of an antiphase multiplet with positive δ 's originates two submultiplets in antiphase. The distance between the submultiplets at the extremes is always the number of δ 's times J_R , i.e., nJ_R . The same result of J doubling in the time domain is achieved with the set of δ 's of (a) and (e).

submultiplets is equal to the number of δ 's used times the real J , i.e., (nJ_R) . The same result of J doubling in the time domain is reached only when 2^m δ 's with alternating signs (where m is an integer) are employed for in-phase multiplets (Fig. 1a). For antiphase multiplets an array of 2^m positive δ 's is needed (Fig. 1e). From the deconvoluted signals described until now, it is not easy to obtain the effectively deconvoluted multiplet, i.e., the one that is positioned at the center of the original signal, that preserves the original integral and the original sw or sw minus the J removed. The deconvoluted multiplet simplifies the analysis for signals with several J 's and it facilitates the spin network analysis.

A set of an even number of antiphase δ 's with \mathcal{C}_2 symmetry that ends with negative δ 's (Fig. 1c) gives rise to two deconvoluted signals at the extremes with negative intensities separated by a gap

of nJ_R , and a new deconvoluted multiplet at the center of the original multiplet. An equivalent pattern is obtained for antiphase multiplets when they are doubled by a set of δ 's with \mathcal{S}_2 symmetry (Fig. 1d). For both sw's, the original and the convoluted one, the zero is defined at $sw/2$. This new signal has four advantages. (1) It is located at the center of the original multiplet, independently of the sw chosen at the beginning of the process. (2) It has the same integral as the original multiplet, as expected for a deconvoluted NMR signal. (3) If $nJ_R > (sw + J_R)$ and all points out of the range $\pm sw/2$ are eliminated, the signal is precisely the effectively deconvoluted multiplet. (4) It is a linear process that allows multistage deconvolution of all the couplings present in a multiplet. This particular modified J doubling not only determines the J_R value but also makes possible a simple effective deconvolution. If the number of δ 's tends to infinity (the signals at

TABLE 1
Subharmonic Coincidences Present in Integral Functions

Number of couplings	Type of multiplet	Position of subharmonics
1	In-phase	$J/(2p - 1)$
1	Antiphase	J/q
2	In-phase	$J_1/(2p - 1), J_2/(2p - 1), J_1 \pm J_2 /(2p - 1)$
2	Antiphase	$J_1/q, J_2/q, J_1 \pm J_2 /q$
3	In-phase	$J_1/(2p - 1), J_2/(2p - 1), J_3/(2p - 1), J_1 \pm J_2 /(2p - 1), J_1 \pm J_3 /(2p - 1), J_2 \pm J_3 /(2p - 1)$
3	Antiphase	$J_1/q, J_2/q, J_3/q, J_1 \pm J_2 /q, J_1 \pm J_3 /q, J_2 \pm J_3 /q$

Note. p is an integer larger than one and smaller than or equal to half the number of deltas used. q is an integer larger than one and smaller than the number of deltas used.

the extremes tend to infinity too and the signal at the center remains unchanged), the whole operation behaves like a formal deconvolution of the multiplet.

Modified J doubling is tested with simulated signals (noise free) to measure a given coupling constant as a function of linewidth (Fig. 2). For an in-phase doublet (Fig. 2a), J_R can be

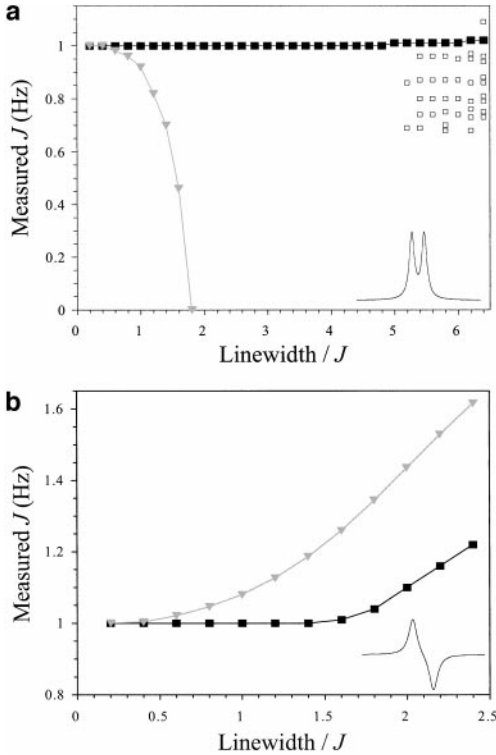


FIG. 2. Measurement of simulated coupling constant of 1 Hz with the direct method (\blacktriangledown) and modified J doubling (\blacksquare) as a function of linewidth. (a) Simulated in-phase doublet of 1 Hz. Digital resolution employed is 0.005 Hz and 128 δ 's. This number of δ 's is used for all the results shown. The direct method starts to fail at 0.6 Hz of linewidth; at 1.8 Hz it is no longer possible to recognize peaks of the two maxima. Modified J doubling is capable of determining the coupling constant until the linewidth reaches a value of 5.0 Hz. Beyond this point, other minima (\square), which do not correspond to subharmonics, start to appear. (b) A simulated doublet with an antiphase coupling of 1.0 Hz is shown. The direct determination is not longer reliable after a linewidth of 1.0 Hz. Modified J doubling is able to measure the constant within an error of 10% up to values of 1.8 Hz linewidth.

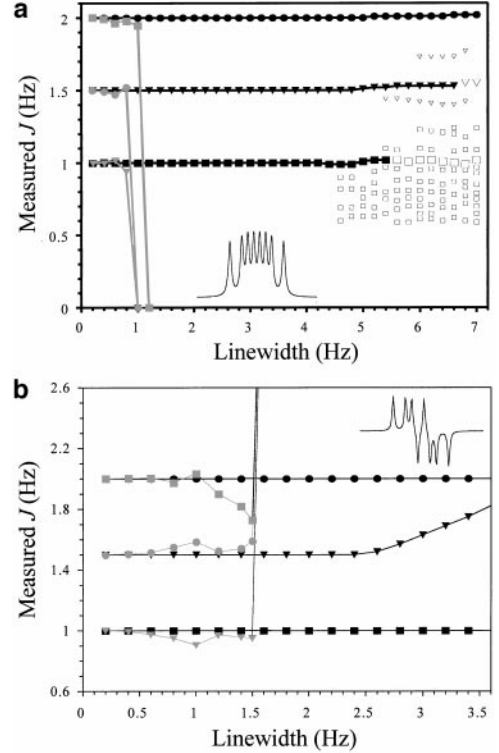


FIG. 3. (a) Determination of coupling constants with the direct method and modified J doubling as a function of linewidth for a simulated multiplet with three in-phase constants of 1, 1.5, and 2.0 Hz. (b) Measurement of coupling constants with the direct method and modified J doubling as a function of linewidth. A simulated multiplet with two in-phase constants of 1 and 2 Hz and an antiphase constant of 1.5 Hz is used. Direct measurements are indicated by $J = 2.0$ Hz, \blacksquare ; $J = 1.5$ Hz, \bullet ; $J = 1.0$ Hz, \blacktriangledown . J doubling measurements are indicated by $J = 2.0$ Hz, \bullet ; $J = 1.5$ Hz, \blacktriangledown ; $J = 1.0$ Hz, \blacksquare . Small empty squares (\square) and triangles (\triangledown) indicate minima which do not correspond to subharmonics. Large empty squares (\square) and triangles (\triangledown) indicate minima which may correspond to the J_R .

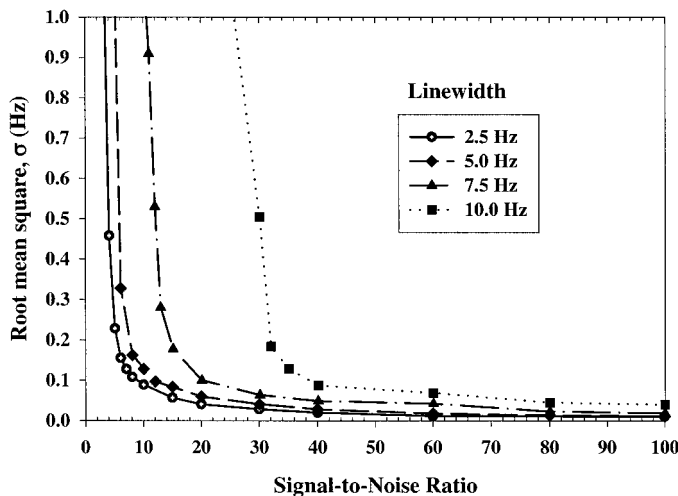


FIG. 4. Results from a simulation showing how the root mean square (σ_J) of a coupling constant of 5.0 Hz is affected by the signal-to-noise-ratio. The σ_J represented by each point is computed by determining the value of the coupling constant from 200 separated spectra in which different levels have been added. Different linewidths (2.5, 5.0, 7.5, and 10 Hz) are represented with different symbols. An sw of 40.96 Hz with 4096 data points is used.

quantified to values of a linewidth/ J_R ratio of approximately 5. Above this value, several minima, which do not correspond to subharmonics, start to emerge. Although there is indeed a minimum at the value of the coupling constant, the appearance of these other local minima complicates a straightforward determination. For an antiphase doublet (Fig. 2b), signal cancellation reduces dramatically the limit of the measurement. When the linewidth is less than twice J_R , a certainty of more than 90% is obtained. It is clearly seen that, in both cases, J doubling yields better results than the direct method.

NMR experimental signals have rarely just one coupling constant. It is more realistic to test the method with multiplets with three or more J 's. For this purpose, it is convenient to perform a multistage process. This one consists of finding the largest passive J , which appears as a deep minimum at high frequencies in the integral function; this is the easiest determination of a coupling because it does not have interference of subharmonics from the other couplings. Thus, the original multiplet is convoluted with this J_R value. The new integral function is obtained from the deconvoluted signal and the whole process is repeated until all passive constants have been

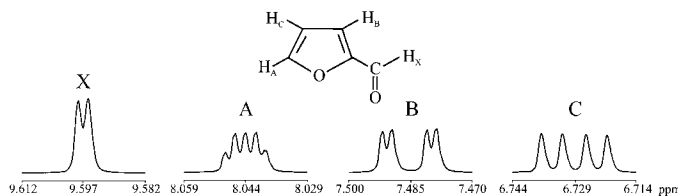


FIG. 5. Multiplets from the 300-MHz spectrum of furan-2-aldehyde with the assignment used in the text.

removed. Figure 3a shows the results for a multiplet with three passive coupling constants of 1.0, 1.5, and 2.0 Hz. Once the linewidth is larger than 1.2 Hz, it is possible to see only one peak instead of eight. However, modified J doubling can still measure the three constants up to a linewidth of 4.6 Hz. At this value, several minima, which do not correspond to subharmonics, start to emerge near the smallest J . Even though a minimum of 1 Hz is still present at a linewidth over 5.4 Hz, it is no longer possible to objectively verify that it is a real coupling

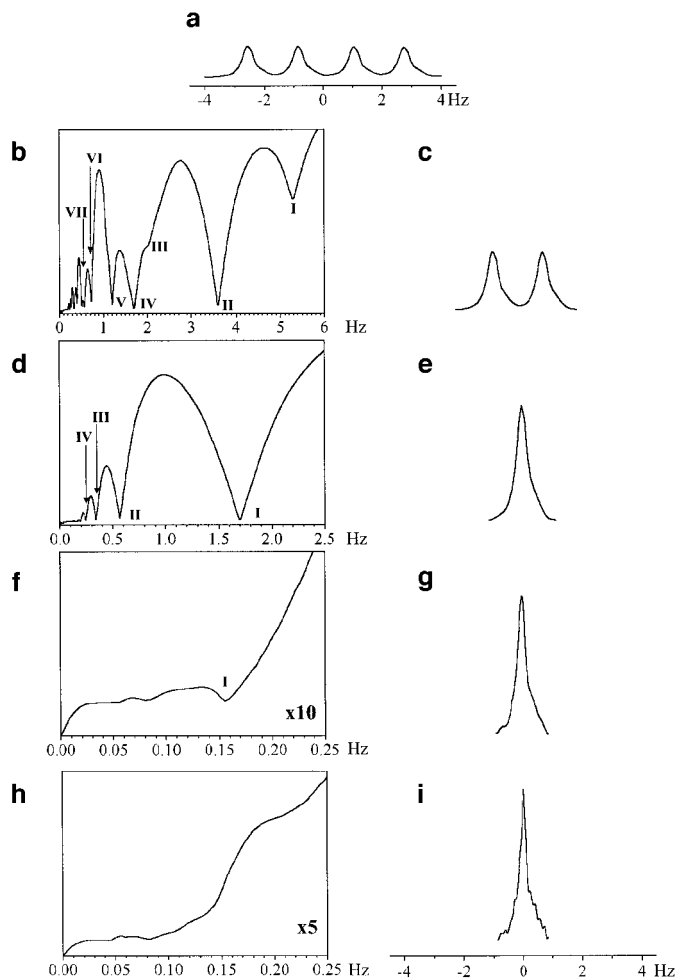


FIG. 6. Multistage deconvolution process of the H_c multiplet of furan-2-aldehyde in $[^2H_6]DMSO$. Three coupling constants are determined: $J_{BC} = 3.60$ Hz, $J_{AC} = 1.70$ Hz, and $J_{CX} = 0.16$ Hz. (a) The H_c multiplet. (b) Integral function of H_c : (I) $J_{BC} + J_{AC} = 5.30$ Hz, (II) J_{BC} , (III) $J_1 - J_2 = 1.90$ Hz, (IV) J_{AC} , (V) $J_{BC}/3 = 1.20$ Hz, (VI) $J_{BC}/5 = 0.72$ Hz, and (VII) $J_{AC}/3 = 0.57$ Hz and $J_{BC}/7 = 0.51$ Hz. (c) Deconvoluted multiplet after removing the splitting J_{BC} and reducing the sw by 3.60 Hz. (d) Integral function of the multiplet in (c): (I) J_{AC} , (II) $J_{AC}/3 = 0.57$ Hz, (III) $J_{AC}/5 = 0.34$ Hz, and (IV) $J_{AC}/7 = 0.24$ Hz. (e) Deconvoluted multiplet after removing the splitting J_{AC} and reduction of the sw; no other splitting is clearly observed. (f) The integral function of the multiplet in (e) shows only a minimum at J_{CX} . (g) Deconvoluted multiplet after removing the splitting J_{CX} and sw reduction. (h) Integral function of the multiplet in (g); one can hardly observe a minimum at 0.08 Hz. (i) Convoluted graph of signal (g) with 0.08 Hz. Digital resolution of the spectra is 0.005 Hz. The reference signal of TMS has a linewidth of 0.35 Hz.

TABLE 2
Coupling Constants for Furan-2-aldehyde (Hz)

Solvent	J_{AB}			J_{AC}			J_{BC}			J_{AX}			J_{BX}			J_{CX}		
	(A)	(B)	(R)	(A)	(C)	(R)	(B)	(C)	(R)	(A)	(X)	(R)	(B)	(X)	(R)	(C)	(X)	(R)
[² H ₆]Benzene	0.78	0.78	0.78	1.70	1.70	1.69	3.59	3.58	3.56	0.65	0.65	0.63	0.12	0.13	0.13	0.31	0.32	0.32
[² H]Chloroform	0.78	0.77	0.79	1.70	1.70	1.69	3.60	3.60	3.58	0.70	0.70	0.68	0.11	0.12	0.11	0.27	0.27	0.27
[² H ₆]DMSO	0.77	0.79	0.80	1.70	1.70	1.69	3.60	3.60	3.60	0.84	0.83	0.84	—	—	0.08	0.16	0.16	0.16

Note. (A) Measured in multiplet A; (B) measured in multiplet B; (C) measured in multiplet C; (X) measured in multiplet X; (R) from Ref. (12).

constant. The constant of 2.0 Hz can be measured without ambiguities until 6.0 Hz, five times the limit of the direct method.

A second test uses a multiplet with two passive constants of 1.0 and 2.0 Hz and an active one of 1.5 Hz (Fig. 3b). Above a linewidth of 0.8 Hz, estimation of J 's by location of peak maxima and minima is random and very difficult to analyze. Moreover, it is no longer possible to measure any realistic coupling after a linewidth of 1.5 Hz; the only measured value is 4.2 Hz. Modified J doubling is capable of determining the antiphase coupling with an error of less than 10% up to values of 3.0 Hz in the linewidth. Generally, the method limits for in-phase J 's in a multiplet are found to be slightly smaller than the ones for a doublet; they go up to 4.6 Hz linewidth for $J = 1.0$ Hz and 6.0 Hz linewidth for $J = 2.0$ Hz. For antiphase multiplets, modified J doubling measurements are better determined if passive constants are first found out and removed, as described above. The active splittings are measured and removed at the end.

These results are expected to vary as noise is introduced. We study how noise affects the determination of a in-phase multiplet with 5.0 Hz, as proposed by Stonehouse and Keeler (5). A noiseless simulated signal is obtained and a predetermined noise level is added to it. Afterward, this signal-noise function is processed by modified J doubling to measure the value of the coupling constant between 3.00 and 7.00 Hz. This procedure is then repeated 200 times with different but similar noise levels. The root-mean-square deviation (σ_J) of the determined values from the true value (5.00 Hz) is plotted against the mean value of the noise levels for four different linewidths in Fig. 4. The σ_J increases as the signal-to-noise ratio (SNR) decreases and as the linewidth increases. Surprisingly, the deviations of the results remain very similar when SNR > 40. When the SNR > 30 for a linewidth of 10 Hz, it is possible to measure the coupling constant within an accuracy larger than 90%. The same result is obtained for SNR > 3 and linewidth < 6. Active splittings have a similar behavior.

Small long-range couplings of furan-2-aldehyde (Fig. 5) are chosen to test the method experimentally. A multistage deconvolution process (Fig. 6) is shown for the H_C multiplet in [²H₆]DMSO (Fig. 6a). Once the modified J doubling processes the signal, the integral function of H_C is obtained (Fig. 6b). A

minimum appears at 3.60 Hz, which corresponds to the largest coupling constant. After this, the multiplet is deconvoluted with this J value (Fig. 6c). Modified J doubling is applied to the deconvoluted multiplet, producing a simplified integral function (Fig. 6d) which clearly shows the presence of 1.70 Hz coupling. Further deconvolution with this value (Fig. 6e) generates a signal that can be taken for a singlet. Nevertheless a third integral function shows that it is indeed a doublet since it shows a minimum at 0.16 Hz (Fig. 6f). This corresponds to the smallest coupling constant known. Figure 6g reveals a singlet with imperfections due to magnetic field inhomogeneties and small artifacts introduced by the filtering. A further stage vaguely suggests the presence of a minimum at 0.08 Hz (Fig. 6h). Such a broad minimum (Fig. 6h) cannot be taken as an indicator of another coupling constant. If we doubt about when to stop the process, we can go further deconvolutioning again with 0.08 Hz. The new signal will contain wiggle artifacts at the edges with a strange lineshape (Fig. 6i). The new lineshape is not preserved as in the previous processes. A broad minimum in the integral function, wiggle artifacts at the edges of the deconvoluted signal, and strange lineshapes are indicators to stop the deconvolution process one step before (Fig. 6g). In this manner, problems due to poor SNR and problems of measuring more coupling constants than the ones present are avoided. It is convenient to decrease the original sw by J , as expected for a formal deconvolution process, since each deconvolution step will increase imperfections and noise present in the extracted signal.

Solutions of furan-2-aldehyde in [²H₆]benzene, [²H]chloroform, and [²H₆]DMSO are examined. Complete results are shown in Table 2. The coupling constants are measured in both signals. The first J_{MN} in a column represents the value obtained from multiplet M , and the second one the value obtained from multiplet N . It is not possible to measure the smallest coupling constant reported, which corresponds to 0.08 Hz in [²H₆]DMSO. All the other results, including the J_{BX} in [²H]chloroform and [²H₆]benzene (0.11 and 0.12 Hz), are in complete agreement with previously reported data (10, 11) within an error of ± 0.03 Hz.

Automatic analysis of 2D NMR signals is easily implemented by taking each row or column and performing the process as it is for a 1D signal. The integral function will be the

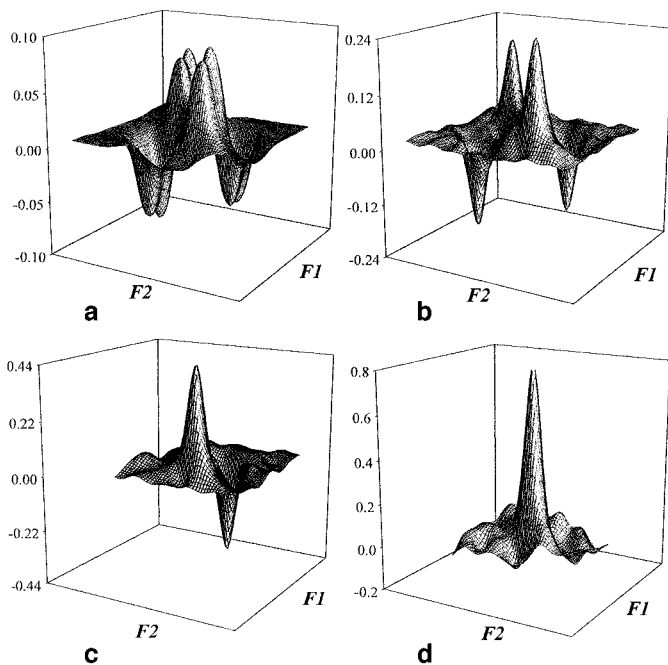


FIG. 7. Progressive deconvolution of splittings in a COSY cross peak (15×15 Hz and 0.165 Hz/point) between H_B and H_C in furan-2-aldehyde. (a) The unprocessed cross peak. (b) After removal of two passive splittings: $J_{AC} = 1.70$ Hz in one dimension and $J_{AC} = 0.78$ Hz in the other one. (c) After removal of the active splitting $J_{AC} = 3.60$ Hz in the F_2 dimension. (d) After removal of the active splittings $J_{AC} = 3.60$ Hz in the F_1 dimension.

sum of all the individual integral functions obtained for each row or column. Figure 7 shows the deconvolution process for 2DQF-COSY cross signal for H_B and H_C (Fig. 7a). First, two successive deconvolutions are performed, removing $J_{AC} = 1.70$ Hz in F_1 and $J_{AB} = 0.78$ Hz in F_2 (Fig. 7b). Active splitting, $J_{BC} = 3.60$ Hz, is then deconvoluted in F_2 (Fig. 7c) and in a second stage in F_1 (Fig. 7d). The same value for the coupling constant J_{BC} is measured in both dimensions. Some artifacts show up as a consequence of 2D signal imperfections in the original multiplet, border effects and noise due to the filtering process.

In order to be implemented, modified J doubling does not require previous knowledge of the linewidth or the number of coupling constants present in the multiplet as other methods do. Nevertheless, a little experience with integral functions

turns out to be very useful for proper interpretation of signals, i.e., complex multiplets from natural products or organic molecules. Modified J doubling makes possible the observation and the measurement of small coupling constants hidden in the linewidth once a good experimental signal is provided. Therefore, the more complex the signal the more useful the method. Modified J doubling also simplifies multiplets by independent successive deconvolutions from 1D and/or 2D experiments.

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