Double-quantum biased covariance spectroscopy: Application to the 2D INADEQUATE experiment

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Abstract

A novel processing scheme is presented that converts a two-dimensional double-quantum NMR spectrum into a single-quantum correlation spectrum. The covariance-like spectrum is computed from the 2D Fourier transform spectrum by emphasizing contributions that fulfill the double-quantum condition resulting in a symmetric spectrum that is easier to analyze. The method is demonstrated for the 2D INADEQUATE experiment.
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1. Introduction

It has been recognized for a long time that a two-dimensional (2D) double-quantum NMR spectrum of two-spin 1/2 systems contains in principle the same information as the corresponding double-quantum filtered single-quantum 2D correlation spectrum. In practice, however, a double-quantum spectrum has certain advantages and drawbacks with respect to its single-quantum correlation counterpart. The main advantages are the excellent suppression of unwanted signals and the distribution of the magnetization over a smaller number of multiplet components. These features form the basis of the 2D INADEQUATE experiment [1–4], which is one of the best known double-quantum NMR experiments. On the other hand, a double-quantum spectrum is more difficult to analyze than a single-quantum correlation spectrum, because the indirect frequency dimension does not directly correspond to the resonance frequencies of single spins, but rather to the

sum of frequencies of pairs of spins. Consequently, tracing the magnetization along the spin-coupling network is not as easy as in a symmetric COSY-type single-quantum correlation spectrum.

In this work it is shown how a 2D double-quantum spectrum can be converted into a 2D single-quantum correlation spectrum by means of covariance spectroscopy [5–8]. The covariance method is combined with the condition that in a 2D double-quantum spectrum a crosspeak at frequency ω_1 along the indirect dimension occurs only if two spins resonate at frequencies ω_2 and ω_2' along the detection dimension fulfilling the condition

$$\omega_1 = \omega_2 + \omega_2'. \tag{1}$$

The method is first explained and then experimentally demonstrated at the example of the 2D INADEQUATE experiment [1–4].

2. Method

A ^{13}C–^{13}C 2D INADEQUATE time-domain data set $s(t_1, t_2)$ is recorded and processed in standard fashion.

2D Fourier transformation and phase correction is applied to $s(t_1, t_2)$ yielding a real 2D spectrum represented by matrix $\boldsymbol{S}$ with elements $S(\omega_1, \omega_2) = \text{Re} \iint dt_1 \, dt_2 \cos(\omega_1 t_1) \exp(-i\omega_2 t_2) s(t_1, t_2)$ where ω_1 and ω_2 denote the discrete indirect double-quantum and single-quantum (detection) axes, respectively. Next, the covariance matrix is calculated in the frequency domain according to [6,7]

$$C(\omega_2', \omega_2) = \boldsymbol{S}^{\text{T}} \cdot \boldsymbol{S} = \sum_{\omega_1} S(\omega_1, \omega_2') S(\omega_1, \omega_2), \qquad (2)$$

where ω_2, ω_2' take all possible single-quantum frequency values and the sum goes over all (discrete) double-quantum frequencies ω_1. All terms in the sum of Eq. (2) are given equal weight. However, because spectrum $\boldsymbol{S}$ is a double-quantum spectrum, the products $S(\omega_1, \omega_2') S(\omega_1, \omega_2)$ that strictly or approximately fulfill Eq. (1) are the only ones that potentially contain useful spin connectivity information. All other terms represent mainly noise. By emphasizing the terms that contain the relevant information, biased correlation matrices $\boldsymbol{C}_1$ and $\boldsymbol{C}_2$ can be constructed as is described in the following.

If no a priori information about the multiplet structure of the single-quantum resonances is available, a symmetric correlation spectrum $\boldsymbol{C}_1$ is obtained by retaining only the terms in Eq. (2) that strictly fulfill Eq. (1)

$$C_1(\omega_2', \omega_2) = S(\omega_2 + \omega_2', \omega_2') S(\omega_2 + \omega_2', \omega_2). \qquad (3)$$

It follows from Eq. (3) that two ^{13}C spins I and S that have INADEQUATE crosspeak multiplets $S(\omega_1, \omega_2)$ at positions $(\omega_I + \omega_S, \omega_I + \pi J)$ and $(\omega_I + \omega_S, \omega_S - \pi J)$ exhibit crosspeaks in $C_1(\omega_2', \omega_2)$ at positions $(\omega_I + \pi J, \omega_S - \pi J)$ and $(\omega_S - \pi J, \omega_I + \pi J)$. Because the two multiplet components $S(\omega_I + \omega_S, \omega_I + \pi J)$ and $S(\omega_I + \omega_S, \omega_S - \pi J)$ have opposite signs, $C_1(\omega_I + \pi J, \omega_S - \pi J)$ and $C_1(\omega_S - \pi J, \omega_I + \pi J)$ are negative. In full analogy, 2D INADEQUATE multiplets at positions $(\omega_I + \omega_S, \omega_I - \pi J)$ and $(\omega_I + \omega_S, \omega_S + \pi J)$ lead to negative crosspeaks in $C_1(\omega_2', \omega_2)$ at positions $(\omega_I - \pi J, \omega_S + \pi J)$ and $(\omega_S + \pi J, \omega_I - \pi J)$.

If the single-quantum resonances are known to be doublets with scalar couplings $2\pi J$ that are similar for all directly bonded carbon pairs, other terms in the sum of Eq. (2) which do not strictly fulfill the double-quantum condition of Eq. (1) contain also correlation information that can be included in a correlation spectrum $\boldsymbol{C}_2$ defined as

$$C_2(\omega_2', \omega_2) = \sum_{x = -2\pi J, 0, 2\pi J} S(\omega_2 + \omega_2' + x, \omega_2')$$
$$\times S(\omega_2 + \omega_2' + x, \omega_2). \qquad (4)$$

In addition to the crosspeaks of $C_1(\omega_2', \omega_2)$, $C_2(\omega_2', \omega_2)$ contains multiplet components originating from the multiplet component pairs $S(\omega_I + \omega_S, \omega_I + \pi J)$ and $S(\omega_I + \omega_S, \omega_S + \pi J)$ as well as $S(\omega_I + \omega_S, \omega_I - \pi J)$

and $S(\omega_I + \omega_S, \omega_S - \pi J)$. Because these peak pairs have the same sign, the resulting multiplet components in $C_2(\omega_2', \omega_2)$ are positive. In case certain 2D INADEQUATE double-quantum frequencies are aliased along the indirect dimension, they can be included in the covariance processing of Eqs. (3) and (4) by modifying the double-quantum condition of Eq. (1) to $\omega_1 = \omega_2 + \omega_2' + 2\pi(SW_1) \cdot m$, where m is an integer and SW_1 is the spectral width along the indirect dimension.

In contrast to matrix $\boldsymbol{C}$, matrices $\boldsymbol{C}_1$ and $\boldsymbol{C}_2$ of Eqs. (3) and (4) are not semi-positive definite and therefore do not fulfill the mathematical definition of a covariance matrix. As a consequence, application of the matrix square-root operation to matrices $\boldsymbol{C}_1$ and $\boldsymbol{C}_2$, which is useful for regular covariance spectra [6,7], has no obvious benefit because the square-root of negative eigenvalues leads to (undesirable) imaginary contributions.

3. Results and discussion

The double-quantum biased covariance method is demonstrated for a ^{13}C–^{13}C INADEQUATE experiment [1–4] of menthol (Fig. 1) at natural ^{13}C abundance dissolved in deuterated chloroform. The time-domain data consists of 2048 $(t_2) \times 1024$ (t_1) complex data points. An expanded region of the 2D Fourier transformed spectrum $\boldsymbol{S}$ is shown in Fig. 2. The spectrum takes the familiar form of a 2D INADEQUATE spectrum [1–4] with antiphase doublets with splittings $2\pi J$ in ω_2 appearing along the double-quantum dimension ω_1 at the sum of the two single-quantum frequencies along ω_2 (Fig. 2). The expanded spectrum shows the one-bond connectivities between carbons 4–5, 4–3, 5–7, and 8–10. Few additional singlet peaks appear, which reflect residual magnetization of isolated ^{13}C spins that have only ^{12}C neighbors.
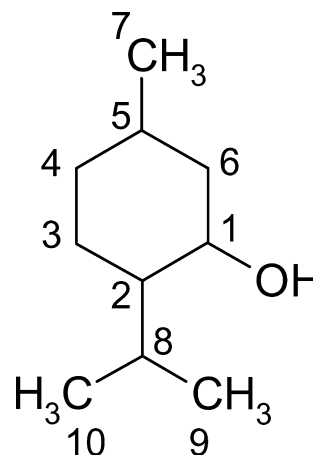

Fig. 1. Chemical structure of menthol with the carbon atoms numbered from 1 to 10.
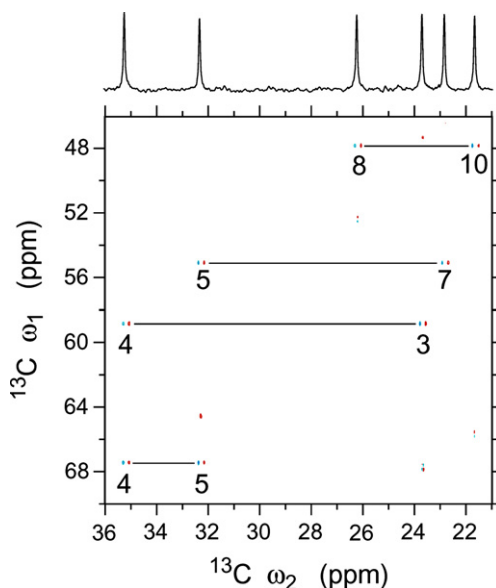
Fig. 2. ^{13}C–^{13}C 2D INADEQUATE spectrum of a 30% by weight menthol solution at ^{13}C natural abundance in CDCl$_3$ in a 5 mm NMR tube obtained by the standard $90°$–Δ–$180°$–Δ–$90°$–t_1–$90°$–t_2 INADEQUATE pulse sequence [1–4]. Positive peaks are in blue and negative peaks are in red. The peaks are labeled according to the corresponding carbon numbers of Fig. 1. A proton-decoupled ^{13}C 1D spectrum is shown on top of the figure. Delay $\Delta = 1/(4J_{CC})$ was set to 6.2 ms. A data set of 2048 (t_2) × 1024 (t_1) complex data points was collected at 600 MHz magnetic field strength and 298 K with a total measurement time of 12 h. The time-domain data matrix was zero filled to 3072 (t_2) × 2048 (t_1) points prior to Fourier transformation.

Fig. 3 shows the INADEQUATE covariance spectrum calculated according to Eq. (3). The spectrum is strictly symmetric and takes the form of a single-quantum correlation spectrum. Thus, the double-quantum axis ω_1 has been replaced by the single-quantum axis ω_2'. The spectrum contains crosspeaks each consisting of two negative multiplet components located on diametrically opposed corners of a square of side length $2\pi J$. As mentioned above, the spectrum of Fig. 3 is constructed without making any assumption about the magnitude of the scalar J couplings. The singlet peaks of Fig. 2 are eliminated because according to Eq. (3) only those spectral pairs $S(\omega_1, \omega_2)$ and $S(\omega_1, \omega_2')$ that fulfill the double-quantum condition are used for the construction of spectrum C_1. The resolution along ω_2' and ω_2 is solely determined by the resolution along the detection dimension ω_2 of the original 2D INADEQUATE experiment. A spectrum similar to the one in Fig. 3 can be obtained by a modified pulse sequence that contains a spin echo prior to acquisition [9,10].

If the J couplings are rather uniformly distributed, the double-quantum condition can be slightly relaxed according to Eq. (4) leading to the occurrence of additional multiplet components as is shown in the spectrum C_2 of Fig. 4. Each crosspeak adopts a COSY-type multiplet structure with four multiplet components arranged
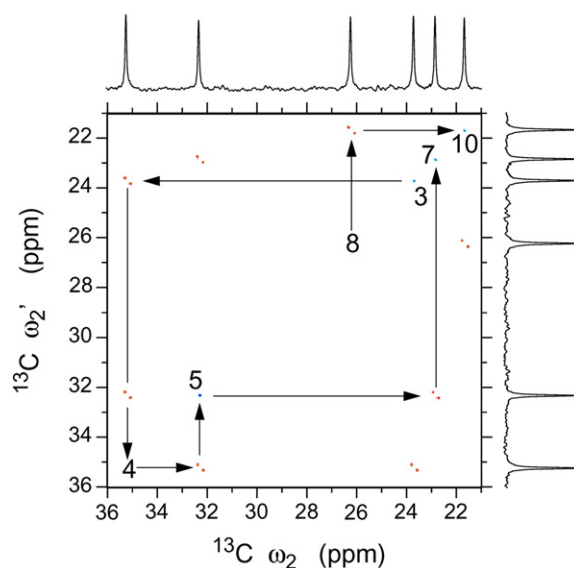

Fig. 3. Section of the symmetric covariance-type NMR spectrum of menthol obtained by application of Eq. (3) to the 2D INADEQUATE spectrum of Fig. 2. Positive peaks are in blue and negative peaks are in red. The peaks are labeled in accordance with the carbon numbers given in Fig. 1.
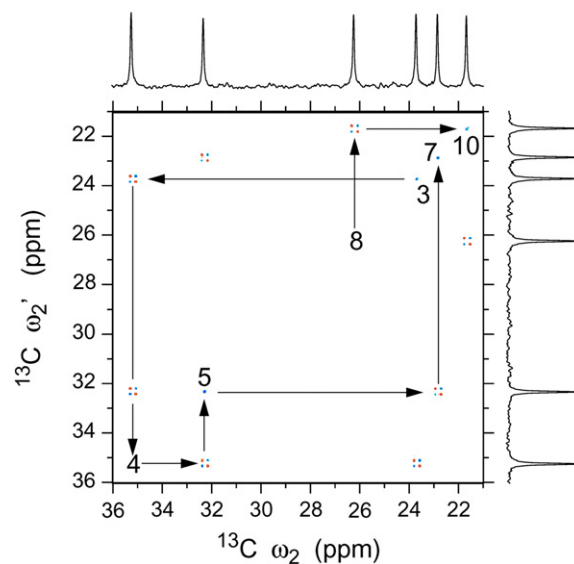

Fig. 4. Section of the symmetric covariance-type NMR spectrum of menthol obtained by application of Eq. (4) to the 2D INADEQUATE spectrum of Fig. 2. Positive peaks are in blue and negative peaks are in red. The peaks are labeled in accordance with the carbon numbers given in Fig. 1.

in an antiphase manner along both frequency axes at the corners of squares with side lengths $2\pi J$. The spectrum in Fig. 4 is the ^{13}C analog of a ^1H–^1H COSY spectrum. It allows the straightforward tracing of the carbon skeleton as is indicated in the figure.

COSY-type crosspeaks are also found when Eq. (3) is applied to an INADEQUATE spectrum with relatively

low resolution along ω_1, for example due to a small number of t_1 increments. Deviations from the infinitely weak coupling limit and ^{13}C-isotope effects can cause slight changes in the average positions of the two doublet components in the 2D INADEQUATE spectrum, which are mirrored in the corresponding resonance positions of the double-quantum biased covariance spectrum.

The double-quantum biasing underlying the spectra calculated using Eqs. (3) and (4) provides higher sensitivity as compared to the covariance spectrum of Eq. (2), because the terms in Eq. (2) that do not fulfill the double-quantum condition, representing mainly noise, have been eliminated. In our experience, the signal-to-noise ratio of the original INADEQUATE spectrum should be about 3 or higher in order to yield a double-quantum biased covariance spectrum that can be analyzed manually. For sensitivity improvement, the original INADEQUATE spectrum S can be first subjected to sensitivity enhancement processing schemes [11–14] prior to the double-quantum biased covariance analysis. Application of the method to the composite refocusing INADEQUATE experiment [15,16], where magnetization transfer from double to single-quantum coherence is restricted to only one multiplet component thereby yielding improved sensitivity, requires a slight modification of Eq. (3)

$$C_1'(\omega_2', \omega_2) = S(\omega_2 + \omega_2' \pm 2\pi J, \omega_2')$$
$$\times S(\omega_2 + \omega_2' \pm 2\pi J, \omega_2). \qquad (5)$$

The sign depends on whether the down-field or up-field multiplet component is selected in the composite refocusing experiment.

In summary, a variant of covariance NMR spectroscopy is applied to 2D double-quantum NMR spectroscopy. The covariance scheme operates on the 2D FT spectrum without requiring pulse-sequence modification. It converts a double-quantum spectrum into a single-quantum correlation spectrum, which combines advantages of double-quantum and single-quantum spectroscopy yielding a symmetric spectrum that is easy to analyze. The method is applicable to other 2D double-quantum spectra as well as to zero-quantum spectra. For zero-quantum spectra both sums $\omega_2 + \omega_2'$ in Eq. (3) are to be replaced by $\omega_2 - \omega_2'$.

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