

TWO-DIMENSIONAL NMR SPECTROSCOPY

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Abstract

Two-dimensional (2D) NMR spectroscopy is a powerful indirect method of studying spin systems. It allows the measurement of NMR parameters that cannot be determined with conventional one-dimensional spectroscopic techniques. Examples of applications of 2D experiments to the exact measurement of coupling constants, to sensitivity enhanced detection of ^{15}N and to removal of chemical shift anisotropy in solids are discussed.

I INTRODUCTION

The concept of two-dimensional (2D) Fourier transformation in NMR was first introduced by Jeener (1) in 1971. Since that time, several hundred different 2D experiments have been proposed. The selection of experiments described here is based on the insight they provide into the basis and generality of two-dimensional NMR.

In general, four different time intervals in a 2D NMR experiment can be distinguished: a preparation period, an evolution period, a mixing period and a detection period (Fig.1a). As an example of a 2D pulse scheme, the oldest 2D experiment (1) is illustrated in Fig.1b. The operation of this pulse sequence will be briefly discussed for the simple case of a set of isolated, non-coupled spins. At the end of the preparation period, which has a duration

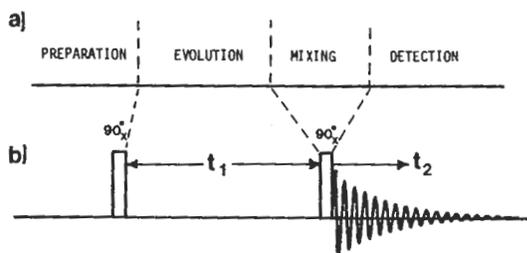


Fig.1 (a) General subdivision of the time axis in a 2D experiment. (b) Schematic representation of the oldest 2D NMR experiment. The sequence is repeated for many different durations of the evolution period, yielding a 2D time signal $s(t_1, t_2)$.

of several times the longitudinal relaxation time T_1 , a 90°_x pulse rotates the thermal equilibrium longitudinal magnetization into a position parallel to the y axis of the rotating frame (Fig.2a). The magnetization then rotates for a time t_1 , with angular chemical shift frequency Ω_1 . A second 90°_x pulse rotates this transverse magnetization into the xz plane, leaving a transverse component with amplitude proportional to $\sin(\Omega_1 t_1)$ parallel to the x axis (Fig.2c). This transverse component is detected during the time t_2 , and gives, neglecting relaxation effects, a signal

$$s(t_1, t_2) = C \sin(\Omega_1 t_1) \cos(\Omega_2 t_2) \quad [1]$$

The experiment is repeated for a large number of different values for the length of the evolution period, t_1 , and the acquired free induction decay (FID) is stored separately for each value of t_1 . In Fig.3a the Fourier transform of a set of FID's, obtained with different lengths of the evolution period is displayed. As expected, the amplitude of the resonance is modulated as a function of t_1 , with frequency Ω_1 . If one now takes slices parallel to the

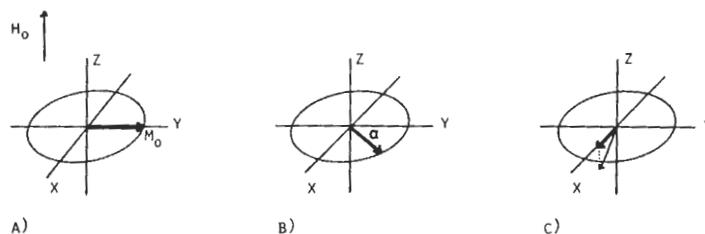


Fig.2 Evolution of the magnetization of a set of isolated spins during the sequence of Fig.1b. (A) Just after the first 90° pulse, (B) just before the second 90° pulse and (C) just after the second 90° pulse.

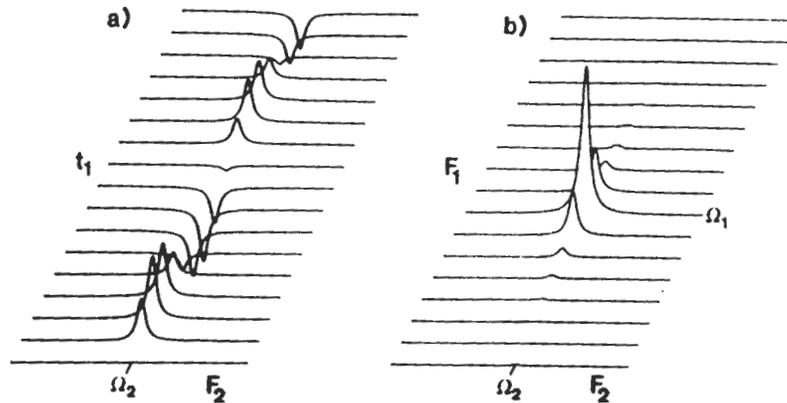


Fig.3. (a) The absorptive part of a set of spectra obtained with the sequence of Fig.1b and modulated in amplitude as a function of t_1 . (b) A 2D spectrum obtained from (a) by Fourier transformation of cross-sections parallel to the t_1 axis.

through the t_1 axis through the data matrix of Fig.3a, all slices except the one taken at $F_2 = \Omega_2$ will show zero intensity. The t_1 -slice, taken at $F_2 = \Omega_2$ shows intensity modulated with frequency Ω_1 . Fourier transformation with respect to t_1 of all those slices will then give a resonance in the final 2D spectrum at $(F_1, F_2) = (\Omega_1, \Omega_2)$ (Fig.3b). In this simple case, Ω_1 and Ω_2 are identical, and the resonance will be on the diagonal of the 2D spectrum. For a system with homonuclear spin-spin coupling, a theoretical treatment becomes more complicated (1,2), as magnetization transfer from one spin to another is introduced by the second pulse in the scheme of Fig.1b, yielding important connectivity information in the final 2D spectrum (1-6). The basic idea however, is clear from the simple example given above: the rotation frequency of the magnetization during the evolution period is measured indirectly by studying the effect of that rotation on the signals observed during the detection period, t_2 . Hence, in a 2D spectrum, the F_2 coordinate of a resonance equals the precession frequency of the transverse magnetization during the detection period. The F_1 coordinate of a resonance equals the average precession frequency during the evolution period.

The major power of 2D NMR is three-fold:

1. The study of how magnetization is transferred from one spin to another during the mixing period, yielding important conformational and structural information.
2. The study of non-equilibrium phase coherences between spin states which are not directly observable because they do not correspond to transverse magnetization (multiple quantum coherences).

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3. The study of the average precession frequency, which allows great simplification over the study of real-time frequencies that are measured in most direct-detection experiments. Examples of various experiments demonstrating those features will be discussed.

II DETECTION OF PROTON-CARBON-13 LONG-RANGE COUPLINGS

Consider a ^{13}C multiplet component in a proton-coupled ^{13}C spectrum at frequency $\nu_{\text{C}}+J/2$, where J is the coupling to an arbitrary proton, P, in the $m=1/2$ spin state. ν_{C} is determined by the chemical shift frequency of the ^{13}C nucleus considered, and by couplings to protons other than P. If the pulse sequence of Fig.4 is applied to this spin system, during the first half of the evolution period the transverse magnetization of the multiplet component considered precesses with frequency $\nu_{\text{C}}+J/2$, and evolves through an angle α , given by

$$\alpha = 2\pi(\nu_{\text{C}}+J/2)t_1/2. \quad [2]$$

This angle is inverted by the ^{13}C 180° pulse, and the spin state of proton P is inverted by the selective proton 180° pulse, to $m=-1/2$. The new precession frequency of the component considered is therefore equal to $\nu_{\text{C}}-J/2$. At the end of the evolution period, t_1 , the total angle precessed through is then given by $-\pi Jt_1$. The average precession frequency during the evolution period is therefore determined by the coupling to proton P, and is independent of the chemical shift frequency and of couplings to protons other than P. In a 2D spectrum we will then get two resonances in the F_1 dimension, at $F_1 = \pm J/2$. This can give a dramatic simplification (7) over studying conventional proton-coupled ^{13}C spectra. Fig.5 gives an example of sections parallel to the F_1 axis of the 2D spectrum taken at the F_2 resonance frequencies of the aromatic carbons in 2-acetonaphtalene, where proton H1 was selectively inverted at the center of the evolution period.

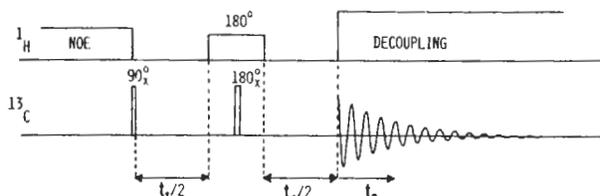


Fig.4 Pulse scheme of the selective proton-flip experiment. The selective proton pulse rotates the magnetization of a preselected proton by 180° . Only couplings to this proton will produce a splitting in the 2D ^{13}C spectrum.

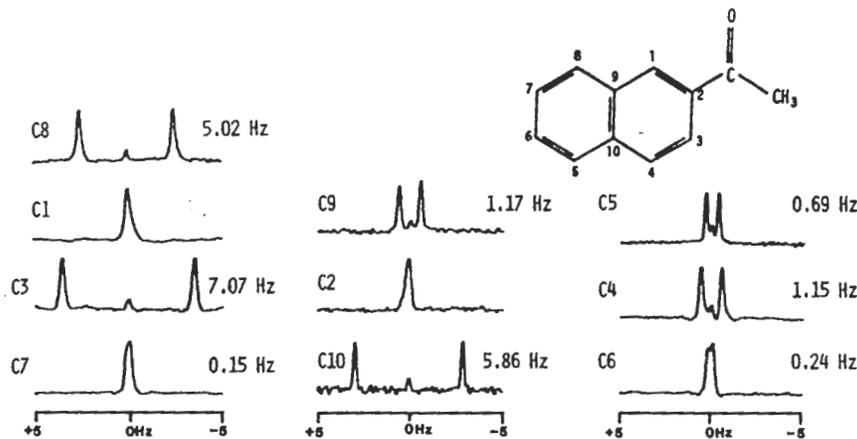


Fig.5 Cross-sections parallel to the F_1 axis through the aromatic resonances of the selective 2D J-spectrum of 2-acetonaphthalene. Proton H1 was selectively inverted at the center of the evolution period, and only couplings to this proton give rise to a splitting in the F_1 dimension.

III INDIRECT DETECTION OF ^{15}N CHEMICAL SHIFTS

Detection of ^{15}N chemical shifts is limited by its low NMR sensitivity, due to its low magnetogyric ratio, and by its low natural abundance (0.37%). A new approach (8-10) to improve ^{15}N sensitivity utilizes the concept of multiple quantum coherence (11-14).

Consider an isolated ^{15}N - ^1H spin system. The appropriate energy level diagram is sketched in Fig.6. The proton resonance frequencies equal $\delta_{\text{H}} \pm J/2$, where δ_{H} is the proton chemical shift frequency and J is the ^{15}N - ^1H scalar coupling constant. The ^{15}N resonance frequencies equal $\delta_{\text{N}} \pm J/2$. It follows immediately from Fig.6, that the double quantum coherence between levels 1 and 4 equals $\delta_{\text{H}} - \delta_{\text{N}}$. If this frequency can be measured indirectly, in a 2D experiment, the value of δ_{N} can be calculated if δ_{H} is known. As NMR sensitivity is several orders of magnitude better for protons than for ^{15}N , and the multiple quantum coherences are detected via the protons, a significant shortening of measuring time can be expected.

One of the most suitable sequences for creation and detection of ^1H - ^{15}N multiple quantum coherences is shown in Fig.7. It has been calculated (15), that a $90^\circ(^1\text{H})-1/(2J)-90^\circ(^{15}\text{N})$ sequence converts all longitudinal proton magnetization into equal amounts of zero and double quantum coherence. Those coherences then evolve during the evolution period, t_1 , and are converted back into transverse proton magnetization by a final $90^\circ(^{15}\text{N})$ pulse. The detected proton magnetization is then modulated as a function of t_1 with the

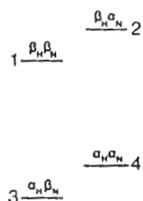


Fig.6 Energy level diagram and wave functions for an isolated $^{15}\text{N}-^1\text{H}$ spin pair.

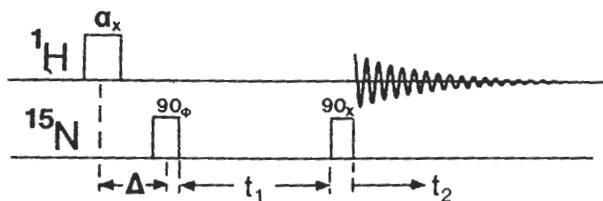


Fig.7 Pulse sequence for the indirect observation of ^{15}N via zero and double quantum coherence. The phase ϕ is cycled in the four steps of the experiment.

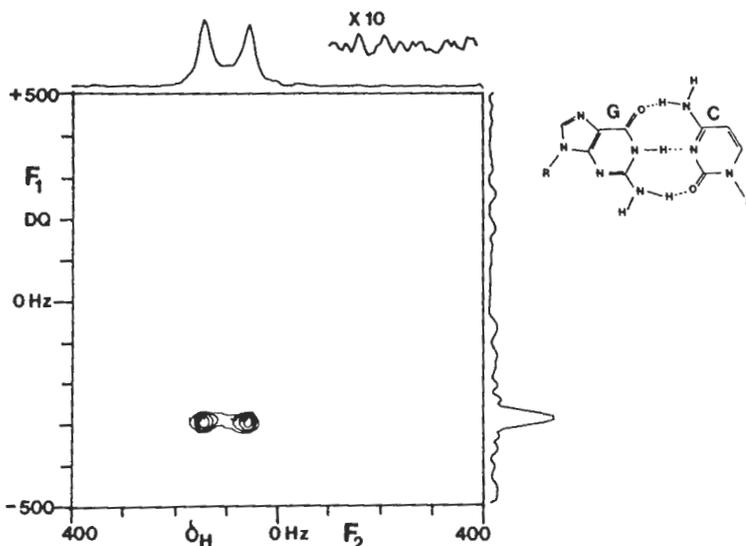


Fig.8 Two-dimensional heteronuclear $^1\text{H}-^{15}\text{N}$ zero quantum spectrum of the molecule shown in the inset. A selective proton pulse (flip angle of 45° on resonance) was used to minimize excitation of resonances of protons not coupled to the nitrogen, avoiding dynamic range problems. A 32×256 data matrix was acquired, and 800 transients were recorded for each t_1 value. The total measuring time was 2 hrs.

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frequencies that existed during t_1 , i.e. the zero and double quantum frequencies. Suitable phase cycling of the first $90^\circ(^{15}\text{N})$ pulse allows the selection of either the zero or the double quantum component (8,10). A 2D Fourier transformation will then show that the two detected proton doublet components are both modulated by the zero or double quantum frequency.

An example of a double quantum spectrum obtained for a 0.25 M sample solution with natural abundance ^{15}N concentration in a 5 mm sample tube, is shown in Fig.8. The total measuring time was 2 hrs. With conventional ^{15}N detection, similar sensitivity would be obtained in a measuring time on the order of 2 years.

IV MAGIC-ANGLE HOPPING

Unlike in liquid samples, in solids the chemical shift anisotropy (CSA) does not average to zero. Therefore, in solid-state NMR, the resonance frequency of a nucleus depends on the orientation of the crystallite with respect to the static magnetic field. In a polycrystalline sample, all possible orientations of crystallites contribute to the spectrum, giving rise to so-called anisotropy powder patterns. Usually, the width of these powder patterns is larger than the difference in isotropic resonance frequencies of the different sites, and overlap will occur. A way around this overlap problem is the use of magic-angle spinning (16-18). One of the problems with this approach is that very high spinning speeds are required for many practical applications, which leads to mechanical difficulties.

A two-dimensional solution to remove the CSA is based on the same principle as magic angle spinning; the resonance frequency of, for example, a ^{13}C nucleus in a certain crystallite is given by (19)

$$\Omega(\beta) = \Omega_T + C_1 \cos(\beta + \phi_1) + C_2 \cos(2\beta + \phi_2) \quad [3]$$

where C_1 , C_2 , ϕ_1 and ϕ_2 are constants, and β is the angle through which the sample has been rotated about the magic angle axis. Ω_T is the isotropic chemical shift frequency. It follows immediately from Eq.[3], that if the magnetization evolves during three consecutive intervals, each of length $t_1/3$, with angles $\beta = 0^\circ$, 120° and 240° respectively, that the average precession frequency during t_1 equals Ω_T . A two-dimensional experiment which is based on this principle is described in reference 20; the detected (t_2) static powder pattern is modulated as a function of t_1 with the isotropic chemical shift frequencies. A projection onto the F_1 axis of such a 2D ^{13}C spectrum, obtained for para-diethoxybenzene, is shown in Figure 9. Also shown are the cross-sections through the 2D spectrum, taken parallel to the F_2 axis, showing the CSA powder

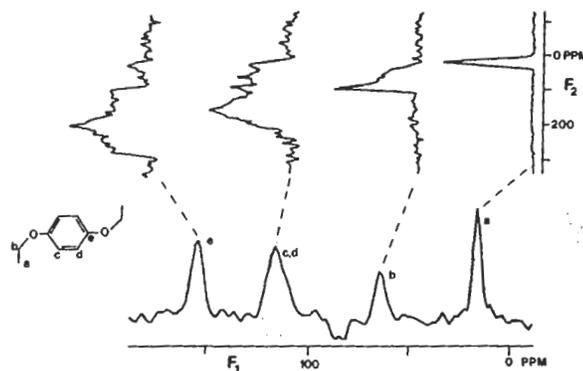


Fig.9 Projection of a 2D magic-angle hopping ^{13}C spectrum of paradiethoxybenzene onto the F_1 axis, and cross-sections parallel to the F_2 axis taken at the isotropic shift frequencies in the F_1 dimension, displaying the CSA powder patterns for the various sites.

patterns for the different sites. This latter information is not directly available from a magic-angle spinning spectrum, nor from a non-spinning spectrum because overlap of the various powder patterns often makes those spectra intractable. Resolution in the isotropic shift dimension (F_1) in Figure 9 is mainly limited by probe design and natural abundance ^{13}C - ^{13}C coupling.

V CONCLUSIONS

Although only a very small number out of the large collection of two-dimensional NMR experiments have been discussed, it is clear that the 2D approach extends the capabilities of NMR enormously. Many applications of 2D NMR in organic chemistry and biochemistry to structural and conformational problems have appeared in the recent literature.

The practical implementation of the various 2D experiments is still rather complicated, but as almost all experiments can be completely automated by proper spectrometer design and support software, it is to be expected that in the future the ease of operation will become much better. The only price one then will have to pay is a large amount of data storage space and calculation time to store and compute the 2D spectrum. However, in view of current developments in computer technology, this probably will not impede a wide-spread use of two-dimensional NMR.

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