High Resolution N.M.R. Measurements in Inhomogeneous Magnetic Fields: Use of the SECSY Pulse Sequence

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The high resolution proton n.m.r. spectra of a D_2O solution of amino acids have been measured in an inhomogeneous B_0-field *via* the conventional SECSY pulse sequence and also a broad band decoupled variant, the latter providing a signature for each component in the mixture; the results obtained are compared with the corresponding zero-quantum coherence spectra.

As part of a general programme to explore the measurement of high resolution n.m.r. spectra of organic molecules in highly inhomogeneous magnetic fields we have already demonstrated[1] the use of zero-quantum coherence (ZQC). Here we present an alternative approach, based on the well known SECSY pulse sequence,[2,3] which has the added advantage that it is potentially applicable to species which have shorter spin–spin relaxation times.

It has previously been demonstrated theoretically,[3] though not experimentally, that some of the cross peaks present in the spectrum produced by a SECSY experiment are independent of magnetic field inhomogeneity. In this study we demonstrate, with an aqueous solution of amino acids, the feasibility of utilising this phenomenon with both the conventional SECSY pulse sequence,[2] Figure 1a, and also with an adaptation which produces broad band decoupled[4—6] SECSY[7] spectra, Figure 1b. The results obtained are compared with those produced by the conventional ZQC pulse sequence,[1,8—10] Figure 1c, and by its broad band decoupled variant,[11] Figure 1d. In this study the degree of magnetic field inhomogeneity across the solution of amino acids was increased from 1 part in 10^8, Figure 2a, to 1 part in 10^6, Figure 2b, so that the conventional spectrum contained no useful information. Clearly, the corresponding SECSY spectra, Figure 2c—d, are still at high resolution and contain peaks which are characteristic of the chemical species producing them. The corresponding ZQC spectra are given for comparison in Figure 2e—f.

Although both the ZQC and the SECSY pulse sequences produce peaks which are independent of magnetic field inhomogeneity they do so for different reasons. A conventionally observed single-quantum coherence (SQC) of a spin k precesses at a frequency ω'_k given by equation (1), where ω_k is the precessional frequency of the spin in the absence of magnetic field inhomogeneity and $\Delta B(x,y,z)$ is the spatially dependent magnetic field inhomogeneity. Clearly the width of the transition will increase as $\Delta B(x,y,z)$ increases across the sample. In contrast, a ZQC[12—15] is a phase coherence between

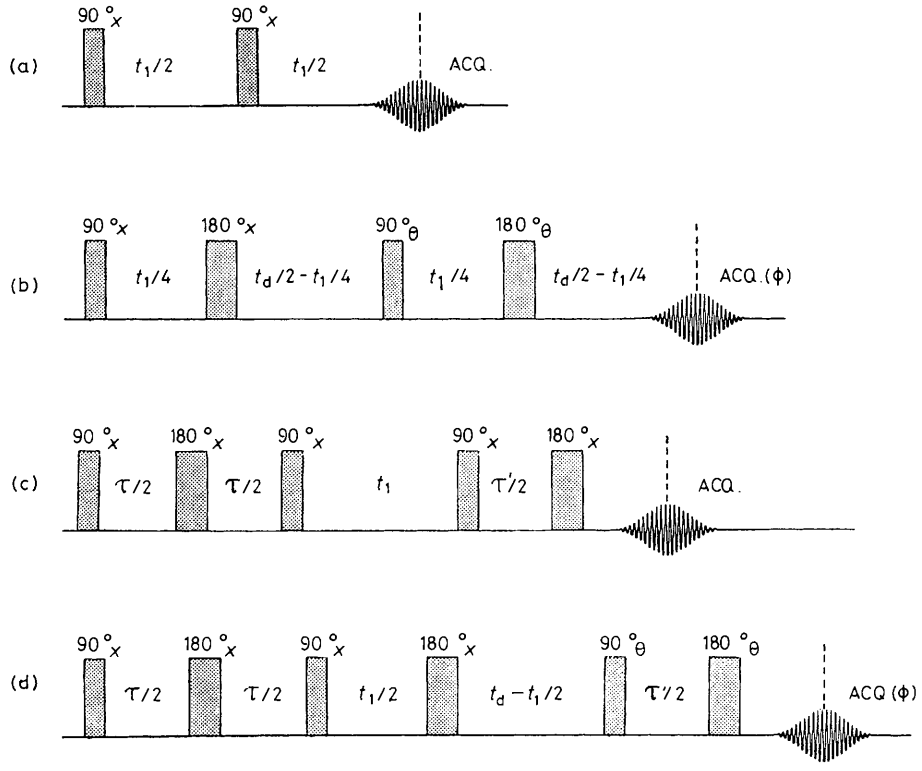

Figure 1. Pulse sequences for producing: (a) SECSY spectra, (b) broad band decoupled SECSY spectra. Phase cycling: θ = $x,y,-x,-y$ and ϕ = $x,-x,x,-x$. (c) Zero-quantum coherence specta, (d) broad band decoupled zero-quantum coherence spectra. Phase cycling: θ, ϕ = $x,y,-x,-y$. For (a) and (c) the use of an inhomogeneous magnetic field makes the use of additional magnetic field gradients or phase cycling redundant.

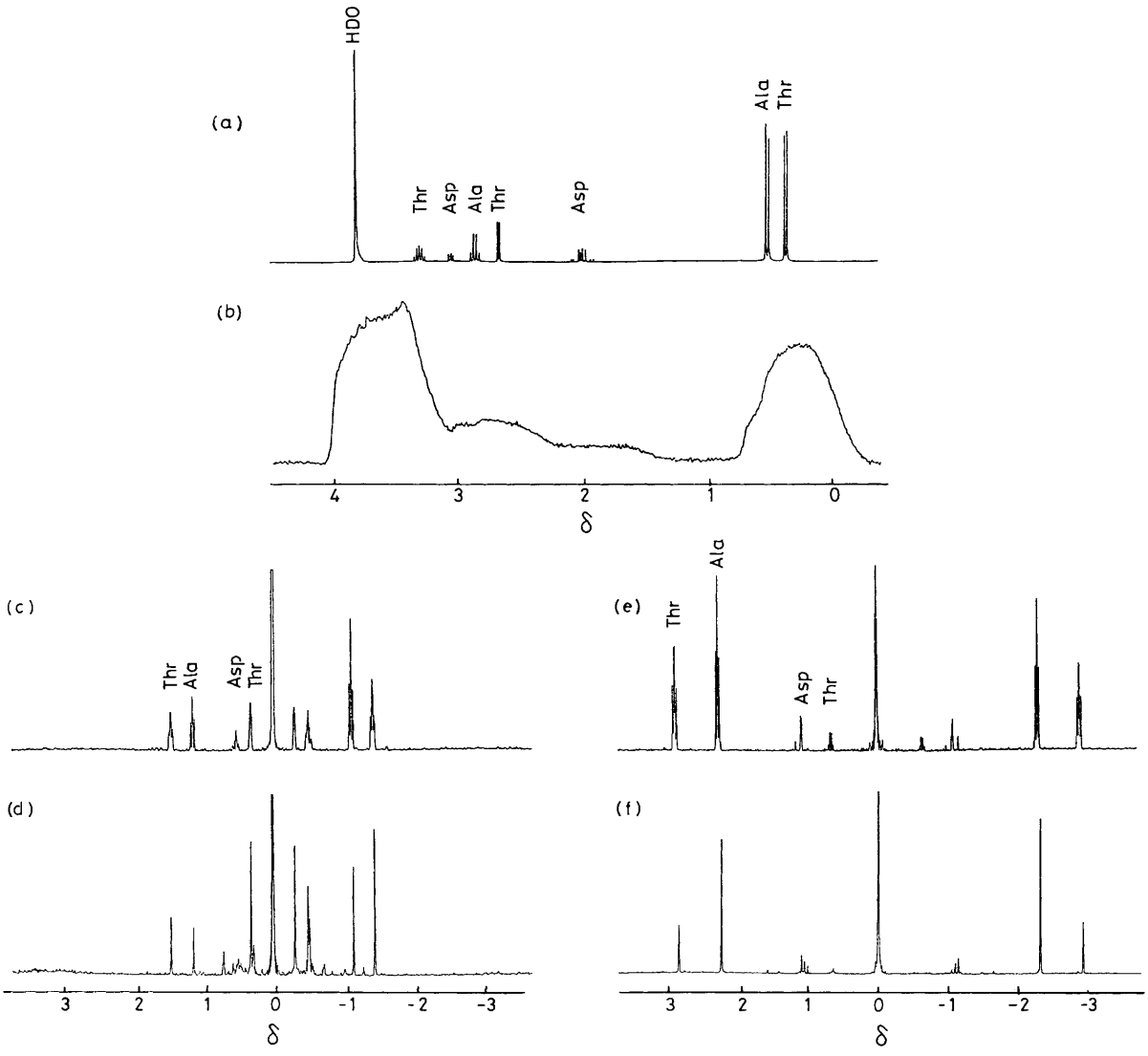

Figure 2. ^{1}H Spectra in D_2O at 300 MHz of a mixture of L-alanine, L-threonine, and L-aspartic acid, (each of 0.1 M). (a) Single-quantum spectrum in an homogeneous magnetic field. (b) Single-quantum spectrum in an inhomogeneous magnetic field. Spectra acquired in the same inhomogeneous field as (b): (c) SECSY spectrum; t_1 increment = 22 μs, 1024 increments used, 1 acquisition per increment. (d) Broad band decoupled SECSY spectrum; t_d = 250 ms, t_1 increment = 22 μs, 1024 increments used, 4 acquisitions per increment. (e) Zero-quantum coherence spectrum; τ, τ′ = 60 ms, t_1 increment = 22 μs, 1024 increments used, 1 acquisition per increment. (f) Broad band decoupled zero-quantum coherence spectrum, τ, τ′ = 60 ms, t_d = 250 ms, t_1 increment = 22 μs, 1024 increments used, 4 acquisitions per increment. All two dimensional data were processed by Fourier transformation in both dimensions (a sine apodization function was applied to the data before Fourier transformation with respect to t_1) and the projection in F1 taken. All measurements were carried out on a Bruker AM 300 MHz spectrometer.

two or more spins for which the transition rule $\Delta M = 0$ applies. A ZQC between two spins will precess at the *difference* in the SQC precessional frequencies of those two spins.

In general, SECSY cross peaks occur at half the frequency of ZQC peaks. This is because whereas a ZQC evolves at the difference in precessional frequencies of the two spins participating in it for the whole of the evolution period, each of the two spins which give rise to a SECSY peak only evolves for half of the evolution period. In both cases the magnetic field inhomogeneity contribution to the spin frequencies (equation 1) in each pair of spins giving rise to a cross peak is the same, and therefore cancels out. This apparent similarity between the SECSY-, and ZQC-spectra, Figures 2c, e respectively, is somewhat superficial; their multiplet structures are generally different, only occasionally coinciding, and, with SECSY, magnetic field inhomogeneity often results in peaks at the same frequency in F1, but not in F2 (which is still susceptible to inhomogeneity), with different phases cancelling each other out.

$$\omega'_k = \omega_k + \gamma\Delta B(x,y,z) \quad (1)$$

By altering the structure of their evolution periods it is possible to produce SECSY and ZQC pulse sequences which produce broad band decoupled spectra, reducing each multiplet to a singlet, Figure 1b, d. Taking into account the scaling down of frequencies in SECSY the spectra produced by these two modified pulse sequences are directly comparable. As with the coupled SECSY spectrum it can be seen that the responses produced are characteristic 'signatures' of the compounds producing them, and as such are diagnostically

useful where the magnetic environment is inhomogeneous as is often the case with *in-vivo* spectroscopy.

A SECSY measurement has a significant advantage over its ZQC equivalent since to produce a spectrum with the same sweep width and digital resolution in F1 it requires $(\tau + \tau')$ milliseconds less because it does not require additional preparation and refocussing periods. In practice this results in a reduction of 100—400 ms, potentially of great significance for *in-vivo* applications as the spin–spin relaxation times found there are usually prohibitively short for ZQC experiments. Both of the SECSY pulse sequences described here are easier to use than their ZQC counterparts. The peak intensities in the conventional SECSY spectrum are not significantly dependent on experimental parameters, whereas those produced in the conventional ZQC experiment are critically dependent on τ and τ', the lengths of the preparation and refocussing periods. For the broad band decoupled sequences the advantage of SECSY is even more marked: ZQC peak intensities are critically dependent on three experimental parameters, τ, τ', and t_d, whereas those for SECSY are only critically dependent on t_d. Finally, whereas ZQC pulse sequences designed to work in inhomogeneous magnetic fields do not produce responses from A_nX_n spin systems the SECSY sequence does. The major disadvantage of SECSY spectra is that they are more crowded than their ZQC counterparts, but if broad band decoupling is used this is not a significant problem.

In conclusion, we note that although in terms of signal-to-noise the SECSY (and ZQC) experiment cannot compete with conventional (SQC) spectra when magnetic field homogeneity is high, when it is low there is no contest because the conventional (SQC) experiment is impossible. Under these circumstances SECSY, particularly in its broad band decoupled form, is superior to the corresponding ZQC experiment in terms both of simplicity of use, and of experiment time (it is 100—400 ms shorter) which may be critical for *in-vivo* applications.

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