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Generation of Pure Phase NMR Subspectra for Measurement of Homonuclear Coupling Constants

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Recently it has been demonstrated that dramatic spectral simplification can be obtained by editing a regular ^{1}H NMR spectrum into a set of subspectra by means of the one-dimensional HOHAHA experiment (*1*). As pointed out earlier, scalar couplings are difficult to measure from such a spectrum because of phase and intensity distortions of the multiplet components in the various subspectra. Here, it is demonstrated that pure phase subspectra can be obtained readily by combination of the 1D HOHAHA experiment with the *z*-filtering technique (*2*). Guidelines for minimizing the number of τ averages, needed for the *z* filter, are also presented.

If the Zeeman term from the Hamiltonian is eliminated, the spin system will evolve solely under the influence of scalar couplings. Braunschweiler and Ernst (*3*) first utilized this concept in isotropic liquids for generating total coherence transfer (TOCSY) 2D spectra of spin systems of coupled protons. A number of more efficient mixing schemes based on the same mechanism have since been reported (*1, 4, 5*). We prefer to use the so-called MLEV-17 sequence (*4*) for mixing because it covers a substantial bandwidth ($\pm 0.3\nu_{\mathrm{rf}}$) and because it is relatively insensitive to exact phase shifting or pulse width adjustment. Moreover, this mixing scheme lengthens the apparent $T_{1\rho}$ (*4, 5*), so that less signal is lost during this mixing period relative to other mixing schemes.

To record pure phase spectra, Braunschweiler and Ernst proposed coadding a number of spectra, recorded with different mixing times. This procedure works satisfactorily when few spins are involved in a *J*-coupled network. For larger spin systems however, this procedure is not perfect because the magnetization transfer appears to lose its oscillatory character, i.e., the oscillation is overdamped. Consequently, the spectra obtained are not 100% absorptive and measurement of scalar couplings may lead to errors. Moreover, in a large network of coupled spins (alkaloids, steroids, etc.) averaging over a number of mixing times, some of which would have to be quite long, gives a subspectrum of nearly the entire molecule and therefore defeats the purpose of the experiment. As demonstrated here, the *z*-filtering technique, proposed by Sørensen *et al.* (*2*), can be implemented easily in the HOHAHA experiments to provide pure phase subspectra.

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The theoretical aspects of isotropic mixing have been discussed elsewhere (*3, 5–7*). For the two-spin case, Braunschweiler and Ernst showed that, if at the start of the isotropic mixing the magnetizations of two coupled protons, I and S, are aligned along the x and $-x$ axes, respectively, this magnetization evolves according to

$$(I_x - S_x) \xrightarrow{J\mathbf{I}\cdot\mathbf{S}t} (I_x - S_x)\cos 2\pi Jt + 2(I_yS_z - I_zS_y)\sin 2\pi Jt. \qquad [1]$$

The second term on the right side of expression [1] contains the product terms I_yS_z and I_zS_y which denote antiphase magnetization components that give rise to the phase distortions in the final spectrum. It is interesting to note that complete *net* magnetization transfer from spin S to spin I takes place in a time $1/2J$ whereas in the pulse interrupted free precession experiments (*8*) a time $1/J$ is required for complete *net* magnetization transfer from S to I. This shows that the isotropic mixing technique can be more efficient at transferring magnetization among coupled spins in the case where the natural linewidth is larger than the J coupling. For systems consisting of more than two coupled spins, analytical equations describing the magnetization transfer process become rapidly more complicated. Results derived for A_2X_2 and AX_n systems have been presented elsewhere (*9*).

During isotropic mixing the sum of the spin magnetizations, $I_\alpha + S_\alpha$ where $\alpha = x, y, z$, is conserved. In practice, however, instrumental imperfections make it very difficult to conserve the magnetization in all three directions simultaneously. The most successful mixing schemes utilize a virtual spin-lock axis (*3–5*) along which the sum of the spin magnetizations is conserved. These mixing schemes can be considered as homonuclear analogs of the Hartmann-Hahn cross polarization experiment (*10*). For magnetization in antiphase along the $\pm x$ axis (as present in expression [1]) this virtual spin-lock axis is chosen to be the x axis and terms $(I_z - S_z)$ and $(I_y - S_y)$ are rapidly defocused during the mixing mainly due to rf inhomogeneity. In the original TOCSY experiment, the axis along which the 180° pulses are applied is the virtual spin-lock axis. In other schemes, an actual spin lock (*1, 5*) is performed or else so-called "trim pulses" provide a short spin-lock duration preceding and following the mixing scheme (*4*). From the nature of the Hamiltonian during isotropic mixing, $J\mathbf{I}\cdot\mathbf{S} = [JI_zS_z + J/2(I^+S^- + I^-S^+)]$, it follows that coherence transfer is limited to eigenstates with the same total magnetic quantum number. It can be shown that a single 90° pulse following the isotropic mixing sequence when applied along an axis perpendicular to the virtual spin-lock axis converts *all* antiphase components into zero-quantum coherences (ZQCs); the $(I_x - S_x)$ components are converted into $(S_z - I_z)$ longitudinal magnetization. During a time τ_z, following this 90° conversion pulse, the ZQCs oscillate with their characteristic frequencies, determined by the differences in chemical shifts of the various protons in the coupling network involved in a particular ZQC; the z magnetizations are stationary. A final 90° pulse, applied at the end of the τ_z interval, converts both the longitudinal magnetization and the ZQCs back into observable magnetization. Magnetization originating from ZQC will oscillate in amplitude as a function of τ_z whereas magnetization originating from longitudinal order monotonically decays with time constant T_1. As proposed by Sørensen *et al.* (*2*), direct coaddition of the results for a large number of different τ_z values removes the ZQC contribution and leaves a pure absorption-mode spectrum.

As recently shown by Waugh (*11*), offset effects in the isotropic mixing sequence can give rise to additional terms in the mixing Hamiltonian. These terms can lead to spurious generation of N-quantum coherence by the 90° pulse at the end of the mixing period. This N-quantum coherence also can be removed by τ_z averaging, but more efficiently by phase cycling of the final pulse of the z filter. Hence, τ_z averaging is only needed to eliminate zero-quantum contributions that could lead to observable transverse magnetization.

Earlier, Rance *et al.* (*12*) have discussed the analogous problem of removing coherent cross peaks in NOESY spectra by systematic variation of the mixing time. They provided recipes for removing ZQC contributions for a range of chemical-shift values. In the application to obtaining purely absorptive 1D HOHAHA spectra for the exact measurement of scalar coupling constants, we may assume that the chemical shifts of the protons involved are known. Analogous to the discussion given by Rance *et al.*, we now can give a recipe for obtaining pure absorption phase 1D HOHAHA spectra. Below, the range of τ_z values to be selected for elimination of the ZQC contribution will be discussed.

By coadding the spectra obtained for two τ_z values for which a particular ZQC differs in phase by exactly 180°, the effect of this ZQC on the summed spectrum is eliminated. Neglecting the effect of scalar coupling on the zero-quantum frequencies, the ZQ frequencies of interest during the τ_z interval are equal to the differences in chemical shifts of coupled nuclei and differences in chemical shifts between nuclei that have a common coupling partner. In practice, we find that a choice of τ_z values that is optimized for elimination of ZQC contributions from directly coupled protons usually is sufficient for providing virtually distortion free subspectra. If the chemical-shift differences expressed in hertz between directly coupled nuclei in a certain spin system are given by $\delta_1, \delta_2, \ldots, \delta_N$, we define delay times $\tau_n = 1/(2\delta_n)$. τ_z averaging is then obtained if the results for the following τ_z values are directly coadded:

$$\tau_z = a\tau_1 + b\tau_2 + \cdots + p\tau_N, \qquad [2]$$

where the coefficients $a - p$ are either zero or one and all possible combinations are used; i.e., $2^N\tau_z$ values have to be used. For systems consisting of more than five spins

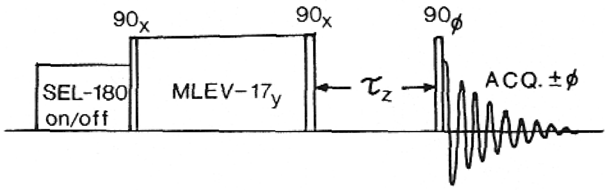

FIG. 1. Pulse sequence of the z-filtered 1D HOHAHA experiment. The selective 180° pulse, inverting a preselected proton multiplet, is applied only in odd-numbered scans. In even-numbered scans this pulse is disabled by switching off the decoupler. Correspondingly, data are added and subtracted. In addition, the phase ϕ of the final 90° pulse is rotated synchronously with the receiver phase along all four axes. Spectra obtained for a number of different τ_z values are directly coadded. The MLEV17$_y$ sequence consists of an integer number of repetitions of the sequence (*ABBA AABB BAAB BBAA* 180_y), where A is $90_x180_y90_x$ and B is $90_{-x}180_{-y}90_{-x}$.

a less complete τ_z averaging will usually also provide satisfactory results. If "random" τ_z values are selected by the spectrometer, these should at least vary over the range of $0\text{–}1/\delta_{\min}$, where $\delta_{\min}$ is the smallest chemical-shift difference between two coupled protons of interest.

The pulse sequence used in the present work is sketched in Fig. 1. The sequence starts with a selective spin inversion ($\gamma H_2/2\pi \approx 10$ Hz) of a preselected proton multiplet

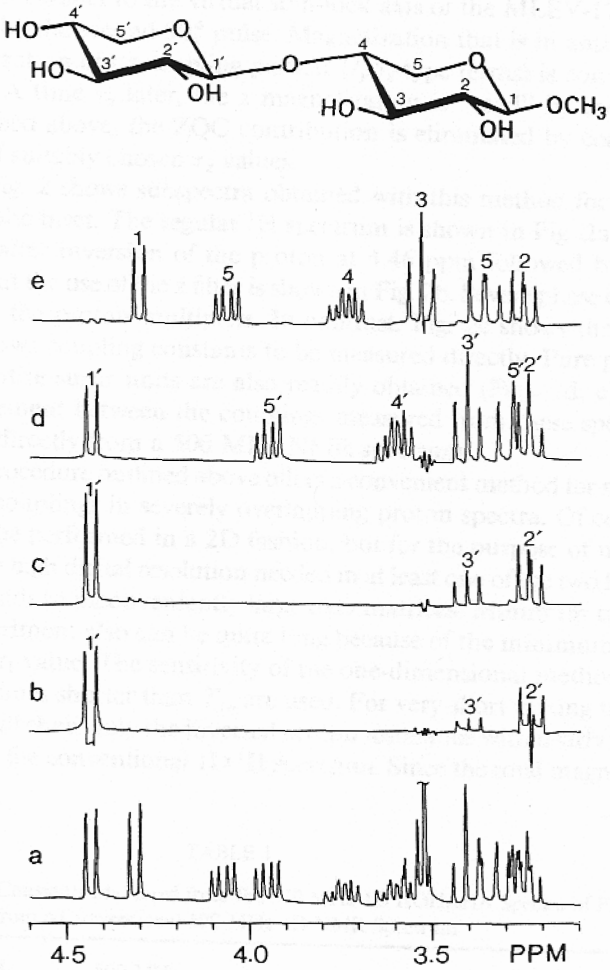

FIG. 2. 270 MHz ^{1}H spectra of the disaccharide shown in the inset. (a) Regular 1D spectrum. (b) Subspectrum obtained without z filtering and a 33 ms mixing period. The spectrum results from 16 scans (30 s). (c) The same spectrum with z filtering, using τ_z values of 0, 1.5, 11.1, and 12.6 ms, as calculated from expression [2]. The spectrum results from 8 scans per τ_z value; measuring time 1 min (d, e) z-filtered subspectra of the entire sugar units, obtained with 195 ms mixing times and 32 τ_z values; 8 scans per τ_z value; measuring time 9 min per subspectrum. For each of the subspectra the C1′ proton (b–d) or the C1 proton (e) was inverted by the selective 180° pulse.

along the virtual spin-lock axis is a constant of motion, magnetization of this single proton is redistributed over all other protons in the spin system during the mixing period. Neglecting relaxation, if the magnetization of one proton is redistributed equally among N protons in the spin system, the sensitivity in the 1D subspectrum will be reduced by a factor of N compared to a 1D spectrum recorded in the same amount of measuring time. Because of the required τ_z averaging and the phase cycling of the final pulse of the z filter, the minimum number of scans needed for a subspectrum is usually at least 32; a larger number of scans usually will result in better suppression of spurious resonances in the final difference spectrum. Therefore, a minimum of several minutes measuring time is generally required per subspectrum.

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