Elimination of Refocusing Pulses in NMR Experiments

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A large number of one- and two-dimensional NMR experiments employ 180° refocusing pulses with the main purpose of obtaining absorption-mode spectra. Imperfections in the flip angle of the refocusing pulse will generally lead to degradation of the sensitivity of the experiment and may give rise to unwanted resonances in 2D spectra. Those problems can sometimes be significantly alleviated by proper use of the composite pulse concept (1-4). However, we will demonstrate that those problems can be completely avoided by omitting the refocusing pulses altogether.

As a first example, consider the refocused INEPT experiment (5-8). Its sequence is sketched in Fig. 1a. A pair of 180° pulses applied simultaneously to the 1H and 13C nuclei at the midpoint of the Δ2 period removes the effect of chemical shift during this period and average evolution during this period is only due to the heteronuclear scalar coupling, J. If, for a methine group, the delay Δ2 is set to 1/2J, the two 13C doublet components will be in phase at the end of this period and will be aligned along the x axis (7, 8). If broadband proton decoupling is started at this time, an absorption mode proton-decoupled 13C spectrum can be recorded. We propose to omit this pair of refocusing pulses in the INEPT experiment and to start data acquisition immediately after the 90° 13C detection pulse (Fig. 1b). Broadband proton decoupling is switched on a time, Δ2', later. This means that during the first 2-3 ms (for 13C) of the data acquisition the signal is in the proton-coupled mode, whereas during the rest of the free induction decay the signal is in the proton-decoupled mode. The envelope amplitude of this signal shows a strong similarity with the time domain signal to which a convolution difference filter (9) has been applied, and consequently, a dip in the baseline will occur after Fourier transformation. For the normal case, where the decay constant $T_2^*$ of the 1H-decoupled 13C signal is much larger than the reciprocal of the coupling constant, $J^{-1}$, the depth, ε, in the baseline is calculated to be

$$\epsilon = \frac{1 - 2/\pi}{2J}$$  \hspace{1cm} [1]

whereas the intensity $I$ of the resonance involved equals $T_2^*$. For a typical case with a nominal line width of 1 Hz (1/2π = 1/π) and a 150 Hz coupling constant, the dip in the baseline is only 0.4% of the peak height and does not cause any serious
problems. However, one should note that the total integrated intensity of the
spectrum, according to the Fourier theory, has to be zero.

As an example, results for the aldrotironic acid derivative sketched at the top of
Fig. 2 are presented. Figure 2a shows the regular NOE-enhanced FID spectrum of
the protonated $^{13}$C nuclei, obtained from 200 scans. Figure 2b shows the INEPT
spectrum obtained with omission of the 180° ($^1$H, $^{13}$C) pulse pair and Fig. 2c shows
the result of the optimized refocused INEPT experiment. The signal intensity is
slightly ($\sim 10\%$) higher without the 180° pulses, but a baseline distortion is visible.
This distortion is much larger than calculated on the basis of Eq. [1], because each
of the $^{13}$C resonances causes a dip in the baseline with a width of several hundred
hertz. Clearly, those dips overlap and reinforce each other.

Another experiment where refocusing pulses can be omitted is the APT sequence
(10). This experiment is widely used for multiplicity determination of $^{13}$C sites and
its sequence is sketched in Fig. 3a. Figure 3b shows our modified sequence, in
which the 180° $^{13}$C refocusing pulse has been omitted and data acquisition is started
immediately after the 90° $^{13}$C pulse. The functional dependence of the observed
resonance intensity on the length of time delay $\Delta$ is unchanged with the original
version of the experiment. However, as the duration of $\Delta$ in this type of experiment
is typically much longer than in the INEPT sequence, the baseline distortion will
be worse, but will usually not obscure any of the information desired. Because only
one $^{13}$C pulse per experiment is applied in our modification, a reduced flip angle
can be used for this pulse, allowing a higher experiment repetition rate and
consequently higher sensitivity (11).

Figure 4a shows the conventional NOE-enhanced high-field $^{13}$C spectrum of the
sugar sketched in the inset of Fig. 2. Figure 4b shows the result obtained with the
nonrefocused APT experiment for a $\Delta$ value of 6.5 ms. Baseline distortion is clearly
visible, but the methylene (up) and methine (down) sites can easily be distinguished.
One might argue that starting data acquisition directly after the observe pulse and switching on the proton decoupling a time, Δ, later produces results very similar to starting data acquisition at time Δ and right shifting the data by a number of data points that would have been acquired during this time (which is identical to applying a strong linearly frequency-dependent phase correction to the final spectrum). However, this approach gives rise to a rather strong undesirable baseline roll (8).

The ideas of right shifting the data points and omission of refocusing pulses can be combined in order to obtain a two-dimensional absorption-mode heteronuclear chemical-shift correlation spectrum. The theoretical basis for the 2D heteronuclear chemical-shift correlation experiment has been discussed in detail in a number of papers (12–17) and will not be repeated here. The regular absorption-mode pulse scheme employs two 180° (1H, 13C) pulse pairs, applied at the midpoints of the Δi
FIG. 3. (a) Pulse sequence of the regular APT sequence. (b) Pulse sequence of the modified APT sequence, where data acquisition is started immediately after the $^{13}$C observe pulse. To optimize sensitivity the flip angle of the $^{13}$C pulse may be chosen smaller than 90°.

and $\Delta_2$ intervals (Fig. 5a). The pulse pair in the center of $\Delta_1$, can be omitted if the data points are right-shifted by $\Delta_1/\Delta t_1$ data points, prior to Fourier transformation with respect to $t_1$. As discussed above for the INEPT experiment, the pulse pair in the center of the $\Delta_2$ interval can be omitted if data acquisition is started immediately after the $^{13}$C detection pulse (Fig. 5b). The experiment has to be performed in the

FIG. 4. (a) Regular NOE-enhanced FID spectrum of the aldotrionic acid derivative sketched in the inset of Fig. 2. (b) Modified APT spectrum obtained with the pulse sequence of Fig. 3b. Each spectrum is the result of 200 accumulations.
amplitude-modulated mode, with the decoupler placed at either the low or the high field side of the $^1\text{H}$ spectrum. The decoupler can be positioned in the center of the proton spectrum if additional (TPPI) phase shifting of the decoupler pulses is used (18–20). For the simplest case where the decoupler frequency is placed at one side of the $^1\text{H}$ spectrum, more decoupler power is needed to decouple effectively the $^1\text{H}$ spectrum. However, with the introduction of the new composite pulse decoupling techniques (21), this usually does not present any practical problems. More data storage space by a factor of two is needed in the amplitude-modulated version compared with the phase-modulated experiment (16) in order to obtain the same digital resolution, but it can be shown (22) that the amplitude-modulated version yields an improvement in sensitivity by $\sqrt{2}$ over the phase-modulated experiment.

Figure 6 shows the 2D absorption-mode heteronuclear chemical-shift correlation spectrum, obtained from a $128 \times 1024$ data matrix on a Nicolet NT-270 spectrometer. Gaussian line broadening is used in both dimensions to avoid truncation and zero filling by a factor of two is used in both dimensions, resulting in a $128 \times 1024$ data matrix for the displayed absorption part of the spectrum. Along the sides of the 2D spectrum the projections onto the $F_2$ and $F_1$ axes are shown, displaying the absorption-mode lineshapes. The resolution in the $F_1$ dimension is limited by the digital resolution (compare with conventional $^1\text{H}$ spectrum recorded on the same sample and shown above the $F_1$ projection) and could be improved by using a longer acquisition time in the $t_1$ dimension.

The idea of omitting refocusing pulses and starting proton decoupling and data acquisition at different times is not limited to the examples presented above, but applies to a large number of heteronuclear 1D and 2D pulse schemes. Sequences with fewer pulses tend to be less sensitive to practical instrumental problems and
FIG. 6. Two-dimensional absorption-mode heteronuclear chemical-shift correlation spectrum of the sugar sketched in the inset of Fig. 2, obtained with the sequence of Fig. 5b. The spectrum results from a $128 \times 1024$ data matrix and 80 accumulations were performed for each $t_1$ value (total measuring time 4 h). Along both axes the projections of the 2D spectrum on these axes are shown. Along the $F_1$ axes the conventional $^1$H spectrum (high resolution) is also displayed.
will generally be less susceptible to possible misadjustments of experimental parameters and may therefore produce more reliable results.

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REFERENCES