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Removal of F_1 Baseline Distortion and Optimization of Folding in Multidimensional NMR Spectra

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Because of finite pulse widths, the evolution periods in multidimensional experiments often start at a finite duration. This "sampling delay" necessitates the use of a linearly frequency-dependent phase correction in the indirectly detected dimensions of 2D, 3D, and 4D NMR experiments, which introduces baseline distortion. As shown previously (*1*), this baseline distortion can be avoided by using linear prediction to calculate the missing data points, provided the sampling delay corresponds to an integral number of dwell times. Here we demonstrate an equally effective approach, requiring that the sampling delay equals exactly half a dwell time. This approach will be illustrated for the simplest case of 1D and 2D NMR, but it is successfully used in our laboratory for the processing of 3D and even 4D (*2*) spectra.

Problems associated with the use of discrete Fourier transforms in NMR have been clearly illustrated by Otting *et al.* (*3*). They suggest the use of a scaling factor equal to $(1 + 2\tau/\Delta t)/2$ for the first data point, in order to minimize baseline distortions, where τ is the time at which the first data point is sampled and Δt is the dwell time. However, as will be discussed below, simple scaling of the first data point is generally insufficient to remove completely the baseline distortion associated with delayed sampling.

As pointed out previously (*4*), to increase the digital resolution of 3D heteronuclear NMR spectra it is desirable to limit the size of the spectral windows in the indirectly detected dimensions (F_1 and F_2) to less than the actual frequency distribution of resonances. For data acquired with the TPPI method (*5*), folding of resonances outside the actual spectral window occurs. For data acquired with the hypercomplex method (*6–8*) aliasing will occur for these resonances, i.e., a resonance just upfield of the frequency window appears at the lowfield side in the spectrum. In the case of folding, a resonance just upfield of the spectral window folds back into the spectrum at the upfield side. A clear discussion of aliasing and folding is given by Freeman (*9*). As will be demonstrated below, extensive aliasing can often be used without introducing any ambiguity.

Both for complex and for real data, use of a scaling factor equal to 0.5 for adjusting the first data point gives good results for sampling delays that are very short relative to the dwell time (*3*). For sampling delays that are not much shorter than the dwell time, the linear phase correction needed for the spectrum causes significant baseline

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undulations, regardless of the scaling factor used for the first data point. This is illustrated in Fig. 1, for a signal that has a sampling delay equal to one quarter dwell time. Figure 1a shows the Fourier transform using a scaling factor of 0.75; Fig. 1b shows the result for a scaling factor of 0.6. Neither of the two Fourier transforms gives a flat baseline, although the scaling factor of 0.6 gives a baseline which is closer to zero. A special case occurs when sampling is delayed by exactly one-half of a dwell time. In this case, it can be shown that the use of no scaling (i.e., a scaling factor of 1) gives a spectrum that after linear phase correction is completely free of baseline distortion (Fig. 1c).

The effective sampling delay in a multidimensional experiment usually can be calculated in a straightforward manner. For example, in the NOESY experiment,

$$90°—t_1—90°—T_{mix}—90°—\text{Acq.}(t_2),$$

the sampling delay, τ, in the t_1 dimension is given by (*10*)

$$\tau = 4\tau_{90}/\pi + t_1(0), \qquad [1]$$

where τ_{90} is the duration of the 90° pulse, the $t_1(0)$ is the programmed duration for the first t_1 increment (usually <2 μs). For the HMQC experiment,

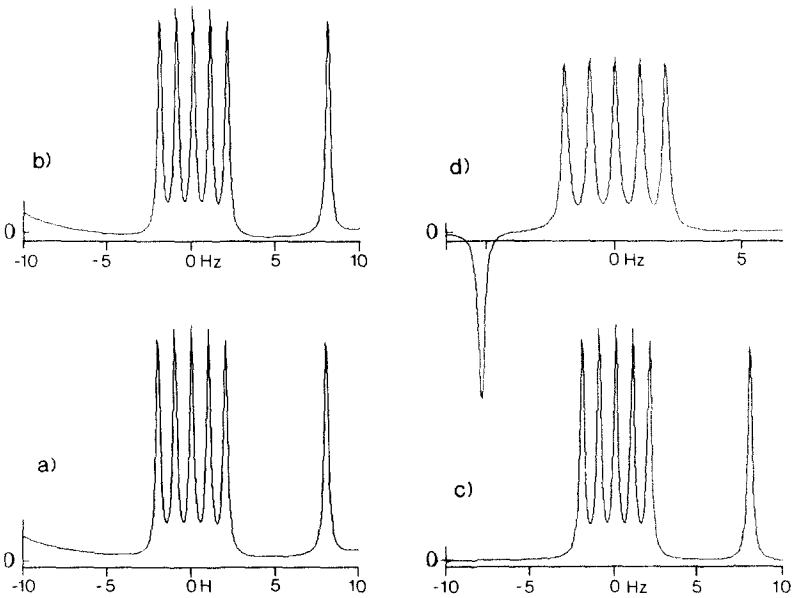

FIG. 1. (a, b) Simulated spectra obtained by Fourier transformation of data with an initial sampling delay of one-quarter dwell time. For (a), multiplication of the first data point by 0.75 has been used, for (b) the scaling factor was 0.6. For both (a) and (b) the linear phase correction was 90° across the spectrum. (c) Spectrum obtained for a sampling delay equal to one-half dwell time, with no scaling of the first data point. (d) Spectrum obtained if the spectral window is narrowed by 33%. The resonance at the right side of the spectrum is aliased and appears inverted at the left hand side. For both (c) and (d) the linear phase correction is 180°.

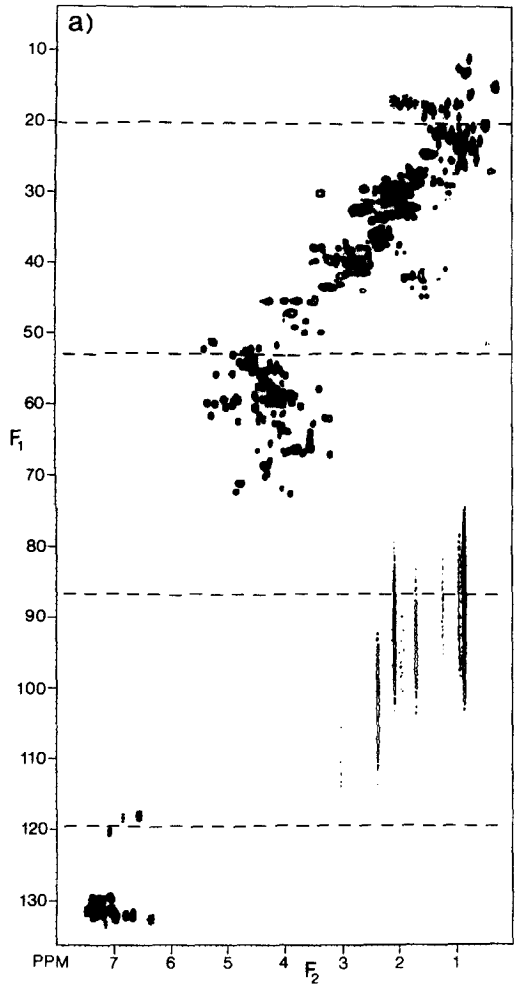

FIG. 2. Heteronuclear multiple-quantum correlation (HMQC) spectra of uniformly ^{13}C-enriched (95%) calmodulin in D_2O, recorded at 500 MHz. (a) Regular correlation spectrum, using a t_1 increment of 60 μs, resulting in a 132 ppm spectral window in the F_1 dimension. The ^{13}C 90° pulse width was 40 μs, the ^1H 180° pulse width was 48 μs, and the first t_1 duration was set to 20 μs, giving an effective first t_1 duration of 120 μs. The broken lines indicate where aliasing occurs when the F_1 spectral window is narrowed fourfold. (b, c) HMQC spectrum recorded under identical conditions, but with a t_1 increment of 240 μs. (b) Positive levels, corresponding to nonaliased resonances and resonances that have been aliased twice in the F_1 dimension. (c) Negative levels, corresponding to resonances that have been aliased once. Because of the relatively strong resolution enhancement digital filtering used in both the F_1 and the F_2 dimensions, the relatively narrow methyl resonances show an "overenhanced" lineshape, resulting in the lobes marked "×" in (b, c).

$$90°(^1H)—1/(2J_{XH})—90°(X)—t_1/2—180°(^1H)—t_1/2—90°(X)—1/(2J_{XH})—\text{Acq.}(t_2),$$

the sampling delay, τ, in the t_1 dimension is given by

$$\tau = 4\tau_{90X}/\pi + \tau_{180H} + t_1(0), \qquad [2]$$

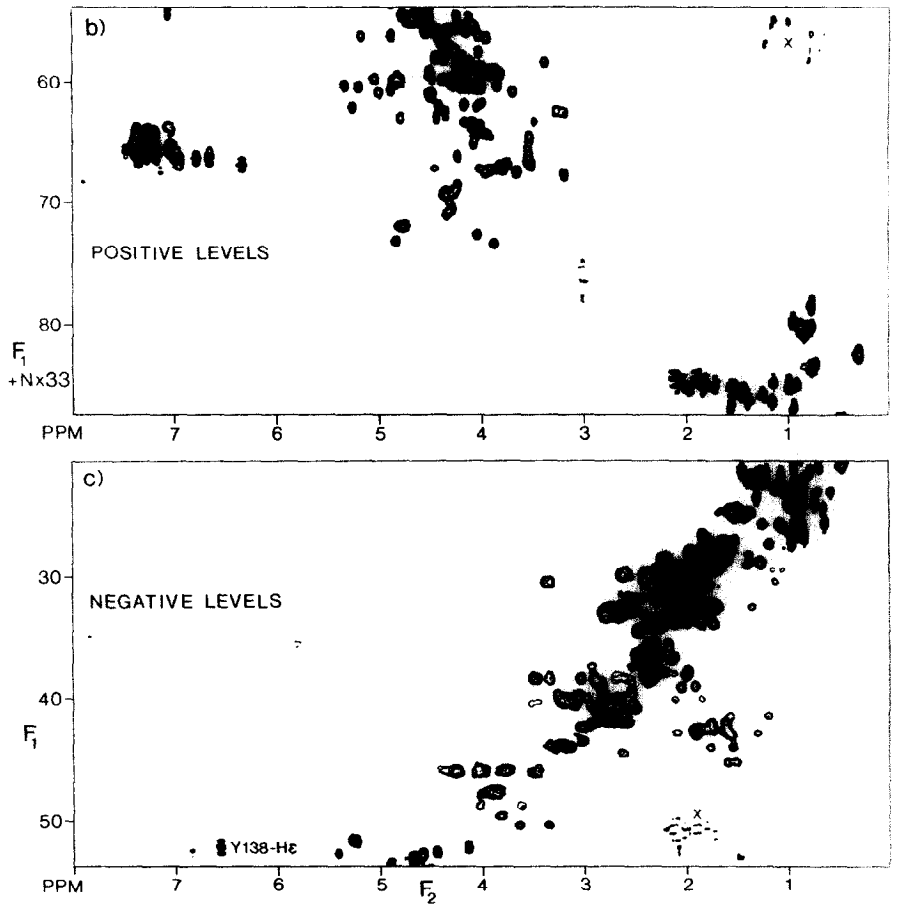

FIG. 2—*Continued*

where τ_{90X} is the duration of a 90°(X) pulse and τ_{180H} is the ^{1}H 180° pulse width. If data are acquired in the States format (*7*), the linear phase correction, ϕ_1, needed in the F_1 dimension is given by

$$\phi_1 = \tau/\Delta t_1 \times 360°, \qquad [3]$$

where Δt_1 is the dwell time in the t_1 dimension (t_1 increment). Thus, if sampling is delayed by exactly half a dwell time, a 180° linear phase correction is needed across the spectrum. As a consequence, resonances that have been aliased appear with opposite phase (*4*), facilitating separation of aliased and nonaliased resonances. This is illustrated in Fig. 1d, where the spectral width has been narrowed down by 33% relative to the spectrum of Fig. 1c. The most upfield resonance now appears aliased and with opposite phase at the lowfield side of the spectrum.

Frequently, extensive aliasing can be used without risking overlap (and cancellation) of aliased and nonaliased resonances, especially for heteronuclear experiments that correlate ^{1}H and ^{13}C chemical shifts. This is illustrated in Fig. 2 for a HMQC spectrum of the protein calmodulin (16.7 kDa), uniformly labeled with ^{13}C. Figure 2a shows

the spectrum recorded with a wide F_1 spectral width (132 ppm). For this large F_1 spectral width, the dwell time in the t_1 dimension is very short (60 μs), and the effective sampling delay was 120 μs. The large (720°) linear phase correction needed in the F_1 dimension results in baseline distortion that is apparent as vertical bands in the spectrum of Fig. 2a. Figures 2b and 2c show positive and negative contour levels of the HMQC spectrum recorded under identical conditions, but with the F_1 spectral width narrowed fourfold, to 33 ppm. In this case the sampling delay (120 μs) corresponds to exactly half a dwell time, and a 180° linear phase correction is used in the F_1 dimension. Figure 2b displays positive resonances, corresponding to resonances that have not been aliased, or that have been aliased twice (aromatic and methyl resonances). Figure 2c displays resonances that have been aliased an odd number of times. With the exception of the Tyr-138-Cϵ resonance, all resonances in this plot correspond to the 53–20 ppm window. Comparison of Figures 2a, 2b, and 2c indicates that no information is lost by the multiple aliasing used in the F_1 dimension, and that the baseline distortion present in the spectrum of Fig. 2a is not present in Figs. 2b and 2c.

Assuming identical acquisition times in the t_1 dimension, use of a narrower F_1 spectral window allows more scans to be taken for each t_1 increment if the spectrum is to be recorded in a given amount of time. Note that this does not increase the sensitivity of the experiment (*11, 12*) but it reduces data storage requirements and allows more extensive phase cycling to be used. Both of these aspects are important for optimizing the recording of 3D and 4D NMR data sets. Because of the undesirable folding properties of real Fourier-transform spectra, data acquisition in the complex format is preferable over the TPPI format if the spectral window is made narrower that the distribution of resonances.

The fact that distortionless baselines can be obtained even if the sampling is delayed by half a dwell time is relevant not only for multidimensional high-resolution NMR but may also be of importance for NMR imaging and for the recording of solid-state NMR and phase-sensitive pulsed ESR spectra.

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REFERENCES

1. D. MARION AND A. BAX, *J. Magn. Reson.* **83,** 205–211 (1989).
2. L. E. KAY, G. M. CLORE, A. BAX, AND A. M. GRONENBORN, *Science* **249,** 411 (1990).
3. G. OTTING, H. WIDER, G. WAGNER, AND K. WÜTHRICH, *J. Magn. Reson.* **66,** 187 (1986).
4. L. E. KAY, D. MARION, AND A. BAX, *J. Magn. Reson.* **84,** 72 (1989).
5. D. MARION AND K. WÜTHRICH, *Biochem. Biophys. Res. Commun.* **113,** 967 (1983).
6. L. MÜLLER AND R. R. ERNST, *Mol. Phys.* **38,** 963 (1979).
7. D. J. STATES, R. A. HABERKORN, AND D. J. RUBEN, *J. Magn. Reson.* **48,** 286 (1982).
8. D. MARION, M. IKURA, R. TSCHUDIN, AND A. BAX, *J. Magn. Reson.* **85,** 393 (1989).
9. R. FREEMAN, "A Handbook of Nuclear Magnetic Resonance," pp. 71–73, Wiley, New York, 1988.
10. A. BAX AND D. MARION, *J. Magn. Reson.* **78,** 186 (1988).
11. M. H. LEVITT, G. BODENHAUSEN, AND R. R. ERNST, *J. Magn. Reson.* **58,** 462 (1984).
12. A. BAX AND T. H. MARECI, *J. Magn. Reson.* **53,** 360 (1983).