

Determination of Spectral Parameters in Broad NMR Lines

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INTRODUCTION

^{17}O nmr studies have been utilized for analyzing amino acids, peptides and related model compounds in solution (1-3) and in the solid state (4-6). ^{17}O nmr lines are broader than ^1H and ^{13}C lines due to the nuclear quadrupolar relaxation of ^{17}O nuclei, the effects of paramagnetic ions, and larger hyperfine coupling constant. The combined effect of broad lines and the low natural abundance of ^{17}O (0.037%) results in a poor signal-to-noise ratio (S/N). Because of the high noise level, parameter values can not be estimated visually. To overcome these difficulties, we usually study ^{17}O enriched materials, and carry out extensive signal averaging. Even with such modifications, visual parameter estimation remains problematic. Least squares curve fitting has been applied to these noisy, distorted and poorly resolved spectra, yielding estimates of the four parameters of interest, i.e., amplitude, peak position, spin-spin relaxation time and phase angle. One of the pivotal factors in determining the validity of the results of least squares estimation is the model equation; significant errors occur if it does not fit the data adequately (7). To test the accuracy of the various nonlinear model equations, we applied them to simulated noisy spectra with known parameters and compared the results.

The least squares fit of Lorentzian lines in the frequency domain is used widely for lines which are the combination of the real and imaginary parts of the Fourier transform of a free induction decay (FID). However, inherent

limitations of the discrete Fourier transform affect nmr spectrum analysis. These limitations include poor resolution and truncation effect for finite acquisition times, spectral distortion as a result of data lost during spectrometer dead time, and aliasing phenomena induced by discrete sampling of the time domain signal (8,9). In this study, we derive a model equation formulated with consideration to the limitations enumerated above.

METHODS

The FID signal $f(t)$ observed in pulsed FT nmr experiment is written as ;

$$f(t) = Ae^{-t/T_2} e^{j(\omega_0 t + \phi)} \quad [1]$$

where A , T_2 , ω_0 and ϕ are maximum amplitude, spin-spin relaxation time, peak position frequency and phase angle, respectively. The Fourier transform of Eq. [1] is ;

$$F(\omega) = \int_{-\infty}^{\infty} f(t) e^{-j\omega t} dt \\ = \frac{A\{T_2 - jT_2^2(\omega - \omega_0)\} \{\cos(\phi) + j\sin(\phi)\}}{\{1 + T_2^2(\omega - \omega_0)^2\}} \quad [2]$$

The detected spectrum in a pulsed FT experiment is the real part of Eq. [2] and is given by

$$R(\omega) = \frac{A\{T_2 \cos(\phi) + T_2^2(\omega - \omega_0) \sin(\phi)\}}{\{1 + T_2^2(\omega - \omega_0)^2\}} \quad [3]$$

where T_2 is $1/(\pi \times \text{Line width})$. Eq.[3] is the most widely used nonlinear model equation in the frequency domain.

FT nmr involves data acquisition of discrete FID values and discrete Fourier transformation of the FID. The finite data acquisition results in truncation effects which can be ignored when the finite acquisition time is longer than $5T_2$. The spectrometer dead time results in phase angle distortion, and improper sampling time over the Nyquist criterion brings about the aliasing effect (10). So, we consider of the effects of selecting sampling time and spectrometer dead time to derive better model equations. The spectrometer dead time introduces distortion into the phase of the signal. This distortion results in changes of the base line, reduction of intensity and broadening of the line width. Phase correction is required to compensate for the transfer function of the detection system. The phase angle results from the hardware filter networks, the finite duration of the pulse and the spectrometer dead time necessary to avoid pulse breakthrough (11). In Eq. [1], phase angle ϕ can be divided into ϕ' which represents an inherent phase shift due to the hardware filter networks and $d\phi$ which varies with dead time. The latter can be shown to have the following form:

$$d\phi = \omega_0 T_d \quad [4]$$

where ω_0 and T_d are peak position frequency and spectrometer dead time, respectively (12). Given this expression for $d\phi$, we can easily find ϕ' from ϕ .

The sampling rate is a significant parameter determining the degree of line shape distortion in the frequency domain. The Nyquist sampling theorem states that the sampling rate must be at least twice the highest frequency of interest to prevent spectral foldovers. These foldovers are called aliasing. The Nyquist frequency for a lorentzian line is infinite and thus the Nyquist theorem does not provide the criterion for choosing a minimum sampling frequency (11). So, for any finite sampling time, the DFT introduces a certain amount of foldovers which alter the line shape; the best one can do is to choose a sampling

time which minimizes severe aliasing. An exact equation for the DFT which takes the above phenomena into consideration can be used as a nonlinear least square model. In practice, the time domain signal $f(t)$ in Eq. [1] is sampled as $f(kT_s)$ where $k=0,1,2,\dots,N-1$ and T_s is sampling time and N is the number of sampling points. The discrete Fourier transform of $f(kT_s)$ is given as follows (12):

$$F[n/NT_s] = T_s \sum f(kT_s) e^{-j2\pi nk/N} \quad [5]$$

To observe the aliasing effect as a function of sampling time, we assume infinite acquisition time. Eq. [5] then reduces to the following:

$$F[\omega] = \frac{T_s A \{1 - RP - jRQ\} \{\cos(\phi) + j\sin(\phi)\}}{\{(1-RP)^2 + (RQ)^2\}} \quad [6]$$

where $R = \exp(-T_s/T_2)$, $P = \cos\{(\omega - \omega_0)T_s\}$ and $Q = \sin\{(\omega - \omega_0)T_s\}$. The real part of Eq. [6] is used for the nonlinear model equation for the exact DFT;

$$R(\omega) = \frac{AT_s \{(1-RP)\cos(\phi) + RQ\sin(\phi)\}}{\{(1-RP)^2 + (RQ)^2\}} \quad [7]$$

When we substitute the spectrometer dead time term (Eq. [4]) into Eq. [1] and [5], and take the discrete Fourier transform of it, we can modify the frequency domain model as following :

$$R(\omega) = \frac{A'' T_s \{(1-RP)\cos(\phi'') + RQ\sin(\phi'')\}}{\{(1-RP)^2 + (RQ)^2\}} \quad [8]$$

where $A'' = A \exp(-T_d/T_2)$ and $\phi'' = \omega_0 T_d + \phi$. This model equation is selected as the proper model equation to fit the nmr spectra irrespective of aliasing and phase distortion phenomena.

To find the best fit parameters, one typically minimizes an objective function, for instance, sum of squared errors between observed and predicted values. This objective function is minimized by the Gauss-Newton method (13).

Simulated data were generated in the

main frame IBM computer at the University of Illinois at Chicago using a time domain FID model equation. Noise was generated by a Gaussian random number generating subroutine (zero mean and variance 0.02). Signal-to-noise ratios, 5 and 10 were used as well as a noise-free signal. To get the proper signal-to-noise ratios, the same noise sequence was multiplied by the appropriate scale factor to ensure the consistency of the noise source. A line width of 2,000 Hz were selected. Peak intensity was fixed

TABLE I

Comparison of model equations for the nmr parameter estimation of simulated broad line noisy spectra. Simulated amplitude, peak position, line width and phase angle are 10,000, 5,000 Hz, 2,000 Hz and 0.2, respectively. Estimated value of the parameter and its standard error are given.

Parameter	S/N	Conventional model (Eq. 3)	Proposed model (Eq. 8)
Amplitude (10000)	∞	9398.92±0.46	9999.95±0.00
	10	9380.57±42.5	9989.90±33.4
	5	9362.84±85.3	9980.07±66.7
Peak position (5000 Hz)	∞	5000.87±0.87	4999.99±0.00
	10	5003.34±3.34	5002.39±2.39
	5	5005.76±5.76	5004.75±4.75
Line Width (2000 Hz)	∞	2001.51±1.51	1999.99±0.00
	10	1995.76±10.7	1996.23±8.93
	5	1990.19±21.6	1992.54±17.8
Phase Angle (0.2 rad)	∞	0.5131±0.000	0.2000±0.000
	10	0.5136±0.004	0.2004±0.004
	5	0.5141±0.008	0.2008±0.008

* () in the parameter column stands for known expected value.

at 10,000 and the inherent phase angle at 0.20 radian with spectrometer dead time of 10 μ sec. The simulated data were Fourier transformed, and zero filling was done to make a total of 8,192 points, exceeding $10T_2$ acquisition time. Curve fitting was performed by the SAS statistical software package; nonlinear regression analysis (NLIN) were used.

RESULTS AND DISCUSSIONS

The conventional model equation (Eq. 3) doesn't give proper parameter values without the additional base line change parameter since base line increases due to the sampling time effect. However, the proposed model equation (Eq. 8) does not require such base line change term because the equation yields that effect. In amplitude and phase angle estimations, distorted parameter values are obtained by the conventional model equation because of spectrometer dead time effect, while the proposed model gives true parameter values. In peak position and line width estimation, both models produce accurate parameter values. Table I presents such results of parameter estimation. As the S/N decreases, the bias of the estimated value from the known expected value increases, and the uncertainty level (confidence limits) proportional to the standard error of estimation increases in both models. The proposed model produces relatively more accurate parameter values than the conventional model equation.

CONCLUSIONS

In broad line spectra where the peak position are well separated from each other, the spectral distortion from the finite acquisition time, aliasing effect from improper sampling time and phase angle distortion due to spectrometer dead time can be reduced by choosing optimal parameter values. For spectral parameter estimation, we can eliminate these phenomena by performing the least squares analysis using a proper model equation. The proposed model equation in this study contains the sampling time and spectrome-

ter dead time terms which significantly affect the spectral distortion. It gives more accurate and non-distorted parameter values than the conventional Lorentzian line model.

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