Author

Before embarking on this project, Kathleen Cao wasn't certain about what to pursue in graduate school. Now that's clearer, after immersing herself in chemistry research with Professor Shaka. Kathleen advises others who have an interest in research not to hesitate in getting started, explaining that professors and other students provide all the necessary guidance and training on projects. In May 2002, she presented her findings at the UCI Undergraduate Research Symposium. She plans to attend graduate school in physical chemistry and hopes to continue research in NMR spectroscopy, a topic that now fascinates her. When Kathleen is not in the lab, she enjoys reading and tutoring high school students.

Key Terms

- Decimated Signal Diagonalization
- Discrete Fourier Transform
- Filter Diagonalization Method
- Spectrum Analysis

A Comparative Study of the Filter Diagonalization Method and Decimated Signal Diagonalization Method for One-Dimensional NMR Data Processing

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Abstract

Two parametric fitting methods for spectrum analysis, the filter diagonalization method (FDM) and decimated signal diagonalization (DSD) method, are compared for processing one-dimensional nuclear magnetic resonance (NMR) spectroscopy time signals. These methods are alternatives to the discrete Fourier transform (DFT) for processing time domain data for NMR. FDM and DSD use pure linear algebra to diagonalize small matrices generated from an NMR time signal in order to extract the inherent spectral parameters, the characteristic frequencies and amplitudes. These techniques have advantages over DFT because they can use smaller data sets and the resolution is not restricted by the Fourier transform time-frequency uncertainty principle. The main difference between FDM and DSD is the method of generating small matrices from a single long signal; FDM filters basis functions whereas DSD filters the time signal. This comparative study shows that the development of DSD is not yet at the level of FDM, particularly for one-dimensional NMR data processing.

Faculty Mentor

There has never been any acceptable alternative to Fourier transform for frequency analysis of the time-dependent signal obtained in nuclear magnetic resonance. Kathleen Cao's work on alternative linear algebraic methods of making the connection between the time and frequency domains shows why this is so. Two very closely related approaches, both of which may seem to be equivalent on paper, give markedly different results. The decimated signal diagonalization method (DSD) is obtained by first "decimating" or reducing the signal size and then extracting frequencies. The filter diagonalization method (FDM) is obtained by constructing a filtered local frequency-domain signal matrix, and then diagonalizing. Rather surprisingly, the latter is far more effective, and now, based on Kathleen's careful investigation, we know exactly why. Making a discovery can be one of the most exciting events in one's life, therefore, it is very important for undergraduates to conduct research and get a taste of the unknown.

Athan J. Shaka
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Introduction

Nuclear magnetic resonance (NMR) spectroscopy is a technique that provides information to determine molecular structure. Currently, spectroscopy analysis is done by utilizing the discrete Fourier transform (DFT). However, alternative methods, such as the filter diagonalization method (FDM) and decimated signal diagonalization method (DSD), have been developed that offer a tremendous time benefit because experiments can be run in significantly less time compared to experiments run using DFT. FDM and DSD are linear algebra methods that diagonalize data matrices to extract eigenvalues and eigenvectors. Exploration of these alternative methods is important because data can be analyzed in a more time-efficient manner and with higher resolution. Thus, structural elucidation of complex macromolecules becomes more rapid.

Large endeavors such as the Human Genome Project will lead to the discovery of thousands of proteins, and their structures will need to be determined before they can be studied further. Fields related to healthcare and drug development will also benefit from these methods. The fundamental step of identifying target molecules will be made simpler and faster, thereby advancing the progress of synthesizing drugs that are selective and specific. In fields where time is of the essence, DSD and FDM have the potential to offer these solutions readily.

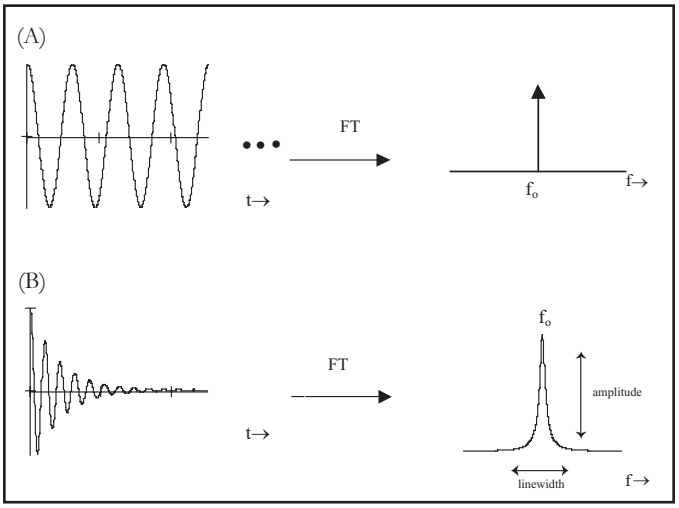

Figure 1
Time signals and their Fourier transformations. (A) A single sinusoid of infinite length transforms to a delta function with an exact frequency and infinite height. (B) A damped sinusoid transforms to a Lorentzian peak with a frequency, linewidth and amplitude.

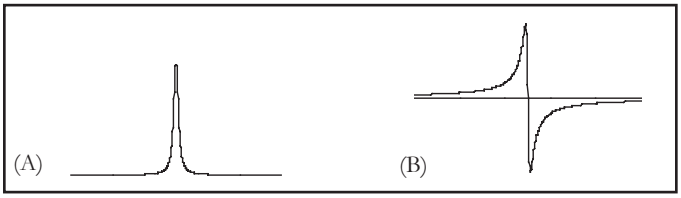

Figure 2
The components of a Lorentzian line: (A) The real, absorptive and (B) the imaginary, dispersive.

Spectral analysis determines the characteristic frequencies, amplitudes and phases of the constituents of a time-varying signal (Hoch and Stern, 1996). This study compares two unconventional methods for spectral estimation, FDM (Wall and Neuhauser, 1995) and DSD (Belkic et al., 1999). In NMR spectroscopy, a time signal is transformed into a frequency spectrum as illustrated in Figure 1. The real component of the frequency signal is called the absorptive Lorentzian line while the imaginary component is called the dispersive Lorentzian line (Figure 2).

Although DFT is the traditional method for NMR data processing, parametric fitting methods such as FDM and DSD require less data. Therefore, throughput can be increased, which is advantageous because the signal length required by DFT can be inconvenient or impossible to obtain, particularly for complex multidimensional spectra, such as those of large biological molecules. Limitations of DFT include its slow convergence and low resolution. In addition, DFT alternatives, FDM in particular, can be used for multi-dimensional data sets, unlike DFT, which is intrinsically one-dimensional (Mandelshtam, 2001). Parametric alternatives to DFT result in shorter experimental data acquisition time and better resolution.

In this investigation, simple model signals and a real, experimentally obtained signal for the protein myoglobin were processed using FDM, DSD and DFT. Because FDM and DSD use windows to process data rather than computing the entire spectrum simultaneously, the effects of window size and window placement were investigated. Observations were made about the consistency of the results for different window sizes chosen for the same region in the spectrum. The reliability of the alternative parametric methods rests on their abilities to produce a spectrum that is at least comparable to the correct DFT.

Theoretical Background

The Discrete Fourier Transform

Experimental data is neither infinite nor continuous. Rather, it is a collection of sampled points measured at every time step, τ. The time domain data, c_k, with N data points, is

transformed to the frequency domain using DFT in Equation 1:

$$f_n = \frac{1}{\sqrt{N}} \sum_{k=0}^{N-1} c_k e^{-2\pi i k n / N} \qquad (1)$$

The time signal and frequency response are both periodic. The period of the signal is the spectral width, or Nyquist range, which is defined as $2\pi/\tau$ radians/sec or $1/\tau$ Hz. Because a finite number of data points are acquired, this is a truncated, infinite time signal. Data is acquired for some time period called the sampling time. Due to the truncation, the data does not decay to zero; therefore zeroes are added to the end of the signal. The DFT of this signal is a sinc function where the width of the center peak is inversely proportional to the sampling time. The DFT of such a signal is shown in Figure 3.

The resolution in the frequency domain is determined by the Fourier transform time-frequency uncertainty principle in Equation 2:

$$\delta F \approx \frac{1}{N\tau} \qquad (2)$$

Hence, frequency separation (δF) is inversely proportional to the number of data points, so as the number of data points increases, the resolution improves. Another way to view this is that resolution increases as acquisition time increases. The best frequency spectrum obtained using DFT comes from time signals that have been sampled at small time intervals (large N, small τ) and over a long time period (until the signal decays to zero), which yields sharp, resolved lines in the spectrum.

Filter Diagonalization Method

The 1-D filter diagonalization method is well developed, reliable, fast, and capable of resolving frequencies beyond the FT uncertainty principle. FDM was originally developed by Neuhauser (1990) for time-dependent quantum dynamics calculations, and was improved and applied to time signal data processing by Mandelshtam and Taylor (1997). FDM is a numerical procedure designed to extract the parameters (peak positions, linewidths, amplitudes, and phases) of time domain data by fitting the data to a sum of damped sinusoids. The time signal is assumed to be in the form of (3)

$$c_n = \sum_{k=1}^{K} d_k e^{-in\tau\omega_k} \qquad (3)$$

where d_k are complex amplitudes and w_k are complex frequencies. The nonlinear summation (3) is converted to a linear algebra problem of the form of (4)

$$\mathbf{U}^{(1)}\mathbf{B}_k = u_k \mathbf{U}^{(0)}\mathbf{B}_k \qquad (4)$$

where $\mathbf{U}^{(1)}$ and $\mathbf{U}^{(0)}$ are matrices, $\mathbf{B}_k$ is an eigenvector (complex amplitude), and u_k is the eigenvalue (complex frequency). $\mathbf{U}^{(1)}$, the matrix representation of the evolution operator (5), is broken into smaller matrices that are easily diagonalized:

$$\hat{U} = e^{-i\tau\hat{\Omega}} \qquad (5)$$

where $\hat{\Omega}$ is the Hamiltonian operator. In an appropriate basis set such as the Fourier basis, $\mathbf{U}^{(1)}$ is constructed from the time signal c_n. This reduction of the original large data matrix is a consequence of the windowing techniques used

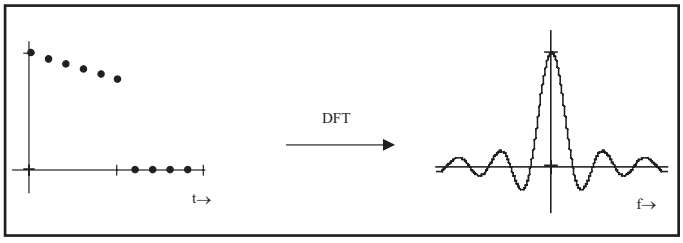

Figure 3
A finite number of data points are acquired, yielding a truncated signal that does not decay to zero and must be zero-filled at the ends. Transforming this signal by DFT results in a sinc function.

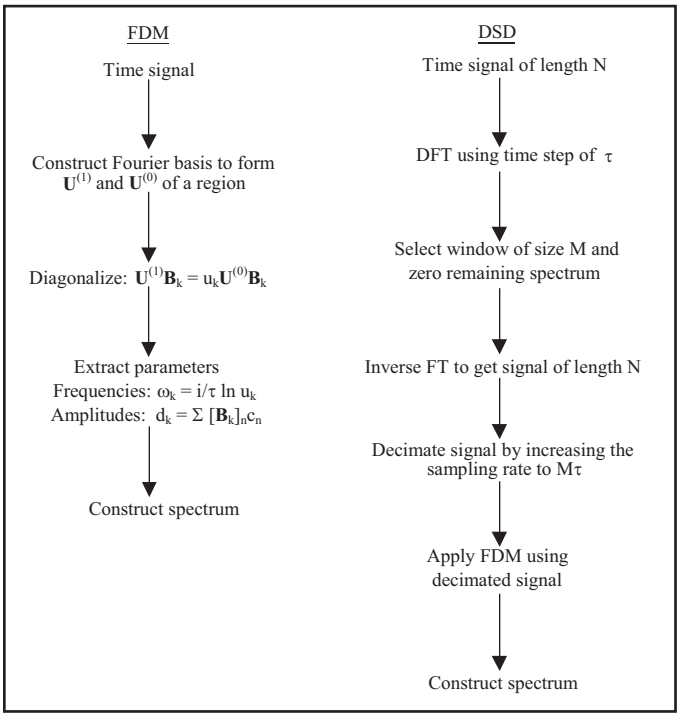

Figure 4
Flow chart illustrates the steps involved in FDM and DSD.

by FDM to filter out basis functions, thus increasing the numerical efficiency of a spectral calculation.

Decimated Signal Diagonalization

Like FDM, DSD uses windows to reduce a large data matrix to a number of simple ones before diagonalization. However, while FDM filters basis functions to create its windows, DSD filters the time signal. A time signal is processed to get a low-resolution spectrum by DFT. This spectrum is divided into M windows containing at most 200 data points. A new signal is created for each window by zeroing the content outside the window and then recentering the window at zero. The inverse DFT is performed to convert the frequency data back into the time domain. The decimation step occurs when this new time signal is sampled at M times greater than the original time step, creating a band-limited decimated signal, which is diagonalized to extract the spectral parameters.

Experimental Methods

The model signals in the published DSD results contained sharp, resolved peaks. In this investigation, experiments were carried out using model signals with resolved peaks but with a definite linewidth and an experimental myoglobin signal. First, a program for DSD was coded using the published algorithm (Belkic et al., 1999).

Various windows, differing in length and position, were used for the first model signal. Varying the window size demonstrates how the signal length affects FDM and DSD in their spectral estimation. By using different window positions, the stability of these methods was analyzed. The model signal was 1,024 points long. Experiments were conducted using window lengths of 32, 64, 128, and 256 grid points, starting at grid points 210, 215, 220, and 225.

The second model signal contained a very broad peak located near a triplet, with the broad peak targeted for study. FDM and DSD were tested to determine whether the spectral parameters of the broad peak could be accurately extracted and the resulting spectra were compared. Window lengths of 32, 64 and 128 grid points were used. These windows varied in position, encompassing more of the broad peak with increasing window length.

The signal for myoglobin was 32,768 points long. Because the endpoints of the myoglobin spectrum are mainly noise, only the dense region was processed with FDM and DSD. The spectrum was initially constructed using 62 windows that were 256 points long. The spectrum was then reconstructed using 63 additional windows that contained 50% overlap of the original 62 windows. In order to smooth out the edge effects contained in each window, a weighting function ($\cos^2(ax)$, where 'a' is an appropriate constant) was applied to each window and all of the windows were added together.

Results and Discussion

The first study of FDM and DSD used a model signal of the form (6)

$$c_n = \sum_{k=1}^{K} d_k e^{-in\tau\omega_k} \qquad (6)$$

with 1,024 points and 2 sinusoids (K = 2). The DFT spectrum for this model signal is shown in Figure 5.

The frequency spectrum consists of two peaks at frequency values -1.7 and -2.0 Hz with a relative intensity of 1:10. The smaller peak at -1.7 Hz is the peak of interest. The spectral parameters (frequencies, widths and amplitudes) extracted using FDM were accurate. The poles (peaks represented by a complex frequency and amplitude pair) calculated by DSD were acceptable, however, the poles not corresponding to true peaks misrepresented the actual spectrum by bunching up at the edges. Hence, the DSD-reconstructed spectra themselves were not of the quality of the FDM or DFT spectra. All of the DSD spectra exhibited severe edge effects that caused distortions in the baseline. The poorest DSD spectrum was observed in the 32-point window positioned at grid point 210. The tail of the peak is affected by the nearby edge effect because it does not return to the baseline. FDM was able to calculate the win-

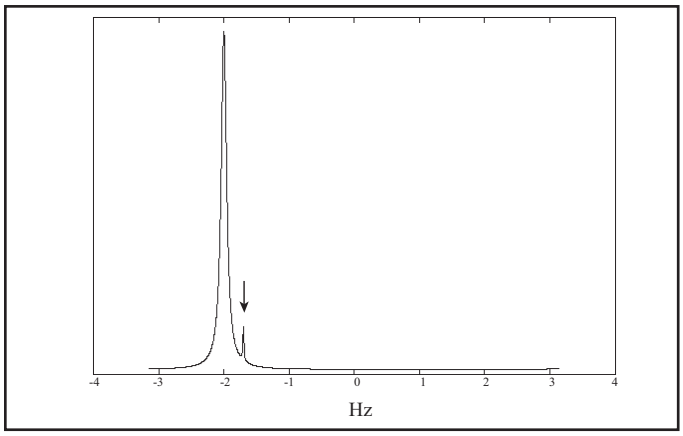

Figure 5
The frequency spectrum of the first model signal obtained using DFT. The peak of interest is the small peak on the shoulder of the larger peak.

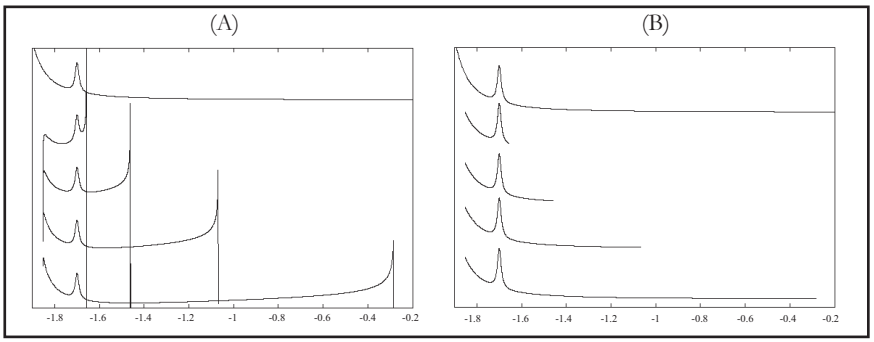

Figure 6
(A) DSD and (B) FDM results are compared using windows of different lengths. The DFT for the window is shown at the top; below the DFT spectrum is the experiment using the 32-point window, followed by the 64-, 128- and 256-point windows.

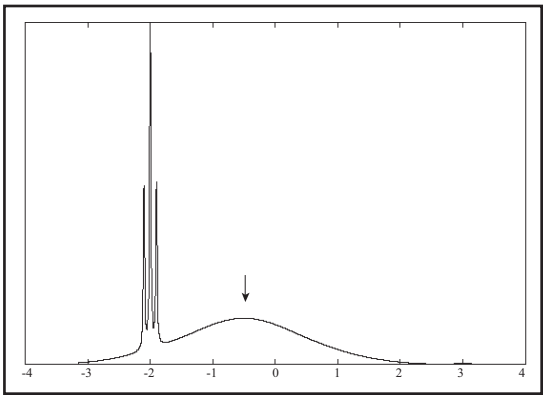

Figure 8
DFT spectrum of the second model signal

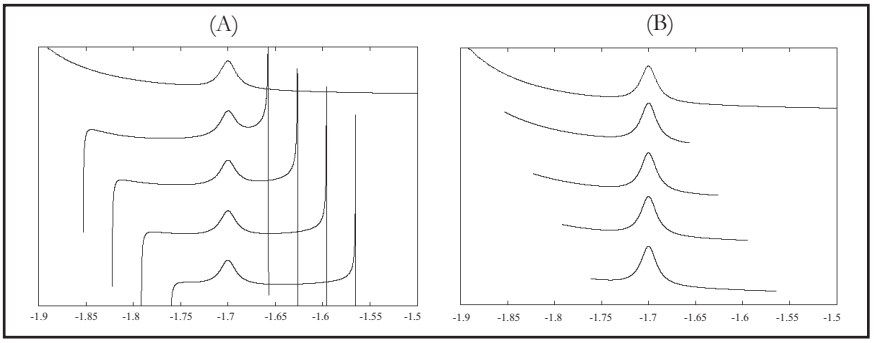

Figure 7
(A) DSD and (B) FDM results for the 32-point window at different positions. For comparison, the DFT spectrum is shown at the top of each figure.

Table 1
Eigenvalues extracted for the broad peak in the second model signal by FDM and DSD.

	32-point window		64-point window		128-point window		
	FDM	DSD	FDM	DSD	FDM	DSD	Actual Value
Frequency Range (Hz)	-0.9081 to -0.1227		-1.3008 to 0.2630		-1.5462 to 1.5953		-----
Frequency (Hz)	-0.5002	-0.4660	-0.5000	-0.4939	-0.5000	-0.4999	-0.5
Width	-1.4968	-0.7976	-1.5000	-1.3076	-1.5000	-1.5007	-1.5
Amplitude	19988	3641	20000	15374	20000	40230	20000

This model signal was processed using 32-, 64-, 128-, and 256-point windows in four different, nearby positions. The objective behind conducting experiments with varying window lengths was to observe whether the window size (i.e., the number of data points used) would effect the results. The second part of the experiment, varying the window position, was carried out to test whether the results were consistent between windows. Figure 6 illustrates the DSD and FDM results for this model signal using 32, 64, 128, and 256 points for a given window position. Figure 7 illustrates the DSD and FDM results using the 32-point window at different positions.

The second model signal, also of form (6), contained 256 points and 4 sinusoids (K = 4). The peak of interest in this spectrum was a large, broad peak with a frequency of -0.5 Hz to the right of a triplet at about -2 Hz (Figure 8). In this experiment, the extracted parameters were not successfully determined as compared to the first model signal (Table 1).

Broad peaks are generally difficult to process. The FDM and DSD spectra for the windows used in this experiment were not perfect, but the DSD results were clearly not comparable to the FDM results. One experiment used a window that was 32 points long and contained only the top of the peak starting at grid point 91. The second experiment used a 64-point window beginning at grid point 75. The third experiment used half the number of total points, 128, with the window beginning at grid point 65. The window began as far to the left as possible to

dows exactly, including the correct contribution of the large neighboring peak located outside the window. This was possible because FDM filters basis functions, and they are not defined. In these windows, FDM calculates a basis set and fits the basis functions to peaks. However, if a basis function best represents a peak outside the window, then that basis function "leaves" the window to fit the outside peak.

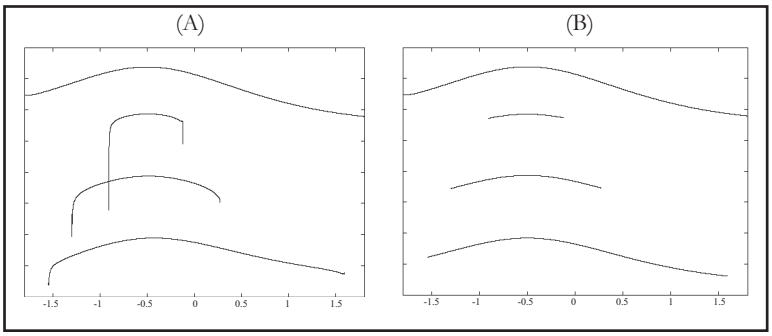

Figure 9
(A) DSD and (B) FDM spectra for the second model signal. The DFT spectrum is at the top of each figure. Below the DFT are the 32-, 64- and 128-point windows.

encompass most of the broad peak while excluding the triplet. The DSD spectra show edge effects and baseline distortions similar to the results from the first model signal, while FDM was able to calculate a good fit for the peak (Figure 9).

The third experiment used a real-time signal, composed of 32,768 data points, of the protein myoglobin in solution. The windows used were 256 points long and spanned a frequency range of 93.75 Hz. For DSD, 137 windows were analyzed. Each window contained 50% of the previous window. For example, the first window ranged from -3700.19531 to -3606.44531 Hz. The second window started at the center of the first window, -3653.32031 Hz and ended at -3559.57031 Hz. A smoothing function, $\cos^2(x/93.75\pi)$, was applied to the windows before summing them. This function was used because it has a value of 1 in the center of the window and 0 at the edges, and it eliminated the edge effects produced by DSD. The period of the smoothing function was equivalent to the window frequency range of 93.75 Hz. Due to the 50% overlap, what is 0 in one window is 1 in the neighboring window. After summation of all the windows, the result is a reasonable-looking spectrum, however, the spectrum is still not better than the FDM spectrum. At best, DSD is comparable to FDM. Figure 10 shows the myoglobin spectra obtained using DFT and FDM. Though the 1-D spectrum for myoglobin is dense, FDM successfully reproduces the DFT result. The DSD results are shown in Figure 11, before and after the application of the $\cos^2(x/93.75\pi)$ weighting function to the windows.

In comparing the DSD spectrum with the FDM or DFT spectra, a glaring problem with the DSD spectrum is evident. At about -800 Hz, near the large water peak, there is a spike in the DSD spectrum that is not present in the DFT or FDM plots.

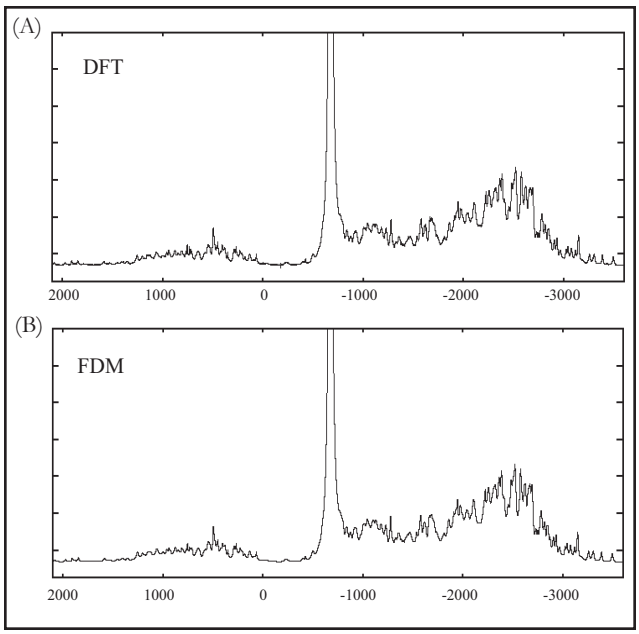

Figure 10
1-D myoglobin spectra obtained using (A) DFT and (B) FDM.

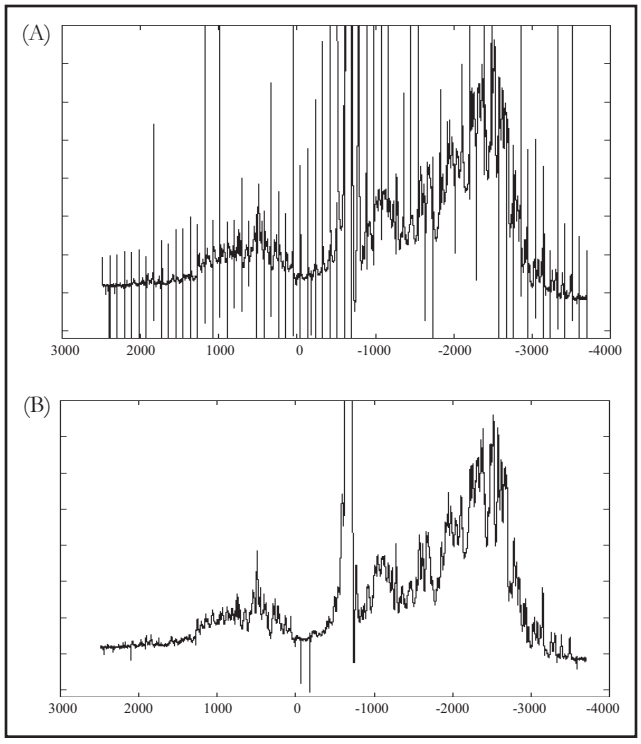

Figure 11
DSD myoglobin spectra obtained (A) before and (B) after the use of overlapping windows and a weighting function.

It has been demonstrated that DSD is capable of extracting the correct eigenvalues for NMR data signals when the peaks are sharp, however, the frequency spectra produced by DSD are incorrect (Belkic et al., 1999). Baseline distortions and edge effects are the main problems encountered

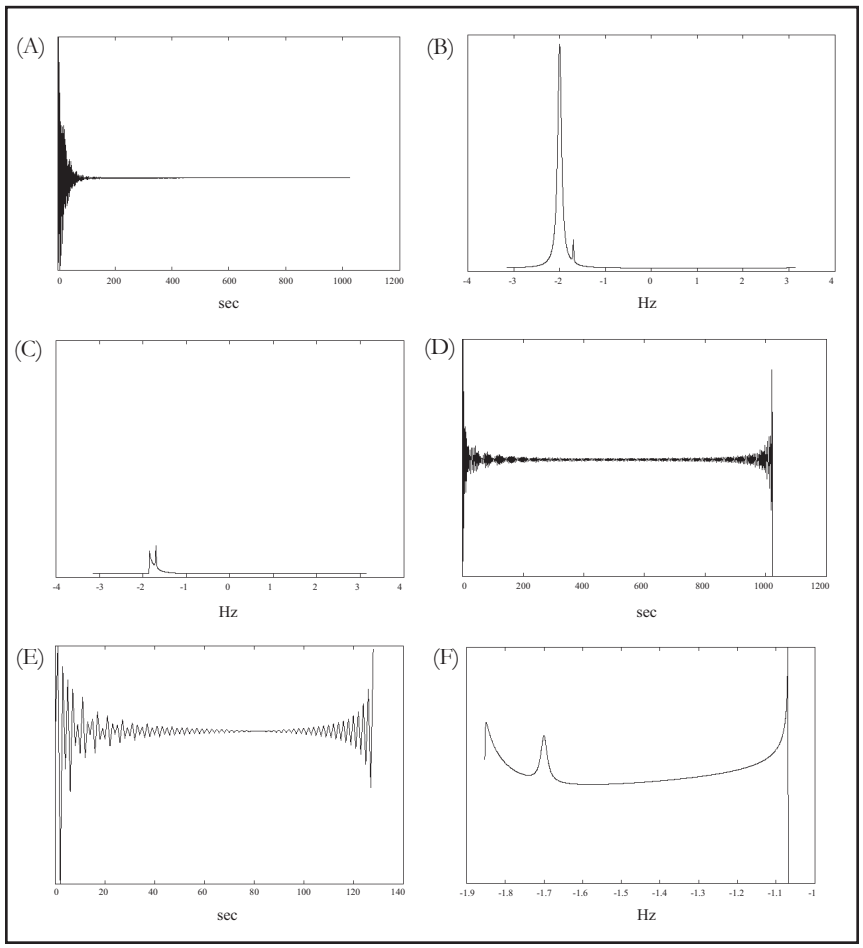

Figure 12

A DSD example. (A) A time signal is processed by DFT to give (B) the frequency spectrum. (C) A window is selected to be analyzed and the spectrum outside the window is set to zero. (D) The new spectrum is converted back to the time domain by inverse DFT. (E) The signal is decimated to produce a new, shorter time signal, which is then diagonalized, giving (F) the resulting frequency spectrum.

in DSD spectra. For dense spectra, the distortions and artifacts can be masked using windows with 50% overlap; this greatly improved the myoglobin signal.

A possible explanation for the obvious problems in the DSD frequency spectrum is that when DSD decimates the time signal, the periodicity of the signal is lost. The time-domain data is a sinusoid that decays to zero. When DSD employs the inverse DFT, the original length of the time signal is still intact. In this step, a serious problem arises; the time signal for this zeroed spectrum is wrong, which results in the ends blowing up instead of decaying to zero. Next, DSD calls for decimation of the signal. When the sampling time is increased by a factor of M, fewer data points are acquired, creating a time signal shorter than the original. This new signal still possesses the divergent ends and is introduced into FDM as the input data signal. Figure 12 illustrates the steps involved in DSD using the first model signal.

Conclusion

The model signals used in this investigation tested the extreme conditions that must be satisfied by any NMR data-processing method. A combination of sharp and broad lines was chosen because they behave differently in time. Sharp lines decay slowly in time while broad peaks decay quickly in time. FDM is able to handle these extreme cases, whereas DSD appears to work only when processing well-resolved, sharp lines. It was also shown that using a $\cos^2(ax)$ smoothing function and overlapping windows results in spectra that are substantially better than the DSD spectra obtained using the published method. However, the quality of the DSD dense parameters and spectra are inferior to those obtained using FDM alone.

Acknowledgements

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