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Craig, Edward Clayton

APPLICATION OF DISPERSION VERSUS ABSORPTION (DISPA) IN FOURIER TRANSFORM NUCLEAR MAGNETIC RESONANCE AND FOURIER TRANSFORM ION CYCLOTRON MASS SPECTROMETRY

The Ohio State University

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APPLICATION OF DISPERSION VERSUS ABSORPTION (DISPA)
IN FOURIER TRANSFORM NUCLEAR MAGNETIC RESONANCE
AND FOURIER TRANSFORM ION CYCLOTRON MASS SPECTROMETRY

DISSERTATION

Presented in Partial Fulfilment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

By

Edward Clayton Craig, B.S., M.S.

* * * * * *

The Ohio State University
1987

Reading Committee:
Professor Alan G. Marshall
Professor Prabir Dutta
Professor Richard L. McCreery

Approved by

Advisor

Department of Chemistry
TO MY MOTHER
I would like to express appreciation to Professor Alan G. Marshall for his guidance and support throughout the course of this work. I would like to give special thanks to Dr. Charles E. Cottrell for his guidance, helpful advice and suggestions. I would like to extend a very heartfelt thanks to the members of Dr. Marshall's research group for their assistance, tolerance, and suggestions.

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I want to express very special gratitude to my mother and step-father, whose love and support has proved to be invaluable to me during the course of my doctoral work.

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with α-Adrenoreceptors," D. Miller, A. Hamada, E. Craig, J. Galluci,
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Magnetic Field Inhomogeneity: Spinning Shims (z, z2 , z3, z4, z5),"

Major Field: Analytical Chemistry
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CHAPTER I
INTRODUCTION

A. INTRODUCTION

This chapter is devoted to the definition of terms and basic concepts necessary for appreciation of those chapters which follow. The three sections which follow immediately are intended as a brief review for readers unfamiliar with Fourier transform theory, Fourier transform nuclear magnetic resonance (FT/NMR), and/or Fourier transform ion cyclotron mass spectrometry (FT/ICR). Readers already familiar with these principles may wish to skip the following sections and proceed directly to the section covering Dispersion versus Absorption (DISPA) plots. The following sections are intended only as a review and the reader should refer to the numerous texts and reviews available on Fourier transform theory (1-8), FT/NMR (9-15) and FT/ICR (16-18) for more detailed discussions.

After DISPA plots are introduced, their basic properties are discussed and a brief survey of their potential applications is provided. The final section of this chapter introduces the aims and goals of this dissertation. As these aims and goals are discussed the organization of the remainder of the dissertation is presented to emphasize the significance of each chapter toward these goals.
1. FT/NMR EXPERIMENT

Nuclei with half integral spin quantum numbers \( I = 1/2, 3/2, 5/2, \ldots \), meaning that the sum of the protons and neutrons in the nucleus is odd, have microscopic magnetic moments associated with them. When these nuclei are placed in a static magnetic field a torque is exerted on their individual microscopic magnetic moments which tends to align their z-components either parallel (low energy state) or anti-parallel (high energy state) with the static magnetic field, \( H_0 \). The energy difference between the upper and lower energy states is very small but at equilibrium the Boltzman distribution results in a small excess of nuclei in the low energy state, sufficient to produce a net macroscopic magnetic moment, \( M_0 \), aligned with the static magnetic field (\( H_0 \) in Figure 1(a)) for the entire assemblage (sample).

Thermal motion prevents all the nuclei from aligning perfectly with the static magnetic field; consequently the nuclei precess about an axis parallel with that of the static magnetic field moment.

The rate of precession (resonance frequency) is called the Larmor frequency, \( \omega_0 \), and is given by

\[
\omega_0 = \gamma H_0 \tag{1.1}
\]

where \( \gamma \) is the magnetogyratic ratio which is characteristic of a particular type of nuclei. \( H_0 \) is the static magnetic field strength.
Fig. 1. The net magnetic moment, \( M_0 \), for a sample tends to align with static magnetic field, \( H_0 \), as shown in (a). When the sample subjected to a short burst of radiation as from an rf transmitter the net magnetic moment is tilted away from the z-axis (b). The tilting of the net magnetic moment away from the z-axis (c) gives it components in the x,y-plane, \( M_x \) and \( M_y \).
A transmitter coil is positioned along an axis perpendicular to the magnetic field usually designated as the x-axis (Figure 1(b)). The sample is subjected to a short burst of radio frequency radiation (called a rf pulse or pulse) from the transmitter coil. The wave train corresponding to the rf pulse has a time varying magnetic field orthogonal to $H_0$, namely $H_1$. $H_1$ (Figure 1(c)) tends to pull the net magnetic moment, $M_0$, of the sample into the x-y plane. This tilting of the magnetic moment away from the z-axis produces a projection in the x-y plane. The projection of the magnetic moment can be decomposed into its x and y components, $M_x$ and $M_y$ respectively.

As the magnetic moment precesses about the z-axis, the magnitude of $M_x$ oscillates at the Larmor frequency of the particular nucleus. A receiver coil placed along the y-axis has an oscillating current induced in it by the oscillating component $M_y$. If there is no time delay between the excitation and the detection the receiver along the y-axis will sense a signal which behaves in time as a perfect cosine function, beginning at a maximum and oscillating continuously thereafter. If a receiver coil is placed along the x-axis then the detected signal is a perfect sine function (same as a cosine function phase shifted by 90°). In practice there is generally some time delay between excitation and detection and the observed signal is neither a pure cosine function (odd function) nor a pure sine function (even function) but some linear combination of the two.

The observed signal decays with time because after termination of the rf pulse the magnetic moment tends to realign with the static
magnetic field (z-axis) and the projection in the x-y plane
disappears. This is generally a first order rate process called $T_1$
relaxation (spin-lattice relaxation or longitudinal relaxation).
The magnitude of the projection in the x-y plane also decays with
time because the magnetization tends to diffuse through the spin
ensemble or stated in another way the spins lose phase coherence with
one another. This process is also a first order decay process called
$T_2$ relaxation (spin-spin relaxation or transverse relaxation).

All the nuclei in the sample are irradiated (excited)
simultaneously and the image current actually observed by the
detection electronics is the composite of several sinusoids with
different phases, frequencies, amplitudes and decay constants. This
experimental signal is the time domain signal often referred to as
the Free Induction Decay or FID. The time domain signal is digitized
and stored in computer memory to await further processing.

2. FT/ICR EXPERIMENT

Ions are generated (by any of several means) and introduced into
a static magnetic field under vacuum. The charged ions circulate
with a characteristic frequency in the magnetic field just as they
would in a cyclotron. To prevent ions from escaping from the
cell,"trapping" plates are positioned at either end of the cell along
the axis of the static magnetic field, $B_0$, (the z-axis). They are
maintained at constant potential (positive for positive ions,
negative for negative ions), effectively sequestering the ions.
The orbital frequency (resonance frequency) for ions of charge $q$ is inversely proportional to their mass ($m$) and directly proportional to the static magnetic field ($B_0$).

$$\omega = \frac{qB_0}{m} \quad [1.2]$$

Equation [1.2] is used to convert the observed resonance frequency of an ion into mass.

The ions are excited to larger radius orbits by applying an electric field which oscillates at a frequency equal to the resonance frequency of the ion. A range of masses is generally excited using a frequency sweep excitation (sometimes called chirp excitation). In frequency sweep excitation a waveform is emitted from the excitation plates for which the radiofrequency increases linearly with time. The slope (rate at which the excitation frequency changes) of the sweep excitation is dependent upon the frequency range that is excited and the rate at which the frequency range is excited. During excitation ions of a given mass achieve phase coherence (by being displaced off center as a packet of ions) with each other.

The circulating charges of the ion packets induce an oscillating image current onto a pair of detection plates located orthogonal to the excitation plates. The observed signal decays exponentially (generally) with time due to first order decay processes such as collision with neutral carrier gas molecules or the cell wall.
Since several ions are observed simultaneously the observed signal is the composite of individual decaying sinusoids for each of the ion masses which have been excited.

3. COMPARISON BETWEEN FT/NMR AND FT/ICR EXPERIMENTS

In FT/NMR the decaying sinusoidal signal arises from the precession of nuclei in a magnetic field whereas in FT/ICR the decaying sinusoidal signal arises from the circulation of ions in an electric field. The primary relaxation methods in both cases, although physically different, are first order rate processes which result in an exponential decay of the time domain signal. The range of frequencies observed for the two experiments is very different. FT/NMR spectral bandwidths are on the order of Hz to kHz while FT/ICR bandwidths require observation of frequencies in the MHz range. This difference in frequency range dictates the different excitation and detection procedures required by the two experiments. Both FT/NMR and FT/ICR generate image currents representing the composite of several exponentially decaying sinusoids. Since the signals obtained for the two experiments are mathematically identical the processing of the data after collection is the same for both experiments.

4. DATA MANIPULATION

Generally, the data is Fourier transformed to obtain the frequency domain spectra. The time domain signal for an individual sinusoid with resonance frequency \( \omega_0 \) is taken to be a causal signal represented as
\[ f(t) = e^{-t/\tau} \cos(\omega_b t), \quad 0 < t < \infty. \]  

where \( t \) is time, \( \tau \) is the decay time for the relaxation and \( \omega_b \) is the resonance frequency.

The complex Fourier transform of \( f(t) \) can be taken as shown in Equation [1.4] to obtain the complex frequency domain spectrum \( F(\omega) \).

\[ F(\omega) = \int_{0}^{\infty} f(t) e^{-i\omega t} dt \]  

The real and imaginary components of \( F(\omega) \) are usually represented separately. \( f(t) \) can be represented as the linear combination of an odd (cosine) function, \( f_0(t) \), and an even (sine) function, \( f_e(t) \).

\[ f(t) = f_0(t) + f_e(t) \]  

In the limit that \( \omega_b > > 1/\tau \), the frequency domain absorption spectrum, \( A(\omega) \), is obtained by taking the cosine Fourier transform of the even portion of \( f(t) \).

\[ A(\omega) = \frac{1}{\pi} \int_{0}^{\infty} f_e(t) \cos(\omega t) dt = \frac{\tau}{1 + \frac{\tau^2}{(\omega_b - \omega)^2}} \]  

The frequency domain dispersion spectrum, \( D(\omega) \), is obtained by taking the sine Fourier transform of the odd portion of \( f(t) \).

\[ D(\omega) = \frac{1}{\pi} \int_{0}^{\infty} f_0(t) \sin(\omega t) dt = \frac{\tau^2 (\omega_b - \omega)}{1 + \frac{\tau^2}{(\omega_b - \omega)^2}} \]
The magnitude spectrum (also called the absolute value spectrum), \( M(\omega) \), is obtained from the absorption and dispersion spectra using the following equation

\[
M(\omega) = \sqrt{A(\omega)^2 + D(\omega)^2} \tag{1.8}
\]

Squaring the magnitude spectrum generates the power spectrum \( P(\omega) \).

\[
P(\omega) = (M(\omega))^2 = A(\omega)^2 + D(\omega)^2 \tag{1.9}
\]

B. DISPERSION VERSUS ABSORPTION (DISPA) PLOTS

Marshall noted by from the analogy between magnetic relaxation and dielectric relaxation that a plot of dispersion, \( D(\omega) \), versus absorption, \( A(\omega) \), was perfectly circular for a true Lorentzian line shape (18, 19). True Lorentzian line shape results from the Fourier transform of an exponential decay. The relationship developed by Marshall is given as

\[
\left( A(\omega) - \frac{\tau}{2} \right)^2 + \left( D(\omega) \right)^2 = \left( \frac{\tau}{2} \right)^2 \tag{1.10}
\]

Simply stated Equation [1.10] says that a plot of dispersion versus absorption (DISPA) is a perfect circle with radius \( \frac{\tau}{2} \) and centered at \( (-\frac{\tau}{2}, 0) \) (vertical coordinate is dispersion, horizontal coordinate is absorption).
Fig. 2. (a) Normalized absorption-mode (even function) and dispersion-mode (odd function) spectrum for a single Lorenzian line shape. (b) Plot of dispersion versus absorption (DISPA) for line shapes in (a). This figure was borrowed from reference (20).
Equation [1.10] is analogous to a semi-empirical relationship established by Cole and Cole (21) for dielectric relaxation data. The Cole-Cole plot is a semi-circular plot, generally with the circle center displaced below the abscissa, obtained by plotting the imaginary component of the complex dielectric relaxation versus its real component. This plot has been used for over 40 years in the analysis of dielectric relaxation mechanisms, and is the most highly cited paper in the field.

Marshall (18) demonstrated theoretically the DISPA plots can be used to distinguish between some seven specific kinds of line broadening mechanisms in radiofrequency (NMR, ICR, NQR) and microwave (ESR, pure rotational) spectrometry. These different line broadening mechanisms are outlined in the list below:

1. Resolution of two closely-shaped Lorentzian lines
2. Gaussian distribution in resonance frequency
3. Resolution of two Lorentzians of different line widths
4. Log-Gaussian distribution in relaxation time
5. Log-Gaussian distribution in correlation time
6. Chemical exchange between two sites at equal population but different resonance frequency
7. Chemical exchange between two sites of equal population with the same resonance frequency but different line width.
Symmetrical distributions in spectral peak position produce experimental DISPA plots which deviate outside the reference circle. Distributions in peak widths for peaks centered at the same position produce experimental DISPA plots which are displaced inside the reference circle.

Many of the distortions predicted for DISPA plots have been observed experimentally. Most of the broadened spectral line shapes which have been examined by constructing DISPA plots have to date resulted from some variation of a distribution in chemical shifts (exchange between sites of different resonance, magnetic field inhomogeneity through the sample, resolution of closely spaced peaks). Only one example has been reported where a DISPA plot was prepared for a broadened spectral line shape resulting from some type of distribution in line widths positioned at the same resonance frequency (22).

The first experimental DISPA plots were constructed by Roe and Marshall in 1978 (18). They confirmed, using $^1$H FT/NMR spectra of HDO, that the DISPA plot of a true Lorentzian line shape is perfectly circular. They also constructed experimental DISPA plots to verify many of the distortions predicted for different line broadening mechanisms. Resolution of two closely spaced peaks ($^{19}$F FT/NMR of 5-fluorouracil artificially broadened by exponential multiplication of the time domain signal); distribution in chemical shifts ($^1$H FT/NMR of benzene absorbed on silica gel); distribution in chemical shift and relaxation time ($^1$H FT/NMR of H2O in Bacto-agar); and a
distribution of different relaxation times at the same resonance frequency ($^{31}$P FT/NMR of 70S ribosomes. deviation of this particular plot inside the reference circle has been attributed to the presence of regions of variable flexibility in the 70S ribosome unit).

DISPA plots have been most useful in the study of deviations from true Lorentzian line shapes. Although aesthetically pleasing, DISPA plots are difficult to interpret and so a variety of linearized plots have been constructed to visualize small deviations from Lorentzian line shape more clearly (23).

One review of DISPA plots has been written (24). A program has been published for the on-line construction of DISPA plots on Bruker FT/NMR instruments suitable for single peak spectra (25). DISPA plots have been prepared for Gaussian line shapes by mathematically transforming Gaussian functions into Lorentzian functions (20).

1. PROPERTIES OF DISPA PLOTS

(1) DISPA plots are perfectly circular for true Lorentzian line shapes (18, 19). This property is a direct consequence of the mathematical form of the absorption and dispersion line shapes. Marshall first noted that the absorption and dispersion line shapes are related by an expression which just exactly describes a circle centered at ($\frac{\tau}{2}, 0$) and having a radius of $\frac{\tau}{2}$.

$$\left[A(\omega) - \frac{\tau}{2}\right]^2 + \left[D(\omega)\right]^2 = \frac{\tau^2}{2}$$ [1.11]
Equation [1.11] is verified by substituting the normalized expressions for absorption and dispersion into Equation [1.11]. This derivation, originally derived by Marshall (18, 19), is reproduced in Appendix A.

(2) DISPA plots deviate from circularity in a predictable fashion based on specific line broadening mechanisms (18, 19). In general when line broadening results from some type of distribution of component peak positions the experimental DISPA plot deviates outside the DISPA reference circle. When line broadening results from a distribution in line widths, for components all centered at the same spectral peak position, the experimental DISPA plot deviates inside the reference circle.

(3) DISPA plots are rotated about the origin by an angle equal to the phase error in the spectral data. This property is verified in Appendix B by demonstrating that a delay in the time domain between the excitation and detection times produces an effect exactly equivalent to a rotation of the complex frequency domain spectrum about the frequency axis. The real and imaginary components are calculated by taking the cosine and sine Fourier transforms of the time shifted by some arbitrary time $t_d$ time domain spectrum.

\[
R(\omega) = \int_{0}^{\infty} f(t-t_d) \cos(\omega t) \, dt \quad [1.12]
\]
\[ I(\omega) = \int_{0}^{\infty} f(t-t_d) \sin(\omega t) \, dt \quad [1.13] \]

As is shown in Appendix B, the real, \( R(\omega) \), and imaginary, \( I(\omega) \), components are related to the true absorption and dispersion components by the following expressions.

\[
R(\omega) = A(\omega) \cos(\omega t_d) - D(\omega) \sin(\omega t_d) \quad [1.14]
\]
\[
I(\omega) = A(\omega) \sin(\omega t_d) + D(\omega) \cos(\omega t_d) \quad [1.15]
\]

Since \( \omega \approx \omega_0 \), for narrow line shapes the substitution

\[
\omega t_d \approx \omega_0 t_d = \phi \quad [1.16]
\]

where \( \phi \) is some constant phase angle can be made and Equations [1.14] and [1.15] become

\[
R(\omega) = A(\omega) \cos(\phi) - D(\omega) \sin(\phi) \quad [1.17]
\]
\[
I(\omega) = A(\omega) \sin(\phi) + D(\omega) \cos(\phi) \quad [1.18]
\]

(4) For a true Lorentzian the square root of the area inside the DISPA circle is proportional to the number of species responsible. This would be the number of nuclei in FT/NMR or the number of ions in FT/ICR. This property allows quantitation without requiring phase correction of the spectral data. Derivation for this property is given in Appendix C.

(5) Circles centered at the origin of the DISPA plot have radii with constant spectral magnitudes. Stated more precisely, the
distance from the origin to any point on the DISPA circle, regardless of phase, is equal to the magnitude value associated with that point. This is verified in Figure 3 by noting that a line, $M(\omega)$, between the origin and a point on the DISPA circle forms the hypotenuse of a right triangle for which the vertical side is the dispersion, $D(\omega)$, and the horizontal side is the absorption, $A(\omega)$. Using the Pythagorean theorem, the length of $M(\omega)$ is given by

$$M(\omega) = \sqrt{(A(\omega))^2 + (D(\omega))^2}$$

which is exactly the definition for the magnitude at an arbitrary frequency $\omega$.

The following list summarizes the properties of DISPA plots discussed in the preceding paragraphs.

**Properties of DISPA Plots**

1. Perfectly circular for true Lorentzians.
2. Deviates from circularity in a predictable fashion based on specific line broadening mechanisms.
3. Rotated about the origin by an angle equal to the phase error.
4. Square root of the area inside the DISPA circle is proportional to the number of species represented.
5. Circles centered at the origin of the DISPA plot have radii with constant spectral magnitudes.
Fig. 3. The distance between any point on the DISPA circle and the origin is equal to the magnitude corresponding to that point.

\[ M = \sqrt{A^2 + D^2} \]
2. RELATION BETWEEN DISPA PLOT AND COMPLEX FREQUENCY SPECTRUM

The DISPA plot is constructed by plotting the mutually orthogonal real and imaginary components of the complex frequency domain spectrum against each other. A DISPA plot then represents the projection of the complex frequency domain spectrum onto a third plane which is mutually orthogonal to both planes containing the real and imaginary components. This relationship is illustrated in Figure 4. Simply stated the DISPA plot is the projection of the complex frequency domain spectrum onto a plane perpendicular to the frequency axis. With this picture of the complex spectrum in mind, the properties of DISPA plots and spectra can be visualized somewhat differently. The magnitude value associated with a particular frequency is simply the perpendicular distance between the frequency axis and the complex frequency spectrum. Phase errors serve to rotate entire complex spectrum about the frequency axis.
Fig. 4. The DISPA plot, the real, and the imaginary spectra are mutually orthogonal projections of the complex frequency domain spectrum.
3. GENERALIZED EXPRESSION FOR DISPA PLOT

Equation [1.21] derived by Marshall for the DISPA plot, is not general since it only correctly applies to spectral data without phase error. Properties number 3 and number 5 from the above list of properties of DISPA plots can be combined to obtain a more general expression for DISPA plots. The mathematical expression for a circle centered at \((x_0, y_0)\) with radius \(r\) is given by

\[
(x-x_0)^2 + (y-y_0)^2 = r^2. \tag{1.20}
\]

Marshall's expression for a DISPA plot

\[
\left(\frac{A(\omega)}{2}\right)^2 + (D(\omega))^2 = \left(\frac{\tau}{2}\right)^2 \tag{1.21}
\]

describes a circle with radius \(\tau/2\) centered at \((\tau/2, 0)\). As the DISPA circle is rotated by phase error \(\phi\) the center of the DISPA circle will trace out a second circle with radius \(\tau/2\) but centered at the origin, (0,0). Stated in another way, regardless of the phase the center of the DISPA circle is described by the relationship

\[
x^2 + y^2 = r^2 \tag{1.22}
\]

where \(r = \tau/2\). Any point, \((x,y)\), on this second circle can be expressed in terms of \(r\), the radius, and \(\phi\), the phase angle.

\[
x = r \cos(\phi) \tag{1.23}
\]
\[
y = r \sin(\phi) \tag{1.24}
\]

The center of the DISPA circle for any phase \(\phi\) is then given by the coordinate pair \(\left(\frac{\tau}{2} \cos(\phi), \frac{\tau}{2} \sin(\phi)\right)\). The DISPA circle is then represented by the expression
where \( R(\omega) \) and \( I(\omega) \) are the real and imaginary components respectively.

Substituting the expressions for \( R(\omega) \) and \( I(\omega) \) from Equations [1.14] and [1.15] into the above equation for the generalized DISPA plot

\[
\left[ A(\omega) \cos(\omega t_d) - D(\omega) \sin(\omega t_d) - \frac{\tau}{2} \cos(\omega_0 t_d) \right]^2 + \left[ A(\omega) \sin(\omega t_d) + D(\omega) \cos(\omega t_d) - \frac{\tau}{2} \sin(\omega_0 t_d) \right]^2 = \left[ \frac{\tau}{2} \right]^2 \tag{1.26}
\]

Equation [1.26] can be simplified somewhat with the approximation that for narrow lines \( \omega \approx \omega_0 \), meaning the phase is approximately constant as \( \omega t_d \approx \omega_0 t_d = \phi \). \tag{1.27}

Using the above approximation Equation [1.26] can be reduced to

\[
\left[ A(\omega) \cos(\phi) - D(\omega) \sin(\phi) - \frac{\tau}{2} \cos(\phi) \right]^2 + \left[ A(\omega) \sin(\phi) + D(\omega) \cos(\phi) - \frac{\tau}{2} \sin(\phi) \right]^2 = \left[ \frac{\tau}{2} \right]^2 \tag{1.28}
\]

4. POTENTIAL APPLICATIONS OF DISPA PLOTS

Now that the different properties of DISPA plots have been outlined the next step is to propose ways in which these unique properties may be used to solve specific problems.
The first application for DISPA plots using property #2 was proposed by Marshall (18, 19) is to use DISPA plots to examine and discriminate between different kinds of line broadening mechanisms.

Property #1 suggests that DISPA plots may offer a useful criterion to assess instrument parameters. Any parameter which, when it is improperly adjusted, causes the observed line shape to be non-Lorenztian can be monitored by observing the difference between the experimental DISPA plot and the reference circle. The parameter can be optimized by varying its value until the observed difference between the experimental DISPA plot and the reference circle is zero (if the line shape becomes perfectly Lorentzian).

Property #3, rotation of the DISPA plot about the origin by phase error, suggests the DISPA plots may be useful for the measurement of phase errors in the frequency domain spectra. The phase error for a given spectral peak can essentially be determined by measuring the angle by which the DISPA plot is rotated about the origin.

Property #4, namely that the square root of the area inside the DISPA plot is proportional to the number of species responsible, suggests that DISPA plots may be useful for quantitation. This application would be most beneficial in situations where phase correction of the spectral data is difficult, e.g., for overlapped peaks.

Property #5, circles centered at the origin of the DISPA plot having radii with constant spectral magnitudes, suggests that data
points may be selected regardless of phase for whatever reason by picking them such that they lie beyond some threshold radius.

C. PURPOSE

The purpose of this dissertation is to develop and test various applications of DISPA plots.

Some very specific goals have been established along with the more general purpose of this dissertation. These goals are outlined in the following list.

1. Demonstrate utility of Lorentz line shape analysis via DISPA and RDDISPA plots.
2. Develop a basis useful for the quantitation of results from experimental DISPA and RDDISPA plots.
3. Develop methodology by which spectral line shapes from different sources can be compared.
4. Define/describe those factors which must be considered/controlled if DISPA is to be used to analyze true, correct experimental FT/NMR and FT/ICR line shapes. These factors can roughly be subdivided into 3 general categories:
   a. Instrumental
   b. Experimental
   c. Computational
5. Develop software routines applicable to DISPA line shape analysis of experimental data.
6. Develop experimental methodology necessary for the implementation of line shape analysis via DISPA
7. Assess the different applications of DISPA plots and determine their attributes and deficiencies.

D. ORGANIZATION OF DISSERTATION

Chapter II presents a program for the construction of on-line DISPA and RDDISPA plots on FT/NMR instruments. Along with this program a discussion is made of those factors which need to be controlled so that systematic errors in the construction of DISPA plots can be avoided.

In Chapter III, a test is made of the applicability of DISPA to parameter optimization by attributing deviations from Lorentzian line shapes to errors in the shim current parameters.

Chapter IV develops a new procedure based on the rotation of a DISPA plot to measure spectral phase directly. Variations of this procedure are applied in routines developed in Chapters V and VII for the automatic on-line phase correction of FT/NMR and FT/ICR data. To date no successful routine has been devised for the phase correction of FT/ICR data. Phase correction of FT/ICR data is complicated by a number of factors which are examined in detail in Chapter VI.

In Chapter VIII, DISPA analysis is used to examine the $^1$H decoupled $^{13}$C FT/NMR line shapes of a series of free and cross-linked dextran polymers. This represents the first application of DISPA line shape analysis to $^1$H decoupled $^{13}$C FT/NMR spectra so the purpose here is two-fold. First the nature of the spectral line broadening mechanism is determined and second those factors which must be
controlled to obtain suitable DISPA plots from $^1$H decoupled $^{13}$C FT/NMR spectra are examined.

Program source routines and less essential derivations are included in the appendices at the end of this dissertation.

Table 1 summarizes the different applications of DISPA plots which are examined and indicates which properties are utilized for each application.
### TABLE 1
APPLICATIONS OF DISPA PLOTS EXAMINED IN THIS DISSERTATION

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<thead>
<tr>
<th>General Application</th>
<th>Property Utilized</th>
<th>Specific Application(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameter optimization</td>
<td>DISPA plot is circular for true</td>
<td>Monitor magnetic field inhomogeneity through FT/NMR samples as a function of shim coil</td>
</tr>
<tr>
<td></td>
<td>Lorentzian</td>
<td>current</td>
</tr>
<tr>
<td>Phase measurement</td>
<td>DISPA plot is rotated about the origin by an angle equal to the phase error</td>
<td>i) Automatic phase correction of FT/NMR data (linear phase correction problem)</td>
</tr>
<tr>
<td>Study line broadening mechanisms</td>
<td>DISPA plot deviates from circularity in a predictable fashion based on the line</td>
<td>ii) Automatic phase correction of FT/ICR data (nonlinear phase correction problem)</td>
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<td></td>
<td>broadening mechanism</td>
<td>Determination of line broadening mechanism in $^1$H decoupled $^{13}$C FT/NMR spectra of dextran polymers</td>
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CHAPTER II

CONSTRUCTION OF EXPERIMENTAL DISPERSION VERSUS ABSORPTION PLOTS (DISPA)

A. INTRODUCTION

This chapter is directed primarily toward construction of DISPA plots from FT/NMR data but most of the considerations which are discussed are equally applicable to other forms of microwave or radio spectroscopy for which DISPA plots can be constructed (FT/ICR, ESR, and FT/NQR). The goal of this chapter is to provide the reader with enough familiarity with the potential sources of distortion in construction of DISPA plots to allow them to be averted and/or to recognize and correct them when they do occur.

The program presented here is written in PASCAL and is applicable to the Bruker Aspect-3000 computer found on the Bruker AM-500 and Bruker MSL-300 FT/NMR instruments. The PASCAL program also constructs what are called radial difference DISPA (abbreviated RDDISPA) plots. The procedure for construction of RDDISPA plots is given. By presenting the program for construction of DISPA plots in this way it is hoped that the reader can use the program to its best potential. A survey of the potential sources of DISPA plot misconstruction is given in the last part of this chapter. Application of this program for the construction of DISPA plots are given in the chapters that follow, particularly Chapters III and VIII where a strong emphasis is placed on the actual application of DISPA and RDDISPA plots to line shape analysis.
B. PROGRAM TO CONSTRUCT DISPA AND RDDISPA PLOTS ON BRUKER AM-500 FT/NMR

1. INTRODUCTION

The program discussed here was written for use on a Bruker AM-500 FT/NMR. The instrument is equipped with an Aspect 3000 computer, color raster, WATANABE digital plotter and had a CDC "Hawk" disk drive. The Aspect 3000 computer has a 24-bit word length (signed integers span the range -8,388,608 to +8,388,607). ADAKOS version 820601 is used to monitor disk and CPU operations. The program should be applicable to other Bruker instruments configured in a similar fashion.

A simplified flow chart for plotting DISPA and RDDISPA plots is given in Figure 5. The program called "DISPA6" is written in PASCAL. T. C. L. Wang has previously written a PASCAL program for use with the Bruker Aspect 2000 computer suitable for construction of DISPA plots (25). Table 2 outlines the differences between T. C. L. Wang's program and DISPA6. DISPA6 has been designed for operation with multipeak spectra and plotting on a color raster and digital plotter.
Fig. 5. Simplified flowchart for Bruker PASCAL program "DISPA6". Designations for lettered superscripts are provided in the text.
The designations corresponding the lettered superscripts of Figure 5 are given in the following list.

a Peak positions are stored in the integer ARRAY POSITION [I] and peak magnitudes are stored in the real array HEIGHT[I].

b File data is unshuffled and stored in the real arrays A[I] and D[I]. A[I] and D[I] are roughly normalized by dividing by HEIGHT[PEAK] where PEAK is the index of the selected peak. The Magnitude spectrum across the spectral window is calculated on the fly from the rough normalized values of A[I] and D[I] and stored in the real array MAG[I].

c The number of data points fit (N), the order of the polynomial fit (M), and the type of weighting used (unit, statistical, or instrumental) are all optional. Provision is made to allow default values (N=7, M=4, and unit weighting) when optional values are not entered.

d DISPA and RDDISPA plots are constructed directly from the normalized, scaled values in the arrays A[I] and D[I]. Note: the RDDISPA plot is constructed independent of the DISPA plot. Axes for both DISPA and RDDISPA plots are constructed from predetermined coordinates. Coordinates for points on the DISPA reference circle are calculated and scaled as they are needed.
<table>
<thead>
<tr>
<th>T. C. L. WANG'S DISPA PROGRAM</th>
<th>DISPA6</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Normalization is based on polynomial fit to the Absorption mode maximum.</td>
<td>Normalization is based on polynomial fit to the Magnitude mode maximum.</td>
</tr>
<tr>
<td>2. Suitable for single peak spectra. Requires input of peak index.</td>
<td>Suitable for multipeak spectra. Has built in peak picking capability.</td>
</tr>
<tr>
<td>3. Designed for CRT and analog plotter.</td>
<td>Designed for color raster and digital plotter.</td>
</tr>
<tr>
<td>4. Constructs only DISPA plot.</td>
<td>Constructs both DISPA and RDDISPA plot.</td>
</tr>
<tr>
<td>5. Window size is automatically set to 20 line widths on either side of selected peak.</td>
<td>Allows variable window size about selected peak.</td>
</tr>
<tr>
<td>6. Mandatory linear baseline correction.</td>
<td>Optional linear baseline correction (better to rely on instrument's baseline correction routine).</td>
</tr>
<tr>
<td>7. Maximum spectrum size is 8K.</td>
<td>No maximum spectrum size.</td>
</tr>
</tbody>
</table>
T. C. L. Wang's program is designed for single peak spectra and plotting on a CRT and an analog plotter. DISPA6 normalizes the data to the interpolated magnitude mode maximum rather than the absorption mode maximum. This avoids small normalization errors that result when attempting to normalize the data to the apparent maximum of a slightly misphased absorption mode peak. The magnitude mode maximum is equal to that of a perfectly phased absorption mode peak but does not vary with the spectral phase as the absorption mode maximum does.

DISPA6 does not store the complete spectrum; rather a spectral "window" is extracted about the desired peak. It is this spectral window which is normalized, scaled and ultimately plotted. The raw data is never modified. After extraction the data is converted to real values and all normalization and scaling are carried out as floating point operations, thereby minimizing truncation and rounding errors.

2. DATA FILE FORMAT

The Aspect 3000 stores data on disk in blocks of 256 words each. The first block of each data file, called the "-1 sector" contains file and spectral information. The sixth word in the -1 sector holds the spectrum size (number of data points collected for FID). For more details about the organization of the -1 sector the reader should refer to the Bruker PASCAL programming manual (26).
Data is stored in the blocks following the -1 sector. Real and imaginary data as it is stored on disk is "shuffled". The first word in a file after the -1 sector belongs to the real spectrum while the second word belongs to the imaginary spectrum. This alternation continues through the rest of the file. DISPA6 assigns an index of 1 to the first word; this essentially assigns all the words with odd indices to the real spectrum and all words with even indices to the imaginary spectrum (this situation would be reversed had the index of the first word been arbitrarily assigned a value of 0).

3. ZEROING VARIABLES AND DATA ARRAYS

At the start of execution DISPA6 assigns all the variables and arrays equal to zero. This step is essential. The PASCAL compiler assigns the address associated with a particular variable but it does not zero that address. After execution commences the program will use whatever value is stored in the address reserved for a given variable. To show the importance of this zeroing step, take for example the sort routine. The sort routine is used in conjunction with the peak search. It assumes that the intensities stored in the addresses reserved for the peak intensities are all initially zero. If some large value is placed in one of these addresses by some operation previous to execution of the program, and the value is not set to zero by the program, when the sort routine is initiated the nonsense value will be retained by the sort routine and the peak search will fail.
This causes the program to have a "random" bug. The program will only fail if by coincidence some operation prior to execution leaves a value at an address which will cause the program to fail. This type of bug is very difficult to locate because it creates a program that works sometimes and sometimes not. This type of bug does not arise for FORTRAN or BASIC programs because addresses are not reserved for variables unless there is a value available to store in the reserved address.

4. PARAMETER INPUT

DISPA6 request the filename for the data file. Any legitimate filename may be entered with or without a decimal point and a three character alphanumeric extension. A disk device code (=D0, =D1, or =D2) can be attached directly to the end of the file name to specify a disk. Attaching "=D2" to the end of the file name allows the program to read data directly from the user's removable hard disk avoiding the necessity of storing the data in the computer's memory space.

The program request the number of peaks in the spectrum (NPK). It is necessary only to enter the number of peaks (NPK) desired. For instance, if there are 15 actual peaks in the spectrum and the operator enters 3 for the number of peaks, the program will locate only the 3 largest peaks in the spectrum. If a 1 is entered for the number of peaks then the program automatically locates the largest peak in the spectrum. DISPA6 is currently dimensioned to allow 20 peaks (this value can be changed by changing the constant SIZEC in the program).
At this point the program will open the data file and read the file size from the sixth word in the -1 sector. The number of data points in the absorption spectrum is determined by dividing the file size by 2. This value is output to the terminal and the operator is requested to enter the number of points to be retained about the absorption maximum (NPT), which becomes the window size. The largest allowed value is 1024 (which can be changed by changing the value of the constant DIM2 in the program). For most purposes window sizes of 100-150 points were found to be adequate.

5. PEAK SEARCH

The peak search routine utilizes a "floating threshold" which is always set equal to whatever value is stored in the address for HEIGHT[1]. As peaks are located their spectral indices and absorption mode peak maxima are stored in integer array POSITION[I] and the real array HEIGHT[I] respectively. The number of elements in these arrays is set by the number of peaks (NPK) designated for the spectrum. Each time a new peak is located a replacement sort is initiated and the indexes for the peaks are assigned such that the least intense peak is always given an index of 1 and its intensity is stored in HEIGHT[1]. Smaller peaks are always displaced by larger ones. The NPK largest peaks found up to a point are always retained. A peak is defined as any spectral point which is larger than the points on either side of it and larger than whatever value is currently stored in HEIGHT[1] (floating threshold value).
As an aside, note that the peak search routine searches the absorption spectrum, a procedure which is valid only when the absorption spectrum is properly phase corrected. To locate peak positions in an unphased absorption spectrum the phase independent magnitude mode spectrum must be generated and searched. DISPA6 expects to receive properly phased spectra.

6. PEAK SELECTION AND WINDOW EXTRACTION

After completion of the peak search the peak indices, their spectral indices, and their absorption intensities are listed and the operator is requested to enter the desired peak index. The entered peak index is set equal to the variable PEAK. When NPK=1 the peak listing is skipped and POSITION[PEAK] is automatically set to the spectral index of the highest point in the absorption spectrum.

The spectrum window is defined with the relationships:

\[
\text{LEFTPT} = \text{POSITION}[\text{PEAK}] - \text{NPT} + 1 \quad [2.1]
\]
\[
\text{RIGHTPT} = \text{POSITION}[\text{PEAK}] + \text{NPT} \quad [2.2]
\]

where LEFTPT is the first spectrum index extracted and RIGHTPT is the last. Since the real and imaginary data are shuffled in the data file LEFTPT must be odd to insure that the first point extracted belongs to the absorption spectrum and the last point extracted belongs to the dispersion. This is accomplished by checking the value of NPT when it is entered. If the entered value for NPT is even then 1 is added to it to make it negative.
The program returns to the beginning of the data file and reads through the spectrum until it reaches the index LEFTPT (the data files must be read sequentially from disk; random access is not possible). As the data is read it is alternately assigned first to the real array A[I] and then to the real array D[I]. The data is automatically normalized to give the absorption peak a maximum value near 1.0 by dividing the raw data values by HEIGHT[PEAK] prior to their assignment to A[I] or D[I]. This “rough” normalization is necessary to avoid obtaining numbers which are too large in the polynomial regression routine used later for interpolation of the exact position and magnitude of the peak maximum. The magnitude spectrum across the spectral window is calculated on the fly from the normalized values of A[I] and D[I] as they are assigned, and stored in the real array MAG[I]. MAG[I] is used for the final peak normalization.

7. LINEAR BASELINE CORRECTION

Prior to the final normalization the program provides for an optional linear baseline correction across the extracted spectral window. Absorption and dispersion data are baseline corrected separately. The values of 10 points at either end of the selected spectral window are averaged. These average values are assigned to “average” data points in the center of the 10 point region at either end of the spectral window. The slope and intercept for the line connecting the average points at either end of the spectral window are calculated.
The resulting line is used to correct the data across the entire spectral window. Caution must be exercised when making a baseline correction since a systematic error will result should a baseline correction be attempted when the chosen spectral window is too narrow and the wings of the dispersion have not dropped to zero at each end of the window.

8. POLYNOMIAL REGRESSION FIT TO MAGNITUDE MODE SPECTRUM

The program utilizes a polynomial regression routine which fits an mth order polynomial to N points nearest the magnitude maximum. The fitted polynomial has the form:

$$MAG(\omega) = a_0 + a_1\omega + a_2\omega^2 + \ldots + a_m\omega^m$$  \[2.3\]

where $MAG(\omega)$ is the magnitude intensity at the frequency $\omega$. $a_i$ are the polynomial coefficients.

The algorithm for the polynomial regression routine was originally developed by Carnahan et. al. (27) and written into a FORTRAN program by Johnston (28). The matrix inversion portion of the FORTRAN program was translated into PASCAL by Cooper (29), and the remainder was later translated into PASCAL by Wang et. al. (25). The routine has been modified to allow optional inputs for the number of data points fit (N), the order of the polynomial fit (M) and the type of weighting used in the regression fit (unit, statistical, or instrumental). Provision has been made to allow optional default values (N=7, M=4, and unit weighting) in place of the optional inputs.
These default values generally provided satisfactory normalization for the constructed DISPA and RDDISPA plots. When difficulties were encountered with the polynomial regression fit and/or the plot normalization the cause was generally poor digital resolution or poor signal-to-noise for the spectrum.

The polynomial regression routine returns the coefficients for the fitted polynomial stored in the array ALFA[I].

9. POLYNOMIAL INTERPOLATION OF MAGNITUDE MAXIMUM

A procedure developed by Wang (25) was used to interpolate for the exact magnitude maximum. The derived coefficients for the polynomial are used to calculate its intensity at 20 equally spaced points over a specific range. The procedure is iteratively repeated using successively narrower ranges centered around the highest polynomial intensity previously calculated. The iteration continues until the difference between the largest and smallest values calculated for a particular spectral range is less than or equal to $1.0 \times 10^{-6}$. The largest intensity found is used to normalize A[I] and D[I].

10. GRAPHIC OUTPUT

Graphic displays for both the color raster and the WATANABE digital plotter are facilitated by generation of a plot file compatible with the Bruker Graphic Display Package Version 841226.1. DISPA and RDDISPA plots are constructed simultaneously from the normalized, scaled values in A[I] and D[I].
Coordinates for the points on the DISPA reference circle are calculated and scaled as they are needed. The equations:

\[ X = 4.0 \cos(\theta) + 4.0 \]  \[2.4\]
\[ Y = 4.0 \sin(\theta) \]  \[2.5\]

generate a series of points \((X,Y)\) on a circle 4.0 cm in diameter centered at \((4.0, 0.0)\).

The radial angle \(\theta\) is varied by constant increments to generate several points on the reference circle.

Axes for both the DISPA and RDDISPA plots are "hard" drawn, based on predetermined coordinates.

Scaling is achieved using the relationships:

\[ \text{SCALEX} = \frac{\text{SXMANYMAX}}{\text{XMAX} - \text{XMIN}} \]  \[2.6\]
\[ \text{SCALEY} = \frac{\text{SYMANYMAX}}{\text{YMAX} - \text{YMIN}} \]  \[2.7\]

where \(\text{SXMANYMAX}\) and \(\text{SYMANYMAX}\) are the maximum length and height respectively (in centimeters) of the screen, onto which the plot is to be displayed. \(\text{XMAX} - \text{XMIN}\) is the maximum length in centimeters for the plotted spectrum. \(\text{YMAX} - \text{YMIN}\) is the maximum height in centimeters for the plot. The plotting subroutines automatically correct for the difference between the number of vertical pixels and the number of horizontal pixels for a given unit of length.
11. CONSTRUCTION OF RADIAL DIFFERENCE DISPA PLOT (RDDISPA)

For many applications the deviation between the experimental DISPA plot and the reference circle is more useful than the experimental DISPA plot. Referring to Figure 6, the radial difference, $d$, is defined as the difference between a line drawn from the center of the DISPA reference circle to the experimental DISPA curve and the radius of the DISPA reference circle. $d$ is evaluated from the right triangle construction shown in Figure 6 (a). The sides of the right triangle as they are defined in Figure 6 (a) are related by the Pythagorean theorem

$$d + \frac{A_{\text{max}}}{2} = A(\omega) - \frac{A_{\text{max}}}{2} + (D(\omega))^2 \quad [2.8]$$

$$\phi = \tan^{-1} \frac{D(\omega)}{A(\omega) - \frac{A_{\text{max}}}{2}} \quad [2.9]$$

The radial difference DISPA plot (RDDISPA) is obtained by plotting $d$ as a function of $\phi$. $d$ is defined such that it is negative (positive) when the experimental DISPA curve is inside (outside) the reference circle. The corresponding RDDISPA plot is shown in Figure 6 (b).
Fig. 6. A radial difference DISPA plot (RDDISPA) (b) is derived from its corresponding DISPA plot as follows. The radial difference, \( d \), is defined as the difference between a line drawn from the center of the DISPA reference circle to the DISPA curve and radius of the reference circle. \( d \) is readily evaluated from the right triangle construction shown in (a). The RDDISPA plot (\( d \) vs. \( \phi \)) is a convenient display of the difference between a DISPA curve and its reference circle, for use in comparing different DISPA curves. \( d \) and \( \phi \) can be computed directly from \( A(\omega) \) and \( D(\omega) \) without intermediate construction of a DISPA plot—see text.
The RDDISPA plot can be constructed directly from the absorption and dispersion data without the intermediate construction of the DISPA plot using Equations [2.10] and [2.11] to calculate $d$ and $\phi$.

$$d = \left[ A(\omega) - \frac{A_{\text{max}}}{2} \right]^2 + (D(\omega))^2 \right]^{1/2} - \frac{A_{\text{max}}}{2} \quad [2.10]$$

$$\phi = \tan^{-1} \left( \frac{D(\omega)}{A(\omega)} - \frac{A_{\text{max}}}{2} \right) \quad [2.11]$$

Equations [2.10] and [2.11] apply to unnormalized data where $A_{\text{max}}$ is the intensity at the absorption peak maximum (= intensity of magnitude peak maximum). For data normalized to 1.0, $A_{\text{max}} = 0.5$ and Equations [2.10] and [2.11] become [2.12] and [2.13].

$$d' = \left[ (A'(\omega) - 0.5) + (D'(\omega))^2 \right]^{1/2} - 0.5 \quad [2.12]$$

$$\phi' = \tan^{-1} \left( \frac{D'(\omega)}{A'(\omega)} - 0.5 \right) \quad [2.13]$$
The prime notation is used to designate that the data is normalized to give a peak maximum with an intensity of 1.0.

The tangent function is only defined for angles in the range

\[-\frac{\pi}{2} \leq \phi < \frac{\pi}{2}\] radians. The relationships below are used to correct \(\phi\) for radial angles other than \(-\frac{\pi}{2}\) radians.

\[
\text{If } A(\omega) < \frac{A_{\text{max}}}{2} \text{ then } \phi = \phi + \pi \text{ radians } [2.14]
\]

\[
\text{If } A(\omega) > \frac{A_{\text{max}}}{2} \text{ then } D(\omega) > 0 \text{ then } \phi = \phi + 2\pi \text{ radians } [2.15]
\]

For normalized data the above relationships are given by Equations [2.16] and [2.17].

\[
\text{If } A'(\omega) < 0.5 \text{ then } \phi = \phi + \pi \text{ radians } [2.16]
\]

\[
\text{If } A'(\omega) > 0.5 \text{ and } D'(\omega) > 0 \text{ then } \phi = \phi + 2\pi \text{ radians } [2.17]
\]

When plotting the RDDISPA plot "d" is scaled to the magnitude maximum.
C. DISPA6 GENERATES UNDISTORTED DISPA AND RDDISPA PLOTS

It is important to verify that DISPA6 does not alter the experimental data which is plotted. To accomplish this a PASCAL program called FAKER was written to simulate spectra with perfect Lorentzian line shapes (see Appendix F for FAKER source code). Spectra simulated by FAKER are exactly compatible with the Bruker AM-500's software package called DISR86 (later version of the software package DISNMRP). To verify that the line shapes created by FAKER are true Lorentzians DISR86's routine to regression fit a Lorentzian line to experimental data was used to fit a Lorentzian line to the simulated spectra. Figure 7 (a) verifies that the simulated spectra are true Lorentzians. The "+" signs in Figure 7 (a) are simulated data and the solid line is the fitted Lorentzian line shape. Figure 7 (b) shows the DISPA and RDDISPA plots obtained when simulated data set is processed by DISPA6. The DISPA and RDDISPA plots constructed by DISPA6 are clearly undistorted as demonstrated in Figure 7.
Fig. 7. (a) Lorentzian line shape simulated by the routine FAKER. "+" signs are calculated values derived from regression fit of a Lorentzian function to the simulated line shape. (b) DISPA and (c) RDDISPA plots constructed by DISPA6 corresponding to simulated line shape in (a).
D. SOURCES OF MISCONSTRUCTION OF DISPA AND RDDISPA PLOTS

Consider next the various sources of misconstruction of DISPA plots (and indirectly RDDISPA plots). The figures which are provided are based on actual DISPA and RDDISPA plots constructed by DISPA6 using spectra of true Lorentzian lineshapes simulated by FAKER with the specific spectral distortions to be studied. For a few of the drawings (poor S/N, poor digital resolution) it was more convenient to base the drawings on actual experimental data.

Aside from true spectral lineshapes, distortions in DISPA plots can be subdivided into three general categories for discussion. These include distortions associated with 1) the instrument and data collection parameters, 2) actual spectral interferences such as peak overlap or baseline anomalies, and 3) computer related and processing errors such as computational and truncation errors as well as improper scaling of the data prior to plotting.

1. DISTORTIONS RELATED TO DATA COLLECTION

a. POOR DIGITAL RESOLUTION

If there are too few points across the peak then it is poorly defined and the DISPA plot will be irregularly shaped (Figure 8), as when the natural linewidth is very narrow. The problem can be corrected by increasing the number of data points collected and/or decreasing the spectral window which is collected. The time domain signal can be zero filled once to interpolate between data points without distortion of the spectral line shape.
Poor digital resolution also causes problems in scaling the data since it is difficult to properly fit a polynomial to the peak maximum and it is unlikely that there will be a point near the peak maximum.

b. POOR SIGNAL-TO-NOISE RATIO

An absorption-to-mode signal-to-noise ratio (S/N) of at least 20:1 is required for useful results (30). Poor S/N causes difficulties in observing the natural line shape distortions (Figure 9). Natural line shape distortions are generally small and scatter in the data hinders interpretation. For FT/NMR the detection coil must be carefully tuned to optimize S/N. Signal averaging can be used to increase S/N. When signal averaging is too time consuming it is permissible, especially for relatively broad lines, to multiply the time domain signal by an exponential to enhance S/N at the expense of a very small amount of spectral line broadening and line shape distortion.

c. POOR MAGNETIC FIELD HOMOGENEITY

In FT/NMR the homogeneity of the magnetic field throughout the sample can greatly affect the observed spectra line shape and consequently the shape of the DISPA plot (Figure 10). This effect is discussed in detail in Chapter III. The analogous effect of electric field inhomogeneities on DISPA plots for FT/ICR has not been examined to date.
Fig. 8. Experimental DISPA plot derived for spectral line shape with poor digital resolution. The above DISPA plot corresponds to the \(^1\)H decoupled \(^{13}\)C FT/NMR spectra of methanol in D\(_2\)O obtained by averaging 6099 transients (4.0 \(\mu\)sec pulse width; 11.75 \(T\) field). Digital resolution was 0.470 Hz/pt and the full spectral width at half height was 0.6192 Hz.
Fig. 9. Experimental DISPA plot derived for spectral line shape with poor signal-to-noise ratio. The above plot corresponds to the $^1$H decoupled $^{13}$C FT/NMR spectra for the C6 carbon ($\delta_C$ 65.958 ppm) of G100 Sephadex gel obtained from 18366 32K transients (4.0 $\mu$sec pulse width; 11.75 T magnetic field). Digital resolution was 0.477 Hz/pt and full width at half height was 7.97 Hz. The signal-to-noise ratio was 7.5.
Fig. 10. Experimental DISPA plot corresponding to a spectral line shape obtained under conditions of poor magnetic field homogeneity. The above plot corresponds to the $^1$H FT/NMR spectra of HDO with a trace of CuSO$_4$ to broaden the line shape (FWHH 11 Hz). The spectra was obtained by deliberately misadjusting the z-gradient shim current away from its optimum value.
d. DATA COLLECTION PARAMETERS

The effects of specific data collection parameters for FT/NMR spectra on line shape and DISPA plots are discussed in the final sections of this chapter.

2. SPECTRAL INTERFERENCES

a. BASELINE ERRORS

Baseline anomalies can be identified by careful examination of the DISPA plot. In general baseline errors tend to displace the origin of the experimental DISPA plot away from the origin of the reference circle. Examination of the points near the DISPA plot origin is very useful toward identifying the nature of the anomaly. For instance, a simple constant offset in the absorption (Figure 11) spectrum will displace the DISPA plot's origin horizontally along the absorption axis. A constant offset in the dispersion (Figure 12) spectrum displaces the origin of the DISPA plot vertically along the dispersion axis. A combination of constant offsets in both the absorption and dispersion (Figure 13) generates a DISPA plot with an origin which lies neither on the vertical or horizontal axis.

A linear slope in the absorption baseline generates a DISPA plot which spirals "out" (Figure 14) or "in" depending on whether the baseline slope is positive or negative. The rate of spiraling depends
Fig. 11. A constant offset in absorption displaces the origin of the DISPA plot horizontally along the absorption axis.
Fig. 12. A constant offset in dispersion displaces the origin of the DISPA plot vertically along the dispersion axis.
Fig. 13. A combination of constant offsets in absorption and dispersion disspaces the origin of the DISPA plot away from the origin of the vertical and horizontal axes.
Fig. 14. DISPA plot corresponding to a simulated spectra with a positive linear baseline slope in the absorption. Note the characteristic "spiral": positive slopes spiral "in" while negative slopes spiral "out".
Fig. 15. DISPA plot corresponding to a simulated spectrum with a positive linear baseline slope in the dispersion. Note characteristic "closing" of the DISPA circle upon itself. Negative slopes in the dispersion result in DISPA plots which open up and drop below the lower portion of the reference circle.
on the magnitude of the slope and the width of the spectral peak. The deciding factor is how much the baseline changes from one side of the peak to the other.

A slope in the dispersion baseline distorts the DISPA plot along the vertical direction. A compression of the DISPA plot along the vertical axis occurs when there is a positive slope in the dispersion (Figure 15). The resulting DISPA plot will be non-circular. The points near the origin will actually overlap one another for a large positive slope. This can be envisioned as having the vertical zero rise across the peak. An elongation of the DISPA plot in the vertical direction is observed when the baseline has a negative slope. This can be envisioned as having the DISPA plot's vertical zero drop from one side of the peak to the other.

In practice constant and linear baseline errors in the absorption and dispersion spectra are not serious problems. They are easily recognized and corrected with simple linear baseline correction routines. They are, however, useful models from which to extrapolate to visualization of the DISPA plot distortions observed for peaks over-lapped by other peaks.

d. SPECTRAL OVERLAP BETWEEN PEAKS

The best way to envision the effects of spectral overlap between two peaks on their DISPA plots is to imagine two peaks which are slowly brought closer together, increasing their overlap with each other. One peak becomes the baseline on top of which the other peak is placed. The magnitude of the effect on the DISPA plot reflects the relative
magnitudes of the two peaks which overlap one another. A small peak has a minimal effect on the observed DISPA plot of a larger peak with which it overlaps. For two overlapping peaks of comparable size the effects of spectral overlap are much more pronounced.

The DISPA plot of a peak overlapped by the tail of another peak is very similar to that obtained for a slight baseline slope (Figure 16). An indentation (Figure 17) in the DISPA plot results when the overlap between the two peaks is increased. A "loop" (Figure 18) results when there is a very large amount of spectral overlap but the maxima of the two peaks are still resolved. The DISPA plot of a large spectral peak with a small shoulder will exhibit a complete loop. If the two components overlap one another so extensively as to be unresolvable then the DISPA plot is distorted without loops or indentations.

c. PHASE ERRORS

For convenience, the portion of the DISPA plot very near the experimental origin will be called the "base" of the DISPA plot. The last few points which are plotted just before the spectrum cuts off on either side of the peak will be called the upper and lower base points, referring to the last few experimental points plotted above and below the horizontal axis of the DISPA plot (refer to Figure 19 for clarification).

If the DISPA plot is circular but the region near the base of the plot is not symmetrical about the origin then there is an anomaly in the plot. Often it is difficult to distinguish small phase errors from
Fig. 16. DISPA plot corresponding to a simulated Lorentzian spectral line shape which is slightly overlapped by the tail of an adjacent Lorentzian of equal width and area located at a lower frequency. If the adjacent peak had occurred at a higher frequency the observed distortion would be in the lower portion of the DISPA plot.
Fig. 17. DISPA plot with "indentation" produced when the observed spectral line shape represents the composite line shape formed by two closely spaced Lorentzian components.
Fig. 18. DISPA plot with "loop" corresponding to spectral line shape with small shoulder peak.
small constant errors in the dispersion baseline. If the upper and lower base points lie on the same vertical line with respect to each other then the anomaly is probably due to an offset in the dispersion baseline. If the line connecting the upper and lower base points is not vertical the anomaly is very likely due to a small phase misadjustment (Figure 19). This type of analysis assumes that the digital resolution is sufficiently high that the spectral line shape is well defined. As will be discussed in Chapter IV the DISPA plot for a peak with spectral phase error is rotated about the origin by an angle equal to the phase error.

3. DISTORTIONS RELATED TO PROCESSING OR COMPUTATIONAL ERRORS

a. IMPROPER NORMALIZATION FACTOR

When the experimental data is normalized with the wrong normalization constant the resulting experimental DISPA plot will remain circular but will be too large or too small depending on the normalization factor which is used to normalize the data (Figure 20). This type of error can most easily be identified by observing that the maximum absorption intensity of the experimental plot does not equal the diameter of the reference circle. Improper normalization generally reflects a poor polynomial fit to the magnitude maximum.

b. SCALING ERRORS

When the reference circle is not circular the scaling factors for the x and y coordinates are wrong (Figure 21). This can easily be corrected by measuring the vertical and horizontal diameters of the
Fig. 19. DISPA plot with slight phase error (a) compared to DISPA plot with slight offset error in the dispersion (b). Note that a line constructed between the "base" points in (a) is not vertical but remains symmetrical with relation to the axes origin, while in (b) the line constructed between the "base" points is vertical but symmetric with respect to the origin.
Fig. 20. DISPA plots corresponding to simulated spectra were the normalization factor is too small (inside reference circle) and too large (outside reference circle). Note the plots remain circular but fail to coincide with the reference circle at the absorption maximum.
Fig. 21. DISPA plots obtained with improper x- and y-scale factors are elliptical. Here the x-scaling is correct but the y-scale is compressed.
reference circle and deriving a proportionality constant equal to the ratio of one coordinate to the other. The proportionality constant can then be used to normalize the scale of one coordinate axis to the other.

c. IMPROPER BASELINE CORRECTION

An error will result if the baseline correction is based on two points not lying in the baseline. Selection of a spectral window which is too narrow will cause the wings of the dispersion to be clipped, and the line constructed between the ends of the dispersion at either side of the spectral window will have a slope other than that of the true baseline. When this line is used to baseline correct the spectrum the result is the same as would be predicted for the DISPA plot of a spectrum with a sloping baseline in the dispersion (Figure 22).

d. NORMALIZATION TO APPARENT MAXIMUM OF MISPHASED ABSORPTION PEAK

Normalization of the DISPA plot to the apparent maximum of a significantly misphased absorption peak results in an artificial expansion of the DISPA plot (Figure 23). This results because the point with the greatest horizontal displacement away from the vertical DISPA axis does not lie on the horizontal axis. As shown in Figure 23 the DISPA circle appears to expand from its true size. The DISPA plot remains circular as shown by plotting the DISPA plot of a slightly misphased peak normalized to its apparent maximum intensity. The solution to this problem is to normalize the data based on the phase
Fig. 22. DISPA plot corresponding to a simulated spectrum which was baseline corrected over a frequency range which was too narrow. Consequently the tails of the dispersion component were clipped and the baseline correction was incorrect. This is entirely equivalent to introducing a slope into the dispersion component.
Fig. 23. Expansion of DISPA plot results when the DISPA plot is normalized to the apparent maximum of a significantly misphased absorption peak.
independent magnitude mode intensity (which equals the absorption intensity of the perfectly phased absorption peak).

e. TRUNCATION AND ROUNING ERRORS

Truncation errors have generally not been serious since the 24 bit word size allows for representation of real values over a broad dynamic range. Rounding errors are avoided by using floating point calculations whenever possible.
E. PRACTICAL EXPERIMENTAL CONSIDERATIONS

Several practical experimental factors must be controlled to obtain genuine, reproducible line shapes/DISPA plots/RDDISPA plots. The considerations mentioned below are directed toward the collection of FT/NMR data for the express purpose of spectral line shape analysis. These considerations can be subdivided into three general categories: chemical, instrumental and computational considerations. Most of the computational considerations have already been discussed in the previous sections of this chapter. The chemical and instrumental factors are discussed below.

1. CHEMICAL CONSIDERATIONS

a. DISSOLVED OXYGEN GAS

Oxygen gas dissolved in an NMR sample broadens the observed line by only a few tenths of one Hz and so is important only for very narrow lines where the relative contribution to the total of line broadening due to dissolved oxygen is large (31).

When necessary, dissolved oxygen can be removed by several different procedures. Oxygen free N₂(g) can be bubbled through the sample solution just prior to data collection. A vacuum may be placed on the sample within a desiccator, possibly followed by back filling the desiccator with oxygen free N₂(g). The sample may also be subjected to a series of freeze-thaw cycles, by repeatedly freezing the sample with liquid nitrogen under vacuum and allowing it to melt (32).
b. METAL IONS

Paramagnetic and ferromagnetic ions can dominate the relaxation process and so must be eliminated from the sample. The NMR tubes should be cleaned with concentrated HNO₃, followed by rinsing with 0.1 M EDTA solution and finally, distilled/deionized water. Aqueous samples, when permissible, should be dialyzed against 0.1 M EDTA solution and then deionized water or the appropriate buffer solution (33).

c. SAMPLE VISCOSITY

High sample viscosity symmetrically broadens NMR spectral signals.

d. SAMPLE HOMOGENEITY

The sample should be free of air bubbles or particles which would produce heterogeneous magnetic susceptibility throughout the sample volume (34).

e. SAMPLE VOLUME

Small sample volume offers the distinct advantage in that they require over a smaller volume which the magnetic field homogeneity and sample temperature control. For this reason it is preferrable to use a 5 mm NMR tube when possible. The lower limit for the sample volume is ~0.4 ml, which is roughly the volume necessary to "cover" the probe coil. The upper limit is set by the weight of the sample. If the sample volume is too large, the additional weight of the sample will prevent the air spinner from functioning properly.
f. VORTEX

The sample should be fitted with a vortex plug, especially for lower viscosity solutions and smaller sample volumes. The presence of a vortex in front of the probe coil will result in a non-uniform magnetic susceptibility through the volume of the sample and thus poor lineshape.

2. INSTRUMENTAL CONSIDERATIONS
a. MAGNETIC FIELD HOMOGENEITY

Magnetic field inhomogeneity is the most common source of inhomogeneous line broadening and consequently the least interesting and the one that requires the greatest attention. A number of precautions must be taken to minimize (if not eliminate) the magnetic field inhomogeneity throughout the sample.

The magnet must be very carefully shimmed, initially manually and finally with an automatic shimming procedure. Automatic shimming is generally based on a SIMPLEX routine that attempts to optimize either the $^2$D lock signal intensity or the absolute area under the observed $^1$H free induction decay, FID, (time domain signal) when the FID is nicely shaped (i.e., symmetrical with smooth exponential decay). When shimming is based on maximizing the absolute area under the FID, it is preferable to collect the data using a data set size large enough to avoid truncation of the FID while not so large as to introduce unnecessary noise into the spectrum.
An internal lock (usually 2D) is always preferable since it is difficult to shim the magnet while using an external lock because the magnetic susceptibility of the observed sample and lock sample can be different when an external lock is used.

b. TEMPERATURE VARIATION

Any shift in temperature will lead to a corresponding chemical shift. Temperature variation is a much more serious concern for nuclei requiring long acquisition times (e.g., 13C). Not only is a constant temperature required, but the temperature throughout the sample volume must also be uniform. For this reason it is preferable to use smaller samples when possible since a smaller volume must be temperature controlled. DISPA plots for 13C NMR require 1H decoupling, causing sample heating, requiring temperature regulation (35).

c. ACQUISITION TIME

Shorter acquisition times are preferable since there is less time/opportunity for variation in the magnetic field or sample temperature to occur.

d. SPECTRAL WIDTH

Smaller spectral widths coupled with smaller data set sizes allow shorter total acquisition times, thus reducing problems associated with longer acquisition times.
e. PHASE CORRECTION

Linear phase corrections are applied in FT/NMR. These phase corrections depend on two separate adjustments: a frequency independent term (zero order phase correction), and a frequency dependent term (first order phase correction). Since the phase correction applied at a specific point in the frequency domain spectrum depends on the contributions of two adjustments, phase errors can generate line shape distortions which will in turn generate distorted DISPA plots. DISPA plots are circular only when the observed phase error is frequency independent.

f. LOCK SIGNAL

The lock signal itself is both a direct and an indirect influence on the observed NMR lineshape. Whenever signal averaging is required, the spectrum must be locked to avoid any shift in the observed line position during acquisition. This requirement is particularly important for samples which need longer acquisition times. The width of the lock signal is important since it dictates the accuracy with which the peak position is determined.

Broader lock signals (i.e., D₂O) permit drift in the spectrometer frequency, whereas a narrower lock signal can be more sharply locked, but suffers the disadvantage of being more easily lost. For the reason just described, a narrower lock signal is preferred for line shape analysis. In a more indirect vein, the intensity and phase of the lock signal are related to how efficiently the field homogeneity can be adjusted by the Autoshim routine, when the lock
signal is used for shim correction. In general, a weak lock signal leads to poor field homogeneity and thus poor line shape.

**g. DATA SET SIZE**

Enough data points should be accumulated for each time domain spectrum to avoid truncation of the FID. Thus, a large number of data points should not be collected, because noise will accumulate and will degrade signal-to-noise ratio in the final frequency domain spectrum.

**h. RECEIVER GAIN**

The FID must not be clipped as this will generate distortions in the lineshape which will ultimately generate distorted DISPA plots.

**i. PULSE WIDTH**

The rf excitation pulse width is related to the signal intensity: if the signal intensity is too large, the pulse width must be reduced to avoid clipping the FID resulting in distorted line shapes and DISPA plots, as described immediately above.

**F. CONCLUSION**

A program has been written for the construction of DISPA and RDDISPA plots and a detailed examination of those factors which produced distorted plots has been made.
A. INTRODUCTION

The resonance frequency of a nucleus in an NMR sample is a function of the magnet field strength sensed by the nucleus. When the magnetic field varies throughout the sample a corresponding variation of resonance frequency for single kind of nucleus results. The NMR line shape is broadened leading to a loss in spectral resolution. Current NMR magnets are equipped with small coils called "shim coils" or "shims" used to generate a compensating magnetic field gradient in an effort to obtain a homogeneous magnetic field throughout the sample.

In a homogeneous magnetic field, the response of a rapidly tumbling isolated nuclear spin to a short resonant radiofrequency pulse is a time-domain exponentially damped sinusoid, whose complex Fourier transform yields real and imaginary spectra which may be phased and normalized to give the absorption and dispersion Lorentzian curves, $A(\omega)$ and $D(\omega)$, expressed in Eqs. [3.1] and [3.2].

$$A(\omega) = \frac{\tau}{1 + (\omega_0 - \omega)^2 \tau^2} \quad [3.1]$$
in which $\omega_0$ is the peak center frequency, $\omega$ is the spectral frequency of interest, and $\tau$ is the time constant for exponential damping of the time-domain signal.

Any deviation from Lorentzian shape is reflected as a displacement of the DISPA curve from its "reference" circle, making the DISPA plot a sensitive probe of NMR lineshape (18).

Since static magnetic field gradients are expected to produce non-Lorentzian NMR spectral line shapes, it is desirable to explore the effects on DISPA plots of the $z^n$-gradients ($n = 1, 2, 3, 4, \text{ and } 5$) compensated by "spinning" shim coil currents. Qualitatively, it is anticipated that the field gradients producing an inhomogeneously broadened spectrum should displace a DISPA curve outside its reference circle, and that the magnitude of the displacement should vary directly with the strength(s) of the gradient(s). In order that the DISPA effects be useful for magnet shimming, it is necessary to determine whether or not the direction and magnitude of DISPA displacements can distinguish uniquely between the various $z^n$-gradient contributions to NMR line shape.
Eqs. [3.1] and [3.2] apply only if the static magnetic field, $B_0$, is homogeneous throughout the NMR sample. If the magnetic field varies throughout the sample, then the Larmor condition ($\omega_0 = \gamma B_0$, in which $\omega_0$ is the Larmor frequency, and $\gamma$ is the magnetogyric ratio) converts a distribution in $B_0$ into a distribution in $\omega_0$. The inhomogeneously broadened spectral line is then described as the convolution of a Lorentzian function with the Larmor frequency distribution function (see below).

1. LINE SHAPES IN THE PRESENCE OF STATIC MAGNETIC Z̄-GRADIENTS

For simplicity, the center of the magnetic field distribution from the main magnet coil, the center of symmetry of the distribution in magnetic field associated with each of the shim coils, and the geometric center of the NMR sample are all assumed to coincide at the same point, A (see Figure 24). Let point B represent an arbitrary off-center location within the sample. The magnetic field at A is simply the static magnetic field, $B_0$, provided that the shim coils are adjusted to give zero magnetic field contribution at A. The Larmor frequency for a nucleus at A is given by Eq. [3.3].

$$\omega_{0,A} = \gamma B_0 = \omega_0$$  \[3.3\]

The static magnetic field at point B in Figure 24 may be represented by a spatial function, $(B(x,y,z))$ corresponding to a Larmor frequency, $\omega_{0,B}$. 
Fig. 24. Schematic diagram of the NMR sample region bounded by the receiver coil. For simplicity, the geometric center of the sample region and the center of symmetry of the static magnetic field profile are assumed to coincide at A, the origin of a cartesian axis system. The field at any other point, B, can be described by cylindrical coordinates, $r$, $z$, and $\theta$. $r_{\text{max}}$ is the sample radius, and $z_{\text{max}}$ is the distance from the center of the receiver coil to the top of the receiver coil.
\[ \omega_{0,B} = \gamma(B_0 + B(x,y,z)) \]
\[ = \gamma B_0 + \gamma B(x,y,z) \]
\[ = \omega_0 + \omega(x,y,z) \]
\[ = \omega_0 + K f(x,y,z) \]  

where \( K \) is a scaling factor, and \( f(x,y,z) \) is a function of Cartesian coordinates only.

The observed NMR lineshape is no longer Lorentzian, but consists of a composite sum of a continuous distribution of Lorentzian components, each of the same linewidth \( (2/\tau) \), but with varying resonant frequency, \( \omega_0 \). The DISPA plot is thus no longer circular and is typically displaced outside of its reference circle. Since the resonant frequency \( \omega_0 \) is a function of spatial location, the composite absorption and dispersion line shapes are obtained by integrating over the entire sample volume within the receiver coil (Eqs. [3.5] and [3.6]).

\[
A(\omega) = \int \int \int_{x,y,z} \frac{\tau}{1 + (\omega_0 - \omega + K f(x,y,z))^2 \tau^2} \, dz \, dy \, dx \]  

\[
D(\omega) = \int \int \int_{x,y,z} \frac{(\omega_0 - \omega + K f(x,y,z))^2 \tau^2}{1 + (\omega_0 - \omega + K f(x,y,z))^2 \tau^2} \, dz \, dy \, dx \]

Since the NMR sample and the desired solenoidal static magnetic field distribution are typically cylindrical, it is convenient to convert from Cartesian to cylindrical coordinates (Eqs. [3.7] and [3.8]).

\[
A(\omega) = \int \int \int_{r,z,\theta} \frac{r \tau}{1 + (\omega_0 - \omega + K f(r,z,\theta))^2 \tau^2} \, d\theta \, dz \, dr \]
\[
D(\omega) = \int \int \int \frac{(\omega_0 - \omega + K f(r,z,\theta)) r \tau^2}{1 + (\omega_0 - \omega + K f(r,z,\theta))^2 \tau^2} \, d\theta \, dz \, dr \tag{3.8}
\]

\(r\) is the Jacobian multiplier needed to convert the triple integral from Cartesian to cylindrical coordinates. The limits for the triple integral are: \(0 < \theta < 2\pi \text{ rad}; 0 < r < r_{\text{max}}\), in which \(r\) is the radius of the sample; and \(-z_{\text{max}} < z < +z_{\text{max}}\), in which \(z_{\text{max}}\) is half of the receiver coil height. For a given function, \(f(r,z,\theta)\), describing the variation of the magnetic field within the sample, the integral expressions (Eqs. [3.7] and [3.8]) can be solved to obtain composite absorption and dispersion line shapes, \(A(\omega)\) and \(D(\omega)\), from which theoretical DISPA plots may be generated.

Magnetic field inhomogeneity will be manifested as a displacement between the DISPA plot and its reference circle. Therefore, an appropriate plot for quantitating the displacement is the RDDISA plot described in the previous chapter.

The magnetic field in a modern FT/NMR superconducting solenoid is made homogeneous by passing small currents through as many as 17 different shim coils, each of which generates a weak magnetic field with a characteristic geometry. It is convenient to express the actual static magnetic field distribution by an expansion in the basis set of spherical harmonics (36). Ideally, each shim coil is then designed to compensate for one of the terms of that expansion (see Table 3). Thus, the effect of each shim current can be simulated by substituting the appropriate \(f(r,z,\theta)\) from Table 3 into Eqs. [3.7] and [3.8] to obtain absorption and dispersion spectra (and hence a DISPA plot).
### TABLE 3
MAGNETIC FIELD GEOMETRIES ASSOCIATED WITH COMMON SHIM COILS

<table>
<thead>
<tr>
<th>Common Name for Shim</th>
<th>Cartesian Coordinates $f(x,y,z)$</th>
<th>Cylindrical Coordinates $f(r,z,\theta)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$z^0$</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>$z^1$</td>
<td>$z$</td>
<td>$z$</td>
</tr>
<tr>
<td>$z^2$</td>
<td>$2z^2-(x^2+y^2)$</td>
<td>$2z^2-r^2$</td>
</tr>
<tr>
<td>$z^3$</td>
<td>$z[2z^2-3(x^2+y^2)]$</td>
<td>$z[2z^2-3r^2]$</td>
</tr>
<tr>
<td>$z^4$</td>
<td>$8z^2[z^2-3(x^2+y^2)]+3(x^2+y^2)$</td>
<td>$8z^2[z^2-3r^2]+3r^4$</td>
</tr>
<tr>
<td>$z^5$</td>
<td>$48z^3[z^2-5(x^2+y^2)]+90z(x^2+y^2)^2$</td>
<td>$48z^3[z^2-5r^2]+9zr^4$</td>
</tr>
<tr>
<td>$x^1$</td>
<td>$x$</td>
<td>$r\cos(\theta)$</td>
</tr>
<tr>
<td>$y^1$</td>
<td>$y$</td>
<td>$r\sin(\theta)$</td>
</tr>
<tr>
<td>$zx^1$</td>
<td>$zx$</td>
<td>$zrcos(\theta)$</td>
</tr>
<tr>
<td>$xy^1$</td>
<td>$xy$</td>
<td>$r^2\cos(\theta)\sin(\theta)$</td>
</tr>
<tr>
<td>$x^2y^2$</td>
<td>$x^2-y^2$</td>
<td>$r^2[\cos^2(\theta)-\sin^2(\theta)]$</td>
</tr>
<tr>
<td>$z^2x$</td>
<td>$x[4z^2-(x^2+y^2)]$</td>
<td>$r\cos(\theta)[4z^2-r^2]$</td>
</tr>
<tr>
<td>$z^2y$</td>
<td>$y[4z^2-(x^2+y^2)]$</td>
<td>$r\sin(\theta)[4z^2-r^2]$</td>
</tr>
<tr>
<td>$zxy$</td>
<td>$zxy$</td>
<td>$zr^2\cos(\theta)\sin(\theta)$</td>
</tr>
<tr>
<td>$z(x^2y^2)$</td>
<td>$z(x^2-y^2)$</td>
<td>$zr^2[\cos^2(\theta)-\sin^2(\theta)]$</td>
</tr>
<tr>
<td>$x^3$</td>
<td>$x(x^2-3y^2)$</td>
<td>$r^3\cos(\theta)[\cos^2(\theta)-3\sin^2(\theta)]$</td>
</tr>
<tr>
<td>$y^3$</td>
<td>$y(3x^2-y^2)$</td>
<td>$r^3[3\cos^2(\theta)-\sin^2(\theta)]$</td>
</tr>
</tbody>
</table>

*a Ref. 36*
2. CALCULATION OF THEORETICAL LINE SHAPES FOR "SPINNING" SHIMS

Table 3 shows that the expressions for the magnetic field geometries for the so-called "spinning" shims \( z^1, z^2, z^3, z^4, z^5 \) are independent of \( \theta \), because sufficiently rapid rotation about the \( z \)-axis will average non-radial magnetic field gradients to zero. For the "spinning" shim gradients, \( f(r,z,\theta) \) is independent of \( \theta \), and the \( \theta \) integral in Eqs. [3.7] and [3.8] reduces to

\[
\int_0^{2\pi} d\theta = 2\pi \quad [3.9]
\]

leaving

\[
A(\omega) = 2\pi \int_0^{+z} \int_0^{r_{\text{max}}} \frac{r^\tau}{1 + (\omega_0 - \omega + Kf(r,z))^2} \tau^2 dr \, dz \quad [3.10]
\]

\[
D(\omega) = 2\pi \int_0^{+z} \int_0^{r_{\text{max}}} \frac{r (\omega - \omega + Kf(r,z)) \tau^2}{1 + (\omega_0 - \omega + Kf(r,z))^2} dr \, dz \quad [3.11]
\]

Eqs. [3.10] and [3.11] were solved analytically for some of the spinning shim gradients; for the remaining shims, Eqs. [3.10] and [3.11] were solved numerically by approximating the integrals as double summations (Eqs. [3.12] and [3.13]).

\[
A(\omega) = 2\pi \sum_{-Z}^{+Z} \left[ \sum_0^{r_{\text{max}}} \left[ \frac{r^\tau}{1 + (\omega_0 - \omega + Kf(r,z))^2} \tau^2 \right] \Delta r \right] \Delta z \quad [3.12]
\]

\[
D(\omega) = 2\pi \sum_{-Z}^{+Z} \left[ \sum_0^{r_{\text{max}}} \left[ \frac{r (\omega - \omega + Kf(r,z)) \tau}{1 + (\omega_0 - \omega + Kf(r,z))^2} \tau^2 \right] \Delta r \right] \Delta z \quad [3.13]
\]
For convenience in calculation, \( \omega \) was scaled in units of \((1/\tau)\); i.e. \( \tau \) was set equal to unity in Eqs. [3.12] and [3.13]. For a typical 5mm sample tube, \( 0 < r < 2.5 \) and \( -5 < z < +5 \). In the double sums, \( \Delta r = 2.5/30 \) and \( \Delta z = 10/60 \) to give a total of \( 30 \times 60 = 1800 \) distinct spatial locations. \( A(\omega) \) and \( D(\omega) \) were each evaluated at 75 different frequencies, centered at \( \omega_0 \), with \(-9/2\tau < (\omega_0 - \omega) < +9/2\tau\). The effect of changing the shim current in each coil was simulated by varying \( K \), the relative amplitude of the corresponding term in Table 3.

C. EXPERIMENTAL

Proton FT/NMR spectra were acquired on a Bruker AM-500 instrument operating at 11.74 T, with a \(^{13}\text{C}/^{1}\text{H} \) dual probe. The sample was HDO to which a trace of CuSO\(_4\) had been added in order to give a somewhat broadened (11 Hz full linewidth at half-maximum height) and thus more Lorentzian peak. The magnet was then shimmed until a circular DISPA plot and a flat radial difference plot indicated that the static magnetic field was essentially homogeneous throughout the detected sample region. Spectra were acquired for \( z^1 \) and \( z^2 \) shim coil current values ranging from well below to well above those which gave optimally homogeneous field.

Detection parameters were: excitation pulse width of 3.0 \( \mu \)s (ca. 6° pulse); 12-bit digitizer; 2K free induction decay with a spectral width of 1253 Hz, to give an acquisition period of 0.8172 s and frequency-domain resolution of 1.224 Hz/point; sample spinning
rate, 20 Hz, temperature, 300 K. Each spectrum was obtained from a single transient (not zero-filled), acquired without field-frequency lock. Each time domain free induction decay was baseline-flattened, and otherwise unapodized before Fourier transformation. The same zero- and first-order phase corrections were applied to all transformed spectra. The DISPA program discussed in Chapter II was then used to generate DISPA and RDDISPA plots in-house. Both the real and imaginary spectra were stored on disk.

D. RESULTS AND DISCUSSION

1. THEORETICAL SIMULATIONS

Absorption-mode spectra for increasing "spinning" shim (z, z^2, z^3, z^4, z^5) coil positive currents are shown in Figure 25. Several qualitative features have been confirmed empirically (37). First, spectral linewidth increases as shim current increases; since peak area is proportional to number of spins (i.e., constant), peak height must therefore decrease with increasing field homogeneity. Second, odd-order shims (z, z^3, and z^5) produce symmetrical line shapes, whereas even-order shims (z^2 and z^4) produce asymmetric line shapes. [Shim coil negative currents produce line shapes which are mirror images (left-to-right) of those plotted in Figure 25 for shim coil positive currents.] Third, the shim current required to produce comparable distortion in the absorption-mode peak shape increases by about an order of magnitude for each higher order in the series
expansion (e.g., from \( z^2 \) to \( z^3 \)). Fourth, the highest-order shims tend to distort the spectrum most at its base in the wings, whereas the lower-order shims tend to distort the spectrum most at its top near the center (e.g., compare \( z^2 \) and \( z^4 \) in Figure 25. A further troublesome feature is that even-order shim misadjustment is easily confused with misphasing, because the asymmetric line shape produced by \( z^2 \) and/or \( z^4 \) yields a baseline that appears to slope when the spectrum is correctly phased. Clearly, it is not especially easy to discriminate between various possible shim misadjustments (compare the spectra in the third-from-left column of Figure 25) by inspection of the absorption-mode spectrum.

Figure 26 shows DISPA plots corresponding to the spectra of Figure 25. For each of the "spinning" shims, the displacement of the DISPA curve from its reference circle increases as the shim current increases. As expected, the odd-order shim currents produce symmetrical DISPA plots, whereas the even-order shims give asymmetric DISPA plots. A shim coil negative current produces a DISPA plot which is a mirror image (reflected about the absorption axis) of the DISPA plot obtained for a positive current through the same shim coil. Each of the five "spinning" shim coil currents produces a characteristic DISPA shape: for example, \( z^3 \), \( z^4 \), and \( z^5 \) (rightmost column in Figure 26) are readily distinguished, whereas their corresponding absorption-mode spectra (rightmost column in Figure 3) are quite similar in shape.
Fig. 25. Theoretical absorption-mode spectra for several values of each of the spinning shim coil currents. In each row, field inhomogeneity increases from left to right. Note that the shim current needed to produce the plotted line shape distortions decreases by about one order of magnitude for each successively higher order term.
Increasing Shim Current Error

Fig. 26. Theoretical DISPA plots corresponding to the absorption-mode spectra of Figure 25.
Fig. 27. Theoretical RDDISPA plots corresponding to the DISPA plots of Figure 25. In each case, the vertical axis is scaled in units of the absorption-mode maximum and the horizontal axis is scaled in rad.
the same sense (e.g., positive in this case). For each of the five "spinning" shims, the RDDISPA plots' displacement from its abscissa increases with increasing shim coil current. Odd- and even-order shims are readily distinguished by their respectively symmetrical or asymmetrical plots, and negative shim currents produce RDDISPA curves that are mirror-images (reflected through their midpoint) of those for positive shim currents of the same magnitude.

2. EXPERIMENT VERSUS THEORY

Figures 28-30 show the respective absorption-mode spectra and their corresponding DISPA and RDDISPA curves, for a range of $z$ or $z^2$ shim coil currents. The experimental absorption spectra and DISPA plots are in good qualitative agreement with those computed for theoretically perfect $z$ and $z^2$ terms (Table 3). As predicted, the most circular DISPA plot (with smallest radial-difference displacement) is obtained for the optimum shim currents which produce the narrowest and most symmetrical absorption-mode spectrum. The longer the sample along the $z$-axis, the more important are the $z$-shims in defining the line shape.
Fig. 28. Experimental absorption-mode spectra as a function of $z^1$ (left) or $z^2$ (right) shim coil current. Proceeding from bottom to top, each shim current increases in constant increments, passing through its optimum value at the middle spectrum.
Fig. 29. DISPA plots corresponding to the experimental absorption-mode spectra shown in Figure 28.
Fig. 30. RDDISPA plots constructed for the experimental spectra in Figure 28.
Attempts to quantify experimental higher-order \((z^3, z^4, z^5)\) contributions were much less successful for several reasons. First and most important, the magnetic field profile produced by each of the higher-order coils is not purely \(z^3\) (or \(z^4\) or \(z^5\)) in symmetry; each of these shims produces a field with other \(z^n\) components. Second, it becomes difficult to distinguish between phase misadjustments and shim misadjustments for the asymmetrical line shapes produced by the even-order shims \((z^2\) and \(z^4\)), although phasing becomes more accurate as the field is iteratively shimmed closer to homogeneity. Third, the shim coil maximum currents for \(z^3\), \(z^4\), \(z^5\) were too small to produce easily visible effects on the line shape of the experimental sample (HDO doped with CuSO\(_4\) to give a nearly perfect Lorentzian shape, but at the cost of relatively broad line width of ca. 11 Hz). For example, in order to obtain observable line shape distortions for the \(Z^3\) or \(Z^4\) shim coils, impractically large shim current errors were required; the field generated by the \(Z^3\) and \(Z^4\) shim currents required significant readjustment of \(z\) and \(z^2\), and it became difficult to isolate effects due solely to \(z^3\) and \(z^4\).

3. APPLICATION: SHIM COIL DESIGN:

As noted above, a well-known problem in magnet shimming is that the various shim coil contributions are not independent: e.g., changing \(z^3\) affects \(z\). All manual or automated iterative shim procedures would be greatly simplified if non-interacting shims could be designed. A major problem in shim coil design and testing is that it has proved difficult to determine an accurate actual spatial
profile of the magnetic field produced by a given shim coil. The present results suggest that a DISPA or RDDISPA plot could offer a sensitive measure for discriminating between the various $z^n$ terms, and should therefore offer a good test for evaluating the "purity" with which a given shim approaches the desired field profile for a particular $z^n$ term.

4. APPLICATION: AUTOMATED SHIMMING

Experimental shimming (manual or iteratively automatic) of an inhomogeneous magnetic field is generally based on several successive techniques. Initially, the various shim coil currents are adjusted to give a maximal field-frequency lock signal. Further manual shimming is based on the qualitative appearance (longest and most exponential decay) of a free-induction decay for a sample with potentially very narrow spectral linewidth. Automated shimming can then be based (for example) on optimizing the area under the absolute-value of the free-induction decay. Finally, the high-order shims (e.g., $z^4$, $z^5$) are usually determined by manually optimizing the symmetry and shape of a frequency-domain spectral peak.

Automated shimming based on maximizing the area under an absolute-value FID (38) has the advantage of simplicity, but can be relatively slow because of the simplex procedures required for searching among possible $z^n$ shim current values. Moreover, this method works best when the FID has already been shimmed to near-exponential shape, and the method is not especially sensitive to misadjustments in higher-order shims.
Alternatively, the root-mean-square error from a best-fit lorentzian to a frequency-domain spectral peak could be used as an index for shimming. However, the shim criterion is again based upon a single spectral shape parameter which conveys no direct clue as to which shim current should be changed, and some sort of simplex search would again be required. Moreover, the regressive fit procedure further slows the analysis.

A DISPA plot, on the other hand, is potentially well suited for line shape analysis for several reasons. First, time-consuming regressive fits are avoided, since the spectral data is used directly in assessing the line shape. Second, because a DISPA plot is automatically scaled to the spectral width, the DISPA plot becomes more sensitive to line shape as the field homogeneity improves (and the spectral line width narrows), whereas the area under an absolute-value FID becomes less sensitive to line shape as the homogeneity improves. Third, the shape of the DISPA plot can suggest which shim term most requires adjustment, and in which direction. As for any shimming method, the DISPA-based method will work best when the sample is well-centered in the receiver coil, so that the geometric center of the shim axis system coincides with the center of the sample and the center of symmetry of the $B_0$ field.

RDISPA plots appear to offer the most sensitive assay of shim fields available. Although the radial difference plot parameters ($d$ and $\phi$ in Figure 6) are defined based on the DISPA plot, as indicated in Chapter II they can be derived directly from the absorption and dispersion data.
Finally, it is worth noting that the area under a RDDISPA plot is a composite value which can be minimized to obtain an optimum field homogeneity. Minimization of a function to a known value (zero) is much easier than optimization to an unknown value (e.g., absorption-mode peak height or width, or the area under an absolute-value FID). It may be possible to utilize an expression such as Eq. [3.14] to approximate the area under the radial difference plot directly; the area could then be subjected to a Newton-Raphs type of minimization procedure in order to provide the basis for an automated shim correction routine.

\[
\text{Area} \equiv \frac{1}{2} \sum d_i(\phi_i+1 - \phi_i-1) \quad [3.14]
\]

E. CONCLUSION

DISPA or radial-difference DISPA plots offer a potentially attractive means for recognizing, discriminating between, and quantitating the magnetic field inhomogeneities corresponding to various "spinning" shim coil currents. Although the imperfections in field profiles of presently available shim coils prevent full implementation of DISPA-based automated shimming, the DISPA method can in principle be used to optimize the shim coil designs themselves, thereby improving the future ease of shimming by DISPA or existing methods.
CHAPTER IV
PHASE MEASUREMENT VIA ROTATION OF DISPA PLOTS

A. INTRODUCTION

A time delay between excitation and detection of signals produces phase shifts in the final frequency domain spectrum (for both FT/NMR and FT/ICR). To compensate for the time delay some form of either time shift in the time domain, or phase shift in the frequency domain, must be undertaken. A problem that is frequently encountered is being able to identify how much phase correction is necessary at a given frequency.

This problem is generally addressed by means of iterative procedures (39) which seek to optimize some characteristic or set of characteristics in the real frequency domain spectrum. Several SIMPLEX procedures have been developed in which phase correction is based on: (1) maximizing intensity of the real peak, (2) maximizing the positive area above the baseline in the real spectrum, (3) minimizing the negative area below the baseline in the real spectrum, (4) minimize the difference in baseline intensity on either side of the real spectrum peak or (5) some combination of these. SIMPLEX routines suffer two disadvantages: they tend to be slow because of the iterative procedures necessary to implement them and they may converge to false optima. On most FT/NMR instruments manual phase correction routines coupled with real time spectrum display are used for phase correction; unfortunately this type of phase correction procedure is difficult to automate.
For a single-peak spectrum, phase correction can be achieved in the time domain by dropping points from the time domain signal until the first point remaining is a maximum. This time shift compensates for experimental delays between excitation and detection. The disadvantage to this technique is that there is not necessarily a point collected exactly at the maximum, thus requiring some form of interpolation.

Another time domain oriented technique is autoregression (40). The basic principle is to fit the time domain signal as a linear combination of time varying sinusoids noting that every data point represents a linear combination of all of the signal amplitudes at that time. The parameters amplitude, frequency, damping factor and phase for each component are derived from the regression analysis. The frequency domain spectrum is then constructed from the amplitudes, frequencies and damping factors while the phases are arbitrarily set to zero. This technique has the added advantage of allowing the resolution for specific peaks in the spectrum to be tailored by arbitrarily varying the damping factors (i.e., line widths) associated with specific components; also, spectral editing is possible by simply neglecting to include specific components in the spectral construction. The major disadvantage of this technique is that the number of components must be known beforehand and it is difficult to resolve low intensity signals in the presence of high intensity ones. This type of spectral analysis has only recently been applied to FT/NMR and remains to be applied to FT/ICR.
Phase correction can also be achieved, at least in principle, via deconvolution (41, 42, 43). Convolution of components in one domain can be removed in the Fourier domain by division.

Two techniques have appeared in the literature in which DISPA is applied to phase correction. One technique iteratively minimizes difference between the lobe heights in a radial difference plot (44). The second procedure measures the angle of rotation by a linear algebra approach which determines points at which the DISPA plot intersects the vertical and horizontal Cartesian coordinate axes. A right triangle construction is then generated to determine the rotation of the DISPA plot about the origin (45).
B. ROTATION OF DISPA PLOT

The shift theorem in Fourier analysis states that a shift in the time domain corresponds to a phase shift in the frequency domain (46).

If \( g(t) \xrightarrow{\text{FT}} G(\omega) \) \[4.1\]

then \( g(t-t_0) \xrightarrow{\text{FT}} G(\omega)e^{-i\omega t_0} \) \[4.2\]

\[ \begin{align*}
&= G(\omega)(\cos(\omega t_0)+i\sin(\omega t_0)) \quad [4.3] \\
&= G(\omega)\cos(\omega t_0)-G(\omega)i\sin(\omega t_0) \quad [4.4]
\end{align*} \]

This corresponds to rotation of the complex spectrum about the frequency axis in the complex frequency domain. The real and imaginary components become mixed. The projection onto the real and imaginary plane (the DISPA plot) is rotated along with the complex-frequency spectrum. The DISPA plot then is rotated by an angle equal to the phase error of the complex spectrum. Appendix B contains a proof demonstrating that the angle of rotation of the DISPA plot about the origin is equal to the phase error. Thus the problem then becomes one of measuring the angle of rotation which the experimental DISPA plot has undergone.
A new method is introduced here for the determination of the angle by which the experimental DISPA plot has been rotated. This procedure allows the direct measurement of the apparent phase error of any Lorentzian lineshape.

C. PHASE MEASUREMENT

Two proofs are presented below, the first is a geometric proof to emphasize the basic principles and the second is an analytical geometry proof used to establish the appropriate mathematical relationships for direct utilization of the raw data in phase determination for a spectral peak.

1. GEOMETRIC PROOF

Consider a DISPA circle as shown in Figure 31 rotated by some angle $\phi$ about its origin. Any two chords (chord A and chord B) may be constructed on the DISPA circle with arbitrary placement and arbitrary length. The perpendicular bisectors of the two chords can then be constructed. The intersection of the two perpendicular bisectors is the center of the DISPA circle, which is a point on the rotated DISPA abscissa.

The vertical ($y$) and horizontal ($x$) coordinates at the center of the DISPA circle can then be used to calculate the angle $\phi$, as shown in Equations [4.5] and [4.6].

$$\tan(\phi) = \frac{y}{x} \quad [4.5]$$

$$\Rightarrow \phi = \tan^{-1} \left( \frac{y}{x} \right) \quad [4.6]$$
Fig. 31. The perpendicular bisectors of any two arbitrary chords formed between points on the DISPA circle intersect each other at the center of the DISPA circle.
Since \( \tan^{-1} \left( \frac{y}{x} \right) \) is only defined in the range \(-\frac{\pi}{2} < \phi < \frac{\pi}{2}\) the signs of \( x \) and \( y \) are examined to determine the quadrant in which the phase angle lies. The following relationships allow the measured apparent phase angles to be corrected for their rotation into the four quadrants.

If \( x < 0 \), then \( \phi = \phi + \pi \) radians 2nd and 3rd quadrants [4.7]

If \( x > 0 \) and \( y > 0 \), then \( \phi = \phi + 2\pi \) radians 4th quadrant [4.8]

Otherwise, \( \phi = \phi \) radians 1st quadrant. [4.9]

2. ANALYTICAL GEOMETRY PROOF

Points on a DISPA plot can be represented as Cartesian coordinate pairs where the horizontal coordinate is the real intensity and the vertical coordinate is the imaginary intensity associated with a particular frequency in the spectrum. Figure 32 is identical to Figure 31 except the points are indicated in terms of their absorption and dispersion coordinate pairs \((A_i, D_i)\) where \( i \) refers to the particular spectrum frequency.

The slope of chord A is given by

\[
M_{1,2} = \frac{D_2 - D_1}{A_2 - A_1}. \tag{4.10}
\]

The slope of the perpendicular to any line is given as the negative reciprocal of the line's slope. The slope \( (S_{1,2}) \) of the perpendicular to chord A is given by
Fig. 32. The center of the DISPA circles lies on the axis of rotation and the ratio of its horizontal and vertical coordinates is equal to the tangent of the angle of rotation, $\phi$. 
\[ S_{1,2} = -\frac{1}{M_{1,2}} = -\left(\frac{A_2-A_1}{D_2-D_1}\right) \] 

[4.11]

The coordinates of the midpoint of chord A are obtained by taking the average of the coordinates of the points at the end of chord A to get

\[
\left(\frac{A_1+A_2}{2}, \frac{D_1+D_2}{2}\right).
\]

From the slope of the perpendicular bisector (Equation [4.11]) and a point on it (the midpoint of the chord) the intersection with the vertical coordinate axis can be calculated as shown below

\[
B_{1,2} = \left(\frac{D_1+D_2}{2}\right) + \left(\frac{A_2-A_1}{D_2-D_1}\right)\left(\frac{A_1+A_2}{2}\right).
\]

[4.12]

The equation of the perpendicular bisector for chord A is given by

\[
y = S_{1,2} x + B_{1,2}
\]

[4.13]

\[
y = \left[-\frac{A_2-A_1}{D_2-D_1}\right] x + \left[-\frac{D_1+D_2}{2} + \frac{A_2-A_1}{D_2-D_1} \frac{A_1+A_2}{2}\right].
\]

[4.14]
By symmetry an equivalent expression is obtained for the perpendicular bisector of chord $B$.

$$y = S_{3,4}x + B_{3,4} \quad [4.15]$$

$$y = \left[ -\left( \frac{A_4-A_3}{D_4-D_3} \right) \right] x + \left[ \frac{D_3+D_4}{2} \right] \left( \frac{A_4-A_3}{D_4-D_3} \right) \left( \frac{A_3+A_4}{2} \right) \quad [4.16]$$

The two perpendicular bisectors have a point in common, $(x, y)$, which is obtained by solving them simultaneously. The set of simultaneous equations

\[
\begin{align*}
y &= S_{1,2}x + B_{1,2} \quad [4.17] \\
y &= S_{3,4}x + B_{3,4} \quad [4.18]
\end{align*}
\]

is obtained. Rearranging the above expressions the following system of simultaneous equations is obtained

\[
\begin{align*}
B_{1,2} &= 1 \cdot y - S_{1,2} \cdot x \quad [4.19] \\
B_{3,4} &= 1 \cdot y - S_{3,4} \cdot x \quad [4.20]
\end{align*}
\]

The system of equations can be expressed in matrix form to obtain

\[
\begin{bmatrix}
B_{1,2} \\ B_{3,4}
\end{bmatrix} =
\begin{bmatrix}
1 & -S_{1,2} \\ 1 & -S_{3,4}
\end{bmatrix} \begin{bmatrix}
y \\ x
\end{bmatrix} \quad [4.21]
\]

Cramer's rule can be used to obtain expressions for $x$ and $y$.

$$y = \frac{B_{1,2}(S_{3,4}) - B_{3,4}(S_{1,2})}{1 \cdot (S_{3,4}) - 1 \cdot (S_{1,2})} \quad [4.22]$$
This derivation may be carried one step further to calculate the actual phase angle, as below.

\[y = \frac{B_{3,4} \cdot S_{1,2} - B_{1,2} \cdot S_{3,4}}{S_{1,2} - S_{3,4}}\]  \hspace{1cm} [4.23]

\[x = \frac{B_{3,4} - B_{1,2}}{S_{1,2} - S_{3,4}}\]  \hspace{1cm} [4.24]

The "average" phase angle (indicated by a bar over \(\tan(\phi)\)) derived from several pairs or chords is given by

\[\bar{\tan}(\phi) = \frac{1}{N} \sum_{j=1}^{N} \left[ \frac{B_{3,4}(S_{1,2}) - B_{1,2}(S_{3,4})}{B_{3,4} - B_{1,2}} \right]_j\]  \hspace{1cm} [4.27]

where \(N\) is the number of pairs of chords and "j" designates a particular pair. Unfortunately, the division of \(y\) by \(x\) results in the loss of sign information necessary to determine which quadrant the phase angle is in.
D. APPLICATION TO EXPERIMENTAL DATA

More than one pair of chords can be constructed to provide more than one intersection of perpendicular bisectors, thus allowing an average center to be determined. The minimum number of data points necessary for application of this phase determination procedure is three. Although it is possible to construct a number of chords for given set of data points, there is a limit to the number of unique intersections which may be obtained. The number \( N \) of possible unique intersections, determined by inspection is given by:

\[
N = \sum_{i=1}^{(n-2)} i \quad \text{[4.28]}
\]

where \( n \) is the number of data points selected for a given peak.

The program selects all possible unique chord combinations. For a set of six data points A, B, C, D, E, and F the possible unique chord pairs are:

- A-B:B-C
- B-C:C-D
- B-C:D-E
- C-D:D-E
- D-E:E-F
- A-B:C-D
- A-B:D-E
- A-B:E-F
- B-C:E-F
- A-B:E-F

where "-" denotes points connected by a chord and ":" chord pairs selected for intersection of their perpendicular bisectors. A nested loop is used to select all the possible chord pairs (Figure 33).
Fig. 33. Flowchart with nested loop used to select all possible unique chord pairs for use in the phase measurement.
E. SELECTION OF DATA POINTS

Although it is theoretically possible to use all of the points in the DISPA plot, it is generally advantageous to select only those points which have magnitudes >60% maximum magnitude, primarily to avoid glancing intersections as indicated in Figure 34. Glancing intersections occur from the intersection of perpendicular bisectors of chords on either side of the DISPA circle. Small errors in their slopes can result in large errors of their intersection as indicated by the dotted lines.

Points can be selected above any threshold fraction, f, of the magnitude maximum. By using the relationship

\[ C = \sqrt{f \cdot \text{MAG}_{\text{max}}} \]  \hspace{1cm} [4.29]

where \( C \) is the magnitude associated with a specific data point and \( \text{MAG}_{\text{max}} \) is the maximum magnitude. This relationship was derived from the right triangle construction in Figure 35. Concentric circles about the origin of a DISPA plot are circles with radii of constant spectral magnitudes.

From the smaller triangle in Figure 35

\[ r^2 = (A-r)^2 + B^2 \]  \hspace{1cm} [4.30]
\[ B^2 = r^2 - (A-r)^2 \]  \hspace{1cm} [4.31]

From the larger triangle in Figure 35

\[ C^2 = A^2 + B^2 \]  \hspace{1cm} [4.32]
\[ = A^2 + (r^2-(A-r)^2) \]  \hspace{1cm} [4.33]
\[ = A^2 + r^2 - (A^2-2Ar+r^2) \]  \hspace{1cm} [4.34]
\[ = 2Ar \]  \hspace{1cm} [4.35]
Fig. 34. When the chosen chords are near parallel to each other, their corresponding perpendicular bisects intersect in almost a "head-on" fashion. Small errors in the slopes for these perpendicular bisects can produce large errors in the measured phases. Such glancing intersections can be avoided by requiring that the selected data points lie at least 60% or more of the total distance away from the origin along the axis of rotation. Such a restriction allows only intersections resulting from acute angles.
\[ r = \frac{\text{MAG}_{\text{max}}}{2} \]  \tag{4.36}

\[ C^2 = 2A \frac{\text{MAG}_{\text{max}}}{2} = A \cdot \text{MAG}_{\text{max}} \]  \tag{4.37}

\[ \Rightarrow C = \sqrt{A \cdot \text{MAG}_{\text{max}}} \]  \tag{4.38}

"A" can be expressed in terms of its fraction, f, of the maximum magnitude

\[ A = f \cdot \text{MAG}_{\text{max}} \]  \tag{4.39}

When the expression for A in Equation [4.39] is substituted into Equation [4.38] the following relationship is obtained

\[ C = \sqrt{f \cdot \text{MAG}_{\text{max}}} \]  \tag{4.40}

Points are chosen by selecting only those with magnitudes > C. Equation [4.40] allows points to be selected beyond any arbitrary fraction, f, of the DISPA circle's diameter independent of the spectral phase.
Fig. 35. Data points can be selected beyond a given fraction "A" of the total distance along the axis of rotation by selecting those points with magnitudes greater than or equal to "C".
F. ERRORS IN PHASE MEASUREMENT

As is always the case DISPA is very sensitive to line shape but fortunately the distortions tend to deviate in a symmetrical fashion about the rotated axis. Averaging tends to compensate for biases resulting from line broadening.

Baseline distortions and offsets cause errors because they displace the circle center, generating errors in the measured phase angle. These types of errors are demonstrated in Figures 35-37.

Figure 38 gives a detailed flow chart for the analysis of the phase of a single Lorentzian by calculating the average circle center directly from the experimental data. The spectral peak of interest is stored in a temporary file called WORK(I). Only those points with magnitudes equal or greater than a manually designated fraction (FRACTION) of the maximum magnitude intensity (INTENSITY) are selected. The calculations are the same as those described in Equations [4.5] to [4.40].
Fig. 36. Linear offset in the absorption leads to incorrect phase measurement by displacing the position of the DISPA circle center.
Fig. 37. Linear offset in the dispersion leads to incorrect phase measurement by displacing the position of the DISPA circle center.
Fig. 38. Flow chart corresponding to the subroutine SACPBC.ASC which selects points and measures the phase for a single Lorentzian peak.
Division by zero errors, although not impossible, are very seldom encountered in practice. To avoid division by zero errors, the values of the denominators $D_1-D_2$, $D_3-D_4$ and $S_{1,2}-S_{3,4}$ are checked prior to division. If a zero value is encountered then the corresponding chord pair is ignored, and the algorithm proceeds to the next chord pair.

The FORTRAN subroutine SACPBC.ASC corresponding to the flowchart in Figure 38 is given in Appendix H. The PASCAL equivalent is given in Appendix E.

When applied to simulated data sets having peaks of known phase error, very good results were obtained for the measured phase errors. Typical errors were on the order of $<1^\circ$ error. For simulated data with superimposed noise, the errors in measuring the phase were somewhat larger, typically $<3^\circ$ error but still acceptable.

Application of this routine to measurement of phase errors in real data sets is reserved for Chapters V and VII, and the reader is referred to those chapters for specific application to FT/NMR and FT/ICR data.

G. EFFECT OF SPECTRAL OVERLAP ON PHASE MEASUREMENT

A systematic error in the phase measurement results whenever the peak for which the phase is being measured is overlapped by one or more other peaks. The effect is equivalent to measuring the phase when the spectral baseline is nonlinear and nonzero.
When two peaks overlap one another, the real \( R(\omega) \) and imaginary \( I(\omega) \) spectral amplitudes at any given frequency \( \omega \) are given by:

\[
R(\omega) = \rho_A \frac{\tau_A}{(1+\tau_A^2(\omega-\omega_A)^2)} + \rho_B \frac{\tau_B}{(1+\tau_B^2(\omega-\omega_B)^2)}
\]

\[
I(\omega) = \rho_A \frac{\tau_A^2(\omega-\omega_A)}{(1+\tau_A^2(\omega-\omega_A)^2)} + \rho_B \frac{\tau_B^2(\omega-\omega_B)}{(1+\tau_B^2(\omega-\omega_B)^2)}
\]

in which \( \rho_A \) and \( \rho_B \) are weight factors relating the relative intensities of the two components. The resonance frequencies corresponding to components A and B are \( \omega_A \) and \( \omega_B \) respectively.

The spectral linewidths of the two components are given by

\[
\frac{2}{\tau_A} \quad \text{and} \quad \frac{2}{\tau_B}
\]

The average linewidth for the two peaks is calculated as
The separation between the two peaks is given by $|\omega_A - \omega_B|$. The number of average linewidths separation ($S$) is given in the following equation.

$$
S = \frac{1}{\frac{1}{\tau_A} + \frac{1}{\tau_B}} 
$$

[4.44]

To measure the systematic phase error introduced by peak overlap a BASIC program was written to calculate the real and imaginary intensities as expressed in Equations [4.41] and [4.42]. The program then plots the corresponding theoretical DISPA plots for both peaks. The phases for both peaks were measured manually from the DISPA plots.

Since the phases of the two peaks A and B are zero by definition, the measured phases are the phase errors. The phase errors were measured as a function of decreasing average linewidths of separation (increasing peak overlap). The phase error as a function of peak separation (overlap) is plotted in Figure 39.
Fig. 39. Effect of spectral overlap between adjacent peaks on the measured phases of each peak. Note that peak B is lies at a higher frequency than does peak A. Peak A and B are identical.
Figure 39 was constructed from the overlap of identical peaks with the same linewidths and intensities. As would be expected the phase errors measured for the two peaks are equal in magnitude but opposite in sign. When the two overlapping peaks are not of the same intensity and/or the same width, the symmetry shown in Figure 39 breaks down. When the two peaks exactly overlap (no separation) the phase error is zero.

Figure 39 indicates that systematic errors are large as 3° (or 0.0083 cycles) are obtained for the phase measurements of identical peaks separated by 20 linewidths. When one peak is very much larger than the other peak, the phase measurement of the larger peak is very much less susceptible to systematic errors resulting from the overlap with the smaller peak. Thus, large, well-resolved peaks should be chosen for phase measurement.

When two peaks of nearly the same intensity and linewidth overlap one another the average of their measured phases is zero. Thus, it is reasonable to take the average of two closely overlapping peaks and apply it at an frequency halfway between the two peaks. Such a situation arises, for example, in $^1$H NMR spectra for multiplets such as doublets.

Returning to the analogy of the nonlinear nonzero baseline, when a peak is symmetrically flanked on both sides by peaks of approximately the same intensities and linewidths the symmetry of the phase errors due the overlapping peaks on either side cancels out and
the measured phase is correct. This corresponds to a situation as is
described in Figure 40 where the offsets in the dispersion and
absorption cancel one another. The center of the DISPA circle shifts
but it remains on the proper rotation axis. An example would be a
triplet or pentet in $^1$H NMR.

The arguments made in the two previous paragraphs can be
combined to phase quartets, which can be visualized as a doublet
flanked symmetrically on either side by peaks of the same intensities
and linewidths. The average of the measured phases for the two
central peaks can be applied to the central frequency of the doublet.
The overlapping effects of the two outer peaks can be ignored since
their effects are cancelled by symmetry.

The previous arguments suggest that phase measurements need not
be limited to single isolated peaks but can be applied to
symmetrically split multiplets or overlapping peaks of similar
amplitudes and linewidths as well. For multiplet with an even number
of peaks, the average measured phase of the two central peaks should
be applied to the frequency at the center of the multiplet. For a
multiplet with an odd number of peaks, it is sufficient to measure
the phase of the central peak while ignoring the overlapping peaks on
either side. This removes some of the restrictions as to which peaks
can be selected for phase measurement in a spectrum.
Fig. 40. Phase errors caused by constant offsets in dispersion and absorption can occasionally cancel one another and the measured phase will be correct.
H. CONCLUSION

The groundwork has been developed to allow the measurement of spectral phase based on the intersection of perpendicular bisectors of arbitrary chords constructed between points on the DISPA circle. The procedure has been successfully tested (Chapters V and VII) with both simulated and actual data. Error in such phase measurements generally results because the center of the DISPA circle has been shifted in some way, either by some type of baseline anomaly or by spectral overlap.

Lorentzian line shape is not required per se, provided the line shape remains symmetrical since averaging effectively compensates for symmetrical distortions in the line shape/DISPA plot. Errors in the phase measurement derived from spectral overlap are predictable and can be compensated. The measured phases for symmetrically split multiplies (often seen in FT/NMR) can be compensated for their spectral overlap thus permitting greater flexibility in choosing spectral peaks for phase measurement.
A. INTRODUCTION

In this chapter the DISPA based phase measurement procedure developed in the previous chapter is tested by applying it to measure phases of peaks in FT/NMR spectra. The measured phases of selected peaks are in turn used to determine the proper phase correction for the entire spectrum. This constitutes a convenient and useful routine for automatic phase correction.

Currently several routines are available for phase correction of FT/NMR frequency domain spectra. Commercially available instruments generally provide manual phase correction aided by some type of pseudo-real time graphics display of the spectrum, allowing the effects of the manually entered phase corrections to be visually as they are entered. The phase corrections are entered either numerically through the instrument keyboard or by means of a potentiometer controlling the voltage reaching an analog-to-digital converter. These manual procedures are very effective, but they are not readily automated.

Automated phase correction routines fall into two general categories. In the first category, pre-stored zero and first order phase corrections are automatically applied to phase correct a spectrum after it has been obtained. The phase corrections must be determined beforehand, generally by manually phase correcting another
spectrum collected with identical data collection parameters. The virtue of this approach for automatic phase correction is lost when a series of spectra are collected, in which different data collection parameters are used. For instance, if a series of spectra are collected where the relaxation delays vary the appropriate phase corrections will be different for each spectrum. The second category comprises iterative procedures which attempt to phase correct the spectrum by trying to optimize some characteristic or set of characteristics associated with the spectrum. These procedures are generally SIMPLEX based routines which try to phase correct the spectrum by trying to: (1) maximize the positive area of the real spectrum; (2) minimize the negative area of the real spectrum; (3) minimize the difference between the baseline intensities on either side of an absorption peak; or (4) some combination of these or similar properties associated with the spectrum. SIMPLEX routines are not always successful at selecting the optimum phase corrections, and they tend to be slow due to the iteration required.

A DISPA based phase correction procedure offers a clear advantage over currently available automatic phase correction procedures because it does not require an iterative procedure to determine the appropriate phase corrections. DISPA based phase correction overcomes the problems encountered by other types of automatic phase correction routines. Since DISPA based phase correction determines the appropriate phase corrections for each individual spectrum, automatic data collection routines, which vary the data collection
parameters between spectra, can be executed without additional
difficulties for phase correction. DISPA based phase correction
does not require iteration, nor is its success reliant upon
optimization of any particular set spectral characteristics.

Difficulties anticipated for DISPA based phase correction are those
outlined in the previous chapter, which represent systematic errors
in the phase measurement stemming either from problems of spectral
overlap and/or baseline distortions. These problems can easily be
overcome by appropriate peak selection, and/or baseline correction.

B. THEORY

The phase across an FT/NMR spectrum typically increases linearly
as a function of frequency. Measurement of the phase at any two
frequencies in the spectrum thus allows the phases at all the other
frequencies to be interpolated/extrapolated linearly. Phase
measurement at a given frequency can be accomplished by measuring the
angle of rotation of a DISPA plot about its origin at that
frequency.

Referring to Figure 41, $\phi_A$ and $\phi_B$ represent the measured
phases for two selected peaks A and B. The spectral point indexes
are $I_A$ and $I_B$ at the peak maxima for peaks A and B, respectively.
Peak A has been chosen arbitrarily as the zero order peak. The
significance for this designation is made evident later in this
discussion. The frequency increases linearly with the spectrum point
index. This is not an arbitrary assumption; for FT/ICR the frequency
Fig. 41. The phase in an NMR spectrum varies linearly as a function of frequency. The phase values $\phi_A$ and $\phi_B$ are "heights" of the phase function at the spectral indices $I_A$ and $I_B$. The upper line describes the phase error while the bottom line describes the phase correction.
and spectral point indices can be parallel or opposed in direction. The frequency difference between the two peaks is related to the difference between their point indexes \((I_B - I_A)\). The phase difference between peak A and peak B is \((\phi_B - \phi_A)\). The slope of the line connecting the two measured phases is given as

\[
\text{slope} = \frac{(\phi_B - \phi_A)}{(I_A - I_B)}. \tag{5.1}
\]

In constructing an equation for the line passing through the two measured phases it is necessary to obtain a point on the line. Either of the measured phases can conveniently be chosen. For the purpose of discussion, the point \((I_A, \phi_A)\) is selected as a pivotal point. So doing, the phase at \(I_A\) will be \(\phi_A\) (the zero order phase correction), and the contribution from the first order phase correction must be set to zero at that point. The equation of the line connecting the two measured phases is

\[
\phi_1 = \phi_A + \frac{(\phi_B - \phi_A)}{(I_B - I_A)} \cdot (I - I_A). \tag{5.2}
\]

Note the second term is forced to equal zero when \(I = I_A\). If peak B had been chosen as the zero order peak, then the equation would be given as

\[
\phi_1 = \phi_B + \frac{(\phi_B - \phi_A)}{(I_B - I_A)} \cdot (I - I_B). \tag{5.3}
\]
The equations [5.2] and [5.3] define the phase development across the FT/NMR spectrum. What is sought is the phase correction across the spectrum. One way to envision phase correction, is to imagine that if a certain phase error rotates the DISPA circle about its origin by a certain angle $\phi_4$, then the phase correction must rotate the DISPA circle back by the same angle. The phase correction is then $-\phi_4$.

After the phase correction has been determined, the phase is corrected using the relationships shown below.

$$\theta_i = -\phi_i \quad [5.4]$$
$$A_i = R_i \cos \theta_i - I_i \sin \theta_i \quad [5.5]$$
$$D_i = R_i \sin \theta_i + I_i \cos \theta_i \quad [5.6]$$

$R_i$ and $I_i$ in equations [5.5] and [5.6] refer to the real and imaginary components at spectrum index $i$. $A_i$ and $D_i$ are the phase corrected absorption and dispersion components. $\theta_i$ is the phase correction.

C. EXPERIMENTAL

Broadband proton decoupled $^{13}$C FT/NMR spectra were acquired on a Bruker AM-500 instrument operating at 11.74 T (125 MHz for $^{13}$C). The sample was a gel prepared by saturating Sephadex G100 in 0.1 M Na$_2$HPO$_4$/D$_2$O buffer (pH 7.80). Sephadex G100 (MW) is a crosslinked dextran polymer obtained commercially from Pharmacia. A trace of methanol ($\delta_c$ 49.3 ppm) was added as an internal reference.
12225 32K transients were averaged for a pulse width of 4.0 μsec (~ 53° flip angle). Prior to Fourier transformation the last 16K data points were dropped from the transient. This improves the signal-to-noise and divides in half the number of data points in the final frequency domain spectrum which must be stored in the computer memory for phase correction. Dropping data points from the end of the transient does not change the phase characteristics of the final frequency domain spectrum, just its digital resolution. The digital resolution of the final frequency domain spectrum was 0.94 Hz/pt. After truncation the time domain data was baseline corrected to give a zero average baseline. The frequency domain spectrum was baseline zeroed and flattened using a linear baseline correction routine. The data was then Fourier transformed and stored on disk as a standard FT/NMR data file.

A PASCAL program for on-line phase correction of FT/NMR data called PHASER was constructed from the principles discussed in the theory section. A simplified flow chart for the phase correction routine is given in Figure 42.

This data file was used as data input for PHASER. PHASER stored the phase corrected spectrum on disk as a new data file compatible with the FT/NMR software package.
Fig. 42. Flow chart for the routine PHASER which performs a linear phase correction of FT/NMR data based on the measured phases of two peaks in the spectrum.
D. RESULTS AND DISCUSSION

PHASER was tested by using it to measure the phases of peaks in simulated spectra with known phase errors and no noise. The measured phases obtained for the simulation were found to be generally less than 0.08 degrees, corresponding to about 0.23% error. The % error is defined as

\[
\% \text{ error} = \frac{(\text{true value} - \text{measured value})}{\text{true value}} \times 100
\]

Figures 43 and 44 show the real and imaginary spectra before and after phase correction. The peaks used for zero and first order phase measurement, A and B respectively, are indicated by small arrows above the peaks. The interpolated phase corrections for the spectral peaks between A and B are quite good. The phase correction extrapolated to peak C was slightly in error.

Peak C was not used here for phase measurement because it was much narrower and the digital resolution of the spectrum was so low that only points above 40% of maximum magnitude could be used in the phase measurement. This was not enough points (translates to phase measurement based on a single intersection) to allow a statistical examination of the final average phase obtained. For comparison peak C was used in place of peak B and the final phase correction was very acceptable. The measured phases were 0.8381 ± 0.005 cycles (= 301.7 ± 9.8 degrees) and 0.8393 ± 0.027 cycles (= 302.1 ± 9.8 degrees) for peaks A and B respectively. The calculated slope between the two peaks (separated by 3244 index pts) is 3.699 x 10\(^{-7}\) cycles/pt.
Fig. 43. Real and imaginary FT/NMR spectra before and after phase correction with PHASER.
Fig. 44. Portion of $^1$H decoupled $^{13}$C spectra of sodium dodecyl sulfate before and after phase correction with PHASER. The spectrum shows how effective PHASER is for the phase correction of spectra with relatively poor signal to noise ratio.
1.332 x 10^{-4} \text{ degrees/pt} \) is statistically insignificant because the two measured phases are well within one complete standard deviation of one another. The error in the phase measurement associated with peak B causes a relatively large uncertainty in the slope measurement.

Figure 45 shows the measured phases from individual intersections (solid lines) as they are computed by PHASER for peaks A and B. Figure 45 also shows the running average phase (dashed lines) as it develops. For both peaks the running average quickly approaches a constant phase value. The scatter for the measured phases associated with peak B \((0.8381 \pm 0.005 \text{ cycles})\) is much worse than the scatter for the measured phases of peak A \((0.8393 \pm 0.027 \text{ cycles})\), because peak B (the smaller of the two) has a poorer signal-to-noise \((S/N = 26)\) than peak A \((S/N = 45)\).

Points lying at 40% or greater of the total DISPA circle diameter or more away from the origin of the experimental DISPA plot were selected. Points selected for the shorter peak B consequently had lower intensities relative to the average noise intensity than did points selected for peak A. This indicates that the fraction of the observed point attributable to noise intensities for points associated with peak B is larger than for peak A. This difference in \(S/N\) for the two peaks translates into a difference in the relative uncertainties for the phase measurements (i.e., as the \(S/N\) of a peak increases so does the certainty of its phase measurement).
Fig. 45. Measured phases for individual intersections (solid lines) and the running average (dashed lines), calculated by PHASER corresponding to the spectra in Figure 43. (a) peak A, (b) peak B, and (c) 5 points nearest maximum of peak B.
PHASER selected 6 points above 40% for peak A and 8 points above 40% for peak B. This occurs simply because peak B is spectrally broader. For the same digital resolution more points are required to define a broad peak than a narrow peak. This may seem trivial except the number of intersections averaged to determine the phase for peak B is over twice that averaged to determine the phase for peak A (21 intersections for peak B, and 10 intersections for peak A). Although more points and more intersections were used for the phase measurement in B the uncertainty is much worse. Closer examination of Figure 45 (b) shows that the largest deviations for peak B occur generally toward either end of the plot. These deviations because of the way the program algorithm picks its chord pairs occurs for intersections which include points farthest from the peak center (maximum). To see the effect of discarding the less intense points the 3 least intense points were dropped from the phase measurement leaving only the most intense points for the phase determination. The calculated average phase (0.8397 cycles) was very close to the calculated average phase originally obtained (0.8393 cycles) but the final standard deviation was much smaller (± 0.0046 cycles versus ± 0.027 cycles). From these results it is clear that it is better to restrict point selection to points near the peak maximum for phase determination even if it means that the number of intersections averaged is reduced.

For convenience of discussion, only one data set was discussed in detail but examination of other data sets has shown that the best
phase correction results are generally obtained when the point selection is restricted to points near the peak maximum. These points are least sensitive to baseline and line shape distortions.

PHASER operates best when the spectrum being phased has good digital resolution, good spectral line shapes (if not Lorentzian at least symmetrical), good signal-to-noise, and at least two peaks which are spectrally isolated. The spectral baseline needs to be flat and zero to avoid systematic errors in phase measurement. as mentioned in the discussion, symmetrically overlapped peaks can be used but isolated peaks are more convenient to use when they are available.

E. CONCLUSION

As described earlier DISPA based phase measurement represents the only procedure which allows direct measurement of spectral phase error. All other phase correction routines rely either on iterative optimization or manual procedures. As expected, the success of DISPA based phase measurement depends strongly on the quality of the spectrum: the phase correction was more successful for better quality spectra (good digital resolution, good S/N, and good line shape). Although averaging several intersections compensates well for scatter in the data, it is more advantageous to restrict the points selected for phase measurement to those points near the peak maximum since these points are generally least affected by baseline and line shape distortions. Restricting the number of points reduces
the number of intersections averaged but the precision of the phase measurement is much improved.
CHAPTER VI

SOURCES OF PHASE ERROR IN FT/ICR SPECTRA

A. INTRODUCTION

In FT/ICR ions are generated and allowed to circulate in a strong magnetic field. The frequency of circulation is inversely proportional to the mass-to-charge ratio and directly proportional to the magnetic field strength.

\[ \omega = \frac{qB}{m} \]  

[6.1]

The resulting circulating charge induces an oscillating charge in the detector plates which is converted to a voltage and then digitized. The time domain signal is Fourier transformed to generate a frequency domain spectrum. Spectral peaks at higher frequencies are associated with ions of smaller mass-to-charge ratios. The angular position of the ion packet in its circular orbit in the FT/ICR cell determines the phase of the oscillating signal induced in the detection electronics.

Current FT/ICR spectra are magnitude mode spectra primarily because of the difficulty in obtaining real and imaginary data via phase correction. Although independent of phase, the magnitude mode spectrum, \( M(\omega) \), defined as

\[ M(\omega) = \sqrt{A(\omega)^2 + D(\omega)^2} \]  

[6.2]
is an inherently broader line shape than the absorption line shape because it represents a mixing of a narrow line shape \( A(\omega) \) and a broad line shape \( D(\omega) \). It is desirable to obtain absorption mode FT/ICR for a number of reasons. Absorption mode FT/ICR offers a theoretical improvement in resolution by a factor of between 2 and \( \sqrt{3} \) for sinc or Lorentz line shapes respectively (42). The predicted gain in resolution is obtainable without a concomitant loss in peak height-to-noise ratio since the signal intensity of a perfectly phased absorption-mode spectrum is exactly equal to the signal intensity of the magnitude-mode signal. Current methods of resolution enhancement (such as exponential multiplication and window apodization techniques) do so at the cost of degraded signal-to-noise ratio.

Absorption-mode FT/ICR offers the additional benefit of simplified quantitation since the number of ions represented by the peak is directly related to the area under the perfectly phased absorption mode peak. Better resolution allows extension of the observable mass range as it becomes possible to resolve higher mass peaks. Foldover peaks resulting as artifacts of the Fourier transformation are more easily detectable in the absorption mode spectra, since foldover peaks have anomalous phases relative to the other peaks in the spectrum. Resolution is proportional to field strength (48). To double the resolution with magnitude mode spectra the field strength must be doubled. For this reason absorption mode FT/ICR offers a financially attractive alternative for improvement of
resolution. Phase correction of the real spectra represents a purely software oriented procedure by which increased resolution is obtained without hardware modification.

For a number of reasons which will be described in detail, it is difficult to phase-correct FT/ICR data. Chapter VII describes a procedure, based on the observed line shape, which has been demonstrated to be useful for the phase correction of FT/ICR data over narrow spectral ranges. The constraint of narrow band widths is not as severe as it may seem, in the sense that generally only narrow bandwidths are of interest when ultra high resolution is required. An additional procedure will be shown to succeed in extending phase correction to slightly wider spectral ranges. This extrapolation is limited in its application, however, since it requires some prior knowledge of the phase wrapping function for the spectrum. Phase wrapping is a term used to describe the accumulation of one or more complete cycles of phase, variation across the spectral bandwidth.

In this chapter a detailed discussion of those factors responsible for the development of phase error in the spectrum is presented. The phase correction is broken down into two general aspects. First, "empirical phase correction" (discussed in this chapter) is based on specific knowledge of the signal transfer characteristics of the instrument itself. Secondly, "line shape based phase correction" (discussed in the following chapter) is based
on phase correcting the observed data after it is has been collected and Fourier transformed, independent of any prior knowledge of the instrumental phase shifts.

B. SOURCES OF PHASE ERROR IN FT/ICR

1. INTRODUCTION

For convenience in future discussion, the term "ion signal" refers to the transient waveform associated with the circulation of a single type of ion within the ICR cell. The image current actually observed represents the sum of all the ion signals coming from all the ions in the ICR cell.

It is important to note that the phase of an ion peak in the final frequency-domain (mass) spectrum is dictated by the phase of the time-domain ion signal actually captured in memory by the analog-to-digital converter (Figure 46). The initial phase of the ion in the ICR cell and the number of cycles that accrue between the time that the ion is excited and the time that the corresponding ion signal is actually digitized by the analog to digital converter is irrelevant since the apparent phase of any ion must be less than one cycle.

However, for comparison of relative phases between different mass peaks in the same spectrum, it is necessary to consider the initial phases of the ions and the number of cycles of phase each ion accrues prior to digitization. The initial phases of different ion signals and the signal transfer characteristics of the instrument dictate the phases of the different ion peaks relative to one another in the final mass domain (frequency domain) spectrum.
Fig. 46. The apparent phase observed for a peak in the frequency domain spectrum reflects the phase of the signal actually captured by the A/D converter associated with that particular peak. It does not indicate the number of total cycles that have accumulated prior to capture by the A/D converter.
Although it may represent several thousand cycles of total phase accumulation, the apparent phase of any given mass peak can never be greater than plus or minus 1 cycle. For this reason, the simple determination of the phases of the ion peaks at different points in the spectrum is not sufficient for description of the phase wrapping function across the spectrum.

In FT/ICR, three basic sources contribute to phase error in the final mass spectrum. These are the excitation function, time delay between excitation and detection, and phase shifts from the detector electronics. Each of these sources of phase error are discussed individually in the following sections.

2. EXCITATION PROCESS

Although the excitation process establishes the initial phase of the ion, the accumulation of phase must progress linearly with time since ions can circulate only at their resonance frequencies.

Two general methods of excitation are available in FT/ICR: single frequency and frequency sweep (also referred to as frequency chirp).

a. SINGLE FREQUENCY EXCITATION

Single frequency excitation is a short sharp burst of radiation at a single frequency, used to excite all the ions simultaneously. The same initial phase is imparted to all the ions. The width of the excitation power spectrum is inversely proportional to the width of
the excitation pulse. The amount of power transmitted to excite the ions is proportional to the area under the excitation pulse.

Ideally, an arbitrarily short burst of radiation could excite an arbitrarily broad range of frequencies (masses) with sufficient power as to be detectable. Unfortunately, the intensity of the pulse becomes prohibitively large for the very narrow pulse widths, which are required to excite broad spectral ranges (\( > 10^4 \, \text{volt over 0.1 \, \mu sec} \) would be required to excite ions in the range 10 a.m.u. < m/e < 500 a.m.u.).

b. FREQUENCY SWEEP EXCITATION

In frequency sweep excitation (48) ions are excited by a linearly increasing frequency ramp, relieving the requirement that the excitation waveform be very intense while still exciting a broad spectral range. The ions are all excited simultaneously and but do not all have the same initial phase.

The linear frequency sweep is described by:

\[
\omega = ct + \omega_0
\]

where \( \omega \) is the frequency of the excitation waveform at time \( t \), \( c \) is the sweep rate (units of frequency/time) and \( \omega_0 \) is the initial frequency at time \( t = 0 \). The phase, \( \phi \), of the excitation waveform corresponding to a particular excitation frequency, \( \omega_0 \), is given by:

\[
\phi = \phi_0 + \omega t
\]
where $\phi_1$ is the initial phase of the excitation waveform at the beginning of the frequency sweep.

The excitation frequency can be expressed as a function of time, $t$,

$$\omega = f(t)$$

$$= ct + \omega_1$$

$$t = \frac{\omega - \omega_1}{c}$$

Substituting equation [6.7] into equation [6.4] gives an expression for the initial phase of the excitation waveform as set by the frequency sweep excitation in terms of the frequency which is excited.

$$\phi_{ex} = \phi_1 + \frac{\omega(\omega - \omega_1)}{c}$$

Equation [6.8] was used to predict the initial phase of the ions by assuming that the circular motion of the ion packet is directly coupled to the oscillations of the excitation waveform. Strictly speaking this is not correct since the initial phase of the ions is obtained from the convolution of the excitation waveform with time:

$$\phi_{ion} = \phi_{1,ion} + \int_0^T \omega(t) \, dt$$

were $\phi_{1,ion}$ and $\phi_{ion}$ is the phase of the ion before and after
excitation. $T$ is the duration of the excitation process. Since the frequency of the excitation waveform varies linearly with time, substitution of equation [6.3] into equation [6.9] gives:

$$
\phi_{\text{ion}} = \phi_{\text{i,ion}} + \frac{T}{0} (ct + \omega_i) \, dt.
$$

[6.10]

Integrating the above expression

$$
\phi_{\text{ion}} = \phi_{\text{i,ion}} + \frac{1}{2} c \cdot T^2 + \omega_i \cdot T
$$

[6.11]

which defines the initial phase of the ion motion in terms of the excitation time, $T$, the initial phase of the ion itself, $\phi_{\text{i,ion}}$, and the initial frequency of the excitation waveform, $\omega_i$.

If the initial phase prior to excitation ($\phi_i$) and the initial frequency prior to excitation are zero, equation [6.8] reduces to:

$$
\phi_{\text{ex}} = \frac{\omega^2}{c}
$$

[6.12]

The initial phase of a given ICR frequency ($\omega_{\text{ex}}$), following the excitation period, is related quadratically to the frequency. For a given frequency the initial phase set by the excitation is directly related to the length of the excitation period (i.e., doubling the sweep rate will cut the initial phase in half) (see Figure 47).
Fig. 47. Theoretical plots indicating the initial phase as a function of frequency, as is established by the frequency sweep excitation. The indicated curves correspond to sweep rates of (a) 0.25, (b) 0.5 and (c) 1.0 MHz/msec respectively.
Fig. 48. Experimental plot verifying the theoretical predictions in Figure 47. The dots were obtained by measuring the frequencies at which zero crossings occurred for the frequency domain spectrum. This spectrum was derived by Fourier transformation of the time domain excitation waveform which was simultaneously detected as it was generated. The solid line corresponds to the theoretical values obtained for a sweep rate of 1.0 MHz/msec.
This relation was verified experimentally (Figure 48) by detecting the time domain excitation waveform simultaneously as it was generated. The time domain waveform was then Fourier transformed and phase accumulation was measured directly by counting the zero-crossings in the frequency domain spectrum. One complete cycle of phase accumulates between each pair of zero crossings.

The derivatives with respect to the frequency and sweep rate yield equations [6.13] and [6.14], which give the rates of change of the initial phase of the ion

\[
\frac{d\phi_{ex}}{d\omega} = \frac{2}{c} \omega \tag{6.13}
\]

\[
\frac{d\phi_{ex}}{dc} = -\frac{\omega}{c^2} \tag{6.14}
\]

with respect to frequency and sweep rate respectively. Equation [6.13] represents the slope of the phase vs. frequency at a given frequency in the spectrum (this represents an estimate of the second order phase correction at a frequency due to the excitation).

The program presented later in this chapter assumes different ions effectively reach resonance absorption at different times during the excitation process they are subject to uniquely different delay times between the time they achieve circular coherence and their ion signals are digitized by the analog to digital converter (Figure 49).
Fig. 49. The phase correction program essentially assumes that different masses achieve circular coherence at different times. This effectively causes each ion to have a different delay time between excitation and detection.
An additional consideration that must be taken into account when determining the initial phase as set by the excitation process is the orthogonality of the excitation plates and the detection plates. The phase of the detected waveform must then be 90° out of phase from that of the exciting waveform (Figure 50). Correction for this constant phase shift, independent of frequency, is simple.

3. TIME DELAYS

The shift theorem in Fourier transform theory states that a delay in the time domain corresponds to a phase shift in the frequency domain. After excitation there is a series of time delays prior to data acquisition. The phase shift that develops during the time delays is a linear function of frequency.

\[ \phi_t = \phi_{\text{initial}} + \omega t \]  

[6.15]

The total phase that develops (accumulates) is directly proportional to the total time between excitation and the data acquisition. Figure 51 shows the different delays involved in excitation and detection and their relative durations.

In Figure 51, TTX shows the delay between the time that an ion actually achieves phase coherence (is excited) and the end of the excitation pulse. DL4 is a time delay between the time when the excitation sweep is turned off and the time that the detection electronics are turned on. DEL is a time delay between the triggering of the detector and the time that the analog to digital converter is turned on. This delay allows the digitizer to settle prior to digitalization.
Fig. 50. The phase of the excitation waveform generated at the excitation plates and the phase of the detected waveform must necessarily be 1/4 cycle (90°) apart because the detection and excitation plates are orthogonal to one another.
Fig. 51. Event diagram associated with the Nicolet FTMS-1000 instrument. Relative durations of the indicated events are approximately correct.
Although as stated previously, the phase accumulation due to time delay is a linear function of the frequency, whose slope depends on the mode of signal detection (direct or heterodyne), the frequency associated with a particular ion signal may be changed between the time that the ion is excited and the time that the corresponding signal is digitized by the analog to digital converter. After the frequency is shifted, the phase accumulation must continue as a function of the new frequency.

In addition to the delays mentioned above, the first 15 data points acquired by the digitizer are discarded (49) (Figure 64), which is equivalent to an additional delay of 14 dwell times prior to data collection. The phase shift that results when 15 data points are dropped (14 dwell times) at the beginning of data digitization depends on the frequency that actually reaches the digitizer. If, as in the case of heterodyne detection, the frequency is transformed to a lower frequency, then phase shift accumulates more slowly over the 14 discarded dwell times.

The dwell time is calculated by dividing the acquisition time by the number of data points (number of samplings) taken for the free induction decay.

\[
\text{Dwell Time} = \frac{\text{Acquisition Time}}{\text{Number of Data Points}}
\]  [6.16]
Drop 15 data points

Fig. 52. The analog-to-digital converter (ADC) discards the first 15 data points (14 dwell times) that it receives. This produces the same effect as a time delay of 14 dwell times prior to capture of the signal by the ADC. The actual phase shift is a function of the frequency which actually reaches the ADC. In heterodyne mode the ion signal associated with a particular mass is shifted to a lower frequency so the corresponding total phase shift will be less.
The phase that accumulates is then given by:

$$\phi = \phi_{\text{initial}} + 14 \cdot \omega \cdot d$$ \[6.17\]

where \(\phi_{\text{initial}}\) is the phase at the moment that the digitizer is turned on, \(\omega\) is the frequency of the ion signal that actually reaches the digitizer, and \(d\) is the dwell time.

4. MODE OF DETECTION

Two modes of detection are available: direct and heterodyne. Figure 53 provides a block diagram of the detector. Depending on the position of the software gate the signal is passed either directly to the analog to digital converter (direct mode), or through a mixer shifting it to lower frequencies and a low pass filter and then on to the analog to digital converter (heterodyne mode).

a. DIRECT MODE

In direct mode, the ICR time-domain signal is sent directly to the analog-to-digital converter. The phase shift from the direct mode electronics was measured by entering signals of known frequency at point A in Figure 53 to mimic the signal coming from the detection plates. The difference in phase between the signal at point A and the signal at point B is then measured directly by means of an oscilloscope. The phase shift due to the direct mode detection electronics was found to be linear (Figure 54) with a slope of 0.22
Fig. 53. Block diagram showing the major components of the Nicolet FTMS-1000 detector.
Fig. 54. Measured phase shifts obtained for the direct mode electronics of the Nicolet FTMS-1000 instrument. The measured phase shifts were made via direct mode detection between points A and B in Figure 53.
cycles/MHz. The phase shift as a function of frequency was measured on two separate occasions with the following results:

<table>
<thead>
<tr>
<th>Slope (Cycles/MHz)</th>
<th>y-Intercept (Cycles)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.218</td>
<td>0.214</td>
</tr>
<tr>
<td>0.225</td>
<td>-0.0667</td>
</tr>
</tbody>
</table>

The different intercepts arise from different cable lengths used to make the two measurements. It, therefore, seems reasonable that the measured slope is correct but the constant offset prevents determination of the true intercept.

To be certain that there were no effects due to the instrument bandwidth, the phase shift for a 1 MHz signal was measured as a function of bandwidth (Figure 55). The instrument bandwidth was found to have no effect on the phase shift characteristics of the instrument.

b. HETERODYNE MODE

In heterodyne mode, the frequencies of the image current are mixed with (beat against) the output of reference oscillator to produce new frequencies representing the sum and difference frequencies between the original image current and the reference frequency. The higher (sum) frequencies are removed by means of a low pass filter. After filtering, the signal is composed of a set of frequencies each of which has a one-to-one correspondence to the actual frequencies derived from the sample signal but shifted to
Fig. 55. Experimental phase shift for a 1.0 MHz signal as a function of bandwidth for direct mode detection for the Nicolet FTMS-1000.
lower frequencies. After the frequency shift the rate of phase accumulation due to time delays is also shifted since lower frequencies accumulate phase delays more slowly as a function of time.

Literature values (50,51) are available for the phase shift as a function of frequency for the signal as it passes through the multiplier (Analog Devices model 429A). These values are presented in Figure 56. Linear regression fit to the plotted values gives a slope of $2.359 \times 10^{-2}$ cycles/MHz and an intercept very near zero of $1.639 \times 10^{-3}$ cycles/MHz.

Figure 56 represents the phase shift for a particular frequency signal as it passes through the multiplier. Essentially the effect is a linear phase shift due to the time delay required for the signal to pass through the device. When two sinusoids, $x$ and $y$, are mixed the resulting waveform is described by (52)

$$ \cos(x) \cdot \cos(y) = \frac{1}{2} \left[ \cos(x + y) + \cos(y - x) \right] . $$

Letting,

$$ x = \omega t + \phi_1 $$

$$ y = \omega_{ref} t + \phi_{ref} $$
Fig. 56. Literature values (50,51) obtained for the phase shift through the multiplier (Analog Devices model 429A) used by the heterodyne detection electronics of the Nicolet FTMS-1000 instrument.
Fig. 57. Published theoretical (53) values for the phase shift as a function of frequency for a 4-pole Butterworth low pass filter as is used by the heterodyne detection electronics of the Nicolet FTMS-1000.
where \( \omega_1 \) and \( \phi_1 \) are the frequency and initial phase for a signal entering the multiplier. \( \omega_{\text{ref}} \) and \( \phi_{\text{ref}} \) are the corresponding frequency and initial phase for the reference signal generated by the frequency synthesizer. When these two signals are mixed according to Equ. [6.18] the resulting signal (53) is given by

\[
a \cdot \cos(\omega_1 t + \phi_1) \cdot \cos(\omega_{\text{ref}} t + \phi_{\text{ref}}) =
\]

\[
= \frac{a}{2} \left[ \cos[(\omega_1 + \omega_{\text{ref}})t + (\phi_1 + \phi_{\text{ref}})] + \cos[(\omega_{\text{ref}} - \omega_1)t + (\phi_{\text{ref}} - \phi_1)] \right] \quad [6.21]
\]

The high frequency components (sum frequencies) are removed by a low pass filter leaving the final term to describe the signal,

\[
\frac{a}{2} \cos[(\omega_{\text{ref}} - \omega_1)t + (\phi_{\text{ref}} - \phi_1)]. \quad [6.22]
\]

The expression inside the square brackets is essentially the phase of the signal coming out of the low pass filter at a time, \( t \). If the time delay through the multiplier is \( t_d \), then the phase of the signal exiting the multiplier is function of the initial phases for both the sample signal and the reference signal as well as a function of difference between the reference frequency and the sample frequency. The total phase shift for the low frequency component exiting the multiplier is given by

\[
(\omega_{\text{ref}} - \omega_1)t_d + (\phi_{\text{ref}} - \phi_1) \quad [6.23]
\]
Figure 57 shows theoretical values obtained from literature (54) for the phase shift characteristics of the 4-pole Butterworth low-pass filter as a function of the cutoff frequency, $f_c$. The function is nearly linear for frequencies below the cutoff frequency (slope $\approx -0.4815$ cycles/$(f/f_c)$, intercept $\approx 0.0180$ cycles).

The absolute frequency associated with a particular ion signal shifts after the multiplier meaning that the phase prior to the multiplier is a function of the natural resonance frequency of the ion. After the multiplier the phase accumulation is a function of the difference between the reference frequency and the ion's natural resonance frequency. As shown in Figure 58 the ion signal in heterodyne mode undergoes several phase shifts as it is transferred from the receiver plate to the analog-to-digital converter. The signal experiences a linear phase shift between the receiver plates and the multiplier; the signal has a linear phase shift as it passes through the multiplier, the phase shift between the multiplier and the low pass filter is linear. The phase shift upon passing through the low pass filter is nonlinear. Finally, the phase shift between the low pass filter and the analog to digital converter is linear.

Attempts to phase data based on empirical knowledge about the instrument are complicated by the additional linear error resulting from the length of the cables used to make the measurement.
Fig. 58. Schematic diagram showing the different sources of phase error as a signal is passed through the FT/ICR detector.
Also in the case of heterodyne detection, the total phase shift through the heterodyne related circuitry is not measureable. Much of the wiring is integral, prohibiting measurement of the exact phase shift, for instance, between the multiplier and the low pass filter or between the low pass filter and the analog-to-digital converter. Admittedly, these phase shift contributions may be small, but even small contributions can lead to relatively large errors in apparent phase of an ion signal.
C. DISCUSSION

Table 4 provides the predicted phase contributions from the various sources of phase error during signal processing. These values are predicted from the various measured and theoretical relationships discussed in the preceding text. For comparison, the predicted phase shift attributable to each source is provided for a 1 MHz signal. The relative phase shift is then given as the fraction of the total phase attributable to a specific source for a 1MHz signal. The total accumulated phase shift is also provided for each step in the signal processing.

From examination of the predicted values in Table 4, for direct mode spectra the largest single contribution to the total phase shift is the total time delay prior to acquisition, accounting for 99.3% of the total phase shift. For heterodyne spectra the major contribution to the total phase shift are the frequency sweep excitation (49.8%) and the total time delay prior to acquisition (49.8%). This would appear to indicate that phase correction could be achieved by correcting for these major contributions and ignoring the smaller ones. Unfortunately, the solution is not that simple because the total phase shift for the direct mode spectra is about 500 cycles and the total phase shift for the heterodyne mode is about 1500 cycles, meaning that one cycle represents 1/500 = 0.2% or 1/1500 = 0.1% of the total observed phase shifts for direct mode and heterodyne modes, respectively. This means that any contribution greater than 0.2% or 0.1% of the total can lead to an error of one or more cycles in the final correction. Any error greater than 1
cycle is significant because, as stated previously, the apparent phase varies only between +1 cycle. Any phase contribution that generates an error representing a significant portion of 1 cycle must then be taken into account making the smaller contributions important. By way of example, note that the predicted phase shift due to the low pass filter is -0.48 cycles, this represents 48% of the apparent phase for that signal in the final spectrum. Although the fractional contribution to the total phase shift is small, the contribution to the final apparent phase shift is relatively large.

Ivan Santos wrote a program (Appendix G) which utilized the above phase shift relationships in an effort to achieve phase correction of FT/ICR spectra. A typical result obtained for this program is shown in Figure 59. As can be seen, although the absorption mode peaks are properly oriented (erect), the baseline is badly distorted; this results when the predicted phase correction is in error by a few cycles for each frequency. The apparent 90° phase shift in the spectrum very probably occurs because the program neglects to correct for the orthogonality between the receiver and detection plates.

Two possible sources of error are indicated by these results: 1) the phase predicted at each data point is incorrect, and/or 2) the exact frequency attributable to a specific data point in the spectrum is incorrect. These are difficult problems to resolve. Since no prior knowledge of the exact phase wrapping function is available function, it is impossible to know if all the sources of phase error
Fig. 59. Typical result obtained by phase correction routine written by Ivan Santos. The spectrum shown corresponds to a mass spectrum of CO$^+$, N$_2^+$, and C$_2$H$_4^+$ ions (all nominal 28 a.m.u.) collected with frequency sweep excitation and heterodyne detection. The apparent phase error of 90° probably results because the routine neglects to correct for the 90° phase shift between the excitation and detection waveforms.
have been taken into account until the proper phase correction has been found experimentally.

The instrument often requires recalibration of frequencies after tuning indicating that small frequency shifts are unavoidable. Any factors responsible for generating frequency shifts in the instrument become important when trying to calculate the exact frequency associated with a specific data point in the spectrum. This is a problem requiring a much more detailed understanding of the ion motion and detector than is presently available. Currently the program attempts to calculate the exact frequency associated with a given data point based on the excitation waveform, a process subject to error. One solution to this problem would be to assign the frequencies for known peak positions manually and have the program interpolate to obtain the appropriate intervening frequencies. This has not been tried.

Some question may arise as to whether the observed baseline distortions could in fact be the result of program errors. This question can not be dismissed entirely but it can be set to rest somewhat by noting that very similar results are obtained when the same phase corrections are applied manually.

It is not possible to generate simulated data to test this program since any proper simulation of the data must account for all sources of phase shift, which are not known.
D. CONCLUSION

Much knowledge has been gained to describe the phase accumulation of ion signals prior to digitization. Although somewhat successful, phase correction of FT/ICR data based purely on empirical knowledge of the phase transfer characteristics of the instrument's detector is still inadequate. A thorough understanding of excitation waveform and the phase transfer characteristics of the detector will probably be required should any future attempts be made to phase correct the data using Fourier deconvolution techniques.
CHAPTER VII
DISPA BASED PHASE CORRECTION OF FT/ICR DATA

A. INTRODUCTION

As described in Chapter VI, a number of problems prevent phase correction of FT/ICR data based on empirical knowledge. This chapter introduces a procedure which utilizes completely directly measured phases to phase correct FT/ICR data. Many of the difficulties encountered in Chapter VI are circumvented. Specifically, these difficulties are the inability to calculate the exact absolute frequency associated with a specific data point, the necessity of knowing the specific signal transfer characteristics of the instrument and a complete and exact knowledge of the phase error contributions of the system.

B. STRATEGY

The strategy adopted to phase correct FT/ICR data begins by determining the apparent phases at selected frequencies in the spectrum. This was accomplished by measuring the rotation of the DISPA plot using the procedure described in Chapter IV and equating the rotation of the DISPA plot of a spectral peak to the apparent phase error associated with the frequency at which the peak occurs. By measuring the phase error occurring at selected peaks throughout the spectrum a sampling of the apparent phase as a function of
frequency is achieved. Figure 60 shows schematically how the rotation of the DISPA plot for different peaks through the spectrum is related to the changing phase through the spectrum.

Once the phase has been sampled as a function of frequency, interpolation is used to determine phases associated with frequencies between the frequencies at which the phases were measured. Two general procedures have been used for phase interpolation. First, the measured values may be used to construct a phase spectrum, pairs \((\omega_i, \phi_i)\). After sampling the phase spectrum the polynomial connecting the sampled points (coordinate pairs \((\omega_i, \phi_i)\)) is then derived. This is accomplished either by solution of simultaneous equations or by polynomial regression fit to the measured values. The generated function of frequency is in turn used for interpolation of the spectrum phase at frequencies between those which have been measured. The interpolated phases are used in conjunction with the phase correction equations:

\[
R_{\text{new}} = R_{\text{old}} \cos (\phi) - I_{\text{old}} \sin (\phi) \quad [7.1]
\]

\[
I_{\text{new}} = R_{\text{old}} \sin (\phi) + I_{\text{old}} \cos (\phi) \quad [7.2]
\]

where \(\phi\) is the interpolated phase defined by the calculated polynomial at the \(i\)th frequency. The derived polynomial represents the measured phase and its negative represents the proper phase correction. This is equivalent to rotating the DISPA plot in a clockwise
direction by $|\phi_1|$ degrees. The phase error causes the DISPA plot to be rotated in one direction so the phase correction must necessarily rotate the DISPA by the same amount but in the opposite direction to undo the effect.

C. FT/ICR PHASE CORRECTION PROGRAM

A FORTRAN program, SACPRC.ASC (Appendix H) based on the strategies described in the previous section has been written to phase correct FT/ICR data. The program is completely on-line and is written explicitly for the Nicolet FTMS-1000 or FTMS-2000 instruments. SACPRC is called as a standard 3 letter command ("PRC") used in conjunction with the normal FTMS software routine.

SACPRC.ASC uses data stored by the standard Nicolet FTMS software. In addition to the main routine, several subroutines are utilized to process the FT/ICR data for phase correction. These subroutines are provided in Appendix H.

The program includes a peak search routine (see Appendix H SACPPI.ASC for source code) which searches for peaks based on the unnormalized magnitude mode intensities. The unphased absorption and dispersion mode data cannot be searched because the observed intensity maxima vary with the phase.
Fig. 60. The phase at selected peaks (frequencies) can be determined by measuring the angle of rotation of the DISPA plots corresponding to the peak (simulated spectra).
Exact peak positions were determined by using the 3-point interpolation procedure developed by Comisarow (55). Exact peak position refers to the exact data point index or fraction of a point index at which the peak maximum occurs. This procedure is incorporated by the procedure SACFRC.ASC (Appendix H).

As described above, the analytical geometry procedure in Chapter IV was used for phase measurement (see Appendix H SACPBC.ASC for source code). Although the phase measurement is based on the rotation of the DISPA plot for a given peak, the actual DISPA plot itself is not constructed, avoiding the necessity of normalizing the data.

Two different routines were written for determination of the phase polynomial. One routine, SACSSE.ASC, calculates the polynomial which solves the set of simultaneous equations corresponding to the measured phases at the selected spectral frequencies. The second routine, SACFIT.ASC, performs an n\textsuperscript{th} order polynomial regression fit to the measured phases as a function of frequency. These procedures will be described and compared in greater detail later in this chapter.
Point selection for the DISPA based phase measurement is carried out by the procedure described on page 112. Only points with magnitude intensities greater than a specified fraction of the peak maximum are used for phase measurement.

All calculations are conducted in the main program and all its subroutines with floating point values in order to minimize potential truncation errors. All phase calculations are in units of cycles and are converted to radians only when they are used in conjunction with trigonometric functions.

D. INTERPOLATION BETWEEN MEASURED PHASES

Two different procedures were developed for interpolation of the phases between the measured phases. As stated previously, these are (1) solution of simultaneous equations, and (2) \( n \)th order polynomial regression fit. To contrast these procedures, their mathematical developments are provided explicitly in the following sections.

1. SOLUTION OF SIMULTANEOUS EQUATIONS

In Figure 61, the frequencies and their corresponding measured phases constitute coordinate pairs of points for some imagined function \( f(\omega) \).

\[
f(\omega_1) = \phi_1 \quad [7.3]
\]

\[
= \alpha + \beta \omega_1 + \gamma \omega_1^2 + \ldots \quad [7.4]
\]
Fig. 61. The frequencies and their corresponding phases can be used as coordinate pairs, \((\omega_i, \phi_i)\), to define the phase spectrum, \(f(\omega)\), as it varies across the spectrum.
As shown in Figure 61, the three frequencies and their measured phases can be used to generate the following system of simultaneous equations:

\[
\begin{align*}
\phi_1 &= \alpha + \beta \omega_1 + \gamma \omega_2^2 \\
\phi_2 &= \alpha + \beta \omega_2 + \gamma \omega_2^2 \\
\phi_3 &= \alpha + \beta \omega_3 + \gamma \omega_3^2
\end{align*}
\]  

[7.5]

In the above system, there are three equations and three unknowns (the coefficients \( \alpha \), \( \beta \), and \( \gamma \)).

In matrix form the system of equations is given by:

\[
\begin{align*}
\phi_1 &= 1 \ \omega_1 \ \omega_1^2 \ \alpha \\
\phi_2 &= 1 \ \omega_2 \ \omega_2^2 \ \beta \\
\phi_3 &= 1 \ \omega_3 \ \omega_3^2 \ \gamma
\end{align*}
\]  

[7.6]

The system of simultaneous equations is solved using Cramer's Rule for the coefficients \( \alpha \), \( \beta \), and \( \gamma \).

\[
\alpha = \frac{\begin{vmatrix}
\phi_1 \ \omega_1 \ \omega_1^2 \\
\phi_2 \ \omega_2 \ \omega_2^2 \\
\phi_3 \ \omega_3 \ \omega_3^2 \\
1 \ \omega_1 \ \omega_1^2 \\
1 \ \omega_2 \ \omega_2^2 \\
1 \ \omega_3 \ \omega_3^2
\end{vmatrix}}{\begin{vmatrix}
1 \ \omega_1 \ \omega_1^2 \\
1 \ \omega_2 \ \omega_2^2 \\
1 \ \omega_3 \ \omega_3^2
\end{vmatrix}}
\]  

[7.7]
Cramer's Rule is entirely general and can be applied to a system of linearly independent equations in $m$ unknowns. For the general system of equations:

\[
\begin{align*}
    a_{11}x_1 + a_{12}x_2 + \ldots + a_{1m}x_m &= b_1 \\
    a_{21}x_1 + a_{22}x_2 + \ldots + a_{2m}x_m &= b_2 \\
    \vdots & \vdots \\
    a_{m1}x_1 + a_{m2}x_2 + \ldots + a_{mm}x_m &= b_m
\end{align*}
\]

or

\[
\sum_{k=1}^{m} a_{1k}x_k = b_1 \quad (i=1, 2, \ldots, m). 
\]
In matrix form
\[
\begin{bmatrix}
a_{11} & a_{12} & \cdots & a_{1m} \\
a_{21} & a_{22} & \cdots & a_{2m} \\
\vdots & \vdots & \ddots & \vdots \\
a_{m1} & a_{m2} & \cdots & a_{mm}
\end{bmatrix}
\begin{bmatrix}
x_1 \\
x_2 \\
\vdots \\
x_m
\end{bmatrix}
= 
\begin{bmatrix}
b_1 \\
b_2 \\
\vdots \\
b_m
\end{bmatrix}
\]  
[7.12]

or \( AX = B \).

\[ X = BA^{-1} \]  
[7.13]

The unknowns \( x_k \) are given by

\[ x_k = \frac{D_k}{D} \]  
[7.14]

where \( D \) is the determinant of the matrix \( A \) and \( D_k \) is the determinant of the matrix formed by replacing the \( k \)th column with column vector \( B \).

Two important points are brought out by the mathematical development of the solution of simultaneous equations. First, the order of the polynomial that is derived for the phase function is set by the number of peaks for which the phase is measured. Second, the derived phase function is guaranteed to have the proper phase only at the frequencies at which the phases are measured, as demonstrated schematically in Figure 62. The dashed line in Figure 62 represents the true function whereas the solid line represents the derived function. The dots represent the phases measured at the indicated
Fig. 62. This schematic diagram describes how the solution of simultaneous equations can only guarantee the correct phase at the frequencies where the phase has been measured (measured phases are indicated by the dots). The function derived by the solution of simultaneous equations (solid line) does not necessarily coincide with the true function describing the phase variation through the spectrum (dashed line).
frequencies. From Figure 62 it is clear that as the true function is sampled more frequently (more peaks are selected) and the distance between the sampled phases (peaks) diminishes, the interpolated function more closely approximates the true function, at the cost of requiring more peaks over the frequency range of interest.

2. POLYNOMIAL REGRESSION

Using the same quadratic functional form as was used in the derivation for the solution of simultaneous equations

\[ \phi_i = a + b \omega_i + c \omega_i^2 \]  \[ 7.15 \]

the quality of the fit is designated by \( \chi^2 \), where \( \chi^2 \) is defined as the sum of the squares of the deviations between the measured values and the calculated values.

\[ \chi = \sum_{i=1}^{n} \frac{\Delta \phi}{\sigma_i} \]  \[ 7.16 \]

\[ = \sum_{i=1}^{n} \left[ \frac{1}{\sigma_i^2} \phi_i - (a + b \omega_i + c \omega_i^2)^2 \right] \]  \[ 7.17 \]
n is the number of frequencies (peaks) at which the phases, $\phi_i$, are measured. The $\sigma_i$'s are standard deviations associated with each measured phase value. For simplicity in the remainder of the derivation, all the summations will be taken from $i = 1$ to $n$.

$$\sum_{i=1}^{n} \sigma_i$$

[7.18]

The best fit to the true phase function is the one that generates the smallest total squared deviation from the measured phase values. Using standard polynomial regression, the partial differentials of total deviation squared are minimized (set equal to zero) with respect to each of the coefficients for the assumed functional form (in this case the assumed functional form is quadratic and the coefficients are $\alpha$, $\beta$, and $\gamma$).

$$\frac{\partial^2}{\partial \alpha} = -2\sum \left[ \frac{1}{\sigma_i^2} \phi_i - (\alpha + \beta \omega_i + \gamma \omega_i^2) \right] = 0 \quad [7.19]$$

$$\frac{\partial^2}{\partial \beta} = -2\sum \left[ \frac{\omega_i}{\sigma_i^2} \phi_i - (\alpha + \beta \omega_i + \gamma \omega_i^2) \right] = 0 \quad [7.20]$$

$$\frac{\partial^2}{\partial \gamma} = -2\sum \left[ \frac{\omega_i^2}{\sigma_i^2} \phi_i - (\alpha + \beta \omega_i + \gamma \omega_i^2) \right] = 0 \quad [7.21]$$
The above partial differential expressions are rearranged to give the following system of simultaneous equations.

\[
\begin{align*}
\sum \left[ \frac{\phi_1}{\sigma_1^2} - \frac{\alpha}{\sigma_1^2} - \frac{\beta \omega_1}{\sigma_1^2} - \frac{\gamma \omega_1^2}{\sigma_1^2} \right] &= 0 \\
\sum \left[ \frac{\phi_1 \omega_1}{\sigma_1^2} - \frac{\alpha \omega_1}{\sigma_1^2} - \frac{\beta \omega_1^2}{\sigma_1^2} - \frac{\gamma \omega_1^3}{\sigma_1^2} \right] &= 0 \quad [7.22] \\
\sum \left[ \frac{\phi_1 \omega_1^2}{\sigma_1^2} - \frac{\alpha \omega_1^2}{\sigma_1^2} - \frac{\beta \omega_1^3}{\sigma_1^2} - \frac{\gamma \omega_1^4}{\sigma_1^2} \right] &= 0 \\
\end{align*}
\]

\[
\begin{align*}
\frac{\phi_1}{\sigma_1^2} &= \alpha \frac{1}{\sigma_1^2} + \beta \frac{\omega_1}{\sigma_1^2} + \gamma \frac{\omega_1^2}{\sigma_1^2} \\
\frac{\phi_1 \omega_1}{\sigma_1^2} &= \alpha \frac{\omega_1}{\sigma_1^2} + \beta \frac{\omega_1^2}{\sigma_1^2} + \gamma \frac{\omega_1^3}{\sigma_1^2} \quad [7.23] \\
\frac{\phi_1 \omega_1^2}{\sigma_1^2} &= \alpha \frac{\omega_1^2}{\sigma_1^2} + \beta \frac{\omega_1^3}{\sigma_1^2} + \gamma \frac{\omega_1^4}{\sigma_1^2} \\
\end{align*}
\]

The matrix form of the system of equations is given below.
\[
\begin{align*}
\sum \frac{\phi_1}{\sigma_1^2} &= n \sum \frac{\omega_1}{\sigma_1^2} \sum \frac{\omega_1^2}{\sigma_1^2} \quad \alpha \\
\sum \frac{\phi_1 \omega_1}{\sigma_1^2} &= \sum \frac{\omega_1}{\sigma_1^2} \sum \frac{\omega_1^2}{\sigma_1^2} \sum \frac{\omega_1^3}{\sigma_1^2} \quad \beta \tag{7.24} \\
\sum \frac{\phi_1 \omega_1^2}{\sigma_1^2} &= \sum \frac{\omega_1^2}{\sigma_1^2} \sum \frac{\omega_1^3}{\sigma_1^2} \sum \frac{\omega_1^4}{\sigma_1^2} \quad \gamma
\end{align*}
\]

Just as Cramer's Rule was used to solve the system of simultaneous equations in the previous section it is again used here. The determinant of the denominator is expressed as \( \Delta \) and is given by the following expression.

\[
\Delta = \begin{vmatrix}
\sum_{i=1}^{n} \omega_i & \sum_{i=1}^{n} \omega_i^2 \\
\sum_{i=1}^{n} \omega_i^2 & \sum_{i=1}^{n} \omega_i^3 \\
\sum_{i=1}^{n} \omega_i^3 & \sum_{i=1}^{n} \omega_i^4
\end{vmatrix} \tag{7.25}
\]

The coefficients are given by the following relationships.

\[
\alpha = \frac{1}{\Delta} \begin{vmatrix}
\sum_{i=1}^{n} \omega_i & \sum_{i=1}^{n} \omega_i^2 \\
\sum_{i=1}^{n} \omega_i \phi_i & \sum_{i=1}^{n} \omega_i^2 \phi_i \\
\sum_{i=1}^{n} \omega_i^2 \phi_i & \sum_{i=1}^{n} \omega_i^3 \phi_i & \sum_{i=1}^{n} \omega_i^4 \phi_i
\end{vmatrix} \tag{7.26}
\]
The same derivation just presented for a quadratic function can be extended to m-dimensional polynomials of the form

$$\phi = A_0 + A_1 \omega + A_2 \omega^2 + \ldots + A_m \omega^m$$  \[7.29\]

where \(n\) is the number of frequencies (peaks) sampled and \(m\) is the order of the polynomial fit to the measured phase values.

The final matrix relationship for the m-dimensional polynomial in Equation [7.29] is given on the following page.
Using Cramer's Rule

\[
\Delta = \begin{vmatrix}
\sum \frac{1}{2} & \sum \frac{\omega_1}{2} & \sum \frac{\omega_1^2}{2} & \sum \frac{\omega_1^3}{2} & \sum \frac{\omega_1^m}{2} \\
\sum \frac{\omega_1}{2} & \sum \frac{\omega_1^2}{2} & \sum \frac{\omega_1^3}{2} & \sum \frac{\omega_1^m}{2} \\
\sum \frac{\omega_1^2}{2} & \sum \frac{\omega_1^3}{2} & \sum \frac{\omega_1^m}{2} & \sum \frac{\omega_1^{m+1}}{2} \\
\sum \frac{\omega_1^m}{2} & \sum \frac{\omega_1^{m+1}}{2} & \sum \frac{\omega_1^{m+2}}{2} & \sum \frac{\omega_1^{m+m}}{2}
\end{vmatrix}
\]

[7.31]
\[
A_0 = \frac{1}{\Delta} \begin{vmatrix}
\sum \frac{\phi_i}{\sigma_1^2} & \sum \frac{\omega_i^2}{\sigma_1^2} & \sum \frac{\omega_i^2}{\sigma_1^2} & \sum \frac{\omega_i^2}{\sigma_1^2} \\
\sum \frac{\phi_i \omega_i}{\sigma_1} & \sum \frac{\omega_i^2}{\sigma_1} & \ldots & \sum \frac{\omega_i^{m+1}}{\sigma_1} \\
\vdots & \vdots & \ddots & \vdots \\
\sum \frac{\phi_i \omega_i^m}{\sigma_1} & \sum \frac{\omega_i^{m+1}}{\sigma_1} & \ldots & \sum \frac{\omega_i^{2m}}{\sigma_1}
\end{vmatrix}
\]
[7.32]
$$A_j = \frac{1}{\alpha} \begin{bmatrix}
n \sum \frac{\omega_1}{\sigma_1} & \sum \frac{\omega_1^2}{\sigma_1} & \ldots & \sum \frac{\omega_1^{j-1}}{\sigma_1} & \sum \frac{\phi_1}{\sigma_1} & \ldots & \sum \frac{\omega_1^m}{\sigma_1} \\
 \sum \frac{\omega_1^2}{\sigma_1} & \sum \frac{\omega_1^3}{\sigma_1} & \ldots & \ldots & \frac{\phi_1 \omega_1}{\sigma_1} & \ldots & \\
 \sum \frac{\omega_1^3}{\sigma_1} & \ldots & \ldots & \ldots & \frac{\phi_1^2 \omega_1^2}{\sigma_1} & \ldots & \\
 \ldots & \ldots & \ldots & \ldots & \ldots & \ldots & \\
 \sum \frac{\omega_1^m}{\sigma_1} & \sum \frac{\omega_1^{m+1}}{\sigma_1} & \sum \frac{\omega_1^{m+2}}{\sigma_1} & \ldots & \frac{\phi_1 \omega_1^m}{\sigma_1} & \ldots & \sum \frac{\omega_1^{2m}}{\sigma_1}
\end{bmatrix}$$
The number of degrees of freedom for the m-dimensional function in Equation [7.33] is given by the expression

\[ \text{degrees of freedom} = n - m - 1. \] [7.34]

When the number of degrees of freedom is negative, the assumed functional form of the polynomial is underdetermined and cannot be solved (this corresponds to having more unknowns than equations). When the number of degrees of freedom is zero, the assumed functional form is just exactly determined. The polynomial derived from the polynomial regression is identical to that obtained from the solution of simultaneous equations in the previous section. When the number of degrees of freedom is greater than zero the assumed functional form is overdetermined.

E. APPLICATION OF PROGRAM TO PHASE CORRECTION OF FT/ICR DATA

Figure 63 shows a typical real FT/ICR spectrum (single frequency excitation with heterodyne detection) before and after phase correction using the DISPA based automatic phase correction routine (SACPRC.ASC). The small anomalies at the base of each peak are artifacts of the data collection process and are not produced by the phase correction routine. This is verified in two ways. First, the unphased peak farthest to the left coincidentally is very nearly 180° out of phase (almost exactly inverted). It shows the same baseline anomaly prior to phase correction. Second, when the phase correction program is applied to simulated spectra which are known to be free of
Fig. 63. Typical real spectrum before and after phase correction for three peaks all nominal mass 28 a.m.u. Corresponding magnitude spectrum is included at the top for comparison.
baseline artifacts or peak shape anomalies, no artifacts are seen in the phase corrected spectra.

The spectrum shown in Figure 63 is of CO⁺, N₂⁺, and C₂H₄⁺ ions, all nominally mass 28 ions. The total mass range is 0.0635 amu (27.9795-28.0430 amu) corresponding to a total frequency range of 3.7291 kHz (1646.7033-1642.9742 kHz).

Although the spectrum in Figure 63 exhibits rather poor line shapes it does exhibit other attributes desirable to have for phase correction. The phase correction program works best when the spectrum that is being phase corrected has the following characteristics:

1) good line shape
2) good digital resolution
3) all peaks selected for the polynomial regression fit are isolated with minimal spectral overlap
4) no phase wrapping occurs across the spectrum.

Phase wrapping refers to the accumulation of one or more complete cycles of phase across the spectrum. This will be discussed in detail in a later section of this chapter.
F. COMPARISON OF PHASE CORRECTION TO OTHER DATA PROCEDURES

PROCESSING

An FT/ICR time domain signal \( f(t) \) can be processed in a number of different ways to obtain a frequency domain spectrum. These procedures are shown in Figure 64. Generally, the magnitude spectrum \( M(\omega_i) \) is generated (Figure 64 (a)).

\[
M(\omega_i) = \sqrt{R(\omega_i)^2 + I(\omega_i)^2} \tag{7.36}
\]

where \( R(\omega_i) \) and \( I(\omega_i) \) are the real and imaginary components of the frequency domain spectrum.

Several techniques are available to enhance the resolution for magnitude mode spectra. One procedure is to multiply the time domain signal by an exponential function of the form \( e^{t/\tau} \), where \( \tau \) is the relaxation time, prior to Fourier transformation (Figure 64 (b)). This is done in an effort to compensate for the natural exponential decay process that the time domain signal experiences, thus making the signal appear to persist longer resulting in a narrower line.
Fig. 64. Procedures for construction of mass domain spectra: (a) magnitude spectrum, (b) magnitude spectrum constructed from time domain spectrum apodized by exponential function, (c) same as (b) except the apodized time domain function has been truncated to reduce the noise, (d) magnitude spectrum constructed from time domain spectrum which has been apodized by the indicated window function, and (e) absorption spectrum obtained by phase correction of real and imaginary spectra.
shape (improved resolution). Unfortunately, this type of multiplication weights the noise at the end of the time domain signal heavily, resulting in degraded signal-to-noise ratio. The time domain signal can be truncated (Figure 64 (c)) to remove the noise, thus improving the signal-to-noise but half the data points in the time domain signal are discarded giving a final frequency domain spectrum with half the digital resolution. Also an abrupt truncation of the time domain signal may generate line shape anomalies in the final frequency domain spectrum.

The time domain signal can be multiplied by what is called a "window apodization function" (Figure 64 (d)). There are many different kinds of window apodization functions. Although their functional forms vary, they all serve the same basic purpose. They make the signal appear to persist as long as possible, while deemphasizing the noise at the end of the time domain signal. Abrupt truncation of the time domain signal is avoided by having the window function gradually approach zero near its end. The particular window function (it does not appear to have been named) which was used increases for the first quarter of the time domain signal, remains constant over the second and third quarters of the time domain signal and decreases over the final quarter of the time domain signal. The window apodization function is shown schematically in Figure 65.
Fig. 65. Apodization function used in conjunction with window apodization. The function increases from 0.0 to 1.0 across the first quarter of the time domain signal; it equals 1.0 during the second and third quarters and decreases back to 0.0 during the fourth quarter.
<table>
<thead>
<tr>
<th>Magnitude Spectrum</th>
<th>Linewidth (FWHH, Hz)</th>
<th>Digital Resolution (Hz/pt)</th>
<th>S/N</th>
<th>Spectral Resolution (M/ΔM)x10^{-3}</th>
<th>(S/N)*(Resolution x10^{-6})</th>
<th>Relative Spectral Resolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.4</td>
<td>0.455</td>
<td>216</td>
<td>477</td>
<td>103.2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Exponentially Apodized Magnitude</td>
<td>2.0</td>
<td>0.455</td>
<td>5.76</td>
<td>725</td>
<td>4.2</td>
<td>1.518</td>
</tr>
<tr>
<td>Truncated Exponentially Apodized Magnitude</td>
<td>2.5</td>
<td>0.228</td>
<td>53.5</td>
<td>635</td>
<td>34.0</td>
<td>1.331</td>
</tr>
<tr>
<td>Window Apodized Magnitude</td>
<td>2.1</td>
<td>0.455</td>
<td>72</td>
<td>833</td>
<td>60.0</td>
<td>1.744</td>
</tr>
<tr>
<td>Phased Real Spectrum</td>
<td>2.0</td>
<td>0.455</td>
<td>133</td>
<td>791</td>
<td>105.2</td>
<td>1.657</td>
</tr>
<tr>
<td></td>
<td>Linewidth FWHH, (Hz)</td>
<td>Digital Resolution (Hz/pt)</td>
<td>S/N</td>
<td>Spectral Resolution (M/ΔM) x 10^-3 (S/N)*(Resolution) (x10^-6)</td>
<td>Relative Spectral Resolution</td>
<td></td>
</tr>
<tr>
<td>----------------------</td>
<td>----------------------</td>
<td>-----------------------------</td>
<td>-----</td>
<td>---------------------------------------------------------------</td>
<td>-----------------------------</td>
<td></td>
</tr>
<tr>
<td>Magnitude Spectrum</td>
<td>4.5</td>
<td>0.455</td>
<td>225</td>
<td>422</td>
<td>95.1</td>
<td></td>
</tr>
<tr>
<td>Exponentially</td>
<td>2.9</td>
<td>0.455</td>
<td>10.7</td>
<td>604</td>
<td>6.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
<td>1.429</td>
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<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.304</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Window Apodized</td>
<td>2.0</td>
<td>0.455</td>
<td>158</td>
<td>818</td>
<td>129.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.937</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phased Real Spectrum</td>
<td>2.5</td>
<td>0.455</td>
<td>314</td>
<td>721</td>
<td>226.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.706</td>
<td></td>
</tr>
<tr>
<td>TABLE 7</td>
<td>COMPARISON OF DIFFERENT DATA PROCESSING PROCEDURES FOR C₂H₄⁺ MASS PEAK</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----------------</td>
<td>-----------------</td>
<td>-----------------</td>
<td>-----------------</td>
<td>-----------------</td>
<td>-----------------</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Linewidth FWHH, (Hz)</td>
<td>Digital Resolution (Hz/pt)</td>
<td>S/N</td>
<td>Spectral Resolution (M/ΔM) x 10⁻³</td>
<td>(S/N)*(Resolution) (x 10⁻⁶)</td>
<td>Relative Spectral Resolution</td>
</tr>
<tr>
<td>Magnitude Spectrum</td>
<td>5.4</td>
<td>0.455</td>
<td>152</td>
<td>461</td>
<td>70.1</td>
<td>1</td>
</tr>
<tr>
<td>Exponentially Apodized Magnitude</td>
<td>3.7</td>
<td>0.455</td>
<td>3.67</td>
<td>423</td>
<td>1.6</td>
<td>0.917</td>
</tr>
<tr>
<td>Truncated Exponentially Apodized Magnitude</td>
<td>2.9</td>
<td>0.228</td>
<td>37.75</td>
<td>585</td>
<td>22.1</td>
<td>1.270</td>
</tr>
<tr>
<td>Window Apodized Magnitude</td>
<td>2.4</td>
<td>0.455</td>
<td>76</td>
<td>838</td>
<td>63.7</td>
<td>1.818</td>
</tr>
<tr>
<td>Phased Real Spectrum</td>
<td>2.4</td>
<td>0.455</td>
<td>123</td>
<td>772</td>
<td>95.0</td>
<td>1.675</td>
</tr>
</tbody>
</table>
Figure 66 shows the spectra which were obtained from the same time domain signal using the different data processing procedures outlined in Figure 64. From Figure 66 it is clear that the phased real spectrum provides the best combination of S/N and resolution obtainable in FT/ICR. Expanded plots for the individual peaks in Figure 66 are provided in Figures 67-69. Tables 5-7 supply the measured linewidth, digital resolution, signal to noise, and spectral resolution for the different peaks shown in Figure 66. Tables 5-7 also provide values for the relative spectral resolution and the product of the signal-to-noise and the spectral resolution. To summarize the observed results, although apodized magnitude mode spectra (exponentially apodized, truncated exponentially apodized and window apodized) generate frequency domain spectra with resolution comparable to that of the phased real spectrum, they must sacrifice signal-to-noise ratio. The resolutions observed for the phased real peaks (CO⁺, 791K; N₂⁺, 721K; N₂H₄⁺, 772K) are better than their magnitude mode counterparts (CO⁺, 477K; N₂⁺, 422K; C₂H₄⁺, 461K).

The improvement factors in resolution (relative spectral resolutions) for the observed real mass peaks over the observed magnitude mass peaks (CO⁺, 1.657; N₂⁺, 1.706; C₂H₄⁺, 1.675) were slightly smaller than the $\sqrt{3}$ (=1.732) factor predicted by theory, probably because the observed line shapes are non-Lorentzian. The theoretical factor of $\sqrt{3}$ corresponds to Lorentzian line shapes.
Fig. 66. Spectra obtained from the same time domain data set using
the data processing procedures indicated in Figure 64. The
designations (a)-(e) are the same as in Figure 64.
Fig. 67. Expanded plots corresponding to the CO$^+$ ion obtained using the procedures outlined in the text and Figure 64. Designations (a)-(e) are the same as in Figure 64.
Fig. 68. Expanded plots corresponding to the $\text{N}_2^+$ ion obtained using the procedures outlined in the text and Figure 64. Designations (a)-(e) are the same as in Figure 64.
Fig. 69. Expanded plots corresponding to the $\text{C}_2\text{H}_4^+$ ion obtained using the procedures outlined in the text and Figure 64. Designations (a)-(e) are the same as in Figure 64.
The product of signal-to-noise ratio and resolution is a more appropriate criterion for evaluating the quality of the spectral peak shapes obtained. As indicated in Tables 5-7, the phased real spectrum generates the largest signal-to-noise ratio and spectral resolution product of all the procedures examined. In the case of CO\textsuperscript{+}, the product of signal-to-noise ratio and spectral resolution is only slightly larger for the phased real peak \((105.2 \times 10^6)\) than that obtained for the magnitude mode peak \((103.2 \times 10^6)\). The signal-to-noise ratio and spectral resolution products for the phased real N\textsubscript{2}\textsuperscript{+} and C\textsubscript{2}H\textsubscript{4}\textsuperscript{+} mass peaks \((226.3 \times 10^6\) and \(95.9 \times 10^6\) respectively) are significantly larger than those obtained for the magnitude mode counterpart \((95.1 \times 10^6\) and \(70.1 \times 10^6\) respectively).

G. STRATEGY FOR RESOLVING PROBLEMS OF SPECTRAL OVERLAP

As indicated in Chapter IV, systematic errors result when the spectral peak used for DISPA based phase measurement overlaps with one or more other peaks. This produces a dilemma: the phase measurement procedure requires isolated peaks for success but the goal of high resolution spectroscopy is discrimination between very closely spaced peaks.
One solution to this problem is to reduce sample pressure. At lower cell pressure the spectral line shape is inherently narrower. This is an attractive solution because the resolution is maximized and the phase correction is applied directly to the spectrum of interest.

A second approach to this problem is to collect separate spectra with identical acquisition parameters. The separately collected spectra would contain isolated peaks over the spectral region of interest. The phases of these isolated peaks from the different spectra could be measured individually free of spectral interference. The measured phases can be used to interpolate the phase as a function of frequency over the spectral region of interest. This function can then be applied to phase correct a spectrum collected with the same acquisition parameters but having unresolved peaks.

Figure 70 shows a series of four spectra all collected with identical acquisition parameters. The phases for all the peaks were measured separately. The measured apparent phases are provided in Table 8. The measured apparent phases for these spectra are plotted against their corresponding frequencies in Figure 71. The solid line in Figure 71 is the interpolated phase as a function of frequency derived from the phases measured in the bottom spectrum in Figure 70. The phase corrected spectra are shown in Figure 72.
Fig. 70. Unphased real spectra containing (a) CO$^+$ only, (b) N$_2^+$ only, (c) C$_2$H$_4^+$ only, and (d) all three ions simultaneously. All spectra were collected with identical data collection parameters.
Fig. 71. Apparent phase as a function of frequency obtained for the peaks in spectrum (d) of Figure 70 (indicated by dark circles) and the peaks in spectra (a)-(c) of Figure 70 (indicated by dark squares). The curve is the regression fit of a quadratic function to the measured phases in spectrum (d) of Figure 70.
Fig. 72. Spectra corresponding to those in Figure 70 which have been phase corrected using the same phase correction function. Designations (a)-(d) are the same as in Figure 70.
Figures 70-72 clearly indicate that the phase associated with a particular frequency is a function of acquisition parameters and is independent of the particular species in the FT/ICR cell. Thus, after the phase correction over a particular spectral range for given set of acquisition parameters has been determined for one spectrum, it can be applied to other spectra having the same acquisition parameters.
TABLE 8
MEASURED APPARENT PHASES FOR ION PEAKS IN SINGLE AND MULTIPEAK SPECTRA

<table>
<thead>
<tr>
<th>Multipeak Spectrum</th>
<th>Spectrum</th>
<th>Ion Peak</th>
<th>Index (pt.)</th>
<th>Frequency (pt.)</th>
<th>Phase (°)</th>
<th>Phase (cycles)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CO⁺</td>
<td>1996</td>
<td>6196</td>
<td>189.353</td>
<td>0.52599</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N₂⁺</td>
<td>3446</td>
<td>4747</td>
<td>284.247</td>
<td>0.78958</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C₂H₄⁺</td>
<td>6687</td>
<td>1505</td>
<td>208.945</td>
<td>0.58040</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Single Peak Spectra</th>
<th>Spectrum</th>
<th>Ion Peak</th>
<th>Index (pt.)</th>
<th>Frequency (pt.)</th>
<th>Phase (°)</th>
<th>Phase (cycles)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CO⁺</td>
<td>1992</td>
<td>6200</td>
<td>186.840</td>
<td>0.51900</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N₂⁺</td>
<td>3441</td>
<td>4751</td>
<td>278.693</td>
<td>0.77415</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C₂H₄⁺</td>
<td>6686</td>
<td>1509</td>
<td>219.053</td>
<td>0.60848</td>
</tr>
</tbody>
</table>

* Values correspond to the spectra in Figure 70.
H. PHASE WRAPPING

Figure 71 demonstrates an additional phenomenon which must be taken into consideration. The phase is expected to be a monotonically increasing function of frequency. This is not the case in Figure 71 because the measured apparent phase for the \( \text{N}_2^+ \) ion peak (0.78958 cycles) is larger than the measured apparent phases for the \( \text{CO}^+ \) or \( \text{C}_2\text{H}_4^+ \) ion peaks on either side of it (0.52599 and 0.58040 cycles, respectively). Apparently at least one complete cycle of phase has accumulated between the \( \text{N}_2^+ \) ion peak and the \( \text{CO}^+ \) ion peak. Since the frequency difference between the \( \text{C}_2\text{H}_4^+ \) peak and the \( \text{N}_2^+ \) peak is larger than the frequency difference between the \( \text{N}_2^+ \) peak and the \( \text{CO}^+ \) peak it is reasonable to believe that one or more cycles of phase accumulate between the \( \text{C}_2\text{H}_4^+ \) ion peak and the \( \text{N}_2^+ \) peak.

This accumulation of one or more full cycles of phase across the spectrum is called phase wrapping or sometimes phase cycling. This indicates that the phase correction which was applied to the bottom multiplex spectrum in Figure 70 to obtain the phased spectrum at the bottom of Figure 72 is not strictly correct because the phase correction polynomial is not monotonically increasing. This occurs because Equations [7.29] do not include terms for phase wrapping. To account for phase wrapping Equations [7.29] should be written as
The system of equations that results when the phase wrapping terms are included is not uniquely solvable because there are more unknowns than equations. Each equation in the system contributes two unknowns, \( x_k \) (the coefficients of the final function) and \( n_i \) (the total number of cycles of phase accumulation associated with a particular frequency).

One remedy for the phase wrapping problem is to add integral multiples of phase cycles to the measured apparent phase until the interpolated function takes on the expected monotonically increasing form as a function of frequency. To do this, however, some a priori knowledge of the functional form for phase accumulation is required. Over a narrow spectral range the phase accumulation can be approximated by a linear function. Nonlinear functions are required over broader ranges. Figure 73 shows how integral multiples of phase cycles can be added to the measured phases to obtain a function that

\[
\sum_{k=1}^{m} (a_{ik} x_k + n_i) = b_i
\]
Fig. 73. "Ladder diagram" constructed by adding integral multiples of phase cycles to the measured apparent phases obtained from the peaks in spectrum (d) of Figure 70.
is linearly increasing with frequency. The term "ladder diagrams" will be coined to refer to plots such as that in Figure 73.

When the linear phase function obtained from the ladder diagram in Figure 73 is used to phase correct the spectrum from which the original apparent phases were measured, the spectra in Figure 74 are obtained.

Over a narrow range the phase accumulation is closely approximated by a linear function. Therefore, for narrow spectral ranges, linear ladder diagrams allow interpolation of the phase correction polynomial. As the spectral range becomes broader, the accounting process by which the number of cycles across the spectrum is tallied becomes more complex and the success of the interpolation declines. Additionally, as the number of cycles added to the measured phases increases, then the apparent relative contribution to the total phase declines and the linear interpolation fails. By way of example, consider a peak with an apparent measured phase of 0.7 cycles, if the ladder diagram indicates that 1.0 cycle must be added to the measured apparent phase to achieve a linear function. The relative contribution of the measured apparent phase to the total phase is given by

$$\frac{0.7}{1.0 + 0.7} = 0.412.$$ [7.38]
Fig. 74. Real and imaginary spectra corresponding to the phase correction of spectrum (d) in Figure 70 using the linear phase correction functions predicted by the "ladder diagram" in Figure 73. The upper real and imaginary spectra were obtained using the phase correction functions labeled (A)-(C), while the lower spectra were obtained using the functions labeled (D) and (E).
When the ladder diagram indicates that 5.0 complete cycles (corresponding to interpolation over a broader spectral range) must be added to the measured apparent phase the relative contribution of the total phase due to the measured apparent phase is given by

\[
\frac{0.7}{5.0 + 0.7} = 0.123
\]  

[7.39]

This reasoning indicates that successful interpolation requires measurement of the phase frequently enough as to avoid accumulation of more than one or two complete cycles of phase between the sampled frequencies (ion peaks). In other words, more peaks are required to extend the phase measurement/correction to broader spectral ranges.

Accounting for phase wrapping is necessary for interpolation of the phase between frequencies at which it is measured. However, in situations such as Figure 71, where the phase correction polynomial is exactly determined, there is no particular advantage in accounting for the phase accumulation between the peaks, simply because there are no intervening peaks which require phase correction. The phase correction equations:

\[
R_{\text{new}} = R_{\text{old}} \cos(\phi_i) - I_{\text{old}} \sin(\phi_i)
\]  

[7.40]

\[
I_{\text{new}} = R_{\text{old}} \sin(\phi_i) + I_{\text{old}} \cos(\phi_i)
\]  

[7.41]

predict that \(R_{\text{new}}\) and \(I_{\text{new}}\) are both zero when \(R_{\text{old}}\) and \(I_{\text{old}}\) are both
zero no matter what phase correction, $\Phi_i$, is applied. This implies that no matter what phase correction function is used, if the particular function generates the proper phases at the frequencies where the spectral peaks occur, the final phased real spectrum will appear correct. On closer examination, although the phased real spectrum appears correct, the phased imaginary spectrum may contain peaks with reversed sign sense. This is an annoyance but it does not affect the line widths or resolution in the real spectrum which is obtained. So, from a practical standpoint, the real spectrum is just as useful as a spectrum which was phase corrected with a rigorously determined phase correction polynomial. In this sense, the procedure does generate the correct phases at the spectral peaks of interest and does so in a smooth fashion. This represents a solution to the phase problem, although not a unique one, nor one which is rigorously correct (in the sense that the exact true total phase is not determined at every frequency in the spectrum).

Phase correction across the entire spectrum is desirable, but from a practical standpoint is not necessary. Although hundreds of cycles may develop across any given spectrum, only fractions of cycles can be used in the final phase correction for any given frequency.

The Nicolet 1280 computer relies on a lookup table when evaluating sines and cosines of the phases. Although the sine and cosine functions can accept angles greater than $2\pi$ radians, it was
determined experimentally that as the number of cycles increases, the computer's sine and cosine functions return values with increasingly larger errors. To circumvent this problem, the calculated phase values (in units of cycles) must be reduced to their fractional part prior to calling the sine or cosine functions. This is achieved by truncating the real value to get the integer value and then subtracting the integer value from the real value. In essence, no matter what the total phase at a given frequency, it must be reduced to the apparent phase before the actual phase correction can be carried out. This means that the phase correction polynomial must be reduced to a piecewise functional form. Figure 75 shows schematically how the phase polynomial is reduced to a piecewise "sawtooth" function which has phase angles lying between 0 and 1 cycle. The discontinuities occur at frequencies where phase cycling occurs. It is this "sawtooth" function which is converted to radians just prior to calling the sine and cosine functions. The end result of this conversion process is that even if the phase polynomial could be determined across the entire spectrum the final phase correction must be applied piecewise. In light of this, it is clear that it is sufficient to define the phase polynomial in a piecewise fashion. Therefore, it is permissible to use the measured apparent phases and to interpolate directly between them as long as no phase cycling occurs between the measured phases. The real problem is in being
Fig. 75. Schematic diagram demonstrating how the phase variation be reduced to a "sawtooth" function were the apparent phase is between one and zero cycles.
able to identify frequencies at which phase cycling occurs. Ladder diagrams are helpful for identifying frequencies at which phase cycling occurs but are applicable only over narrow spectral ranges.

I. COMBINED INSTRUMENT AND DISPA BASED PHASE CORRECTION

One of the original motivations for attempting the instrument based phase correction of the previous chapter was to remove enough of the phase error based on the intrinsic phase transfer properties of the instrument that the DISPA based phase correction routine could remove the remaining phase error. In a sense the instrument based phase correction was to be used as a "rough phase correction" removing on the order of thousands of cycles of phase error and the DISPA based phase correction was to be used as a "fine phase correction" removing phase errors smaller than one cycle. The poor results obtained by the instrument based phase correction routine have prevented this possibility. Also when an effort was made to phase correct data which had already been "roughly" corrected with the instrument based procedure it was observed that the DISPA based program was unable to remove baseline anomalies which were introduced by the rough phase correction. It is worth noting however that if in the future a procedure (instrument based or otherwise) is developed for the removal of major portions of the phase error, then the DISPA based phase correction can be extended to wider spectral ranges.
J. CONCLUSION

A DISPA based phase correction routine has been successfully demonstrated for the phase correction of FT/ICR data. The phase correction is not rigorous because the total phase accumulation for any given frequency cannot be determined, only the apparent phase. Since the instrument computer’s sine/cosine lookup table precludes the use of rigorously determined phases, from a practical perspective, the phase correction which is obtained is adequate.

As predicted an increase in resolution very near the factor of \( \sqrt{3} \) was obtained over conventional magnitude-mode spectra when the properly phased real spectrum (absorption) was created.
CHAPTER VIII
DISPA BASED LINE SHAPE ANALYSIS OF DEXTRAN POLYMERS

A. INTRODUCTION

Line broadening mechanisms in FT/NMR spectra may be evaluated by using DISPA plots. This application is tested in this chapter as well as developing methodology suitable for the analysis of line broadening mechanisms in $^{13}$C FT/NMR spectra via DISPA plots.

Specifically, DISPA is applied to study the line broadening mechanisms in free and cross-linked dextran polymers. Dextran polymers were selected because they satisfied a number of specific requirements outlined below:

1) Viscous solutions of polymers have broad spectral lines which suggest potentially "interesting" line broadening mechanisms.

2) Compounds available at high purity, with good quality and reproducible physical properties.

3) Uniform and narrow molecular weight distribution for polymers.

4) Free of metal ions.

5) Exhibit isolated $^{13}$C NMR peaks.

6) Commercially available.

7) Well-studied motional behavior.

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1. STRUCTURE OF DEXTRAN POLYMERS

Dextrans are defined structurally as polymers of D-glucose (1) having linkages other than α(1°4). The dextrans (2) in this study have α(1°6) linkages for backbone formation with occasional α(1°3) linkages to provide branching (58). Included in this study is a series of Sephadex® gels, which are dextrans with glycerol cross-linking (3) to give them rigidity.
Dextran Structure with α(1→6) Backbone Linkages and α(1→3) Branching

Sephadex Structure Showing Glycerol Cross Linking Between C2 Carbons of Monomers from Different Chains.
The degree of crosslinking is strongly associated with the rigidity and the level of water absorption characteristic of a particular gel. Sephadex gels which are commercially designated by lower numbers are much more rigid (more highly cross linked) and absorb lower levels of water (Table 9). For instance, Sephadex G-25 is much more rigid than is Sephadex G-100. Also the more rigid structures do not require as much time to swell as do the flexible structures.

$^{13}$C NMR spectra of dextrans have 6 peaks corresponding to the 6 carbon nuclei in the glucose monomers. Peak assignment for C1 and C6 has been made by Dorman and Roberts (59). Tentative assignment for the remaining peaks was made by Colson, Jennings, and Smith (60) based on analogy to oligomers of D-glucose. The anomeric carbon (C1) is shifted well down field providing a potentially excellent candidate for DISPA analysis. The C6 carbon is shifted upfield from the remaining peaks and was expected to be sufficiently resolved in a 11.75 tesla field as to permit DISPA analysis of its line shape as well.
## TABLE 9
RECOMMENDED SWELLING TIMES AND PERCENT HYDRATIONS FOR DEXTRAN POLYMERS

<table>
<thead>
<tr>
<th>Dextran</th>
<th>% Hydration</th>
<th>Weight % After Drying For</th>
<th>Recommended Swelling Time</th>
<th>Particle Size</th>
<th>1 Gram Swells To</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>15 hr. a</td>
<td>36 hr. b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T40</td>
<td>12.24</td>
<td>9.4</td>
<td>------</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
<tr>
<td>T2000</td>
<td>6.35</td>
<td>7.7</td>
<td>------</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
<tr>
<td>G100sf</td>
<td>9.36</td>
<td>---</td>
<td>72 hr.</td>
<td>20-50 μm</td>
<td>15-20 ml</td>
</tr>
<tr>
<td>G75sf</td>
<td>3.68</td>
<td>5.0</td>
<td>24 hr.</td>
<td>20-50 μm</td>
<td>12-15 ml</td>
</tr>
<tr>
<td>G25f</td>
<td>9.43</td>
<td>---</td>
<td>3 hr.</td>
<td>20-80 μm</td>
<td>4-6 ml</td>
</tr>
</tbody>
</table>

a Measured values
b From Benesi and Gerig (64)

C From manufacturer (Pharmacia Fine Chemicals)

N.A. = Not Applicable

sf = Super fine

f = Fine
2. EVIDENCE FOR MOTIONAL BEHAVIOR OF DEXTRAN POLYMERS

The presence of sharp spectral lines in the spectra of dextran polymers implies that there must be at least some motional narrowing of the line shapes taking place, due to some type of rapid segmental motion of the polymer chains since rigid rotation (reorientation) of the macromolecules would be too slow to account for the observed narrow line widths (61,62). Moreover, sharp spectral line shapes are observed for peaks in spectra of crosslinked dextran gels where the molecules are covalently bound and cannot rotate freely in solution.

Tvaroska, Perez, and Marchessault (63) have calculated the three-dimensional space available to α-D-(1-6)-linked glycans and have found that polysaccharides such as dextran are permitted a much larger space than polysaccharides having (1-4) linkages. Polysaccharides with (1-4) linkages have two bonds (one atom) about which rotation can occur between rigid monomeric ring structures. Dextrans with (1-6) linkages have three bonds (two atoms) about which rotation between the rigid monomeric ring structures can occur. Tvaroska, Perez, and Marchessault noted that the presence of an additional rotationally labile bond between the monomeric units affords much greater flexibility than related structures such as cellulose or amylose which have (1-4) linkages (64). The presence of this additional labile bond of rotation also prevents dextrans from forming regular helical segments as observed for cellulose and amylose type structures. Dextrans consequently tend to be more flexible and more extended in solution, as borne out by the much higher solubilities observed for dextrans relative to structures like cellulose and amylose.
Benesi and Gerig (64) have measured the $T_1$ and $T_2$ values for a series of free dextran polymers (Pharmacia T-40, T-250, and T-2000) and one cross linked dextran (Pharmacia G-75). $T_1$'s and $T_2$'s for all $^{13}$C nuclei were essentially equal except for slightly larger values for C6. Thus, relaxation for all of the carbon nuclei associated with the ring structure (Cl through C5) occurs at the same rate, indicating that motion leading to relaxation must involve movement of the ring moiety as a whole. Slightly larger $T_1$'s observed for C6 suggest that some other motion may be responsible for the relaxation of the C6 nuclei. Since C6 occupies a "hinge" point between bonds where rotations or librations are allowed, such motion can reasonably be attributed to the hinging that occurs between the rigid ring monomers. Benesi and Gerig measured the nuclear Overhauser enhancements and found them to be consistent with dipole-dipole relaxation (Cl through C5 had average n.O.e. values of $1.7 \pm 0.3$ while the average n.O.e. value for C6 was $2.0 \pm 0.3$). Benesi and Gerig noted that relaxation data obtained for dextran polymers in solution is similar to that observed for other polymers in solution (65). For a model in which the monomeric unit was taken as an ellipsoid of revolution undergoing anisotropic reorientation, Benesi and Gerig found their best fit to the data with rotational diffusion constants of $4 \times 10^9$ and $2.5 \times 10^8$ rad./sec for motion around the symmetry axis ($R_{||}$) and tumbling around axes perpendicular to the symmetry axis ($R_{\perp}$).
In general the relaxation has been ascribed to rapid local motion of small segments of the polymer relatively independent of the overall polymer conformation. An example of this type of motion has recently appeared in a paper by Helfand (66) who noted that "crankshaft" type motions of portions of the backbone allow rapid motion without requiring complete reorientation of the entire macromolecule.

Scherrer, Lundin, Benson and Wit (67) examined a series of crosslinked dextran hydrogels (Sephadexes G-15, G-25, G-50, G-75, G-100, and G-200). They collected $^1$H decoupled $^{13}$C FT/NMR spectra for each of the gels at 29°C and 90°C. A progressive broadening of the spectral line shapes and a progressive decline in signal-to-noise ratio was correlated to increasing rigidity (crosslinking) of the dextran structures. These observations were attributed to a gradual loss of mobility of the gel carbon atoms as the structures were increasingly hindered by crosslinking agents. The spectra of the more highly crosslinked gels contained additional peaks which were assigned to the "glyceryl" crosslinking agents (crosslinking is facilitated by condensation reaction between the dextran polymer and epichlorohydri in the presence of concentrated sodium hydroxide).
B. THEORY

1. DISPA PLOTS AND LINE SHAPE

As stated previously, DISPA generates a perfectly circular plot only when the observed spectral line shape is truly Lorentzian (i.e., results from a single exponential decay process in the time domain). Each Lorentzian component contributes a single term to the observed absorption and dispersion line shapes:

\[
A_i(\omega) = \frac{\tau_1}{1 + \tau_1^2 (\omega - \omega_i)}/3
\]

\[
A_i(\omega) = \frac{\tau_i^2 (\omega - \omega_i)}{1 + \tau_1^2 (\omega - \omega_i)}/2
\]

where \( \tau_1 \) and \( \omega_0,1 \) are the relaxation time and resonance frequency corresponding to the reference circle. Line shapes which result from the summation of Lorentzian components all having the same resonance frequency but with different linewidths (relaxation times), produce DISPA plots displaced inside the reference circle. Line shapes representing the sum of two or more Lorentzian components all having the same width but different peak
positions (different $\omega_{0,1}$) are expected to generate experimental DISPA plots which are displaced outside the reference circle (18). Such a line shape would be defined mathematically as:

$$A(\omega) = \sum_{i=1}^{m} \frac{\rho_i \tau_i}{(1+\tau_i^2(\omega_{0,1}-\omega))}$$  \hspace{1cm} \text{[8.3]}

$$D(\omega) = \sum_{i=1}^{m} \frac{\rho_i \tau_i}{(1+\tau_i^2(\omega_{0,1}-\omega))}$$  \hspace{1cm} \text{[8.4]}

where $\omega_{0,1}$ designates the resonance frequency of the individual components and $\rho_i$ is the corresponding weighting factor.

Line shapes which result from the summation of Lorentzian components all having the same resonance frequency but with different linewidths (different $\tau_i$'s) are expected to produce experimental DISPA plots which are displaced inside the reference circle (18) These line shapes are described mathematically as:

$$A(\omega) = \sum_{i=1}^{m} \frac{\rho_i \tau_i}{(1+\tau_i^2(\omega_{0}-\omega)^2)}$$  \hspace{1cm} \text{[8.5]}

$$D(\omega) = \sum_{i=1}^{m} \frac{\rho_i \tau_i^2 (\omega_{0}-\omega)}{(1+\tau_i^2(\omega_{0}-\omega)^2)}$$  \hspace{1cm} \text{[8.6]}
A continuous distribution of Lorentzian components all having the same line width but different peak positions generates line shapes which are given by:

\[
A(\omega) = \int \frac{G(\omega) \tau}{1 + \tau^2(\omega - \omega_0)^2} \, d\omega_0 \tag{8.7}
\]

\[
D(\omega) = \int \frac{G(\omega) \tau^2}{1 + \tau^2(\omega - \omega_0)^2} \, d\omega_0 \tag{8.8}
\]

where \(G(\omega_0)\) is the density function relating the relative intensity of a particular component at a given resonance frequency. Likewise, an expression for the line shape resulting from a continuous distribution of Lorentzian components all having the same resonance frequency but different line widths may be represented by the integral expressions:

\[
A(\omega) = \int \frac{G(\tau) \tau}{1 + \tau^2(\omega - \omega_0)^2} \, d\tau \tag{8.9}
\]

\[
D(\omega) = \int \frac{G(\tau) \tau^2 (\omega - \omega_0)}{1 + \tau^2(\omega - \omega_0)^2} \, d\tau \tag{8.10}
\]
The integral expressions in Equations [8.7]-[8.10] are quite general, in the sense that no specific density functions have been given for \( G(\omega) \) or \( G(\tau) \). The line broadening mechanism described by Equations [8.7] and [8.8] occur in situations such as that described in Chapter III where magnetic field inhomogeneity through the sample generates a distribution of resonances associated with a specific nucleus throughout the sample. Line broadening described by Equations [8.9] and [8.10] arises when for some reason a single type of nucleus is offered a variety of relaxation mechanisms or rates through which loss of magnetization can occur. In the situation of polymers in viscous solutions, such a distribution of relaxation times reflects a distribution of correlation times, \( \tau_c \).
2. RELATION BETWEEN LINE SHAPE AND MOLECULAR MOTION

For molecules such as dextran polymers the primary relaxation mechanism for a \(^{13}\)C nucleus is the dipole-dipole interaction with one or more directly bonded \(^1\)H nuclei (65). There are several possible other relaxation mechanisms (quadrupole, scalar, spin-rotational) but none of the others as large as the dipole-dipole interaction.

The dipolar relaxation rate is proportional to the number of protons covalently bonded to the \(^{13}\)C nuclei and the rate at which the orientation of the intranuclear vector between the \(^{13}\)C and \(^1\)H nuclei changes relative to the static magnetic field. As molecules "tumble" in solution due to Brownian motion the \(^{13}\)C and \(^1\)H nuclei reorient themselves relative to the static magnetic field. The tumbling rate is generally described in terms of the correlation time, \(\tau_c\), which can be thought of as the amount of time required for the intranuclear vector colinear with the \(^{13}\)C-\(^1\)H bond to change its angular orientation relative to the static magnetic field by one radian. As the molecules tumble in solution, the covalently bonded \(^{13}\)C and \(^1\)H nuclei impose positive or negative contributions on the apparent magnetic fields sensed by each other. These apparent magnetic fields fluctuate randomly at a rate related to the rate at which the intranuclear vector reorients relative to the static magnetic field. The randomly fluctuating magnetic field created by the motion of the \(^1\)H nucleus provides an effective mechanism for the relaxation of the \(^{13}\)C nucleus.
The magnitude of the magnetic field contribution imposed by one nucleus onto another is proportional to the square of the product of the gyromagnetic ratios for both nuclei. The effect declines rapidly when the distance between the nuclei increases (involving a factor of \( r^{-6} \) where \( r \) is the distance between the nuclei). Only covalently bonded nuclei are sufficiently close to one another to facilitate relaxation.

For isotropic motion, in the extreme narrowing limit (i.e., \( \tau_c^2 (\omega_H + \omega_C^2)^2 \ll 1 \)) the longitudinal, \( T_1 \), and transverse, \( T_2 \), relaxation times are related to the correlation time, \( \tau_c \), by the following equations (66). \( n \) is the number of protons covalently bonded.

\[
\frac{1}{n T_1} = \frac{\gamma_H^2 \gamma_C^2 h^2}{2\pi r^6} \tau_c \tag{8.11}
\]

\[
\frac{1}{n T_2} = \frac{\gamma_H^2 \gamma_C^2 h^2}{2\pi r^6} \tau_c \tag{8.12}
\]

Typically macromolecules do not undergo isotropic motion and more complicated expressions are needed to describe the observed relaxation times. One model approximates the motion of macromolecules by envisioning them as ellipsoids which contain "embedded" intranuclear vectors which reorient themselves as functions of rotational diffusion constants \( R_1 \) and \( R_2 \) for motion parallel and perpendicular...
to some fixed axis for the molecule. In the motional narrowing limit such a model gives the following relationships (67).

$$\frac{1}{n T_1} = \frac{1}{n T_2} = \frac{\gamma^2 \gamma_C h^2}{2\pi \gamma_0^2 r^6} \tau c \left[ \frac{A}{6R_1^2} + \frac{B}{R_1^2 + 5R_1^2} + \frac{C}{4R_1^2 + 2R_1^2} \right]$$

[A, B, and C are constants relating to the orientation of the fixed axis to the static magnetic field.]

Other models attempt to describe molecular motion of macromolecules in terms of two or more correlation times, while some describe the motions of the internuclear vectors in terms of random jump rotations between sites with either equivalent or nonequivalent probabilities.

Distributions of correlation times, \(P(C)\), have also been proposed to explain molecular motion of macromolecules although there is no theoretical basis for such a proposal (68,69). Some controversy has developed as to which particular distribution function is most appropriate to describe experimental relaxation data. The spectral densities are given by

$$J(\omega) = \int_{0}^{\infty} G(\tau)P(\tau_C)e^{-i\omega\tau} \, d\tau_C$$
where
\[ \tau_{c \text{ max}} \int_{\tau_{c \text{ min}}}^{\tau_{c \text{ max}}} G(\tau_c) d\tau_c = 1 \quad . \] [8.14]

Regardless of which model is correct it is clear that when motion is favored about one or more axes relative to another (anisotropic motion) the relaxation process occurs at two or more rates reflecting the particular motions involved. The observed time domain signal is then the composite of two or more exponential decays and the final frequency domain spectrum represents the composite of two or more Lorentzian components centered at the same resonance frequency. The observed frequency domain line shape depends on the relative populations (areas under the component Lorentzians; related to the number of nuclei involved) and relaxation times (widths of the component Lorentzians).

When the observed line shape is a composite of one or more Lorentzian components centered at the same resonance frequency it is non-Lorentzian and the corresponding DISPA plot deviates symmetrically inside the reference circle (18). Likewise the corresponding RDDISPA plot will have symmetrical deviations below the horizontal axis.

The exact nature of the deviations must be related to the number of components involved (i.e., the number of relaxation mechanisms), the widths of the individual components (i.e., the rate of relaxation associated with a specific component), and the area under the individual components (i.e., the number of nuclei relaxing at a particular rate).
To date nearly all the studies of the molecular motion of macromolecules have commonly involved the measurement of longitudinal (T₁) and transverse (T₂) relaxation times and correlation of the observed experimental data to specific models. Nuclear Overhauser effects (nOe) are generally measured as well. The correlation between the theoretical and experimental nOe values indicates how well the dipole-dipole mechanism applies to the data.

Simple measurement of relaxation times may not be adequate or proper for the study of the molecular motion of macromolecules since in the presence of more than one relaxation rate the measured relaxation times may be biased in favor of faster relaxation times (69). Examination of the actual line shape avoids this type of bias and affords some insight into the true nature of the molecular motion. DISPA plots offer a particularly attractive and convenient method by which the spectral line shape can be examined.

The specific aim of this work is not to verify a specific line broadening mechanism but rather to demonstrate the application of DISPA and RDDISPA plots to the examination of broadband ¹H decoupled ¹³C spectral line shapes for the purpose of assessing molecular motion. DISPA has several key advantages over other procedures for studying macromolecular motion. By examining the entire line shape rather than just its width (or relaxation time) the inherent biases associated with such methods can be avoided. DISPA does not require the collection of multiple spectra as is required for the measurement of T₁'s via inversion recovery type experiments. Careful examination of the
deviations of DISPA plots can then reveal the most appropriate model for analysis of the data (isotropic motion versus anisotropic versus distribution of correlation times).

C. EXPERIMENTAL

1. SAMPLE PREPARATION

A series of free and crosslinked dextrans (Sephadex gels) was purchased from Pharmacia. All samples were prepared in a 0.1 M Na$_2$HPO$_4$/D$_2$O buffer which was adjusted to pH meter reading 7.80 with D$_3$PO$_4$. Prior to use, the buffer was degassed for 2-3 hr. by pulling a vacuum on it with an aspirator.

Free dextrans (T40 and T2000) were prepared by weighing the solid dextran and buffer solution directly into 5 mm NMR tubes. The solutions were then mixed with a vortex stirrer. The gels (G25, G75, and G100) could not be prepared in the same manner as the free dextrans, since swelling of the gels inside the NMR tubes trapped air bubbles within the tubes. The gels were "poured" into the NMR tubes in much the same manner that gel chromatography columns are poured. Prior to pouring, the gels were allowed to swell for a period of time in excess of that recommended by the manufacturer (Table 9). After pouring, excess buffer was removed from the tubes with a pipette. Sample volumes of 3 ml were used. Methanol (0.2 ml) was added to each sample as a reference and an internal check for magnetic field homogeneity. When not in use, samples were stored in
a refrigerator to prevent bacterial contamination. Prior to use, samples were allowed to warm up to room temperature. The water contents of the various dextrans were determined gravimetrically and considered to be insignificant (Table 9), so no correction was made to the sample concentrations to allow for hydration.

2. VISCOSITY MEASUREMENT

Viscosities for the two free dextran solutions (T40 and T2000 dextrans) were measured with a falling ball viscosimeter. The viscosities were related to the time required for a stainless steel ball to drop at terminal velocity between two sets of fiduciary lines. The apparatus was calibrated with glycerol/water solutions of known concentrations using literature values as reference viscosities. The calibration data is provided in Table 10 and experimentally measured free dextran viscosities are provided in Table 11. The temperature was 23°C. Since a water bath with a glass window was not available into which the device could be placed while making the necessary measurements, no effort could be made to control the temperature. For this reason, the measurements are probably accurate only to within ± 5%. These measurements were made to indicate the viscosity range over which the $^{13}$C FT/NMR data was collected, more exact measurements were not considered necessary.
### Table 10
CALIBRATION OF FALLING BALL VISCOMETER WITH GLYCEROL/WATER SOLUTIONS

| Glycerol/Water (% w/w) | Average Droptime (min.)<sup>a</sup> | Solution Density (g/ml)<sup>b</sup> | Kinematic Viscosity (cS) | Absolute Viscosity (cP) | Relative Viscosity (unitless) | Calculated K  
|------------------------|------------------------------------|-------------------------------------|-------------------------|------------------------|-----------------------------|----------------
| 64                     | 2.161                              | 1.16617                             | 11.73                   | 13.68                  | 13.65                       | 0.92   
|                        |                                    | 1.1663<sup>b</sup>                 |                         |                        |                             |        
| 76                     | 4.394                              | 1.1842                              | 33.88                   | 40.65                  | 40.56                       | 1.35   
|                        |                                    | 1.1997<sup>b</sup>                 |                         |                        |                             |        
| 84                     | 16.120                             | 1.2135                              | 69.18                   | 84.49                  | 84.33                       | 0.77   
|                        |                                    | 1.2214<sup>b</sup>                 |                         |                        |                             |        
| Average                | ---                                | ---                                 | ---                     | ---                    |                             | 1.02   

<sup>a</sup> Average of 3 values  
<sup>b</sup> Measured at 23°C  
<sup>c</sup> Literature value obtained at 20°C (CRC)

\[
K = \frac{\mu_{\text{absolute}}}{(P_f - P) \cdot \tau}
\]

where:  
\( \mu \) = absolute viscosity (cP)  
\( P_f \) = 8.02 g/ml density of stainless steel ball  
\( P \) = density of solution (g/ml)  
\( \tau \) = drop time minutes
### TABLE 11
MEASURED VISCOSITIES FOR FREE
DEXTRAN SAMPLES AT 23°C

<table>
<thead>
<tr>
<th>Sample</th>
<th>Average Drop Time (min.)</th>
<th>Solution Density (g/ml)</th>
<th>Absolute Viscosity (cP)</th>
<th>Relative Viscosity</th>
</tr>
</thead>
<tbody>
<tr>
<td>T40 Dextran</td>
<td>7.809</td>
<td>1.1836</td>
<td>54</td>
<td>54</td>
</tr>
<tr>
<td>19.8% w/w</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T2000 Dextran</td>
<td>9.826</td>
<td>1.1422</td>
<td>69</td>
<td>69</td>
</tr>
<tr>
<td>9.77% w/w</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\[ \mu = K (\rho - \rho_f) \tau \]

where \( \mu \) = absolute viscosity (cP)

\[ K = 1.02 \text{ (ml/g. min)} \]

\( \rho_f = 8.02 \text{ g/ml for stainless steel} \)

\( \rho = \text{density of solution (g/ml)} \)

Relative Viscosity = \( \frac{\text{Absolute Viscosity}}{\text{Absolute Viscosity of Water at 20°C}} \)

Absolute Viscosity Water at 20°C = 1.002 cP
3. DATA COLLECTION

Prior to data collection, the FT/NMR spectrometer was carefully shimmed. Initially, the intensity of the $^2$H-lock signal was maximized by manually adjusting the shim coil currents for the $z^1$ and $z^2$ shim coils. Then, the $z^1$, $z^2$ and $z^3$ shim coils were corrected using an Autoshim routine which is a SIMPLEX based on optimization routine which maximizes the absolute area under the $^1$H time domain signal. The procedure was repeated several times alternating between the optimization of two shim coil currents ($z^1$ and $z^2$) and three shim coil currents ($z^1$, $z^2$, and $z^3$), while checking the line width of the $^{13}$C methanol peak after each optimization procedure. After the line width of the $^{13}$C methanol peak ceased to narrow, small manual corrections of the $z^4$ shim coil current were made until an optimally symmetrical methanol $^{13}$C peak as possible could be obtained. The signal-to-noise ratio was generally poor requiring that several acquisitions be averaged to observe the $^{13}$C line shapes.

For the chosen samples, the reference peak served two purposes. First, it provided an internal reference to which the chemical shifts of the polymer peaks could be related. Second, it provided a narrow peak which was known to be Lorentzian that could be used to monitor the magnetic field homogeneity, from its line width and line shape.

Initially, the $^{13}$C peak for acetone was selected as a reference peak ($\approx 30.4$ ppm) but was later rejected when it was found to be split by $^{13}$C satellites. The $^{13}$C peak for methanol was then selected as a reference peak. In addition to being unsplit, the methanol $^{13}$C
peak (\& 49.0 ppm) was located closer to the polymer peaks than was the acetone \(^{13}\text{C}\) peak allowing a narrower spectral range to be collected, thus permitting better digital resolution over the spectral range of interest.

Data was collected on a Bruker AM-500 FT/NMR with a 11.75 tesla magnet (500 MHz \(^1\text{H}\) and 125 MHz \(^{13}\text{C}\)). Spectra were collected with a dual \(^{13}\text{C}/^1\text{H}\) 5 mm probe at 303K. Data collection parameters are provided in Table 12.
### TABLE 12

**DATA ACQUISITION PARAMETERS**

<table>
<thead>
<tr>
<th>Parameter and Instrument Abbreviation</th>
<th>T40 Dextran</th>
<th>T2000 Dextran</th>
<th>G100 Sephadex</th>
<th>G75 Sephadex</th>
<th>G25 Sephadex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spectrometer Frequency (MHz) SF</td>
<td>125.759</td>
<td>125.759</td>
<td>125.759</td>
<td>125.759</td>
<td>125.759</td>
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<tr>
<td>Synthesizer Frequency (Hz) SY</td>
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<td>93.0</td>
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<td>78811.070</td>
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<td>78759.058</td>
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<tr>
<td>Spectrum Size (points) SI</td>
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<td>32768</td>
<td>32768</td>
<td>32768</td>
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<tr>
<td>Spectral Width (Hz) SW</td>
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<td>7692.308</td>
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<tr>
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<td>0.470</td>
<td>0.477</td>
<td>0.477</td>
<td>0.477</td>
</tr>
<tr>
<td>Pulse Width (sec) PW</td>
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<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Relaxation Delay (sec.) RD</td>
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<td>0.0</td>
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<td>0.0</td>
</tr>
<tr>
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<tr>
<td>Receiver Gain (A.U.) RG</td>
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<td>400</td>
<td>200</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Parameter and Instrument Abbreviation</td>
<td>T40 Dextran</td>
<td>T2000 Dextran</td>
<td>G100 Sephadex</td>
<td>G75 Sephadex</td>
<td>G25 Sephadex</td>
</tr>
<tr>
<td>--------------------------------------</td>
<td>-------------</td>
<td>---------------</td>
<td>---------------</td>
<td>--------------</td>
<td>--------------</td>
</tr>
<tr>
<td>Number of Scans</td>
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<td>12225</td>
<td>18366</td>
<td>10001</td>
<td>12179</td>
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<tr>
<td>Filter Width (Hz)</td>
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<td>9700</td>
<td>9800</td>
<td>9800</td>
<td>7600</td>
</tr>
<tr>
<td>Decoupler Frequency Offset (Hz)</td>
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<td>9000.00</td>
<td>7500.00</td>
<td>9000.00</td>
<td>9000.00</td>
</tr>
<tr>
<td>Decoupler Power (Watts)</td>
<td>DP 13 H CPD</td>
<td>13 H CPD</td>
<td>13 H CPD</td>
<td>13 H CPD</td>
<td>13 H CPD</td>
</tr>
<tr>
<td>Line Broadening (Hz)</td>
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<td>0.0</td>
<td>0.0</td>
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<tr>
<td>Spectrum Reference (Hz)</td>
<td>SR 69464.07</td>
<td>69464.07</td>
<td>69464.07</td>
<td>69464.07</td>
<td>69464.07</td>
</tr>
</tbody>
</table>
4. DATA PROCESSING

After collection, the raw time domain data was stored on disk for later analysis. As many acquisitions as time permitted were collected for each data set. Prior to Fourier transformation, all time domain data was baseline corrected. Only one spectrum was linebroadened to enhance the signal-to-noise ratio (G-25, LB= 1.0 Hz).

DISPA and RDDISPA plots were generated on-line on the Bruker Aspect 2000 computer using the PASCAL program, DISPA6, described in Chapter II. DISPA and RDDISPA plots were generated for all the polynomial peaks in the accumulated $^{13}$C FT/NMR spectra. A fourth order polynomial regression fit was made to the seven points nearest the highest observed point for each peak. The calculated magnitude maximum for the fitted polynomial was then used to normalize the data prior to generation of the DISPA plot. Zero order phase corrections were applied to some of the peaks prior to construction of their corresponding DISPA plots. RDDISPA plots were generated directly from the real and imaginary data without the intermediate generation of a DISPA plot.
D. RESULTS

Proton decoupled $^{13}$C FT/NMR spectra for the dextran structures are provided in Figures 110 through 114 (Appendix K). Expanded plots of individual peaks are provided in Figures 115 through 142 (Appendix L).

In general the spectral line widths were observed to broaden progressively in proceeding to more rigid (more highly crosslinked) structures. Spectral line widths are summarized in Table 13. The observed signal-to-noise ratios (Table 14) became progressively poorer requiring more scans for the more rigid structures. Each spectrum contained six peaks corresponding to the six different carbon nuclei in the glucose moiety. A typical spectrum with the positions of the carbon peaks labeled is shown in Figure 76. Peak positions for the individual peaks remained relatively constant (Tables 15 and 16).

Although the peak positions remained unchanged the amount of spectral overlap becomes progressively greater as the line widths themselves become broader. This is indicated in Table 17 where the number of line widths between adjacent peaks is shown.

Peaks associated with the crosslinking agents are more apparent in the spectra of the more highly crosslinked materials. These peaks are most pronounced in the spectrum of G-25.

DISPA and RDDISPA plots corresponding to the individual spectral peaks are provided in Figures 77 through 105. Two general trends are observed for the DISPA plots. First, they show progressively greater compression (i.e. deviation inside the reference circle) in the more highly crosslinked structures and second there is a progressive
TABLE 13

$^{13}$C FT/NMR SPECTRAL PEAK LINEWIDTHS FOR DEXTRAN POLYMERS (FWHH) (Hz)

<table>
<thead>
<tr>
<th>Peak</th>
<th>T40 Dextran</th>
<th>T2000 Dextran</th>
<th>G100 Sephadex</th>
<th>G75 Sephadex</th>
<th>G25 Sephadex$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>4.5514</td>
<td>3.5852</td>
<td>6.2855</td>
<td>8.2899</td>
<td>90.21</td>
</tr>
<tr>
<td>C2</td>
<td>4.3165</td>
<td>3.4256</td>
<td>5.8078</td>
<td>7.1879</td>
<td>-----</td>
</tr>
<tr>
<td>C3</td>
<td>4.7885</td>
<td>3.6184</td>
<td>5.9416</td>
<td>7.3528</td>
<td>-----</td>
</tr>
<tr>
<td>C4</td>
<td>4.6050</td>
<td>3.4819</td>
<td>5.5972</td>
<td>7.4074</td>
<td>-----</td>
</tr>
<tr>
<td>C5</td>
<td>5.3524</td>
<td>3.8550</td>
<td>6.6955</td>
<td>7.9594</td>
<td>-----</td>
</tr>
<tr>
<td>C6</td>
<td>7.8113</td>
<td>6.0165</td>
<td>7.9721</td>
<td>9.7065</td>
<td>-----</td>
</tr>
<tr>
<td>CH$_3$OH</td>
<td>0.6192</td>
<td>&lt;0.470$^c$</td>
<td>2.0954</td>
<td>3.3634</td>
<td>9.34</td>
</tr>
</tbody>
</table>

$^a$ Based on regression fit to Lorentzian

$^b$ Measured manually.

$^c$ Less than digital resolution of the spectrum.
### TABLE 14

**SIGNAL-TO-NOISE RATIOS**

**FOR SPECTRA OF DEXTRAN POLYMERS**

<table>
<thead>
<tr>
<th>Peak</th>
<th>T40</th>
<th>T2000</th>
<th>G100</th>
<th>G75</th>
<th>G25</th>
<th>a</th>
<th>b</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>60.486</td>
<td>60.158</td>
<td>44.585</td>
<td>44.083</td>
<td>4.74</td>
<td>8.377</td>
<td></td>
</tr>
<tr>
<td>C2</td>
<td>75.835</td>
<td>53.298</td>
<td>32.252</td>
<td>26.702</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C3</td>
<td>73.766</td>
<td>66.040</td>
<td>43.790</td>
<td>43.990</td>
<td>9.862</td>
<td>14.716</td>
<td></td>
</tr>
<tr>
<td>C4</td>
<td>26.999</td>
<td>34.239</td>
<td>30.600</td>
<td>41.898</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C5</td>
<td>31.396</td>
<td>34.707</td>
<td>15.829</td>
<td>16.424</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CH$_3$OH</td>
<td>272.911</td>
<td>339.377</td>
<td>216.838</td>
<td>149.801</td>
<td>2.573</td>
<td>?</td>
<td></td>
</tr>
</tbody>
</table>

- a Sephadex G25 without line broadening.
- b Sephadex G25 with 1.0 Hz line broadening.

**Note:** $S/N = \text{rms}(S/N) \times 0.5$. 
Fig. 76. Typical $^1$H decoupled $^{13}$C FT/NMR spectrum obtained for dextran polymers (this particular spectrum is for T2000 dextran). The corresponding carbon nuclei are indicated.
## TABLE 15

C\textsuperscript{13} FT/NMR SPECTRAL PEAK POSITIONS FOR DEXTRAN POLYMERS (ppm)

<table>
<thead>
<tr>
<th>Peak</th>
<th>T40 Dextran</th>
<th>T2000 Dextran</th>
<th>G100 Sephadex</th>
<th>G75 Sephadex</th>
<th>G25 Sephadex</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>98.002</td>
<td>98.091</td>
<td>98.107</td>
<td>98.095</td>
<td>98.111</td>
</tr>
<tr>
<td>C2</td>
<td>71.728</td>
<td>71.795</td>
<td>71.809</td>
<td>71.798</td>
<td>71.864</td>
</tr>
<tr>
<td>C3</td>
<td>73.735</td>
<td>73.794</td>
<td>73.806</td>
<td>73.795</td>
<td>73.766</td>
</tr>
<tr>
<td>C4</td>
<td>70.495</td>
<td>70.571</td>
<td>70.584</td>
<td>70.574</td>
<td>70.834</td>
</tr>
<tr>
<td>C5</td>
<td>69.900</td>
<td>69.959</td>
<td>69.972</td>
<td>69.960</td>
<td>69.942</td>
</tr>
<tr>
<td>C6</td>
<td>65.891</td>
<td>65.955</td>
<td>65.968</td>
<td>65.957</td>
<td>66.000</td>
</tr>
<tr>
<td>CH\textsubscript{3}OH</td>
<td>49.300</td>
<td>49.301</td>
<td>49.300</td>
<td>49.298</td>
<td>49.420</td>
</tr>
</tbody>
</table>
### TABLE 16

$^{13}$C FT/NMR SPECTRAL PEAK POSITIONS FOR DEXTRAN POLYMERS (Hz)

<table>
<thead>
<tr>
<th>Peak</th>
<th>T40 Dextran</th>
<th>T2000 Dextran</th>
<th>G100 Sephadex</th>
<th>G75 Sephadex</th>
<th>G25 Sephadex$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>12330.4</td>
<td>12334.7</td>
<td>12336.5</td>
<td>12336.8</td>
<td>12338.4</td>
</tr>
<tr>
<td>C2</td>
<td>9026.2</td>
<td>9028.0</td>
<td>9028.6</td>
<td>9029.5</td>
<td>9037.6</td>
</tr>
<tr>
<td>C3</td>
<td>9278.8</td>
<td>9279.6</td>
<td>9281.3</td>
<td>9281.4</td>
<td>9276.7</td>
</tr>
<tr>
<td>C4</td>
<td>8871.1</td>
<td>8874.4</td>
<td>8875.6</td>
<td>8875.6</td>
<td>8947.9</td>
</tr>
<tr>
<td>C5</td>
<td>8796.5</td>
<td>8797.4</td>
<td>8798.7</td>
<td>8798.4</td>
<td>8795.9</td>
</tr>
<tr>
<td>C6</td>
<td>8292.5</td>
<td>8293.8</td>
<td>8295.0</td>
<td>8294.6</td>
<td>8300.2</td>
</tr>
<tr>
<td>CH$_3$OH</td>
<td>6206.0</td>
<td>6199.6</td>
<td>6199.4</td>
<td>6199.9</td>
<td>6215.0</td>
</tr>
</tbody>
</table>

$^a$ Based on regression fit to Lorentzian.

$^b$ Measured manually with screen cursor.
<table>
<thead>
<tr>
<th></th>
<th>C1--C3</th>
<th>C3--C2</th>
<th>C2--C4</th>
<th>C4--C5</th>
<th>C5--C6</th>
<th>C6--CH$_3$OH</th>
</tr>
</thead>
<tbody>
<tr>
<td>T40 Dextran</td>
<td>653.45</td>
<td>55.49</td>
<td>34.77</td>
<td>14.98</td>
<td>76.57</td>
<td>494.99</td>
</tr>
<tr>
<td>T2000 Dextran</td>
<td>848.21</td>
<td>71.44</td>
<td>44.47</td>
<td>20.99</td>
<td>102.03</td>
<td>694.73</td>
</tr>
<tr>
<td>G100 Sephadex</td>
<td>499.74</td>
<td>42.84</td>
<td>27.01</td>
<td>12.52</td>
<td>68.68</td>
<td>416.31</td>
</tr>
<tr>
<td>G75 Sephadex</td>
<td>390.65</td>
<td>34.65</td>
<td>21.09</td>
<td>10.01</td>
<td>57.04</td>
<td>320.54</td>
</tr>
</tbody>
</table>

* Linewidth is taken as average of linewidths (FWHH) for each pair of adjacent peaks.*
worsening of the line shape distortions due to increased spectral overlap for the rigid structures. More details about the spectra are obtained by examining the DISPA plots more closely. Proceeding from left to right in the spectrum the DISPA plots corresponding to each peak are considered in order in the following paragraphs.

The DISPA plot for Cl has an "indentation" on its low frequency side which occurs at larger angles away from the vertical axis. Some component is apparently becoming more significant when going to more rigid structures. Excluding the indentation due to the additional component, the DISPA plot of peak A proceeds from near-circular to very definite compression going from less rigid to more rigid structures.

The DISPA plots associated with peak C3 are nearly circular for the less rigid structures with a noticeable increase in compression when going to the more rigid structures. For all the spectra examined the magnitude of the observed compression is less than that observed for Cl in the same spectrum.

DISPA plots for C2 show progressively larger amounts of compression corresponding to increasingly rigid structures. Although the compression is symmetrical, it is difficult to ascertain whether the observed compression is the result of spectral overlap from peaks on either side of it or the result of some genuine non-Lorenzian line shape.

C4 and C5 are too close together to allow for examination of their line shapes. The mutual overlap between these two peaks creates reciprocal distortions in their DISPA plots. The DISPA plot of C4 is
distorted on its high frequency side resulting from overlap with C5, while the DISPA plot of C5 has a distortion on its low frequency side corresponding to the overlap with C4.

C6 is roughly twice as broad as the other peaks, as a consequence of having twice as many covalently bonded protons attached to it (i.e., relaxes twice as fast). The broader peak C6 shows some distortion resulting from the spectral overlap with peak C5.

DISPA plots for the methanol reference peak associated with each spectrum were found to be relatively poor. These spectral peaks were narrower and the poor DISPA plots resulted because at constant sampling rate the number of points defining the methanol peaks was less than the number defining the polymer peaks. The narrow line shapes were much more sensitive to small variations in the magnetic field homogeneity than were the broader polymer peaks.
FIG. 77. Experimental DISPA and RDDISPA plots for the Cl peak of T40 Dextran Solution.
FIG. 78. Experimental DISPA and RDDISPA plots for the C3 peak of T40 Dextran Solution.
FIG. 79. Experimental DISPA and RDDISPA plots for the C2 peak of T40 Dextran Solution.
FIG. 80. Experimental DISPA and RDDISPA plots for the C4 peak of T40 Dextran Solution.
FIG. 81. Experimental DISPA and RDDISPA plots for the C5 peak of T40 Dextran Solution.
FIG. 82. Experimental DISPA and RDDISPA plots for the C6 peak of T40 Dextran Solution.
Fig. 83. Experimental DISPA and RDDISPA plots for the methanol peak of T40 Dextran solution.
FIG. 84. Experimental DISPA and RDDISPA plots for the Cl peak of T2000 Dextran Solution.
FIG. 85. Experimental DISPA and RDDISPA plots for the C3 peak of T2000 Dextran Solution.
FIG. 86. Experimental DISPA and RDDISPA plots for the C2 peak of T2000 Dextran Solution.
FIG. 87. Experimental DISPA and RDDISPA plots for the C4 peak of T2000 Dextran Solution.
FIG. 88. Experimental DISPA and RDDISPA plots for the C5 peak of T2000 Dextran Solution.
FIG. 89. Experimental DISPA and RDISPA plots for the C6 peak of T2000 Dextran Solution.
Fig. 90. Experimental DISPA and RDDISPA plots for the methanol peak of T2000 Dextran solution.
FIG. 91. Experimental DISPA and RDDISPA plots for the Cl peak of G100 Sephadex gel.
FIG. 92. Experimental DISPA and RDDISPA plots for the C3 peak of G100 Sephadex gel.
FIG. 93. Experimental DISPA and RDDISPA plots for the C2 peak of G100 Sephadex gel.
FIG. 94. Experimental DISPA and RDDISPA plots for the C4 peak of G100 Sephadex gel.
FIG. 95. Experimental DISPA and RDDISPA plots for the C5 peak of G100 Sephadex gel.
FIG. 96. Experimental DISPA and RDDISPA plots for the C6 peak of G100 Sephadex gel.
Fig. 97. Experimental DISPA and RDDISPA plots for the methanol peak of G100 Sephadex gel.
FIG. 98. Experimental DISPA and RDDISPA plots for the Cl peak of G75 Sephadex gel.
FIG. 99. Experimental DISPA and RDDISPA plots for the C3 peak of G75 Sephadex gel.
FIG. 100. Experimental DISPA and RDDISPA plots for the C2 peak of G75 Sephadex gel.
FIG. 101. Experimental DISPA and RDDISPA plots for the C4 peak of G75 Sephadex gel.
FIG. 102. Experimental DISPA and RDDISPA plots for the C5 peak of G75 Sephadex gel.
FIG. 103. Experimental DISPA and RDDISPA plots for the C6 peak of G75 Sephadex gel.
Fig. 104. Experimental DISPA and RDDISPA plots for the methanol peak of G75 Sephadex gel.
Fig. 105. Experimental DISPA and RDDISPA plots for the C1 peak of G25 Sephadex gel.
E. DISCUSSION

Clearly the body of information currently available describing dextran polymers indicates these molecules are highly flexible in aqueous solution with portions capable of rapid segmental motion nearly completely independent of the overall polymer conformation (i.e. isotropic). The introduction of crosslinking agents necessarily inhibits this flexibility and the motions responsible for the relaxation of $^{13}$C nuclei consequently become more dependent on the polymer conformation (i.e. anisotropic). The spectral line shapes would be expected to become less Lorentzian as the structures become more highly crosslinked.

The experimental DISPA plots obtained in this study appear to support the above model. As indicated in the results section the DISPA plots in general show small but progressively larger deviations in going from less rigid (more flexible) to more rigid (less flexible) structures. The interpretation of these results is complicated by the larger distortions due to spectral overlap and decreased signal-to-noise observed for the more highly crosslinked structures. Of the six spectral peaks in each of the dextran spectra examined, those associated with the C1 ($\delta_C$ 97.8 ppm) and C6 ($\delta_C$ 65.8 ppm) nuclei were best spectrally resolved.

As indicated in the results section the DISPA plot corresponding to C1 shows an indentation on its low frequency side. This minor component may arise from a small percentage (~6%) of $\alpha$-D-(1-3) linkages typically found in dextran polymers (68). The DISPA and RDISPA plots
corresponding to C1 in each of the dextrans are provided in Figures 106 and 107 respectively. Ignoring the distortion due to the additional component by concentrating on the lower half of the DISPA plot and the left side of the RDDISPA plot, a slight compression of the DISPA plot is observed at the bottom of Figure 106 (the rigidity/amount of crosslinking increases toward the bottom of the Figure). The proportion of the points below the horizontal axis, indicating a larger deviation inside the DISPA circle, appears at the bottom of Figure 107. The DISPA and RDDISPA plots observed for the free dextrans (T40 and T2000) are very nearly circular and therefore consistent with Lorenzian line shape. The DISPA plots for the crosslinked gels (Sephadex G100 and G75) show some compression (particularly G75) inside the reference circle and the corresponding RDDISPA plots exhibit an increased proportion of points below the horizontal axis. In spite of the poor signal-to-noise observed for the highly crosslinked G-25 Sephadex it is apparent that the DISPA plot deviates greatly inside the reference circle and consequently the line shape must be highly non-Lorentzian.

The presence of the additional component overlapping the C1 peak complicates the interpretation of the previous paragraph. This complication is avoided by examining the DISPA and RDDISPA plots associated with the C6 peak in each spectrum. Unfortunately the C6 peak in the Sephadex G25 spectrum is too poorly resolved to afford construction of a DISPA or RDDISPA plot and so that spectrum cannot be included in this comparison. The DISPA and RDDISPA plots for the C6 peak in each of the remaining spectra examined are provided in Figures
Fig. 106. Experimental DISPA plots corresponding to C1 peaks in the spectra of each dextran polymer examined. Note that the degree of cross-linking (rigidity) increases toward the bottom of the figure.
Fig. 107. Experimental RDDISPA plots corresponding to C1 peaks in the spectra of each dextran polymer examined. Note that the degree of cross-linking (rigidity) increases toward the bottom of the figure.
108 and 109 respectively. As was observed for Cl, the DISPA plots show greater compression for the more highly crosslinked structures. The effect is more apparent when examining the associated RDDISPA plots which have a larger proportion of points below the horizontal axis for the more highly crosslinked structures.

The compression of the DISPA plots appears to correlate well with the rigidity of the structures. As the motions responsible for relaxation become more hindered (more anisotropic) the line shapes become less Lorentzian and the corresponding DISPA plots show deviations from circularity inside the reference circle. The observed experimental DISPA plot deviations (RDDISPA plots had displacements below the horizontal line) correspond to spectral line shape representing the composite of two or more Lorentzian components centered at the same resonance frequency but with different line widths (18). The magnitudes of these experimental deviations are generally small but the compressions were still detectable particularly for the RDDISPA plots which showed only displacements below their horizontal axes.

The DISPA plots of the free dextrans (T-40 and T-2000) had DISPA plots which were most nearly circular. Since these molecules were not crosslinked their motions would predictably be most nearly isotropic and their line shapes would be expected to be very nearly Lorentzian so it is consistent that their DISPA plots are very nearly circular.

The DISPA plots for the crosslinked gels Sephadex G-100 and G-75 are definitely compressed but the magnitudes of these deviations are
Fig. 108. Experimental DISPA plots corresponding to C6 peaks in the spectra of each dextran polymer examined. Note that the degree of cross-linking (rigidity) increases toward the bottom of the figure.
Fig. 109. Experimental RDDISPA plots corresponding to C6 peaks in the spectra of each dextran polymer examined. Note that the degree of cross-linking (rigidity) increases toward the bottom of the figure.
small and interpretation of the results is complicated by the increased spectral overlap resulting from the broadening of the peaks. The peaks were so broad for G-25 gel that the DISPA plot for only Cl could be constructed. Although the signal-to-noise for G-25 gel was extremely poor there is a very definite deviation of the DISPA plot inside the reference circle indicating a high degree of anisotropic motion as expected for the dextran backbone.

The primary difficulties associated with the use of DISPA plots to examine motional behavior of polymers stem from difficulties encountered in ensuring that the observed spectral line shapes are genuine and undistorted. Truely anisotropic motion leads to symmetrical deviations inside the DISPA plot. When the DISPA plots show some type of asymmetry then some other type of behavior besides motional anisotropy is contributing to the observed line shape. For this reason symmetrically distorted DISPA plots are most reliable for assessing motional flexibility.

As discussed in Chapter III the spectral line shape is dependent on the magnetic field homogeneity through the sample. Since none of the polymer peaks showed any deviations outside the reference circles it is clear that the magnetic field homogeneity was not obscuring the effects on the spectral line shapes due to anisotropic motion. Methanol was added to the dextran samples in hopes that its line shape could be used to gauge the magnetic field homogeneity in the sample. The narrower methanol line shape was much too sensitive to small deviations in the magnetic field homogeneity, so much so in fact that a truely
perfect line shape could not be obtained for any of the methanol peaks. Rather than try to get a purely Lorentzian line shape for the methanol peak it was determined that adequate magnetic field homogeneity was obtained when the instrument was shimmed until the methanol peak was as narrow as possible. Shimming the instrument was most successful when the area under the time domain proton signal was maximized as a criterion for optimum field homogeneity. The lock signal was too broad (consequence of the high solution viscosity) and the signal-to-noise ratio for the time domain carbon signal was too low to be used for shimming the instrument. A $^1\text{H}/^{13}\text{C}$ dual probe is required so the instrument can be shimmed on the proton time domain signal, while data collection is made for the carbon spectra.

Distortions due to spectral overlap were generally made obvious by examining the separation between adjacent peaks and noting that distortions on the high (or low) frequency side of a DISPA plot could be attributed to near neighbor peaks positioned at higher (or lower) frequencies. The lower half of the DISPA plot corresponds to the higher frequencies. One clue to recognizing these types of distortions is to note that the corresponding DISPA plots are asymmetrical.

Even though the amount of dextran material inside the NMR samples was relatively constant, far more scans were required to obtain the same level of signal-to-noise for the rigid structures as for the flexible structures, because there is a smaller fraction of "liquid-like" segments in the dextran structures (65) of the more highly crosslinked materials. This behavior complicates the data
collection process since several hours (at least 8 to 10 hours) of signal averaging is required before the actual experimental line shape can be observed. If for instance there are errors in the data collection parameters or the magnetic field homogeneity these may not be detectable until after much time is consumed signal averaging.

F. CONCLUSION

DISPA plots provide a means to examine the relative flexibilities of the dextran polymers. The exact degree of crosslinking (concentration of the crosslinking agent) is not available limiting this study to a qualitative assessment of the flexibilities. As indicated in the introduction section of this chapter the motional behavior of dextran polymers has been studied in some detail and is well established.

The significant advantage that DISPA has over current methods commonly used for studying motional behavior of polymers in solution is that it requires data from only a single spectra whereas current methods generally require data from several different spectra. Polymer motion is generally studied by measuring T1's for several different nuclei at several different positions in the polymer. A "map" is then pieced together showing which parts of the polymer are moving (relaxing) more or less rapidly than other parts of the polymer. To this end, "inversion-recovery" type experiments (71) which require collection of several spectra with varying relaxation delay times are
conducted. Clearly DISPA offers a more convenient approach to assess flexibility.

As indicated in the discussion the primary difficulties encountered in the use of DISPA plots to assess motional behavior involve those problems which must be overcome to ensure that the observed spectral line shapes are genuine. For the most part these problems can be minimized by carefully shimming the instrument and carefully selecting peaks to be examined.
A. DISCUSSION OF VARIOUS APPLICATIONS OF DISPA

The purpose of this dissertation has been to find and examine applications for DISPA (and the associated RDDISPA) plots. The primary applications which have been examined fall into three general categories:

(1) Parameter optimization
(2) Phase measurement/correction
(3) Line shape analysis

Each of these categories is discussed separately below.

1. PARAMETER OPTIMIZATION

As an example of parameter optimization the deviations from circularity of the experimental DISPA plots were correlated to errors in the shim coil currents for the \( z^n \) labeled shim coils (Chapter III) of a FT/NMR instrument. The magnitudes of the deviations from circularity were verified both theoretically (for all the \( z \) through \( z^5 \) shim coils) and experimentally (for the \( z \) and \( z^2 \)) to be proportional to the parameter requiring correction, the shim coil current. The shapes of the DISPA and RDDISPA plots were shown to be indicative of the particular shim coil whose current was in error. Additionally the handedness (left or right handed) was shown to be
related to the sign of the error current (positive or negative) for those shim coils which produced asymmetric DISPA and RDDISP4 plots.

Since each \( z^n \) labeled shim coil must necessarily generate a magnetic field gradient which has other \( z^n \) components associated with it, it is not possible to modify any one shim coil without affecting the gradients established by the remaining \( z^n \) coils (i.e. no single shim coil can be varied independent of the others). Therefore although the deviation from circularity for the DISPA plot has been successfully demonstrated for measuring the error in the appropriate shim current, it is not possible to correct these coils independent of one another because of the geometry of present day shim coils. This limitation of the shim coil designs does not invalidate the application of DISPA or RDDISP4 plots for parameter optimization.

The deviations from circularity effectively reflect the three dimensional geometry of the spin distribution through the sample, so that any parameter which affects the spin distribution throughout the sample can be monitored in the same manner as the shim coil currents. Other sample parameters associated which may be examined/monitored include temperature gradients and sample homogeneity.

2. PHASE MEASUREMENT/CORRECTION

The procedure introduced in Chapter V has been successfully demonstrated for the direct measurement of spectral phase for both FT/NMR and FT/ICR. This phase measurement procedure has been incorporated into on-line phase correction routines for phase correction of FT/NMR spectra (solution of linear phase problem), and
FT/ICR spectra over a narrow mass range (solution of non-linear phase problem). The phase measurement is very accurate (less than 0.3% error for spectra with good line shape and S/N) and rapid (requires only a few seconds for execution).

The major sources of error in the phase measurement were found to be poor line shape, spectral overlap, and baseline anomalies. These problems can be overcome by careful magnet shimming (in FT/ICR the shape is dependent on the potential of the trapping plates, and excitation amplitude, the sample pressure), careful selection of spectral peaks, and proper baseline correction.

The resolution in the FT/ICR mass domain which was obtained by phase correction of the real spectrum is improved by a factor very near the theoretical value of 3 over the conventional magnitude spectrum. At present the limit to phase correction of FT/ICR data appears to be the number of peaks available over a narrow spectral range to define the phase correction function. The "ladder diagrams" described in Chapter VII have been demonstrated for the interpolation of the proper phases between measured values but they are still only usable over a narrow spectral range.

In all DISPA has proven quite effective for phase measurement.

3. LINE SHAPE ANALYSIS

DISPA and RDDISPA plots were shown to be useful for the analysis of broadband $^1$H decoupled $^{13}$C FT/NMR spectral line shapes of dextran polymers. The observed deviations although small, could be detected
for well isolated peaks free of overlapping components. Of the six peaks in the dextran spectra only one, C6, was sufficiently isolated and free of minor overlapping components as to permit an unambiguous assessment of its spectral line shape. The rigidity of the dextran structures (degree of crosslinking) was correlated with compression of the corresponding DISPA plot. These results indicate that DISPA and RDDISPA plots may be useful for the assessment of motional behavior of polymers. Ensuring that the observed line shapes are genuine is the primary problem encountered in utilizing DISPA and RDDISPA plots in this manner. In general this involves being certain that the sample is properly prepared, the magnet is correctly shimmed, and the observed spectral peaks are not overlapped by other components.

B. FUTURE WORK

Future work associated with the application of DISPA and RDDISPA plots should involve more specific experimental applications. From this work the analysis of spectral line shapes associated with dextran polymers was made difficult by the small deviations which were actually observed. High resolution $^1$H FT/NMR line shape studies (77) of several swollen crosslinked polymer gels (poly(methyl methacrylate), polystyrene, poly(ethylene oxide), and poly(dimethyl-siloxane)) indicate that these gels will provide more easily observable deviations from Lorentzian line shapes.
If a polymer system can be found in which the degree of crosslinking can be varied synthetically in a reproducible fashion then it may be possible to quantitatively associate the observed distortions in the DISPA and RDDISPA plots to the relative rigidity of the polymer structure.

The average measured phase errors due to spectral overlap between peaks with the same areas and widths was found to be the correct phase for the position half way between the peak maxima. Thus, the phase errors for overlapped peaks of different intensity and line width may be some type of weighted average dependent on the relative overlap between the peaks and their relative intensities and widths. If such a relationship can be determined (either by computer modeling or analytically) then the phase measurement routine described in Chapter V can be applied with much greater flexibility.

The phase measurement routine can be improved by including a provision that any individual intersection which results in a measured phase that is greater than two standard deviations away from the overall average phase be omitted in a second calculation of the overall average phase. This would remove the occasional erroneous intersection that sometimes enters into the phase measurement.

The current phase correction routines must be told which peaks to use for the phase correction. Provision can be included to have the program select the most appropriate peak(s) and to compensate for spectral overlap for symmetrical multiplets.
The FT/NMR phase correction program, PHASER, described in Chapter V can benefit greatly by the incorporation of a linear regression routine to allow the measured phases of several peaks to be used for the determination of the phase correction function rather than having the calculated function depend on the measured phases of two peaks only as it does now.

The development of a routine which can display the DISPA and/or RDDISPA on-line as a pseudo-real time display would be very useful for applications of manual parameter optimization or phase correction since the effects of whatever adjustments could be visualized as they are entered into the instrument.

C. CONCLUSION

Throughout the course of this work, emphasis has been placed on the examination of properties and applications of DISPA plots. One might claim that the DISPA plot is merely a rerepresentation of the data which does not provide additional information. This attitude blindly ignores the inherent sensitivity DISPA plots have demonstrated for small deviations in line shape and the obvious applications for phase measurement. The results in Chapter III of this dissertation also suggest that DISPA may have legitimate applications for parameter optimization which would ultimately allow maximization of spectral properties such as resolution and signal-to-noise ratio. From this work and the work of several others it is apparent that the DISPA plot is more than a rerepresentation of the data, it is a new way to look at the data.
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APPENDIX A

VERIFICATION THAT DISPA PLOTS FOR TRUE LORENTZIANS ARE PERFECTLY CIRCULAR

To verify Equation [1.11] the normalized expressions for absorption and dispersion,

\[ A(\omega) = \frac{\tau}{(1+\tau^2(\omega_0-\omega)^2)} \]  \hspace{1cm} [A.1]

\[ D(\omega) = \frac{\tau^2(\omega_0-\omega)^2}{(1+\tau^2(\omega_0-\omega)^2)} \]  \hspace{1cm} [A.2]

may be substituted into Equation [1.11].

\[ \left[ \frac{\tau}{(1+\tau^2(\omega_0-\omega)^2)} - \frac{\tau}{2} \right]^2 + \left[ \frac{\tau^2(\omega_0-\omega)^2}{(1+\tau^2(\omega_0-\omega)^2)} \right]^2 = ? \]  \hspace{1cm} [A.3]

substituting \( \Delta \) for \((1+\tau^2(\omega_0-\omega)^2)\) leaves the following expression.

\[ \left[ \frac{\tau}{\Delta} - \frac{\tau}{2} \right]^2 + \left[ \frac{\tau^2(\omega_0-\omega)^2}{\Delta} \right]^2 = ? \]  \hspace{1cm} [A.4]
Rearranging the above expression in the following manner shows that the expression on the right hand side is equal to the constant value \( \pi^2 \cdot (-) \). And therefore Equation [1.11] describes a circle.

\[
\begin{align*}
\left( \frac{-2\pi - \tau \Delta}{2\Delta} \right)^2 + \left( \frac{-\tau^2 (\omega_0 - \omega)}{\Delta} \right)^2 &= \ ? \\
\frac{(2\pi - \tau \Delta)(2\pi - \tau \Delta)}{4\Delta^2} + \frac{\tau^4 (\omega_0 - \omega)^2}{\Delta^2} &= \ ? \\
\frac{4\tau^2 - 4\tau^2 \Delta + \tau^2 2 \Delta^2}{4\Delta^2} + \frac{\tau^4 (\omega_0 - \omega)^2}{\Delta^2} &= \ ? \\
\frac{4\tau^2 - 4\tau^2 \Delta + \tau^2 2 \Delta^2}{4\Delta^2} + \frac{4\tau^4 (\omega_0 - \omega)^2}{4\Delta^2} &= \ ? \\
\frac{4\tau^2 (1 + \tau^2 (\omega_0 - \omega)^2) - 4\tau^2 \Delta + \tau^2 2 \Delta^2}{4\Delta^2} &= \ ? \\
\frac{4\tau^2 \Delta^2 - 4\tau^2 \Delta + \tau^2 2 \Delta^2}{4\Delta^2} &= \ ? \\
\frac{\tau^2 \Delta^2}{4\Delta^2} &= \left[ \frac{-\tau}{\Delta} \right]^2 \\
\end{align*}
\]

Q.E.D.
APPENDIX B

VERIFICATION THAT THE DISPAP PLOT IS ROTATED ABOUT THE ORIGIN BY AN ANGLE EQUAL TO THE SPECTRAL PHASE ERROR

The time domain signal is obtained as

\[ f(t) = e^{-t/\tau} \cos(\omega_0 t), \quad 0 < t < \infty. \] \[ \text{(B.1)} \]

When there is a time delay, \( t_d \), the time domain signal becomes

\[ f(t) = e^{-(t-t_d)/\tau} \cos(\omega_0(t-t_d)). \] \[ \text{(B.2)} \]

Using standard practice the real and imaginary frequency domain components are obtained by taking the cosine and sine Fourier transforms of the time domain signal respectively.

\[ R(\omega) = -\int_{-\infty}^{\infty} f(t) \cos(\omega t) \, dt \] \[ \text{(B.3)} \]

\[ I(\omega) = -\int_{-\infty}^{\infty} f(t) \sin(\omega t) \, dt \] \[ \text{(B.4)} \]

Consider the cosine Fourier transform when there is a time delay

\[ R(\omega) = \int_{-\infty}^{\infty} e^{-(t-t_d)/\tau} \cos(\omega_0(t-t_d)) \cos(\omega t) \, dt \] \[ \text{(B.5)} \]

substituting the following relationships

\[ t = t - t_d + t_d \] \[ \text{(B.6)} \]

\[ dt = d(t-t_d) \] \[ \text{(B.7)} \]
gives the following relationship

\[ R(\omega) = \frac{1}{\pi} \int_{0}^{\infty} e^{-(t-t_d)/\tau} \cos(\omega_0(t-t_d))\cos(\omega(t-t_d) + \omega t_d) d(t-t_d). \]  

[B.7]

Using the trigonometric identity:

\[ \cos A \cos B = \frac{1}{2} \left[ \cos(A-B) + \cos(A+B) \right] \]  

[B.8]

the following relationship is obtained

\[ R(\omega) = \frac{1}{2\pi} \int_{0}^{\infty} e^{-(t-t_d)/\tau} \left[ \cos(\omega_0(t-t_d) - (\omega(t-t_d) + \omega t_d)) + \cos(\omega_0(t-t_d) + (\omega(t-t_d) + \omega t_d)) \right] d(t-t_d) \]  

[B.9]

\[ = \frac{1}{2\pi} \int_{0}^{\infty} e^{-(t-t_d)/\tau} \left[ \cos(\omega_0 - \omega)(t-t_d) + \omega t_d) + \cos(\omega_0 + \omega)(t-t_d) + \omega t_d) \right] d(t-t_d) \]  

[B.10]

\[ = \frac{1}{2\pi} \int_{0}^{\infty} e^{-(t-t_d)/\tau} \cos((\omega_0 - \omega)(t-t_d) + \omega t_d) d(t-t_d) \]

\[ + \frac{1}{2\pi} \int_{0}^{\infty} e^{-(t-t_d)/\tau} \cos((\omega_0 + \omega)(t-t_d) + \omega t_d) d(t-t_d) \]  

[B.11]

Using the trigonometric identity:

\[ \cos(A+B) = \cos A \cos B - \sin A \sin B. \]
\[ R(\omega) = \frac{1}{2\pi} \int_{-\infty}^{\infty} e^{-\frac{(t-t_d/\tau)}{2}} \cos((\omega_0-\omega)(t-t_d)) \cos(\omega t_d) \, d(t-t_d) \]

\[ - \frac{1}{2\pi} \int_{0}^{\infty} e^{-\frac{(t-t_d/\tau)}{2}} \sin((\omega_0-\omega)(t-t_d)) \sin(\omega t_d) \, d(t-t_d) \]

\[ + \frac{1}{2\pi} \int_{0}^{\infty} e^{-\frac{(t-t_d/\tau)}{2}} \cos((\omega_0+\omega)(t-t_d)) \cos(\omega t_d) \, d(t-t_d) \]

\[ - \frac{1}{2\pi} \int_{0}^{\infty} e^{-\frac{(t-t_d/\tau)}{2}} \sin((\omega_0+\omega)(t-t_d)) \sin(\omega t_d) \, d(t-t_d) \]

Pulling the constant terms out of the integrals gives the following relationship.

\[ R(\omega) = \frac{1}{\pi} \left[ \cos(\omega t_d) \int_{0}^{\infty} e^{-\frac{(t-t_d/\tau)}{2}} \cos((\omega_0-\omega)(t-t_d)) \, d(t-t_d) \right. \]

\[ - \sin(\omega t_d) \int_{0}^{\infty} e^{-\frac{(t-t_d/\tau)}{2}} \sin((\omega_0-\omega)(t-t_d)) \, d(t-t_d) \]

\[ + \cos(\omega t_d) \int_{0}^{\infty} e^{-\frac{(t-t_d/\tau)}{2}} \cos((\omega_0+\omega)(t-t_d)) \, d(t-t_d) \]

\[ - \left. \sin(\omega t_d) \int_{0}^{\infty} e^{-\frac{(t-t_d/\tau)}{2}} \sin((\omega_0+\omega)(t-t_d)) \, d(t-t_d) \right] \]

\[ [B.12] \]

\[ [B.13] \]
Combining like terms gives the following relationship.

\[
R(\omega) = \frac{1}{2\pi} \left[ \cos(\omega t_d) \left[ \int_0^\infty e^{-(t-t_d/\tau)} \cos((\omega_0-\omega)(t-t_d)) \, dt \right] 
+ \int_0^\infty e^{-(t-t_d/\tau)} \cos((\omega_0+\omega)(t-t_d)) \, dt \right] 
- \sin(\omega t_d) \left[ \int_0^\infty e^{-(t-t_d/\tau)} \sin((\omega_0-\omega)(t-t_d)) \, dt \right] 
- \int_0^\infty e^{-(t-t_d/\tau)} \sin((\omega_0+\omega)(t-t_d)) \, dt \right] \tag{B.14}
\]

Using the integrals

\[
\int_0^a e^{-at} \cos(bt) \, dt = \frac{a}{a^2 + b^2} \tag{B.15}
\]

\[
\int_0^a e^{-at} \cos(bt) \, dt = \frac{a}{a^2 + b^2} \tag{B.16}
\]

the following relations are obtained.

\[
R(\omega) = \frac{1}{2\pi} \left[ \cos(\omega t_d) \left[ \frac{\tau}{(\frac{1}{\tau})^2 + (\omega_0-\omega)^2} + \frac{\tau}{(\frac{1}{\tau})^2 + (\omega_0+\omega)^2} \right] 
- \sin(\omega t_d) \left[ \frac{\omega_0-\omega}{(\frac{1}{\tau})^2 + (\omega_0-\omega)^2} + \frac{\omega_0+\omega}{(\frac{1}{\tau})^2 + (\omega_0+\omega)^2} \right] \right] \tag{B.17}
\]
Since in NMR \( \omega = \omega_0 \), the terms with \((\omega_0 + \omega)^2\) in the denominator are very small and may be dropped.

\[
R(\omega) = \frac{1}{2\pi} \cos(\omega t_d) \left[ \left( \frac{1}{\tau} \frac{1}{(\tau)^2 + (\omega_0 - \omega)^2} \right) - \sin(\omega t_d) \left( \frac{(\omega + \omega_0)}{(\tau)^2 + (\omega_0 + \omega)^2} \right) \right] \tag{B.18}
\]

The factors in the smaller square brackets are the absorption, \( A(\omega) \), and the dispersion, \( D(\omega) \), line shapes.

\[
R(\omega) = \frac{1}{2\pi} \left[ \cos(\omega t_d) \cdot A(\omega) - \sin(\omega t_d) \cdot D(\omega) \right] \tag{B.19}
\]

The above equation shows that real spectrum which is observed when there is a time delay represents a mixture of absorption and dispersion components. The exact proportions of which are established by the weighting factors \( \frac{1}{2\pi} \cos(\omega t_d) \) and \( -\frac{1}{2\pi} \sin(\omega t_d) \).

As a final approximation the relationship

\[
\omega t_d = \omega t_d = \phi \tag{B.20}
\]

can be used to obtain

\[
R(\omega) = \frac{1}{2\pi} \left[ \cos(\phi) \cdot A(\omega) - \sin(\phi) \cdot D(\omega) \right] \tag{B.21}
\]
Using the same logic as was just applied to obtain $R(\omega)$, the imaginary lineshape is obtained by taking the sine Fourier transform of the time domain signal.

$$I(\omega) = \frac{1}{\pi} \int_{0}^{\infty} f(t) \sin(\omega t) \, dt$$

$$f(t) = e^{-(t-t_d)/\tau} \cos(\omega_d(t-t_d))$$

$$I(\omega) = \frac{1}{\pi} \int_{0}^{\infty} e^{-(t-t_d)/\tau} \cos(\omega_d(t-t_d)) \sin(\omega t) \, dt$$

Substituting

$$t = t - t_d + t_d$$

$$dt = d(t-t_d)$$

$$I(\omega) = \frac{1}{\pi} \int_{0}^{\infty} e^{-(t-t_d)/\tau} \cos(\omega_d(t-t_d)) \sin(\omega(t-t_d) + \omega_d) \, d(t-t_d)$$

Using the trigonometric identity

$$\cos A \sin B = \frac{1}{2} \left[ \sin(A-B) + \sin(A+B) \right]$$

the following relation is obtained

$$I(\omega) = \frac{1}{2\pi} \int_{0}^{\infty} e^{-(t-t_d)/\tau} \left[ \sin(\omega_d(t-t_d)-(\omega(t-t_d)-\omega_d)) \ight.$$  

$$+ \sin(\omega_d(t-t_d) + \omega(t-t_d) + (\omega_d)) \right] \, d(t-t_d)$$

$$I(\omega) = \frac{1}{2\pi} \int_{0}^{\infty} e^{-(t-t_d)/\tau} \left[ \sin((\omega_0-\omega)(t-t_d) + \omega t_0) \ight.$$  

$$+ \sin((\omega_0-\omega)(t-t_d) + \omega t_d) \right] \, d(t-t_d)$$
Applying the identity

\[ \sin (A + B) = \sin A \cos B + \cos A \sin B \]  

the following is obtained

\[
I(\omega) = \frac{1}{2\pi} \int_0^\infty e^{-(t-t_d)/\tau} \left[ \sin((\omega_0-\omega)(t-t_d)) \cos(\omega t_d) 
+ \cos((\omega_0-\omega)(t-t_d)) \sin(\omega t_d) \right] d(t-t_d) 
+ \frac{1}{2} \int_0^\infty e^{-(t-t_d)/\tau} \left[ \sin((\omega_0+\omega)(t-t_d)) \cos(\omega t_d) 
+ \cos((\omega_0+\omega)(t-t_d)) \sin(\omega t_d) \right] d(t-t_d)
\]

Pulling the constant terms out in front of the integrals gives

\[
I(\omega) = \frac{1}{2\pi} \left[ \cos(\omega t_d) \int_0^\infty e^{-(t-t_d)/\tau} \sin((\omega_0-\omega)(t-t_d)) d(t-t_d) 
+ \sin(\omega t_d) \int_0^\infty e^{-(t-t_d)/\tau} \cos((\omega_0-\omega)(t-t_d)) d(t-t_d) 
+ \cos(\omega t_d) \int_0^\infty e^{-(t-t_d)/\tau} \sin((\omega_0+\omega)(t-t_d)) d(t-t_d) 
+ \sin(\omega t_d) \int_0^\infty e^{-(t-t_d)/\tau} \cos((\omega_0+\omega)(t-t_d)) d(t-t_d) \right]
\]

\[ [B.33] \]
Combining like terms,

\[
I(\omega) = \frac{1}{2\pi} \left[ \cos(\omega t_d) \left[ \int_0^\infty e^{-\tau (t-t_d)/\tau} \sin((\omega_0-\omega)(t-t_d)) \, d(t-t_d) \right] + \int_0^\infty e^{-\tau (t-t_d)/\tau} \sin((\omega_0+\omega)(t-t_d)) \, d(t-t_d) \right] \\
+ \sin(\omega t_d) \left[ \int_0^\infty e^{-\tau (t-t_d)/\tau} \cos((\omega_0-\omega)(t-t_d)) \, d(t-t_d) \right] + \int_0^\infty e^{-\tau (t-t_d)/\tau} \cos((\omega_0+\omega)(t-t_d)) \, d(t-t_d) \right] \]  

[B.34]

Integrating,

\[
I(\omega) = \frac{1}{2\pi} \left[ \cos(\omega t_d) \left[ \frac{(\omega_0-\omega)}{(-\tau)^2 + (\omega_0-\omega)^2} + \frac{(\omega_0-\omega)}{(-\tau)^2 + (\omega_0+\omega)^2} \right] \right] \\
+ \sin(\omega t_d) \left[ \frac{1}{(-\tau)^2 + (\omega_0-\omega)^2} + \frac{1}{(-\tau)^2 + (\omega_0+\omega)^2} \right] \]  

[B.35]
The terms with \((\omega_0 - \omega)^2\) in the denominator are very small and may be ignored.

\[
I(\omega) = \frac{1}{2\pi} \left[ \cos(\omega t_d) \left[ \frac{1}{(\frac{1}{2})^2 + (\omega_0 - \omega)^2} \right] \right.
\]

\[
+ \sin(\omega t_d) \left[ \frac{1}{(\frac{1}{2})^2 + (\omega_0 - \omega)^2} \right] \left. \right]
\]

[B.36]

The terms in square brackets are the true absorption, \(A(\omega)\), and dispersion, \(D(\omega)\), lineshapes.

\[
I(\omega) = \frac{1}{2\pi} \left[ \cos(\omega t_d) \cdot A(\omega) + \cos(\omega t_d) \cdot D(\omega) \right] \quad [B.37]
\]

Substituting, \(\omega t_d \equiv \omega_0 t_d = \phi\)

\[
I(\omega) = \frac{1}{2\pi} \left[ \sin(\phi) \cdot A(\omega) + \cos(\phi) \cdot D(\omega) \right] \quad [B.38]
\]

As was found for \(R(\omega)\), \(I(\omega)\) is a weighted mixture of true absorption and dispersion lineshapes when there is a delay in the time domain signal.

The relationships

\[
R(\omega) = \cos(\phi) A(\omega) - \sin(\phi) D(\omega) \quad [B.39]
\]

\[
I(\omega) = \sin(\phi) A(\omega) + \cos(\phi) D(\omega) \quad [B.40]
\]
are exactly equivalent to the transformation relating the \((x, y)\) coordinates of a point for one set of cartesian axes to another set of coordinates \((x', y')\) corresponding to a different set of cartesian axes rotated by an angle \(\theta\). The transformation for axis rotation from non-prime to prime coordinates is described by

\[
\begin{align*}
x' &= x \cos \theta + y \sin \theta \\
y' &= -x \sin \theta + y \cos \theta
\end{align*}
\]

where \(x = D(\omega), y = A(\omega), x' = I(\omega), y' = R(\omega)\).

The jist of this discussion is that phase error (delays in the time domain) result in a rotation of the DISPA plot by an angle equal to the phase error.
APPENDIX C

RELATION BETWEEN THE AREA INSIDE THE DISPA CIRCLE AND THE NUMBER OF SPECIES REPRESENTED

The number of species, \( m \), and the diameter of the DISPA circle, \( d \), are both related to the intensity maximum of the absorption line, \( A_{\text{max}} \), by the following equations

\[
m = k' A_{\text{max}} \quad \text{[C.1]}
\]
\[
d = k'' A_{\text{max}} \quad \text{[C.2]}
\]

where \( k' \) and \( k'' \) are the corresponding proportionality constants.

Solving for \( d \) in terms of \( m \), the following equation is obtained.

\[
d = \frac{k''}{k'} m \quad \text{[C.3]}
\]

Equation [C.3] can be simplified by combining the constants into a single constant, \( k \).

\[
d = km \quad \text{[C.4]}
\]

The area inside the DISPA circle, \( R \), is related to the diameter by

\[
R = \frac{\pi}{4} (km)^2 \quad \text{[C.6]}
\]

By combining all the constants into a single constant, \( c \), and solving for \( m \) in terms of \( R \) the following relation is obtained.

\[
m = c \sqrt{R} \quad \text{[C.7]}
\]

The number of species is proportional to the square root of the area inside the DISPA circle.
APPENDIX D

PROGRAM DISPA6

DISPA6 constructs DISPA and RDDISPA plots on-line using standard Bruker disk files as input. DISPA6 was written expressly for application on the Bruker AM-500 FT/NMR (ADAKOS Version 820601) with a color raster and a WATANABE Instruments digital plotter (model WX4636R). Graphic output to both the raster and the digital plotter is facilitated by subroutines obtained with the Bruker Graphic Display Package for PASCAL Version 84 12 26.1.

The program request the file name, number of peaks in absorption spectrum, and the number of points extracted about the peak as a window. The peak maximum is determined by fitting an $n^{th}$ order polynomial to the points nearest the magnitude maximum. If desired, default values may be used for the polynomial regression fit. Specifically these default values will fit a 4$^{th}$ order polynomial to the 7 points nearest the peak maximum with unit weighting.

There is no limit to data set size. The largest allowed window is dimensioned as 1024. Satisfactory results are generally obtained from the default values and a window size of 100 to 200 points.

A more detailed discussion of DISPA6 is given in Chapter II.
PROGRAM DIASPAS

CONST

PWIDTH=101
PTS=2561
SIZEA=111
SIZEB=31
SIZEC=201
NUMBER=1001
DIMX=10241
SECSIZE=2561
TOL=1.0E-351
SIZE=61
FACTOR=1.3333333
PI=3.14159271
SFFHI=10.01
SFRD=5.01

VAR

F1:FILE
F2:FILE
RESPONSE, ANSWER:CHAR
J, K, L, M, N, NP, NPTS:INTEGER
COUNT, PEAK, RIGHTPT, LEFTPT:INTEGER
H, N, IUT, NUMOPS:INTEGER

MAT A=ARRAY(1..DIM1, 1..DIM2) OF REAL
MAT B=ARRAY(1..DIM2) OF REAL
MAT C=ARRAY(1..DIM1) OF REAL

FILE:STRING(201)

(*******************************************************************************)

(*******************************************************************************)
PROCEDURE INITIAL;
BEGIN
SMO:=0.0;
FOR I:=1 TO 20 DO
BEGIN
POSITION[i]:=01
HEIGHT[i]:=0.01
END; (* FOR I *)
FOR I:=1 TO 21 DO
BEGIN
&I:=0.01
ALPHA[I]:=0.01
IFVi,3]:=0;
FOR J:=1 TO 11 DO
AH[I,]J]:=0.01;
END; (* FOR I *)
END; (* INITIAL *)
PROCEDURE SORT;
BEGIN
IF NPK = 1 THEN
BEGIN
FOR I:=1 TO (NPK - 1) DO
BEGIN
LITTLE := HEIGHT[I];
SAVE := I;
FOR K:= (I + 1) TO NPK DO
IF HEIGHT(K) < LITTLE THEN
BEGIN
SAVE:=K;
LITTLE:=HEIGHT(K);
END; (* IF HEIGHT(K) < LITTLE *)
TEMP:=HEIGHT[I];
HEIGHT[I]:=HEIGHT(SAVE);
HEIGHT(SAVE):=TEMP;
ITEM:=POSITION[I];
POSITION[I]:=POSITION(SAVE);
POSITION(SAVE):=ITEM;
END; (* IF HEIGHT(K) < LITTLE *)
END; (* FOR I *)
END; (* SORT *)

(* INITIAL *)
PROCEDURE INPUTII;
BEGIN
WRITE(' ENTER INPUT FILE NAME: ');
READLN(FILEIN);
WRITE(' ENTER THE NUMBER OF PEAKS IN THE ABSORPTION SPECTRUM: ');
READLN(NPK1);
RESET(F1, FILEIN);
SECS:=BLOCKREAD(F1, BUF, 1, -1);
SIZE:=BUF[6];
ASS:=(SIZE DIV 2);
WRITELN(' THERE ARE ' , ASS, ' POINTS IN THE ABSORPTION SPECTRUM. ');
WRITELN(' ENTER THE NUMBER OF POINTS TO BE RETAINED ABOUT ');
WRITE(' THE ABSORPTION MAXIMUM: ');
READLN(NPT1);
IF ((NPT DIV 2)*2 = NPT1) THEN NPT1:=NPT1 + 1;
SECSUM:=SIZE DIV SECSIZE;
SECTOR:=01;
HEIGHT(1):=0.01;
HX:=BLOCKREAD(F1, BUF, 1, SECTOR);
I1:=BUF[31];
I2:=BUF[31] + 0;
FOR J:=3 TO (SECSIZE DIV 2) DO
BEGIN
I3:=BUF[(J+2)-1] + 0.01;
IF (I2 > HEIGHT(I1)) THEN BEGIN
   HEIGHT(I1):=I2;
   POSITION(I1):=(I1+2);
   SORT;
END;
IF (I2 > HEIGHT(I1)) THEN BEGIN
   I1:=I2;
   I2:=I3;
   COUNT:=COUNT + 1;
END;
END;
FOR J:=1 TO (SECSIZE DIV 2) DO
BEGIN
I2:=BUF[(J+2)-1] + 0.01;
IF (I2 > HEIGHT(I1)) THEN BEGIN
   HEIGHT(I1):=I2;
   POSITION(I1):=COUNT;
   SORT;
END;
END;
UNTIL SECTOR >= SECSUM;
END;
(*INPUT II*)
PROCEDURE INPUT2;
BEGIN
  IF (HPK > 1) THEN
    BEGIN
      WRITELN(' PEAK POSITION HEIGHT');
      FOR I:=1 TO HPK DO
        WRITELN(' ',I,' ',POSITION[I],', ',HEIGHT[I]);
      WRITE(' SELECT THE DESIRED PEAK (ENTER PEAK NUMBER): ');READLN(PEAK);
      END;
    ELSE
      PEAK:=1;
      RESET(FILE1);
      LEFTPT := POSITION[PEAK] - NPT + 1;
      RIGHTPT := POSITION[PEAK] + NPT - 1;
      KOUNT := 0;
      K := 1;
    FOR I := 1 TO (SECMUN + 1) DO
      BEGIN
        SECTOR:=(I-1);
        KK:=BLOCKREAD(FILE1,BUF,1,SECTOR);
        FOR J:=1 TO SECSIZE DO
          BEGIN
            KOUNT:=KOUNT + 1;
            IF ((KOUNT >= LEFTPT) AND (KOUNT <= RIGHTPT)) THEN
              BEGIN
                KK:=Buf[J]/HEIGHT[PEAK];
                K:=K+1;
              END;
            ELSE
              BEGIN
                A[KK]:=Buf[J]/HEIGHT[PEAK];
              END;
          END;
      END;
    END;
  ELSE
    BEGIN
      MP1:=H + 1;
      END (# INPUT2 #)
  END (# INPUT2 #)
PROCEDURE DEFAULTS;
BEGIN
  WRITELN(' DO YOU WANT DEFAULT VALUES FOR THE?');
  WRITE(' POLYNOMIAL FIT TO THE MAGNITUDE MAXIMUM (Y/N): ');READLN(ANSWER);
  IF ANSWER = 'N' THEN
    BEGIN
      WRITE(' ENTER THE DEGREE OF POLYNOMIAL FOR FIT: ');READLN(MP);
      M = MP;
      WRITE(' ENTER THE NUMBER OF POINTS USED FOR CURVE FIT: ');READLN(MN);
      WRITE(' PICK WEIGHTING OPTION: 1...UNIT WEIGHTING');
      WRITE(' 0...STATISTICAL WEIGHTING');
      WRITE(' -1...INSTRUMENTAL WEIGHTING');READLN(IWT);
      END;
  ELSE
    BEGIN
      M:= 4; MP:= 5; MN:= 7; IWT:= 11;
      WRITELN(' FORTH ORDER POLYNOMIAL FIT TO MAGNITUDE MAXIMUM');
      WRITELN(' SEVEN HIGHEST POINTS USED FOR POLYNOMIAL FIT');
      WRITELN(' UNIT WEIGHTING');
    END; (# DEFAULTS #)
END (# DEFAULTS #)
PROCEDURE WEIGHTING;
BEGIN
  IF (IWT=1) THEN
    BEGIN
      FOR I:=1 TO N DO
        WI[I]:=1.0;
    END; (# WEIGHTING #)
END (# WEIGHTING #)
PROCEDURE BASELINE;
BEGIN
INDEX:=(NPT DIV 2)+11
RESPONSE:='N' I
IF RESPONSE='Y' THEN
BEGIN
FOR I=1 TO NPT DO
BEGIN
IF (IN(*2) OR (NC=NPI) OR (M)11)) THEN
WRITE('illegal data input')
LHV:='0.0';
RHV:='0.01';
LM:=O1;
RM:='01;
IF (D[I] = LHV) THEN
BEGIN
LHV:=D[I];
LM:=I;
END
IF (D[I] <= RHV) THEN
BEGIN
RHV:=D[I];
RM:='I';
END
END
IF RHV:='0.01
BEGIN
SW:=ABS(RH-LN); LE:=ROUND(INDEX - (20*BW));
IF (LE(I) THEN LE:='1
RE:=ROUND(INDEX + (20*BW));
IF (RE) D I 2 THEN RE:='321;
NPTS:='RE-LE+11'
IF (NPTS DIV 2) (INDEX=11) THEN
NPTS:=2*(INDEX-11)
FLAG:=0
REPEAT (# UNTIL FLAG > I
LS:='0.01;
RS:=0.01;
FOR I=1 TO 10 DO
BEGIN
K:=(I-I1 + LE);
L: RE-I11;
IF (FLAG=0)
THEN
BEGIN
LS:=LS+K;
RE:=RS+K;
END
ELSE
BEGIN
LS:=LS+D[K];
RE:=RS+D[I];
END
END
END
IF (FLAG=0)
BEGIN
LS:=LS/10.01;
RA:=RS/10.01;
SLOPE:=(RA-LA)/RE-LE-P1
E1:=RA-SLOPE*RE-4.51
LE:=ROUND(LE); RE:=ROUND(RE);
FOR I:=LE TO RE DO
BEGIN
CY:=SLOPE*I+911
IF (FLAG=0)
ELSE D[I]=D[I]-CY
END
END
FLAG:=FLAG+11
UNTIL (FLAG=11)
END
IF RESPONSE='N'
END
PROCEDURE SELECTPTS:
BEGIN
NP:=(N-1) DIV 2;
NL:=INDEX-NP;
NR:=INDEX-NP;
FOR I:=NL TO NR DO
BEGIN
J:=-NL+1;
YAIJ:=YAI[NJ];
END;
FOR I:=1 TO N DO
BEGIN
J:=-I;
YAIJ:=YAI[NJ];
END;
FOR I:=1 TO N DO
BEGIN
IF (YAI[I]=0.0) THEN
BEGIN
IF (YAI[I]=0.0) THEN
WRITE(' TERMINAL ERROR, Y[I] CANNOT BE 0.0!');
Y[I]:=1.0/Y[I];
END;
END;
END;
PROCEDURE POLREC:
(* POLREC,HATINV,VECGFA FIT DATA TO THE POLYNOMIAL:
(*
(* Y= A0+A1X+A2X2+...+ANXN:M
(* *
(* BY THE METHOD OF LEAST SQUARES, FOLLOWING THE PROCEEDURE
(* OF K. J. JOHNSON.
(* *
(* FIRST, AX=........CALCULATE B
(* *
BEGIN
FOR I:=1 TO N DO
BEGIN
B[I]:=B[I]+W[I]*YAI[I];
.:.VAL:=A[I];
FOR J:=2 TO NP1 DO
BEGIN
.:.VAL:=XYVAL*XAI[I];
B[I]:=B[I]+XYVAL*W[I];
END;
END;
END;
END;
BEGIN
(* THEN, CALCULATES THE UNIQUE 2M+1 ELEMENTS OF AM. THIS
(* INCLUDES THE UNIQUE OFF DIAGONAL ELEMENTS AND THE FIRST AND
(* LAST DIAGONAL ELEMENTS OF AN[I,1] AND AN[M+1,M+1] OF AM.
(* K:=2M+1
FOR I:=1 TO N DO
BEGIN
AM[I,1]:=AM[I,1]+W[I];
XVAL:=1.0;
FOR J:=2 TO K DO
BEGIN
XVAL:=XVAL*XAI[I];
IOVER:=J-MP11;
IF (IOVER = 0.0) THEN AM[I,OVER+1,MP11]:=AM[I,OVER+1,MP11]+W[I]*XVAL;
ELSE AM[I,J]:=AM[I,1]+W[I]*XVAL;
END;
END;
END;
BEGIN
(* THEN
(* ASSIGNS THE UPPER RIGHT TRIANGLE (INCLUDING DIAGONAL ELEMENTS)
(* FOR J:=2 TO M DO
BEGIN
FOR I:=1 TO M DO
AM[I,J]:=AM[I-1,J+1];
END;
BEGIN
(* ASSIGN SYMMETRIC ELEMENTS OF AN
(* FOR J:=1 TO M DO
BEGIN
FOR I:=J+1 TO MP1 DO
AM[I,J]:=AM[J,I];
END;
END;
END;
END;
END;
END;
PROCEDURE HATINV_VIVAR AM:MATRICEFA)

(* THIS SUBROUTINE INVERTS REAL, SQUARE MATRICES *1)
(* AM IS A NPM1 MATRIX *1)
(* DETERM IS THE DETERMINANT OF THE MATRIX. IF THE *1)
(* MATRIX IS SINGULAR THE PROGRAM IS STOPPED. *1)
BEGIN
DETERM:=1.0;
(* MAIN LOOP ELIMINATE ONE ROW AT A TIME *)
I:=1:1 (* DO FOR I TO N *)
REPEAT (* UNTIL I>N *)
(* SEARCH THE REMAINING MATRIX FOR MAXIMUM ELEMENT (PIVOT) *)
(* TEST FOR A SINGULAR COEFFICIENT MATRIX. *)
AMAX:=0.0;
FOR J:=1 TO N DO
BEGIN
IF (IPV[J,3]>1.0) THEN
BEGIN
FOR K:=1 TO N DO
BEGIN
IF (IPV[K,3]>1.0) THEN
BEGIN
IF AMAX<ABS(IPV[K,J]) THEN
BEGIN
IPV[ROW,J]:=IPV[ROW,K];
AM[ROW,J]:=AM[ROW,K];
END;
END;
END;
END;
END;
IF AMAX<TOL THEN DETERM:=0.0;
ELSE (* DO ONLY IF MATRIX IS NONSINGULAR *)
IPV[ICOL,3]:=IPV[ICOL,3]+1;
IPV[I,J]:=IPV[I,J];
IPV[I,2]:=IPV[I,2];
(* INTERCHANGE ROWS TO PUT PIVOT ELEMENT ON DIAGONAL *)
IF (IPV[ROW,ICOL]) THEN
FOR L:=1 TO N DO
BEGIN
SWAP:=AM[ROW,L];
AM[ROW,L]:=AM[ICOL,L];
AM[ICOL,L]:=SWAP;
END;
(* UPDATE DETERMINANT AND NORMALIZE PIVOT ROW *)
PIVOT:=AM[ICOL,ICOL];
DETERM:=DETERM*PIVOT;
AM[ICOL,ICOL]:=T;
(* FOR L:=1 TO N DO *)
AM[ICOL,L]:=AM[ICOL,L]/PIVOT;
(* REDUCE THE NON-PIVOT ROWS BY SUBTRACTION *)
FOR L:=1 TO N DO
IF (L<ICOL) THEN
BEGIN
T:=AM[L,ICOL];
AM[L,ICOL]:=AM[L,ICOL]/T;
FOR I:=1 TO N DO
AM[L,I]:=AM[L,I]-AM[ICOL,I]*T;
END;
END;
END;
END;
END (* ELSE IF AMAX<TOL *)
ELSE (* IF AMAX<TOL *)
BEGIN
(* INTERCHANGE COLUMN AND MODIFY DETERMINANT *)
NSWAF:=0i; (* THIS IS SIGN FLAG *)
IF DETERM>0.0 THEN
BEGIN
FOR I:=1 TO N DO
BEGIN
L:=N-I+1;
IF (IPV[L,1]<IPV[L,2]) THEN
BEGIN
ROW:=IPV[L,1];
ICOL:=IPV[L,2];
NSWAF:=NSWAF+1;
FOR K:=1 TO N DO
BEGIN
SWAP:=AM[K,ROW];
AM[K,ROW]:=AM[K,ICOL];
AM[K,ICOL]:=SWAP;
END;
END;
END;
END;
PROCEDURE VECALFA
(* THIS SUBROUTINE FINDS THE COEFFICIENT VECTOR, ALFA *)
BEGIN
  FOR I := 1 TO MPI DO
    BEGIN
      FOR J := 1 TO H DO
        ALFA[I,J] := ALFA[I,J] + AM[I,J]*HI[J];
    END;
  END;
END VECALFA;

PROCEDURE DATAOUT;
(* THIS SUBROUTINE CALCULATES THE VARIANCE AND PRINTS THE TABLE *)
BEGIN
  FOR I := 1 TO H DO
    BEGIN
      YCALC := YCALC + ALFA[I];
      XVAL := 1.0;
      FOR J := 2 TO MPI DO
        BEGIN
          XVAL := XVAL * XAI[I];
          YCALC := YCALC + ALFA[I] * XVAL;
        END;
    END;
  END;
  FOR I := 1 TO MPI DO
    BEGIN
      SIGAII := SIGMA(SORT(ABS(AM[I,J])))
      FOR J := 1 TO H DO
        BEGIN
          SIGALII := SIGMA(ALFA[I,J])
        END;
    END;
END DATAOUT;

END.
PROCEDURE NORM
(* THIS SUBROUTINE DETERMINES THE MAGNITUDE MAXIMUM AND USES IT TO NORMALIZE THE ABSORPTION AND DISPERSION CURVES. *)
BEGIN
HA:=0.0;
PM:=(HA-1.0)/2.0;
INT:=-0.5;
REPEAT (* UNTIL ABS(HA-MAX)<1.0E-06 *)
LHP:=PM-(2.0*INT);
RHP:=PM+(2.0*INT);
MAX:=HA;
INT:=(RHP-LHP)/20.0;
FOR I:=1 TO 21 DO
BEGIN
MAX(I):=LHP+(I-1.0)*INT;
VMAX(I):=ALFA(I);
FOR J:=1 TO N DO
VMAX(I):=VMAX(I)+ALFA(J)*EXP(J*X(I))
IF (VMAX(I)>MAX) THEN
BEGIN
MAX:=VMAX(I);
PM:=MAX(I);
END (* IF VMAX(I)>MAX *)
END (* FOR I *)
UNTIL (ABS(HA-MAX)<1.0E-06)
(* HA:=MAHEIGHT(PEAK) *) (* CONVERTS BACK TO ORIGINAL HEIGHT *)
PH:=PH+L-1.0;
WRITELN(' ABSORPTION MAXIMUM AT POINT NO. ',PH,');
WRITELN(' ABSORPTION MAXIMUM = ',MA);
FOR I:=1 TO NPT DO
BEGIN
AI:=AI/MA;
DI:=DI/MA;
MAG:I:=MAG(I)/MA;
END (* FOR I *)
END (* NORM *)

(******************************************************************
PROCEDURE RADLPLT;
BEGIN
FOR I:=1 TO NPT DO
BEGIN
AY:=(AI-0.5);
IF (AY<0.0) THEN
BEGIN
PHI:=ATAN(DI/AY);
IF (AY<0.0) THEN
BEGIN
PHI:=PHI+PI;
END (* IF AY<0.0 *)
IF (AY<0.0 AND DI>0.0) THEN
BEGIN
PHI:=PHI+2*PI;
END (* IF AY<0.0 AND DI>0.0 *)
RD:=SORT(AY*AY+DI*DI)-0.5;
END (* IF THEN *)
ELSE
BEGIN
RD:=0.0;
RD:=RD+0.0;
END (* ELSE *)
IF (AY<0.0) THEN
BEGIN
PHI:=PHI+PI;
RD:=RD+0.0;
END (* IF AY<0.0 *)
FOR I:=1 TO NPT DO
BEGIN
IF (ABS(RD)>RDMAX) THEN
BEGIN
RDMAX:=ABS(RD);
PHITEMP:=PHI*360.0/(2.0*PI);
END (* IF AY<0.0 *)
END (* FOR I *)
WRITELN(' MAXIMAL RADIAL DIFFERENCE IS ',RDMAX,' Tau.');
WRITELN(' LOCATED AT PHI ',PHITEMP,' DEGREES.');
FOR I:=1 TO NPT DO
BEGIN
PHI:=BDPHI+PHI*2*PI/9.0;
RD:=BDRD+RD;
END (* FOR I *)
END (* RADLPLT *)
(*****************************************************************)
PROCEDURE GENERATEPLOT;
VAR
XMIN, XMAX, YMIN, YMAX, SCALEX, SCALEY, X, Y, DX, DY: REAL;
TERM: CHAR;
BEGIN
PROCEDURE PUTCOORDINATES(X, Y: REAL);
VAR
XI, YI: INTEGER;
BEGIN
XI: = ROUND(X-XMIN)*SCALEX;
YI: = ROUND((Y-YMIN)*SCALEY*FACTOR);
WRITE(XI, ' ', YI, TERM);
END;
PROCEDURE PDRAW(X, Y: REAL);
BEGIN
WRITE('P', X, Y, 'D');
PUTCOORDINATES(X, Y);
END;
PROCEDURE PMOVE(X, Y: REAL);
BEGIN
WRITE('P', X, Y);
PUTCOORDINATES(X, Y);
END;
PROCEDURE AXES;
BEGIN
PMOVE(0.0, -5.0);
PDRAW(0.0, 5.0);
PMOVE(0.0, 0.0);
PMOVE(8.0, 0.0);
PDRAW(8.0, 0.25);
PMOVE(8.0, 0.25);
PDRAW(8.0, -0.25);
PMOVE(4.0, 0.0);
PDRAW(4.0, -0.25);
PMOVE(-0.25, 4.0);
PMOVE(-0.25, 0.0);
PDRAW(-0.25, 0.25);
PMOVE(9.0, 0.0);
PDRAW(9.0, 0.25);
PMOVE(9.0, 0.25);
PDRAW(9.0, -0.25);
PMOVE(16.5, 0.0);
PDRAW(16.5, 0.25);
PMOVE(16.5, 0.25);
PDRAW(16.5, -0.25);
PMOVE(11.0, -0.25);
PDRAW(11.0, -0.25);
PMOVE(9.25, -0.25);
PDRAW(9.25, 0.01);
PMOVE(9.25, 0.01);
PDRAW(9.25, 0.25);
PMOVE(9.25, 0.25);
PDRAW(9.25, 0.01);
PMOVE(9.25, -0.25);
PDRAW(9.25, -0.25);
PMOVE(8.75, 0.01);
PDRAW(8.75, 0.01);
PMOVE(8.75, 0.01);
PDRAW(8.75, -0.01);
PMOVE(8.75, -0.01);
PDRAW(8.75, 0.01);
PMOVE(8.75, 0.01);
PDRAW(8.75, 0.01);
PMOVE(8.75, 0.01);
PDRAW(8.75, -0.01);
PMOVE(8.75, -0.01);
PDRAW(8.75, 0.01);
PMOVE(8.75, 0.01);
PDRAW(8.75, 0.01);
PMOVE(8.75, 0.01);
PDRAW(8.75, 0.01);
PMOVE(8.75, 0.01);
END;
PROCEDURE CIRCLE:
BEGIN
  THETA:=0.0;
  INCREM:=2.0*3.149/NUMBER;
  FOR I:=1 TO (NUMBER * 1) DO
    BEGIN
      X:=(4.0*COS(THETA)+4.0)%(0.61546);
      Y:=(4.0*SIN(THETA))%(5.6);
      PDRAW(X,Y);
      THETA:=THETA + INCREM;
    END
  END
END (* CIRCLE *)

BEGIN (* GENERATEPLOT *)
XMIN:=-1.01; XMAX:=20.01; YMIN:=-7.5; YMAX:=13.5;
SCALEX:=(XMAX/XMIN-XMIN));
SCALEY:=(YMAX/YMIN-YMIN));
TERM:=CHR(3); (* CTRL/C *)
REWRITE(PF,'PLOTFILE.ASC');
AXES: CIRCLE;
DX:=0.01;
DY:=0.007725;
FOR I:=1 TO NPT DO
  BEGIN
    X:=0.04[I];
    Y:=0.04[I];
    PDRAW(X,Y);
    X:=X-DX;
    Y:=Y-DY;
  END
FOR I:=1 TO NPT DO
  BEGIN
    X:=X-DX;
    Y:=Y-DY;
    PDRAW(X,Y);
  END
END (* GENERATEPLOT *)

PROCEDURE TOSCREEN:
VAR
  FIRST,BLOCK: INTEGER;
  PAGEBUF: ARRAY[1..SECSIZE] OF INTEGER;
BEGIN
  GDCLEAR(ALLPL);
  WITH PP DO
    BEGIN
      WPANE:=DO; DPANE:=ALLPL;
      BGCOLOR:=GREEN;
    END (* WITH *);
  FIRST:=1; BLOCK:=0;
  RESET(PFB,'PLOTFILE.ASC');
  REPEAT
    BLOCK:=BLOCK+BLOCKREAD(PFB,PAGEBUF,1,BLOCK);
    GDPAGE(PAGEBUF[1],FIRST,SECSIZE);
  UNTIL FIRST=0;
  CLOSE(PFB,LOCK);
END (* TO SCREEN *)

PROCEDURE TOPLOTTER:
VAR
  FO,FP,FR: INTEGER; (* FACTORS FOR WATANABE PLOTTER *)
  CHX,CHY: REAL;
  C,P: CHARI
  ASCALE,ASPACE: INTEGER;
  TERMINATORS,TERMEPS: SET OF CHARI
  DX,DT: REAL;
BEGIN
(*--------------------------------------------------------------------------*)
PROCEDURE INIPLOTTER;
BEGIN
   CMX:=201; CMY:=151; DX:=41; DY:=31;
   FG:=ROUND(100*CMX$/CMX$SYMX$/SYMX$); FP:=ROUND(100*CMX$);
   FR:=SYMX$;
   ASPACE:=ROUND(100*CHARINC$/CHARINC$/CMX$); ASCALE:=ASPACE;
   USTRING(PUNCH,'\0,0,11'); (" LOWER LEFT ");
   UCHAR(PUNCH, '*'); (" OFFSET [DX,DY] ");
   UNUM(PUNCH,DX*100,5,01); UCHAR(PUNCH,'*');
   UNUM(PUNCH,DY*100,5,01); UCHAR(PUNCH,'*');
   UCHAR(PUNCH,'Z'); (" UPPER RIGHT ");
   UNUM(PUNCH,CMX*100,6,01); UCHAR(PUNCH,'*');
   UNUM(PUNCH,CMY*100,6,01); UCHAR(PUNCH,'*');
   UCHAR(PUNCH,'O'); (" ALFA SPACE ");
   UNUM(PUNCH,ASPACE,5,01); UCHAR(PUNCH,'*');
   UCHAR(PUNCH,'B'); (" ALFA SCALE ");
   UNUM(PUNCH,SCALE,3,3); UCHAR(PUNCH,'*');
   TERMINATORS:=[CHR(3)];
   TERMSEPS:=[CHR(3),',','!',CHR(10),CHR(13),'*','-'];
END; ( " INIPLOTTER ");
(**********************************************************************)
PROCEDURE SETPLOTFACTOR;
BEGIN
   UCHAR(PUNCH,'*');
   UNUM(PUNCH,FP,9,01); UCHAR(PUNCH,'*');
   UNUM(PUNCH,FR,9,01); UCHAR(PUNCH,'*');
   END; ( " SETPLOTFACTOR ");
(**********************************************************************)
PROCEDURE SETEXTFACTOR;
BEGIN
   USTRING(PUNCH,'L1,1,1');
END; ( " SETEXTFACTOR ");
(**********************************************************************)
BEGIN ( " TOPLOTTER ");
INIPLOTTER;
SETPLOTFACTOR;
SETEXTFACTOR;
RESET(PF,'PLOTFILE.ASC');
REPEAT
   REPEAT
      READ(PF,C);
      UNTIL NOT (C IN TERMSEPS);
      IF C='P' THEN SETEXTFACTOR;
      UCHAR(PUNCH,C);
      UNTIL (C IN TERMINATORS) OR EOF(PF);
      IF C='P' THEN SETPLOTFACTOR;
      UNTIL (C='F') OR EOF(PF);
      CLOSE(PF,LOCK);
END; ( " TO PLOTTER ");
("#5 DPPROC.UTXT")
(**********************************************************************)
(**********************************************************************)
BEGIN (* MAIN PROGRAM *)
WRITE(' DISPA6.ASC 06 10 86.01' !)
INITIAL;
SORT;
INPUT1;
INPUT2;
DEFAULTS;
WEIGHTING;
BASELINE;
SELECTPTS;
POLREG;
MATINV(A);;
VECALFA;
DATAOUT;
NORM;
RADLPLT;
MAKEPORT(PP);;
GENERATEPLOT;
TOSCREEN;
WRITE(' DO YOU WANT A HARD COPY? (Y/N) ');
READLN(RESPONSE);
IF (RESPONSE = 'Y') THEN
   BEGIN
      WRITE(' HOW MANY HARD COPIES DO YOU WANT? ');
      READLN(NHCOPS);
      REPEAT
         WRITE(' INSERT A FRESH SHEET OF PAPER INTO THE PLOTTER' );
         WRITE(' HIT A (RETURN) WHEN YOU ARE READY');
         READLN(RESPONSE);
         TOPLOTTER;
         NHCOPS:=NHCOPS-1;
      UNTIL NHCOPS <= 0;
   END;
WRITE(' PROGRAM COMPLETED ' !)
END. (* MAIN PROGRAM *)
APPENDIX E

PROGRAM PHASER

PHASER operates on the same instrument described in Appendix D to phase correct FT/NMR data based on the rotation of the DISPA plots corresponding to two selected peaks in the spectrum. The maximum data set size is 16K. The program requests the file name and the spectral indices for the two peaks used for zero and first order phase correction.
PHASER.ASC

------ FILE: PHASER.ASC

( $ PHASER.ASC 9/28/86.01 $ )

PROGRAM PHASER

CONST ( $ GLOBAL $ )

SECSIZE=256 ( $ SECTOR SIZE FOR CDC DISK--ARRAY[0..255] $ )

( $ CONSTANTS IN THE -1 SECTOR $ )

ID=0; NH=1; NH2=2; EXT=3
SEC=4; SIZ=5; PBA=6; URL=7
UBI=8; MLA=9; DAT=10; AXS=11
AUTH=12; COMMNTS=16

TH=4000.01 ( $ MINIMUM THRESHOLD FOR PEAK PICKING $ )

ME=201

VAR ( $ GLOBAL $ )

I,KK,NTP,NPK,SECSIZE,SECTOR,MAXPOINT:INTEGER
INDEXO,INDEX1:INTEGER ( $ SELECTED PEAK INDEXES $ )
INTENSITY:REAL ( $ INTENSITY OF PEAK MAXIMUM $ )
AMAX:REAL
RESPONSE,FLAG:CHAR
INFILE,OUTFILE:STRING[20]; ( $ DISK FILE NAMES $ )
F1,F2:FILE; ( $ UNTYPED DATA FILES $ )
A,D:ARRAY[1..192] OF INTEGER; ( $ ALLOWS 16K FID's $ )
WORK:ARRAY[1..60] OF INTEGER; ( $ ALLOWS WINDOW OF 20 PTS $ )
POSITION:ARRAY[1..201] OF INTEGER; ( $ ALLOWS 20 PEAKS $ )
HEIGHT:ARRAY[1..20] OF REAL; ( $ ALLOWS 171 INTERSECTIONS $ )
X:ARRAY[1..171] OF INTEGER; ( $ 20 PTS ON CIRCLE $ )
Y:ARRAY[0..SECSIZE] OF INTEGER;

**********************************************************************

PROCEDURE SORT1

VAR

I,KK,INDEXO,INDEX1:INTEGER
MIN,TEMP:REAL
BEGIN ($ SORT $)
WRITE('.');
IF (NPK=1) THEN
FOR I=1 TO NPK DO
BEGIN
MIN=HEIGHT[I];
INDEXO=I;
FOR K=(I+1) TO NPK DO
IF (HEIGHT[K] < MIN) THEN
BEGIN
INDEXO=K;
MIN=HEIGHT[K];
END;
ITEM=HEIGHT[I];
HEIGHT[I]=HEIGHT[INDEXO];
HEIGHT[INDEXO]=ITEM;
POSITION[INDEXO]=POSITION[I];
POSITION[I]=POSITION[INDEXO];
END ($ FOR $)
END ($ SORT $)

**********************************************************************
PROCEDURE PSORTI
VAR
I, K, IMIN, ITEMP, INDEX: INTEGER;
TEMP: REAL;
BEGIN (# PSORT I)
(* WRITELN(' PSORT 05/20/86.01';) *)
  IF (HPK > I) THEN
    FOR I:=1 TO HPK DO
      BEGIN
        IMIN:=POSITION(I);
        INDEX:=I;
        FOR K:= [I+1] TO HPK DO
          IF (POSITION(K) < IMIN) THEN
            BEGIN
              ITEMP:=POSITION(K);
              POSITION(K):=POSITION(INDEX);
              POSITION(INDEX):=ITEMP;
              TEMP:=HEIGHT(I);
              HEIGHT(I):=HEIGHT(INDEX);
              HEIGHT(INDEX):=TEMP;
            END;
          END;
        END;
      END; (* FOR I *)
END; (* PSORT I *)

PROCEDURE BASELINI
VAR
I, ASL, ASR, DSL, DSR: INTEGER;
AXA, DDXA, AAL, AAR, DAL, DAR, ASL, DSSL, DSLOPE, AI, DI: REAL;
BEGIN
(* WRITELN(' BASELIN 05/20/86.01';) *)
(* WRITELN(' BASELINE CORRECTION IN PROGRESS...'); *)
  ABL:=0; ASL:=0; DSR:=0;
  FOR I:=1 TO NE DO
    BEGIN
      ABL:=AI + ABL;
      ASL:=AI + NTPE + ABR;
      DSSL:=DI + DSL;
      DSR:=DI + NTPE + DSR;
    END; (* FOR I *)
  AXA:= (NE + 1)/2.0;
  DDXA:= (NE + 1)/2.0;
  AAL:= ASL/NE;
  AAR:= ASR/NE;
  DAL:= DSL/NE;
  DAR:= DSR/NE;
  ASLOPE:= (AAR-AAL)/(NTPE/NE);
  DSLOPE:= (DAR-AAL)/(NTPE/NE);
  AI:= AAL-ASLOPE*AXA;
  DI:= DAL-DSLOPE*DDXA;
  FOR I:=1 TO NTPE DO
    BEGIN
      AI[I]:= ROUND(AI[I]-ASLOPE*(I-1)+AI[I]);
      DI[I]:= ROUND(DI[I]-DSLOPE*(I-1)+DI[I]);
    END; (* FOR I *)
END; (* BASELIN I *)
PROCEDURE WINDOW(VAR PINDEX: INTEGER);
VAR
  I:INTEGER;
  AREAL,DREAL,HAB:REAL;
BEGIN (*WINDOW #)
  (* WRITELN(" WINDOW 05/20/86.01 IN...") #)
  (LEFT= PINDEX-ROUND(NPTS/2.0))
  FOR I= 1 TO NPTS DO
    BEGIN
      WORK[I]= B[LEFT*I]I
      AREA= A[LEFT*I]I
      DREAL= D[LEFT*I]I
      HAB= HAB (AREA*AREAL*DREAL*DREAL)
      WORK[I]= 2*NPTS= ROUND(HAB)
    END; (*FOR I #)
END; (*WINDOW #)
--------------------------------------------------------------------------------------------------
PROCEDURE PPICK(VAR)
VAR
  I,J,K:INTEGER;
  AREAL,DREAL:HAB:REAL;
BEGIN (*PPICK #)
  (* WRITELN(" PPICK 05/20/86.01") #)
  (PEAK SEARCH IN PROGRESS..."")
  AREA= A[I]I
  DREAL= D[I]I
  SORT(AREAL*AREAL*DREAL*DREAL)
  AREA= A[I]I
  DREAL= D[I]I
  SORT(AREAL*AREAL*DREAL*DREAL)
  WRITELN(" NUMBER TRANSFORMED POINTS= ",NTP:6)
  FOR J= 3 TO NTP DO
    BEGIN
      AREA= A[I]I
      DREAL= D[I]I
      SORT(AREAL*AREAL*DREAL*DREAL)
      IF (12>HEIGHI AND (12>11 AND (12>13)) THEN
        BEGIN
          HEIGHI= 12
          POSITION= (J-1)
          SORT
        END; (*IF 12 #)
      END; (*FOR J #)
      AMAX= 0.01
      FOR J= 1 TO NPK DO
        IF (HEIGHI) AMAX THEN
          AMAX= HEIGHI
      FOR J= 1 TO NPK DO
        HEIGHI= HEIGHI*100.0/AMAX
      SORT
    WRITELN(" ");
  END; (*PPICK #)
--------------------------------------------------------------------------------------------------
PROCEDURE PBCPHASE(VAR PHASE:REAL);
VAR
  I,J,K,KP1,JP1,INDEX,LIMIT:N:INTEGER;
  XSUM,YSUM,A1,A2,A3,A4,B1,B2,B3,B4:XAVG,YAVG:REAL;
  XMIN,YMIN,XMAX,YMAX;STX,STY,FRACTION:REAL;
  POINT:ARRAY[1..100] OF INTEGER; (*MAX OF 100 PTS USED#)
  XC,YC:ARRAY[1..100] OF REAL;

------------------------------------------------------------------------------------------
PROCEDURE PICKPTS
BEGIN (# PICKPTS $)
  'WRITELN(' PICKPTS OS/14/86.01');$)
  WRITELN('-----------------------------------------------------------------------');$)
  WRITE(' HIGHEST DESIRED FRACTION OF PEAK');$)
  READLN(FRAC);$)
  WRITE(' LIMI';$)
  LIMIT := ROUND(SORT(FRACTION)#INTENSITY#AMAX/100.0);$)
  FRACTION := LIMIT/AMAX;$)
  WRITE(' LOMEST ACCEPTABLE ABSOLUTE INTENSITY: ',LIMIT);$)
  WRITE(' LOMEST ACCEPTABLE RELATIVE INTENSITY: ',FRACTION);$)
  INDEX := 01;$)
  FOR I := 1 TO NPTS DO
    IF (WORK[I+2*NPTS] > LIMIT) THEN BEGIN
      INDEX := INDEX + 11
      POINT(INDEX) := I
      END; ( # IF...FOR I $)
  WRITELN(' POINTS SUITABLE FOR PHASE CALCULATION: ');$)
  FOR J := 1 TO INDEX DO
    BEGIN
      POINT(J) := J12;
      WRITE(' ',WORK[I+2*NPTS]:10);$)
      WRITE(' ',WORK[I]:10);$)
      END; ( # FOR J $)
  WRITELN(' ');$)
  END (# PICKPTS $)
  (***********************************************************************$)
PROCEDURE SETUP1
BEGIN (# SETUP $)
  WRITELN(' SETUP OS/20/86.01');$)
  A1 := WORK[POINT[1]];$)
  A2 := WORK[POINT[1+1]];$)
  A3 := WORK[POINT[2]];$)
  A4 := WORK[POINT[3+1]];$)
  D1 := WORK[POINT[1] + NPTS];$)
  D2 := WORK[POINT[1+1] + NPTS];$)
  D4 := WORK[POINT[3+1] + NPTS];$)
  DMAX := D1 - D2;$)
  DMIN := D3 - D4;$)
  END (# SETUP $)
  (***********************************************************************$)
PROCEDURE CALCULATE1
VAR
  TPHASE: REAL;$)
BEGIN (# CALCULATE $)
  WRITELN(' CALCULATE OS/20/86.01');$)
  S12 := (D1-D2-S128(A1+A2))/2.0;$)
  S34 := (D3-D4-S348(A3+A4))/2.0;$)
  XCI[KOUNT] := (S34-S12)/S12HS34;$)
  YCI[KOUNT] := (S34-S12-S12HS34)/S12HS34;$)
  XSUM := XCI[KOUNT] + XSUM;$)
  YSUM := YCI[KOUNT] + YSUM;$)
  XAVG := XSUM/KOUNT;$)
  YAVG := YSUM/KOUNT;$)
  TPHASE := ATAN(YCI[KOUNT]/XCI[KOUNT])/4001;$)
  PHASE := ATAN(XAVG/YAVG)/4001;$)
  IF (XAVG < 0.0) THEN
    IF (YAVG > 0.0) THEN BEGIN
      PHASE := PHASE + 0.51 ($ 2ND AND 3RD QUADRANTS $)
      IF (XAVG < 0.0) AND (YAVG < 0.0) THEN
        PHASE := PHASE + 0.51 ($ 4TH QUADRANT $)
      END; ( # IF...THEN $)
  IF (XCI[KOUNT] < 0.0) THEN
    TPHASE := TPHASE + 0.51 ($ 2ND AND 3RD QUADRANTS $)
  IF (YCI[KOUNT] > 0.0) AND (XCI[KOUNT] < 0.0) THEN
    TPHASE := TPHASE + 0.51 ($ 4TH QUADRANT $)
  KPI := K11;$)
  JP1 := J11;$)
  WRITE(KOUNT:2,' ',K12,' ----',KPI12,' ',J12,' ----',JP12,' '');$)
  WRITELN(XCI[KOUNT],',',YAVG,' ',YAVG);$)
  WRITE(' PHASE= ',PHASE:6:3,' cycles');$)
  WRITELN(' AVG. PHASE= ',PHASE:6:3,' cycles');$)
  KOUNT := KOUNT + 11;$)
  END (# CALCULATE $)
  (***********************************************************************$)
BEGIN (# PBCPHASE 8)
(S "WRITEH(" PBCPHASE 05/20/86.OI" "")
PICKPTS;
XSUM:= 0.01
YSUM:= 0.01
KOUNT:= 11
H:= INDEX-11
FOR K:= 1 TO #KOUNT DO
BEGIN
FOR J:= (K+1) TO #KOUNT DO
BEGIN
IF (D1MD2 < 0.0) AND (D3MD4 < 0.0) THEN
BEGIN
S12:= (A2-A1)/(D1MD2)
S34:= (A4-A3)/ID1MD4)
S12M34:= S12-S34
IF (S12M34 < 0.0) THEN
CALCULATE
ELSE
BEGIN
WRITEH(" INTERSECTION IGNORED..." "")
WRITEH(" S12 - S34 = 0.0 " "")
END (# S12M34 ELSE #)
END (# IF D1MD2 ELSE #)
ELSE
BEGIN
WRITEH(" INTERSECTION IGNORED..." "")
WRITEH(" D1-D2 OR D3-D4 = 0.0 " "")
END (# D1MD2 ELSE #)
END (# FOR J #)
END (# FOR K #)
IF (KOUNT = 0) THEN
BEGIN
WRITEH(" INSUFFICIENT NUMBER OF POINTS ABOVE LIMIT: " "")
WRITEH(" INCREASE DIGITAL RESOLUTION OF SPECTRUM OR " "")
WRITEH(" DECREASE LIMIT (i.e. FRACTION: " "")
END (# IF KOUNT #)
XSBS:= 0.01
YSBS:= 0.01
FOR I:= 1 TO #KOUNT DO
BEGIN
XSBS:= (XCI[I] - XAVG) * (XCI[I] - XAVG) + XSBS;
YSBS:= (YCI[I] - YAVG) * (YCI[I] - YAVG) + YSBS;
END (# IF I #)
STDX:= SORT(XSBS/(KOUNT-1))
STDY:= SORT(YSBS/(KOUNT-1))
WRITEH(" AVE. CENTER COORDINATES: " "")
WRITEH(" X= \(XAVG; 12:3\), \(STDX; 12:3\)")
WRITEH(" Y= \(YAVG; 12:3\), \(STDY; 12:3\)")
WRITEH(" finaL AVERAGE APPARENT PHASE ',PHASE:6:4', cycley")
WRITEH("--------------------------------------------------------------------------------")
END (# PBCPHASE 8)
(*******************************************************************************)
PROCEDURE INPUT:
VAR
I, J, N, M: INTEGER;
SLOPE, S, Cycles, ACycles, BCycles, SIN, COSINE: REAL;

BEGIN (# INPUT #)
(' WRITE(' ' INPUT 09/06/84') '
SLOPE := 0.01;
FLAG := 'N';
WRITE(' ENTER NUMBER OF PEAKS IN SPECTRUM: ');
READLN(NPK);
WRITE(' ENTER NUMBER OF POINTS IN PEAK WINDOW (max 20 min 3): ');
READLN(NPTS);
WRITE(' DO YOU WISH TO ENTER PEAK POSITIONS? (Y/N) ');
READLN(RESPONSE);
IF (RESPONSE = 'Y') THEN
BEGIN
  AMAX := 0.01;
  FOR I := 1 TO NPK DO
  BEGIN
    WRITE(' ENTER INDEX FOR PEAK (' , I , ') ');
    READLN(POSITION[I]);
    HEIGHT[I] := SORTA[I]*A[I]+D[I]*D[I];
    IF (HEIGHT[I] > AMAX) THEN
      AMAX := HEIGHT[I];
  END;
  FOR I := 1 TO NPK DO
  BEGIN
    POSITION[I] := I;
    WRITE(' , POSITION[I] = ' , I , ', ' , HEIGHT[I] ;
    IF (FLAG = 'N') THEN
    BEGIN
      WRITE(' SELECT PEAK FOR ZERO ORDER PHASE CORRECTION: ');
      READLN(INDEX);
      IF (INDEX = 0) THEN
        WRITE(' SELECT PEAK FOR FIRST ORDER PHASE CORRECTION: ');
        READLN(INDEX);
      END;
      WRITE(' DO YOU WANT TO BASELINE CORRECT? (Y/N) ');
      READLN(RESPONSE);
      IF (RESPONSE = 'Y') THEN BASELIN;
    END;
  END;
END;
END;
PROCEDURE LINPHASE;
VAR
  I, RENEW, INNEW: INTEGER;
  PHASE, SLOPE, ACYCLES, BCYCLES, SINE, COSINE: REAL;
BEGIN (* LINPHASE *)
  (* WRITELN(' LINPHASE 09/06/86:01') *)
  INPUT:
    MAXPOINT:=POSITION(INDEXO);
    WINDOW(MAXPOINT);
    INTENSITY:= HEIGHT(INDEXO);
    PBCPHASE(ACYCLES);
  IF (FLAG = 'N') THEN
    BEGIN
      MAXPOINT:=POSITION(INDEXO);
      WINDOW(MAXPOINT);
      INTENSITY:= HEIGHT(INDEXO);
      ACYCLES:= BCYCLES - ACYCLES;
      SLOPE:= CYCLES/POSITION(INDEXO)-POSITION(INDEXO);
    END;
  END (* IF FLAG *)
  WRITELN(' FINAL VALUES: '
  WRITELN(' INDEX ZERO ORDER... ',POSITION(INDEXO):6)
  WRITELN(' FIRST ORDER... ',POSITION(INDEXO):6)
  WRITELN(' ZERO ORDER PHASE... ',ACYCLES:4, ' cycles')
  WRITELN(' FIRST ORDER PHASE... ',SLOPE:6, ' cycles/dt')
  WRITELN(' PHASE CORRECTION IN PROGRESS... ')
  FOR I:= 1 TO NTP DO
    BEGIN
      PHASE:= (1 - POSITION(INDEXO))
      PHASE:= -1.0* (PHASE-ABS(TRUNC(PHASE)))/TUOP;
      SINE:= SIN(PHASE);  
      COSINE:= COS(PHASE);
      RENEW:= ROUND(A[I]*COSINE-D[I]*SINE);
      INNEW:= ROUND(A[I]*SINE+D[I]*COSINE);
      A[I]:= RENEW;
      D[I]:= INNEW
    END;
  END (* FOR I *)
END (* LINPHASE *)
(*$$$$$$$$$$$$$$$$$$$$$$$$$$$$$$$$$$$***********)
(*$$$$$$$$$$$$$$$$$$$$$$$$$$$$$$$$$$$***********)
BEGIN (* PHASER *)
WRITE(' PHASER.ASC 9/28/84.01 ')
READ(INFILE)
WRITE( ' ENTER OUTPUT FILE NAME: ')
READ(OUTFILE)
RESET(INFILE)
RESET(OUTFILE)
SEC1 = BLOCKREAD(IF1,X1,-11)
WRITE(' PARAMETER BLOCK HAS BEEN READ... ')
SIZE1 = X(SIZ) DIV SECSIZE1
NPI = X(SIZ) DIV 21
SECTOR = 01
REPEAT
SEC1 = BLOCKREAD(IF1,X1,SECTOR1)
FOR I = 0 TO 255 DO
  IF ((I+1) DIV 2) = (I+1) THEN
    BEGIN
      KK = ((I+1) DIV 2) + SECTOR1281
      XI1 = XI11
    END
  ELSE
    BEGIN
      KK = ((I+2) DIV 2) + SECTOR1281
      XI1 = XI11
    END
SECTOR = SECTOR + 11
UNTIL SECTOR > SIZE1
WRITE(' READING OF DATA COMPLETE... ')
LIPHASE1
WRITE(' PHASE CORRECTION COMPLETED. ')
END (* PHASER *)
FAKER is used on the Bruker AM-500 (described in Appendix D) to generate simulated data sets which are entirely compatible with the software package. FAKER provides for the simulation of single peak spectra with constant, linear and quadratic modifications to the spectral baselines of the real and imaginary spectra.

Note the instrument software can be used to modify the spectral phase.

The program requests an input file which is used as a surrogate to hold the simulated spectrum after it is generated. The data set size and parameter block is identical to that of the surrogate file. The simulated data file containing the simulated spectrum and surrogate parameters is stored on disk in the same fashion as a standard data file.
---- FILE: FAKER.ASC

PROGRAM FAKER;(* FAKER.ASC 07/06/86.01 *)
(* THIS PROGRAM READS IN A FILE AND THEN CHANGES IT BY ALLOWING THE GENERATION OF A FAKE DATA SET IN PLACE OF THE ORIGINAL DATA SET WHICH RETAINS THE SAME -1 SECTOR AS THE ENTERED DATA SET. *)

CONST
SECSIZE=256;(* FOR CDC--Note 255 for ARRAY(0..255) *) MAX= 2000000.01 (* MAXIMUM PEAK HEIGHT *)

(* CONSTANTS IN THE -1 SECTOR *)
ID=0; HI=1; HM=2; E=3; SEC=4; SIZ=5; PSA=6; URL=7; USZ=8; MLA=9; DAT=10; AXS=11;
AUTH=12; COMMENTS=16;

VAR
F1,F2:FILE;(* UNTYPED DATA FILES *)
'Y':ARRAY[0..SECSIZE] OF INTEGER;
INFILE,OUTFILE:STRING[20];
TEMP,TAU:REAL;
A,B,C:REAL;(* BASELINE POLYNOMIAL: *)

Y(I) = A + B*I + C*I^2

BEGIN
  WRITELN('FAKER.ASC 7/6/86.01');
  WRITE('ENTER INPUT FILE NAME: ');
  READLN(INFILE);
  WRITE('ENTER OUTPUT FILE NAME: ');
  READLN(OUTFILE);
  A:=0.01;B:=0.01;C:=0.01;TAU:=1.01;O0:=512;TEMP:=0.01;
  WRITELN('BASELINE POLYNOMIAL: Y(I) = A + B*I + C*I^2');
  WRITE('ENTER A: ');
  READLN(A);
  WRITE('ENTER B: ');
  READLN(B);
  WRITE('ENTER C: ');
  READLN(C);
  WRITE('ENTER TAU: ');
  READLN(TAU);
  WRITE('ENTER INDEX FOR PEAK CENTER: ');
  READLN(00);
  RESET(F1,INFILE);
  (* READ IN -1 SECTOR TO FIND OUT FILE SIZE *)
  SECS:=BLOCKREAD(F1,X,1,1);
  SIZE:=(X*SIZ) DIV SECSIZE;
  WRITE(F2,OUTFILE);
  SECTOR:=0;(* # CURRENT SECTOR *)
  REPEAT
    SECS:=BLOCKREAD(F1,X,1,SECTOR);(* # READ IN EACH SECTOR *)
    FOR I:= 0 TO (SECSIZE-1).DO
      BEGIN
        IF ((I+1) DIV 2) = (I+1) THEN (* ODD "I"==) DISPERSION *)
          BEGIN
            KK:= ((I+1) DIV 2) + SECTOR*128;
            TEMP:= (O0-KK)*TAU/(1.0+TAU*TAU*(O0-KK)*(O0-KK));
            Y[I]:= ROUND(MAX*TEMP+(A+KK*B+KK*C));
          END;
        ELSE (* EVEN "I"==) ABSORPTION *)
          BEGIN
            KK:= ((I+1) DIV 2) + SECTOR*128;
            TEMP:= 1.0/(1.0+TAU*TAU*(O0-KK)*(O0-KK));
            Y[I]:= ROUND(MAX*TEMP+(A+KK*B+KK*C));
          END;
        END;
    END;
  UNTIL SECTOR = SIZE;
  END;
(\* THEN CLOSE FILES \*)
CLOSE(F2, Lock);
(\* KF: OPEN FILES AND COPY USER-INFO BLOCK \*)
RESET(F1, INFILE);
RESET(F2, OUTFILE);
SECS:=BLOCKREAD(F1, X, 1, -1); (* READ IN ORIG -1 SECTOR *)
SECS:=BLOCKREAD(F2, Y, 1, -1); (* READ IN NEW -1 SECTOR *)
(* COPY COMMENTS AND USER INFO BLOCK *)
FOR I:=PSA TO SECSSIZE DO
   Y(I-1)=X(I-1); (* REWRITE USER-INFO SECTOR *)
SECS:=BLOCKWRITE(F2, Y, 1, -1);
CLOSE(F2, LOCK); ENDF (* FILETEST *)
APPENDIX G

PROGRAM PRDECPHA

PRDECPHA was written by Ivan Santos to phase correct FT/ICR data based on the phase transfer characteristics of the instrument. The program was written expressly for the Nicolet FTMS-1000 instrument and corrects for many of the sources of phase error discussed in Chapter VI.
C **** PRDECPHA ****
C **** THIS CORRECTS DATA FOR THE PHASE ACCUMULATION
C DURING EXCITATION, DEL, DEL, AND THE ADC SKIP
C **** FTRX-- DUP ****
C **** 9 06.07 ****
INTEGER SDATA, RENEW, SPN, DROSWFLG, ENLOFDFLAG, PTPRINTV
REAL HIFREQ, LOFREQ, IMOLD, HIENDFREQ, LOENDFREQ, LOFPHA
CHARACTER EXT(3), SUBSTR(5), COPY(3), PRTYPE(3), T1BUFF(50)
COMMON /SDATA/ SDATA(8192)
COMMON /NTP/ NTP
COMMON /HIFREQ/HIFREQ
COMMON /LOFREQ/LOFREQ
COMMON /HEFFRO/HEFFRO
COMMON /DELAYS/ DELAYS
COMMON /SUWBF/ SUWBF
COMMON /T1MEX/T1MEX
COMMON /MODEK/MODEK
COMMON /SUWBF/BIAS
COMMON /BWIDTH/BWIDTH
COMMON /DEL1/DEL1
COMMON /SUPRA/SUPRA
COMMON /EXTIME/EXTIME
COMMON /IFRTEO/IFRTEO
COMMON /LOFREQ/LOFREQ
COMMON /REFFREO/REFFREO
COMMON /DELAY/DELAY
COMMON /SUWBF/BIAS
COMMON /T1MEX/T1MEX
COMMON /DEL1/DEL1
COMMON /SUPRA/SUPRA
COMMON /EXTIME/EXTIME
COMMON /IFRTEO/IFRTEO
COMMON /LOFREQ/LOFREQ
COMMON /REFFREO/REFFREO
COMMON /DELAY/DELAY

THIS SECTION ASSIGN'S DEVICE CODE FOR OUTPUT

1 PRINT *, 'Do you want a hard copy of the data?'
   READ(1,2500) COPY
   IF (SUBSTR(COPY, 1,1).EQ. 'Y') THEN
   2 PRINT *, 'What type of printer do you have LP or CP?'
   READ(1,2500) PRTYPE
   IF (SUBSTR(PRTYPE, 1,1).EQ. 'C') THEN
      J=9
   ELSEIF (SUBSTR(PRTYPE, 1,1).EQ. 'L') THEN
      J=10
   ELSE
      PRINT *, 'Please answer printer type again'
      GOTO 2
   ENDIF
   3 ELSEIF (SUBSTR(COPY, 1,1).EQ. 'N') THEN
      J=2
   ELSE
      PRINT *, 'Please answer again'
      GOTO 1
   ENDIF
C
C ****** HIFRED = EMSPAR  LOFRED = ELSPAR  ******

C LOENDFRA = ELSPAR
IF (MODEKP, EQ. 0) THEN
  FRAME = HIFRED - LOFRED
  HIENDFRA = HIFRED
  ELSEIF (MODEKP, EQ. 1) THEN
    FRAME = REFFRO - ELSPAR
    HIENDFRA = REFFRO
  ELSE
    PRINT *, ' MOD NOT CORRECTLY SET'
    PRINT *, ' ENDING PROGRAM'
    STOP
  ENDIF
  END
ENDIF

PRINT *, ' Enter P for FREQ SENSE or S for SINGLE FREQ'
READ(1,2500) EXT
IF (SUBSTR(EXT, 1, 1). EQ. 'P') THEN
  FROSWFLG = 1
ELSEIF (SUBSTR(EXT, 1, 1). EQ. 'S') THEN
  FROSWFLG = 0
  SGLFOFLG = 1
ELSE
  PRINT *, ' Please answer again'
  GOTO 5
ENDIF

PRINT *, ' Enter PINDEX print interval, i.e. "200" will print every 200 pts'
PRINT *, ' 0 or (RE) will print only first'
PRINT *, ' point. Entering a "1" will print all points. '
READ(1,800) PIPRINT
PRINT *, ' Enter STARTING PTE RETURN defaults to 1 '
READ(1,800) SPN
IF (SPN. EQ. 0) THEN
  SPN = 1
ENDIF

N = SPN
ETSTRA = FLOAT(100)
WRITE(J,2100)
WRITE(J,5000)
WRITE(J,3000) TIMUF
WRITE(J,2000)
WRITE(J,1100) ELSPAR, EMSPAR
WRITE(J,2200) REFFRO, BWIDTH
WRITE(J,2900) IDWELL
WRITE(J,1000) MODEKP
WRITE(J,2400) IBIAS
WRITE(J,9000) TIMEZ
WRITE(J,1100) FRAME, HIENDFRA, LOENDFRA
WRITE(J,2300) FROSWFLG, SGLFOFLG
WRITE(J,1975) PSTRA
WRITE(J,1985) PIPRINT
WRITE(J,2000)

C THE READCLK IS THE TIME SHIFT DUE TO THE ADC SKIPPING THE
C FIRST 15 PTE OR 14 INTERVALS XDWELL TIME BETWEEN SAMPLES
ADSKIP = 0
PRINT *, ' PHASE CALCULATIONS HAVE STARTED'
IF (MODEKP, EQ. 0) THEN
PTN = FLOAT(NTP)
OMEGA = HENFDO - (PTN-1.0)*FRANGE/(PTBRA-1.0)
ELSEIF(MODEK,EQ.1) THEN
PTN = FLOAT(1)
OMEGA = LOENDFDO + (PTN-1.0)*FRANGE/(PTBRA-1.0)
ENDIF
PINI = FLOAT(NTP)
RHODEKP = FLOAT(MODEK)
RIBIAS = FLOAT(RIBIAS)
OMEGA = REFRO-PNI*(SWIDTH+RHODEK*0.5+RIBIAS)/PTBRA
OMEGARRAD = THPI+OMEGA
PEIX = OMEGARRAD+ETIME + (SWPRAT+ETIME**2)*FROSFLG
PEXL = OMEGARRAD+EXTIME - FROSFLG+OMEGARRAD/SWPRAT
POL4 = DELAY+OMEGARRAD
PDEL = DELA+OMEGARRAD
PADC = ADCSKIP+OMEGARRAD
PDECPIHA = PEIX + POL4 + PDEL + PADC
LDFPHA = PDECPIHA
WRITE(J,2600) LDFPHA
DO 100 I = 508,NTP
IF(MODEK,EQ.0) THEN
PTN = FLOAT(1)
OMEGA = HENFDO - (PTN-1.0)*FRANGE/(PTBRA-1.0)
ELSEIF(MODEK,EQ.1) THEN
PTN = FLOAT(NTP-1.0)
OMEGA = LOENDFDO + (PTN-1.0)*FRANGE/(PTBRA-1.0)
ENDIF
PINI = FLOAT(1)
RHODEKP = FLOAT(MODEK)
RIBIAS = FLOAT(RIBIAS)
OMEGA = REFRO-PNI*(SWIDTH+RHODEK*0.5+RIBIAS)/PTBRA
OMEGARRAD = THPI+OMEGA
PEIX = OMEGARRAD+ETIME + (SWPRAT+ETIME**2)*FROSFLG
PEXL = OMEGARRAD+EXTIME - FROSFLG+OMEGARRAD/SWPRAT
POL4 = DELAY+OMEGARRAD
PDEL = DELA+OMEGARRAD
PADC = ADCSKIP+OMEGARRAD
PDECPIHA = PEIX + POL4 + PDEL + PADC - LDFPHA
PDECPIHA = -PDECPIHA
CPHASE = COS(PDECPIHA)
BIPHASE = SIN(PDECPIHA)
HLOL = FLOAT(SDATA(I,1))
IMOLD = FLOAT(SDATA(I,1)+NTP)
REWNEW = MINT(REOLD+CPHASE+IMOLD+BIPHASE)
INNEW = MINT(REOLD+BIPHASE+IMOLD+CPHASE)
SData(I) = RENew
SData(I,1+NTP) = INNEW
IF(1,LE.N) THEN
WRITE(J,1000) OMEGA, OMEGARRAD
WRITE(J,1010) UNMEGARRAD
WRITE(J,1020) PEIX, POL4
WRITE(J,1040) PDEL, PADC
WRITE(J,1050) ADCSKIP, EXTIME
WRITE(J,1070) PDECPIHA
WRITE(J,1080) CPHASE, BIPHASE
APPENDIX H

PROGRAM SACPRC

SACPRC is the main program used to phase correct FT/ICR data based on the measured rotation of a series of DISPA plots for specified spectral peaks. The program is written specifically for the Nicolet FTMS-1000 instrument equipped with a Nicolet 1280 computer.

SACPRC is called as a three letter command "PRC" from the standard Nicolet FTMS software routine. The file FMSFOR.PRC must be in residence for SACPRC to function. SACPRC will perform a phase correction on whatever spectrum is resident in the array SDATA.

The program request the number of peaks in the spectrum, the number of points in the spectral window for each peak used for phase measurement and order of polynomial fit to the function.

Included in this appendix is a load file used in conjunction with RELOAD (the FORTRAN linker) and a series of subroutines required by the main program for operation. The function of each subroutine is provided on the following page.
SUBROUTINE - FUNCTION

SACPRL - Main program
SACPPK - peak search
SACBAS - linear baseline correction
SACWNC - extracts window of specified width about peak maximum
SACPBC - measures phase of peak currently in the spectral window based on intersection of perpendicular chord bisectors
SACSOR - sorts found peaks as function of relative intensity
SACASO - sorts 2-D array
SACFRC - performs 3 point interpolation to determine exact frequency of peak maximum
SACPSO - sorts found peaks as function of peak position
SACFIT - performs least squares fit of nth order polynomial to measured phases as a function of spectrum index.
SACDET - calculates the determinate of square matrix necessary for the least squares fit
SACPCO - carries out actual spectrum phase correction after the phase correction function has been determined
:E
FTMS.EXT:J
:B=1000-177777
SACPAC. REL: L
SACPPL. REL: L
SACBAS. REL: L
SACWNC. REL: L
SACPBC. REL: L
SACSGR. REL: L
SACASO. REL: L
SACFRC. REL: L
SACPSO. REL: L
SACFIT2. REL: L
SACDET. REL: L
SACPCO. REL: L
CXLIB.LIB: R
DPLIB.LIB: R
FORRUN. LIB: R
NICAPL. LIB: R
DPSYS.LIB: R
TIOS16. LIB: R
NICSYS.LIB: R
/- TT:U
/FMSFOR. PRC: S
:Q
MASTER PHASE CORRECTION ROUTINE

Allows phase correction via whatever phase routine is used in the subroutine phaser.

NOTE: This program does not calculate the magnitude, and so is compatible with magnitude independent routines only.

INTEGER SDATA, ORDER, WORK, POSITION, ERROR
REAL HEIGHT

CHARACTER RESPONSE*2, SUBSTR*5
COMMON /SDATA / SDATA(8192)
COMMON /NTP/ NTP
COMMON /WORK/ WORK(2048)
COMMON /NPTS/ NPTS

DIMENSION C(10), POSITION(10), HEIGHT(10), X(10), FRO(10)

PRINT *, 'SACPRC.ASC Version 1 (03 27 86.01)'*
PRINT *,
T=0.0
NPK=0

PRINT *, 'ENTER NUMBER OF PEAKS IN SPECTRUM:'
READ(1,1100) NPK

PRINT *, 'ENTER NUMBER OF POINTS IN PEAK WINDOW:'
READ(1,1000) NPTS

PRINT *, 'ENTER HIGHEST ORDER OF PHASE CORRECTION:'
READ(1,1100) ORDER

NTERMS = ORDER + 1

PRINT *, 'DO YOU WISH TO ENTER PEAK POSITIONS? (Y/N)'
READ(1,1400) RESPONSE

IF(SUBSTR(RESPONSE,1,1).EQ. 'Y') THEN
  AMAX=0.0
  DO 30 I=1,NPK
    WRITE(2,1500) I
    READ(1,1000) POSITION(I)
    HEIGHT(I)=SORT(FLOAT(SDATA(POSITION(I))))*
    * FLOAT(SDATA(POSITION(I)))*
    * FLOAT(SDATA(POSITION(I+NTP)))*
    IF(HEIGHT(I).GT.AMAX) AMAX=HEIGHT(I)
  CONTINUE
  DO 35 I=1,NPK
    WRITE(2,1300) I, POSITION(I), HEIGHT(I)
  CONTINUE
ELSE
  CALL PPIC(KTH,POSITION,HEIGHT,NPK,AMAX)
ENDIF

PRINT *,
PRINT *, I, POSITION(I) HEIGHT(I)
PRINT *,

DO 50 I=1,NPK
  WRITE(2,1300) I, POSITION(I), HEIGHT(I)
  PRINT *,
  PRINT *, 'DO YOU WANT BASELINE CORRECTION? (Y/N)'
  READ(1,1400) RESPONSE
  IF(SUBSTR(RESPONSE,1,1).EQ. 'Y') CALL BASEL(10,0)
DO 200 I=1,NPK
   C(I)=0.0
   WRITE(2,1600) I
   CALL WINDOW(POSITION(I),AMAX)
200   CALL PBCPHASE(HEIGHT(I),C(I))
   CALL CALCFRQ(FRQ,POSITION,C,NPK)
DO 300 I=1,NPK
300   C(I)=-C(I)
   CALL POLFIT(FRQ,C,SIGMA,NPK,NTERMS,O,X,CHISQR)
   CALL PDCORRECT(NPK,NTERMS,X,O)
   PRINT *, ''
   CALL FCOM('FPL',ERROR)
1000  FORMAT(15)
1100  FORMAT(12)
1200  FORMAT(11)
1300  FORMAT(4X,15,4X,110,4X,F10.3)
1400  FORMAT(42)
1500  FORMAT(' ENTER INDEX FOR PEAK('','12','') ')
1600  FORMAT(' INSTANT PHASE CALCULATION FOR PEAK('','12','') ')
   STOP
END
SUBROUTINE PICK(TH, POSITION, HEIGHT, NPK, AMAX)
REAL II, I2, I3, HEIGHT
INTEGER SDATA, POSITION
COMMON /SDATA/ SDATA(8192)
COMMON /NTP/ NTP
DIMENSION POSITION(20), HEIGHT(20)
PRINT *, ' SACPPK.ASC (03 23 86.01) ',
PRINT *, ' PEAK SEARCH IN PROGRESS...',
HEIGHT(1)=TH
II=SQRT(FLOAT(SDATA(1))*FLOAT(SDATA(1)) +
       FLOAT(SDATA(1+NTP))*FLOAT(SDATA(1+NTP)))
I2=SQRT(FLOAT(SDATA(2))*FLOAT(SDATA(2)) +
       FLOAT(SDATA(2+NTP))*FLOAT(SDATA(2+NTP)))
I3=SQRT(FLOAT(SDATA(J))*FLOAT(SDATA(J)) +
       FLOAT(SDATA(J+NTP))*FLOAT(SDATA(J+NTP)))
DO 100 J=3, NTP
IF (I2.GT.HEIGHT(1) .AND. I2.GT.I1 .AND. I2.GT.I3) THEN
   HEIGHT(1)=I2
   POSITION(1)=(J-1)
   CALL SORT(NPK, POSITION, HEIGHT)
END IF
WRITE(2,1100) (J-1), SDATA(J-1), SDATA(J-1+NTP), I2
   I1=I2
   I2=I3
100 CONTINUE
AMAX=0.0
DO 200 J=1, NPK
   IF (HEIGHT(J).GT.AMAX) AMAX=HEIGHT(J)
DO 300 J=1, NPK
   HEIGHT(J)=HEIGHT(J)*100.0/AMAX
   CALL PSORT(NPK, POSITION, HEIGHT)
300 CONTINUE
WRITE(8,1000)
1000 FORMAT(18)
1100 FORMAT(18,4X,18,4X,18,4X,F8.3)
RETURN
END
C SACBAS.ASC (03 08 86.01)
C LINEAR BASELINE CORRECTION OF REAL AND IMAGINARY SPECTRA
C
C SUBROUTINE BASELN(NE,FLAG)
INTEGER ASL, ASR, DSL, DSR, WORK, SDATA, FLAG
COMMON /SDATA/ SDATA(8192)
COMMON /NTP/ NTP
COMMON /WORK/ WORK(2048)
COMMON /NPTS/ NPTS
C PRINT *, ' SACBAS (03 08 86.01)'
PRINT *, ' BASELINE CORRECTION IN PROGRESS...'
ASL=0
ASR=0
DSL=0
DSR=0
IF (FLAG.EQ.0) THEN
N=NTP
DO 50 I=1,NE
ASL=SDATA(I)+ASL
ASR=SDATA(I+N-NE)+ASR
DSL=SDATA(I+N)+DSL
DSR=SDATA(I+2*N-NE)+DSR
ELSE
N=NPTS
DO 100 I=1,NE
ASL=WORK(I)+ASL
ASR=WORK(I+N-NE)+ASR
DSL=WORK(I+N)+DSL
DSR=WORK(I+2*N-NE)+DSR
END IF
AXA=FLOAT(NE+1)/2.0
DXA=FLOAT(NE+1)/2.0
AAL=FLOAT(ASL)/FLOAT(NE)
AAR=FLOAT(ASR)/FLOAT(NE)
DAL=FLOAT(DSL)/FLOAT(NE)
DAR=FLOAT(DSR)/FLOAT(NE)
ASLOP= (AAR-AAL)/FLOAT((N-NE))
DSLOP= (DAR-DAL)/FLOAT((N-NE))
AI=AAL-ASLOP*AXA
DI=DAL-DSLOP*DXA
IF (FLAG.EQ.0) THEN
DO 150 I=1,N
SDATA(I)=NINT(FLOAT(SDATA(I)))-(ASLOP*(I-1)+AI))
ELSE
DO 200 I=1,N
WORK(I)=NINT(FLOAT(WORK(I)))-(ASLOP*(I-1)+AI))
END IF
RETURN
END
SUBROUTINE WINDOW(PINDEX, AMAX)
INTEGER SDATA, WORK, PINDEX
COMMON /SDATA/ SDATA(8192)
COMMON /NTP/ NTP
COMMON /WORK/ WORK(2048)
COMMON /NPTS/ NPTS
PRINT *, ' ' SACWNC.ASC (03 26 86.01)
ILeft=PINDEX-NINT(NPTS/2.)
WRITE(2,1100) PINDEX, ILEFT, NPTS
PRINT *, ' ' SACWNC.ASC (03 26 86.01)
DO 100 I=1,NPTS
WORK(I)=SDATA(ILeft+I-1)
WORK(I+NPTS)=SDATA(ILeft+I-1+NTP)
MAG=NINT(SORT(FLOAT(WORK(I)))+FLOAT(WORK(I)))+
*          FLOAT(WORK(I+NPTS))+FLOAT(WORK(I+NPTS)))*100.0/AMAX)
100 WORK(I+2*NPTS)=MAG
WRITE(2,1100) I, WORK(I), WORK(I+NPTS), J, SDATA(J),
#SDATA(J+NTP)
1100 FORMAT(4X,15,4X,110,4X,110,4X,15,4X,110,4X,110)
1100 FORMAT(' ',PINDEX=' ',I6,' ILEFT=' 'I6,' NPTS=' ',I6)
1200 FORMAT(4X,'I',4X,'WORK(I)',4X,'WORK(I+NPTS)',4X,'J',
*4X,'SDATA(J)',4X,'SDATA(J+NTP)')
RETURN
END
DETERMINATION OF INSTANTANEOUS PHASE ERROR VIA INTERSECTION OF PERPENDICULAR CORD BISECTS OF A DISPA CIRCLE

SUBROUTINE PBCPHASE(INTENSITY, PHASE)
INTEGER WORK, POINT
REAL INTENSITY
COMMON /WORK/ WORK(2048)
COMMON /NPTS/ NPTS
DIMENSION POINT(100), XC(100), YC(100)

PRINT *, ' ENTER MINIMUM FRACTION OF PEAK MAGNITUDE
PRINT *, ' MAXIMUM DESIRED FOR POINT SELECTION:
READ(1,1600) FRACTION
LIMIT=INT(SORT(FRACTION)*INTENSITY)
INDEX=0
DO 100 I=1, NPTS
   IF(WORK(I+2*NPTS).GE.LIMIT) THEN
      INDEX=INDEX+1
      POINT(INDEX)=I
   ENDIF
100 CONTINUE
PRINT *, ' POINTS SUITABLE TO USE FOR PHASE CORRECTION:
PRINT *, ' POINT MAG. ABS. DISP.'
DO 200 J=1, INDEX
   WRITE(2,1000) J, WORK(I+2*NPTS), WORK(I), WORK(I+NPTS)
200   PRINT *, ' XSUM=0.0
   YSUM=0.0
   KOUNT=1
   NM1=INDEX-1
   DO 310 K=(K+1), NM1
      DO 310 J=(K+1), NM1
         C CALCULATE VALUES FOR X AND Y AT CENTER OF DISPA CIRCLE
         A1=FLOAT(WORK(POINT(K)))
         A2=FLOAT(WORK(POINT(K+1)))
         A3=FLOAT(WORK(POINT(J)))
         A4=FLOAT(WORK(POINT(J+1)))
         D1=FLOAT(WORK(POINT(K)+NPTS))
         D2=FLOAT(WORK(POINT(K+1)+NPTS))
         D3=FLOAT(WORK(POINT(J)+NPTS))
         D4=FLOAT(WORK(POINT(J+1)+NPTS))
         C AVOID DIVISION BY ZERO
         DIM2=D1-D2
D3MD4=D3-D4
IF (D1MD2.EQ.0.0.OR.D3MD4.EQ.0.0) THEN
  PRINT *, 'INTERSECTION IGNORED...'
  PRINT *, 'D1-D2 or D3-D4 equal to zero. '
  PRINT *, 'Subroutine avoiding division by zero. '
  GO TO 310
ENDIF

C S12=(A2-A1)/D1MD2
S34=(A4-A3)/D3MD4

C AVOID DIVISION BY ZERO
C S12M34=S12-S34
IF (S12M34.EQ.0.0) THEN
  PRINT *, 'INTERSECTION IGNORED...'
  PRINT *, 'S12-S34 equal to zero. '
  PRINT *, 'Subroutine avoiding division by zero. '
  GO TO 310
ENDIF
C
B12=(D1+D2-S12*(A1+A2))/2.0
B34=(D3+D4-S34*(A3+A4))/2.0

C XC(KOUNT)=(B34-B12)/S12M34
YC(KOUNT)=(B34*S12-B12*S34)/S12M34

C XSUM=XC(KOUNT)+XSUM
YSUM=YC(KOUNT)+YSUM
XAVG=XSUM/FLOAT(KOUNT)
YAVG=YSUM/FLOAT(KOUNT)

300 PHASE=ATAN(YAVG/XAVG)*180.0/3.1415927
C SECOND AND THIRD QUADRANTS
IF (XAVG.LT.0.0) THEN
  PHASE=180.0-PHASE
ENDIF
C FOURTH QUADRANT
IF (XAVG.GT.0.0. AND. YAVG.LT.0.0) THEN
  PHASE=360.0-PHASE
ENDIF
KP1=K+1
JP1=J+1
C WRITE(2,1300) KOUNT,K,KP1,J,JP1
C WRITE(2,1310) XC(KOUNT),YC(KOUNT),XAVG,YAVG,PHASE
KOUNT=KOUNT+1
310 CONTINUE
IF (KOUNT.EQ.0) THEN
  PRINT *, 'INSUFFICIENT NUMBER OF POINTS ABOVE LIMIT'
  PRINT *, 'INCREASE DIGITAL RESOLUTION OF SPECTRUM '
  PRINT *, 'OR DECREASE LIMIT.'
  PRINT *, '
RETURN
ENDIF
SXSQ=0.0
SYSO=0.0
DO 320 I=1,KOUNT
   SXSO=(XC(I)-XAVG)*(YC(I)-YAVG) + SXSO
320   SYSO=(YC(I)-YAVG)*(YC(I)-YAVG) + SYSO
   STDX=SORT(ABS(SXSO/FLOAT(KOUNT-1)))
   STDY=SORT(ABS(SYSO/FLOAT(KOUNT-1)))
WRITE(2,1500) XAVG,STDX,YAVG,STDY
WRITE(2,1400) PHASE
PHASE=PHASE+3.1415927/180.0
RETURN
1000 FORMAT(2X,I3,3(2X,I10))
1100 FORMAT(' CORD SET #',I3)
1200 FORMAT(I3)
1300 FORMAT(2X,13,4X,12,'-----',12,4X,12,'-----',12)
1310 FORMAT(4X,2(2(2X,F10.3)),F10.3)
1400 FORMAT(I1 ' FINAL PHASE= ',F10.3,' DEGREES',/)
1500 FORMAT(I1 ' AVERAGE X= ',F12.3,' +/- ',F12.3,/,I1 ' AVERAGE Y= ',F12.3,' +/- ',F12.3)
1600 FORMAT(F5.3)
2000 FORMAT(4(4X,F10.3))
END
C SACSOR.ASC (12 06 85.01)
C SACSOR.ASC <12 06 85.01)
C SORTS AN ARRAY Y(I) SUCH THAT Y(1) IS OF SMALLEST INTENSITY
C
SUBROUTINE SORT(NPK, X, Y)
INTEGER X
DIMENSION X(20), Y(20)
C
PRINT *, 'SACSOR *
C IF(NPK.LE.1) GO TO 300
DO 200 I=1,NPK
MIN=Y(I)
INDEX=I
DO 100 K=(I+1),NPK
IF(Y(K).GE.MIN) GO TO 100
INDEX=K
MIN=Y(K)
CONTINUE
100 TEMP=Y(I)
Y(I)=Y(INDEX)
Y(INDEX)=TEMP
ITEM=X(I)
X(I)=X(INDEX)
200 X(INDEX)=ITEM
300 RETURN
END
SUBROUTINE ASORT(A, ISTART, ISTOP)
DIMENSION A(3,3)
PRINT *, "SACASO.ASC (02 07 86.01)"
DO 200 J = ISTART, (ISTOP-1)
   M = J
   SMALL = A(J,3)
   DO 100 K = (J+1), ISTOP
      IF (A(K,3).LT.SMALL) THEN
         M = K
         SMALL = A(K,3)
      END IF
   100 CONTINUE
   TEMP = A(J,II)
   A(J,II) = A(M,II)
   A(M,II) = TEMP
200 RETURN
END
C SACFRC.ASC Version 1 (03 23 86.01)
C
C Performs a 3 point interpolation to determine the exact
c C frequency of the peak maximum.
C
C SUBROUTINE CALCFRQ(FRQ,POSITION,C,NPK)
INTEGER POSITION, SDATA
COMMON /SDATA/ SDATA(8192)
COMMON /NTP/ NTP
DIMENSION FRQ(10),C(10),POSITION(10)
C
PRINT *, SACFRC.ASC Version 1 (03 23 86.01)'
TWOP1=6.2831853
PRINT *, ' I  POSITION FREQUENCY PHASE'
PRINT *, ' -
DO 100 I=1, NPK
   MM1=POSITION(I)-1
   M = POSITION(I)
   MP1=POSITION(I)+1
   CPI=1.0/(FLOAT(SDATA(MP1))+FLOAT(SDATA(MP1)) +
      FLOAT(SDATA(MP1+NTP))+FLOAT(SDATA(MP1+NTP)))+
   CMP=2.0/(FLOAT(SDATA(M)+FLOAT(SDATA(M)) +
      FLOAT(SDATA(M+NTP))+FLOAT(SDATA(M+NTP)))+
   CMI=1.0/(FLOAT(SDATA(MM1))+FLOAT(SDATA(MM1)) +
      FLOAT(SDATA(MM1+NTP))+FLOAT(SDATA(MM1+NTP)))+
   EXACT=FLOAT(M)-((CPI-CMI)+
   ./(CM1-CMAX+CPI)+FLOAT(NTP))/2.0
   FRQ(I)=(FLOAT(NTP)-EXACT)
100 CONTINUE
DO 200 I=1, NPK
   PHASE=C(I)*360.0/TWOP1
200 WRITE(2,1000) I,POSITION(I),FRQ(I),PHASE
RETURN
1000 FORMAT(4X,12,4X,17,4X,F9.3,4X,F7.3)
END
C SACPSO.ASC (12 06 85.01)
C Sorts an array Y(J) such that Y(J) is of smallest intensity
C Used to resort the position and heights as function of
C position
C
SUBROUTINE PSORT(NPK, X, Y)
INTEGER X
DIMENSION X(20), Y(20)
C PRINT *, ' SACPSO '
IF (NPK.LE.1) GO TO 300
DO 200 I=1, NPK
   IMIN=X(I)
   INDEX=I
   DO 100 K=(I+1), NPK
      IF (X(K).GE.IMIN) GO TO 100
      INDEX=K
      IMIN=X(K)
100 CONTINUE
   TEMP=X(I)
   X(I)=X(INDEX)
   X(INDEX)=TEMP
   TEMP=Y(I)
   Y(I)=Y(INDEX)
200 Y(INDEX)=TEMP
300 RETURN
END
C SACFIT2.ASC Version 1 (03 25 86.01)
C NOTE: Same as SACFIT.ASC (03 25 86.01) only with different
C error message.
C
C Polynomial Regression see P.R. Bevington (p. 140)
C
C Purpose:
C Make a least-squares fit to data with polynomial curve
C
C Usage:
C CALL POLFIT(X,Y,SIGMA,Y,NPTS,TERMS,MODE,A,CHISQR)
C
C Description of parameters:
C X  - Array for independent variable
C Y  - Array for dependent variable
C SIGMA - Array for standard deviations of Y's
C NPTS - Number of coordinate pairs (X,Y)
C TERMS - Number of coefficients (degree of polynomial + 1)
C MODE - Defines type of weighting for least squares fit
C +1 (instrumental) WEIGHT(I) = 1./SIGMA(I)**2
C 0 (no weighting) WEIGHT(I) = 1.
C -1 (statistical) WEIGHT(I) = 1./Y(I)
C A - Array of polynomial coefficients
C CHISQR - Reduced Chi Square for fit
C
C Subroutines and function subprograms required:
C DETERM(ARRAY,NORDER)
C Matrix or order NORDER
C
C Modifications for FORTRAN II:
C Omit double precision specifications
C
C Comments:
C Dimension statement valid for TERMS up to 10
C
C SUBROUTINE POLFIT(X,Y,SIGMA,Y,NPTS,TERMS,MODE,A,CHISQR)
C DOUBLE PRECISION SUMX, SUMY, XTERM, YTERM, ARRAY, CHISQR
C DIMENSION X(I), Y(I), SIGMA(I), A(I)
C DIMENSION SUMX(I), SUMY(I), ARRAY(10,10)
C
C Accumulate Weighted Sums
C
C PRINT *, 'SACFIT.ASC Version 1 (03 25 86.01)'
C 11 NMAX=2+TERMS-1
C 13 DO 15 I=1,NMAX
C 15 SUMX(I)=0.0
C 15 DO 15 J=1,TERMS
C 15 SUMY(J)=0.0
C 21 CHISQR=0.0
C 21 DO 50 I=1,NPTS
C 31 IF(MODE)32,37,39
C
C 32 X=I
C 37 Y=I
32     IF(YI) 35,37,33
33     WEIGHT=1.0/YI
34     GO TO 41
35     WEIGHT=1.0/(-YI)
36     GO TO 41
37     WEIGHT=1.0
38     GO TO 41
39     WEIGHT=1.0/SIGMA(I)**2
40     XTERM=WEIGHT
41     DO 44 N=1,NMAX
42           SUMX(N)=SUMX(N) + XTERM
43     XTERM=XTERM*X1
44     YTERM=WEIGHT*YI
45     DO 48 N=1,NTERMS
46           SUMY(N)=SUMY(N) + YTERM
47     YTERM=YTERM*X1
48     CHISQ=CHISQ+WEIGHT*YI**2
49     CONTINUE

C Construct matrices and calculate coefficients

50     DO 54 J=1,NTERMS
51           DO 54 K=1,NTERMS
52                 N=J*K-1
53                 ARRAY(J,K)=SUMX(N)
54             END DO
55             DELTA = DETERM(ARRAY,NTERMS)
56             IF(Delta) 61,57,61
57             CHISQR=0.0
58             DO 59 J=1,NTERMS
59           A(IJ)=0.0
60           GO TO 80
61     DO 70 L=1,NTERMS
62           DO 66 J=1,NTERMS
63               DO 66 K=1,NTERMS
64                 N=J*K-1
65                 ARRAY(J,K)=SUMX(N)
66               END DO
67                 ARRAY(J,L)=SUMY(J)
68             END DO
69             A(L)=DETERM(ARRAY,NTERMS)/DELTA
70     CONTINUE

C Calculate Chi Square

71     DO 75 J=1,NTERMS
72           CHISQ=CHISQ+2.0*A(J)*SUMY(J)
73     END DO
74     CHISQ=CHISQ+A(J)*A(K)*SUMX(N)
75     CONTINUE
76     FREE=NPTS-NTERMS
77     IF(FREE.EQ.0.0) THEN
78           PRINT *,** ***************
79           PRINT *,** WARNING!!!
80           PRINT *,** POLYNOMIAL NOT OVERDETERMINED!!!
81           PRINT *,** Reduce ORDER of polynomial fit or
C    PRINT e, 'e Increase the number of peaks entered. e'
C    PRINT e, 'e
C    PRINT e, '************************************************
C    PRINT e, 'NOTE: Polynomial is just determined. ' 
C    PRINT e, 'GO TO 80
GO TO 80
ENDIF
77 CHISO=CHISO/FREE
80 RETURN
END
Calculate the Determinant of a Square Matrix.

* see P.R. Bevington (p. 294)

Usage: DET = DETERM(ARRAY, NORDER)

Description of parameters:

ARRAY - Matrix
NORDER - Order of determinant (degree of matrix)

Modifications for FORTRAN II:

Dmit double precision specifications

Comments:

This subroutine destroys the input matrix ARRAY
Dimension statement is valid for NORDER up to 10

FUNCTION DETERM(ARRAY,NORDER)
DOUBLE PRECISION ARRAY,SAVE
DIMENSION ARRAY(10,10)

10 DETERM=1.0
11 DO 50 K=1,NORDER

Interchange Columns if Diagonal Element is Zero.

21 DO 23 J=K,NORDER
22 IF(ARRAY(K,J)) 41,21,21
23 CONTINUE
DETERM=0.0
GO TO 60

Subtract Row K from lower Rows to get Diagonal Matrix.

41 DETERM = DETERM*ARRAY(K,K)
42 IF(K=NORDER) 43,50,50
43 K1=K+1
44 DO 46 I=K1,NORDER
45 DETERM = DETERM*ARRAY(I,K)
46 CONTINUE

50 CONTINUE
60 RETURN

END
SUBROUTINE PCORRECT(NPEAKS, NTERMS, A, FLAG)
INTEGER SDATA, FLAG, WORK, RNEW
REAL PHASE
COMMON /SDATA/ SDATA(8192)
COMMON /NTP/ NTP
COMMON /WORK/ WORK(2048)
COMMON /NPTS/ NPTS
DIMENSION A(10)

READ(1,1100) IFLAG
IF IFLAG.EQ.0 THEN
PRINT *, ' Polynomial Coefficients for:
PRINT *, Y = A(1) + A(2)*X + A(3)*X**2 + ...
DO 50 I-1, NTERMS
WRITE(2,1200) 1, A(I)
50 CONTINUE
ELSE
DO 400 J=1, NPTS
PHASE = 0.0
DO 300 I=1, NPEAKS
K=NTP-J
PHASE=A(I)*((FLOAT(NTP-J))**(I-1)) + PHASE
300 CONTINUE
END IF
400 CONTINUE
ELSE
DO 300 J=1, NPTS
PHASE = 0.0
DO 200 I=1, NPEAKS
K=NTP-J
PHASE=A(I)*((FLOAT(NTP-J))**(I-1)) + PHASE
200 CONTINUE
END IF

ELSE
    WORK(J+NPTS)=NINT(PHASE)
ENDIF

CONTINUE

FORMAT(2X,I5,2X,15,2X,F13.6)

FORMAT(I1)

FORMAT(4X,'A(\',I1,\')=',E12.5)

PRINT *, 'SDATA phasing completed'
RETURN

END
APPENDIX I

PROGRAM SACMAS

SACMAS is the same as SACPRC except SACMAS interpolates between measured phases using a solution of simultaneous equations. Most of the subroutines required by SACMAS have already been presented in the previous appendix so only the load file and those routines unique to SACMAS are included in this appendix.

SACMAS is called from the Nicolet FTMS-1000 instrument software by the command "SAC". The file FMSFOR.SAC must be present to use SACMAS.

SUBROUTINE - FUNCTION

SACMAS - Main program
SACMAG - generates magnitude spectrum from real and imaginary spectra
SACPPI - searches magnitude spectrum for peaks
SACWIN - extracts spectral window about selected peak maximum
SACSSE - solution of simultaneous equations; sets up matrices for use by SACSIM and interprets results from SACSIM
SACSIM - Gauss-Jordan elimination with maximum pivot strategy
:E
FTMS.EXT:J
IB=1000-177777
SACMAS.REL:L
SACMAG.REL:L
SACPPI.REL:L
SACBAS.REL:L
SACWIN.REL:L
SACPBC.REL:L
SACSOR.REL:L
SACASO.REL:L
SACPSO.REL:L
SACSSE.REL:L
SACSIM.REL:L
SACPCO.REL:L
CXLIB.LIB:R
DPLIB.LIB:R
FORRUN.LIB:R
NICAPL.LIB:R
DPSYS.LIB:R
TIOS16.LIB:R
NICSYS.LIB:R
/-TT:U
/-FMSFOR.SAC:S
:Q
SACMAS.ASC Version 1 (02 05 86.01)

MASTER PHASE CORRECTION ROUTINE

ALLOWS PHASE CORRECTION VIA WHATEVER PHASE ROUTINE IS USED IN THE SUBROUTINE PHASER

INTEGER SDATA, ORDER, WORK, POSITION
REAL MAG, HEIGHT
CHARACTER RESPONSE*2, SUBSTR*5
COMMON /SDATA / SDATA(8192)
COMMON /NTP/ NTP
COMMON /WORK/ WORK(2048)
COMMON /NPTS/ NPTS
COMMON /MAG/ MAG(8192)
COMMON /TIMET/ TT
COMMON /DELAY/ DL
COMMON /SWPRT/ SWR
COMMON /MODEKP/MODEKP
DIMENSION C(10), POSITION(10), HEIGHT(10), X(10), A(10, 10)
MODEKP=0
PRINT *, 'SACMAS.ASC Version 1 (02 05 86.01)'
PRINT *,
TH=0.0
NPK=0
PRINT *, 'ENTER NUMBER OF PEAKS IN SPECTRUM: ',
READ(1,1100) NPK
PRINT *, 'ENTER NUMBER OF POINTS IN PEAK WINDOW: ',
READ(1,1000) NPTS
PRINT *, 'ENTER HIGHEST ORDER OF PHASE CORRECTION: ',
READ(1,1100) ORDER
PRINT *, 'CALCULATING MAGNITUDE SPECTRUM'

CALL MAGNITUDE

PRINT *, 'DO YOU WISH TO ENTER PEAK POSITIONS? ',
READ(1,1400) RESPONSE
 IF (SUBSTR(RESPONSE,1,1).EQ.'Y') THEN
  DO 30 I=1,NPK
     WRITE(2,1500) I
     30 READ(1,1000) POSITION(I)
  ELSE
     CALL PPICK(TH,POSITION,HEIGHT,NPK)
     ENDIF
     PRINT *, '
     PRINT *, '
     PRINT *, I  
     PRINT *, 
     DO 50 I=1,NPK
     WRITE(2,1300) I, POSITION(I), HEIGHT(I)
     50 WRITE(2,1300) I, POSITION(I), HEIGHT(I)
     PRINT *, '
     PRINT *, '
     CALL BASELN(10,0)
     DO 200 I=1,NPK
     C(I)=0.0
     WRITE(2,1600) I
     CALL WINDOW(POSITION(I))
C CALL PLOT
200 CALL PBCPHASE(HEIGHT(I),C(I))
  CALL BUILD(A,POSITION,C,NPK)
  CALL POLSOL(A,NPK,1,1.0E-20,X)
C CALL POLSOL(NPK,C,POSITION,X)
  CALL PCorrect(NPK,X,0)
1000 FORMAT(I3)
1100 FORMAT(I2)
1200 FORMAT(I1)
1300 FORMAT(5X,15,4X,110,4X,F10.3)
1400 FORMAT(A2)
1500 FORMAT('ENTER INDEX FOR PEAK('',I2,''))
1600 FORMAT('INstant PHASE CALCuLATION FOR PEAK('',I2,''))
STOP
END
C SACMAG.ASC (02 05 86.01)
C Calculation of Magnitude mode spectrum from SDATA
C
SUBROUTINE MAGNITUDE
INTEGER S
REAL MAG
COMMON /SDATA/ S(8192)
COMMON /NTP/ N
COMMON /MAG/ MAG(8192)
C PRINT *, ' SACMAG.ASC (02 05 86.01)',
AMAX=0.0
DO 100 I=1,N
   MAG(I)=SORT(FLOAT(S(I))+FLOAT(S(I))
   * +FLOAT(S(I+N))+FLOAT(S(I+N))
   IF(MAG(I).GT.AMAX) AMAX=MAG(I)
100 CONTINUE
DD 110 I=1,N
110 MAG(I)=100.0*MAG(I)/AMAX
RETURN
END
SUBROUTINE PICK(TH, POSITION, HEIGHT, NP, N)
REAL MAG, I1, I2, I3, HEIGHT
INTEGER SDATA, POSITION, Y
COMMON /SDATA/ Y(8192)
COMMON /NTP/ N
COMMON /MAG/ MAG(8192)
DIMENSION POSITION(20), HEIGHT(20)
PRINT ' SACPI
HEIGHT(1)=TH
DO 100 J=1, (N-2)
   I1=MAG(J)
   I2=MAG(J+1)
   I3=MAG(J+2)
   IF(I2.GT.HEIGHT(1), AND, I2.GT.11, AND, I2.GT.13) THEN
      HEIGHT(1)=I2
      POSITION(1)=(J+1)
      CALL SORT(NP, POSITION, HEIGHT)
   ENDIF
WRITE(2,1100) J, Y(J), Y(J+N), MAG(J)
100 CONTINUE
CALL PSORT(NP, POSITION, HEIGHT)
1100 FORMAT(18)
RETURN
END
C CARRIES OUT A PEAK EXTRACTION FROM SDATA(I) TO CREATE WORK(I)
C NPTS...NUMBER OF POINTS RETAINED ABOUT PEAK MAXIMUM
C WORK...ARRAY RETURNED CONTAINING REAL AND IMAGINARY PEAK
C SPECTRA
C PINDEX..INDEX IN SDATA WHERE PEAK ABSORPTION OCCURS
C
SUBROUTINE WINDOW(PINDEX)
INTEGER SDATA, WORK, PINDEX
REAL MAG
COMMON /SDATA/ SDATA(8192)
COMMON /NTP/ NTP
COMMON /MAG/ MAG(8192)
COMMON /WORK/ WORK(2048)
COMMON /NPTS/ NPTS
PRINT *, ' 
PRINT *, 'ILEFT-PINDEX-NINT(NPTS/2.)
WRITE(2,1100) PINDEX, ILEFT, NPTS
PRINT *, ' 
C WRITE(2,1200)
DO 100 I=1,NPTS
   WORK(I)=SDATA(ILEFT+I-1)
   WORK(I+NPTS)=SDATA(ILEFT+I-1+NTP)
100    WORK(I+2*NPTS)=NINT(MAG(ILEFT+I-1))
C DO 110 I=1,NPTS
C     J=LEFT+I-1
C WRITE(2,10000) I,WORK(I),WORK(I+NPTS),J, SDATA(J),
C @SDATA(J+NTP)
1100 FORMAT(' PINDEX= ',I6,' ILEFT= ',I6,' NPTS= ',I6)
1200 FORMAT(4X,'I',4X,'WORK(I)',4X,'WORK(I+NPTS)',4X,'J',
      $4X,'SDATA(J)',4X,'SDATA(J+NTP)')
RETURN
END
SUBROUTINE POLSOL(A,N,INDIC,EPS,X)

IMPLICIT REAL (A-H,O-Z)

DIMENSION X(10),A(10,10)

PRINT *, 'SACSSE.ASC Version 1 (01 27 86.01)

WRITE(2,200) N,INDIC,EPS

MAX=N

IF(INDIC.GE.0) MAX=N+1

DO 4 I=1,N

WRITE(2,201) (A(I,J),J=1,MAX)

CALL SIMUL

DETER = SIMUL(N,A,X,EPS,INDIC,10)

PRINT SOLUTIONS

IF(INDIC.GE.0) GO TO 8

WRITE(2,202) DETER

DO 7 I=1,N

WRITE(2,201) (A(I,J),J=1,N)

RETURN

8 WRITE(2,203) DETER,N,(X(I),I=1,N)

IF(INDIC.NE.0) RETURN

9 WRITE(2,204)

DO 10 I=1,N

WRITE(2,201) (A(I,J),J=1,N)

RETURN

FORMATS FOR INPUT AND OUTPUT STATEMENTS

200 FORMAT(4X, 'N = ',I4,4X,'INDIC = ',I4,4X,'EPS = ',

*E10.1,/', ' THE STARTING MATRIX IS: ',/)

201 FORMAT(7F15.3)

202 FORMAT(4X,'DETER = ',F15.3,/', ' THE INVERSE MATRIX IS: '/)

203 FORMAT(4X,'DETER = ',E15.3,/', ' THE SOLUTIONS X(1),..X(10) ARE: ',/)

204 FORMAT(4X,'THE INVERSE MATRIX IS: ',/)

END
FUNCTION THAT USES GAUSS-JORDAN ELIMINATION TO SOLVE A SYSTEM OF SIMULTANEOUS EQUATIONS

FUNCTION SIMUL(N, A, X, EPS, INDIC, NRC)

IMPLICIT REAL (A-H,O-Z)
DIMENSION IROW(10), JCOL(10), JORD(10), Y(10), A(NRC,NRC)
DIMENSION X(N)
MAX=N
IF(INDIC.GE.0) MAX=N+1

IS N LARGER THAN 10?
IF(N.LE.10) GO TO 5
PRINT *, 'ERROR! ARRAY CANNOT HAVE MORE THAN 20 ELEMENTS'
SIMUL=0.0
RETURN

BEGIN ELIMINATION PROCEDURE

5 DETER=1.0
DO 10 K=1,N
KMI=K-1

SEARCH FOR PIVOT ELEMENT

PIVOT=0.0
DO 11 I=1,N
DO 11 J=1,N

SCAN IROW AND JCOL ARRAYS FOR INVALID PIVOT SUBSCRIPTS

IF(K.EQ.1) GO TO 9
DO 8 ISCAN =1,KMI
DO 6 JSCAN =1,KMI
IF(I.EQ.IROW(ISCAN)) GO TO 11
IF(J.EQ.JCOL(JSCAN)) GO TO 11
8 IF(DABS(A(I,J)).LE.DABS(PIVOT)) GO TO 11
PIVOT=A(I,J)
IROW(K)=I
JCOL(K)=J
CONTINUE

INSURE THAT SELECTED PIVOT IS LARGER THAN EPS
IF(DABS(PIVOT).GT.EPS) GO TO 13
SIMUL = 0.0
RETURN

UPDATE DETERMINANT VALUE

13 IROWK=IROW(K)
JCOLK=JCOL(K)
DETER=DETER*PIVOT
NORMALIZE PIVOT ROW ELEMENTS

DO 14 J=1,MAX
    A(IROWK,J)=A(IROWK,J)/PIVOT

CARRY OUT ELIMINATION AND DEVELOP INVERSE

A(IROWK,JCOLK)=1.0/PIVOT
DO 18 I=1,N
    AIJCK=A(I,JCOLK)
    IF (I.EQ.IROWK) GO TO 18
    A(I,JCOLK)=-AIJCK/PIVOT
    DO 17 J=1,MAX
    IF (J.NE.JCOLK) A(I,J)=A(I,J)-AIJCK*A(IROWK,J)
    CONTINUE

ORDER SOLUTION VALUES (IF ANY) AND CREATE JORD ARRAY

DO 20 I=1,N
    IROWI=IROW(I)
    JCOLI=JCOL(I)
    JORD(IROWI)=JCOLI
    IF (INDIC.GE.0) X(JCOLI)=A(IROWI,MAX)

ADJUST SIGN OF DETERMINANT

INTCH=0
NM1=N-1
DO 22 I=1,NM1
    IP1=I+1
    DO 22 J=IP1,N
        IF (JORD(J).GE.JORD(I)) GO TO 22
        JTEMP=JORD(J)
        JORD(J)=JORD(I)
        JORD(I)=JTEMP
        INTCH=INTCH+1
    CONTINUE
    IF (INTCH/2.E+2).NE.INTCH) DETER=-DETER

IF INDIC IS POSITIVE RETURN WITH RESULTS

IF (INDIC.LE.0) GO TO 26
SIMUL=DETER
RETURN

IF INDIC IS NEGATIVE OR ZERO, UNSCRAMBLE THE INVERSE FIRST BY ROWS...

DO 28 J=1,N
    DO 27 I=1,N
        IROWI=IROW(I)
        JCOLI=JCOL(I)
        Y(JCOLI)=A(IROWI,J)
        DO 28 I=1,N
            A(I,J)=Y(I)
THEN BY COLUMNS...

DO 30 I=1,N
   DO 29 J=1,N
      IROWJ=IROW(J)
      JCOLJ=JCOL(J)
      29 Y(IROWJ)=A(I,JCOLJ)
   DO 30 J=1,N
30 A(I,J)=Y(J)

RETURN FOR INDIC NEGATIVE OR ZERO

SIMUL=DETER
RETURN
END
APPENDIX J

UTILITY PROGRAMS FOR NICOLET FTMS-1000 INSTRUMENT

FORTRAN utility programs are provided. The purpose of each of these programs is indicated in the following list. Both the load file and the source code are provided. The following programs are called as 3 letter commands from the standard Nicolet FTMS software unless specified as a stand-alone program.

FROGGER - accepts manually inputted peak index and performs a zero order phase correction on that peak only.
INTEGER SDATA, WORK
REAL IMNEW
CHARACTER RESPONSE(1, SUBSTR*5)
COMMON /SDATA/ SDATA(8192)
COMMON /INDEX/ NTP
COMMON /WORK/ WORK(24*6)
COMMON /NPTS/ NPTS
AMAX=0.0
TOMP=5.2831853
50 PRINT *, 'ENTER INDEX OF PEAK:', INDEX
READ(1,1000) INDEX
PRINT *, 'ENTER NUMBER POINTS IN WINDOW:', INDEX
READ(1,1000) NPTS
CALL INDEX(INDEX, 100.0)
DO 100 I=1+2*NPTS, 3*NPTS
100 WORK(1)= NINT (FLOAT (WORK(1))*100.0/AMAX)
CALL PBCPHASE(100., RADIANS)
SINE=SIN(-RADIANS)
COSINE=COS(-RADIANS)
LEFT=INDEX-NINT (FLOAT (NPTS)/2.) -1
DO 200 I=1, NPTS
200 IMNEW = FLOAT (SDATA(LEFT*I)) + COSINE
- FLOAT (SDATA(LEFT*I+NTP)) + SINE
IMNEW = FLOAT (SDATA(LEFT*I)) + SINE
+ FLOAT (SDATA(LEFT*I+NTP)) + COSINE
SDATA(LEFT*I) = NINT (IMNEW)
300 SDATA(LEFT*I+NTP) = NINT (IMNEW)
C CYCLES=RADIANS/TOMP
C DEGREES=CYCLES/360.0
WRITE(2,1100) INDEX, CYCLES, RADIANS, DEGREES
PRINT *, 'WOULD YOU LIKE TO PHASE ANOTHER PEAK? (Y/N)', RESPONSE
READ(1,1300) RESPONSE
IF (SUBSTR(RESPONSE, 1, 1) .EQ. 'Y') GO TO 50
STOP
1000 FORMAT (15)
1100 FORMAT (5X, 'PEAK INDEX:', 1B, /)
1 3X, 'PHASE (CYCLES):', 'F10.6/',
2 3X, 'RADIANS:', 'F10.6/',
3 3X, 'DEGREES:', 'F10.6'
1200 FORMAT (110)
1300 FORMAT (A1)
END
APPENDIX K

$^1$H DECOUPLED $^{13}$C FT/NMR SPECTRA OF DEXTRAN POLYMERS
Fig. 110. $^1$H decoupled $^{13}$C FT/NMR spectrum of T40 Dextran.
Fig. 111. $^1$H decoupled $^{13}$C FT/NMR spectrum of T2000 Dextran.
Fig. 112. $^1$H decoupled $^{13}$C FT/NMR spectrum of G100 Sephadex.
Fig. 113. $^1$H decoupled $^{13}$C FT/NMR spectrum of G75 Sephadex.
Fig. 114. $^1$H decoupled $^{13}$C FT/NMR spectrum of G25 Sephadex.
APPENDIX L

EXPANDED PLOTS OF INDIVIDUAL PEAKS FROM SPECTRA IN APPENDIX K
Fig. 115. Expanded plot of the methanol peak from $^1$H decoupled $^{13}$C spectrum of dextran T40 solution.
Fig. 116. Expanded plot of the Cl peak from $^1$H decoupled $^{13}$C spectrum of dextran T40 solution.
Fig. 117. Expanded plot of the C3 peak from $^1$H decoupled $^{13}$C spectrum of dextran T40 solution.
Fig. 118. Expanded plot of the C2 peak from $^1$H decoupled $^{13}$C spectrum of dextran T40 solution.
Fig. 119. Expanded plot of the C4 peak from $^1$H decoupled $^{13}$C spectrum of dextran T40 solution.
Fig. 120. Expanded plot of the C5 peak from $^1$H decoupled $^{13}$C spectrum of dextran T40 solution.
Fig. 121. Expanded plot of the C6 peak from $^1$H decoupled $^{13}$C spectrum of dextran T40 solution.
Fig. 122. Expanded plot of the methanol peak from $^1$H decoupled $^{13}$C spectrum of dextran T2000 solution.
Fig. 123. Expanded plot of C1 peak from $^1$H decoupled $^{13}$C spectrum of dextran T2000 solution.
Fig. 124. Expanded plot of the C3 peak from $^1$H decoupled $^{13}$C spectrum of dextran T2000 solution.
Fig. 125. Expanded plot of the C2 peak from $^1$H decoupled $^{13}$C spectrum of dextran T2000 solution.
Fig. 126. Expanded plot of the C4 peak from $^1$H decoupled $^{13}$C spectrum of dextran T2000 solution.
Fig. 127. Expanded plot of the C5 peak from $^1$H decoupled $^{13}$C spectrum of dextran T2000 solution.
Fig. 128. Expanded plot of the C6 peak from $^1$H decoupled $^{13}$C spectrum of dextran T2000 solution.
Fig. 129. Expanded plot of the methanol peak from $^1$H decoupled $^{13}$C spectrum of sephadex G100 gel.
Fig. 130. Expanded plot of the Cl peak from $^1$H decoupled $^{13}$C spectrum of sephadex G100 gel.
Fig. 131. Expanded plot of the C3 peak from $^1$H decoupled $^{13}$C spectrum of sephadex G100 gel.
Fig. 132. Expanded plot of the C2 peak from $^1$H decoupled $^{13}$C spectrum of sephadex G100 gel.
Fig. 133. Expanded plot of the C4 peak from $^1$H decoupled $^{13}$C spectrum of sephadex G100 gel.
Fig. 134. Expanded plot of the C5 peak from $^1$H decoupled $^{13}$C spectrum of sephadex G100 gel.
Fig. 135. Expanded plot of the C6 peak from $^1$H decoupled $^{13}$C spectrum of sephadex G100 gel.
Fig. 136. Expanded plot of the methanol peak from $^1$H decoupled $^{13}$C spectrum of sephadex G75 gel.
Fig. 137. Expanded plot of the Cl peak from $^1$H decoupled $^{13}$C spectrum of sephadex G75 gel.
Fig. 138. Expanded plot of the C3 peak from $^1$H decoupled $^{13}$C spectrum of Sephadex G75 gel.
Fig. 139. Expanded plot of the C2 peak from $^1$H decoupled $^{13}$C spectrum of sephadex G75 gel.
Fig. 140. Expanded plot of the C4 peak from $^1$H decoupled $^{13}$C spectrum of sephadex G75 gel.
Fig. 141. Expanded plot of the C5 peak from $^1$H decoupled $^{13}$C spectrum of sephadex G75 gel.
Fig. 142. Expanded plot of the C6 peak from $^1$H decoupled $^{13}$C spectrum of sephadex G75 gel.