# ITRCAL

## Iteration of Calculated Nmr Spectra Using Least Squares Criteria
## Instructions for Use

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# ITRCAL

## Iteration of Calculated Nmr Spectra Using Least Squares Criteria

## I. INTRODUCTION

The analysis of complex coupled nmr spectra has been reported by Swalen and Reilley[1] and Castellano and Bothner-By,[2] as well as by Hoffman[3] and Arata et. al.[4] In all cases, a theoretical spectrum is calculated using standard techniques[5,6] and then iterated for a best fit with a set of experimentally observed lines. In the ITRCAL program, the method of Castellano and Bothner-By is used. This program is the first reported implementation of the LAOCN3 algorithm on a minicomputer, and it should provide a powerful new scientific tool for laboratory real time analysis of experimental nmr spectra. ITRCAL requires a 12K Nicolet 1080 computer system and a NIC-294 Disk Memory.

The calculation of theoretical nmr spectra from chemical shifts and coupling constants has been well described in several places[5-7] and will not be detailed here. Essentially, a high resolution Hamiltonian matrix is constructed as follows.

The diagonal elements are constructed according to the equation

$$H_{uu} = \sum_{i=1}^{n} \left[ \nu_i I_z(i) + \sum_{j=1}^{n} J_{ij} T_{ij} \right]$$

where $\nu_i$ is the chemical shift of nucleus i
$I_z(i) = +1/2$ for spin $\alpha$ and $-1/2$ for spin $\beta$
$J_{ij}$ is the coupling constant between nuclei i and j
$T_{ij} = +1/4$ if spins i and j of the basic function corresponding to that diagonal element are aligned and $-1/4$ if they are opposed.

The off-diagonal elements are calculated by

$$H_{uv} = 1/2 J_{ij} U$$

where U = 1 if and only if basis function u and basis function v differ only in the interchange of spins i and j
and U = 0 otherwise
and $J_{ij}$ is the coupling constant between the two spins which differ in the two basis functions.

Each such matrix is diagonalized to yield energy levels or eigen values as the diagonal elements. A matrix of eigenvectors is also produced by the diagonalization.

The energies of the transitions, in Hz, are calculated by subtracting each energy level in the previous H-matrix diagonal from each energy level in the current H-matrix diagonal. The probability or the intensity of these transitions is given by

$$I_{km} = \left( \sum_{i=1}^{n} \sum_{j=1}^{n} C_{ik} C'_{jm} X_{ij} \right)^2$$

where $I_{km}$ is the intensity of the transition between energy level k of the last H-matrix and energy level m of this H-matrix

$C_{ik}$ is the $i^{th}$ coefficient of the $k^{th}$ column of the last set of eigenvectors

$C'_{jm}$ is the $j^{th}$ coefficient of the $m^{th}$ column of this set of eigenvectors

$X_{ij}$ = 1 if and only if basis function i of the last set and basis function j of the current set differ only in one spin

= 0 in all other cases.

Iteration for a best fit between a theoretical and an experimental spectrum requires that a set of close guessed parameters be obtained from which to iterate. The actual iteration procedure is a least squares method which tends to minimize the rms error. The amounts which parameters are varied is calculated from a set of partial differentials which are used to minimize the differences between the observed and calculated frequencies. This is discussed in detail in the paper by Castellano and Bothner-By.[2]

Loading and Startup

To load ITRCAL, place the tape in either the high speed or low speed reader, start the DEMON monitor and call the binary loader by typing BIN. The loader will be loaded and started automatically, and if a high speed reader is in use, the tape will begin reading in. If a low speed reader is in use, turn it on after calling BIN and the tape will begin to read in.

When the tape has read in to the rubout in the trailer section of the tape, the computer will stop. Then, restart the monitor at 7600 and store the memory blocks as follows:

```
STORE ITRCL1 0-7577:P

STORE ITRCL2 114225 - 115777:P
```

Then, if you wish to make use of the 7-spin calculation feature, load the NMRCL7 overlay tape and read it in by typing BIN again. When this tape has read in, the computer will stop. Restart DEMON at 7600, and store this program by the following commands:

```
STORE NMRCL1 0-7577:P

STORE NMRCL2 115461 - 115656:P
```

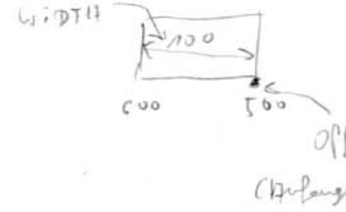
To start ITRCAL, load the two ITRCAL files by the following commands:

```
LOAD ITRCL1

LOAD ITRCL2

GO
```

The GO command will start the program at location 0, and cause it to type out its title and begin accepting data. Note that the RUN command of DEMON II cannot be used, since non-contiguous storage is involved. Once ITRCAL has been used, it modifies its start-up routine so that it no longer goes through the questions for the calculation of a spectrum before awaiting additional commands. The program can then be restarted using the R command.

## Initial Dialogue

Upon startup the first time or whenever the program is restarted, ITRCAL will ask the following questions to obtain parameters for the calculation of a spectrum.



```
ITRCAL V1B  04/73

NUMBER OF SPINS =
SPECTROMETER FREQ (MHZ) =
WIDTH OF DISPLAY (HZ) =
OFFSET OF DISPLAY (HZ) =
INTENSITY THRESHOLD =
```

~ 0.01

This is the beginning of an actual calculation of a guessed or theoretical spectrum. ITRCAL must have an approximate spectrum from which to iterate for a best fit with the observed spectrum. The display frequency convention is that of all other Nicolet programs: the right end is considered to be 0 Hz and the frequency increases in the positive direction to the left. This conforms to the most common scale for both pmr and cmr measurements.

The intensity threshold is the minimum intensity transition that is to be saved in the table of transitions and intensities. Up to 300 transitions can be saved; however for cases involving fewer transitions this threshold can be entered as zero. If no number is entered and a Return is typed, the last entered value for the threshold is assumed. For cases involving 2, 3 or 4 spins, no threshold is necessary.

The spectrometer frequency is used if and only if chemical shifts are entered in ppm or tau units, and is otherwise ignored completely. In the case where shifts are entered only in Hz, the spectrometer frequency may be set to zero by simply typing Return.

The entire spectrum is calculated regardless of the setting of the display region, and the offset and width may be changed after the calculation to examine any portion of the spectrum desired. If no values are entered for offset and width, an offset of zero and a width of 2048 are assumed.

## Entry of Shifts and Coupling Constants

The program will next ask for the number of chemical shifts and coupling constants which would be expected from the number of spins which were entered. For example, if 3 is entered for NUMBER OF SPINS =, the program will type:

```
CHEMICAL SHIFTS
V(1)=
V(2)=
V(3)=

COUPLING CONSTANTS
J(1,2)=
J(1,3)=
J(2,3)=
```

The chemical shifts are assumed to be in Hz if their value is terminated with a Return. If only a Return is entered, a zero value is assumed. If the chemical shift is terminated with a P, the shift is assumed to be in parts per million, where the right end is zero Hz and the ppm scale increases positively downfield. If a chemical shift is terminated with a T, the tau scale is assumed, in which TMS is assumed to be at 10τ, and the spectrum decreases in positive value to the left. If no spectrometer frequency has been entered, the use of the tau or ppm scales will result in all shifts being assigned to zero Hz. The coupling constants, of course, are always entered in Hz.

Following the entry of all shifts and coupling constants, the program will type:

```
SET HORIZ DISPLAY SCALE TO 4K
SET VERT DISPLAY SCALE TO 1K (READJUST AS NEEDED).
```

On 1080 data systems these operations should be performed before proceeding. On BNC-12 and NMR-80 systems, simply set the Size buttons to 4K and the vertical display scale to 1K. The program will print a > sign and is then ready to begin calculation of the spectrum. Any of the commands described below can then be entered.

## II. DESCRIPTION OF BASIC COMMANDS

Each command is one character in length and is executed when a Return is typed. The program is ready for a new command when a new > is printed. The commands may be entered in a string and are executed by typing one return after the entire string. Each command letter is printed and the command is then executed. When all commands are completed a new > is printed. If an illegal command is typed, such as X, the program will print

X IS ILLEGAL

and, if a command string has been entered, go on to the next command. For example, if the string VVVXJ is entered, followed by a Return, the program will allow entry of three V chemical shifts, type X IS ILLEGAL, and allow entry of one coupling constant. A number of commands can be stopped in midstream by typing Q. This will always produce the question

ABORT QUEUE?

If Y is answered, the rest of the current command string is also aborted. If N is answered, the rest of the string is completed. If no string exists at the time the answer is immaterial.

### ITRCAL Commands

The following commands are legal in ITRCAL:

| | |
|---|---|
| A | Assign previously unassigned transitions |
| B | Begin iteration sequence again |
| C | Change a transition assignment |
| E | Print energy levels during next calculation |
| G | Go - perform a non-iterated calculation |
| I | Iterate |
| J | Change a J-value |
| K | Print out the current shifts and coupling constants |
| L | Lorentzian line shape calculation |
| ~~M~~ | Monitor call - jumps to disk monitor |
| ~~N~~ | Enter Nicobug II |
| O | Change Offset |
| Q | Quit current routine and return to command decoder |
| R | Restart program at beginning - clear all arrays |
| T | List Transitions on Teletype |
| V | Change chemical shift |
| W | Change sweep width |
| Z | Zero all transition assignments |

The commands D, F, H, P, S, U, X and Y are illegal

### G - Go Calculate a Spectrum

The G command takes the currently entered values of V and J, and calculates the stick spectrum. The display is active, showing the lines produced at the end of each calculated matrix, so that one can actually watch the spectrum grow as it is calculated. As calculation proceeds, the numbers of the individual matrices are printed, giving the user a feeling for how far along the calculation has proceeded. For N spins, there are always N + 1 matrices, so for example a 3 spin case would print out

```
G 0 1 2 3
```

If a degeneracy is encountered, the degenerate energy levels are printed out during the calculation.

### L - Calculate a Lorentzian line shape for the stick spectrum

After L is typed, the program will print

```
LINE WIDTH =
```

and allow entry of the line width of Hz. If a 0 is entered, the stick spectrum will be displayed. If any positive non-zero number is selected, the program will begin calculating the line shape, displaying the result as each line in the spectrum is calculated.

### J - Change a coupling constant

The J command causes the printing of J followed by a left parenthesis. The program awaits entry of a number designating the actual nuclei between which coupling occurs. The nucleus numbers must be entered in ascending order. The program will not permit illegal numbers to print when they are typed. For example, to change $J_{2,3}$ you would type only the J, the 2 and the 3. The punctuation is added by the program producing J(2,3)=. Any number can then be entered.

### V - Change a chemical shift

The V command causes the printing of a V(. The user enters the number of the nucleus whose shift he wishes to change, followed by the actual value of the shift. Thus, in entering V(3)=118.2, the user types V, 3 and 118.3 and the program types (, and )=. As before, shifts can be entered in tau or ppm units by terminating the entry with a T or P.

### O - Change display offset, W - change the display width

The offset and width of the display can be changed using these commands; if an entire recalculation is performed following this change, the complete spectrum of any region may be displayed in this way. However, the offset and width change the display immediately after the entry of these commands, and the new display will be constructed from the stored transitions. Since only 300 transitions are stored, the display may not appear exactly correct in the case of 5, 6 or 7 spin systems. The offset and width cannot be changed unless ITRCAL has performed a calculation (G or I) since it was loaded.

### E - Print energy levels

This command turns a "switch" on and off controlling the printing of energy levels during a calculation. When E is typed the first time it turns this switch on, and when it is typed again, it turns it off. When the E switch is on, the energy levels are printed out as calculated as the eigenvalues of each set of matrices. These values are not saved between matrices so they are not sorted by increasing value.

### R - Restart program at beginning

This command causes a complete restart of the program. All shift and transition arrays are cleared. It is entirely equivalent to pushing Stop and then starting at address 0.

### M - Monitor call

M calls the disk monitor by jumping to 7600.

### N - Call Nicobug II

N causes a jump to Nicobug II, at 114700. This program is contained in the ITRCAL tape and is always present for your use. It should only be required for actual user modifications of ITRCAL.

### T - List Transitions on Teletype

This command will cause the actual listing of all of the saved transitions and their intensities. Transitions above the selected threshold will have been saved. Only 300 transitions can be stored or listed. The listing can be stopped by typing Q.

### Q - Quit current routine

The Q command will stop the transition listing and will also allow exit from the iteration and transition assignment modes.

## III. COMMANDS USED IN ITERATION

### I - Iterate

The Iteration command assumes that a calculation is to be made using guessed, chemical shifts and coupling constants, followed by an assignment of actual observed frequencies for least squares iteration. Following program startup, the V and J values are entered. Instead of actually calculating the spectrum by typing G, typing I will allow spectrum calculation as well as iteration.

Immediately after the I command is entered, the questions

```
DESIRED VALUE OF RMS ERROR =
MAXIMUM # OF ITERATIONS =
```

are printed. Answer the rms error question with some small number, such as 0.1. This number can be varied empirically after some experience is gained with a particular case. If no value is entered, the last entered value is assumed. The ITRCAL iteration technique converges very strongly if it converges at all, and probably no more than 5 to 10 iterations will ever be needed.

When the above two questions are answered, the messages

```
ENTER VARIABLES
SET 1:
SET 2:
etc.
```

are printed. These variable sets are those groups of V's and J's which are to be varied. If no sets are entered, no iteration is performed since no parameters are to be changed. In the case of symmetrical molecules, several shifts or coupling constants might be varied as a group so that they will not become non-equivalent by iteration. In this case, all those which are to be varied together are entered on the same line. It is not possible to enter both V's and J's as part of the same set. They must be entered as separate groupings.

For example, in iterating an $A_2X$ system, the parameters to be varied are V(1), V(2), V(3), J(1,2), J(1,3) and J(2,3). If all of these are to be varied separately, they are entered on separate lines as different variable sets. If, however, nuclei 1 and 2 are actually both A nuclei, they should be varied together. Furthermore, J(1,3) and J(2,3) would then also be varied together. It should also be noted that in this case, J(1,2) would probably be zero and should not be varied at all.

To accomplish this, the following set assignments are made:

```
ENTER VARIABLES
SET 1:  V(1) V(2)
SET 2:  J(1,2) J(2,3)
SET 3:  V(3)
SET 4:
```

Terminate each entry with a Return. To terminate the list of sets, type Return without entering any value for the last set. The program will ask

```
ARE YOU SURE?
```

This gives you a chance to inspect the list for omissions, redundancies and incorrect groupings. If none are found, type Y followed by a Return. If some are found, typing any other character will allow you to enter a new list. Once you are sure, the program checks for redundancies, such as having the same parameter as a member of two groups. Should it find such an error, it will type a question mark, ring the Teletype bell and print

```
**INCONSISTENCY!
```

This message has been generally found to be emphatic enough to catch the user's notice.

Once a consistent set of variables has been entered, the iteration process will begin. Iteration 0 is simply a calculation of the spectrum based on the guessed J's and V's. The program prints

```
ITERATION 0:  0  1  2  3
```

for the matrices involved in the calculation. It then begins the process of transition assignment. This is done by printing out the calculated transition and intensity and allowing entry of the observed value. The observed values are those actually measured in your nmr spectrum either by peak printout or by ruler measurement. A typical line assignment is shown below:

```
7 8     8.671     0.656  =
```

The numbers are the energy level numbers between which the transition occurs, the calculated frequency and its intensity. The observed value is entered following the equals sign. It is not necessary to assign all the lines in a spectrum. In fact, it is generally more desirable to assign the strong well-defined lines and omit the less intense ones. It is desirable to use intensities as a guide in line assignment, since the actual frequencies may be out of order because of degeneracies. Since the least squares analysis is usually performed on an overdetermined system, only some lines need be assigned.

The assignment questions can thus be answered not only with a line frequency but also with a Return, meaning do not assign this line, or with a " mark, meaning assign this line the same frequency as the line above.

w

When all frequencies have been assigned, the iteration will begin. For each iteration, the program will print out the iteration number and display the number of lines calculated after each matrix is complete. Iteration will halt when (a) the rms error is less than the desired value, (b) when the change in rms error between iteration is less than 3%, or (c) when the number of iterations is exhausted. Iteration can be halted at any time using the Q command. When iteration stops, the program will print out the rms error and the calculated chemical shifts and coupling constants.

### T - Type out the transitions and intensities

In the iterated case, when the T command is given, the final iterated transitions will be printed out in ascending order, with their assigned frequencies printed in the last column for examination.

### A - Assign previously unassigned transitions

If rough convergence is not sufficient, it can sometimes be improved by assigning the weaker transitions and continuing with the iteration. This command will print out the value of all unassigned transitions and allow assignments. The I command will then allow additional iteration.

### K - Print out current chemical shifts and coupling constants

This command simply lists the current V's and J's for inspection. It indicates what values the program will iterate from and how far from the original guessed values they have been changed.

### C - Change a transition assignment

The C command allows changing of any given transition assignment. This can be done by typing C followed by a Return. The program will await entry of the energy level numbers of the transition whose assignment is to be changed. Each level should be followed by a space. When two level numbers have been entered, the program types an equals sign and awaits entry of the new value. Typing Q will allow exit without entering any value if the wrong transition was inadvertently chosen.

### B - Begin iteration sequence again

Preceeding the I command with a B command allows the program to start at the beginning of the iteration sequence. It zeroes the iteration counter, allows entry of a new rms error and iteration count and new parameter sets.

### Z - Zero all transition assignments

This command allows the zeroing of all transition assignments. Thus, ZA strung together would allow a complete new entry of all observed frequencies.

## Plotting

Because of the extreme core limitations imposed by writing the ITRCAL program to perform in a 12K minicomputer, no plotting routine is provided. However, both the stick and Lorentzian spectra can be plotted using routines in the FT-Nmr and T1PRGM packages. The Lorentzian spectrum is stored in the first 1K of data memory and the stick spectrum in the seventh 1K of data memory. Plotting of the stick spectrum can best be accomplished using the interpolating plot routines. It will produce extremely narrow lines.

In going to and from the plot routines, it is sometimes desirable to have ITRCAL remember its current values. To do this, store ITRCAL upon re-entry to the monitor as follows:

```
>M
*STORE ITRCL1  0-7577: P
 REPLACE?  Y
```

(Set Display to Starting = 0 and Size = 8K)

```
*STORE ITRCL2: B
 REPLACE? Y
*LOAD T1PRGM
*GO
& PL
```

## Error Modes

If ITRCAL finds itself performing an "impossible" calculation, such as division by zero, infinite looping, or inversion of a singular matrix, it may exit from the program to Nicobug II, which is stored at 114700. It will print out

```
ERR @ nnnn
```

where nnnn indicates the address of the calculation inconsistency. ITRCAL will not "self-destruct," however and can be restarted from Nicobug by typing G.

## IV. EXAMPLES OF USES OF ITRCAL

### A. An ABX System

Let us consider a simple example of a spectrum for iteration. We will analyze the simple ABX system shown in Figure 1. This spectrum produces the FT-Nmr peak printout which is shown below, and we can, from this information, deduce sufficient information for some guessed parameters to enter in ITRCAL. We can also use this frequency information to enter as the observed transition values during iteration.

```
.PP
    136        78.1425        0.8682   PPM      284
    214        71.2864        0.7920   PPM      480
    228        70.0558        0.7783   PPM      654
    306        63.1996        0.7022   PPM      828
    553        41.4885        0.4609   PPM      444
    611        36.3903        0.4043   PPM     1000
    645        33.4017        0.3711   PPM      236
    704        28.2157        0.3135   PPM      468
    793        20.3926        0.2265   PPM      846
    851        15.2944        0.1699   PPM      420
    870        13.6244        0.1513   PPM      596
    928         8.5262        0.0947   PPM      266
```

It is always necessary to have some sort of external information about the compound in order to perform such an iteration. For instance, coupling constants and chemical shifts of simpler, model compounds are always of a great deal of value in deconvolving a closely coupled spectrum. In this case, however, we will assume that we have little or no such information other than that shown in the peak printout.

Actually, of course, one can analyze an ABX system by a series of complex mathematical manipulations, such as are described by Pople[5] and Becker[6]. However, with ITRCAL, we will discover that most of this complexity becomes unnecessary, as the simplest guesses will be sufficient for analysis.

We will first extract the information that comes easily from ABX spectra. The values $\nu_X$ and $J_{AB}$ are easily obtained. $\nu_X$ is simply the center of the X multiplet, and can be found by adding half the line separation to the lower frequency lines. Thus,

$$\nu_X = (78.1425 - 63.1996)/2 + 63.1996 = 70.67$$

or

$$\nu_X = (71.2864 - 70.0558)/2 + 70.0558 = 70.67$$

```
.PP
    136     78.1425      0.8682  PPM     284
    214     71.2864      0.7920  PPM     480
    228     70.0558      0.7783  PPM     654
    306     63.1996      0.7022  PPM     828
    553     41.4885      0.4609  PPM     444
    611     36.3903      0.4043  PPM    1000
    645     33.4017      0.3711  PPM     236
    704     28.2157      0.3135  PPM     468
    793     20.3926      0.2265  PPM     846
    851     15.2944      0.1699  PPM     420
    870     13.6244      0.1513  PPM     596
    928      8.5262      0.0947  PPM     266
```

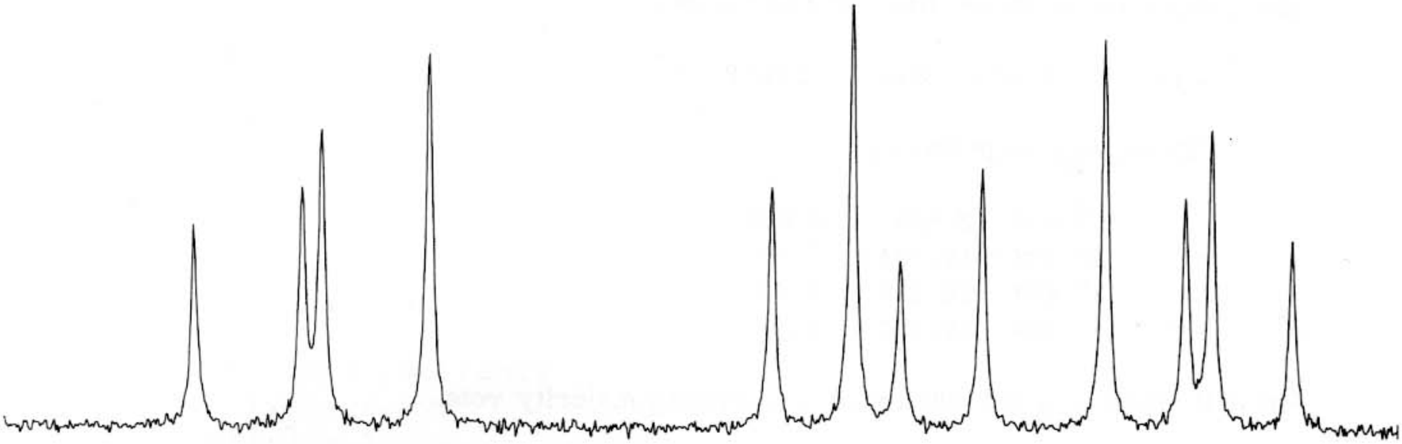


Figure 1. Experimental ABX spectrum

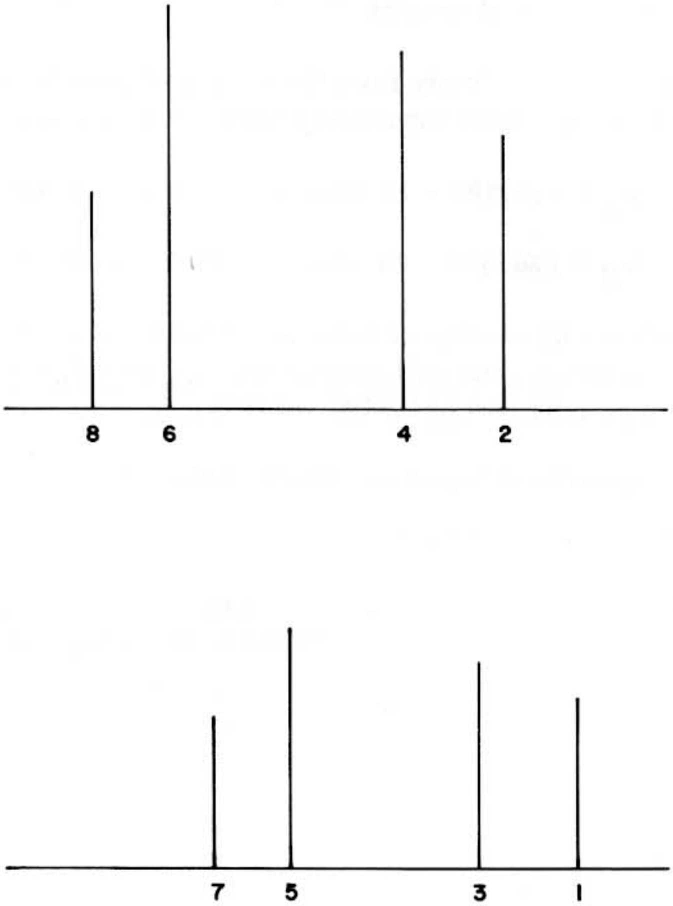


Figure 2. The two quartets in the AB part of the ABX

The AB coupling constant JAB is almost as easy to obtain. It is found by dividing the AB part of the ABX spectrum into two symmetrical quartets. This can usually be done by inspection. The ones of one quartet are labelled 1, 3, 5 and 7 and of the other quartet 2, 4, 6 and 8. Note that these lines may or may not be exactly alternate. This is illustrated in Figure 2. It turns out that $J_{AB}$ is given by the differences between lines in the quartets, or

$J_{AB}$ = 3 - 1 or 4 - 2 or 7 - 5 or 8 - 6.

Thus, $J_{AB}$ is given by

$13.624 - 8.526 = 5.098$
or $20.392 - 15.294 = 5.098$
or $33.401 - 28.215 = 5.186$
or $41.488 - 36.390 = 5.098$

We will adopt $J_{AB} = 5.098$ by overwhelming majority vote.

Now the remaining parameters, $J_{AX}$, $J_{BX}$, $\nu_A$ and $\nu_B$ are considerably harder to extract. Instead of plowing through the tedious equations necessary to find them, we will simply make some semi-naive, semi-educated guesses and try to iterate towards a spectrum of similar character.

As a first guess, let us simply take the center of gravity of the two groups of four lines (not of the two quartets mentioned earlier) as $\nu_A$ and $\nu_B$.

We will simply call $\nu_A = (15.294 - 13.624)/2 + 13.624 = 14.459$

$\nu_B = (36.390 - 33.401)/2 + 33.401 = 34.896$

These will at least allow calculation of some spectra to see if we can come close. As our second naive assumption, we will simply set $J_{AX}$ and $J_{BX}$ to 5 Hz, too. Then, let us calculate the spectrum and see what we find.

The actual parameters entered are shown below.

```
~~*LOAD ITRCL1~~

~~*LOAD ITRCL2~~

~~*GO~~
```

Start

```
ITRCAL   V1B   04/73

NUMBER OF SPINS= 3
SPECTROMETER FREQ (MHZ)=
WIDTH OF DISPLAY (HZ)= 90
OFFSET OF DISPLAY (HZ)=
INTENSITY THRESHOLD= 0

CHEMICAL SHIFTS
V(1)= 14.459
V(2)= 34.896
V(3)= 70.67

COUPLING CONSTANTS
J(1,2)= 5.098
J(1,3)= 5
J(2,3)= 5

SET HORIZ DISPLAY SCALE TO 4K.
SET VERT DISPLAY SCALE TO 1K (READJUST AS NEEDED).

>GL
G  0  1  2  3
LINE WIDTH= .5
>M
M
*LOAD T1PRGM

*GO

&PL
```

The actual calculated spectrum is shown in Figure 3 and obviously bears little resemblance to our experimental one. It is, in fact, one of the "deceptively simple" ABX spectra which the literature always warns us about.

For our next least naive assumption, we will simply assume that we can approximate $J_{AX}$ and $J_{BX}$ from the spacing of the quartet. Thus, we will call both $J_{AX}$ and $J_{BX}$ about 7 Hz. There is no need to supply further significant figures, since these are just guesses. The parameters entered below produce the spectrum shown in Figure 4.

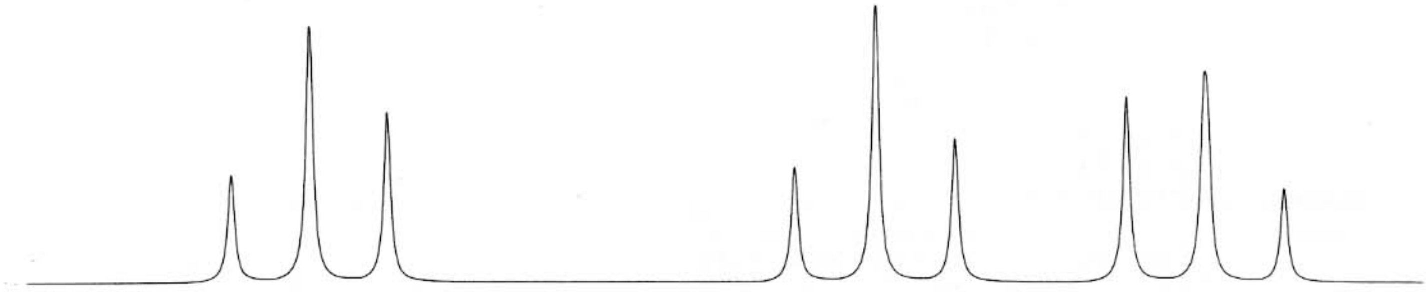


Figure 3. Deceptively simple ABX produced by injudicious choice of parameters.

```
ITRCAL    V1B    04/73

NUMBER OF SPINS= 3
SPECTROMETER FREQ (MHZ)=
WIDTH OF DISPLAY (HZ)= 90
OFFSET OF DISPLAY (HZ)=
INTENSITY THRESHOLD= 0

CHEMICAL SHIFTS
V(1)= 14.459
V(2)= 34.896
V(3)= 70.67

COUPLING CONSTANTS
J(1,2)= 5.098
J(1,3)= 7
J(2,3)= 7

SET HORIZ DISPLAY SCALE TO 4K.
SET VERT DISPLAY SCALE TO 1K (READJUST AS NEEDED).

>GL
G  0  1  2  3
LINE WIDTH= .5
>M
M
*LOAD T1PRGM

*GO

&PL
&
```

This last spectrum looks pretty close to the experimental one. In fact, the line shift differences are only minimal and their ordering about the same. We need only go into iteration mode to calculate the best possible spectrum fit. We should emphasize again here, that the assignment of lines is not necessarily a one-to-one procedure. The guessed spectrum may look similar but have some of the lines out of order. In this case, it is important to go by relative intensities rather than by strict frequency order.

Let us now proceed to iteration. The actual program output is shown on the facing page. The guessed values for the chemical shifts and coupling constants are entered and the Iterate command is given. Note that one can start at the iterate command if a theoretical spectrum has been calculated with the parameters which are to be the guessed ones for iteration. An rms error of .01 is selected along with a maximum iteration count of 10. The variables are entered as six separate groups and confirmed by answering Y to ARE YOU SURE?.

The first calculation proceeds and lines are assigned to all theoretical transitions. Iteration continues with an rms error which decreases during the first three iterations and which stabilizes at .0254 after three iterations. Since there is no change after this many iterations, the program stops and lists the chemical shifts and coupling constants that have been calculated. A line width of 0.5 Hz is added and the calculated vs. observed transitions are printed out. Finally, the Monitor is called and the resulting spectrum plotted out using the PL routine of T1PRGM. The calculated spectrum is shown in Figure 5. The iteration has clearly converged to a meaningful solution, since the spectra are virtually superimposable. The actual spectrum used was generated by adding noise to a theoretical spectrum having the values

V(1) = 15
V(2) = 35
V(3) = 70
J(1,2) = 5
J(1,3) = 7
J(2,3) = 8

so the calculated values clearly agree quite well.

It should be noted that the signs of coupling constants in an ABX are not determined and that the same spectrum could be produced using some negative coupling constants. The actual values used must be selected with careful attention to chemical criteria.

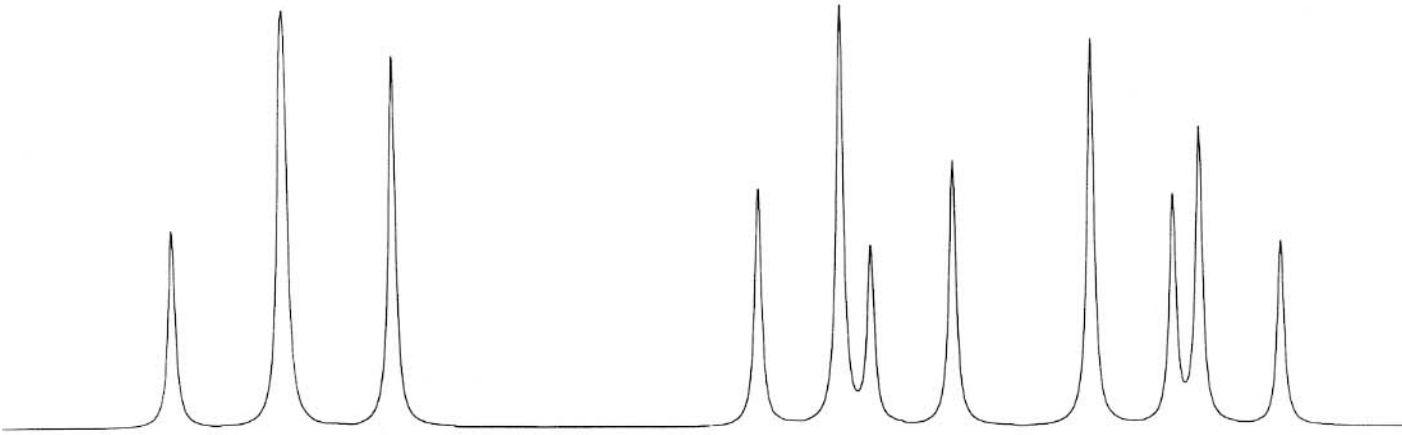


Figure 4. Spectrum produced by close guesses for analysis of ABX case.

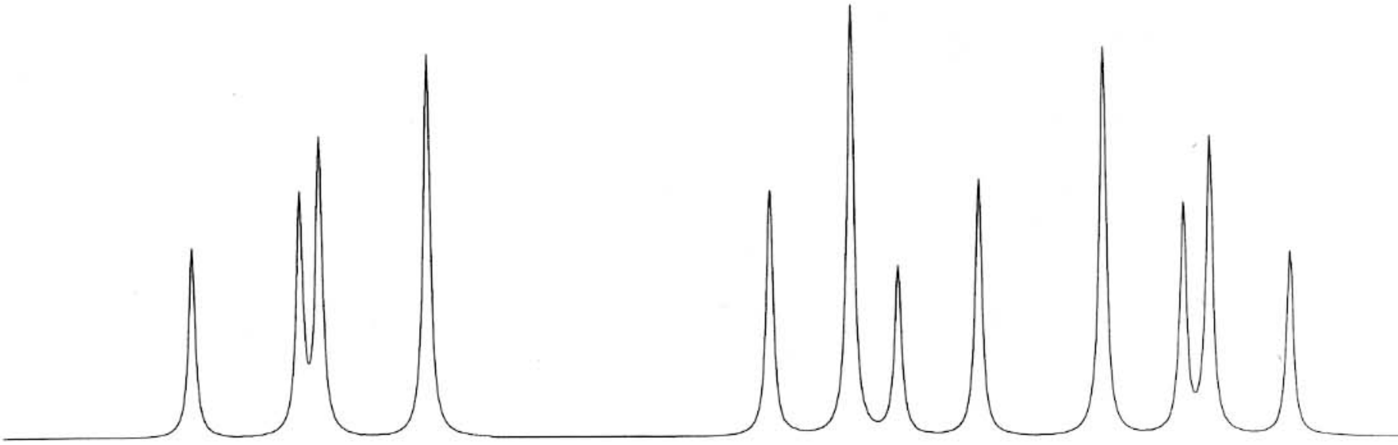


Figure 5. Iterated result of ABX spectrum.

```
ITRCAL    V1B    04/73

NUMBER OF SPINS= 2\3
SPECTROMETER FREQ (MHZ)=
WIDTH OF DISPLAY (HZ)= 90
OFFSET OF DISPLAY (HZ)=
INTENSITY THRESHOLD= 0

CHEMICAL SHIFTS
V(1)= 14.459
V(2)= 34.896
V(3)= 70.67

COUPLING CONSTANTS
J(1,2)= 5.098
J(1,3)= 7
J(2,3)= 7

SET HORIZ DISPLAY SCALE TO 4K.
SET VERT DISPLAY SCALE TO 1K (READJUST AS NEEDED).

>I
I
DESIRED VALUE OF RMS ERROR= .01
MAXIMUM # OF ITERATIONS= 10

ENTER VARIABLES

SET  1: V(1)
SET  2: V(2)
SET  3: V(3)
SET  4: J(1,2)
SET  5: J(1,3)
SET  6: J(2,3)
SET  7:
ARE YOU SURE? Y

ITERATION  0:    0  1  2  3
  7  8      7.928     0.681  =8.526
  4  6     13.110     1.077  =13.624
  3  5     14.746     0.828  =15.294
  1  2     19.929     1.411  =20.392
  6  8     28.730     0.968  =28.215
  4  7     33.913     0.641  =33.401
  2  5     35.826     1.522  =36.390
  1  3     41.009     0.868  =41.488
  5  8     64.268     1.350  =63.199
  3  7     71.087     1.039  =70.055
  2  6     71.365     0.889  =71.286
  1  4     78.183     0.719  =78.142
ITERATION  0:    0  1  2  3
RMS ERROR =  6.07254E-1
ITERATION  1:    0  1  2  3
RMS ERROR =  2.64898E-2
ITERATION  2:    0  1  2  3
RMS ERROR =  2.54033E-2
ITERATION  3:    0  1  2  3
RMS ERROR =  2.54033E-2

CHEMICAL SHIFTS
V(1)=     14.990
V(2)=     35.017
V(3)=     69.994

COUPLING CONSTANTS
J(1,2)=     5.0128
J(1,3)=     7.0146
J(2,3)=     8.0134
>L
LINE WIDTH= .5
 >T
 T
   7  8      8.492      0.677      8.526
   4  6     13.613      1.076     13.624
   3  5     15.305      0.829     15.294
   1  2     20.425      1.415     20.392
   6  8     28.258      0.931     28.215
   4  7     33.379      0.614     33.401
   2  5     36.368      1.560     36.390
   1  3     41.488      0.893     41.488
   5  8     63.210      1.390     63.199
   3  7     70.022      1.062     70.055
   2  6     71.319      0.854     71.286
   1  4     78.131      0.691     78.142
 >M
 M
 *LOAD T1PRGM

 *GO

 &PL
```

### B. An $A_2B_2$ system – ortho-dichlorobenzene

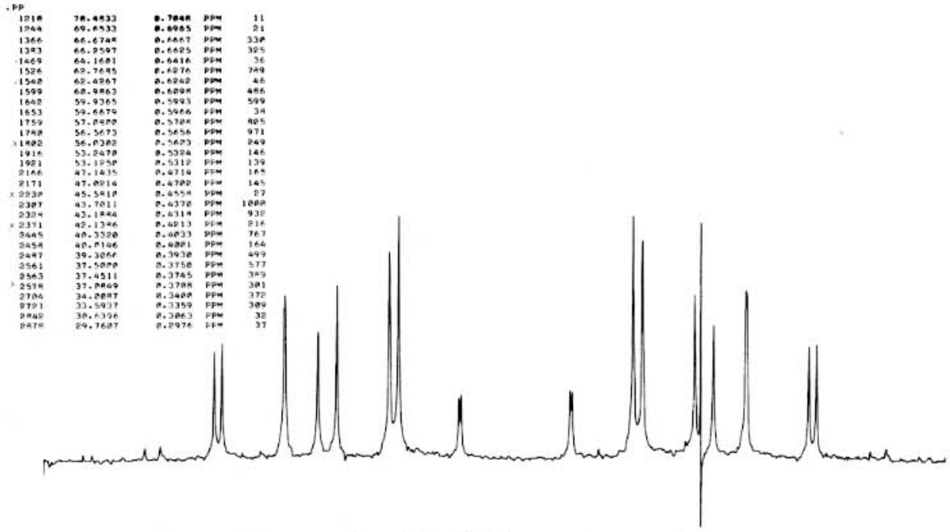
As a second example, let us consider an experimental spectrum of ortho-dichlorobenzene obtained in acetone-$d_6$ solvent. The chemical shifts and coupling constants for this species will vary somewhat from those reported[8] for neat ODCB. The spectrum shown in Figure 6 was obtained from a single pulse followed by an accumulation of 8192 points over 40.96 seconds.[9] The region plotted out is the center 50 Hz of this region. Peak printout is as shown, with x's indicating spurious noise peaks. The line which is out of phase is the folded back TMS line.

To analyze this spectrum, the reported shifts were converted to 100 MHz and entered as the guessed values. The spectrum which was produced is shown in Figure 7. Note the degeneracy messages printed during calculation which warn the user to be careful in making line assignments.

To begin iteration, the I command is given as shown in the printout on the following pages. An rms error of .01 is entered along with a maximum of ten iterations. The variables sets are then etered. Note that in this case V(1) and V(4) are equivalent as are V(2) and V(3), J(1,2) and J(3,4) and J(1,3) and J(2,4). This statement of equivalence is necessary so that these parameters will not become nonequivalent. The variables J(1,4) and J(2,3) are entered on separate lines to indicate that they are to be varied but not together. Set 7 is terminated with a Return, causing an end to the parameter set entry. The set segments are confirmed by answering Y to ARE YOU SURE?.

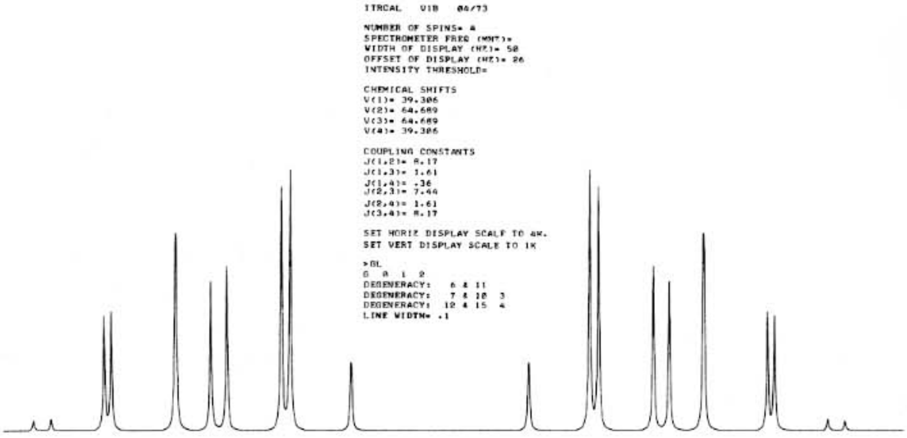
The first iteration is merely a calculation based on the guessed parameters. It is accompanied by the warnings regarding degeneracy. Then the line assignments are made based on the peak printout shown in Figure 6. Note the use of the ditto mark to indicate the assignment should be the same as the previously entered one. Iteration begins automatically following the entry of the ditto mark transitions 5-7. Convergence is achieved in three iterations when an rms error of .1010 is obtained. Iteration ceases here because the difference in rms error is less than 3% from one iteration to the next.

The iterated shifts and coupling constants are shown along with the calculated and observed transition frequencies.



```
.PP
 1210    70.4833    0.7048  PPM    11
 1244    69.6533    0.6965  PPM    21
 1366    66.6748    0.6667  PPM   330
 1393    66.2597    0.6625  PPM   325
 1469    64.1601    0.6416  PPM    36
 1526    62.7685    0.6276  PPM   789
 1540    62.4267    0.6242  PPM    46
 1599    60.9863    0.6098  PPM   486
 1642    59.9365    0.5993  PPM   599
 1653    59.6679    0.5966  PPM    38
 1759    57.0800    0.5708  PPM   805
 1780    56.5673    0.5656  PPM   971
 1802    56.0302    0.5603  PPM   249
 1916    53.2470    0.5324  PPM   146
 1921    53.1250    0.5312  PPM   139
 2166    47.1435    0.4714  PPM   165
 2171    47.0214    0.4702  PPM   145
 2230    45.5810    0.4558  PPM    27
 2307    43.7011    0.4370  PPM  1000
 2324    43.1884    0.4318  PPM   932
 2371    42.1386    0.4213  PPM   216
 2445    40.3320    0.4033  PPM   767
 2458    40.0146    0.4001  PPM   164
 2487    39.3066    0.3930  PPM   499
 2561    37.5000    0.3750  PPM   577
 2563    37.4511    0.3745  PPM   389
 2578    37.0849    0.3708  PPM   301
 2704    34.0087    0.3400  PPM   372
 2721    33.5937    0.3359  PPM   309
 2842    30.6396    0.3063  PPM    32
 2878    29.7607    0.2976  PPM    37
```

Figure 6. ODCB in acetone-$d_6$



```
ITRCAL   V1B   04/73

NUMBER OF SPINS= 4
SPECTROMETER FREQ (MHZ)=
WIDTH OF DISPLAY (HZ)= 50
OFFSET OF DISPLAY (HZ)= 26
INTENSITY THRESHOLD=

CHEMICAL SHIFTS
V(1)= 39.306
V(2)= 64.689
V(3)= 64.689
V(4)= 39.306

COUPLING CONSTANTS
J(1,2)= 8.17
J(1,3)= 1.61
J(1,4)= .36
J(2,3)= 7.44
J(2,4)= 1.61
J(3,4)= 8.17

SET HORIZ DISPLAY SCALE TO 4K.
SET VERT DISPLAY SCALE TO 1K

>BL
G  0  1  2
DEGENERACY:    6 8 11
DEGENERACY:    7 8 10  3
DEGENERACY:   12 4 15  4
LINE WIDTH= .1
```

Figure 7. Uniterated ODCB spectrum

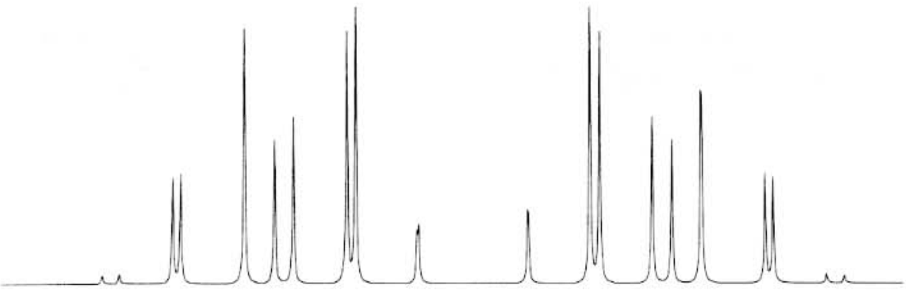


Figure 8. Iterated ODCB spectrum

```
ITRCAL   V1B   04/73

NUMBER OF SPINS= 4
SPECTROMETER FREQ (MHZ)=
WIDTH OF DISPLAY (HZ)= 50
OFFSET OF DISPLAY (HZ)= 26
INTENSITY THRESHOLD=

CHEMICAL SHIFTS
V(1)= 39.306
V(2)= 64.689
V(3)= 64.689
V(4)= 39.306

COUPLING CONSTANTS
J(1,2)= 8.17
J(1,3)= 1.61
J(1,4)= .36
J(2,3)= 7.44
J(2,4)= 1.61
J(3,4)= 8.17

SET HORIZ DISPLAY SCALE TO 4K.
SET VERT DISPLAY SCALE TO 1K (READJUST AS NEEDED).

>I
I
DESIRED VALUE OF RMS ERROR= .01
MAXIMUM # OF ITERATIONS= 10

ENTER VARIABLES

SET  1: V(1) V(4)
SET  2: V(2) V(3)
SET  3: J(1,2) J(3,4)
SET  4: J(1,3) J(2,4)
SET  5: J(1,4)
SET  6: J(2,3)
SET  7:
ARE YOU SURE? Y

ITERATION  0:    0  1  2
DEGENERACY:    6 & 11
DEGENERACY:    7 & 10  3
DEGENERACY:   12 & 15  4

   7 14      29.671      0.060   =29.7607
  10 14      29.671      0.064   ="
   4  6      30.611      0.146   =30.6936
  12 16      33.506      1.280   =33.5937
   9 12      33.924      1.325   =34.009
   3 11      37.422      1.485   =37.4511
   7 13      37.450      0.756   =37.5
  10 13      37.450      0.704   ="
  11 14      39.370      1.680   =39.3066
   4 10      40.263      0.893   =40.0146
   4  7      40.263      0.959   =40.3320
   1  5      43.286      2.719   =43.1884
   5  8      43.754      2.913   =43.7011
   6 13      47.101      0.538   =47.0214
   3 10      47.121      0.243   =47.1425
   3  7      47.121      0.226   ="
   7 12      56.873      0.226   =53.1250
  10 12      56.873      0.243   ="
   7 15      56.893      0.278   =53.2470
  10 15      56.893      0.259   ="
   8 14      60.240      2.913   =56.5673
  14 16      60.708      2.719   =57.0800
   2  6      63.731      1.853   =59.9363
   5 11      64.624      1.680   =60.9862
   6 15      66.545      1.461   =62.4267
  11 12      66.572      1.485   ="
   3  9      70.070      1.325   =66.2597
   1  3      70.488      1.280   =66.6748
   2 10      73.382      0.070   =69.6533
   2  7      73.382      0.075   ="
   5 10      74.323      0.064   =70.4833
   5  7      74.323      0.060   ="
```

```
ITERATION  0:     0  1  2
DEGENERACY:    6 & 11
DEGENERACY:    7 & 10  3
DEGENERACY:   12 & 15  4
RMS ERROR =   1.21419E1
ITERATION  1:     0  1  2
DEGENERACY:    6 & 11
DEGENERACY:    7 & 10  3
DEGENERACY:   12 & 15  4
RMS ERROR =   1.02095E-1
ITERATION  2:     0  1  2
DEGENERACY:    6 & 11
DEGENERACY:    7 & 10  3
DEGENERACY:   12 & 15  4
RMS ERROR =   1.01065E-1

CHEMICAL SHIFTS
V(1)=      39.397
V(2)=      60.821
V(3)=      60.821
V(4)=      39.397

COUPLING CONSTANTS
J(1,2)=     7.9426
J(1,3)=     1.6150
J(1,4)=     0.3581
J(2,3)=     7.5361
J(2,4)=     1.6150
J(3,4)=     7.9426
>T
T
   7 14      29.647      0.000      29.760
  10 14      29.729      0.107      29.760
   4  6      30.677      0.124      30.693
  12 16      33.594      0.592      33.593
   9 12      34.061      0.616      34.009
   7 13      37.530      1.416      37.500
   3 11      37.564      1.455      37.451
  10 13      37.612      0.000      37.500
  11 14      39.194      1.620      39.306
   4 10      40.164      0.000      40.014
   4  7      40.246      1.875      40.332
   1  5      43.158      2.814      43.188
   5  8      43.672      3.087      43.701
   3 10      47.028      0.495      47.142
   6 13      47.099      0.583      47.021
   3  7      47.111      0.000      47.142
   7 15      53.113      0.292      53.247
   7 12      53.113      0.290      53.125
  10 15      53.195      0.247      53.247
  10 12      53.195      0.247      53.125
   8 14      56.545      3.087      56.567
  14 16      57.060      2.814      57.080
   2  6      59.971      1.875      59.936
   5 11      61.024      1.620      60.986
  11 12      62.659      0.731      62.426
   6 15      62.681      0.711      62.426
   3  9      66.162      1.233      66.259
   1  3      66.617      1.185      66.674
   2 10      69.458      0.000      69.653
   2  7      69.540      0.124      69.653
   5 10      70.488      0.107      70.483
   5  7      70.570      0.000      70.483
>L
LINE WIDTH= .1
>
```

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9. This spectrum was provided by L. F. Johnson of Transform Technology, Inc.

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