MEXICO
THE McMASTER PROGRAM FOR EXCHANGE LINESHAPE CALCULATIONS

VERSION 3

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INTRODUCTION

The effects of chemical exchange on NMR spectra are familiar.[1,2] As the rate of exchange increases, the lines broaden, coalesce and sharpen into a single average peak.

For two equally-populated sites without coupling, the calculation of the lineshape can be done with a modified set of Bloch equations.[3,4] The equations are quite simple, and are covered in all the standard texts. However, for more general systems, the calculations can become quite complex. If the spectral parameters are different in the two sites of a coupled system, a single transition in one site may map into parts of several transitions in the other site. A full density matrix calculation may be required.[5]

To analyse experimental data on exchanging systems, we need to implement the theory in a computer program, which simulates the exchange lineshape. Several groups have done this, but the most popular is the durable classic DNMR3/DNMR5.[6,7] Recently, we have reformulated the theory of chemical exchange,[8] and the MEXICO suite of programs represents this new approach, implemented with modern computing techniques.

There are different types of problems when dealing with exchanging systems. One is whether the spin system is coupled or not. The approach is the same, but the uncoupled simulation is much simpler, since there is a one-to-one correspondence between the sites and the lines in the spectrum. What is the output – a text file which is portable, or something designed to be directly compared to experimental data. Once the simulation has been done, we usually want to vary the rates and other parameters to obtain a good fit to experimental data. Therefore, an iterative program that varies the parameters automatically, to get a best fit, is also needed. The MEXICO suite of programs offers both iterative and non-iterative versions of both the coupled and uncoupled calculation.

WHAT'S NEW IN VERSION 3

This version offers iterative and non-iterative versions of both the coupled and uncoupled simulations. Executables are available for a number of platforms, operating systems and data formats. If the data are in Bruker's XWINNMR format, there are versions that run under Linux on a PC (RedHat 7.2), Windows NT on a PC, and IRIX 6.5 on an SGI. If the experimental data

are available in a text (ASCII) format, then the program will compile and run on almost any platform with a C compiler. Executables are distributed for SGI, Sun, Linux and Windows. A version that accepts Varian VNMR data is close to release. Source code for the program is also available for the enthusiast.

The format of the input (`.mch`) file has been modified slightly from previous versions. Old files will have to be updated to the new format.

The mechanics of the simplex iteration have been improved, so that it is more forgiving of errors in the offset and scaling.

For each operating system, there are four executable programs: `mexicnon, mexicnonc` and `mexiter, mexiterc.` Remember that the Windows programs will have an `.exe` extension. These programs are, respectively, the uncoupled and coupled versions of the non-iterative program, and the uncoupled and coupled versions of the iterative program.

THEORY

Static NMR spectra are made up of many individual transitions. These transitions appear at different frequencies, and each has an intensity governed by a transition probability. The reformulated theory[8,9] of chemical exchange shows that this is true for dynamic spectra as well. The difference is that in a dynamic spectrum, both the frequency and the transition probability are *complex numbers*, with real and imaginary parts. The imaginary and real parts of the frequency give the position and the width of the line, and the real and imaginary parts of the transition probability give the intensity and the phase. Even if it is not obvious, any dynamic spectrum is simply a sum of transitions.

The transition probability (in general) also has a simple, physical interpretation.[8] The intensity of a transition is the product of how much coherence it received from the total magnetization at the start, times how much that transition contributes to the detected signal. For a static spectrum, these two numbers are complex conjugates and we retrieve the normal expression for the transition probability. For a dynamic spectrum these two quantities are no longer complex conjugates, so we get the richness and the complications of dynamic NMR.

IMPLEMENTATION

The problem with the computer calculation is doing the input and output. The calculation of the transitions and their probabilities is a large eigenvalue problem in Liouville space, but the numerical methods for doing that are readily available. The challenge is to allow the user to enter the spectral parameters conveniently, and to inspect the calculated spectrum in a quick, visual way. Ideally, comparison with experimental data should also be simple.

The approach taken in MEXICO is to tailor the output to look as much like experimental data as possible. Excellent programs are available which process, display, manipulate and plot

NMR spectra, so it makes sense to use them for displaying the MEXICO output. These programs now run on modern computers, and have well-defined data structures, so it is fairly straightforward to fool them into thinking that MEXICO output is real data.

The engine of the MEXICO program is designed to produce a simple list of transitions and their probabilities. This list can then be used to produce a spectrum or an FID in whatever format is required. At the moment, there is a version which will produce the spectrum as a simple ASCII file on any Unix-based computer, or on a Windows-based PC. The other available version runs on an SGI computer, and is designed to interface closely with Bruker's XWINNMR program. The input format is the same for all the versions - only the output is different.

If experimental data are available on the computer, a non-iterative version of the program will calculate a goodness-of-fit function of the calculation to the experiment. This allows a crude manual iteration of the parameters, in order to achieve a best fit. Once a crude fit is achieved, an iterative version of the program will refine it.

PRESENT CAPABILITIES

The current version (3.0) of the coupled program is restricted to two-site exchange, with equal or unequal populations. The spins are all assumed to have spin-1/2. All the memory allocation in the program is dynamic, so the size of the spin system is limited only by the power of the computer. However, for current computers, six spins exchanging between unequally-populated sites represents a practical limit. The program will test for, and recognise, mutual exchange, since that reduces the size of the matrices. In the calculation, the efficiencies afforded by weak coupling, and the so-called F_z factoring are used. However, no account is yet taken for magnetically equivalent spins.

The uncoupled program can handle up to 8 sites, and this is an arbitrary number, set in the program. There is no limit to the number of processes that connect the sites, since all the memory is allocated dynamically.

INPUT DATA

The program clearly needs the spin parameters of each site, and the rate of exchange between them. These parameters, along with others are contained in an input file, called a mechanism file, with an extension of `.mch`. The input file is a text file, which can be handled with the editor of your choice. The file is written in as user-friendly a fashion as is possible in a text file, using the style of the matrix package called meschach [10] (which is also used extensively for the numerical part of the program). The program assumes that the mechanism file is in the directory from which the program is run. The input files are text files, created by any text editor, and the format is somewhat free. In technical terms, they are read in using the C function `fscanf`. This means that entries need to be separated by "whitespace" - spaces, tabs or carriage returns. In between lines of input, comments can be placed on lines starting with the character '#', and blocks of whitespace are ignored. Some error checking is done, but be sure that the

number of entries for each site is correct. If there is a wrong number of entries, interesting things may result. The exception to the free format is that the title occupies the first non-comment line of the mechanism file. Here, the line is read in (up to 80 characters) until a carriage return, so the title must occupy a single line. Here is a particularly well-commented example of such a file. This file is for the coupled version of the text-data program. The lines that are actually read by the program are highlighted.

```
#data title (<80 characters, all on one line)

N-14 decoupled formamide at low temperature

#Data file for the results
#The program checks the extension for the presence of ".txt"
#and uses that as a flag for text output. It will also work backwards to the
#final directory separator (\ or /, Windows or Unix) and define that as the
#data directory

C:\home\alex\Formam\11\formcalc.txt

#data directory
#for the Bruker XWINNMR version this is ignored,
#since the calculated data are just put into a different procno
#for other versions, this is where the output goes
#note that this is new in version 3

C:\home\alex\Formam\11\

# low and high frequency limits for calculation
# The program only calculates a defined region of the spectrum
# remember low field = high frequency and vice versa

3525.0    3745.0

#natural linewidth (in Hz)
#this is applied equally to all the lines

1.0

#absolute vertical scale of the spectrum, used if there are multiple files

24.0

#Number of sites and number of rate processes (both integers)
#The program allows only two sites and one process at the moment.
#note that the equilibrium constant has been moved away from this line
#in version 3 (it is now down with the rates)

2    1

# Character definition of the spin system.  The number of characters defines
#the number of spins, and spaces between the characters implies weak coupling.
#Therefore, 'abc' defines three strongly coupled spins, whereas 'ab c' defines
#an ABX spin system.

abc

#Permutation of the spin system.  These are integers from zero to (nspins-1),
#and indicate how the spin parameters (shifts and couplings may be permuted.
```

```
#This is designed mainly for mutual exchange, since there the spin parameters
#are identical, and we define the exchange by the permutation.
0   1   2

#Values of 1/T1 (in s-1) for each spin. This allows a more rigorous definition
#of linewidth, since now each transition may have a different width, according
#to random-field relaxation. This complements the natural linewidth parameter.
3.0   0.7   0.7

#Tableau of chemical shifts and couplings, in a triangular form.  The elements
#along the diagonal are the chemical shifts (in Hz, as read off the
#experimental spectrum), and the off-diagonal elements are the couplings.
 3957.7
    13.5     3588.0
     1.5        2.7      3671.8

#Second system, same formats
abc

#Permutation
0   2   1

#Values of 1/T1
3.0   0.7   0.7

#shifts and couplings
3957.7
    13.5     3588.0
     1.5        2.7      3671.8

#The equilibrium constant is used to define the reverse rate
#from the rate that is entered.

1.0

# Rate of the exchange (in s-1) from site 1 to site 2.  In mechanism files
#that the program creates, the reverse rate will also appear here for
#information, but the input ignores it.

1.7
```

You can see that the `.mch` file is divided roughly into three sections. First is the logistics of where the file is to go, and what regions to simulate, etc. The second section gives the static properties of the sites, and the third section defines the kinetics. The format of the first section is the same for all the programs, but the spin system and the kinetics sections are different for the coupled and uncoupled exchange simulations. However, the same file can be used for both the iterative and non-iterative versions.

SPECTRAL PARAMETERS

There is another file needed for input. This is automatically located in the XWINNMR version, but must be created in the text version. It is needed in the text version even if a simple simulation is done and there is no real data. In dealing with real data, it is convenient to have the full spectrum as a file, but only to simulate a small region corresponding to the exchange lineshape. We need to know the frequencies to make the simulation appear in the right place in the file. In order to make this convenient, the sweep width, number of data points, and so on are read from a file. In this case, we need to define them for the program as follows. For the text

version, create a file in the data directory named `procs` (Bruker users will recognise this). This is a somewhat ugly kludge to get the spectrum parameters into the program, and will be replaced with something more elegant. The `procs` file is again a text file containing the sweep width (SW) in Hz, the spectrometer frequency (SF) in MHz, the chemical shift (OFFSET) of the left hand (high frequency) edge of the spectrum (in ppm), and and the number of points in the real part of the spectrum (SI). A typical file looks like this:

```
##$SW_p= 5208.333
##$SF= 300.134916
##$OFFSET= 12.89265
##$SI= 32786
```

The format must be exactly as shown, including the uppercase and the presence or absence of spaces (a space must follow the equal sign, since the program looks for spaces to separate parts). The four parameters are as follows:

`##SW_p=`	sweep width of the whole spectrum (in Hz)
`##SF=`	spectrometer frequency (in MHz)
`##OFFSET=`	chemical shift of the left (lowest field, highest frequency point (in ppm)
`##SI=`	number of digital points in transformed spectrum.

The program will use these numbers to calculate the region of the spectrum over which the calculation will run. It will calculate the frequency (in Hz) of the left-hand edge (from `SF` and `OFFSET`) and then reference the frequencies in the mechanism file (for chemical shifts and display areas) from this value. For a straight simulation, this is somewhat complicated, but it simplifies the comparison with a real spectrum.

PREPARING THE EXPERIMENTAL DATA

The program will do a straightforward simulation, given spin parameters and rates. However, it is also capable of taking experimental data and iterating (either manually or automatically) to achieve a best fit, and extract a value for the rate. In order to do the iteration, the data must be carefully prepared.

In the NMR processing program used, make sure the phasing and the baseline are as good as possible. All processing packages have good routines for doing this. Since the simulation works only on a specific region, concentrate the effort on this region. The simulation assumes perfect phase and baseline, so errors in the original data will interfere with the iteration.

If Bruker's XWINNMR program is being used, process the data in `procno=1`. Then create another `procno` using the `edc` command. Process the FID identically as in the first `procno`. MEXICO will overwrite this second spectrum, so that it can be compared, using dual display, with the real data. Even though the spectrum will be overwritten, it is important to use the same processing, so that the scaling is set correctly. During the iteration, the program will look for the experimental data in `procno=1`.

For the text-based version, the program will prompt for the name of the data file in text format. For example, the program MestRe-C (`http://qobrue.usc.es/jsgroup/MestRe-C/MestRe-C.html`) has an Export command that produces this format. This should be a text file of the whole spectrum, with two columns of data: the first is the frequency, and the second is the intensity. The frequency of each point is ignored by the program – it is calculated internally from data in the `procs` file. Also, the first 11 lines of the file are skipped. This is to allow a title and some sort of self-documentation to be included in the file. This header should not be longer than 11 lines, but if it is shorter, this is not a problem. Any horizontal shifts of the simulated spectrum and the real data are handled automatically.

FORMAT OF THE INPUT FILE

Coupled spin system – remember all blank lines and lines starting with # are ignored.

```
First Line
      #data title (<80 characters, all on one line)

      N-14 decoupled formamide at low temperature

Second Line
      #Data file for the results (including directory)
      #The program checks the extension for the presence of ".txt"
      #and uses that as a flag for text output. It will also work
      #backwards to the final directory separator (\ or /,
      #Windows or Unix) and define that as the directory for the
      #procs file

      C:\home\alex\Formam\11\formcalc.txt

Third Line
      #note that this is new in version 3
      # directory of experimental data
      #for the Bruker XWINNMR version this is ignored,
      #since the calculated data are just put into a different procno

      C:\home\alex\Formam\11\

Fourth Line
      # low and high frequency limits for calculation
      # The program only calculates a defined region of the spectrum
      # remember low field = high frequency and vice versa

      3525.0   3745.0

Fifth Line
      #natural linewidth (in Hz)
      #this is applied equally to all the lines

      1.0

Sixth Line
      #absolute vertical scale of the spectrum, arbitrary if no
      #experimental data present

      24.0

Seventh Line
      #Number of sites and number of rate processes (both integers)
```

```
      #The program allows only two sites and one process at the moment.
      #note that the equilibrium constant has been moved away from this line
      #in version 3 (it is now down with the rates)

      2    1
```

Eighth Line
```
      # Characters definition of the spin system.  The number of characters
      # defines the number of spins, and spaces between the characters implies
      # weak coupling. Therefore, 'abc' defines three strongly coupled spins,
      # whereas 'ab c' defines an ABX spin system.

      abc
```


Ninth Line
```
      #Permutation of the spin system.  These are integers from zero to
      #(nspins-1), and indicate how the spin parameters (shifts and couplings
      #may be permuted. This is designed mainly for mutual exchange, since
      #there the spin parameters are identical, and we define the exchange by
      #the permutation.
0  1  2
```

Tenth Line
```
      #Values of 1/T1 (in s-1) for each spin. This allows a more rigorous
      #definition of linewidth, since now each transition may have a
      #different width, according to random-field relaxation. This complements
      #the natural linewidth parameter.
      3.0  0.7  0.7
```

Eleventh Line
```
      #Tableau of chemical shifts and couplings, in a triangular form.  The
      #elements along the diagonal are the chemical shifts (in Hz, as read
      #off the experimental spectrum), and the off-diagonal elements are the
      #couplings. The numbers in parentheses are for iteration.
      3957.7
        13.5    3588.0(2)
         1.5       2.7    3671.8(1)
```

Next Lines

```
      #Second system, same formats
      abc

      #Permutation
      0  2  1

      #Values of 1/T1
      3.0  0.7  0.7

      #shifts and couplings
      3957.7
        13.5    3588.0(2)
         1.5       2.7    3671.8(1)
```

Next Line
```
      #The equilibrium constant is used to define the reverse rate
      #from the rate that is entered. This has migrated to this line
      #in Version 3

      1.0
```

```
Last Line
     # Rate of the exchange (in s-1) from site 1 to site 2.  In mechanism
     #files that the program creates, the reverse rate will also appear here
     #for information, but the input ignores it.

     1.7(3)

Anything after this is ignored
```

The format for the uncoupled system is similar. Here the sites are defined by single frequencies (no couplings), but more complex kinetic schemes are handled (multiple processes)

```
First Line
     #data title (<80 characters, all on one line)

     Theresa's data at low temperature

Second Line
     #Data file for the results (including directory)
     #The program checks the extension for the presence of ".txt"
     #and uses that as a flag for text output. It will also work
     #backwards to the final directory separator (\ or /,
     #Windows or Unix) and define that as the directory for the
     #procs file

     C:\NMR\niroso\4\ther.txt

Third Line
     #note that this is new in version 3
     # directory of experimental data
     #for the Bruker XWINNMR version this is ignored,
     #since the calculated data are just put into a different procno

     C:\NMR\niroso\4

Fourth Line
     # low and high frequency limits for calculation
     # The program only calculates a defined region of the spectrum
     # remember low field = high frequency and vice versa

-500.0   -100.0

Fifth Line
     #natural linewidth (in Hz)
     #this is applied equally to all the lines

     1.0

Sixth Line
     #absolute vertical scale of the spectrum, arbitrary if no
     #experimental data present

     150.0

Seventh Line
     #Number of sites and number of rate processes (both integers)
     #this is a simple two-site exchange
     2       1

Eighth Line
     #1/T2's (in s-1) for each site
```

```
      1.0    1.0

Ninth Line
      #frequencies of each site
      -348.0   -265.0(2)

Tenth Line
      #M(infinity) for each site
      #This provides a cross-check for the kinetic scheme, since
      #the principle of detailed balance must be obeyed. This means
      #the M(inf) calculated from the rates must match the measured ones
      1.0  1.0

Eleventh line
      #there is a set like this for each process (number of processes
      #from line seven)

      #rate
      88.0(1)

      #The kinetic matrix is presented in sparse format
      #number of non-zero off-diagonal elements of the
      #kinetic matrix
      2

      #Then the elements themselves
      #The row and column addresses (start the numbering from 0,
      #not 1) and the value. Two integers and a floating point
      0  1      1.0
      1  0      1.0
```

For iteration, parameters are designated as variable by putting a parameter number in parentheses directly after the parameter (no space). For instance in the file for the uncoupled case, the rate and one of the frequencies will be varied. The non-iterative version of the program will ignore these parameter numbers. In some cases, two apparently different parameters should be varied together. For example, the file for the coupled case represents mutual exchange, so the chemical shifts should not change independently. This is indicated by using the same parameter number for the shifts in the two sites.

RUNNING THE PROGRAM

It is convenient to have three windows open to run the non-iterative version of the program. One is for the display of the results, one for editing the mechanism file, and one for running the program. The program is designed to be run from the command line, and it supports command-line arguments. Under Windows, run the program in a DOS shell, if you want to use command-line arguments, *i.e.* having the name of the mechanism file included. The command

```
      mexicnon test
```

will make the program look for a file named `test.mch` as the mechanism file. If no name follows `mexicnon` on the command line, then the user will be prompted individually for the name of the file. The command-line usage assumes the extension `.mch`, but this are not required if the filename are entered explicitly. In this case, the program will look for the exact filename entered.

A mechanism file for a simple two-site equally-populated exchange is given here.

```
#theresa's data
N,N dimethyl data at -30 degrees

#data file to overwrite
D:\alex\mestdata\bainth\4\ther.txt

# directory of experimental data
D:\alex\mestdata\bainth\4\

# low and high frequency limits for calculation
-500.0   -100.0

#natural linewidth
1.0

#absolute scale
150

#number of sites, rates
2    1

#1/T2's
1.0   1.0

#frequencies
-348.0    -265.0

#M(infinity)
1.0  1.0

#rate
88.0


2
0  1      1.0
1  0      1.0
```

MEXICO will then do the calculations as specified in the mechanism file, and prompt for a command. A typical console dialog is as follows. This is for the text-based program – the only difference in the XWINNMR version is that the user will be prompted for the procno to be overwritten.

```
D:\alex\codewar\mexico>mexicnon
MEXICO 3.0  uncoupled TEXT
Mechanism file:ther
Calculating line parameters
Line  Frequency     Width  Real Part  Imag Part Equilibrium Equil (calc)
0        -345.57      14.66   1.000000  -0.358519  1.000000   1.000000
1        -267.43      14.66   1.000000   0.358519  1.000000   1.000000
2 lines above threshold
Commands:(r)e-read/(x)toggle chi^2/(w)write and exit/(q)uit
Enter a command :w

D:\alex\codewar\mexico>
```

The possible commands are as follows.

```
r:      re-read the mechanism file. This can be edited in a separate
        window, so the parameters can be changed without re-running the
        program.

x:      toggle the calculation of chi squared - the sum of the squares of
        the differences between the calculated and observed spectra. This
        requires that you have a text form of the spectrum. One way is to
        Export the spectrum (File menu)as an ASCII file from MestRe-C. In
        the XWINNMR version, this uses procno = 1

w:      write out the final results and quit the program. This is the
        normal exit.

q:      quit, without writing the results.
```

The program will print out a summary of the exchange mechanism, then calculate (and print out) all the lines in the spectrum. The calculated spectrum will be output as an ASCII file for plotting and examination in a spreadsheet or other plotting program.

The program will print out the line numbers, their frequency, width and the real and imaginary parts of the intensity. The last two numbers define the intensity and the phase of the line. In the calculation of the lineshape itself, only those transitions above a certain threshold are included. If you have the mechanism file open in an editing window, you can change values, save the new version, and then ask MEXICO to re-read the mechanism file. In this way, you can generate a series of spectra (remember to change the output filename).

In this simple simulation mode of the text version, the output will just be an ASCII list of numbers, which represent the intensity of the spectrum at each point (no frequency information is printed). A simple line plot of the file in a spreadsheet will give the spectrum, and the position and scale are determined by the contents of the `procs` file. If experimental data are available (in text form) and used to calculate the goodness-of-fit, then the output file has a different format. There are now three columns. First is the frequency of each point, second is the experimental spectrum point and third is the calculated point. The columns extend only over the frequency region defined by the mechanism file – only the relevant portion of the experimental data is put in the file, in order to avoid very large files. This file can then be plotted with a spreadsheet.

The following is a console dialog where experimental data are used. The file with the data is `therexp.txt`, but the program does not need the extension - `.txt` is assumed.

```
D:\alex\codewar\mexico>mexicnon
MEXICO 3.0  uncoupled TEXT
Mechanism file:ther
Calculating line parameters
Line  Frequency      Width  Real Part  Imag Part Equilibrium Equil (calc)
0        -345.57      14.66  1.000000  -0.358519  1.000000  1.000000
1        -267.43      14.66  1.000000   0.358519  1.000000  1.000000
2 lines above threshold
Commands:(r)e-read/(x)toggle chi^2/(w)write and exit/(q)uit
Enter a command :x
Turning on chi^2 calculation
Enter a command :r
Calculating line parameters
```

```
Line   Frequency      Width  Real Part  Imag Part Equilibrium Equil (calc)
0         -345.57      14.66  1.000000  -0.358519  1.000000   1.000000
1         -267.43      14.66  1.000000   0.358519  1.000000   1.000000
2 lines above threshold
Experimental file: therexp
Chi^2 =      0.0018898285, offset =  -0.2427 Hz., scale =   1.0101
Commands:(r)e-read/(x)toggle chi^2/(w)rite and exit/(q)uit
Enter a command :
```

At this stage, the `.mch` file was edited (and saved to disk), to change the rate from 88.0 s^{-1} to 90.0. The command `r` was typed, to re-read the file. Note that the chi^2 value has increased.

```
Enter a command :r
Calculating line parameters
Line   Frequency      Width  Real Part  Imag Part Equilibrium Equil (calc)
0         -345.45      14.98  1.000000  -0.367755  1.000000   1.000000
1         -267.55      14.98  1.000000   0.367755  1.000000   1.000000
2 lines above threshold
Chi^2 =      0.0027127832, offset =  -0.2390 Hz., scale =   1.0133
Commands:(r)e-read/(x)toggle chi^2/(w)rite and exit/(q)uit
Enter a command :
```

The rate was changed to 86.0, and `r` was typed again.

```
Enter a command :r
Calculating line parameters
Line   Frequency      Width  Real Part  Imag Part Equilibrium Equil (calc)
0         -345.68      14.35  1.000000  -0.349364  1.000000   1.000000
1         -267.32      14.35  1.000000   0.349364  1.000000   1.000000
2 lines above threshold
Chi^2 =      0.0014887537, offset =  -0.2461 Hz., scale =   1.0063
Commands:(r)e-read/(x)toggle chi^2/(w)rite and exit/(q)uit
Enter a command :w

D:\alex\codewar\mexico>
```

This can be repeated, but is better handled by the iterative program.

In the XWINNMR version, a simulated spectrum will be created in the XWINNMR binary format (overwriting the `1r` file), which can be compared to the real data using dual display.

To do an iterative fit, the mechanism file was modified to put parameter numbers after the rate and one of the frequencies. In this case, the rate and the shift difference are varied. There is no point in making both frequencies iterative, since the program automatically shifts the spectrum horizontally to get the best fit. Note that the non-iterative program will read this file as well - it will just ignore the parameter numbers.

```
#theresa's data
N,N dimethyl data at -30 degrees

#data file to overwrite
D:\alex\mestdata\bainth\4\ther.txt

# directory of experimental data
D:\alex\mestdata\bainth\4\
```

```
# low and high frequency limits for calculation
-500.0    -100.0

#natural linewidth
1.0

#absolute scale
150

#number of sites, rates
2    1

#1/T2's
1.0    1.0

#frequencies
-348.0    -265.0(2)

#M(infinity)
1.0  1.0

#rate
88.0(1)

2
0  1       1.0
1  0       1.0
```

The program will set up and then optimise the simplex (in this case the command-line parameter was used for the mechanism file).

```
D:\alex\codewar\mexiter>mexiter ther
MEXICO iterative 3.0  uncoupled TEXT
Rate =      86.00
Freqencies =    -348.00       -265.00
Generating 3 points for initial simplex, parameters 2 and 1
Rate =      70.65
Freqencies =    -348.00       -266.82
Rates:      70.649
Frequencies:        -348.00         -266.82
Experimental file: therexp
Chi^2 =     0.5236882593, offset =  -1.3499 Hz., scale =   0.7311
Rate =      92.12
Freqencies =    -348.00       -296.61
Rates:      92.121
Frequencies:        -348.00         -296.61
Chi^2 =     0.4374588433, offset =  -4.4769 Hz., scale =   0.6169
Rate =      92.66
Freqencies =    -348.00       -248.73
Rates:      92.663
Frequencies:        -348.00         -248.73
Chi^2 =     0.1100136410, offset =  -8.2716 Hz., scale =   1.0148
2 lines above threshold

Optimizing the simplex
Iteration 0
Rate =     108.54
Freqencies =    -348.00       -318.16
Rates:      108.543
Frequencies:        -348.00         -318.16
```

```
Chi^2 =      0.5773727123, offset =  -8.5783 Hz., scale =    0.3586
Trial failed
*****Contracting
Rate =       84.32
Freqencies =    -348.00       -249.93
Rates:        84.317
Frequencies:          -348.00          -249.93
Chi^2 =      0.1253489792, offset =  -7.6842 Hz., scale =    0.9813
***Improving
Iteration 1
Rate =       84.86
Freqencies =    -348.00      -202.05
Rates:        84.859
Frequencies:          -348.00          -202.05
Chi^2 =      0.6107198867, offset =   1.9954 Hz., scale =    0.7034
Trial failed
*****Contracting
Rate =       90.31
Freqencies =    -348.00      -272.97
```

The program will continue until it converges or reaches a mximum number of iterations. The simplex is slow and inefficient, but quite robust.

```
***Improving
Rate =       88.77
Freqencies =    -348.00       -263.48
Rates:        88.765
Frequencies:          -348.00          -263.48
Chi^2 =      0.0007800691, offset =  -1.0060 Hz., scale =    1.0179
Trial failed
*****Expanding
Iteration 20
Rate =       88.57
Freqencies =    -348.00      -263.55
Rates:        88.573
Frequencies:          -348.00          -263.55
Chi^2 =      0.0007767273, offset =  -0.9715 Hz., scale =    1.0173
Converged, Chi^2 =   0.00077673
Final relaxation parameters
Natural linewidth (in Hz)
     1.0000
```

The values are printed out on the screen, and the final values (in the form of a mechanism file, exchange.mch, are written to disk.

MULTIPLE INPUT FILES

Samples for chemical exchange are seldom pure, and overlaps with interesting regions of the spectrum are common. MEXICO allows for multiple input files on the command line, *e.g.*

```
mexicnon test1 test2 test3
```

In this case, the program will accumulate the transitions associated with the various input files, and calculate the total spectrum. The relative intensity of each system is controlled by the absolute scale parameter in each mechanism file. Also, the region to be calculated is read from the *first* mechanism file. It is probably good practice to keep the region to be calculated

consistent over all the files.

OUTPUT

The program can produce output either as an ASCII file, or as a binary file that can be read directly into Bruker's XWINNMR program on an SGI computer. In the latter case, the program simply overwrites the spectrum in a defined `procno`, so that it can be inspected using dual display. If the output file in the mechanism has a `.txt` extension, the program uses that as a flag for text output. For an ASCII file, it is simply a list of integers - their frequencies are to be inferred from the data in the `procs` file. When real data is available, however, the output ASCII file consists of three columns: each row contains the frequency, the calculated data and the real data. This can be imported into a spreadsheet for further processing and display.

If the user selects the `w` command, which is the normal exit, the program will write a file called `exchange.mch` into the output directory. This has the format of a mechanism file, and can be used directly as such. It contains the final values of all the input parameters. Below is an example.

```
#Output file from MEX(I)CO exchange lineshape program
#Created at14:36:26, Apr  4 2002
dimethylnitrosoaniline at 0

#Filename for calculated lineshape
D:\alex\codewar\nitroso\10\meth10out.txt

#Experimental directory
D:\alex\codewar\nitroso\10\

#Low and high frequencies for calculated region
    2500.000        3700.000

#Natural linewidth (in Hz)
      0.5000

#Absolute scale
    490.0000

#Number of sites, Number of rates
 2    1

#Spin system definition
abcd

#Permutation of the spins
    0     1     2     3

#1/T2 of each site
       0.700          0.700          0.700          0.700

#Frequencies of site1
    3475.000
        9.100      2765.462
        2.300          0.100      2697.843
        0.100          2.500          9.500      2664.840
```

```
#Spin system definition
abcd

#Permutation of the spins
   2    3    0    1

#1/T2 of each site
      0.700         0.700         0.700         0.700

#Frequencies of site1
    3475.000
       9.100     2765.462
       2.300        0.100     2697.843
       0.100        2.500        9.500     2664.840

#Equilibrium constant
    1.000000

#Rate forward and reverse
#Program actually uses forward rate and equlibrium constant
      209.2997
#Reverse rate only for information
#        209.2997
```

In the version for XWINNMR, the program produces a file designed to be read in and displayed against the experimental data, using the dual display routine. The program assumes that a spectrum in a separate `procno` has been created, using exactly the same processing parameters. The program will then overwrite the `1r` file in that directory. The two different sets of processed data (the experimental spectrum, and the calculated spectrum, which has overwritten the separate `procno`) can then be compared. The program will ask for confirmation before the data are overwritten, and it will not overwrite `procno = 1` in any circumstances.

MANUAL ITERATION

The non-iterative version of the program has a crude manual iteration. The program will calculate the sum of the squares of the difference between the calculated and the observed spectrum, which then can be minimised.

If the χ^2 calculation is turned on (this is a toggle command - the program will continue to calculate this until it is turned off), you will be prompted for the filename of the experimental spectrum. This is assumed to have a `.txt` extension, in the text version. The XWINNMR version automatically looks at `procno = 1`. The value of χ^2 will be printed out, along with an offset and a scale. These numbers arise because the program optimises χ^2 by shifting the whole spectrum left and right, and by scaling the spectrum over a limited range. This automatically removes two degrees of freedom in fitting the spectrum. However, the range over which the offset and scale can be varied is limited, so the user is warned if the program bumped into those limits. The value of χ^2 is calculated by the following equation.

$$\chi^2 = \frac{\sum (observed - calculated)^2}{\sum (observed)^2}$$

Values close to 1.0 usually mean that the calculated spectrum is scaled very small, whereas very large values mean that the scaling of the calculated spectrum is far too large. With a good spectrum and a good fit, values of χ^2 of less than 0.01 can be achieved.

SIMPLEX ITERATION

Once an approximate fit is obtained by hand, the iterative version of the program, `mexiter`, can be used.[11] The format of the mechanism file is identical, except that parameters to be iterated are indicated by parameter numbers in parentheses immediately after the parameter (no space between parameter and parenthesis). An example is given below, with the parameters highlighted. Note that the non-iterative version will accept (and ignore) these numbers in parentheses, so that an identical mechanism file can be used for both programs.

The following is a mechanism file for the mutual coupled exchange in *N,N*-dimethyl-4-nitrosoaniline. Three of the four shifts are to be varied, along with the rate. Note that the shifts in the two sites must be varied together (since this is a mutual exchange), so they must be given the same parameter numbers. Note also that varying the fourth shift is redundant, since the program automatically optimises the vertical scale and horizontal offset (within limits) before judging the fit.

Most of the parameters can be varied: shifts, couplings, rates, the natural linewidth and the relaxation rates. The exception is the equilibrium constant, for non-mutual exchange. At the moment, this is fixed by the mechanism file, and must be varied manually. Up to 8 parameters can be varied simultaneously, but it is usually better to break them up into groups of 3 or 4.

Running the program `mexiter` is identical to the non-iterative version. Once the program has started, it will iterate on the parameters, using a simplex algorithm [12], until it converges. Intermediate results will be put out on the console. This algorithm is slow (often >30 iterations) but quite robust. When it converges, it will write the final values in a file called `exchange.mch` in the output directory. This file is in the correct format so that it could be used as input to the program.

The program will automatically adjust the vertical scale, and the horizontal offset (the computer equivalent of sliding the two pieces of paper around as you hold them to the window), and print them out. For good fits, the chemical shifts and the scale in the mechanism file should be adjusted so that the offset comes close to zero (within a few Hz), and the scale is close to 1.0 within a few %). If these numbers get dangerously out of range, the results may be unreliable, and the program will print a warning.

The simplex algorithm starts with a random set of initial conditions, so running the program twice will not give exactly the same result. However, repeating the fit is useful, since the randomness may get out of a local minimum.

EXAMPLES

Here is an example of the non-iterative coupled version, applied to
N,N-dimethyl-4-nitrosoaniline. There are parameter numbers in the file, for future iterations,
but they are ignored. The parameters that will be used for iteration are highlighted

```
#N,N-dimethyl, 4-nitrosoaniline in methanol
#Gord's work at Guelph
dimethylnitrosoaniline at 0

#data file to overwrite
D:\alex\codewar\nitroso\10\meth10out.txt

#data file to overwrite
D:\alex\codewar\nitroso\10\

# low and high frequency limits for calculation
2500.0     3700.0

#natural linewidth (in Hz)
0.5

#absolute scale
245.0

#number of sites, rates, equilibrium constant
2    1

#first system
abcd
#permutation
0   1   2   3
# values of 1/T1
0.7   0.7   0.7   0.7
#shifts and couplings
 3475.0
    9.1     2765.5(4)
    2.3        0.1     2697.9(3)
    0.1        2.5       9.5      2664.9(2)

#second system
abcd
#permutation
```

```
  2   3   0   1
  # values of 1/T1
  0.7   0.7   0.7   0.7
  #shifts and couplings
   3475.0
      9.1    2765.5(4)
      2.3       0.1     2697.9(3)
      0.1       2.5        9.5     2664.9(2)


  #equilibrium constant
  1.0


  #rates
  210.2(1)
```

The console dialog is as follows

```
MEXICO 3.0   coupled TEXT
Mechanism file:gkh620nitro10

                          Spin system: abcd
Site 1 (1:)                        210.2000      Site 2 (:1.000   )
 3475.00                           ------->       2697.90

    9.1000  2765.50                210.2000         9.5000  2664.90

    2.3000     0.1000  2697.90     <-------         2.3000     0.1000  3475.00

    0.1000     2.5000    9.5000  2664.90            0.1000     2.5000     9.1000  2765.50

Calculating line parameters
Line   Frequency     Width  Real Part   Imag Part
0        3467.84     67.77   0.253989    0.022004
1        3467.84     68.16  -0.000000   -0.000000
2        2746.89     65.73   0.257787    0.240346
3        2746.89     70.21  -0.000000   -0.000000
4        1891.68     68.19   0.000000   -0.000000
5        1891.68     68.19   0.000000   -0.000000
6        1952.40     68.38   0.000000    0.000000
7        1952.40     67.99   0.000000    0.000000
```

The program will print out all the line parameters and prompt for a command, as in the uncoupled case. As is typical in a coupled system, not all the lines are above a minimum observable threshold.

```
49       2705.55        67.16    0.107153   -0.025260
50       2703.36        68.16    0.102626   -0.035389
51       2733.84        67.33    0.000019    0.000011
52       3479.34        68.16    0.123020    0.010595
53       2670.59         1.71    0.147431    0.016369
```

```
54      2705.63      67.12   0.105765  -0.027652
55      2771.34       1.06   0.123784   0.000687
Maximum block size = 24
38 lines above threshold
Commands:(r)e-read/(x)toggle chi^2/(w)write and exit/(q)uit
Enter a command :w
```

First, the program sets up the simplex optimization, by randomly choosing 5 sets of parameters for the 4 parameters to be optimized. The program will then go through many iterations, as it varies the parameters and seeks a minimum in χ^2. For each step it will print out the parameters, and some diagnostics of how the simplex is doing. Eventually (note the iteration numbers!), it will converge, and print out the final values.

```
Iteration 52
                        Spin system: abcd
Site 1 (1:)                        217.6498    Site 2 (:1.000   )
 3475.00                           -------> 2691.01

    9.1000  2767.28               217.6498    9.5000  2667.80

    2.3000     0.1000  2691.01    <-------    2.3000    0.1000  3475.00

    0.1000    2.5000    9.5000  2667.80       0.1000    2.5000    9.1000  2767.28
Chi^2 =     0.0005388880, offset =  -0.1170 Hz., scale =   1.0142
Converged, Chi^2 =   0.00053889
Final relaxation parameters
Natural linewidth (in Hz)
     0.5000
1/T2 of each site
     0.700       0.700       0.700       0.700
```

Note that the scale and the offset are close to 1.0 and 0.0, respectively, so the fit is reliable.

The spectrum was acquired on a Bruker spectrometer, so the console dialog is slightly different. A dummy spectrum (processed under identical conditions to that in procno 1), was created. This will be overwritten by the program.

```
bain@transition /usr/people/bain/programs/dnmr/nitroso 27 %!!mexiterc gkh620nitro10
MEXICO iterative 3.0  coupled XWINNMR
                        Spin system: abcd
Site 1 (1:)                        210.2000     Site 2 (:1.000   )
 3475.00                           -------> 2697.90

    9.1000  2765.50               210.2000    9.5000  2664.90

    2.3000     0.1000  2697.90    <-------    2.3000    0.1000  3475.00

    0.1000    2.5000    9.5000  2664.90       0.1000    2.5000    9.1000  2765.50
Output to /u/data/bain/nmr/gkh010620/10
Output procno: 2
```

```
Procno 2 will have its real part overwritten! OK(y/n)?:y
Output to /u/data/bain/nmr/gkh010620/10/pdata/2/1r
Mutual Exchange
Generating 5 points for initial simplex, parameters 4 3 2 and 1
                              Spin system: abcd
Site 1 (1:)                        246.0011     Site 2 (:1.000   )
 3475.00                          -------> 2697.72
    9.1000  2760.26                246.0011    9.5000  2657.95
    2.3000    0.1000  2697.72      <-------    2.3000    0.1000  3475.00
    0.1000    2.5000    9.5000  2657.95        0.1000    2.5000    9.1000  2760.26
Chi^2 =     0.0042843781, offset =   1.2485 Hz., scale =   1.0119
                              Spin system: abcd
Site 1 (1:)                        243.5995     Site 2 (:1.000   )
 3475.00                          -------> 2689.22
    9.1000  2760.03                243.5995    9.5000  2674.17
    2.3000    0.1000  2689.22      <-------    2.3000    0.1000  3475.00
    0.1000    2.5000    9.5000  2674.17        0.1000    2.5000    9.1000  2760.03
Chi^2 =     0.0300623403, offset =  -4.1331 Hz., scale =   0.9362
                              Spin system: abcd
Site 1 (1:)                        187.4508     Site 2 (:1.000   )
 3475.00                          -------> 2698.12
    9.1000  2759.92                187.4508    9.5000  2670.74
    2.3000    0.1000  2698.12      <-------    2.3000    0.1000  3475.00
    0.1000    2.5000    9.5000  2670.74        0.1000    2.5000    9.1000  2759.92
Chi^2 =     0.0065506374, offset =  -2.3454 Hz., scale =   0.9459
                              Spin system: abcd
Site 1 (1:)                        247.0764     Site 2 (:1.000   )
 3475.00                          -------> 2704.76
    9.1000  2770.59                247.0764    9.5000  2655.66
    2.3000    0.1000  2704.76      <-------    2.3000    0.1000  3475.00
    0.1000    2.5000    9.5000  2655.66        0.1000    2.5000    9.1000  2770.59
Chi^2 =     0.0072905799, offset =  -2.3720 Hz., scale =   1.0641
                              Spin system: abcd
Site 1 (1:)                        176.3768     Site 2 (:1.000   )
 3475.00                          -------> 2699.45
    9.1000  2772.39                176.3768    9.5000  2666.21
    2.3000    0.1000  2699.45      <-------    2.3000    0.1000  3475.00
    0.1000    2.5000    9.5000  2666.21        0.1000    2.5000    9.1000  2772.39
Chi^2 =     0.0146210925, offset =  -2.5338 Hz., scale =   1.0076
50 lines above threshold


Optimizing the simplex
Iteration 0
                              Spin system: abcd
```

```
Site 1 (1:)                      184.8531      Site 2 (:1.000   )
 3475.00                             -------> 2710.81
    9.1000  2771.55                  184.8531      9.5000  2651.11
    2.3000     0.1000  2710.81       <-------      2.3000     0.1000  3475.00
    0.1000     2.5000     9.5000  2651.11          0.1000     2.5000     9.1000  2771.55
Chi^2 =     0.0401147266, offset =  -2.5643 Hz., scale =   1.0517
Trial failed
*****Contracting

                         Spin system: abcd
Site 1 (1:)                      228.9129      Site 2 (:1.000   )
 3475.00                             -------> 2694.62
    9.1000  2762.91                  228.9129      9.5000  2668.41
    2.3000     0.1000  2694.62       <-------      2.3000     0.1000  3475.00
    0.1000     2.5000     9.5000  2668.41          0.1000     2.5000     9.1000  2762.91
```

The iteration will continue until converged.

```
Iteration 32
                         Spin system: abcd
Site 1 (1:)                      212.6875      Site 2 (:1.000   )
 3475.00                             ------->    2696.96
    9.1000  2765.63                  212.6875      9.5000  2664.91
    2.3000     0.1000  2696.96       <-------      2.3000     0.1000  3475.00
    0.1000     2.5000     9.5000  2664.91          0.1000     2.5000     9.1000  2765.63
Chi^2 =     0.0003578374, offset =  -1.6584 Hz., scale =   1.0052
Trial failed
*****Contracting
                         Spin system: abcd
Site 1 (1:)                      211.4855      Site 2 (:1.000   )
 3475.00                             ------->    2696.94
    9.1000  2765.72                  211.4855      9.5000  2665.21
    2.3000     0.1000  2696.94       <-------      2.3000     0.1000  3475.00
    0.1000     2.5000     9.5000  2665.21          0.1000     2.5000     9.1000  2765.72
Chi^2 =     0.0003561666, offset =  -1.7544 Hz., scale =   1.0042
***Improving
Iteration 33
                         Spin system: abcd
Site 1 (1:)                      211.7763      Site 2 (:1.000   )
 3475.00                             ------->    2696.84
    9.1000  2765.58                  211.7763      9.5000  2664.97
    2.3000     0.1000  2696.84       <-------      2.3000     0.1000  3475.00
    0.1000     2.5000     9.5000  2664.97          0.1000     2.5000     9.1000  2765.58
Chi^2 =     0.0003558289, offset =  -1.6090 Hz., scale =   1.0046
Converged, Chi^2 =    0.00035583
Final relaxation parameters
Natural linewidth (in Hz)
     0.5000
1/T2 of each site
     0.700        0.700        0.700        0.700
bain@transition /usr/people/bain/programs/dnmr/nitroso 29 %
```

COMPILING THE PROGRAM

The program is written in fairly standard ANSI-style C, and it should compile under most C compilers. It uses a style and many functions from a package of matrix routines called meschach,[10] available through netlib (www.netlib.org). The tar file for meschach is included with the distribution file. Alternatively, meschach can be obtained through the web at ftp://ftpmaths.anu.edu.au/pub/meschach/meschach.html or via anonymous ftp to ftpmaths.anu.edu.au in the pub/meschach directory. There are only two source files - one for the

non-iterative and one for the iterative version. The coupled/uncoupled, Windows/Unix and Bruker/text distinctions are handled by preprocessor flags.

Before mexico is compiled, a meschach archive must be created. Uncompress and un-tar the meschach file as follows.

```
uncompress mesch12b.tar.Z
tar xvf mesch12b.tar
```

Before the package is compiled on an SGI machine, it may be necessary to delete or comment out line 43 in matrix.h, which defines u_int.

```
typedef unsigned int  u_int;    /* line 43, delete or comment out */
```

Now construct the meschach archive, meschach.a, with the following UNIX commands.

```
configure --with-all
make all
make clean
```

On a SGI Indigo running IRIX 6.5, the following command for compiling the program is used. This sets optimization level 2, and links in the meschach archive.

```
cc mexicnon.c -O2 -o mexicnon meschach.a –lm
```

If you are using gcc under RedHat Linux 7.2 (and it may be more general), this version object to the use of the data type complex used in Meschach (since it has a complex data type now). This conflict can be avoided by replacing all the complex references in Meschach and Mexico with Complex. The source code for Mexico does this, but the Meschach files will need to be edited.

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CONTACT

Users will have many suggestions, and may well find many bugs. Please contact Alex Bain at bain@mcmaster.ca.