the minispec

BRUKER

Relaxation Time (T_1 and T_2) Measurements
Bruker minispec Relaxation Time Manual

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Relaxation Time (T_1 and T_2) Measurements
minispec Operator's Manual

1. INTRODUCTION

The Bruker minispec is a low-field pulsed NMR spectrometer used for a wide range of routine NMR applications. Data is usually (but not necessarily) analyzed in the time domain form. This guide describes the use of the minispec with Bruker pre-programmed applications to determine relaxation parameters T_1 and T_2.

In particular, this manual discusses the use of Bruker pre-programmed applications *t1_ir_mb*, *t1_sr_mb*, *t2_cp_mb* and *t2_se_mb*. The fitting routines included in these applications are suitable for processing data that are described by, or that can be simplified to, mono- and bi-exponential decay. Thus, appropriate samples are simple single component systems (neat liquids, solutions) and two component systems (dispersions, suspensions). Three and more component systems can sometimes be treated if some of the components are relatively fast decaying and can be ignored by using suitable experimental parameters. More complex systems that cannot be so simplified may require tri- and quad-exponential decay fits (available in 'minispec\TestApps\Additionals' and named *fit_rel*) or other third party modeling software in order to analyze the experimental data.

Instructions in this manual were developed for the minispec mq series software. Depending upon the versions of the minispec software, different variables or other setup options may be used.

2. COMPONENT LIST

2.1. System

This manual refers to the use of the minispec mq series.

2.2. Probe head

In principle, any probe head can be used to make relaxation measurements. However, due to the dependence of relaxation on sample temperature, a probe head that permits variable temperature control (type .../V) is recommended. This is especially true for T_1 measurements, which require a relatively long time to complete compared to T_2 experiments.

A room temperature probe head may also be used if the temperature of the sample is preconditioned to a defined value before the measurement. However, the duration of the measurement should be short to avoid temperature change during the measurement.

2.3. Circulating Heater / Cryostat Bath

If a type .../V probe head is used, then an external circulating heater / cryostat bath is needed to control the temperature of the sample in the magnet sample chamber. Many manufacturers exist. Some of the prominent brand names include Haake, Julabo and Neslab.

2.4. Software

The following Bruker pre-programmed applications are discussed in this manual:

- Application *t1_ir_mb* for determination of T_1.
- Application *t1_sr_mb* for determination of T_1.
- Application *t2_cp_mb* for determination of T_2.
- Application *t2_se_mb* for determination of T_2.

3. INSTALLATION OF the minispec

3.1. Electrical Requirements

The electrical requirements of the minispec are as follows:

Input	110 V:	090 V - 132 V AC, 50 Hz - 60 Hz, 4.0 A
	230 V:	220 V - 250 V AC, 50 Hz - 60 Hz, 2.5 A
Power	400 Watts maximum	

Grounding is vital for safe operation of the instrument. Never operate the minispec with the grounding conductor disconnected. If local wiring does not provide a ground, an earth-to-chassis connection must be added at the user's location.

For best performance:

- Do not locate the minispec near high voltage power cables.
- Avoid plugging the minispec into the same circuit as appliances that have a heavy and variable draw on the line voltage (e.g. heating ovens, microwaves, air conditioners, etc.).
- Ensure a stable, clean power supply. If power conditions are suspect due to the potential for brownouts, sudden power outages, voltage spikes, etc. consider the installation of a line filter and/or UPS. The UPS unit should be at least a 500W type preferably based on the saturated transformer principle.

3.2. Operating Environment

The minispec should be located so as to avoid exposure to direct sunlight and direct heating/cooling sources (e.g. heating/cooling vents).

Recommended Operating Temperature Range	17 ^{o}C to 28 ^{o}C
Recommended Operating Relative Humidity Range	20 % to 80 % (non-condensing)

For proper ventilation, a minimum distance of 10 cm should be allowed between the back of the electronics box and adjacent objects.

> DO NOT OPERATE the minispec WITH THE CLEAR PLASTIC DUST COVER OVER THE ELECTRONICS BOX.

Place the two units on a flat, level surface capable of supporting their weight (110 kg). Placing the minispec magnet on insulating rubber pads can dampen excessive floor vibration.

Since iron-containing objects can influence the magnetic field inside the magnet box, do not locate the magnet box within 2 m of moving or moveable metallic objects.

Vibration caused by a circulating heater/cryostat bath located on the same bench-top as the magnet can degrade performance. Suggested location for the bath is on the floor below and to the side of the magnet. The bath should be on a separate electrical circuit from the minispec.

4. SAMPLE TEMPERATURE CONTROL

4.1. General

It is recommended that T_1 and T_2 be measured at a defined temperature. A calibrated thermometer is recommended for measuring the actual temperature in the probe head sample compartment. Sample temperature can be controlled in two ways:

1. For magnet temperature probe heads, precondition the temperature in an external heating device such as a heating block or circulating heater/cryostat bath. WARNING: the duration of the measurement should be short to avoid temperature change during the measurement. A large sample will resist temperature change better than a small sample, due to heat capacity. If a magnet temperature probe head will be used, the sample temperature is most conveniently conditioned to 40 °C, since this is mostly the temperature inside the magnet box, and the temperature to which this probe head sample compartment will passively be heated if the cap is left on the sample chamber. Note, however, that after successive measurements the chamber of a probe head will cool off as room temperature air is introduced during sample exchange.

2. For variable temperature probe heads, a circulating heater / cryostat bath is used to control the temperature in the probe head sample compartment. Samples can be preconditioned in an Aluminum block placed in the bath reservoir, or in the probe head chamber (leave 5 to 20 minutes for the sample to equilibrate to temperature, depending on the volume and temperature differential).

4.2. Circulator Fluid

Variable temperature probe heads are equipped with a dewar that is shielded from the NMR coil. These probe head accept all types of circulating fluids, such as protic circulating fluids (e.g. distilled water / ethylene glycol mixtures). However the viscosity of the liquids should be always comparable to distilled water.

4.3. VT Connections

The fluid necessary to heat or cool the variable temperature probe head is carried from an external connector, situated on the back of the magnet box, to the probe head dewar by flexible tygon tubing. The external brass fittings are designed for slip-on connection to hoses that carry fluid to and from the circulating water bath. Rubber tubing can be slipped over the fitting and clamped into place.

Tubing from the circulator should be insulated to prevent thermal loss, and should be long enough to allow the circulating heater/cryostat to be located at least 0.5 m from the magnet.

If the circulator has flow rate control, adjust the flow rate to obtain the necessary temperature stability.

4.4. Temperature Range

The normal operation range of the variable temperature probe head is from -10 to 70 °C. Operation outside this temperatures range is possible, but the minispec may not be able to properly regulate magnet temperature.

5. PREPARATION FOR MEASUREMENTS

5.1. Preparation of the minispec

Check cable connections before powering ON and operating the minispec.

Turn ON the minispec at least three hours prior to use. The temperature in the magnet chamber must be at stable operating temperature (40.0 °C) for best results. If temperature is not yet stabilized, the minispec Status Box (Instrument Status) will indicate the temperature error (magnet temperature not check marked). The actual magnet temperature is displayed there as well.

> NOTE: When restarting the minispec, do not toggle the POWER switch OFF and ON too quickly or the power supply will not have a chance to reset and may not come on. Allow about 5 seconds for a total reset.

The minispec is normally left ON if it will be used from time-to-time during the course of a work week. It is recommended to turn OFF the power to the minispec when it will not be in use for a number of weeks.

The minispec power should be left OFF when power interruptions are likely (*e.g.* during building maintenance, heavy thunder storms) or if the minispec is being cleaned.

5.2. Preparation of the Temperature Conditioner

Turn on the temperature conditioning apparatus at least 30 minutes before use and let the temperature stabilize. If a circulating heater / cryostat bath is used with a variable temperature probe head to regulate temperatures below 10 °C, consider ventilating the probe head sample compartment with dry, temperature conditioned air or Nitrogen gas to prevent condensation in the sample compartment.

5.3. Instrument Tuning and Pulse Length Calibration

Before using relaxation time applications for measurements, the instrument must be tuned and the 90° and 180° pulse lengths must be calibrated using a representative sample**, ideally at a Gain that will be appropriate for experimental samples.** T_1 experiments in particular require very accurate setting of 90° and 180° pulse lengths because miss-set values result in incomplete inversion. Residual signal that results in the X-Y plane will undergo T_2 or T_2* relaxation, which decreases the signal intensity by a mechanism other than T_1 relaxation. Since the length of time for this to occur is variable because of the variable tau, a variable error in signal amplitude will result.

5.3.1. Tuning / Calibration Sample

For rigorous measurement of **absolute** values of T_1 or T_2, samples should be restricted to the homogeneous field region of the magnet (B_o field) and of the coil (B_1 field). This is accomplished by ensuring the proper positioning of the probe head in the magnet pole gap (*see Section 3.2*), and by under-filling the probe head coil. Recommended under-filling heights for the following probe heads are:

... / R / ...	(ratio type)	≤ 1 cm
... / A / ...	(absolute type)	≤ 3 cm

If **relative** values of T_1 or T_2 are sufficient, and absolute relaxation values are not really important, it is advantageous to use an over-filled coil because less care needs to be take in preparing samples and optimizing conditions. Suggested over-filling heights are:

... / R / ...	(ratio type)	≅ 4 cm
... / A / ...	(absolute type)	≤ 6 cm

For measurements of **aqueous** samples, the calibration sample should have a composition similar to the samples of interest, especially with regards to the molarily of salt or buffer. The following sample is suggested:

> Phosphate buffer or physiologic saline with approximately 1.25 g/L $CuSO_4 \cdot 5H_2O$ to shorten T_1 relaxation time.
> Use the same sample tube filling height as will be used with real samples
> *e.g.* 1 cm for underfilled coil, 4 cm for overfilled coil

For **oil** samples, the following sample is recommended:

> White, light mineral oil.
> Use the same sample tube filling height as will be used with real samples
> *e.g.* 1 cm for under-filled coil, 4 cm for over-filled coil

5.3.2. Tuning / Calibration Procedure

For **under-filled coils**, samples should be centered in the coil so that the maximum amplitude is obtained using the test application FID. Position the under-filled sample for maximum signal amplitude by observing the signal with the test application FID. If a consistent sample height is used all the time, the sample lower limit can be set by adjusting the pedestal inside the probe head chamber so that the (*e.g.*) 1 cm sample is in the optimum position. This adjustment has been preset in the factory for a 4 cm sample (over-filled coil) on ratio type probe heads.

Sample positioning is not an issue for over-filled coils.

Start the tuning/calibration procedure by selecting 'Update Settings' from the 'the minispec' menu. Follow the instructions that come up. The update routine automatically sets the correct Gain, Magnetic Field, Detection Angles, and Pulse Lengths while the calibration sample is in the sample chamber. Make a note of the correct Gain setting found by the update routine for later use.

5.4. Application Selection and Curve Fitting Rules

5.4.1. Application Selection

1. T1 Relaxation Time Analysis
   As indicated later, the data points of the t1_ir_mb application are spread upon a range that is a factor 2 bigger than the dynamic range of the t1_sr_mb data points. Therefore fitting the data points will cause more accurate results in the case of the t1_ir_mb application. On the other side the repetition delay of the t1_sr_mb application needs to be only half compared to the delay for the t1_ir_mb application. Thus the measurement time of the t1_ir_mb application is twice the time for the t1_sr_mb analysis. Therefore the user has to select whether accuracy (t1_ir_mb) or short measurement time (t1_sr_mb) is required.

2. T2 Relaxation Time Analysis
   Liquids with T2 relaxation times in the msec range are usually investigated with the t2_cp_mb application. Whereas the t2_se_mb application requires analysis times which are comparable to T1 applications, the t2_cp_mb investigations require only a few seconds. This makes the t2_cp_mb application very attractive. However it is known that samples with shorter T2 relaxation times (like polymers or solids) cannot be measured with the fast t2_cp_mb software, but have to be analyzed with the t2_se_mb application.

5.4.2. Curve Fitting Rules

The following recommendations are valid for all relaxation time applications:

1. Data Points Spacing
   It has been shown in an unchanged time period that always those data point fits have delivered best accuracy and stability which could operate with the highest number of data points. Therefore the operator should always try to chose a high number of data points.

2. Bi-Exponential Fitting

Multiple experiments have proven that the results for samples with two components and small differences in relaxation times may scatter. Two components with significant differences in relaxation times (maybe factor 10) can be analyzed very reproducible.

3. Fitting Offset and Dynamic Range of Data Points
   Adjust parameters in a way that the dynamic range of the data points becomes as big as possible. Adjust the time base so that the remaining offset on the right side of the time axis becomes a minimum. Note that small dynamic ranges and big offsets lead to unstable relaxation time results.

4. Comparison of Different Samples
   If a number of samples should be analyzed with the minispec and distinguished e.g. by their relaxation times, fix the parameters of the application in a way that makes them suitable for all samples of interest. Analyze all samples with one and the same set of parameters. This allows to check for differences in relaxation time parameters best.

5. Amplitudes of Components
   All relaxation time applications deliver also amplitudes of components. They can be used for quantitative analysis. Note that those amplitudes depend also upon sample weights and tube positions in the probe. Therefore calculations with amplitudes require the knowledge of the sample weights and the tubes have to be positioned in the linear responding area of the probe head. It is essential that for comparisons of amplitudes the application parameters remain unchanged - otherwise no comparisons are possible.

5.5. Preparing the Applications

Application files are available to the user via the button 'minispec Applications'. Browsing to the directory 'APPV400x' (later versions 'Application Pool Version x.x') will display the complete pool of minispec applications. In this pool also the relaxation time applications are included.

The sub-directory 'Additionals' in 'minispec\TestApps' contains applications which are mainly intended for programming language ExpSpel teaching purposes.

Application files (**.app*) are viewed and edited by pressing the <EXPSPEL> editor button (empty sheet of paper).

5.5.1. Preparing the Application *t1_ir_mb*

It is highly recommended that you work with a copy of an application. Do not work with the original file *t1_ir_mb*.

1. **Open a Copy of the Original Application *t1_ir_mb***

To copy an existing application to a new name, make sure that the application that should be copied is active. Use the 'File' 'Copy Application File as' function and select the new name.

Return to the minispec software and load the new copy of your application as described in the minispec applications manual.

The minispec is now ready for adjustment and calibration with this application.

2. **Set Parameters for the Working Application**

After the new application is loaded, prepare it for use through the following steps (see below for details):

- Adjust the application parameters ('Acquisition Parameter Table' from the 'Parameter' menu).
- Configure the pulse sequence variables and other settings through the 'Application Configuration Table' from the 'Parameter' menu.

Adjust the application parameters through the 'Acquisition Parameter Table':

Number of Scans:	normally 1 to 4 but depends on signal-to-noise
Recycle delay:	set to 5 to 10 times longer than the anticipated longest T_1
Analogue filter:	narrow to reduce noise, broad if early sampling is required (see Delay Sample Window below)
Digital filter:	20000
Detection mode:	real
Offset compensation:	off

Configure pulse sequence variables and user options through the 'Application Configuration Table'.

5.5.2. Preparing the Application *t1_sr_mb*

See preparation procedure of t1_ir_mb application (chapter 5.5.1.).
Adjust the application parameters through the 'Acquisition Parameter Table':

Number of Scans:	normally 1 to 4 but depends on signal-to-noise
Recycle delay:	set to 5 to 10 times longer than the anticipated longest T_1
Analogue filter:	narrow to reduce noise, broad if early sampling is required (see Delay Sample Window below)
Digital filter:	20000
Detection mode:	magnitude
Offset compensation:	off

Configure pulse sequence variables and user options through the 'Application Configuration Table'.

5.5.3. Preparing the Application *t2_cp_mb*

See preparation procedure of t1_ir_mb application (chapter 5.5.1.).
Adjust the application parameters through the 'Acquisition Parameter Table':

Number of Scans:	normally 1 to 4 but depends on signal-to-noise
Recycle delay:	set to 5 to 10 times longer than the anticipated longest T_1
Analogue filter:	narrow to reduce noise, broad if early sampling is required (see Delay Sample Window below)
Digital filter:	20000
Detection mode:	real
Offset compensation:	off

Configure pulse sequence variables and user options through the 'Application Configuration Table'.

5.5.4. Preparing the Application *t2_se_mb*

See preparation procedure of t1_ir_mb application (chapter 5.5.1.).
Adjust the application parameters through the 'Acquisition Parameter Table':

Number of Scans:	normally 1 to 4 but depends on signal-to-noise
Recycle delay:	set to 5 to 10 times longer than the anticipated longest T_1
Analogue filter:	narrow to reduce noise, broad if early sampling is required (see Delay Sample Window below)
Digital filter:	20000
Detection mode:	magnitude
Offset compensation:	off

Configure pulse sequence variables and user options through the 'Application Configuration Table'.

Note that for all applications (minimum application version 5.0) an enabled 'Phase Cycling Mode' (see 'Application Configuration Table') may have influence on the number of scans performed. If 'Phase Cycling' is active, the number of scans is always a multiple of 4.

6. T1 MEASUREMENT PROCEDURES

6.1. Application t1_ir_mb Options

Application *t1_ir_mb*: Inversion Recovery Pulse Sequence

$\{[RD - 180_0 - IR\ Delay - 90_0 - RDT - asd]_{NS}\}_{N}$ with increment in IR Delay (IR delay = IR Delay * Factor)

where:

RD is the recycle delay (user-defined in the PARAMETER menu)

IR Delay is an incremented inversion recovery delay interval (user-defined starting value in the Application Configuration Table)

RDT is a delay for receiver dead time (user-defined value in the Application Configuration Table)

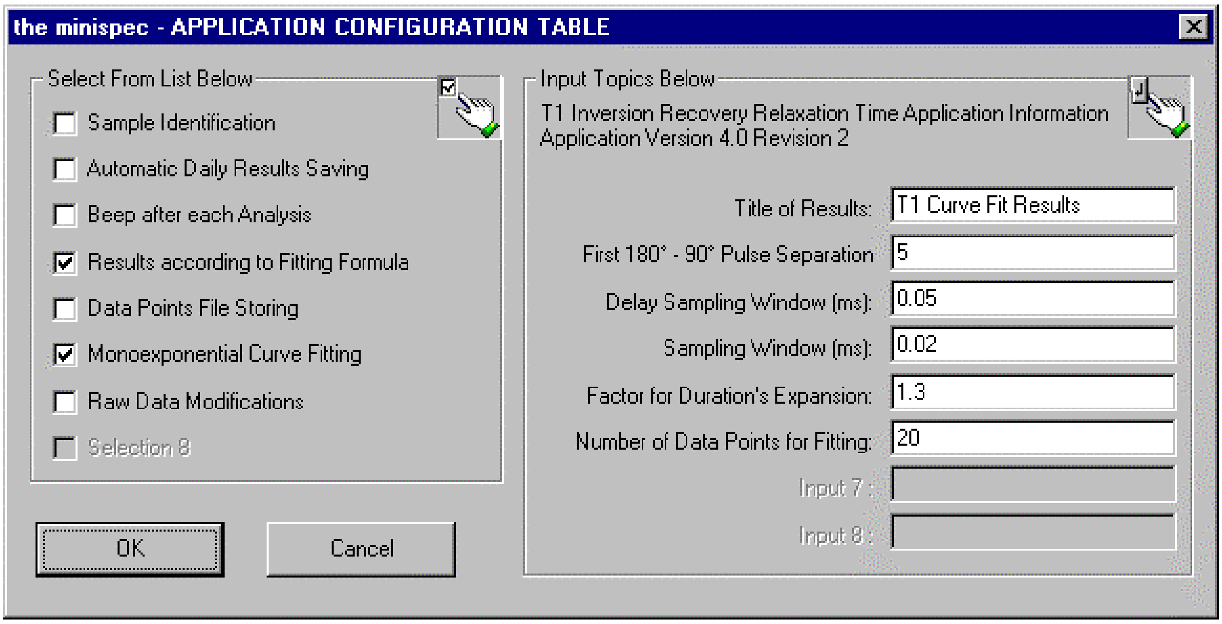
NS is the number of scans for signal averaging (user-defined in PARAMETER <F4> menu)

N is the number of points to be collected (user-defined value in the Application Configuration Table)

FACTOR: After each measurement, IR Delay is multiplying by the duration factor to determine the IR Delay for the next experiment.

6.2. Configuring t1_ir_mb Options

Configure pulse sequence variables and user options through the 'Application Configuration Table'. This table looks as follows (might look different in later application versions):



the minispec - APPLICATION CONFIGURATION TABLE

Select From List Below

☐ Sample Identification
☐ Automatic Daily Results Saving
☐ Beep after each Analysis
☑ Results according to Fitting Formula
☐ Data Points File Storing
☑ Monoexponential Curve Fitting
☐ Raw Data Modifications
☐ Selection 8

Input Topics Below

T1 Inversion Recovery Relaxation Time Application Information
Application Version 4.0 Revision 2

Title of Results:	T1 Curve Fit Results
First 180° - 90° Pulse Separation:	5
Delay Sampling Window (ms):	0.05
Sampling Window (ms):	0.02
Factor for Duration's Expansion:	1.3
Number of Data Points for Fitting:	20
Input 7 :	
Input 8 :	

" Sample Identification:"

Check mark this option if the user should be prompted for a corresponding sample ID at the time of measurement. The sample ID will be printed with the results and, if data storing is chosen, will be used as the data filename.

Do not check mark *this option* to use the default sample numbering. The first sample measured will be labeled sample 1, and the sample number will be incremented after each sample is measured.

"Automatic Daily Results Saving:"

To control results storing. If this option is selected, the program automatically creates a saved copy of the results on the hard disk. This option is useful for archiving after acquisition and results output.

"Beep after Analysis:"

To generate a PC noise after termination of the measurement. This option is useful if not too much time after measurements should be wasted.

"Results according to Fitting Formula:"

Check mark this option to cause the fit amplitude results (the amplitudes at the origin for each component) to be listed according to the signal reading at the origin as projected by the fit, regardless of the fraction of signal this component constitutes in the total signal.

Do not check mark to cause the fit amplitude results to be given as a fraction of the total signal. i.e. if two components A and B are detected at a ratio of 2:1 respectively, Component A will be listed as 66.6 %, component B will be listed as 33.3 %.

"Data Points File Storing:"

To control data points storing. If this option is selected, the program automatically creates a saved copy of the X-Y data pairs on the hard disk. This option is useful for archiving after acquisition, and/or for fitting with other functions not available in *t1_ir_mb* (refer to the test application *fit_rel*).

"Monoexponential Curve Fitting:"

To select either mono- or bi-exponential fitting. If no exponential decay is detected, the average value of the amplitudes is calculated. If bi-exponential is selected (monoexponential curve fitting is not check marked) and the fitting routine detects only one exponential, the routine defaults to mono-exponential fit.

"Raw Data Modifications:"

To select whether or not the fit routine should allow the user to preview data points, decide if any data points should be deleted, and/or re-fit the data.

"Phase Cycling:" (not in Application Versions lower than V5.0)

Phase cycling is a feature to reduce instrumental errors to a minimum. Therefore it is recommended to enable this option. Note that the number of scans will always be a multiple of 4 when phase cycling is active.

" Title of Results (not more than 24 digits):"

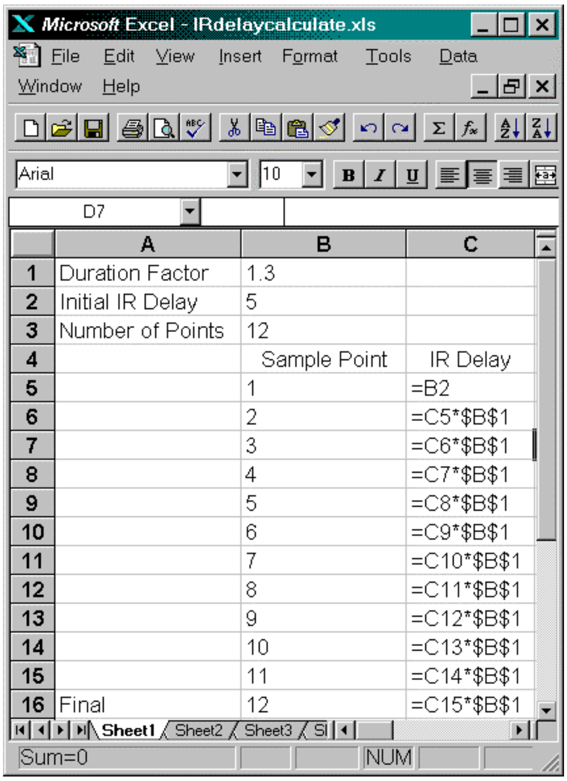
If desired, a custom title can be entered. Up to 24 characters will fit in the space allotted. Normally, accept the default: T1 Curve Fit Results.

"First 180° - 90° Pulse Separation: (values in ms)"

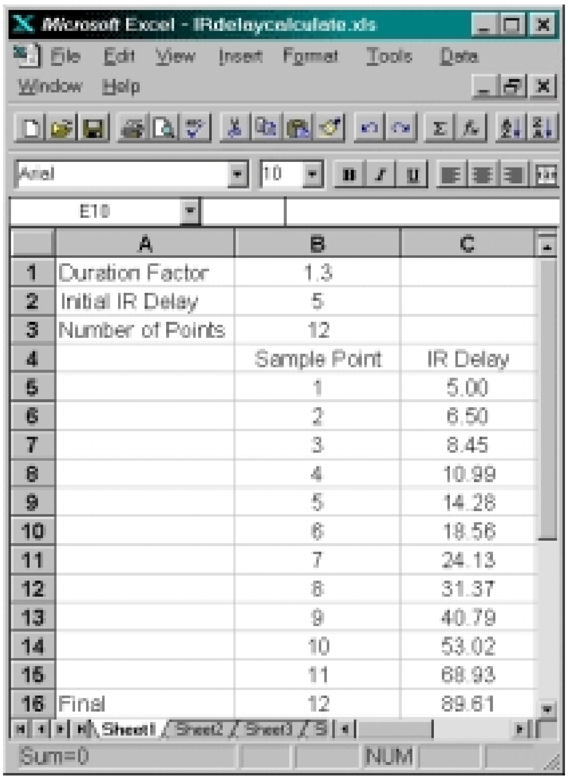
To set the initial Inversion Recovery (IR) Delay (delay between the 180° and 90° pulse); default value is 5 ms. The first inversion recovery pulse sequence is executed using the initial IR Delay. After the first measurement and each time the pulse sequence is repeated, the IR Delay is lengthened and the new value is used in the next determination.

e.g. First IR Delay =initial IR Delay
Second IR Delay = initial IR Delay * Factor
all points thereafter:
Next IR Delay = present IR Delay ∗ Factor

Calculate the final delay by considering the Initial IR Delay, the Factor, and the Number of Points collected. This could be accomplished with a simple spreadsheet that determines the next entry in a column of values by multiplying the present value by a constant value:



	A	B	C
1	Duration Factor	1.3	
2	Initial IR Delay	5	
3	Number of Points	12	
4		Sample Point	IR Delay
5		1	=B2
6		2	=C5*B1
7		3	=C6*B1
8		4	=C7*B1
9		5	=C8*B1
10		6	=C9*B1
11		7	=C10*B1
12		8	=C11*B1
13		9	=C12*B1
14		10	=C13*B1
15		11	=C14*B1
16	Final	12	=C15*B1



	A	B	C
1	Duration Factor	1.3	
2	Initial IR Delay	5	
3	Number of Points	12	
4		Sample Point	IR Delay
5		1	5.00
6		2	6.50
7		3	8.45
8		4	10.99
9		5	14.28
10		6	18.56
11		7	24.13
12		8	31.37
13		9	40.79
14		10	53.02
15		11	68.93
16	Final	12	89.61

"Delay Sampling Window:"

To set the Delay of Sampling Window, in ms. From within this window, 16 data points are collected and averaged to produce the data point for that particular IR Delay.

"Sampling Window:"

To set the Sampling Window width, in ms. From this window, 16 data points are collected and averaged to produce the data point for that particular IR Delay.

"Factor for Duration's Expansion:"

To set the inversion recovery delay multiplication Factor. This factor controls how closely spaced in time the data points will be. Note that, while the Factor does not have limits, it impacts on the calculation of the IR Delay, and the IR Delay does have a limit. Please refer to the discussion on IR Delay above for guidelines.

"Number of Data Points for Fitting:"

To set the Number of Points, which is equal to the number of experiments to be repeated and corresponding points that will be collected. Fitting as few as 10 points is common for a mono-exponential decay. For bi-exponential decays, 20 to 100 points may be beneficial. The maximum number of points is 256. Note that the Number of Points impacts on the number of IR Delays that must be calculated, and the IR Delay does have a maximum value. Please refer to the discussion on IR Delay above for guidelines.

In Application Versions V5.0 or higher the 'Factor for Duration's Expansion' is no longer requested. Instead the value for the last desired duration has to be entered. Afterwards the program calculates the duration factor and also the total measurement time is displayed. The operator has to confirm the total measurement time and can therefore avoid running into too long experiments.

6.3. Routine Measurements with Soft-EDM t1_ir_mb

Load the application that contains the parameters that are most nearly suitable for the T_1 in your sample. If you would like a hard copy of results, set the blue Windows banner on the results window and select 'Print' from the 'File' menu.

Remember to condition the samples to a defined temperature.

Care must be taken to use the correct 90 and 180° pulse. Samples of similar type (*e.g.* all aqueous *vs.* organic, volume, and molarity) can be measured all together without tuning in between. Samples that differ considerably will require prior tuning of the instrument using 'Update Settings'.

Incorrectly set pulse lengths will result in X-Y magnetism and an error in the estimated T_1 due to super-imposed T_2 relaxation. Tune/calibrate the instrument by running 'Update Settings' using a suitable test sample or an experimental sample if T_1 is reasonable short (e.g. 400 ms or less). If no sample is available with suitably short T_1, then the Recycle Delay for 'Update Settings' must be set longer (5 to 10 times) than the estimated T_1 of the available sample, and 'Update Settings' should be run with this longer delay.

Press MEASURE to start the sample series. If the sample ID option is turned on, the user will be prompted to enter a two-part identifier. The first part accepts alphanumeric entries of up to ten characters. The second part must be a numeric entry of up to 3 characters.

If T_1 is poorly known, it is normal to perform a survey measurements to decide what conditions will be needed to best determining the relaxation time constant. Initial values for IR Delay and Factor should be set so that approximately half of the measured amplitudes are negative and the rest are positive.
Recall that the maximum IR Delay = 100 sec. Refer to section 6.2. for tips on how to ensure that the IR Delay will not exceed the 100 sec limit.

PARAMETERS can be changed to suit the particular sample. For example, Recycle Delay may need to be adjusted, normally at least 5 times the anticipated value of the longest T_1. If Gain needs to be changed because of a SIGNAL CLIP error, it is recommended to tune the detection angles at the new gain settings (select 'Update Settings' and uncheck 'Update all Settings' - afterwards check mark 'Detection Angles').

Progress during measurements is given by message in the MESSAGEBOX, "Measuring Point No. <#>" and by the Number of Scans countdown in the right lower corner of the display.

If data storing is selected (see section 6.2.), but sample ID labels are not, data is stored with time coordinates in the filename YYMMDDHRMNSD. The actual title will be listed in the Resultbox after measurement is complete.

If data storing and sample ID labels are turned on then the file name is set to the two part sample ID. The actual title will be listed in the Resultbox after measurement is complete.

6.4. Application t1_sr_mb Options

Application *t1_sr_mb*: Saturation Recovery Pulse Sequence

$\{[\mathrm{RD} - 90_0 - \mathrm{SR\ Delay} - 90_0 - \mathrm{RDT} - \mathrm{asd}]_{\mathrm{NS}}\}_{\text{N with increment in SR Delay (SR delay = SR Delay * Factor)}}$

where:

RD is the recycle delay (user-defined in the PARAMETER menu)

SR Delay is an incremented saturation recovery delay interval (user-defined starting value in the Application Configuration Table)

RDT is a delay for receiver dead time (user-defined value in the Application Configuration Table)

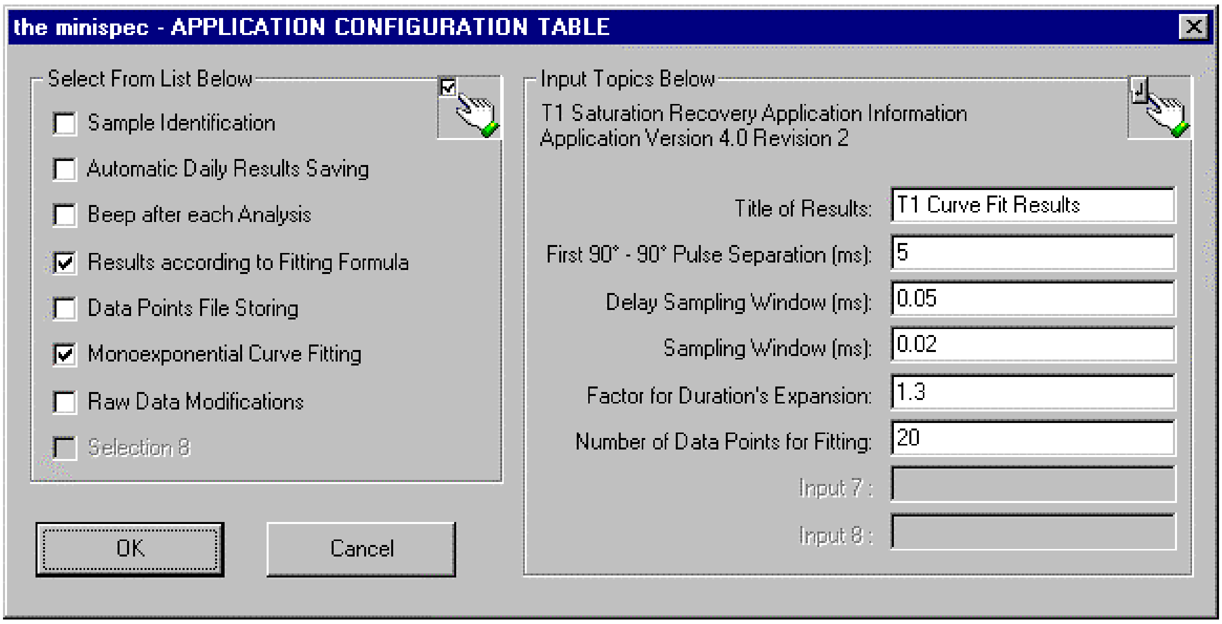
NS is the number of scans for signal averaging (user-defined in the PARAMETER menu)

N is the number of points to be collected time (user-defined value in the Application Configuration Table)

FACTOR: After each measurement, SR Delay is multiplying by the duration factor to determine the SR Delay for the next experiment.

6.5. Configuring t1_sr_mb Options

Configure pulse sequence variables and user options through the 'Application Configuration Table'. This table looks as follows:



the minispec - APPLICATION CONFIGURATION TABLE

Select From List Below

- ☐ Sample Identification
- ☐ Automatic Daily Results Saving
- ☐ Beep after each Analysis
- ☑ Results according to Fitting Formula
- ☐ Data Points File Storing
- ☑ Monoexponential Curve Fitting
- ☐ Raw Data Modifications
- ☐ Selection 8

Input Topics Below

T1 Saturation Recovery Application Information
Application Version 4.0 Revision 2

Title of Results: T1 Curve Fit Results
First 90° - 90° Pulse Separation (ms): 5
Delay Sampling Window (ms): 0.05
Sampling Window (ms): 0.02
Factor for Duration's Expansion: 1.3
Number of Data Points for Fitting: 20
Input 7 :
Input 8 :

" Sample Identification:"

Check mark this option if the user should be prompted for a corresponding sample ID at the time of measurement. The sample ID will be printed with the results and, if data storing is chosen, will be used as the data filename.

Do not check mark *this option* to use the default sample numbering. The first sample measured will be labeled sample 1, and the sample number will be incremented after each sample is measured.

"Automatic Daily Results Saving:"

To control results storing. If this option is selected, the program automatically creates a saved copy of the results on the hard disk. This option is useful for archiving after acquisition and results output.

"Beep after Analysis:"

To generate a PC noise after termination of the measurement. This option is useful if not too much time after measurements should be wasted.

"Results according to Fitting Formula:"

Check mark this option to cause the fit amplitude results (the amplitudes at the origin for each component) to be listed according to the signal reading at the origin as projected by the fit, regardless of the fraction of signal this component constitutes in the total signal.

Do not check mark to cause the fit amplitude results to be given as a fraction of the total signal. i.e. if two components A and B are detected at a ratio of 2:1 respectively, Component A will be listed as 66.6 %, component B will be listed as 33.3 %.

"Data Points File Storing:"

To control data points storing. If this option is selected, the program automatically creates a saved copy of the X-Y data pairs on the hard disk. This option is useful for archiving after acquisition, and/or for fitting with other functions not available in *t1_ir_mb* (refer to the test application *fit_rel*).

"Monoexponential Curve Fitting:"

To select either mono- or bi-exponential fitting. If no exponential decay is detected, the average value of the amplitudes is calculated. If bi-exponential is selected (monoexponential curve fitting is not check marked) and the fitting routine detects only one exponential, the routine defaults to mono-exponential fit.

"Raw Data Modifications:"

To select whether or not the fit routine should allow the user to preview data points, decide if any data points should be deleted, and/or re-fit the data.

" Title of Results (not more than 24 digits):"

If desired, a custom title can be entered. Up to 24 characters will fit in the space allotted. Normally, accept the default: T1 Curve Fit Results.

"First 90° - 90° Pulse Separation (ms):"

To set the initial Saturation Recovery (SR) Delay (delay between the 90° and 90° pulse); default value is 5 ms. The first saturation recovery pulse sequence is executed using the initial SR Delay. After the first measurement and each time the pulse sequence is repeated, the SR Delay is lengthened and the new value is used in the next determination.

e.g. First SR Delay = initial SR Delay
Second SR Delay = initial SR Delay * Factor
all points thereafter:
Next SR Delay = present SR Delay * Factor

Calculate the final delay by considering the Initial SR Delay, the Factor, and the Number of Points collected. This could be accomplished with a simple spreadsheet that determines the next entry in a column of values by multiplying the present value by a constant value (see also section 6.2.).

"Delay Sampling Window:"

To set the Delay of Sampling Window, in ms. From within this window, 16 data points are collected and averaged to produce the data point for that particular SR Delay.

"Sampling Window:"

To set the Sampling Window width, in ms. From this window, 16 data points are collected and averaged to produce the data point for that particular SR Delay.

"Factor for Duration's Expansion:"

To set the saturation recovery delay multiplication Factor. This factor controls how closely spaced in time the data points will be. Note that, while the Factor does not have limits, it impacts on the calculation of the SR Delay, and the SR Delay does have a limit. Please refer to the discussion on SR Delay above for guidelines.

"Number of Data Points for Fitting:"

To set the Number of Points, which is equal to the number of experiments to be repeated and corresponding points that will be collected. Fitting as few as 10 points is common for a mono-exponential decay. For bi-exponential decays, 20 to 100 points may be beneficial. The maximum number of points is 256. Note that the Number of Points impacts on the number of SR Delays that must be calculated, and the SR Delay does have a maximum value. Please refer to the discussion on SR Delay above for guidelines.

In Application Versions V5.0 or higher the 'Factor for Duration's Expansion' is no longer requested. Instead the value for the last desired duration has to be entered. Afterwards the program calculates the duration factor and also the total measurement time is displayed. The operator has to confirm the total measurement time and can therefore avoid running into too long experiments.

6.6. Routine Measurements with Soft-EDM t1_sr_mb

Load the application that contains the parameters that are most nearly suitable for the T_1 in your sample. If you would like a hard copy of results, set the blue Windows banner on the results window and select 'Print' from the 'File' menu.

Remember to condition the samples to a defined temperature.

Care must be taken to use the correct 90° pulse. Samples of similar type (*e.g.* all aqueous *vs.* organic, volume, and molarity) can be measured all together without tuning in between. Samples that differ considerably will require prior tuning of the instrument using the automatic 'Update Settings' routine.

An incorrectly set pulse length will result in unexpected X-Y magnetism and an error in the estimated T_1 due to super-imposed magnetization. Tune / calibrate the instrument by running 'Update Settings' using a suitable test sample or an experimental sample if T_1 is reasonable short (e.g. 400 ms or less). If no sample is available with suitably short T_1, then the Recycle Delay for

'Update Settings' must be set longer (5 to 10 times) than the estimated T_1 of the available sample, and 'Update Settings' should be run with this longer delay.

Press MEASURE to start the sample series. If the sample ID option is turned on, the user will be prompted to enter a two-part identifier. The first part accepts alphanumeric entries of up to ten characters. The second part must be a numeric entry of up to 3 characters.

If T_1 is poorly known, it is normal to perform a survey measurements to decide what conditions will be needed to best determining the relaxation time constant. Initial values for SR Delay and Factor should be set so that approximately half of the measured amplitudes are negative and the rest are positive.

PARAMETERS can be changed to suit the particular sample. For example, Recycle Delay may need to be adjusted, normally at least 3 times the anticipated value of the longest T_1. If Gain needs to be changed because of a SIGNAL CLIP error, it is recommended to tune the detection angles at the new gain settings (select 'Update Settings' and uncheck 'Update all Settings' - afterwards check mark 'Detection Angles').

Progress during measurements is given by message in the RESULTBOX, "Measuring Point No. <#>" and by the Number of Scans countdown in the right lower corner of the display.

If data storing is selected (see section 6.5.), but sample ID labels are not, data is stored with time coordinates in the filename YYMMDDHRMNSD. The actual title will be listed in the Resultbox after measurement is complete.

If data storing and sample ID labels are turned on then the file name is set to the two part sample ID. The actual title will be listed in the Resultbox after measurement is complete.

7. T2 MEASUREMENT PROCEDURES

7.1. Application t2_cp_mb Options

The pulse sequence employed is the Carr-Purcell-Meiboom-Gill (CPMG) spin echo method:

CPMG Pulse Sequence:

$\{RD - 90_0 - tau - [(180_{90} - tau -)_{DE}\ (180_{90} - tau -)\ asd - tau -]_N\}_{NS}$

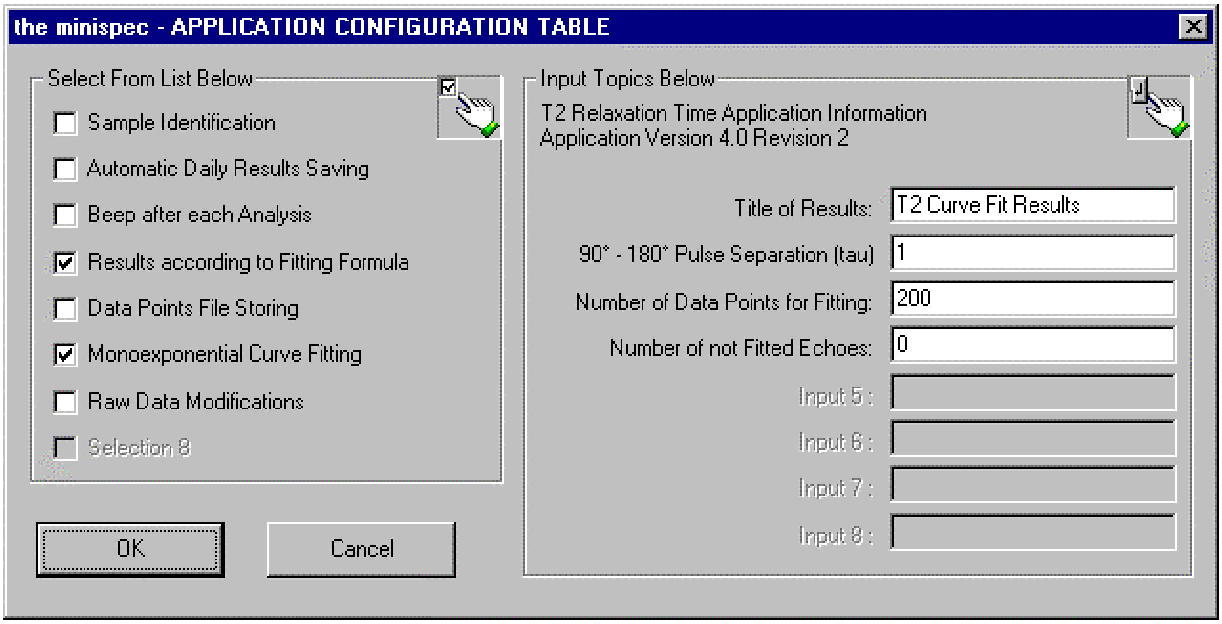
where:

RD is the recycle delay (user-defined in the PARAMETER menu)
tau is a delay interval (user-defined value in the Application Configuration Table)
DE is the number of dummy echoes before an echo is sampled (user-defined value in the Application Configuration Table)
e.g. if DE is 1 every second echo is sampled, if DE is 3 every fourth echo is sampled, etc.
N is the number of points to be collected (user-defined value in the Application Configuration Table)
NS is the number of scans for signal averaging (user defined in the PARAMETER menu)

Further explanation: The pulse sequence begins with a 90° excitation pulse, followed by a waiting interval (referred to as *tau*) in which spins evolve. A 180° pulses that is phase shifted in the X-Y plane by 90° relative to the 90° excitation pulse, reverses the sense of the evolving vectors, and after another period tau, causes an echo to form. Acquisition of a single sample point occurs at sampled echoes. This sequence of phase shifted 180° pulses, with evolution interval tau, and sampling is repeated to collect all the points necessary for describing the signal decay. In most cases the data is not sampled at each echo. Dummy echoes are echoes in which no sampling point is collected. Use of dummy odd number echoes causes the program to sample at every (e.g.) 2nd, 4th, 6th … or 20th echo. The user may set the number of dummy echoes in the Application Configuration Table.

7.2. Configuring t2_cp_mb Options

Configure pulse sequence variables and user options through the 'Application Configuration Table'. This table looks as follows:



the minispec - APPLICATION CONFIGURATION TABLE

Select From List Below

☐ Sample Identification
☐ Automatic Daily Results Saving
☐ Beep after each Analysis
☑ Results according to Fitting Formula
☐ Data Points File Storing
☑ Monoexponential Curve Fitting
☐ Raw Data Modifications
☐ Selection 8

Input Topics Below

T2 Relaxation Time Application Information
Application Version 4.0 Revision 2

Title of Results: T2 Curve Fit Results
90° - 180° Pulse Separation (tau): 1
Number of Data Points for Fitting: 200
Number of not Fitted Echoes: 0
Input 5 :
Input 6 :
Input 7 :
Input 8 :

“ Sample Identification:”

Check mark this option if the user should be prompted for a corresponding sample ID at the time of measurement. The sample ID will be printed with the results and, if data storing is chosen, will be used as the data filename.

Do not check mark *this option* to use the default sample numbering. The first sample measured will be labeled sample 1, and the sample number will be incremented after each sample is measured.

“Automatic Daily Results Saving:”

To control results storing. If this option is selected, the program automatically creates a saved copy of the results on the hard disk. This option is useful for archiving after acquisition and results output.

“Beep after Analysis:”

To generate a PC noise after termination of the measurement. This option is useful if not too much time after measurements should be wasted.

“Results according to Fitting Formula:”

Check mark this option to cause the fit amplitude results (the amplitudes at the origin for each component) to be listed according to the signal reading at the origin as projected by the fit, regardless of the fraction of signal this component constitutes in the total signal.

Do not check mark to cause the fit amplitude results to be given as a fraction of the total signal. i.e. if two components A and B are detected at a ratio of 2:1 respectively, Component A will be listed as 66.6 %, component B will be listed as 33.3 %.

"Data Points File Storing:"

To control data points storing. If this option is selected, the program automatically creates a saved copy of the X-Y data pairs on the hard disk. This option is useful for archiving after acquisition, and/or for fitting with other functions not available in *t1_ir_mb* (refer to the test application *fit_rel*).

"Monoexponential Curve Fitting:"

To select either mono- or bi-exponential fitting. If no exponential decay is detected, the average value of the amplitudes is calculated. If bi-exponential is selected (monoexponential curve fitting is not check marked) and the fitting routine detects only one exponential, the routine defaults to mono-exponential fit.

"Raw Data Modifications:"

To select whether or not the fit routine should allow the user to preview data points, decide if any data points should be deleted, and/or re-fit the data.

"Phase Cycling:" (not in Application Versions lower than V5.0)

Phase cycling is a feature to reduce instrumental errors to a minimum. Therefore it is recommended to enable this option. Note that the number of scans will always be a multiple of 4 when phase cycling is active.

" Title of Results (not more than 24 digits):"

If desired, a custom title can be entered. Up to 24 characters will fit in the space allotted. Normally, accept the default: T2 Curve Fit Results.

" 90° - 180° Pulse Separation (tau):"

To set the delay *tau* in ms. This factor controls how closely spaced in time the data points will be. Note that *tau* does have limits: $0.04 < tau < 100$.

" Number of Data Points for Fitting:"

To set the Number of Points, which is equal to the number of echoes to be sampled. Fitting as few as 100 points is common for a mono-exponential decay. For bi-exponential decays, 200 to 250 points may be beneficial. The maximum number of points is 256.

" Number of not Fitted Echoes:"

To set the number of Dummy Echoes. A combination of the size of tau, the number of dummy echoes, and the number of points controls the length of the acquisition period.

7.3. Routine Measurements with Soft-EDM t2_cp_mb

The CPMG pulse sequence is used in order to eliminate the effect of slightly miss-set 180° pulse widths. The sequence also minimizes errors due to diffusion effects and J-modulation. However, care must be taken not to set *tau* too long relative to the likely rate of diffusion.

A tau limit of 1 msec is recommended to eliminate J-mod and diffusion effects. However, tau is not actually limited in the application and can be set much longer if desired.

This is particularly true when measuring long T_2s in aqueous solutions, but can also be a problem in organic solvents that have high self diffusion coefficients (see also table of self diffusion coefficients in the Bruker Almanac). Diffusion within the sample between pulses causes the signal to decay more rapidly than decay due to T_2 alone. Hence, diffusion causes an error (lower value) for the estimated T_2.

Load the application that contains the parameters which are most nearly suitable for the T_2 in your sample. If you would like a hard copy of results, set the blue Windows banner on the results window and select 'Print' from the 'File' menu.

Remember to condition the samples to a defined temperature.

Tune / calibrate the instrument by running the 'Update Settings' routine using a suitable test sample or an experimental sample if the T_1 is reasonable short (e.g. 400 ms or less). If no sample is available with a suitably short T_1, then the Recycle Delay for 'Update Settings' must be set longer (5 to 10 times) than the estimated T_1 of the available sample, and 'Update Settings' should be run with this longer delay.

Like samples can be measures all together without tuning. Very different samples should be run after tuning the instrument.

Press Measure to start the sample series. If the sample ID option is turned on, the user will be prompted to enter a two-part identifier. The first part accepts alphanumeric entries of up to ten characters. The second part must be a numeric entry of up to 3 characters.

If T_2 is poorly known, it is normal to perform a survey measurement to decide what conditions will be needed to determine the true relaxation time constant.

PARAMETERS can be changed to suit the particular sample. For example, Recycle Delay may need to be adjusted, normally to at least 5 times the anticipated value of the longest T_1. If Gain needs to be changed because of a SIGNAL CLIP error, and real detection mode is being used, it may be necessary to tune the detection angles at the new gain settings (select 'Update Settings' and uncheck 'Update all Settings' - afterwards check mark 'Detection Angles').

It is recommended that, for unknown systems, the CPMG data should be acquired using several tau values in order to detect chemical exchange or diffusion. See also M.C. Vackier, D.N. Rutledge, Journal of Magnetic Resonance Analysis, 2, 1996, 311-32, 311-320.

If data storing is selected (see section 7.2.), but sample ID labels are not, data is stored with time coordinates in the filename YYMMDDHRMNSD. The actual title will be listed in the Resultbox after measurement is complete

If data storing and sample ID labels are turned on then the file name is set to the two part sample ID. The actual title will be listed in the Resultbox after measurement is complete.

General Rules of Thumb for T2 set-up:
For most reproducible results:

Tau and number of points should be chosen so that each sample is measured over the same intensity range (i.e. if the first data point collected is measured at 80 % full scale, the last point measured should be at the same % intensity (offset) for each sample. Results may be best for offsets from 1 to 5 % - not higher than 5 %.).

Start by doing a set-up run where the last signal collected will be much longer than T2 (at least 5 x T2).

Note the Y-axis intensity on the left side of the display (Y-left). Move to the data point where the Y-axis intensity is only 1 to 5 % of the Y-left intensity (Y-right). Take note of the time distance between Y-left and Y-right. This is the required sampling period (X-diff).

Calculate the tau value:
Decide how many points will be collected.
Decide what working application copy will best fit the time X-diff.

Calculate the tau value from the appropriate equation:

Tau = sampling period / number of points x (dummy echos+1) x 2

e.g. if dummy echoes = 3, every 4th echo is sampled.

If set-up T2 determination shows a sampling period of 800 ms is necessary, and 100 points will be collected, tau = 1 ms

If tau is longer than 3 ms, you may have to shorten tau and collect more points instead (avoid diffusion, cross relaxation).

T2*: When analyzing a component that decays within 100 us (solids) the effect of the magnet inhomogeneity is minimal. In this case T2 = T2*. For this component, the FID measured after a 90 degree pulse provides the T2 decay constant by direct fitting.

To find appropriate parameters:

Tau must be short enough to avoid errors due to diffusion between pulses. Set tau appropriately.

The time taken by the total number of echoes must describe most of the decay curve.
The maximum number of points is 256. Fitting is faster with fewer points. Fitting 100 points is commonly enough for a mono-exponential decay. For bi-exponential decays, 200 to 250 points may be beneficial.

Set the DE so that the number of points collected adequately describes the relaxation curve.

The user may select either mono- or bi-exponential fitting. If bi-exponential is selected and the fitting routine detects only one exponential, the routine defaults to mono-exponential fit. In the case of mono- and bi-exponential fits, if no exponential decay is detected, the average value of the amplitudes is calculated.

7.4. Application t2_se_mb Options

The pulse sequence employed is the well-known Hahn Spin Echo method:

Hahn Spin Echo Sequence:

$\{[\mathrm{RD} - 90_0 - \mathrm{tau} - 180_{90} - \mathrm{tau} - \mathrm{asd} - \mathrm{tau}]_{\mathrm{NS}}\}_{\mathrm{N\ with\ increment\ in\ tau\ (tau\ =\ tau\ *\ Factor)}}$

where:

RD is the recycle delay (user-defined in the PARAMETER menu)

tau is an incremented delay interval (user-defined starting value in the Application Configuration Table)

NS is the number of scans for signal averaging (user-defined in the PARAMETER menu)

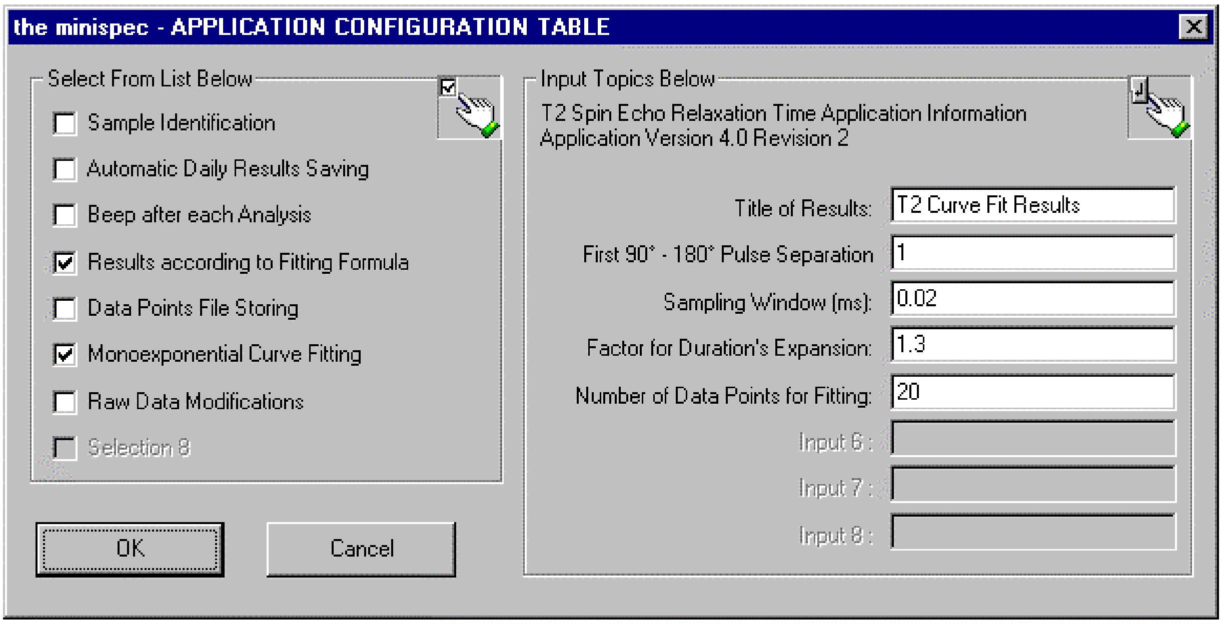
N is the number of points to be collected (user-defined value in the Application Configuration Table)

Factor: After each measurement, *tau* is multiplying by the duration factor to determine the *tau* for the next experiment.

Further explanation: The pulse sequence begins with a 90° excitation pulse, followed by a waiting interval (referred to as *tau*) in which spins evolve. A 180° pulses that is phase shifted in the X-Y plane by 90° relative to the 90° excitation pulse, reverses the sense of the evolving vectors, and after another period tau, causes an echo to form. Acquisition of a single sample point occurs at sampled echoes. Afterwards the recycle delay time passes and the same experiment is repeated *NS* times. Now the first data point is acquired and the measurement continues with the same sequence, but increased *tau* value. The number of data points defines the number of experiments. This means that the execution of this measurement with 20 data points will take approximately 20 times longer than a corresponding CPMG pulse sequence. However for polymers this sequence is a must.

7.5. Configuring t2_se_mb Options

Configure pulse sequence variables and user options through the 'Application Configuration Table'. This table looks as follows:



the minispec - APPLICATION CONFIGURATION TABLE

Select From List Below

☐ Sample Identification
☐ Automatic Daily Results Saving
☐ Beep after each Analysis
☑ Results according to Fitting Formula
☐ Data Points File Storing
☑ Monoexponential Curve Fitting
☐ Raw Data Modifications
☐ Selection 8

Input Topics Below

T2 Spin Echo Relaxation Time Application Information
Application Version 4.0 Revision 2

Title of Results: T2 Curve Fit Results
First 90° - 180° Pulse Separation: 1
Sampling Window (ms): 0.02
Factor for Duration's Expansion: 1.3
Number of Data Points for Fitting: 20
Input 6 :
Input 7 :
Input 8 :

" Sample Identification:"

Check mark this option if the user should be prompted for a corresponding sample ID at the time of measurement. The sample ID will be printed with the results and, if data storing is chosen, will be used as the data filename.

Do not check mark *this option* to use the default sample numbering. The first sample measured will be labeled sample 1, and the sample number will be incremented after each sample is measured.

"Automatic Daily Results Saving:"

To control results storing. If this option is selected, the program automatically creates a saved copy of the results on the hard disk. This option is useful for archiving after acquisition and results output.

"Beep after Analysis:"

To generate a PC noise after termination of the measurement. This option is useful if not too much time after measurements should be wasted.

"Results according to Fitting Formula:"

Check mark this option to cause the fit amplitude results (the amplitudes at the origin for each component) to be listed according to the signal reading at the origin as projected by the fit, regardless of the fraction of signal this component constitutes in the total signal.

Do not check mark to cause the fit amplitude results to be given as a fraction of the total signal. i.e. if two components A and B are detected at a ratio of 2:1 respectively, Component A will be listed as 66.6 %, component B will be listed as 33.3 %.

"Data Points File Storing:"

To control data points storing. If this option is selected, the program automatically creates a saved copy of the X-Y data pairs on the hard disk. This option is useful for archiving after acquisition, and/or for fitting with other functions not available in *t1_ir_mb* (refer to the test application *fit_rel*).

"Monoexponential Curve Fitting:"

To select either mono- or bi-exponential fitting. If no exponential decay is detected, the average value of the amplitudes is calculated. If bi-exponential is selected (monoexponential curve fitting is not check marked) and the fitting routine detects only one exponential, the routine defaults to mono-exponential fit.

"Raw Data Modifications:"

To select whether or not the fit routine should allow the user to preview data points, decide if any data points should be deleted, and/or re-fit the data.

"Phase Cycling:" (not in Application Versions lower than V5.0)

Phase cycling is a feature to reduce instrumental errors to a minimum. Therefore it is recommended to enable this option. Note that the number of scans will always be a multiple of 4 when phase cycling is active.

" Title of Results (not more than 24 digits):"

If desired, a custom title can be entered. Up to 24 characters will fit in the space allotted. Normally, accept the default: T2 Curve Fit Results.

"First 90° - 180° Pulse Separation:"

To set the delay *tau* between the two pulses. See explanations above.

"Sampling Window (ms):"

To set the window which is used for data acquisition.

As both values (sampling window and *tau*) are not independent from each other, the software always verifies whether the entered combinations are possible.

"Factor for Duration's Expansion:"

To define the duration factor and therefore the durations between the two RF pulses of the different experiments. The default value of the duration factor is 1.30.

"Number of Data Points for Fitting:"

To set the number of data points or experiments. This value fixes the number of Hahn-Echo sequences to be executed. Therefore a rough estimation of the total time of analysis can be done as follows:

$$\text{Total Time of Analysis} \cong \text{Number of Scans} \times \text{Recycle Delay Time} \times \text{Number of Points}$$

In Application Versions V5.0 or higher the 'Factor for Duration's Expansion' is no longer requested. Instead the value for the last desired duration has to be entered. Afterwards the program calculates the duration factor and also the total measurement time is displayed. The operator has to confirm the total measurement time and can therefore avoid running into too long experiments.

7.6. Routine Measurements with Soft-EDM t2_se_mb

The spin echo pulse sequence is used in cases where the CPMG sequence cannot be applied. Please note that the sequence is sensitive to sample diffusion. Therefore liquids will be examined with CPMG. In the case of fast relaxation diffusion and other effects can be neglected and the t2_se_mb software can be executed.

Load the application that contains the parameters which are most nearly suitable for the T_2 in your sample. If you would like a hard copy of results, set the blue Windows banner on the results window and select 'Print' from the 'File' menu.

Remember to condition the samples to a defined temperature.

Tune / calibrate the instrument by running the 'Update Settings' routine using a suitable test sample or an experimental sample if the T_1 is reasonable short (e.g. 400 ms or less). If no sample is available with a suitably short T_1, then the Recycle Delay for 'Update Settings' must be set longer (5 to 10 times) than the estimated T_1 of the available sample, and 'Update Settings' should be run with this longer delay.

Like samples can be measures all together without tuning. Very different samples should be run after tuning the instrument.

Press Measure to start the sample series. If the sample ID option is turned on, the user will be prompted to enter a two-part identifier. The first part accepts alphanumeric entries of up to ten characters. The second part must be a numeric entry of up to 3 characters.

If T_2 is poorly known, it is normal to perform a survey measurement to decide what conditions will be needed to determine the true relaxation time constant.

PARAMETERS can be changed to suit the particular sample. For example, Recycle Delay may need to be adjusted, normally to at least 5 times the anticipated value of the longest T_1. If Gain needs to be changed because of a SIGNAL CLIP error, and real detection mode is being used, it may be necessary to tune the detection angles at the new gain settings (select 'Update Settings' and uncheck 'Update all Settings' - afterwards check mark 'Detection Angles').

If data storing is selected (see section 7.5.), but sample ID labels are not, data is stored with time coordinates in the filename YYMMDDHRMNSD. The actual title will be listed in the Resultbox after measurement is complete

If data storing and sample ID labels are turned on then the file name is set to the two part sample ID. The actual title will be listed in the Resultbox after measurement is complete.

General Rules of Thumb for T2 set-up:

For most reproducible results:

Tau and number of points should be chosen so that each sample is measured over the same intensity range (i.e. if the first data point collected is measured at 80 % full scale, the last point measured should be at the same % intensity (offset) for each sample. Results may be best for offsets from 1 to 5 % - not higher than 5 %.).
Start by doing a set-up run where the last signal collected will be much longer than T2 (at least 5 x T2)

Note the Y-axis intensity on the left side of the display (Y-left). Move to the data point where the Y-axis intensity is only 1 to 5 % of the Y-left intensity (Y-right). Take note of the time distance between Y-left and Y-right. This is the required sampling period (X-diff).

Calculate the tau value:
Decide how many points will be collected.
Decide what working application copy will best fit the time X-diff.

To find appropriate parameters:

The time taken by the total number of echoes must describe most of the decay curve.
The maximum number of points is 256. Fitting 100 points is commonly enough for a mono-exponential decay & for bi-exponential decays, 200 to 250 points may be beneficial. However due to long experimental times, 20 respectively 40 data points should be measured at least.

Set the pulse sequence parameters so that the number of points collected adequately describes the relaxation curve.

The user may select either mono- or bi-exponential fitting. If bi-exponential is selected and the fitting routine detects only one exponential, the routine defaults to mono-exponential fit. In the case of mono- and bi-exponential fits, if no exponential decay is detected, the average value of the amplitudes is calculated.

8. ADVANCED TOPICS

8.1. T2 Relaxation Time Physical Principles

For a sample containing hydrogen atoms in a magnetic field B_0 the NMR resonance frequency (ω) is given by

$$\omega = \gamma \cdot B_0 \qquad (1)$$

where γ is the gyromagnetic ratio of the hydrogen nucleus.

If all the nuclei are in the same field (i.e. in a completely homogeneous field) then the NMR signal after a 90° pulse decays exponentially with time constant T_2 according to the equation

$$M_t = M_0 \,.\, \exp(-t / T_2) \qquad (2)$$

In practice, the perfect field condition is almost never achieved. The field is much more often inhomogeneous i.e. some nuclei “see” a higher field ($B_0 + \Delta B$) and some a lower field ($B_0 - \Delta B$) than the average B_0. Thus, within the sample there are nuclei with various resonance frequencies ($\omega, \omega + \Delta\omega, \omega - \Delta\omega$). After the 90° pulse is switched off all nuclei have the same phase. However, after a time t, phase differences are present as a result of the different precession speeds. These differences reduce the signal since this depends on the vector sum of the magnetic moments of all the rotating nuclei and the maximum signal is only obtained when all the individual components are parallel.

If the field distribution is rectangular (i.e. there is a linear field gradient across the sample) then the signal is described to a first approximation by

$$M_t = M_0 \;.\; \frac{\sin(\gamma.\Delta B.t)}{\gamma.\Delta B.t} \qquad (3)$$

where ΔB is the largest deviation of the field from the average value.

Provided that the individual nuclei do not change their positions there is a fixed relationship between their phases. In other words, the dephasing of the magnetization is, under the above condition, a reversible process. With a 180° pulse applied after a time τ one can reverse the motion of the spins and cause the nuclear moments to refocus after a time 2τ. They then dephase again. This process is referred to as a “spin-echo”.

For non-viscous liquids, the condition that the nuclei do not change their positions in order to ensure that they are in-phase again at time 2τ is often not met. As a result of diffusion the nuclei move in the time between the 90° and 180° pulses and may, in so doing, reach a position at which the field strength is different. The precession frequency is thus changed and the phase relationship irreversibly altered. After 2τ these nuclei are no longer in phase with the others and the signal amplitude is therefore reduced.

Under this condition the measured T_2 is smaller than the true T_2. To minimize this error, the sample size should be restricted to 1 cm so the sample is restricted to the area of best B_o and B_1 field homogeneity.

This is most simply accomplished by calibration using a substance of known T_2 (e.g. water at 25°C).

Application *t2_cp_mb* is the application most commonly used to measure NMR relaxation due to spin-spin interactions, as characterized by the time constant T_2.

Equations:

Mono-exponential fit

$$y = A * \exp -(x/\tau) + O$$

where:

A: amplitude at time zero

τ: T_2 decay constant

O: offset

Bi-exponential fit

$$y = A_1 * \exp -(x/\tau_1) + A_2 * \exp -(x/\tau_2) + O$$

where:

A_1, A_2: amplitudes of components 1 and 2 at time zero

τ_1, τ_2: T_2 decay constants of components 1 and 2

O: offset

9. PULSE PROGRAMMING

9.1. T2 Relaxation Time Pulse Program

```
program setup();

int     CNT;            # counter                                 #
int     count;          # counter                                 #
int     loop_count;     # loop counter, number of data points  #
int     ExpOrder;       # exponential order                    #
int     store;          # data pairs storing                      #
int     dummy_echos;    # dummy echoes                            #
real    duration;       # duration                                #
real    wait;           # waiting for data acquisition            #

loop_count = 50;
duration = 1.5;
dummy_echos = 1;

par;
   scans        (1);
   rd      (2.00);
   gain         (56);
   dbw          (100.00);
   abw          ("narrow");
   off_comp      ("off");
   det_mode     ("magnitude");
   dig_res      ("high");
endpar;

echo = input_int( "Input Number of Echoes without Data Acquisition (0...20).", echo );
   if ( ESC )
         return( FALSE );
   endif;

wait = duration / 10;
CNT = 0;
```

```
pulses;
    sp  ( 90, 0, -1 );
    sd  ( duration );
    while ( CNT < loop_count )
             count = 0;
             while ( count < echo )
                 sp  ( 180, 90, -1 );
                 sd  ( 2 * duration );
                 count = count + 1;
             endwhile;
             sp  ( 180,  90, -1 );
             sd  ( duration - wait / 2 );
             asd ( wait );
             sd  ( duration - wait / 2 );
             CNT = CNT + 1;
    endwhile;
endpulses;
```

9.2. T1 Relaxation Time Test Program

```
program setup ();                    # T1 Experiment 180 - 90 Pulse sequence      #

par;
    scans       (1);
    rd          (3.000000);
    gain        (88);
    dbw         (20000.00);          # digital filter bandwidth (kHz)             #
    abw         ("broad");           # analogue bandwidth                         #
    off_comp    ("off");
    det_mode    ("real");
    dig_res     ("high");
endpar;

program measure ();

int   no;                        # number of data                #
int   Loop_Count;                # loop counter                  #
int   cnt;                       # help variable                 #
int   CNT;                       # number of loops               #
int   ExpOrder, Order;           # exponential order             #
int   dec;                       # results mode                  #
int   store, loop;               # data pairs storing - help     #
int   id;                        # sample identification         #
int   out;                       # output actual data point      #
int   sample;                    # sample identification         #
int   modi;                      # data modifications            #
```

```
real  sx[10], sy[10];             # data points                        #
real  x[500], y[500];             # measured data                      #
real  a, b, c, f, g, h;           # variables for determinations       #
real  DUR, D;                     # pulse sequence parameters          #
real  DF;                         # pulse sequence parameter           #
real  WIN;                        # pulse sequence parameter           #
real  DSW;                        # pulse sequence parameter           #
real  Result[5];                  # result from fit                    #
real  Error[5];                   # result errors                      #
real  helptau, helpamp;           # help variables                     #
char ResS[16], ErrS[16];          # rounded results                    #
char text1[100];                  # resultbox title                    #
char str_buf[200];                # sample identification              #
char name[20];                    # datapairs filename                 #
pointer textp;                    # resultbox title                    #
pointer    txt;                   # sample identification              #

strcpy( str_buf, "1");

id            = 2;      sample       = 1;      CNT          = 20;
Loop_Count    = 10;     ExpOrder     = 1;      DUR          = 5;
DF            = 1.3;    WIN          = 0.01;   DSW          = 0.05;
dec           = 1;      store        = 0;      ExpOrder     = 1;
modi          = 2;      loop         = 0;      out          = 1;

print_line( CALIBRATION_FILE );
if ( ERROR )
      DUR        = get_real( CALIBRATION_FILE, "DUR" );
      CNT        = get_int( CALIBRATION_FILE, "LC" );
      ExpOrder   = get_int( CALIBRATION_FILE, "EO" );
      WIN        = get_real( CALIBRATION_FILE, "WIN" );
      DSW        = get_real( CALIBRATION_FILE, "DSW" );

while ( TRUE )
   while ( Loop_Count < CNT and Loop_Count < 500 )

      pulses;                     # pulse sequence generation      #
         sp  ( 180, 0, -1 );      # a 180 degree pulse             #
         sd  ( D );               # 'dur' milliseconds             #
         sp  ( 90,  0, -1 );      # a 90 degree pulse              #
         sd  ( DSW );             # wait for receiver dead time    #
         asd ( WIN );             # get a single data point        #
         D = D * DF;              # next duration                  #
      endpulses;                  # pulse sequence end             #

      is_sample_in;
```

```
        if ( ERROR )
            print_line( FLASHBOX, "No Sample Inserted !!!" );
            return( TRUE );
        endif;

            print_line( RESULTBOX, "Measuring Point No. <#>", Loop_Count + 1 );
        endif;

        measure;
        abscissa(-1, -1, sx);
        ordinate(-1, -1, sy);

        x[Loop_Count] = sx[0];
        y[Loop_Count] = sy[0];

        Loop_Count = Loop_Count + 1;

    endwhile;
    sample = sample + 1;

endwhile;
```

9.3. Program the Data Fitting

The command ***fit_monodecay*** fits a mono-exponential decay curve to the input data. The fit equation has the form:

$$y = A * \exp -(x/\tau) + O$$

where:

A: amplitude
τ: decay constant
O: offset

Prior to the command, the program must declare the necessary input and output arrays, and assign values to the input arrays. Pre-assign the Order to 1.

Previous variable declarations:

```
int order;                                      # Order of the fit, see explanation below.      #
real result[10], error[10];                     # Arrays for results output and their uncertainties,
                                                size of the arrays in brackets                  #
real x_array[positions], y_array[positions];    # Arrays for input data                         #
int number_of_data;                             # Number of data pairs                          #
```

Assign values to the input parameter number_of_data , and input arrays by direct copy of the NMR signal into x- and y-array OR from some combination of user input and signal evaluation (i.e. known concentration for x-array vs. weight-normalized NMR signal amplitude for y-array).

The default is to allow the function to include the offset as a fit variable. One can assign the fit Offset to a pre-defined value using:

off_presetting(0, *fixed_offset_value)**;*

To release the fixed Offset fit use:

off_presetting(-1, 0 ***);***

Syntax of the command:

order = ***fit_monodecay(*** x_array, y_array, number_of_data, result, error ***);***

'ERROR' is set if determination fails (not enough data pairs, matrix singularity ...).

After the fit, the routine sets a return value to order which is the actual order used. If mono-exponential fit is selected but no exponential decay is detected, the average of ordinate values will be calculated, and order will be set to 1 after the fit.

order = 1 means: fit function: $y = A * \exp -(x/\tau) + O$ was used
order = 0 means: the average value of the amplitude was calculated

Results array:

for order:	1	0
result[0] =	A	average value
result[1] =	τ	-
result[2] =	O (Offset)	-

Error array: Uncertainty errors corresponding to each position in the results array

The command ***fit_bidecay*** fits a bi-exponential curve to the input data:

$$y = A_1 * \exp -(x/\tau_1) + A_2 * \exp -(x/\tau_2) + O$$

where:

A_1, A_2: amplitudes of components 1 and 2
τ_1, τ_2: decay constants of components 1 and 2
O: offset

Prior to the command, the program must declare the necessary fit parameters, input and output arrays, and assign values to the fit parameter and input arrays.

Previous variable declarations:

```
int order;                                      # Order of the fit, see explanation below.          #
real result[10], error[10];                     # Arrays for results output and their uncertainties #
real x_array[positions], y_array[positions];    # Arrays for input data                              #
int number_of_data;                             # Number of data pairs                               #
```

Assign values to the input parameter number_of_data, and input arrays by direct copy of the NMR signal into x- and y-array OR from some combination of user input and signal evaluation (i.e. known concentration for x-array vs. weight-normalized NMR signal amplitude for y-array).

The default is to allow the function to include the offset as a fit variable. One can assign the fit Offset to a pre-defined value using:

off_presetting(0, *fixed_offset_value)****;***

To release the fixed Offset fit use:

off_presetting(-1, 0 ***);***

Syntax of the command:

order = ***fit_bidecay(*** x_array, y_array, number_of_data, result, error ***);***

'ERROR' is set if determination fails (not enough data pairs, matrix singularity)

After the fit, the routine sets a return value to order which is the actual order used. If bi-exponential fit is selected but only one component is detected order will be set to 1 after the fit.

order = 2 means: the data fit a bi-exponential function
order = 1 means: the data fit a mono-exponential function
order = 0 means: the average value of the amplitude was calculated

Results array:	order:	2	1	0
	result[0] =	A_1	A	average value
	result[1] =	τ_1	τ	-
	result[2] =	A_2	O (Offs.)	-
	result[3] =	τ_2	-	-
	result[4] =	O (Offs.)	-	-

Error array: Uncertainty errors corresponding to each position in the result array

The command ***fit_tridecay*** fits a tri-exponential curve to the input data. The fit equation has the form:

$$y = A_1 * \exp -(x/\tau_1) + A_2 * \exp -(x/\tau_2) + A_3 * \exp -(x/\tau_3) + O$$

where: $A_{\#}$: amplitude of component # (1 to 3)
$\tau_{\#}$: decay constant, component # (1 to 3)
O: offset

Prior to the command, the program must declare the necessary input and output arrays, and assign values to the input arrays. To assist in the fitting process, the user should be prompted for estimates of the component amplitudes and corresponding relaxation times (A_1, A_2, A_3 and corresponding $\tau_{\#}$: starting values) and the estimated signal offset.

Previous variable declarations:

```
int order;                                   # Order of the fit, see explanation below.          #
real result[10], error[10];                  # Arrays for results output and their uncertainties #
real x_array[positions], y_array[positions]; # Arrays for input data                             #
int number_of_data;                          # Number of data pairs                              #
```

Assign values to the input parameter number_of_data, and input arrays by direct copy of the NMR signal into x- and y-array.

Pre-assign starting values for component amplitudes and relaxation time constants, *i.e.:*

```
# Tri-exponential Fit pre-definitions #
Result[0] = input_real( "Input Amplitude Start Value of FIRST Component", 20, 1 );
if (ESC) return(TRUE); endif;
Result[1] = input_real( "Input Relaxation Time Start Value of FIRST Component", 100, 1 );
if (ESC) return(TRUE); endif;
Result[2] = input_real( "Input Amplitude Start Value of SECOND Component", 20, 1 );
if (ESC) return(TRUE); endif;
Result[3] = input_real( "Input Relaxation Time Start Value of SECOND Component", 100, 1 );
if (ESC) return(TRUE); endif;
Result[4] = input_real( "Input Amplitude Start Value of THIRD Component", 20, 1 );
if (ESC) return(TRUE); endif;
Result[5] = input_real( "Input Relaxation Time Start Value of THIRD Component", 100, 1 );
if (ESC) return(TRUE); endif;
Result[6] = input_real( "Input Offset Start Value", 0, 1 );
if (ESC) return(TRUE); endif;
```

The default is to allow the function to include the offset as a fit variable. One can assign the fit Offset to a pre-defined value using:

```
off_presetting( 0, fixed_offset_value);
```

To release the fixed Offset fit use:

```
off_presetting( -1, 0 );
```

Syntax of the command:

```
order = fit_tridecay( x_array, y_array, number_of_data, result, error );
```

'ERROR' is set if determination fails (not enough data pairs, matrix singularity)

After the fit, the routine sets a return value to order which is the actual order used; e.g. if tri-exponential fit is selected but only one component is detected order will be set to 1 after the fit:

order = 3 means: the data fit a tri-exponential function
order = 2 means: the data fit a bi-exponential function
order = 1 means: the data fit a mono-exponential function
order = 0 means: the average value of the amplitude was calculate

Results array: order:	3	2	1	0
result[0] =	A_1	A_1	A	average value
result[1] =	τ_1	τ_1	τ	-
result[2] =	A_2	A_2	**O** (Offs.)	-
result[3] =	τ_2	τ_2	-	-
result[4] =	A_3	**O** (Offs.)	-	-
result[5] =	τ_1	-	-	-
result[6] =	**O** (Offs.)	-	-	-

Error array: Uncertainty errors corresponding to each position in the result array

The command ***fit_quaddecay*** fits a 4-exponential curve to the input data. The fit equation has the form:

$$y = A_1 * \exp -(x/\tau_1) + A_2 * \exp -(x/\tau_2) + A_3 * \exp -(x/\tau_3) + A_4 * \exp -(x/\tau_4) + O$$

where: $A_\#$: amplitude of component # (1 to 4)
$\tau_\#$: decay constant, component # (1 to 4)
O: offset

Prior to the command, the program must declare the necessary input and output arrays, and assign values to the input arrays. To assist in the fitting process, the user can be prompted for estimates of the component amplitudes and corresponding relaxation times (A_1, A_2, A_3 , A_4 , and corresponding $\tau_\#$: starting values) and the estimated signal offset.

Previous variable declarations:

```
int order;                                   # Order of the fit, see explanation below          #
real result[10], error[10];                  # Arrays for results output and their uncertainties #
real x_array[positions], y_array[positions]; # Arrays  for input data                           #
int number_of_data;                          # Number of data pairs                             #
```

Assign values to the input parameter number_of_data, and input arrays by direct copy of the NMR signal into x- and y-array.

Pre-assign starting values for component amplitudes and relaxation time constants, *i.e.:*

```
# Quad-exponential Fit pre-definitions #
Result[0] = input_real( "Input Amplitude Start Value of FIRST Component", 20, 1 );
if (ESC) return(TRUE); endif;
Result[1] = input_real( "Input Relaxation Time Start Value of FIRST Component", 100, 1 );
if (ESC) return(TRUE); endif;
Result[2] = input_real( "Input Amplitude Start Value of SECOND Component", 20, 1 );
if (ESC) return(TRUE); endif;
Result[3] = input_real( "Input Relaxation Time Start Value of SECOND Component", 100, 1 );
if (ESC) return(TRUE); endif;
Result[4] = input_real( "Input Amplitude Start Value of THIRD Component", 20, 1 );
if (ESC) return(TRUE); endif;
Result[5] = input_real( "Input Relaxation Time Start Value of THIRD Component", 100, 1 );
if (ESC) return(TRUE); endif;
Result[6] = input_real( "Input Amplitude Start Value of FOURTH Component", 20, 1 );
if (ESC) return(TRUE); endif;
Result[7] = input_real( "Input Relaxation Time Start Value of FOURTH Component", 100, 1 );
if (ESC) return(TRUE); endif;
Result[8] = input_real( "Input Offset Start Value", 0, 1 );
if (ESC) return(TRUE); endif;
```

The default is to allow the function to include the offset as a fit variable. One can assign the fit Offset to a pre-defined value using:

off_presetting(0, *fixed_offset_value****);***

To release the fixed Offset fit use:

off_presetting(-1, 0 ***);***

Syntax of the command:

order = ***fit_quaddecay(*** x_array, y_array, number_of_data, result, error ***);***

'ERROR' is set if determination fails (not enough data pairs, matrix singularity)

After the fit, the routine sets a return value to order which is the actual order used; e.g. if quad-exponential fit is selected but only three component are detected order will be set to 3 after the fit:

order = 4 means: the data fit a quad-exponential function
order = 3 means: the data fit a tri-exponential function
order = 2 means: the data fit a bi-exponential function
order = 1 means: the data fit a mono-exponential function
order = 0 means: the average value of the amplitude was calculate

Results array:	order:	4	3	2	1
	result[0] =	A_1	A_1	A_1	A
	result[1] =	τ_1	τ_1	τ_1	τ
	result[2] =	A_2	A_2	A_2	***O*** (Offset)
	result[3] =	τ_2	τ_2	τ_2	-
	result[4] =	A_3	A_3	***O*** (Offs.)	-
	result[5] =	τ_3	τ_3	-	-
	result[6] =	A_4	***O*** (Offs.)	-	-
	result[7] =	τ_4	-	-	-
	result[8] =	***O*** (Offs.)	-	-	-

Error array: Uncertainty errors corresponding to each position in the result array

The command ***display_fit*** ***(*** *FIT...* ***)*** calculates the specified fit, displays the input data pairs with the fit, and executes a utility that provides cursor control and menu options so that the user may highlight and delete data points from the input data and fit the remaining pairs again. The utility may be used in connection with the fit functions discussed in Section ##.

Syntax of the command:
order = ***display_fit*** ***(*** *FIT, result, error, x, y, n, "x_unit", "y_unit"* ***);***

where: FIT can be one of the following fit types:
MONODECAY, BIDECAY, TRIDECAY or QUADDECAY

"x_unit" / "y_unit" These are the units of the data pair arrays in quotations. The maximum number of characters for each unit is nine.

Additionally 'order = -1' indicates that no fit has been executed finally when this utility has been left.
The Error array value for LIN_REG is of no meaning, but the above syntax still has to be fulfilled.

These commands operate the same as the previously described forms used for calculation only. Therefore, previous variable declarations are required, values must be assigned to the input arrays, and where applicable, values must be pre-assigned to the fixed input parameters or starting value assigned to the variable input. Results and Error arrays are also follow the same pattern. Please see Section ## for details of each command.

Menu Items:

<DEL POINT>	Deletes one point on cursor position and executes the fitting procedure. The results of the fit are displayed in the result box (not available on decay fits).
<MARK REGION>, <DEL REGION> and <FIT>	Handles data points deletion and refitting of decay curves using a graphic cursor.
<CONTINUE>	Leaves the display of the data pairs and executes the next ExpSpel command.

9.4. T1 Relaxation Time Program Fitting

```
label calc;
   no = Loop_Count;

   if (modi == 2)
      if (ExpOrder == 2)
            Order = fit_bidecay( x, y, no, Result, Error );
            Order = display_fit( SHOW_FIT, Result, Error, x, y, no, "ms", "%" );
      else
            Order = fit_monodecay( x, y, no, Result, Error );
            Order = display_fit( SHOW_FIT, Result, Error, x, y, no, "ms", "%" );
      endif;
   endif;

   if (modi == 1)
      if (ExpOrder == 2)
         Order = display_fit( BIDECAY, Result, Error, x, y, no, "ms", "%" );
      else
         Order = display_fit( MONODECAY, Result, Error, x, y, no, "ms", "%" );
      endif;
   endif;

   if (modi == 0)
      if (ExpOrder == 2)
         Order = fit_bidecay( x, y, no, Result, Error );
      else
         Order = fit_monodecay( x, y, no, Result, Error );
      endif;

      if (Order == 0)
         print_line( FLASHBOX, "No Exponential Functions - Switch to 'Modifications = 1' !" );
      endif;


      if (Order == 1)
         Order = display_fit( MONODECAY, Result, Error, x, y, no, "ms", "%" );
      endif;
      if (Order == 2)
         Order = display_fit( BIDECAY, Result, Error, x, y, no, "ms", "%" );
      endif;
   endif;
```

10. REFERENCE VALUES AND T1 PULSE PARAMETER COMBINATIONS

Refer also to the Contrast imaging agent literature for a known relaxation standard. Check with Nycomed, Mass Gen Hospital, Epix, for standard recipe.

NOTE: A calibrated thermometer is recommended for measuring the actual temperature in the probe chamber.

90 % formamide in DMSO:	T2 approx. 70 msec
Dioxane in 60 % C6D6:	T2 approx. 5.4 sec
Dodecane 40 deg.C:	T2 approx. 1.2 msec

Examples of allowed combinations for DUR, DURFACTOR and NP (see T1 applications):

Starting DUR (ms)	DURFACTOR	NP
5.0	1.5	25
5.0	5.3	5
3.5	1.1	108
3.5	1.5	25
0.5	5.3	7
0.5	1.5	30
0.5	1.3	46
0.05	5.3	8
0.05	1.5	35
0.05	1.3	55
0.05	1.1	152

11. TROUBLE SHOOTING

This chapter deals with typical errors that may be encountered specifically with relaxation time analysis. A user might refer to the troubleshooting part of this manual only after encountering some problems with relaxation time results. If this is the case, it is recommended that the user read this entire manual and the minispec User's Manual for full information regarding operation of the minispec.

Problem A / Results are unstable

In this section, the expected stability of relaxation results is discussed.

The accuracy of a fit depends upon the number of data points to be fitted, the shape of the decay curve and the available dynamic range of the data points. For all Bruker relaxation time applications, relaxation time standard deviations below ± 5% are possible. The default values of the Bruker applications are optimized for doped water samples (distilled water with 0.5% CuSO4 * 5 H2O - filling height 1 cm) and this type of sample can be measured with relaxation time standard deviations below ± 3%. T2 measurements, on the doped water sample mentioned above, by the CPMG sequence using Bruker Soft-EDM t2_cp_mb with default parameters (or more data points), should produce even better results (± 2%).

If the relaxation time results of numerous analyses have greater scatter about the mean value than mentioned above, the measurement parameters or the sample condition may have to be optimized.

1. First, the user must ensure that the sample inside the probe compartment is kept at a constant temperature. The temperature should be checked for stability at the actual sample position (i.e. inside the probe head). A thermometer can be placed inside the sample chamber and should be checked over a period of time (a few hours). If no external water bath is connected, the temperature is stable as long as there is no 'T!' symbol on the lower left corner of the minispec LC display (an indication of wrong temperature). If an external bath is connected, the temperature of the bath and the probe head chamber should be checked.

   If, prior to measurement, the sample is not pre-conditioned to the temperature of the sample compartment, a delay of at least 15 minutes should be allowed after the sample is inserted into the minispec before the first acquisition is started.

2. Non-optimized parameters are the main cause of unstable relaxation results.

   Parameters that must be considered include those set in the instrument calibration table (pulse lengths, magnetic field, and detection angle), those set using the <Parameter> menu, and those set through the menu <Calibrate> <Sample> in order to vary the pulse sequence.

   Instrument calibration parameters depend on the type of sample and may need to be optimized using the sample of interest or a representative sample. Detection angles are gain dependent and, therefore, need to be tuned at the gain to be used for measuring the sample of interest. Refer also to any directions listed in the corresponding application notes for more information in this matter.

The parameters of an application that are accessible through the <Parameter> menu (particularly receiver gain, recycle delay, or number of scans) have to be adjusted as well. The recycle delay time allows for the nuclei (e.g. Hydrogen nucleus) in the sample to develop initial magnetization and return to this initial state between measurements (i.e. repeat scans for signal averaging). In effect, the recycle delay makes sure that the second scan will give the same signal as the first one, and so on. Usually, at least 5 times the T1 relaxation time must be entered in order to fulfill this condition. The number of scans must be set so as to ensure adequate signal-to-noise is attained before the data points are fitted. Number of scans will have to be adjusted according to signal strength and according to the user's expectation for result repeatability. In general, more scans will need to be collected a higher gains.

The receiver gain fixes the dynamic range of the data points. The operator has to maximize the gain (without allowing the signal to clip) in order to maximize dynamic range, otherwise results will be unstable.

Very important also are the parameters used to vary the pulse sequence of the relaxation time applications (<Calibrate> <Sample> routine). The number of data points and the distances between them have to be adjusted to ensure that the analyzed time period is matched to the decay of the signal. For T2 measurements, it is important to reach almost zero signal on the right side of the time window. If this is not the case, the fit returns an offset that might vary from one fit to the next, altering the real value of T2. Therefore the pulse sequence parameters have to be carefully adjusted. It should also be kept in mind that the larger the number of data points, the more accurate the relaxation values will be delivered by the fitting routine. Note also that, in the case of T2 measurements, delay values longer than 2 msec should be avoided because external sources might disturb the NMR signals.

In case mono-exponential fit is chosen and the sample contains more than one component, unstable and inaccurate results will be received. If bi-exponential fit is active and both components have quite close relaxation times, the fitting results can be unstable. Results will also vary if bi-exponential fit is selected and the sample contains more than two components.

It was discovered that sample tubes filled more than 1 cm and therefore not located in the center of the probe coil might show unusual behavior on the left side of the time period investigated. Fill height should be reduced in those cases.

Instruments not operated on resonance have shown decay curves (T2 analysis) that contain negative data points (real detection mode) or that reflect from the zero line (magnitude detection).

Of course also a defective instrument could be responsible for scattered results. In one case a total collapse to zero of the data points somewhere in the time period was reported. The problem was investigated by running repeat measurements in LIVE mode(set <SETUP> <PROGRAM> "Acquisition mode" to LIVE, <PARAMETER> "Number of Scans" to 1, and initiate repeat scans with <SHIFT> R). This will show if the above described collapse of the signal appears at random, or systematically. A defective probe head could lead to such a behavior.

Problem B / Results are drifting

If results are stable in the short term, but drift to higher or lower values over time, this usually indicates that one part of the experiment set-up has not stabilized.

Often this will be the temperature of the sample itself. Relaxation times are strongly temperature dependent and therefore sample temperature is quite important. If an external water bath is connected the temperature stability of this bath should be verified.

Also a non-stabilized magnet temperature / magnetic field change could cause a results drift. Non-stabilized magnetic fields are indicated on the lower left side of the LC display by a T! symbol.

Last but not least, if multiple CPMG experiments are repeated in a short period of time, significant RF energy can be absorbed by the sample cause heating. Therefore increased relaxation times are to be expected. However a few minutes later the sample should have recovered to its usual temperature and then the correct relaxation time should be found again.

Problem C / Expected relaxation time values cannot be reached

In case relaxation time values are expected from previous measurements or other information sources and those values cannot be reached, care has to be taken on different aspects:

Was the sample previously analyzed at exactly the same temperature and also at the same field / frequency ? If not, the same relaxation times should not be expected.

Also it is important to be sure that the sample investigated is the same as before. For instance mineral oils will have significant relaxation time differences. An unsealed, doped water sample will change its relaxation time when some of the water evaporates.

Care has to be taken also in the choice of the applications and their parameters. Results from a sample previously analyzed with inversion recovery t1_ir_mb sequence should not be compared directly with results from a saturation recovery t1_sr_mb sequence. The CPMG application t2_cp_mb should always be executed with the same pulse sequence parameters (like tau value, number of data points etc.), if results must be closely compared.

If absolute relaxation time numbers are expected, the sample has to be placed in the middle of the probe head coil and the fill height should be around 1 cm. Higher fill heights will lead to relaxation time shifts.

Another trouble might occur if someone measures T2 times with application t2_se_mb. If the sample has is characterized by significant diffusion, this application will lead to shortened relaxation times. Samples that may exhibit diffusion behavior should be examined with the CPMG application t2_cp_mb. On the other hand, scientists have shown that polymer samples cannot be analyzed with CPMG, but require the Hahn-Echo pulse sequence as used in the application t2_se_mb.

Besides this it is also possible that the measurement parameters are not fixed perfectly. More details about choosing the parameters properly are listed above.

Problem D / T2 relaxation times bigger than T1 relaxation times

According to the definition of the relaxation times, the T1 times are always longer or at least equal to the T2 relaxation times. If this is not the case, parameters of the two applications are not correctly adjusted. The user might refer to the above described parameter adjustments.

Problem E / Only a mean value is presented as the result

When the measured data points (decay curve) cannot be fitted with the expected number of components, the fitting software automatically reduces the fit order. For example, if a bi-exponential fit (order = 2) does not detect two components, the order will be reduced to 1 and a mono-exponential fit will be calculated instead. A mono-exponential fit, if no exponential decay is found, will be reduced to a calculation of mean value. Such a result indicates that either no sample is inserted, the signal is too low (receiver gain requires increased value) or the time period is not chosen correctly. In most cases the correct adjustment of the parameters will help. It is also obvious that samples must contain the investigated nuclei in order to yield an NMR signal.

Problem F / Results make no sense - Multiple component analysis do not work

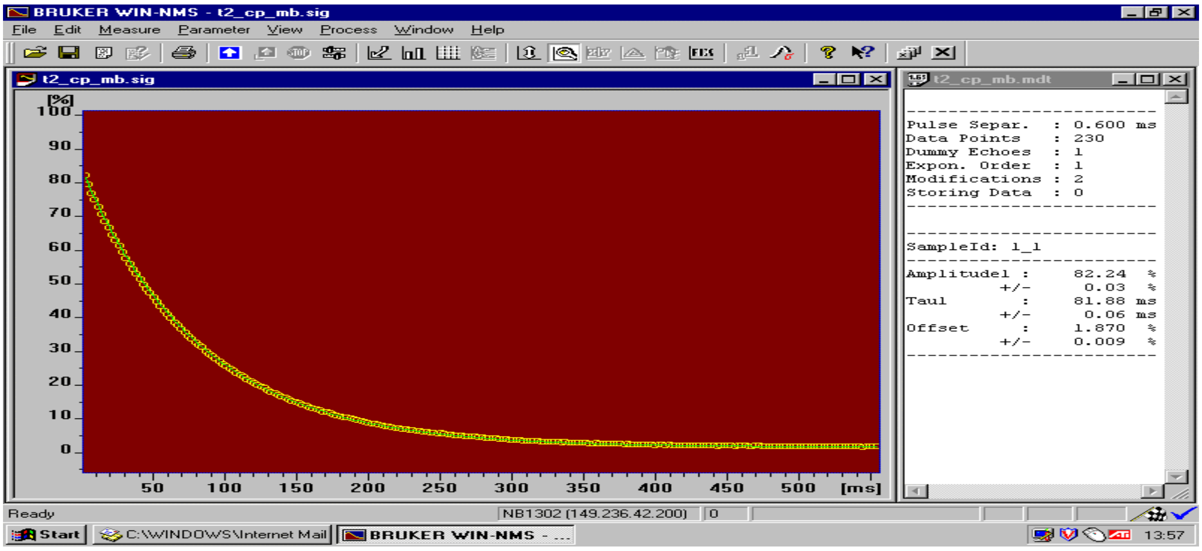
When samples with more than one component are examined, the user must judge whether the results really make sense. The more components the software considers, the more fitting parameters are available. A fit might have a number of different solutions although only one makes sense.

It was already indicated above that problems might also appear when the relaxation times of the different components are too close. Unstable results might be caused.

Fitting routines for more than two components are included in the application fit_rela found in the directory Test_Applications. For the previously mentioned reason, the user is expected to enter the fitting start parameters. This increases the chance of finding meaningful results. However, the results of fits on signals with more than one component should always be checked by the operator whether they make really sense. As different combinations of relaxation times and their amplitudes might lead to comparable fit qualities, it is possible to measure a multi-component sample several times and receive completely different results.

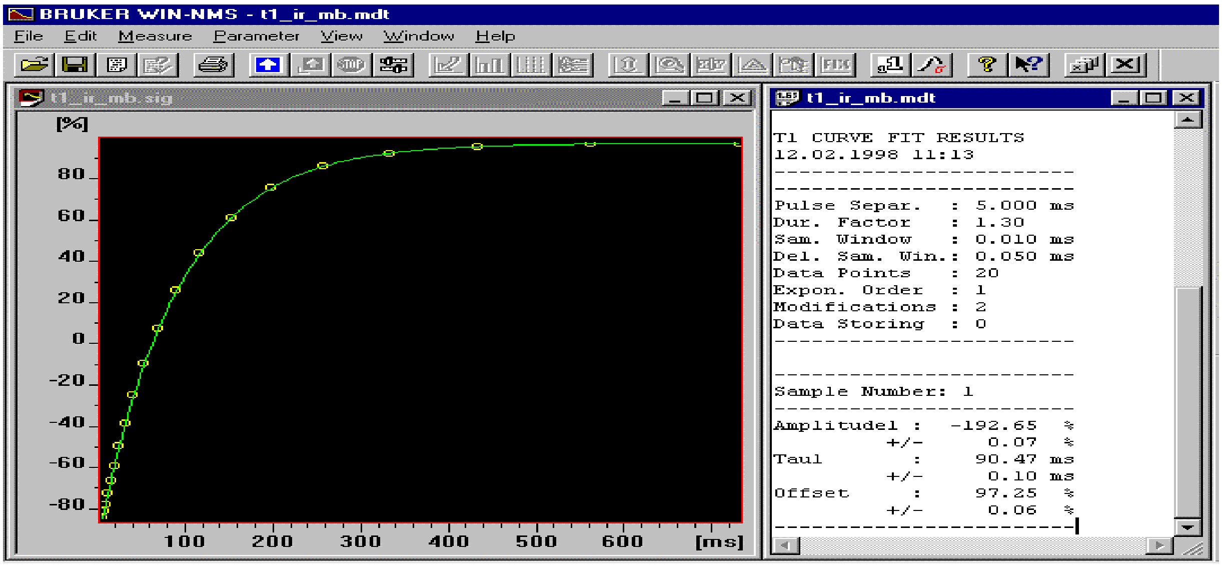
Problem G / NMR Signals do not show expected curve behavior

In the following example a couple of NMR relaxation time curves are presented. Whereas the first curves show the desired and expected behaviors, the rest exhibit unexpected relaxation behaviors. Explanation is given regarding the sources of the errors.



```
-------------------------------
Pulse Separ.   : 0.600 ms
Data Points    : 230
Dummy Echoes   : 1
Expon. Order   : 1
Modifications  : 2
Storing Data   : 0
-------------------------------

-------------------------------
SampleId: 1_1
-------------------------------
Amplitude1 :     82.24  %
       +/-        0.03  %
Tau1       :     81.88  ms
       +/-        0.06  ms
Offset     :     1.870  %
       +/-       0.009  %
-------------------------------
```

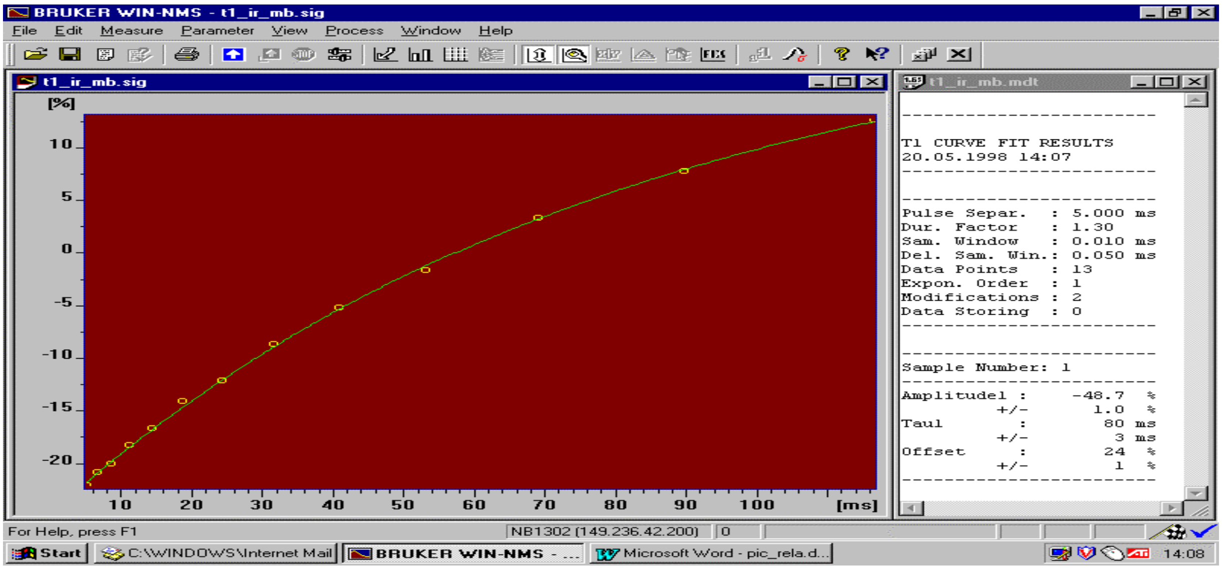
Above is shown a t2_cp_mb application with well-chosen parameters. The gain is chosen so that the full dynamic range of the system is used (signal on the left side starts at about 80 - 90 % of the full display). The total acquisition time is adjusted so that data is sampled from the signal over the entire decay period as the signal approaches base line. A high number of data points with short inter-pulse durations is used. The decay behaves fully mono-exponential. These conditions should result in stable and reproducible T2 relaxation time analysis.



```
T1 CURVE FIT RESULTS
12.02.1998 11:13
------------------------
------------------------
Pulse Separ.   : 5.000 ms
Dur. Factor    : 1.30
Sam. Window    : 0.010 ms
Del. Sam. Win.: 0.050 ms
Data Points    : 20
Expon. Order   : 1
Modifications : 2
Data Storing   : 0
------------------------

------------------------
Sample Number: 1
------------------------
Amplitude1 :  -192.65  %
         +/-     0.07  %
Tau1       :    90.47 ms
         +/-     0.10 ms
Offset     :    97.25  %
         +/-     0.06  %
------------------------
```

The screen dump above shows a t1_ir_mb analysis. As described for the T2 analysis before, parameters are selected optimally. Again the full dynamic range of the system is used and the T1 signal can reach the M_∞ magnetization near the end of the sampling period. Note that for both above shown measurements the same sample has been used. As expected from theories, the T1 value of this liquid (distilled water with CuSO4) is longer than the T2 value.

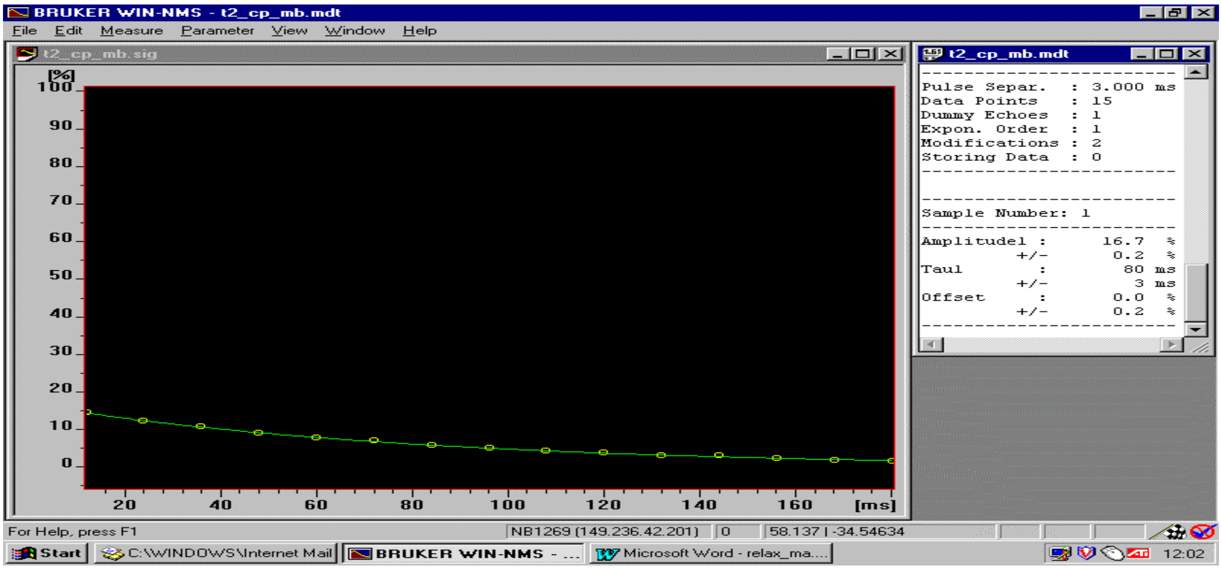
Below you will find a few examples of non-optimized analysis parameters.



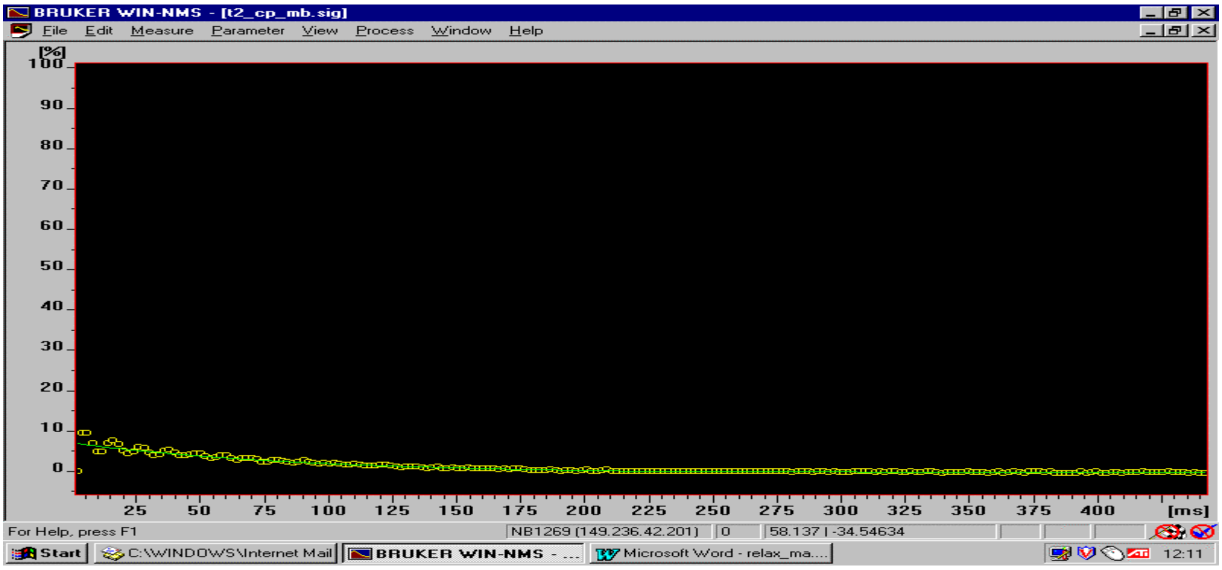
```
-----------------------------

T1 CURVE FIT RESULTS
20.05.1998 14:07
-----------------------------


-----------------------------
Pulse Separ.   : 5.000 ms
Dur. Factor    : 1.30
Sam. Window    : 0.010 ms
Del. Sam. Win.: 0.050 ms
Data Points    : 13
Expon. Order   : 1
Modifications : 2
Data Storing   : 0
-----------------------------


-----------------------------
Sample Number: 1
-----------------------------
Amplitude1 :     -48.7  %
         +/-       1.0  %
Tau1       :        80 ms
         +/-         3 ms
Offset     :        24  %
         +/-         1  %
-----------------------------
```

The same T1 application and the same sample analyzed with non-optimized parameters. The dynamic range of the system is poorly used - the time scale does not reach the M_∞ magnetization - the number of data points acquired is rather low. As a result of those non-optimized settings, a big T1 error range is received: 80 msec with an error of 3 msec. On the optimized analysis before an error of only 0.1 msec could be reached !!!

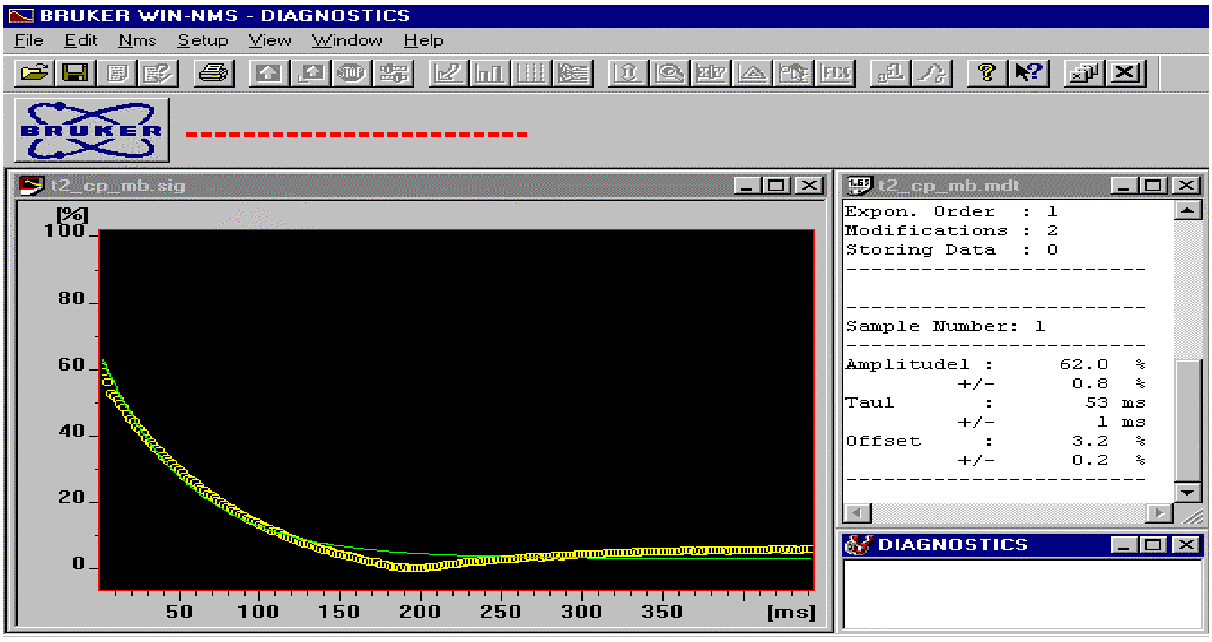
A non-optimized T2 screen is presented below. Explanations as above in the case of non-perfect T1 parameters.



```
------------------------------
Pulse Separ.   :  3.000 ms
Data Points    :  15
Dummy Echoes   :  1
Expon. Order   :  1
Modifications  :  2
Storing Data   :  0
------------------------------

------------------------------
Sample Number: 1
------------------------------
Amplitude1 :       16.7   %
         +/-        0.2   %
Tau1       :         80  ms
         +/-          3  ms
Offset     :        0.0   %
         +/-        0.2   %
------------------------------
```

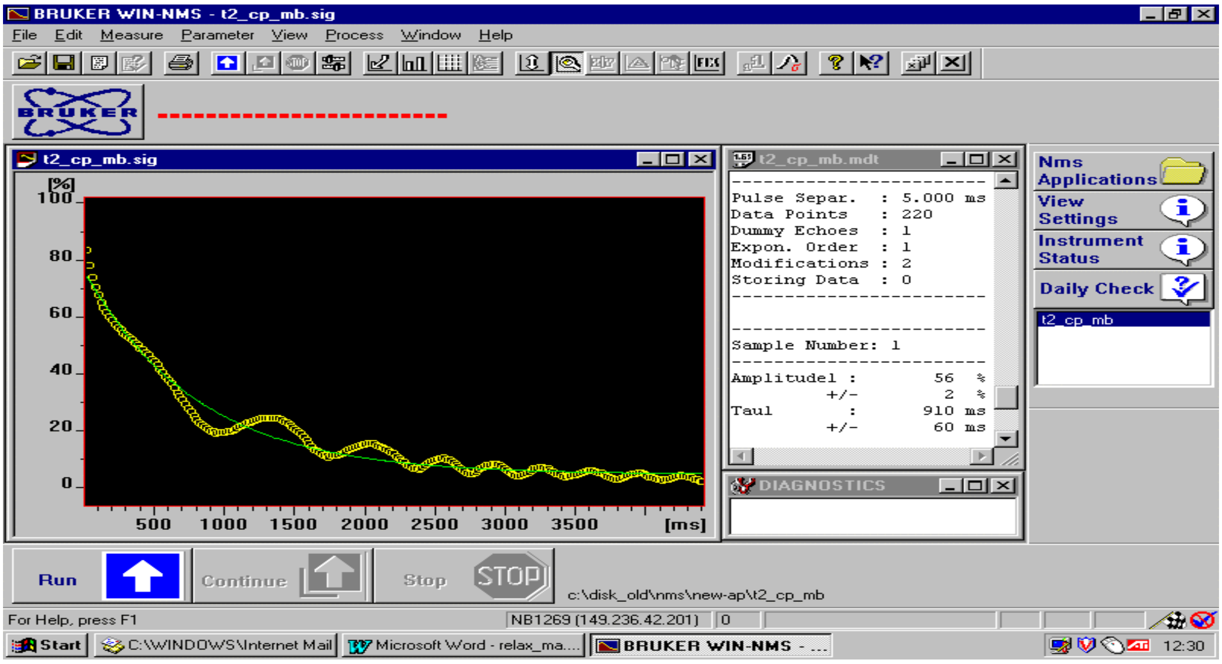


Above: T2 Determination by t2_cp_mb application - Detection Mode: real - minispec out of Resonance. Besides the unusual behavior at the signal beginning, signal strength is significantly lost.



```
Expon. Order  : 1
Modifications : 2
Storing Data  : 0
-------------------------

-------------------------
Sample Number: 1
-------------------------
Amplitude1 :     62.0  %
         +/-      0.8  %
Tau1       :       53 ms
         +/-        1 ms
Offset     :      3.2  %
         +/-      0.2  %
-------------------------
```

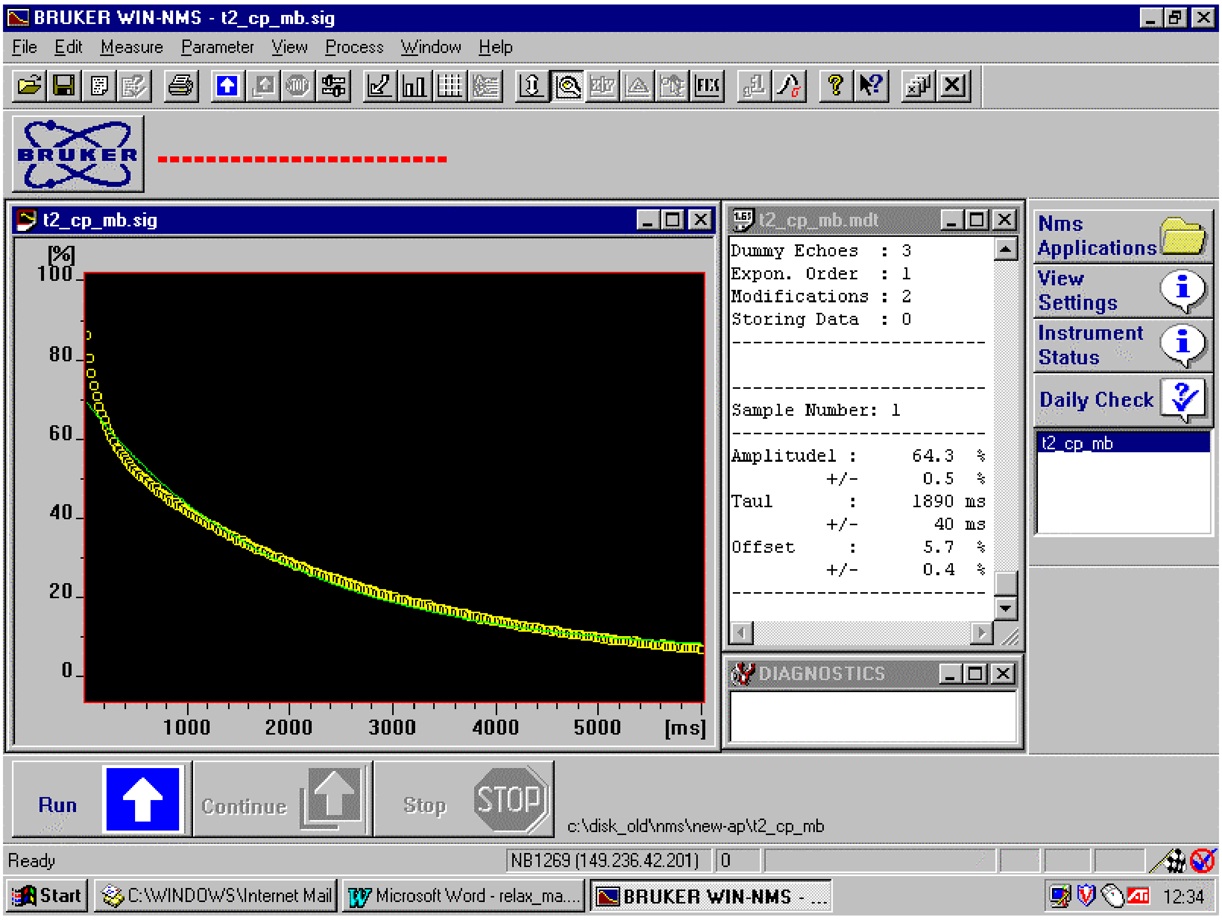
Above: T2 Determination by t2_cp_mb - Detection Mode: magnitude - minispec out of Resonance Besides the totally unusual behavior of the signal, signal strength is partially left.



```
-------------------------
Pulse Separ.   :  5.000 ms
Data Points    :  220
Dummy Echoes   :  1
Expon. Order   :  1
Modifications  :  2
Storing Data   :  0
-------------------------

-------------------------
Sample Number:  1
-------------------------
Amplitude1 :        56  %
         +/-         2  %
Tau1       :       910  ms
         +/-        60  ms
```

c:\disk_old\nms\new-ap\t2_cp_mb

In the example above quite long values for tau have been chosen. External sources influence the NMR signal significantly. Reduction of the tau value is absolutely necessary. In order to reach the base line on the right side of the signal, more data points or dummy echoes have to be acquired.



```
Dummy Echoes  : 3
Expon. Order  : 1
Modifications : 2
Storing Data  : 0
-----------------------

-----------------------
Sample Number: 1
-----------------------
Amplitude1 :     64.3  %
         +/-      0.5  %
Tau1       :     1890 ms
         +/-       40 ms
Offset     :      5.7  %
         +/-      0.4  %
-----------------------
```

The NMR signal fit above indicates that the mono-exponential fitting function is not suitable for this decay curve. In this case a oil / water mixture has been measured - therefore bi-exponential fitting should be used. The results of the fit above will vary from one measurement to the other.

The examples above always demonstrated the influence of one certain error. However it might be possible that the signals of primary analyses will be influenced by multiple errors. Therefore inexperienced users should follow this trouble shooting guide point by point in order to get rid of all unexpected signal behavior.