minispec mq-Series

Oil and Water Droplet Size Measurements using Gradient Strength Variation (G-Var)

User Manual
Version 001

Innovation with Integrity

AIC

This manual was written by

Bruker BioSpin AIC

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1 About This Manual

This manual enables safe and efficient handling of the device.

This manual is an integral part of the device, and must be kept in close proximity to the device where it is permanently accessible to personnel. In addition, instructions concerning labor protection laws, operator regulations tools and supplies must be available and adhered to.

Before starting any work, personnel must read the manual thoroughly and understand its contents. Compliance with all specified safety and operating instructions, as well as local work safety regulations, are vital to ensure safe operation.

The figures shown in this manual are designed to be general and informative and may not represent the specific Bruker model, component or software/firmware version you are working with. Options and accessories may or may not be illustrated in each figure.

1.1 Policy Statement

It is the policy of Bruker to improve products as new techniques and components become available. Bruker reserves the right to change specifications at any time.

Every effort has been made to avoid errors in text and figure presentation in this publication. In order to produce useful and appropriate documentation, we welcome your comments on this publication. Support engineers are advised to regularly check with Bruker for updated information.

Bruker is committed to providing customers with inventive, high quality products and services that are environmentally sound.

1.2 Symbols and Conventions

Safety instructions in this manual and labels of devices are marked with symbols. .

The safety instructions are introduced using indicative words which express the extent of the hazard.

In order to avoid accidents, personal injury or damage to property, always observe safety instructions and proceed with care.

⚠ DANGER

DANGER indicates a hazardous situation which, if not avoided, will result in death or serious injury.

This is the consequence of not following the warning.

1. This is the safety condition.

▶ This is the safety instruction.

⚠ WARNING

WARNING indicates a hazardous situation, which, if not avoided, could result in death or serious injury.

This is the consequence of not following the warning.

1. This is the safety condition.

▶ This is the safety instruction.

⚠ CAUTION

CAUTION indicates a hazardous situation, which, if not avoided, may result in minor or moderate injury or severe material or property damage.

This is the consequence of not following the warning.

1. This is the safety condition.

▶ This is the safety instruction.

NOTICE

NOTICE indicates a property damage message.

This is the consequence of not following the notice.

1. This is a safety condition.

▶ This is a safety instruction.

SAFETY INSTRUCTIONS

SAFETY INSTRUCTIONS are used for control flow and shutdowns in the event of an error or emergency.

This is the consequence of not following the safety instructions.

1. This is a safety condition.

▶ This is a safety instruction.

This symbol highlights useful tips and recommendations as well as information designed to ensure efficient and smooth operation.

1.3 Font and Format Conventions

Type of Information	Font	Examples
Shell Command, Commands, "All what you can enter"	Arial bold	Type or enter **fromjdx zg**
Button, Tab, Pane and Menu Names "All what you can click"	Arial bold, initial letters capitalized	Use the **Export To File** button. Click **OK**. Click **Processing**…
Windows, Dialog Windows, Pop-up Windows Names	Arial, initial letters capitalized	The Stacked Plot Edit dialog will be displayed.
Path, File, Dataset and Experiment Names **Data Path Variables** **Table Column Names** **Field Names (within Dialog Windows)**	Arial Italics	*$tshome/exp/stan/nmr/lists* *expno, procno,*
Parameters	Arial in Capital Letters	VCLIST

Program Code
Pulse and AU Program Names
Macros
Functions
Arguments
Variables
	Courier	`go=2`
		`au_zgte`
		`edmac`
		`CalcExpTime()`
		`XAU(prog, arg)`
		`disk2, user2`

AU Macro	Courier in Capital Letters	`REX` `PNO`

Table 1.1: Font and Format Conventions

About This Manual

2 Introduction

In Bruker´s original Droplet Size application (**D-Var**), the experiment was performed as a function of the duration (*sdel*) of the pulsed gradient field. In that application, for each value of *sdel* the balance of the pulse gradient field had to be calibrated during the measurement procedure, therefore this calibration had to be done for each sample to be measured. As result, the overall experiment was relatively time consuming, typically from 12 to 15 minutes.

In the new Droplet Size application (**G-Var**), the duration of the pulsed gradient field is kept constant, and the parameter that is varied during the experiment is the strength of the pulsed gradient field. Another difference is that the balance for each pulsed gradient field is calculated through a fitting function, determined in the calibration procedure for a set of fixed values of pulsed gradient fields. Therefore, during the measurement the balance does not have to be calibrated, resulting in a faster method compared to the original one, with measurement and data processing typically shorter than 5 minutes.

As the new method relies on the gradient strength variation, this method will be referred in this document as **G-Var**, while the original method will be referred to as **D-Var** (variation of the duration of the pulsed gradient field).

The new G-Var method is described in detail in chapters 3 – 6 of this User Manual, while the existing D-Var methods are explained in chapters 9 and 10. Chapters 7 and 8 apply for both methods.

Introduction

3 The G-Var Application Features

The G-Var application has many new features, here are the main ones:

- The application combines the former Oil Droplet Size and Water Droplet Size applications, whereas it is now possible to interchange between the measurements without the need of loading a different application.
- The application has 2 different user modes: *Research* (R&D) and *Routine* (Quality Control/ QC). To change from *Routine* to *Research*, the administrator password has to be entered.
- The *Routine* operator can only change a few settings in the application, while the *Researcher* operator can change all parameters available in the configuration table.
- The calibration file generated by the application is universal, i.e., the calibration data does not depend on the operator mode (R&D or QC) nor on the experimental method (*Oil Droplets in Water* or *Water Droplets in Oil*). Therefore, once a calibration is generated, it can be used for any user mode and experimental method. Moreover, the name of the calibration file does not depend on the application name, therefore even duplicated applications located in the same folder as the original can be used without the need to recalibrate the instrument.
- The calibration is robust to changes in the parameters in the measurement part, therefore the same calibration can be used for different settings.
- The application stores the results in the folder *G-Var_results* created in the same folder where the application is. Depending on the experimental method (water droplets or oil droplets) a subfolder is created (*Water Droplets* or *Oil Droplets*) and all results will be stored in the pertinent subfolder.
- The user can conveniently define the subfolder name where the results will be stored and also the name of the ASCII file which stores the same information displayed in the database table. This file is formatted in such way that the user can import it directly to Excel or other similar software.

Specific for Research (R&D) user mode:

- Option to sort the gradient strengths in logarithmically/linearly fashion. Moreover the user can manually enter the gradient strengths [T/m] in a table displayed after defining the parameters in the configuration table.

Specific for Routine (QC) user mode:

- The parameter tree is a “light version” of the *Research* mode operation. Only meaningful/ common modified parameters for *Routine* operator are accessible.

4 The G-Var User Interface

As is with other Bruker standard applications, the G-Var application is composed of 3 main parts:

- The Configuration table.
- The Calibration.
- The Measurement.

In the following sections each of these parts will be described in detail.

4.1 The Configuration Table

In the configuration table all of the parameters used during the measurement can be defined. Note that most of the settings related to the calibration are fixed, and the ones that can be user-defined are accessible after pressing the button **Calibrate**.

The configuration table is divided into 2-3 parts in this application (depending on the operation mode).

In the first window the user can define the experimental method (*Water Droplets* or *Oil Droplets*) and the operation mode (*Research* or *Routine*). Whenever any of these settings are changed, the table can be refreshed by pressing **OK**, showing the accessible parameters for these combination of choices. Parameters that are not accessible for the selected combination of operation mode and experimental method, will be grayed out using the standard value. Note that under the circumstances explained above, changes in the remaining parameters will be disregarded after clicking **OK**.

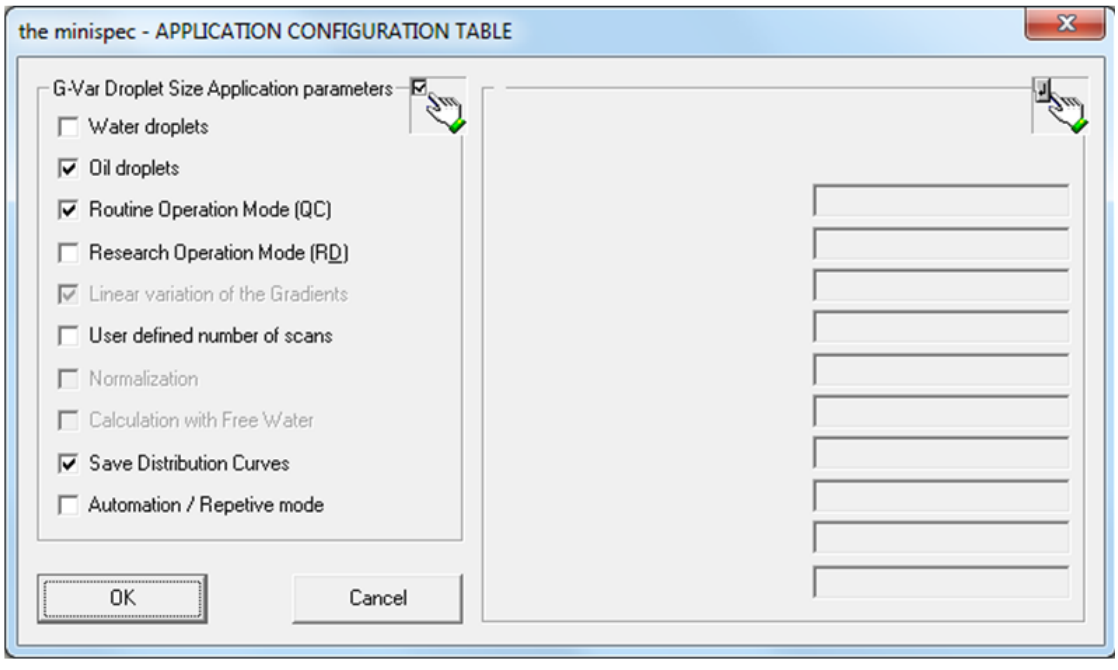


Figure 4.1: First Configuration Table: Defining Parameters for the Measurement.

When the user changes from *Routine* operation mode to *Research* operation mode, they will be prompted to enter the administrator password.

After clicking **OK**, a window will be displayed, whose content will depend on the combination of operator mode and experimental method.

In the case that the experimental method is *Oil Droplets* and the operation mode is *Routine*:

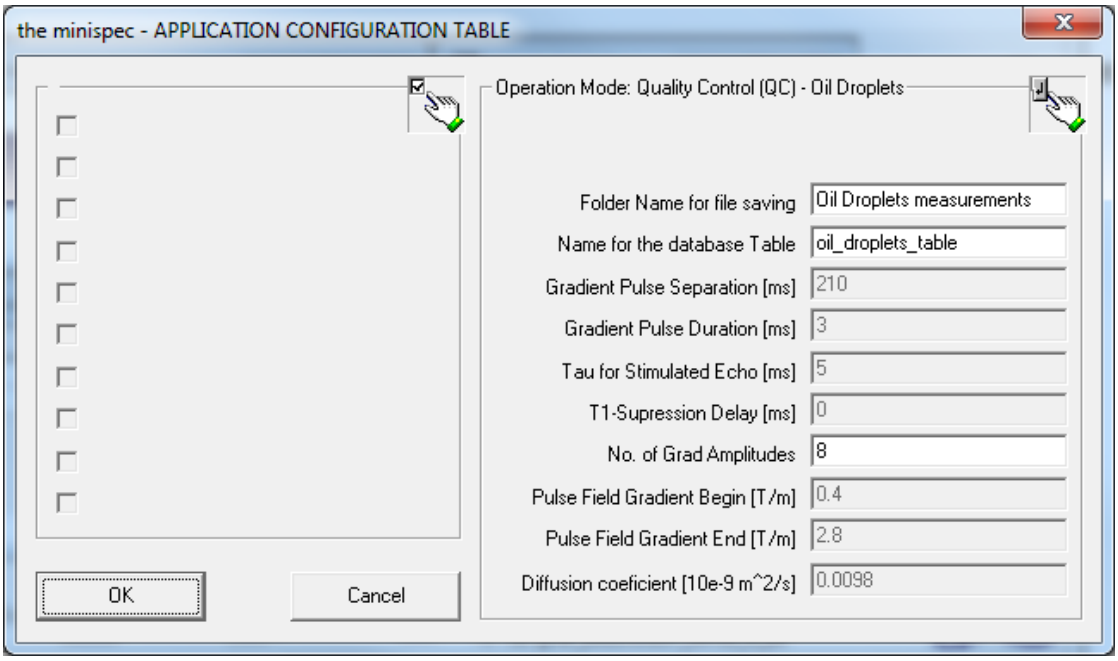


Figure 4.2: Second Configuration Table Shown when the Experimental Method is Oil Droplets and the Operation Mode is Routine.

When the experimental method is *Oil Droplets* and the operation mode is *Research*:

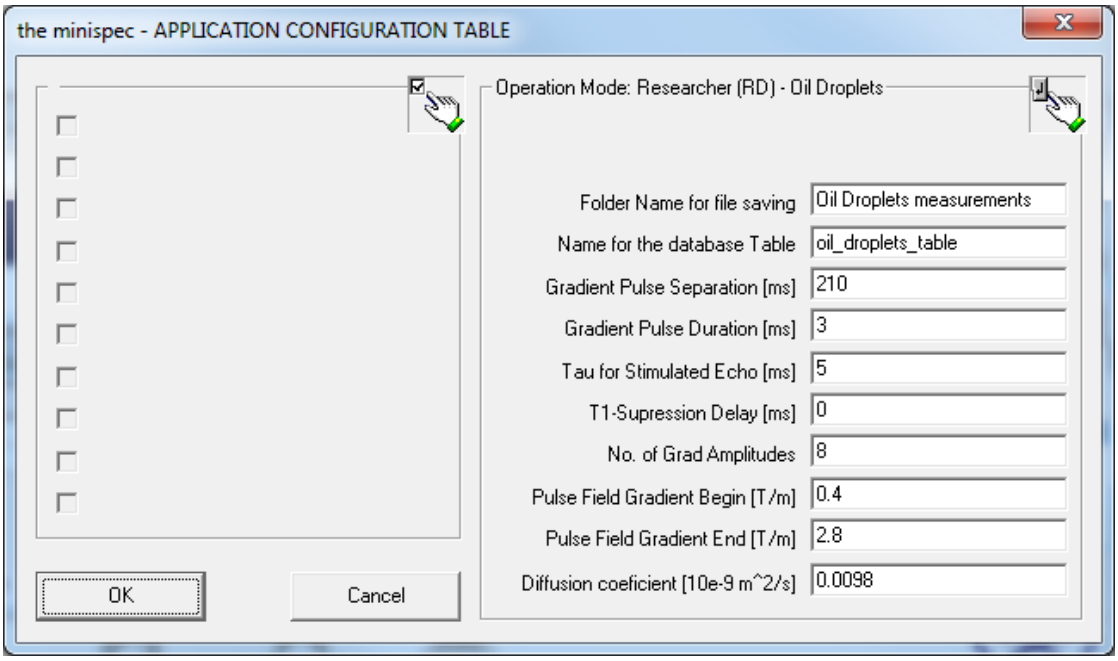


Figure 4.3: Second Configuration Table Shown when the Experimental Method is Oil Droplets and the Operation Mode is Research.

Note that the user can check which configuration is selected by looking in the headline of the dialog.

For example, in the case where the experimental method is *Water Droplets* and the operation mode is *Routine*:

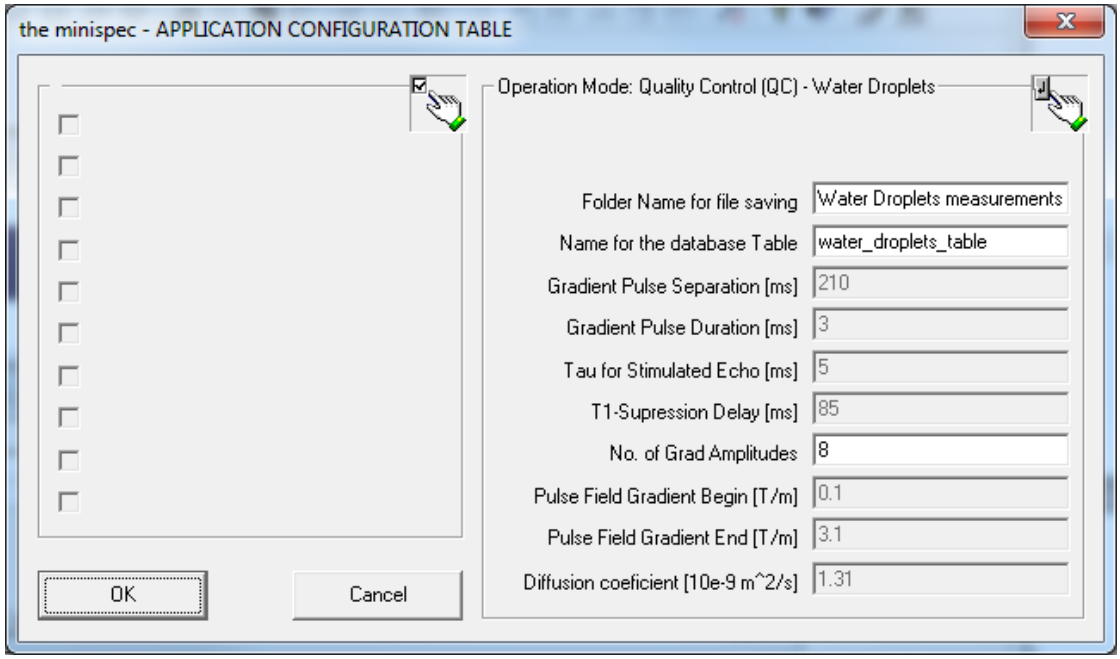


Figure 4.4: Second Configuration Table Shown when the Experimental Method is Water Droplets and the Operation Mode is Routine.

Finally, when the experimental method is *Water Droplets* and the operation mode is *Research*:

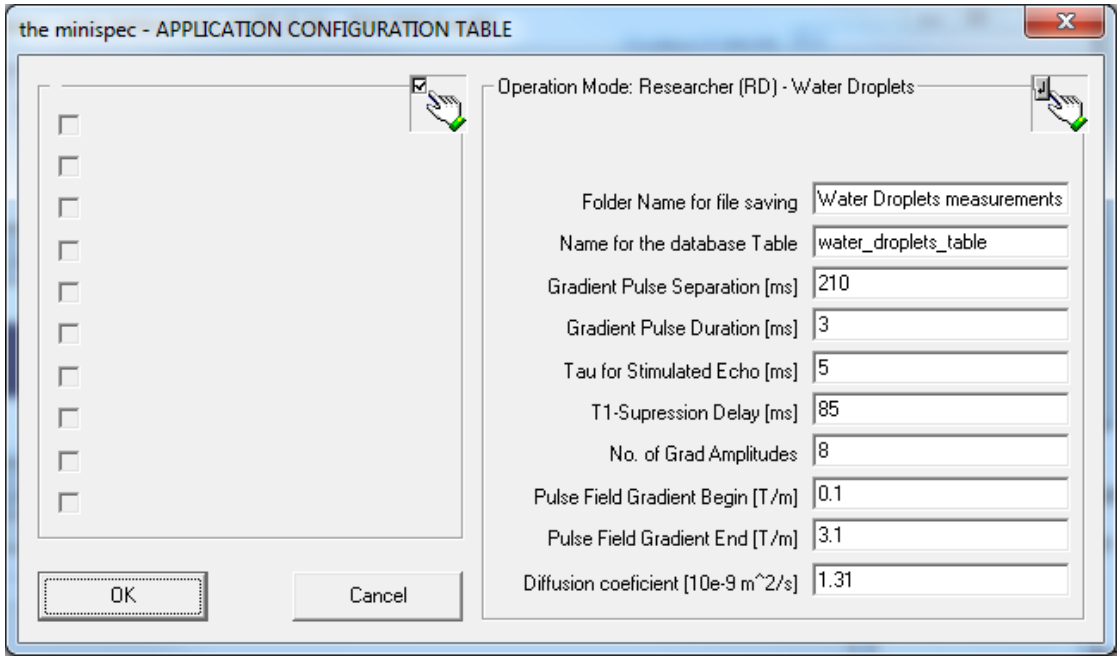


Figure 4.5: Second Configuration Table Shown when the Experimental Method is Water Droplets and the Operation Mode is Research.

In the particular case that the operation mode selected is *Research*, after the second configuration table, a third one will be displayed, where the user can visualize and edit the gradient strengths (T/m) that will be used in the experiment. The values will be sorted based on the options selected in the first and second configuration table. The next two figures illustrate the standard values displayed for *Oil Droplets* and *Water Droplets*, respectively. Note in both figures the user can change individual values of gradient strength to be used for the experiments.

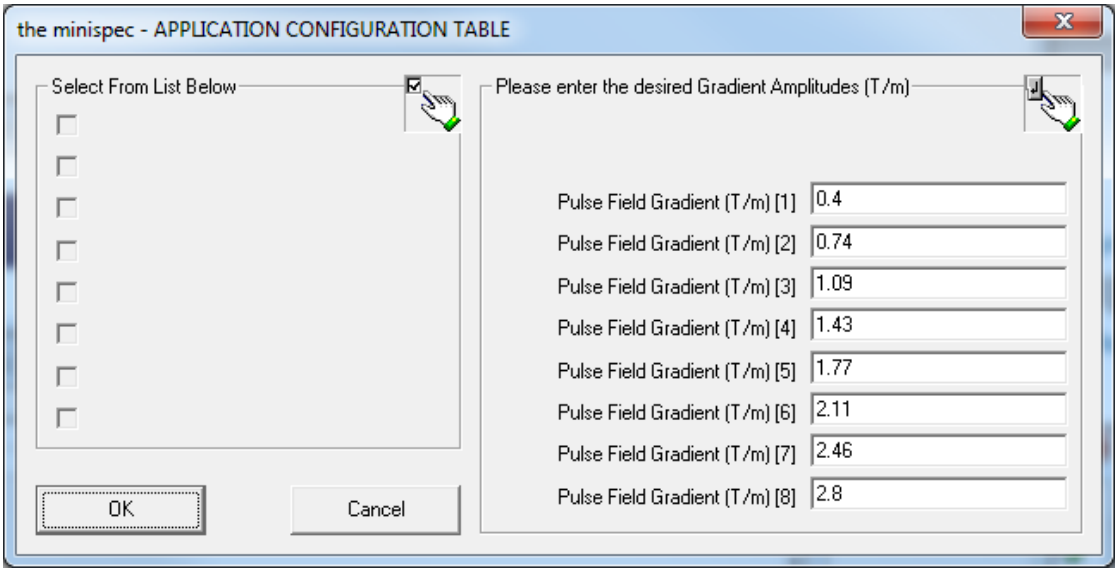


Figure 4.6: Third Configuration Table Shown when the Experimental Method is Oil Droplets and the Operation Mode is Research.

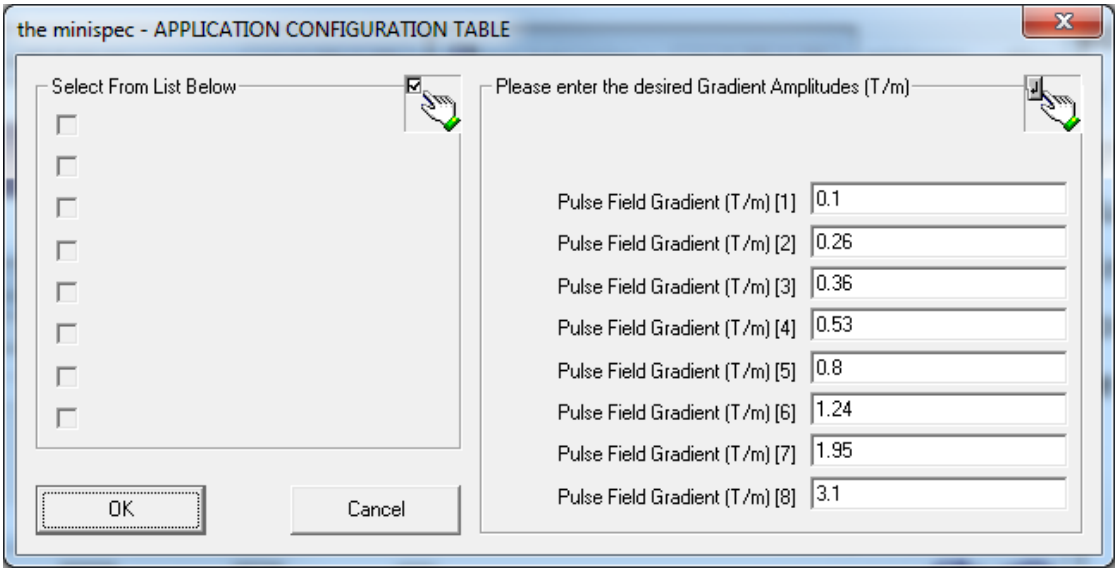


Figure 4.7: Third Configuration Table Shown when the Experimental Method is Water Droplets and the Operation Mode is Research.

One point to note is that all subsequent experiments will in principle use these values for the gradients strengths, as long as that for the sample in study the NMR signal intensity for the strongest gradient is higher than the minimum NMR signal intensity defined after pressing the button *Measure* (discussed in detail below in *The Measurement Procedure [▶ 22]*).

In the case that it fails to fulfill this criteria, the strongest gradient will be automatically calculated by a pilot experiment and then all gradient values will be recalculated considering the weakest and maximum gradient strengths accordingly to the selection: *linear variation of the gradient* displayed in the first window of the *First Configuration Table [13]*. This recalculation is disregarded when another experiment is started afterwards (serial measurement) or when terminating the measurements and starting a new one by pressing *Measure* again. In this case a new verification for the strongest gradient strength will be done and the whole procedure repeated. This topic is discussed in more details below in *The Measurement Procedure [22]* under *Advanced Measurement Settings* and also in *The Gradient Pulse duration and the Pulse Field Gradient End [34]*.

Moreover, when the table is reopened, the values will be refreshed accordingly to the parameters set in the first two configuration table windows.

4.2 The Calibration Procedure

The settings used for the calibration are not related to the settings chosen in the configuration tables. Regardless of the operator mode, most of the settings are fixed and cannot be changed.

The calibration procedure is divided into three steps, each of which the user can decide whether or not to use, as long as the whole calibration procedure has been previously executed once. When performing the first calibration of the instrument, the user will not be able to select individual steps, as the steps will be grayed out. The following figure displays the window which pops up when the **Calibrate** button is pressed.

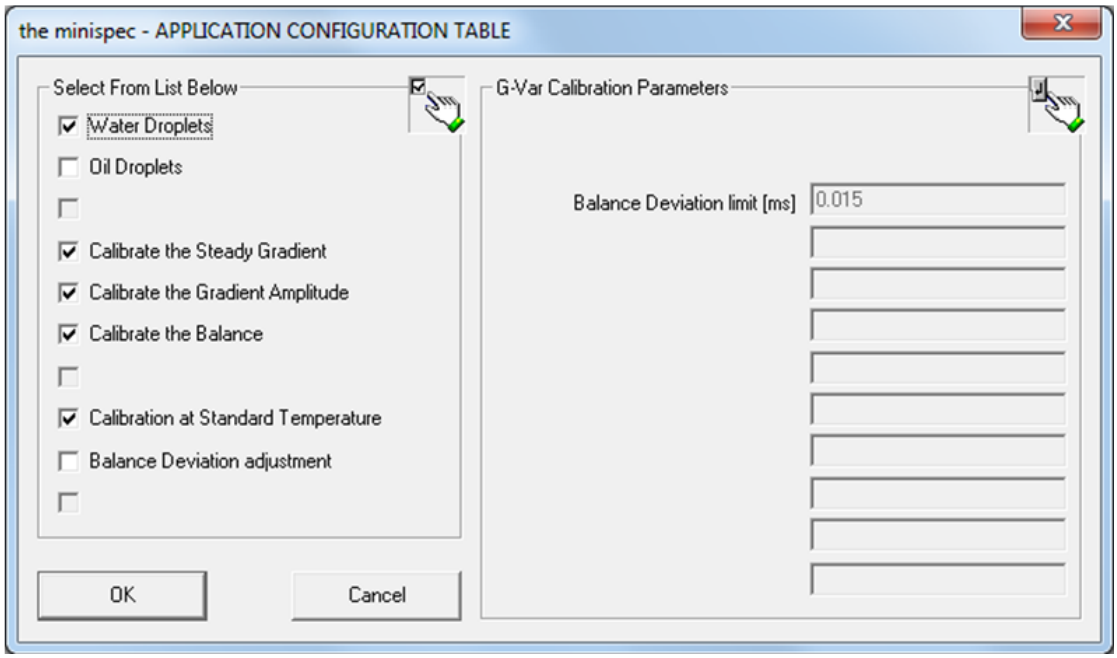


Figure 4.8: Options Available during the Calibration Procedure.

When the instrument is calibrated for the first time, the options *Calibrate the Steady Gradient*, *Calibrate the Gradient Amplitude* and *Calibrate the Balance* will be checked and grayed out.

The first two steps in the calibration procedure (*Calibrate the Steady Gradient* and *Calibrate the Gradient Amplitude*) are the same regardless the experimental method selected. The only difference is that when the option *Calibrate at Standard Temperature* is selected, a pop up window will appear asking if the probe and sample are at 20°C (for oil droplets) or at 5°C (for water droplets). For both steps the recommended sample is (0.5 % $CuSO_4$ 5·H_2O). Details about the sample preparation can be found in *Sample Preparation and Remarks [37]*.

The third calibration step is also the same regardless if the *Water Droplets* or the *Oil Droplets* option has been selected. The only difference is that for oil droplets a sample with D_{33} (average droplet size) of 4 µm is requested, while for water droplets a sample with D_{33} of 6 µm is requested. As explained before, the default temperature for the calibration is 20°C for oil droplets and 5°C for water droplets.

The options *Calibrate at Standard Temperature* and *Balance Deviation adjustment* are changeable upon entering the administrator password. When the first one is unselected, the user is prompt to enter the diffusion coefficient of the sample to be used in the second calibration step, and temperature at which the calibration will be done. When the second option is selected, the user will be able to edit the balance deviation limit in the right side of the window. This parameter is related to the balance adjustment in the second and third calibration steps, being the acceptable deviation limit between the theoretical and experimental echo tops during the calibration. Details about this parameter will be discussed in *Calibration of the Balance [▶ 20]*.

After the calibration has been successfully completed, it will store all pertinent data in the file *G-Var_calibration.cdt*, located in the *NFxxxx* folder (where *xxxx* represents the instrument´s serial number), created where the application is located. This file does not depend on the name of the application which has generated it, the operation mode or the experimental method. Therefore, once this file is successfully created, the user can measure either oil droplets or water droplets in any operation mode. Moreover, even duplications of the application (as long as they are in the same folder as the original one) can be used without the need of recalibrating the instrument. This is particularly interesting for *Research* operation mode, where one can create duplications of the application, with each file having a different parameter tree. Each calibration step is explained in detail in the upcoming sections.

4.2.1 Calibration of the Steady Gradient

During the measurements a steady field gradient is applied to guarantee a defined magnetic field homogeneity of 0.5 ms. This homogeneity value provides stable gradient echoes, without disturbing the measurements.

This calibration step adjusts the steady gradient in order to obtain a signal width (homogeneity) of 0.5 ms. During the calibration the user can visualize the changes on the NMR signal (width of the Free Induction Decay) as the steady gradient is changed. After reaching the homogeneity of 0.5 ms, the corresponding steady gradient is displayed in the result box and stored in the calibration file. The following figure shows a typical NMR signal when this calibration step is completed.

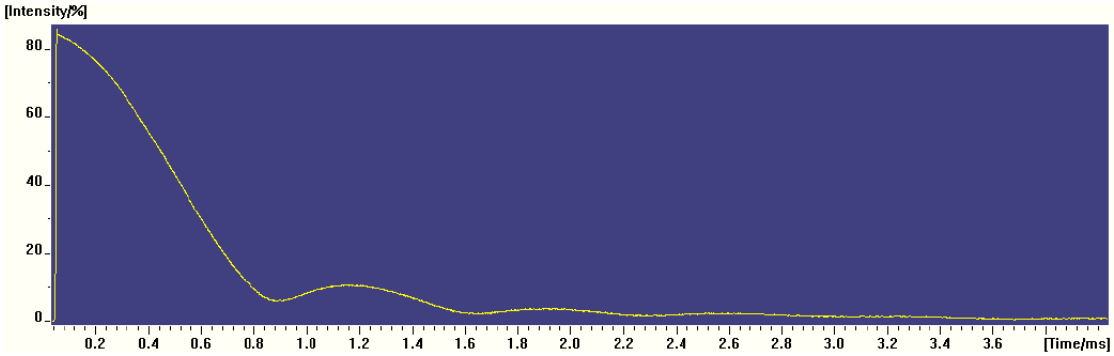


Figure 4.9: NMR Signal Typically Displayed after the Steady Gradient Adjustment.

Recommended Setup:

Method	Standard Temperature	Sample
Oil Droplets	20°C	$CuSO_4$ solution 0.5 % $CuSO_4 \cdot 5{\cdot}H_2O$
Water Droplets	5°C	$CuSO_4$ solution 0.5 % $CuSO_4 \cdot 5{\cdot}H_2O$

Table 4.1: Recommended Settings for the 1st Calibration Step: The Calibration of the Steady Gradient.

4.2.2 Calibration of the Gradient Amplitude

For the calculation of the droplet size distribution, knowledge of the gradient strength [T/m] used during the measurement is necessary. On the other hand, the gradient strength is not a parameter directly controlled by the instrument, in the sense that it is generated and controlled by adjusting electronic currents, being these the accessible parameter by the Instrument, under the name of Gradient Amplitude [%], which can be set from 0 to 100%.

Therefore, it is necessary to determine the correspondence between the Gradient Amplitude [%] (accessible parameter) and the Gradient Strength [T/m] (parameter of interest). For that end, the second step in the calibration procedure is done: the Calibration of the Gradient Amplitude.

This step requires the use of a sample whose diffusion coefficient is known at the temperature at which the calibration is being performed.

During this step several experiments are performed as function of the Gradient Amplitude [%]. For each experiment the Gradient Balance [%] is adjusted in order to get the NMR signal (echo) at the theoretical position. Then the set: {Gradient Amplitude, Gradient Balance, NMR signal amplitude} is saved.

After finishing the experiments for all internally defined values of the Gradient Amplitude [%], one fitting is done to correlate the NMR signal amplitude to the Gradient Amplitude [%], accordingly to Fick´s law for the self-diffusion. From this fitting, the correlation between the Gradient Amplitude [%] (*pfg_amp*) and the Gradient Strength [T/m] (*pfg*) is determined:

*pfg[T/m] = alpha * pgf_amp[%]*, being all relevant information displayed in the result box.

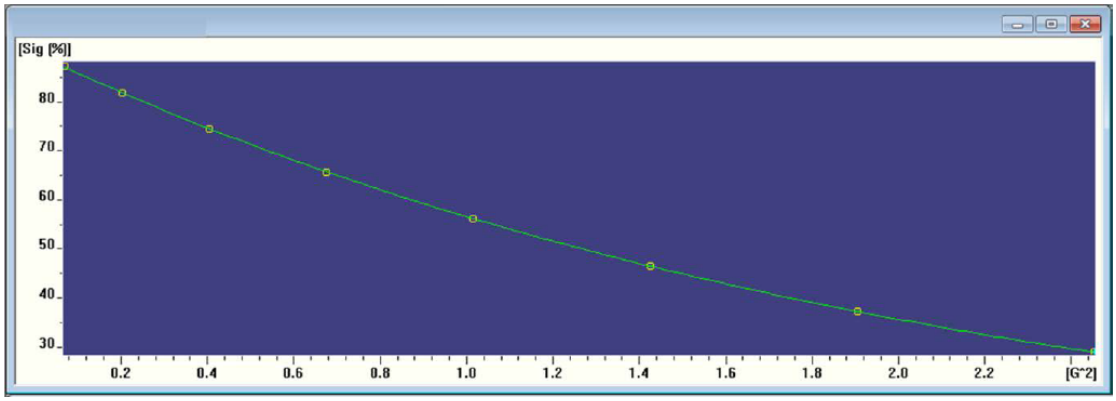


Figure 4.10: Determination of the Relation between the Gradient Amplitude [%] and the Gradient Strength [T/m].

Recommended Setup

Method	Standard Temperature	Sample
Oil Droplets	20°C	$CuSO_4$ solution 0.5 % $CuSO_4 \cdot 5 \cdot H_2O$
Water Droplets	5°C	$CuSO_4$ solution 0.5 % $CuSO_4 \cdot 5 \cdot H_2O$

Table 4.2: Recommended Settings for the 2nd Calibration Step: The Calibration of the Gradient Amplitude.

4.2.3 Calibration of the Balance

As described in *The Measurement Procedure [▶ 22]*, the pulse sequence measures a stimulated echo under the influence of 2 pulsed gradient fields (see the figure *Pulse Sequence Used for the Measurements in the G-Var Application. [▶ 22]*). It is well known that in order to obtain the echo signal close to the theoretical position, both pulsed field gradients must be identical. To ensure that they are as close as possible from each other, a fine tuning of the gradient strength of the second pulsed gradient is done and the position of the echo top is verified. This fine tuning, known as balance, is repeated until the deviation between the theoretical and experimental position of the echo top is smaller than the parameter Balance Deviation Limit shown in the *Options Available during the Calibration Procedure. [▶ 17]*.

As mentioned above, during the second calibration step (*Calibration of the Gradient Amplitude [▶ 19]*), the Balance is also calculated. However, during that step the $CuSO_4$ solution is used, and due to the fast relaxation time and diffusion coefficient, many parameters differ considerably from the standard settings typically used for the measurement. Moreover, the gradient strength range used for the calibration does not cover the whole range used for the measurement. Therefore the third calibration step becomes necessary, where the Balance is adjusted for 8 values of Gradient Strengths, varying from 0.1 T/m to 3.1 T/m (range that cover the typical measurements) by using the same pulse sequence structure to be used in the measurements.

When executed for the first time, this step will use the balance fitting from the second calibration step to determine a starting point for the fine balance adjustment for the several Gradient Strengths [T/m] to be calibrated. In the case that this step is being repeated, i.e. a complete calibration has been previously performed; the application will use the stored data from the third calibration step (fitting curve) to determine the starting point for the fine balance adjustment. Notice that this is the typical case, since the instruments are delivered pre-calibrated.

The first time that the calibration is performed, it might take around 15 minutes to have this step completed, however when the calibration has been previously performed, this step is considerably faster, usually taking less than 5 minutes.

After the end of the calibration, the balance as a function of the Gradient Strength [T/m] is displayed and the 3 different fitting functions are used for the fitting of the displayed data:

- Mono-exponential decay.
- Bi-exponential decay.
- Fourth order Polynomial.

The quality of the fitting is evaluated and the fitting curve that best reproduces the data is selected and displayed in the screen.

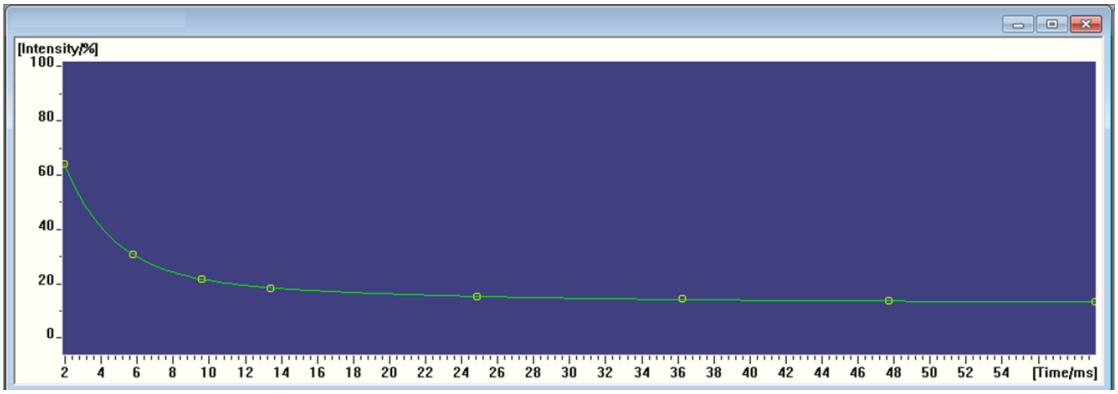


Figure 4.11: Third Calibration Step: Calibration of the Balance vs. the Gradient Amplitude [%].

The quality of the fitting (Fit Error) can be seen in the result box, among all relevant information from this calibration step. The application verifies if the Fit Error is lower than 2. If not, a message is displayed letting the user know that the third calibration step should be repeated; typically this is the case when the calibration is done for the first time.

In the case that repeating the calibration does not improve the Quality of the fitting, one can reduce the Balance Deviation limit, which should lead to an improvement to the Quality of the fitting.

After the calibration ends, the user will see the location of the file which stores all calibration parameters in the result box: /NFxxxx/G-Var_calibration.cdt

Recommended Setup

The calibration requires 2 samples which can be purchased from Bruker: $CuS0_4$ solution and G-Var Balance Calibration sample. Alternatively, the user can produce their own calibration samples, as described in this section.

Method	Standard Temperature	Sample
Oil Droplets	20 °C	G-Var Balance Calibration sample or Mayonnaise with average Oil droplets (D_{33}) smaller than 4µm
Water Droplets	5 °C	G-Var Balance Calibration sample or Margarine with average Water droplets (D_{33}) smaller than 6µm

Table 4.3: Recommended Settings for the 3rd Calibration Step: The Calibration of the Balance.

Despite being necessary to use different temperatures when selecting oil droplets or water droplets for the calibration, it is worth it to remark that the calibration generated can be used for any of both experimental methods. In the last calibration step the only requirement is to use a sample which has NMR signal for the whole range of gradient strengths to be calibrated. In this step the user is prompted to decide which sample will be used for this calibration step. There are few advantages in using the G-Var Balance Calibration sample:

- Can be purchased directly from Bruker, not being necessary to search for samples which fit in the average droplet size requirement when using Margarine or Mayonnaise.
- The sample has no special requirements for storage.
- The sample is stable for long periods of time: 3 years.
- For Water droplets, when using the G-Var Balance Calibration sample the application sets the recycle delay for the third calibration step to 2 seconds, against 5 seconds when Margarines are used, making the calibration considerably faster.

4.3 The Measurement Procedure

4.3.1 The Pulse sequence

As explained in *The Configuration Table [▶ 13]*, the user can define the parameters to be used in the measurement by accessing the Configuration Table. In this section, the meaning of each parameter will be explained in detail. The next figure displays the pulse sequence for water droplets; for oil droplets T1-Supression Delay should be set to 0 and automatically the application removes the T_1-filter (180° pulse and tau_null delay).

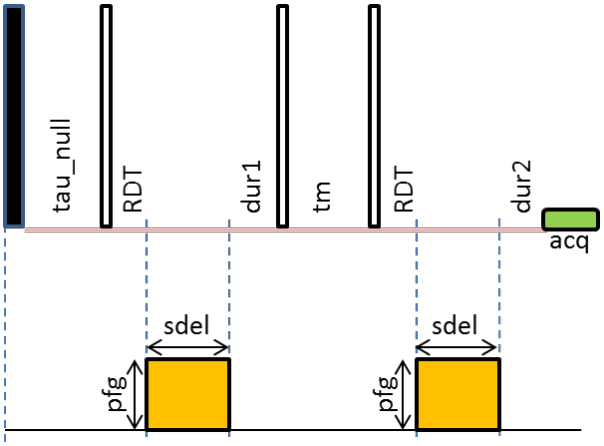


Figure 4.12: Pulse Sequence Used for the Measurements in the G-Var Application.

The open rectangles represent 90° pulses while the black rectangle represents a 180° pulse and the orange rectangle a gradient pulse.

4.3.2 The Configuration Table and the Parameters for the Experiment

As shown before, the accessible parameters in the configuration table are:

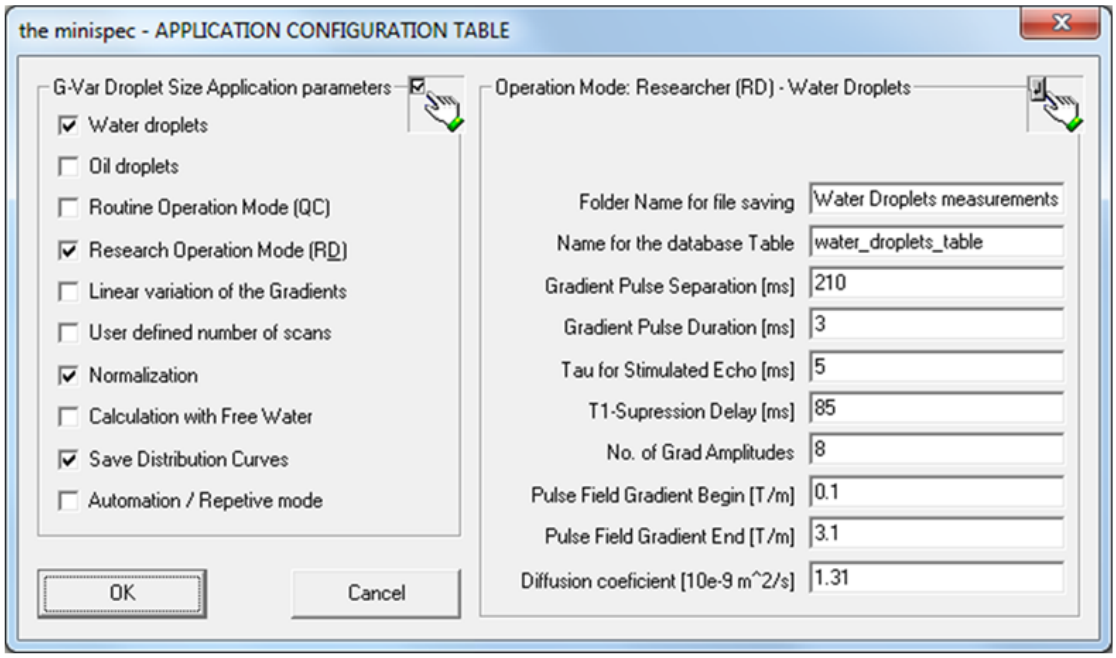


Figure 4.13: Merging all Parameters Available in the two Configuration Tables

Below each option and parameter is discussed in detail.

Water Droplets/Oil Droplets

In the left side one can choose between *Water Droplets* (water in oil emulsions) or *Oil Droplets* (oil in water emulsions).

Research Operation Mode (RD)/Routine Operation Mode (QC)

This option will determine which parameters are accessible to the user. The figure above exemplifies the case of *Research* operation mode, being all parameters accessible in all windows displayed in the configuration table. The administrator password is required to change from QC to RD.

Linear variation of the Gradients

When this option is selected, the gradients to be used in the experiment will be linearly spaced in the range determined by the user. This is typically the case when oil droplets are analyzed, while for the water droplets typically the gradients are distributed in a logarithmic fashion. This option is editable only for the *Research* operation mode, being grayed out for *Routine* operation mode. The default value is set accordingly to the experimental method: *Oil Droplets* (checked) or *Water Droplets* (unchecked).

User defined number of scans

During the measurement procedure the receiver gain is automatically adjusted for each sample to be measured. Based on the receiver gain, the optimal number of scans is calculated. If the user wants to use a different number of scans, he should check this option. In this case, after clicking **OK** in the configuration table, the user will be prompted to enter the number of scans which will be used for all subsequent sample measurements.

Note that when the application automatically adjusts the number of scans, the experiment which has the weakest gradient strength will have 4 fold the value entered, since this one is used to normalize the intensities, being preferable to have its signal with a better signal to noise ratio.

This parameter is available for both operation modes, for routine users the only restriction is that the number of scans must be equal or higher than half of the number of steps in the phase cycling, i.e., higher than 4. For better performance, it is recommended to make the number of scans multiple of 8.

Normalization

For the data evaluation and droplet size determinations, the NMR intensities acquired as function of the gradient strength must be normalized ideally by the NMR intensity when the gradients are not applied. One can understand this intensity as a reference value.

When the *Normalization* option is selected, one additional experiment will be performed without applying gradients and the corresponding signal intensity will be used to normalize all the remaining NMR data. If this option is not selected, the NMR intensities will be normalized by the measurement using the weakest gradient strength.

When studying oil droplets, one must suppress the NMR signal coming from the water, which is achieved by applying a minimum of gradient strength. Therefore, in the case of oil droplets, one has to do the normalization of the data by the experiment performed with the minimum gradient strength (strong enough to suppress the water NMR signal and still weak enough to not disturb the signal from the oil droplets). Therefore the *Normalization* option should be unselected.

On the other hand, when studying water droplets, one does not have this limitation. Since one can use a T_1-filter to remove the signal coming from the oil phase as shown in *Pulse Sequence Used for the Measurements in the G-Var Application. [22]*, nearly without affecting the signal from the water. A more detailed description of how to adjust the T_1-filter is given in *The T1-Supression Delay [34]*. Therefore, when studying water droplets, one should select the *Normalization* option.

Due to the reasons stated above, the *Normalization* option is not selected when the experimental method is oil droplets, being not editable for none of the operation modes; while it is editable by any operation mode when the experimental method is water droplets, being selected by default.

Calculation with Free Water

When this option is selected, the fitting function for the droplet size calculation will include one additional parameter which will represent a NMR signal coming from the free water (continuous phase) in the sample.

When studying oil droplet sizes, usually one performs all the measurements with a minimum of gradient strength in order to suppress the NMR signal from the continuous phase (water). Therefore, one typically would not use the *Calculation with Free Water* in this case. On the other hand, when water droplets are being studied, this option for the data evaluation becomes interesting, since bigger droplets will be taken as part of the free water present on the sample.

Due to the reasons explained above, the *Calculation with Free Water* is not selected by default when the experimental method is set to oil droplets, and it is not editable for none of the operator modes. On the other hand, when the experimental method is set to water droplets, this option becomes available for selection for both operator modes.

Another relevant point is that this parameter is a post-processing option, i.e., the experiment itself and the raw data stored don't depend on this option. Therefore, one can recalculate the droplet size distributions either or not including free water, by choosing this option, without having to repeat the measurement itself.

Finally, when this option is used in samples that have very low concentration of free water (below 5%), typically the fitting function (with free water term) is not the most suitable for the data evaluation. In these cases, the free water calculated is typically 0% and the associated error (Free water error) is 100%. Moreover, the Quality of the fitting (F-statistics) is considerably lower than the one obtained without the Free water term in the fitting function. Therefore, for these samples it is recommended to not use the Free Water calculation.

Save Distribution Curves

When this option is selected, the droplet size distributions will be saved in ASCII format with user defined name and location.

This option is editable in any operation mode and experimental method, being selected by default.

Measurement using Automation/Repetitive mode

This option allows the user to make the measurements using the automation software and hardware.

This option is editable in any operation mode and experimental method, being not selected by default.

In the case that the user wants to make repetitive measurements (for the same sample) and does not have an automation solution, he can select this option and run the measurements in the repetitive mode. To do so, firstly he has to refresh the database table (e.g. by opening the configuration table). Afterwards the user can run the application in the Repetitive mode as usual, being worth it to point out that in the very beginning the user will be prompted to enter the sample name, and from this moment on the application will run in the repetitive mode without any further prompt window.

Folder Name for file saving and Name for the database table

As pointed out in *The G-Var Application Features [▷ 11]*, the application stores the results in the folder *G-Var_results* created in the same folder where the application is. Depending on the experimental method (water droplets or oil droplets) a subfolder is created (*Water Droplets* or *Oil Droplets*) and all results will be stored in a subfolder with the name provided in the field *Folder Name for file saving*. Moreover, in this same folder all information printed in the database table will be saved in ASCII format (table separated) with the name provided in the field *Name for the database Table*. This file can conveniently be imported to Excel or similar software by simply dragging and dropping the file in the desired software. Moreover, when using the same name for this file, the new results will be added at the end of the file.

Gradient Pulse Separation [ms]

The Gradient Pulse Separation, often referred to as *Idel* or *Large Delta*, is the parameter that controls the time between the two pulsed gradient fields (see *Pulse Sequence Used for the Measurements in the G-Var Application. [▷ 22]*).

In *The Gradient Pulse Separation [▷ 33]* it is discussed how to properly adjust this parameter. The default value for this parameter is 210 ms, being possible to change it only in the *Research* operator mode.

The mixture time shown in the *Pulse Sequence Used for the Measurements in the G-Var Application. [▷ 22]* can be calculated in terms of:

Idel, TauW and the 90° pulse length (*p90*): *tm = Idel - (tauw + p90)*

Ideally it should be long enough to allow that the Droplet´s content diffuse inside the whole Droplet during the mixture time (*tm*), however this is not always possible to be achieved for all droplets in the distribution, mainly for the bigger ones.

Gradient Pulse Duration [ms]

The Gradient Pulse Duration, often referred as *Small Delta* or *sdel*, is the parameter that controls the duration of the pulsed gradient field (see *Pulse Sequence Used for the Measurements in the G-Var Application. [22]*). The default value for this parameter is 3 ms, and can be changed only when the operator mode is set to *Research*.

There is an interdependence among *sdel*, *ldel* and the gradient strength (*pfg*) which will be discussed in *The Gradient Pulse Separation [33]* and *The Gradient Pulse duration and the Pulse Field Gradient End [34]*.

Tau for Stimulated Echo [ms]

One can see in the *Pulse Sequence Used for the Measurements in the G-Var Application. [22]* that this sequence uses a stimulated echo, composed by the combination of the three 90° pulses and the delays in between and after them. In analogy to "*tau*" in the Hahn echo which corresponds to half of the echo time, Tau for the stimulated echo corresponds to half of the stimulated echo time. To differentiate both, from now on Tau for the stimulated echo time will be referred as *TauW*.

In the *Pulse Sequence Used for the Measurements in the G-Var Application. [22]* *TauW* is calculated as function of *dur1*, *sdel*, *p90* and the Receiver Dead Time (RDT):

TauW = dur1 + sdel + RDT + p90

Which also has influence in the delay dur2, calculated as:

dur2 = dur1 - acq/2 + 1000.

This parameter is accessible only in the *Research* operation mode, and its default value is 5 ms for both experimental methods.

T1-Supression Delay [ms]

As previously discussed, the pulse sequence starts with a T_1-filter, which is used when water droplets are being studied in order to filter out the signal coming from the oil phase, see *Pulse Sequence Used for the Measurements in the G-Var Application. [22]*. In this figure, the filter time is defined by the variable "*tau_null*". When this parameter is set to 0, the filter is not applied and the sequence starts with the first 90° pulse.

This parameter is accessible only in the *Research* operation mode, and its default value is 0 ms for oil droplets and 85 ms for water droplets.

No. of Gradient Amplitudes

This parameter is related to the number of different values of gradient strengths that will be used for the experiment.

This parameter is editable by any operator mode and its default value is 8 for both experimental methods.

Pulse Field Gradient Begin [T/m]

This parameter defines the first pulse gradient strength to be used in the experiment, which is illustrated as *pfg* in the *Pulse Sequence Used for the Measurements in the G-Var Application. [22]*. This parameter can be changed only when the *Research* operator mode is selected. The default value is 0.4 T/m for oil droplets and 0.1 T/m for water droplets.

Pulse Field Gradient End [T/m]

Similarly to the previous parameter, this one defines the last gradient strength to be used in the experiment. When starting the experiment, after the gain adjustment one experiment using this gradient strength will be performed and the application will check if the signal intensity obtained is above the limits defined by the user (in *Research* mode) or internally defined (in *Routine* mode). More details about this procedure are provided in *The Gradient Pulse duration and the Pulse Field Gradient End [▷ 34]***.**

This parameter can be changed only when the *Research* operator mode is selected. The default value is 2.8 T/m for oil droplets and 3.1 T/m for water droplets.

Diffusion Coefficient [10e-9 m^2/s]

For the droplet size evaluation, it is necessary to know beforehand the diffusion coefficient of the liquid confined in the droplet. One can measure it, by using for instance the diffusion application provided by Bruker.

This parameter can be changed only when the *Research* operation mode is selected, and one must keep in mind that the diffusion coefficient is temperature dependent. The default value for this parameter is 1.31 10^{-9} m^2/s when *Water Droplets* is selected and 0.0098 10^{-9} m^2/s when *Oil Droplets* is selected, which are the water diffusion coefficient at 5°C and the typical oil diffusion coefficient at 20°C, respectively.

Therefore, if the user intends to make the experiment at different temperatures, they must operate the instrument in the *Research* mode, to be able to redefine the diffusion coefficient.

4.3.3 The Measurement

When a new measurement is started by clicking on the button **Measure**, unless the *Automation*/*Repetitive* option was selected, the following window will pop up:

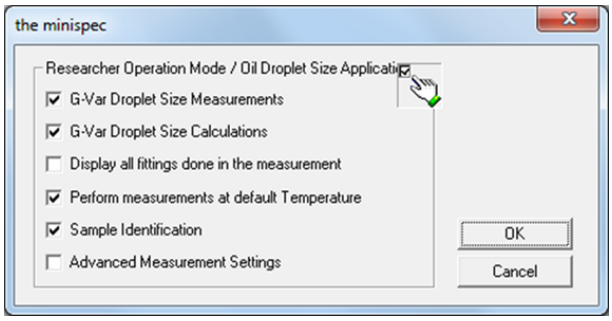


Figure 4.14: Window Which Prompts when Pressing the Button Measure.

G-VAR Droplet Size Measurements

When this option is selected, measurements will be performed.

G-VAR Droplet Size Calculations

When this option is selected, calculations will be performed. It is possible to make only recalculations by selecting this option and unselecting the first one.

Display all fittings done in the measurement

This option allows the user to check every fitting done over the whole measurement, e.g., Gaussian fittings for the echo top determination for each gradient strength; the calibration curves being used for the measurement etc. This option is by default unselected in order to save time. When selected, each fitting will be displayed for around 3 seconds and the experiment resumes automatically.

Perform measurements at default Temperature

This option allows the user to perform the experiment at a different temperature than the default. If not selected, the user will be prompted to enter the temperature and diffusion coefficient of the sample in study. This allows the user to later on check in the log files the temperature that the experiment was performed and diffusion coefficient used.

This option is selected and locked for *Routine* operation mode, while for the *Research* operation mode it is possible to unselect it.

Sample Identification

When selected, the user will be prompt to enter the sample identification, which will be written in the database table and also on the file logs created by the application, which contain all relevant parameters used in the measurement. If not selected, *NoID* will be assigned as the sample identification.

Advanced Measurement Settings

This option appears only when the experiment is started in the *Research* mode, and allows the user to define the minimum NMR signal intensity [%] allowed during the measurements. This value is used to adjust the strongest gradient amplitude in the experiment, procedure done at the beginning of the measurement for each sample (even when serial measurement are done without pressing the button measure again), which is described in *The Gradient Pulse duration and the Pulse Field Gradient End [34]*.

This value is written in the log files and also at the end of the result box under the name *NMR Signal limits*, whose default value is 5% for the minimum and 95% for the maximum, being the last one not changeable regardless the operation mode.

4.3.4 The Database Table

When the application is loaded, three different windows will appear in the minispec software: one for the NMR signal (blue background), one for the result box and one for the results. The third one is a table, often referred as Database Table, which can be saved or loaded in the minispec Software. The following table illustrates the information displayed in such table.

No	Sample ID	Date/Time	D3_3	D0_0	Sigma	exp(sigma)	2_5%	50%	97_5%	Free Water	Fstatistics

Table 4.4: Database Table

This table will be automatically filled out after finishing each experiment, and its content is also saved automatically as described earlier in this section, when the parameters *Folder Name for File Saving* and *Name for the Database Table* were discussed.

The user can also save the table by going to **File** | **Save as** and selecting the minispec – **Spread Sheet Files (*.mdb)** in the field *Save as type*. This file format can also be loaded later on by clicking on the configuration table and browsing to the saved file. It is important to remark that whenever the configuration table or parameter table is accessed, the database table will be refreshed by a blank one. The physical meaning of each column in this table is discussed below.

No

This column stores the number of experiments done. It is important to remark that if the user refreshes the database table (getting a blank one), this counter will restart from 1. Moreover, if the same file is used to save the table content, i.e., the *Folder Name for file saving* and *Name for the database Table* were not modified after refreshing the table, the next results will be appended at the end of this ASCII file.

Sample ID

The Sample ID is the sample identification provided by the user. If this option is not selected when starting the measurement, this column will be filled out with *NoID*.

Date/Time

The date (Day.Month.Year) and time (Hour:Minutes:Seconds) when the experiment was done.

D3_3 and D0_0

Among the parameters that one can calculate from the droplet size distribution, there are 2 particularly interesting: the geometric average of the droplet size in terms of number of occurrences; and the geometric average of the volume distribution of the droplet size.

D3_3 represents the geometric average of the Droplet Size [µm] in the volume distribution, being 50% of the droplets smaller than this value and 50% bigger.

D0_0 represents the geometric average of the Droplet Size [µm] in the frequency or number distribution.

The calculation of these two parameters is explained in details in *Mathematical Aspects of the Data Processing [▶ 39]*.

Sigma and exp(Sigma)

Sigma represents half of the width of the distribution (standard deviation) of droplet sizes, while exp(Sigma) is simply e^{sigma}.

2_5% , 50% and 97_5%

These columns indicate how many (in %) of the droplets have smaller diameters than the value written in each column.

Free Water

When measuring water droplet sizes, the user can choose to include free water in the calculations. When this option is selected, the percentage of free water in the sample will be written in this column; when this option is not selected, the symbol "**-**" will be printed instead.

Fstatistics

This is a parameter that measures the quality of the fitting. Typically it is few thousands for water droplet size measurements and few tens of thousands for oil droplet size measurements.

5 The G-Var Output Data

After successfully finishing the measurement, the results displayed in the database table will be saved as described in the previous section, under the option *Folder Name for File Saving* and *Name for the Database Table*. In the case that these two entries are not renamed, the new data will be added at the end of this file.

Moreover, the raw data containing the NMR signal amplitude and the corresponding gradient strength (T/m) will be saved in the specified folder with the following format:

`sampleID_yyyy_mm_dd_hhH_ttM.dps`

Where:

- `sampleID` is defined by the user when this option has been selected in the beginning of the measurement;
- `yyyy` is the year when the data was created,
- `mm` is the month,
- `dd` the day,
- `hh` the hour, and,
- `tt` the minutes.

Similarly, another two files are created in the same folder:

`sampleID_yyyy_mm_dd_hhH_ttM_log.cdt`

`sampleID_yyyy_mm_dd_hhH_ttM.cdt`

The first file contains the information necessary for the application to make recalculations; and the second file contains the most important points printed out in the result box during the measurement, being the file where the user can find all parameters used for that specific measurement.

Furthermore, the application creates in the specified folder a subfolder: *Distributions*, where the droplet size distributions and respective integrals are saved when this option is selected in the configuration table: *Save Distribution Curves*. The name format is very similar to the files above:

- `sampleID_yyyy_mm_dd_hhH_ttM_volume distribution.dps`
- `sampleID_yyyy_mm_dd_hhH_ttM_volume distribution_integrated.dps`,

The first file is for the droplet size distribution and the second for its integration.

6 Fine Tuning of the G-Var Parameters

This section is reserved for the *Research* operation mode, and it describes how to fine tune some of the parameters used for the measurement.

6.1 The Gradient Pulse Separation

For setting up the gradient pulse separation, one must know the range of the droplet sizes to be measured. Physically, this is the time when the diffusion of the liquid takes place inside the droplet. Therefore, ideally it should be long enough to guarantee that during this time in average the molecules have diffuse inside the whole droplet size volume (being more critical for the bigger droplets in the distribution), otherwise the results will underestimate the droplet sizes. On the other hand, this parameter cannot be too long, otherwise the T_1 relaxation will interfere in the measurement.

One experimental way to determine the optimal range of values for this parameter is plotting the normalized NMR signal Mg/M(0) as function of the gradient pulse separation, where Mg is the NMR signal measured when applying a certain gradient strength and M(0) is the intensity measured under the same conditions but without gradients applied.

The result is typically a curve like the one shown in the figure below. The initial fast decay shows that the molecules didn't met the boundaries of the droplet yet for this diffusion time, behaving as a "free diffusion". As the gradient pulse separation increases, the decay becomes smoother, reaching at certain point a plateau that physically means that for this diffusion time all molecules which diffuse inside the different droplets have met the boundaries of them, being this range the most suitable to perform the experiment.

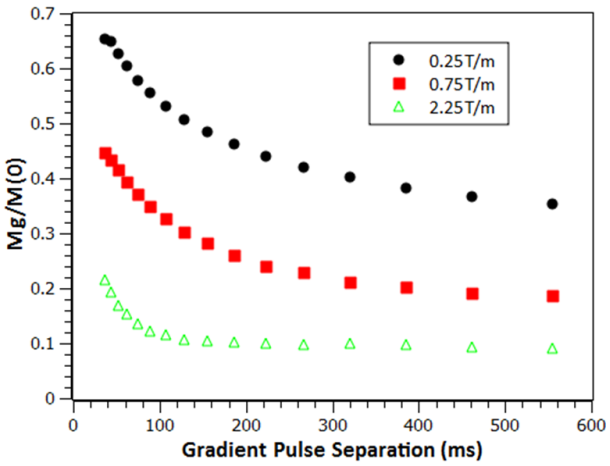


Figure 6.1: Determining the Optimal Value for the Gradient Pulse Separation.

6.2 The Gradient Pulse duration and the Pulse Field Gradient End

There is an interdependency between these two parameters: as one can see in *The Experimental Parameters and the Mathematical Model [41]*, the NMR signal decays (approximately) exponentially with the product of square power of the gradient strength times the duration of the gradient strength.

For certain samples, it might be that the NMR signal disappears after increasing the gradient amplitude. In order to prevent running into this kind of problem in a later stage of the experiment, the application automatically checks at the beginning of each experiment the NMR intensity obtained when the last (strongest) gradient is used. When the NMR signal intensity is below the specifications (available for the *Research* operator, 5 % for the *Routine*), the gradient strength is automatically reduced and further tested. The procedure is repeated until the NMR intensity specification is reached. Afterwards the whole gradient range to be used in the experiment is automatically redefined, keeping the original number of desired points in the final curve.

When the user wants to perform measurements for the original range, they can try to reduce the gradient pulse duration and retry to run the experiment.

Another possible configuration is that the changes in the intensity as function of the gradient strength are not big enough for a suitable fitting. In this case the user either can increase the Pulse Field Gradient end value or increase the Pulse Gradient Duration.

6.3 The T1-Supression Delay

As discussed in *The Configuration Table and the Parameters for the Experiment [23]* under the description of the normalization option, the experiments can be done following a T_1-filter, which is commonly used when water droplets are studied in order to suppress the signal from the oil phase.

To fine adjust this parameter, one can use the application *t1_invrec_table_mq_nf*, which is an inversion-recovery sequence, unselecting the option *mono-exponential fitting* in the configuration table. The curve obtained for the emulsions will be typically a bi-exponential like curve, having a short T_1 component (T_{1oil}) and one longer T_1 component (T_{1water}):

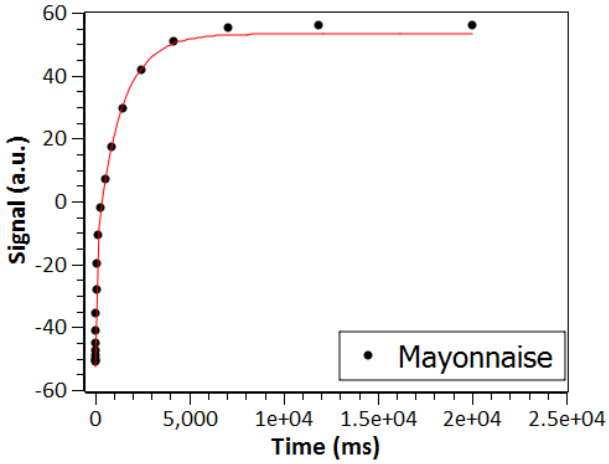


Figure 6.2: Inversion-Recovery Curve for a Mayonnaise Sample.

From this experiment, one can determine the optimal value for the T_1-filter:

$T1\_filter = \ln(2)*T_{1\_oil}$

This corresponds to the time for which the NMR signal from the oil cross the 0% intensity, as exemplified in the following figure, where the deconvolution of the Inversion-Recovery curve was done to illustrate the signal from each component (oil and water).

From this experiment one can also define the recycle delay for the experiment, by using $5*T_{1\_water}$.

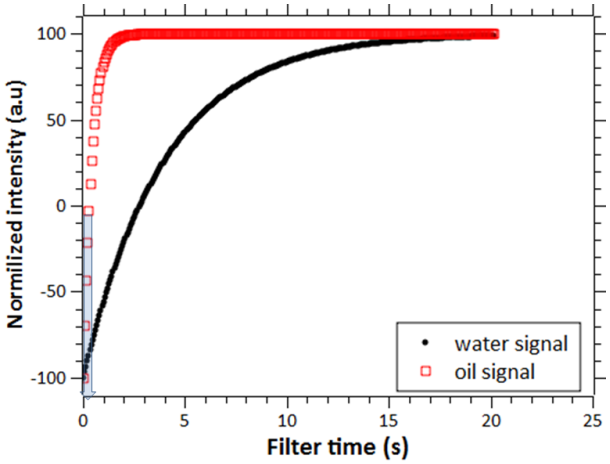


Figure 6.3: Deconvolution of an Inversion-Recovery Curve having 2 Distinct T1 Relaxation Times.

6.4 The Diffusion Coefficient

In order to make the calculation of the droplet size distribution, one must know beforehand the diffusion coefficient of the liquid confined in the droplet at the temperature that the experiment will be carried out.

The estimation of the diffusion coefficient can be done by using one of the standard Bruker applications: *self_diffusion_coefficient_mq_nf*, which can be found in the *Diffusion Pool*. However this application assumes a free diffusion for the calculation, being recommended for the user to prepare a solution of the liquid confined in the droplets and make a measurement of it at the target temperature.

Fine Tuning of the G-Var Parameters

7 Sample Preparation and Remarks

In order to perform precise experiments, it is recommended that the whole sample volume to be analyzed is in the homogeneous B1 - field region of the probe coil. Therefore the sample tubes should always be filled up to 1.5 cm (probe PH H20-10-25(33)-AVGX(Y)), independent of which sample should be analyzed; including the doped water sample (0.5 % $CuSO_4 \cdot 5 \cdot H_2O$) and the samples used for the calibration procedure.

7.1 Accurate Sample Temperature Control

The droplet size experiments are typically done either at 5 °C or 20 °C, for water droplets or oil droplets, respectively. In any case, one must make sure that the samples are at the target temperature during the whole measurement. It is important to remark that it is expected to have deviations between the temperature set in the thermostat/cryostat bath and the actual temperature at the sample position, being this deviation a critical point for the quantification of the droplet size distribution, since the diffusion coefficient is temperature dependent.

Therefore, before starting the measurements and even the calibration procedure, it is recommended to measure the temperature at the sample position inside the probe. To do so, one can either use a suitable thermometer or a sample tube with a liquid inside, making a hole in the tube´s cap and inserting a thermometer inside to measure the temperature of such sample. In both cases, one should adjust the temperature in the thermostat/cryostat bath in order to achieve the target temperature at the sample position inside the probe.

7.2 Low Temperatures and N2 Additional Air Flow

Whenever an experiment is performed at low temperatures (lower than 7°C), it is recommended to use an additional N_2 air flow of 3 liter/hour in the probe to prevent water condensation inside the probe.

Sample Preparation and Remarks

8 Mathematical Aspects of the Data Processing

Droplet size distributions of water-in-oil-emulsions (like margarine and low-calorie spreads) or oil-in-water-emulsions (like mayonnaise and dressings) are assumed to be log-normal. Experimental data show that this mathematical function is most suitable to describe particle size distributions of these products.

In the figure below the droplet diameter *d* is plotted on the x-axis and the relative frequency of a droplet *q(d)* with a specific diameter is shown on the y-axis (frequency distribution curve). The integration of frequency distribution leads to the sum distribution *Q(d)* that gives the fraction of droplets being smaller than or equal to the diameter *d*. The values of *Q(d)* are between 0 for the smallest droplet diameter d_{min} and 1 for the largest diameter d_{max}.

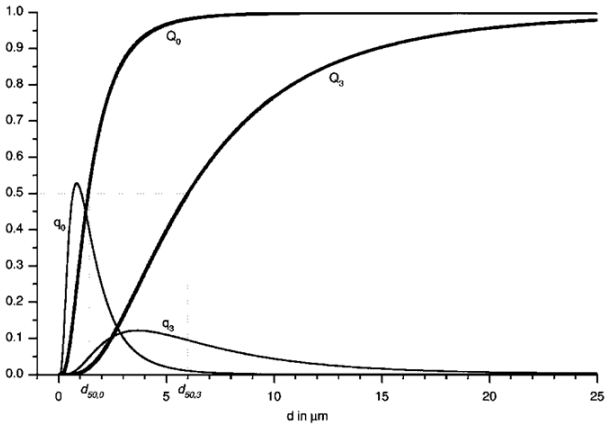


Figure 8.1: Droplet Size Distributions ($d_{50,3}$ = 6.0 µm, $d_{50,0}$ = 1.4 µm, σ = 0.7).

These distributions can be related to different sorts of quantities, which is specified by an index *'i'* at *q* and *Q*. Volume and number are the mainly used sorts of quantities; the index 3 is written for volume and the index 0 for number.

Log-normal distributions are not symmetric, because on one hand droplets will never be smaller than 0 µm and on the other hand the natural limit at large droplets will be much vaguer. If diameters are plotted logarithmic, as shown in the next figure, the frequency distribution turns into the bell-shaped Gaussian normal distribution.

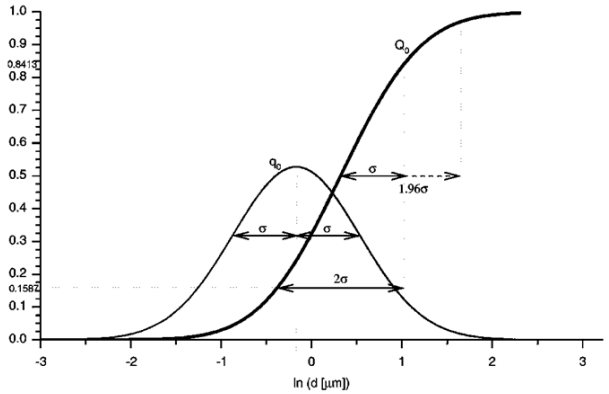


Figure 8.2: Droplet Size Distributions Q_0 and q_0 ($d_{50,0}$ = 1.4 µm, σ = 0.7) in Logarithm Scale.

Mathematically log-normal distributions are described as follows:

$$q_i(d) = \frac{1}{d \cdot \sigma \cdot \sqrt{2\pi}} \cdot e^{\left(-\frac{(\ln(d) - \ln(d_{50,i}))^2}{2\sigma^2} \right)}$$

Therefore, the Particle Size Distribution is characterized by two parameters:

- The geometric mean diameter $d_{50,i}$: 50 % of droplets are smaller and 50 % larger than this diameter, so the area under the distribution curve is divided into equal halves by the geometric mean diameter. This parameter is denoted as $d_{3,i}$.
- The standard deviation σ: width of the distribution.

The quite different shape of volume (*q3*) and number (*q0*) distribution are explained by considering the following facts: Small droplets are present in very large numbers, but they do not contribute a lot to the total volume of water. Droplets with high diameters do not occur in a great quantity, but they represent the main part of volume. So one droplet with *d* = 10 µm occupies the same volume as thousand droplets with *d* = 1 µm. Expressed by distribution parameters the geometric mean diameter of number distribution ($d_{50,0}$) is smaller than that of volume distribution ($d_{50,3}$).

Note that the standard deviations of both distributions are equal.

$d_{50,0}$ can be calculated from $d_{50,3}$:

$$d_{50,0} = d_{50,3} / e^{3\sigma^2}$$

For microbial keeping properties the width of the volume distribution and especially the largest droplets are important. So it is useful to determine distribution intervals. They are derived from the graph in log-scale using values of standardized normal distribution:

For example 95 % of total volume of the droplets of the above sample ($d_{50,3}$ = 6 µm, σ = 0,7) are in the following range (log-scale):

lower limit: $\ln(d_{50,3}) - 1.96 \cdot \sigma$

upper limit: $\ln(d_{50,3}) + 1.96 \cdot \sigma$

Or transferred to linear scale:

lower limit: $d_{50,3} / e^{1.96 \cdot \sigma}$ = 1.5 µm

upper limit: $d_{50,3} \cdot e^{1,96 \cdot \sigma}$ = 23.7 µm

In other words 2.5 % of droplet volume is smaller than 1.5 µm and 97.5 % of droplet volume is smaller than 23.7 µm.

8.1 The Experimental Parameters and the Mathematical Model

During the experiment, the NMR signal is acquired as function of the gradient strength, which will be denoted by *g* for the sake of simplicity. These amplitudes are them normalized by the NMR amplitude obtained using the weakest gradient (or no gradient at all when the Normalization option is selected, as described in *The Measurement Procedure [▶ 22]*). Such normalized amplitudes will be denoted by *R*, being a function of the Pulse Gradient Separation (Δ), the Pulse Gradient Duration (δ), the self-diffusion coefficient D_s, the Gradient Strength g and – due to the effect of restricted diffusion – of the droplet radius *d*:

$$R(\Delta,\delta,D_s,g,d)=\exp\left[-2\gamma^2g^2\sum_{m=1}^{\infty}\frac{\frac{2\delta}{\alpha_m^2D_s}-\frac{2+e^{-\alpha_m^2D_s(\Delta-\delta)}-2e^{-\alpha_m^2D_s\Delta}-2e^{-\alpha_m^2D_s\delta}+e^{-\alpha_m^2D_s(\Delta+\delta)}}{(\alpha_m^2D_s)^2}}{\alpha_m^2(\alpha_m^2d^2-2)}\right]$$

a_m is the m^{th} positive root of the Bessel function equation:

$$\frac{1}{\alpha d}J_{3/2}(\alpha d)=J_{5/2}(\alpha d)$$

g : gyromagnetic ration (=$2.675.10^8$(Ts)$^{-1}$ for protons)

The above function is valid for uniform droplets. For calculation a droplet size distribution is divided into 8 classes assuming uniform droplets for each class.

In the **D-Var** application the Pulse Gradient Duration δ is varied and all other parameters are constant, while in **G-Var** the parameter which is varied is the Pulse Gradient Strength (*g*). Then the parameters of the Droplet Size Distribution *d50,3* and σ can be calculated from the measured data R by a non-linear regression fit (Levenberg-Marquart).

9 D-Var Application Software for Water Droplet Size Determination

If the application software was not originally licensed on your PC by Bruker BioSpin GmbH, but the license arrived separately or later as an upgrade, the license need to be entered into the minispec.exe software. Load the minispec application file *water_droplet_size_mq_nf.app* from the *mq NF Application Pool V8.0 Diffusion* and start the application by pressing **Calibrate**. In case the license is missing, the software will prompt the user to enter the license number. This needs to be done only once.

Measurements are performed at 5 °C and field gradients of 2.0 T/m. For a specific sample appropriate δ values between 0.05 and 5.0 ms are chosen automatically by the application.

9.1 Calibration

- Probe and sample have to be cooled to a constant temperature of 5 °C.
- Update Settings (magnetic field, detection angle and pulse length or alternatively daily check if Update Settings has been done before) with doped water sample. These data can be written into a new instrument settings table.
- Calibrate Button (Calibration Routine for Droplet Size Determination): This procedure is completely carried out with a doped water sample. The calibration is divided into three parts. First it is possible to decide between *Automatic* or *Manual* calibration. In *Automatic* calibration the tuning routines are carried out one after the other. In *Manual* mode the parts can be selected individually from the calibration menu.
- Adjust Steady Gradient (Homogeneity)

  During measurements a steady field gradient is applied to guarantee a defined magnetic field homogeneity of 0.5 ms. This homogeneity value provides stable gradient echoes, but does not disturb this kind of measurements at all. A tuning routine is used to find the steady gradient amplitude necessary for the desired homogeneity. In *Manual* calibration it is possible to change the desired homogeneity of 0.5 ms.
- Adjust Gradient Balance for Calibration

  The value for Pulsed Gradient Balance is determined by a tuning routine to achieve the optimal echo position. In *Manual* calibration the user can set a start value for balance adjustment.
- Calibrate Pulsed Gradient

  The Pulsed Gradient Strength is determined measuring echo amplitudes with and without a gradient of a sample with known self-diffusion coefficient, usually water at 5 °C (D_s = 1.31 10^{-9} m²/s). But it is also possible to use a different sample with a known self-diffusion coefficient. A tuning routine finds the Pulsed Gradient Amplitude required to produce the previously defined gradient strength of 2.0 T/m. This value is a sensible default setting for this application close to the maximal gradient strength of some minispec system configurations. So normally no modification of gradient strength is necessary, although it is possible in *Manual* calibration for special purposes. As there is a slight dependence of the Gradient Balance on the Gradient Amplitude, a balance check is applied after each measurement with a gradient. If this check fails, the balance is re-adjusted (*Automatic* calibration) or unbalance is displayed in ms, and it is possible to decide whether to re-adjust the balance or to continue the gradient calibration (*Manual* calibration). The gradient calibration is finished, when 3 measurements in succession are within the limits 1.99 and 2.01 T/m.

If a calibration is performed automatically, the default settings are used in each calibration part.

It is also possible to change other parameters/durations of this application by opening the application configuration menu. The following dialog will appear first:

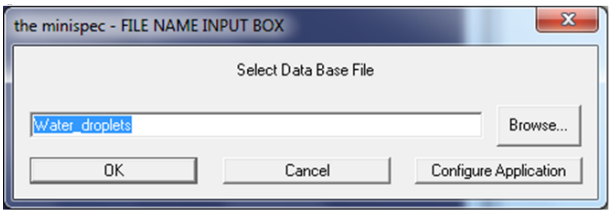


Figure 9.1: File Name Input Box

Press **Configure Application** to alter the parameters or durations. Another table appears on the screen:

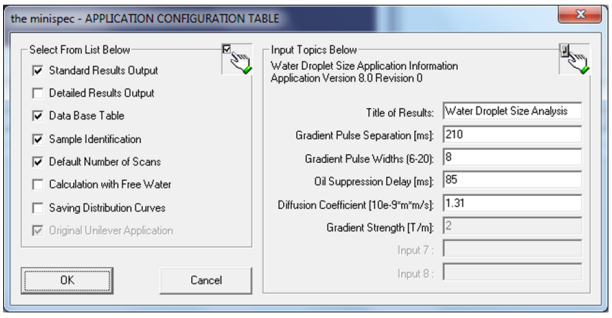


Figure 9.2: Application Configuration Table

- Standard Results Output versus Detailed Results Output

  The *Standard Results Output* will display the information about the results of the measured sample mainly. The detailed output shows more than that: also the signal strengths during the different measurement steps etc. can be followed by checking the detailed results output.

- Data Base Table

  If *Data Base Table* is activated, all results are additionally protocoled into a Microsoft Access database table. This database table is located below the result box and can also be de-activated by un-checking this option.

- Sample Identification

  If *Sample Identification* is activated, it is possible to input an individual expression consisting of maximal 8 characters and 3 digits for each sample. If *Sample Identification* is not selected, samples are numbered automatically. The default setting is *Sample Identification*.

- Default Number of Scans

  Uncheck *Default Number of Scans* to define the number of scans before starting the measurement. Otherwise the default number of scans is set, which is determined according to the receiver gain value for each sample.

- Calculation with Free Water

  Samples with high amounts of water, or samples that have not been treated correctly, it may have certain areas of free water. In this case the water is no longer trapped into droplets, but behaves like free water (non-restricted diffusion). The application can determine such a sample behavior and can calculate the amount of free water accordingly.

- Saving Distribution Curves

  Distribution curves are displayed after the results calculation. Uncheck this option if these curves should not be saved on the PC hard disk.

- Title of Results

  Here the headline of the result box data is defined.

- Gradient Pulse Separation/Ldelta (Δ):

  Ldelta (Δ), is the time between the two gradient pulses, usually 210 ms. This value is suitable for common margarines and low-calorie spreads, and thus does not need to be varied for these products.

- Number of Gradient Pulse Widths

  This parameter can be varied between 6 and 20 and is analog to the number of measurement points. With a low value only a short time for measurement is needed. But increasing the number of measurement points leads to more precise results. With these aspects you can choose the optimal number of gradient pulse widths for your special purpose. The default setting is 8.

- Oil Suppression Delay/Tau_null (τ_0)

  The Tau_null (τ_0) is the duration between the 180° pulse at the beginning of the pulse sequence (which is used to suppress fat signal) and the first 90° pulse. The default value of 85 ms is suitable for common products.

- Diffusion Coefficient for Calculation

  The default setting is the self-diffusion coefficient of pure water at 5 °C of $1.31 \cdot 10^{-9}$ m^2/s. Additives may reduce the diffusion coefficient of water phase in emulsions. So it is possible to use the real diffusion coefficient for calculation.

9.2 Measurement and Calculation

Before a measurement is performed be sure that the system is calibrated and the probe and sample are at 5 °C. If the system has not been used for a longer time, a new calibration is recommended.

The final results will be also written automatically into the Microsoft Access database. This results presentation is a nice platform for printing the results of numerous samples.

The application is structured in a way, that the user is free to perform measurements and calculations in arbitrary sequence.

Before the first measurement a list of important application parameters is printed to the result box:

- Ldelta Δ, τ_0.
- The Number of δ or data points.
- The Strength of the Gradient in T/m.
- The Pulsed and the Steady Gradient Amplitude.
- The Balance for measurement.
- The Number of Scans (user-defined or default)
- The Result Output (Sample Id or Standard).

If *Sample Identification* is selected, the next step is to input it in two parts: the first part may consist of 8 characters at maximum, the second of 3 digits. These parts are connected by a "_". Additionally, the date is appended automatically to get the full sample identification, which is equal to the *Data Pairs* file name (where the measurement data are stored). Examples of *Data Pairs* file names measured on October 23rd, 1996:

- Sample Identification: name_001_231096
- Standard Result Output: 1_231096

If the *Data Pairs* file name already exists, it is possible to set a different name or to overwrite the existing file.

After that it is necessary to insert the sample if it was not done before.

As pulse lengths may vary between different samples, it is possible to check them. The tuning routine finds the pulse lengths that cause minimal signal after a double 90°- and after a 180°-pulse. If **ESC** is pressed during adjustment, the measurement is started immediately.

Before a measurement a test is performed, if receiver gain is suitable to the sample, and if necessary, it is adjusted automatically. According to the gain value the number of scans is set (in default mode) or proposed (in user-defined mode).

Then measurement starts. M_0 is independent from the Gradient Pulse Width δ, so it is measured only at the first and the last δ with the double number of scans as the measurements with gradient. If the difference between the two M_0 values is greater than 3 %, a warning occurs at the end of the measurement. In this case it is recommended to repeat the measurement.

Before the scans with gradient are done, dummy-shots are necessary to avoid unstable echoes during the measurement.

After each scan with gradient balance is checked (at large values for receiver gain after each double or triple scan). If the echo position deviates more than 30 µs from optimal position at the first scan of the first δ the balance adjustment is started. If any unbalance occurs during the measurement, it is printed to the result box (in *Detailed Result Box Output*) and the previous scan is repeated (in every case). After 2 unbalanced echoes in succession, the balance is readjusted. The balance adjustment can be stopped by pressing **ESC**; then the following echo is accepted independent from its position.

The first δ is always 1.0 ms. From the R-value of this measurement the following δ's are determined automatically. R-values above 96 % or below 15 % are rejected, because such extreme values are only of little use.

If the scans for all δ are done, measurement is finished.

For calculation the first step is to input the *Data Pairs* file name. If a measurement was done before the belonging *Data Pairs* file name is proposed.

When fitting is finished, the measured data and the calculated curve are displayed. Here some tools are available for several operations, for example deleting a data point and repeating the fit. If **CONTINUE** is pressed, the calculated values are printed to the result box. The detailed result box output has a different appearance and includes additionally the distribution intervals.

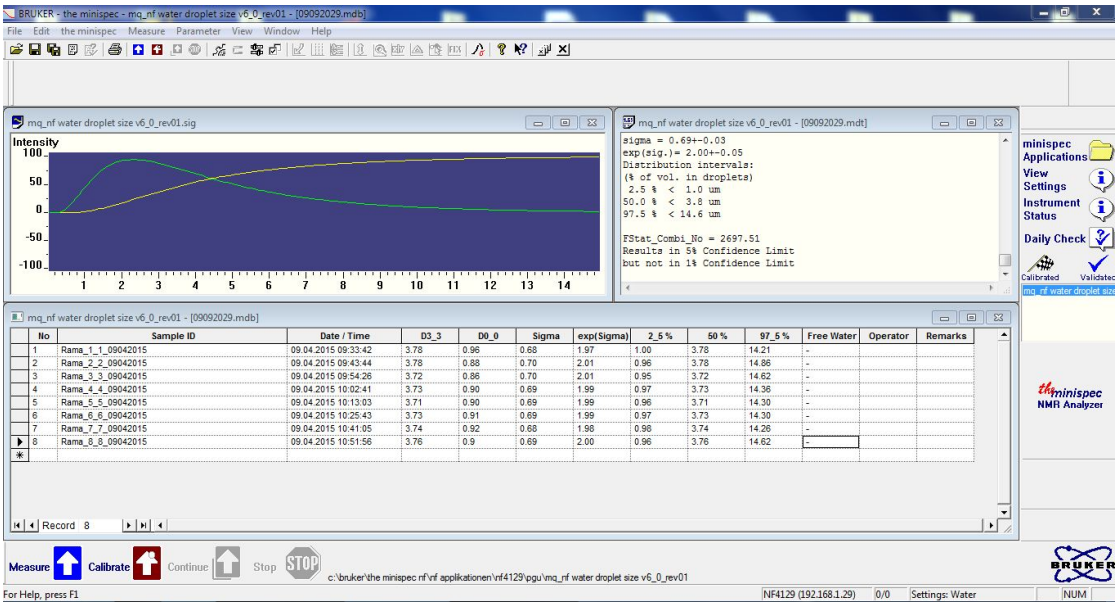


Figure 9.3: The Detailed Result Box with Calculated Curve

The calculated volume distribution curves (q_3 and Q_3) can be optionally displayed (if the application is left here, it is possible to move along sum distribution Q_3 with the cursor [ms = µm]).

Otherwise, further measurements and/or calculations can be performed.

10 D-Var Application Software for Oil Droplet Size Determination

If the application software was not originally licensed on your PC by Bruker BioSpin GmbH, but the license arrived separately or later as an upgrade, the license need to be entered into the minispec.exe software. Load the minispec application file *oil_droplet_size_mq_nf.app* as usual from the *mq NF Application Pool V8.0 Diffusion* and start the application by pressing **Calibrate**. In case the license is missing, the software will prompt the user to enter the license number. This needs to be done only once.

Measurements are performed at 20 °C and field gradients of 2.0 T/m or higher. For a specific sample appropriate δ values between 0.05 and 5.0 ms need to be selected (in later software versions this will be done automatically by the application software).

10.1 Calibration

- Probe and sample have to be tempered to a constant temperature of 20 °C.
- Update Settings (magnetic field, detection angle and pulse length or alternatively daily check if Update Settings has been done before) with doped water sample. These data can be written into a new instrument settings table.
- Calibrate Button (Calibration Routine for Droplet Size Determination): This procedure is completely carried out with a doped water sample. The calibration is divided into three parts. First it is possible to decide between *Automatic* or *Manual* calibration. In *Automatic* calibration the tuning routines are carried out one after the other. In *Manual* mode the parts can be chosen individually from the calibration menu.
- Adjust Steady Gradient (Homogeneity)

  During measurements a steady field gradient is applied to guarantee a defined magnetic field homogeneity of 0.5 ms. This homogeneity value provides stable gradient echoes, but does not disturb this kind of measurements at all. A tuning routine is used to find the steady gradient amplitude necessary for the desired homogeneity. In *Manual* calibration it is possible to change the desired homogeneity of 0.5 ms.
- Adjust Gradient Balance for Calibration

  Value for Pulsed Gradient Balance is determined by a tuning routine to achieve the optimal echo position. In *Manual* calibration the user can set a start value for balance adjustment.
- Calibrate Pulsed Gradient

  The Pulsed Gradient strengths are determined measuring echo amplitudes without and with gradients of a sample with known self-diffusion coefficient, usually water at 20°C (D_s = 2.02 10^{-9} m²/s). But it is possible (but no recommended) to use a different sample with known self-diffusion coefficient, too. A tuning routine finds the Pulsed Gradient Amplitudes required to produce various gradient strengths. There will be a linear relationship between minispec gradient amplitudes and gradient strengths, thus allowing the slope and intercept of such a relation to be calculated. As there is a slight dependence of Gradient Balance from Gradient Amplitude, a balance check is applied after each measurement with gradient. If this check fails, the balance is readjusted (*Automatic* calibration) or unbalance is displayed in ms, and it is possible to decide to re-adjust the balance or to continue the gradient calibration (*Manual* calibration).

If a calibration is performed automatically, the default settings are used in each calibration part.

It is also possible to change further parameters / durations of this application by opening the application configuration menu. The following dialog will appear first:

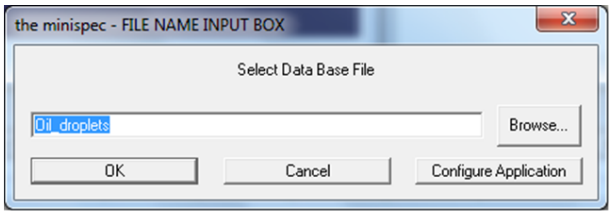


Figure 10.1: File Name Input Box – Oil Droplets

Press **Configure Application** to alter the parameters or durations. Another table appears on the screen:

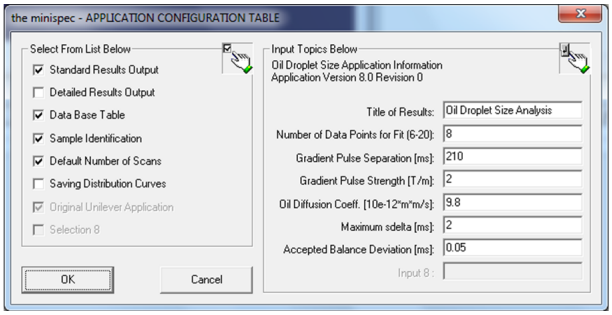


Figure 10.2: Application Configuration Table

- Standard Results Output versus Detailed Results Output

  The *Standard Results Output* will display the information about the results of the measured sample mainly. The detailed output shows more than that: also the signal strengths during the different measurement steps etc. can be followed by checking the detailed results output.

- Data Base Table

  If *Data Base Table* is activated, all results are additionally protocolled into an Microsoft Access database table. This database table is located below the result box and can also be de-activated by un-checking this option.

- Sample Identification

  If *Sample Identification* is activated, it is possible to input an individual expression consisting of maximal 8 characters and 3 digits for each sample. If *Sample Identification* is not selected, samples are numbered automatically. The default setting is *Sample Identification*.

- Default Number of Scans

  Uncheck *Default Number of Scans* to define the number of scans yourself before start of measurement. Otherwise the default number of scans is set, which is determined according to receiver gain value for each sample.

- Saving Distribution Curves

  Distribution curves are displayed after results calculation. Uncheck this option if these curves should not be saved on the PC hard disk.

- Title of Results:

  Here the headline for the result box data is defined.

- Number of Data Points for Fit

  This parameter can be varied between 6 and 20 and is analogue to the number of measurement points. With a low value only a short time for measurement is needed. But an increasing number of measurement points leads to more precise results. With these aspects you can choose the optimal Number of Data Points for Fit for your special purpose. Default setting is 8.

- Gradient Pulse Separation / Ldelta (Δ)

  Ldelta (Δ), is the time between the two gradient pulses, is usually 210 ms. This value is suitable for common mayonnaise or dressing products, and thus does not need to not be varied for these products.

- Gradient Pulse Strength for measurement:

  The default setting is 2 T/m. If the R value cannot be sufficiently reduced even with sdelta = 5msec (maybe down to 10% – 15%), a higher gradient strengths can be selected. The maximum gradient strength should not exceed 3 T/m. See also the maximum sdelta value below.

- Oil Diffusion Coefficient for calculation

  The default setting is the self-diffusion coefficient of a typical oil at 20°C of $9.8 \cdot 10^{-12}$ m^2/s. Special types of oils may have different diffusion coefficients, so it is possible to use the real diffusion coefficient for the result calculation.

- Maximum sdelta value

  The default setting is 2 msec and the maximum sdelta value is fixed to 5 msec. This standard value of 2 msec may be enlarged in order to get R-values in a wider range. The R-values should go down to 10% - 15%. It is recommended to start the analysis with the default settings. If the R-values do not reach 10% - 15%, first re-run the application with a bigger value for the maximum sdelta (e.g. 5 msec). If the alteration of maximum sdelta is not sufficient in order to get low R-value, increase gradient strengths in steps of 0.5T/m. See also gradient pulse strength above.

- Accepted Balance Deviation

  The default setting is 0.05 msec. This value should be suitable for most analysis. In order to accelerate the application, a bigger value maybe selected.

10.2 Measurement and Calculation

Before measurement is performed, be sure that the system is calibrated and the probe and sample are at 20 °C. If the system has not been in used for a longer time, a new calibration is recommended.

The final results will also be written automatically in the Microsoft Access database. The resulting presentation is a nice platform for printing the results of numerous samples.

The application is structured in a way, that the user is free to perform measurements and calculations in arbitrary sequence.

Before the first analysis a list of important application parameters is printed to the result box:

- Ldelta Δ
- Minimum and maximum δ
- The number of δ or data points.
- The Strength of the Gradient in T/m.
- The Pulsed and the Steady Gradient Amplitude.
- The Balance for measurement.
- The Number of Scans (user-defined or default).
- The Result Output (Sample Id or Standard).

If *Sample Identification* is selected, the next step is to input it in two parts: the first part may consist of 8 characters at maximum, the second of 3 digits. These parts are connected by "_". Additionally, the date is appended automatically to get the full sample identification, which is equal to the *Data Pairs* file name (where the measurement data are stored). Examples for *Data Pairs* file names measured on October 23rd, 1996:

- Sample Identification: name_001_231096
- Standard Result Output: 1_231096

If the *Data Pairs* file name already exists, it is possible to set a different name or to overwrite the existing file.

After that it is necessary to insert the sample if it was not done before.

As pulse lengths may vary between different samples, it is possible to check them. The tuning routine finds the pulse lengths that cause minimal signal after a double 90°- and after a 180°-pulse. If **ESC** is pressed during adjustment, the measurement is started immediately.

Before measurement a test is performed, if receiver gain is suitable to the sample, and if necessary, it is adjusted automatically. According to the gain value the number of scans is set (in default mode) or proposed (in user-defined mode).

Then measurement starts. M_0 is analyzed with the minimum δ - value and is only measured at the first beginning and at the end with a higher number of scans as the other data acquisitions. If the difference between the two M_0 values is greater than 3 % a warning occurs at the end of the measurement. In this case it is recommended to repeat the measurement.

Before scans with gradient are done, dummy-shots are necessary to avoid unstable echoes during measurement.

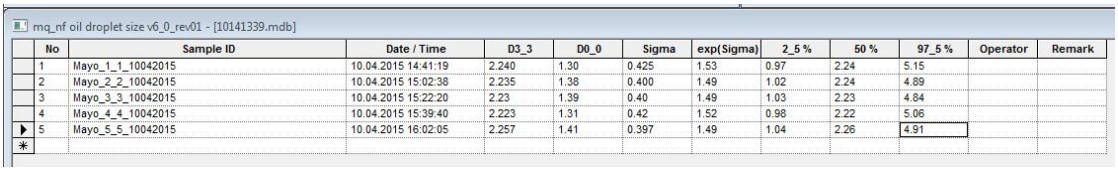
After each acquisition the balance is checked (at large values for receiver gain after each double or triple scan). If echo position deviates more than x µs (defined through the configuration menu) from optimal position at the first scan, the balance adjustment is started. If any unbalance occurs during the measurement, it is printed to the result box (in *Detailed Result Box Output*) and the previous scan is repeated (in every case). After 2 unbalanced echoes in succession, balance is re-adjusted. The balance adjustment can be stopped by pressing **ESC**; then the following echo is accepted independent from its position.

The δ values are calculated automatically by the program, depending upon the selection of the number of sdeltas and the maximum sdelta value. The start value of sdelta is related to a perfect suppression of the water signal.

If the scans for all δ are done, measurement is finished.

For calculation the first step is to input the *Data Pairs* file name. If a measurement was done before the belonging *Data Pairs* file name is proposed.

When fitting is finished, the measured data and the calculated curve are displayed. Here some tools are available for several operations, for example deleting a data point and repeating the fit. If **CONTINUE** is pressed, the calculated values are printed to the result box. The detailed result box output has a different appearance and includes additionally the distribution intervals.



mq_nf oil droplet size v6_0_rev01 - [10141339.mdb]

No	Sample ID	Date / Time	D3_3	D0_0	Sigma	exp(Sigma)	2_5 %	50 %	97_5 %	Operator	Remark
1	Mayo_1_1_10042015	10.04.2015 14:41:19	2.240	1.30	0.425	1.53	0.97	2.24	5.15		
2	Mayo_2_2_10042015	10.04.2015 15:02:38	2.235	1.38	0.400	1.49	1.02	2.24	4.89		
3	Mayo_3_3_10042015	10.04.2015 15:22:20	2.23	1.39	0.40	1.49	1.03	2.23	4.84		
4	Mayo_4_4_10042015	10.04.2015 15:39:40	2.223	1.31	0.42	1.52	0.98	2.22	5.06		
5	Mayo_5_5_10042015	10.04.2015 16:02:05	2.257	1.41	0.397	1.49	1.04	2.26	4.91		

Figure 10.3: The Detailed Result Box

Make sure that the factor calculated (*fac* into the fit menu box or *Oil Droplet Factor* into the result box) is always bigger than 1. If this factor is smaller than 1, reject this result and measure this sample again.

The calculated volume distribution curves (q_3 and Q_3) can be displayed optionally (if the application is left here, it is possible to move along sum distribution Q_3 with the cursor [ms = µm]).

Otherwise further measurements and/or calculations can be performed.

11 Contact

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