BRUKER

Avance GRASP

Installation/User Manual

Version 002

BRUKER

The information in this manual may be altered without notice.

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Introduction $\boldsymbol{1}$

Introduction *1.1*

There are two accessories for Gradient Spectroscopy (GRASP) for use with the Avance series of spectrometers (DMX, DSX, DRX and DPX): a single axis (z-only) and a three axes system. The single axis system uses a GREAT1/10 amplifier whereas the amplifier system for the three axes accessory is the Acustar. Both systems use self screening gradient coils and allow for pre-emphasis compensation of residual Eddy currents. All gradient pulses are generated by the Gradient Control Unit (GCU) which is an intelligent processor located in the AQX32 acquisition computer which can calculate gradient values „on the fly" during the execution of an experiment.

In this manual many of the setup procedures used during installation and in the course of implementing new experiments are identical irrespective of the chosen gradient direction. The corresponding sections are therefore common for both accessories. Wherever the procedures for the single axis and the three axes accessories differ this will be indicated in the text.

Introduction

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Hardware Description 2

Hardware for both Single and Three Axes Accessories. *2.1*

For both single and three axes accessories the following hardware is delivered:

H5577	Acquisition bus extension board[+]
HZ2969	Ribbon cable ACQ bus extension[+]
H2546	Gradient Control Unit (GCU)
3033	30 Jumpers for VME bus
H5517	Cable 40 MHz for TCU-GCU, 2 times SMB
H5517	Cable increment for TCU-GCU, 2 times SMB
HZ04360	Cable data GCU-Amplifier, 50 pin Scotchflex/SCSI, 5m.
HZ0548	RS232 cable CCU-Amplifier, 9 pin male/9 pin female, 5m.
3000	Continental European mains cable
	Cable lock hold TCU-BSMS, burndy pin/SMB*
	Cable external DW TCU - RCU, burndy pin/SMB*
	Cable external EP TCU - RCU, burndy pin/SMB*

[+] (needed only if more than 3 FCU's are present, see section 3.1)
* (Included in console wiring from ECL05)

Hardware for the Single Axis (z-only) GRASP Accessory *2.2*

Additional hardware for z-only GRASP is:

W1207698	BRUKER 10 A, 1 channel gradient amplifier (GREAT 1/10)
W1208167	Cable gradient GREAT 1/10 - Gradient coil, twisted pair
	A Z-Gradient probehead

Hardware for the Three Axes GRASP Accessory (DRX and DMX only). *2.3*

For three axes GRASP the following is delivered:

BM5850HD	Acustar gradient power amplifier
401-117142-02	Cable gradient Acustar-RF filter
431-116991-01	RF filter box
401-117640-02	Cable gradient RF Filter-Gradient coil
	An XYZ-Gradient probehead

Hardware Description

Hardware Installation

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Consoles with an engineering change level of 04 or lower may not have been wired for external address advance. On these consoles connect **TCU/T3/N** to **RCU/Ext.DWCLK** and **TCU/T3/R** to **RCU/Ext.EP** using the cables supplied.

ACQ bus extension (DRX and DMX only). *3.1*

This section applies only to systems where the existing configuration of the AQX32 fills all the available positions on a non-extended ACQ bus. Typically this is true for a system with four FCU boards or more. On systems with three FCU's or less the GCU can be fitted without the need of adding the ACQ extension board.

Switch off the AQX32 and any connected external digitizers. Open the back panel of the AQX32 and locate the end of the ACQ 8 slot bus (see figure 3.1), ACQ Back Panel Overview.

Figure 3.1.   ACQ Back Panel Overview (DRX, DMX)
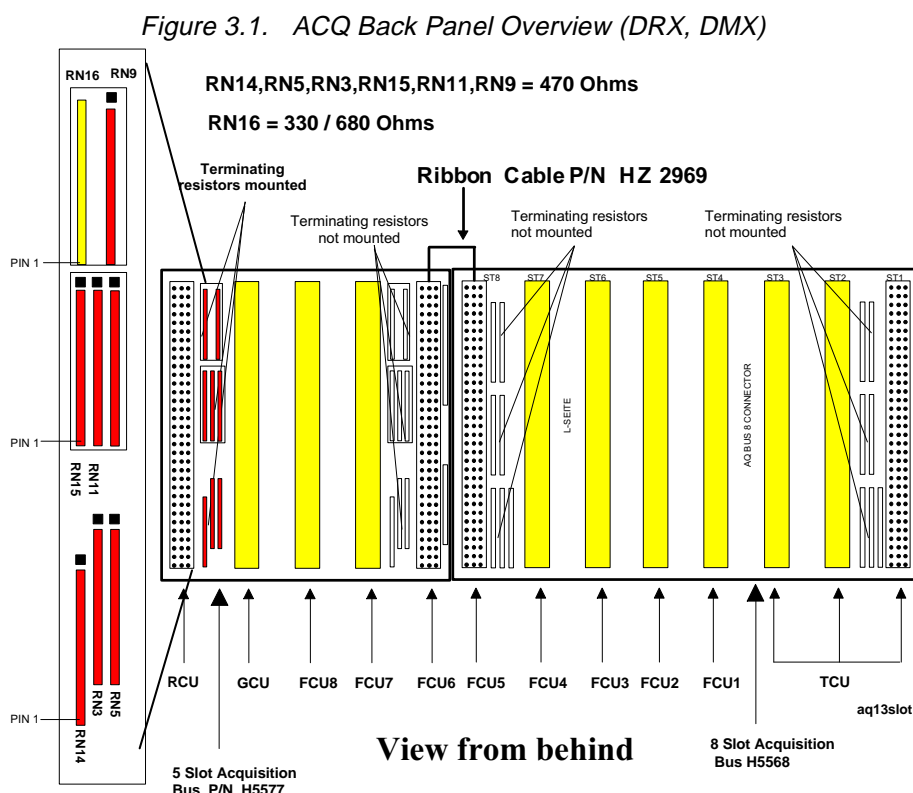
ACQ Back panel indicating standard board positions, jumper settings and 5 slot Acquisition Bus extension Board.

Hardware Installation

Note the positions of the terminating resistors on the last position on the bus and remove them (see figure 3.2). Place the ACQ 5 slot bus extension board onto the next five free connectors and push into place firmly. Connect the last position on the old bus to the first position of the bus extension by means of the ribbon cable HZ2969. Verify the positioning of the terminating resistors on the ACQ 5 slot bus extension board.

Figure 3.2. Standard 8 Slot Acquisition Bus (DRX, DMX)

RN3,RN7,RN8,RN12,RN13,RN14 = 470 Ohms

RN4 = 330 / 680 Ohms
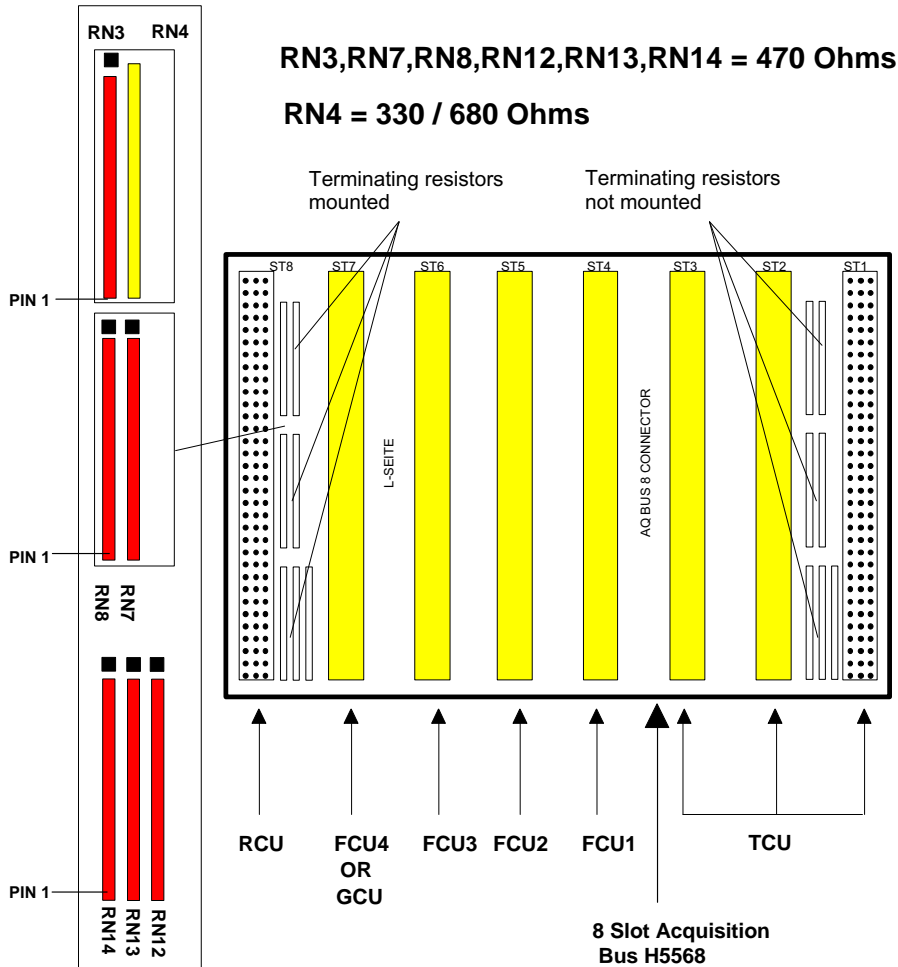
Acquisition bus with indication of standard board allocation and terminator resistor layout for operation without 5 slot extension board.

GCU Installation   *3.2*

At the front of the AQX32, locate the RCU board (see figure 3.3). This should be the right most board on the ACQ bus and, if the ACQ bus had to be extended, this board should be moved: Remove the cover plate from the slot on the end of the extended ACQ bus. Disconnect the RCU and move it to this slot. Reconnect the RCU.

On the GCU board, ensure that jumpers W1 and W2 are connected. Remove the cover plate from the slot to the left of the RCU and insert the GCU into this slot. Cover any open slots. On the back panel of the AQX32, place jumpers on any vacant slots on the VME bus and, if necessary, remove them from any slots

now occupied. Close the back panel of the AQX32.

On the front of the AQX32, locate the right most FCU board (figure 3.3) and disconnect the jumpers W2 and W3. Use the cables H5517 to connect the outputs **40MAO** and **AQSO** on this FCU to the inputs **40MAI** and **AQSI** on the GCU.

Figure 3.3.   ACQ Front Panel Overview (DRX, DMX)
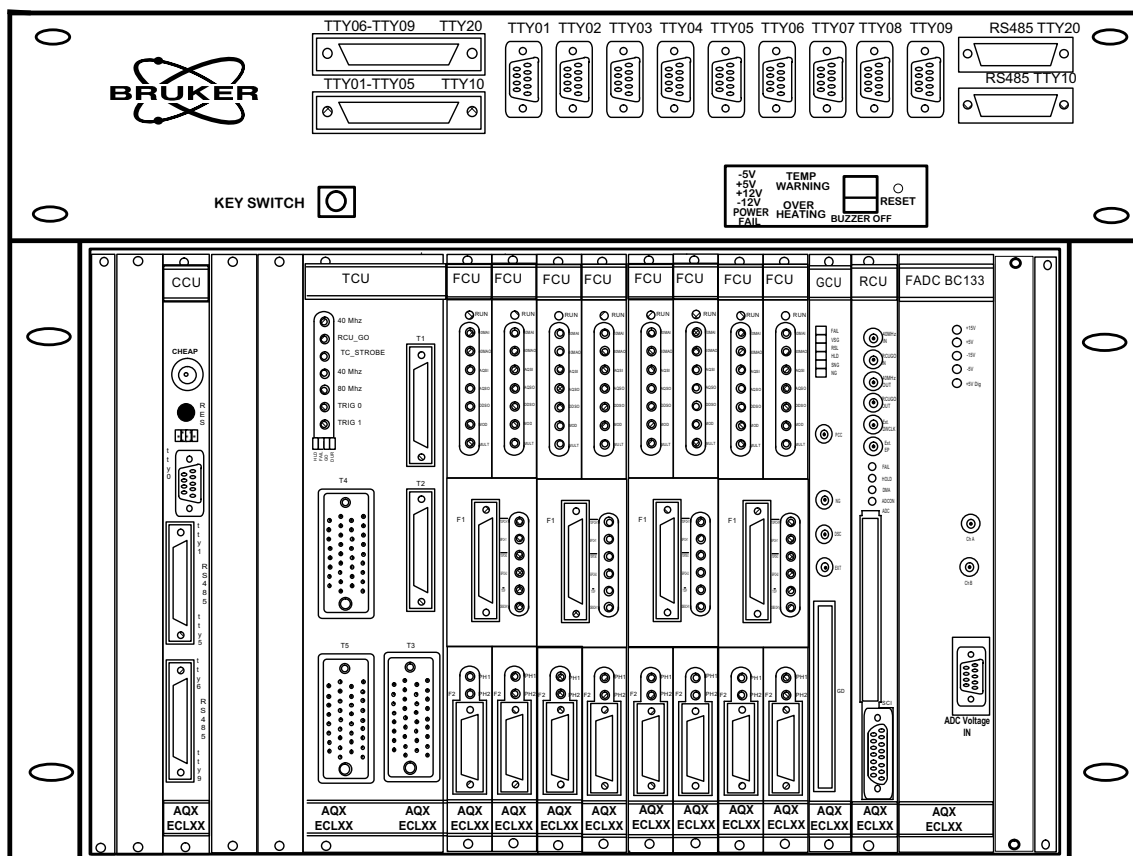
ACQ Front Panel indicating standard board positions.

Gradient Amplifier Installation   *3.3*

On DPX systems, position the gradient amplifier (GREAT 1/10 or Acustar) on top of the spectrometer cabinet. On DRX/DMX systems try to find a free position in the left hand half of the spectrometer cabinet. On systems with up to three RF channels this is often below the PTS620 synthesizer. Place the amplifier in this free position. If no free position is available, place it on top of the spectrometer cabinet. Connect the case of the amplifier to the cabinet by means of an earth lead.

GREAT 1/10 Connections   *3.3.1*

Refer to figure 3.4: Locate the gradient input connector **GRAD IN** on the front of the GREAT 1/10 and connect it to the connector **GD** on the GCU using cable H5510. Locate the connector **RS232** and connect it to a free RS232 position on the AQX RS232/485 extension unit using the RS232 cable HZ0548. Note the position chosen (in the case of a CCU, the default position is **TTY06**). Connect one end of the gradient

cable W1208167 to the output terminal **GRAD OUT** on the front of the amplifier and the other end to the burndy connector on the gradient probe. Finally, connect the GREAT 1/10 to one of the spectrometer mains sockets by means of the mains cable supplied.

Figure 3.4.   GREAT 1/10 Front Panel

Serial I/O to CCU P/N HZ04053

Gradient Input from GCU P/N HZ04360

P/N W1208167 Gradient Output to Gradient Coil
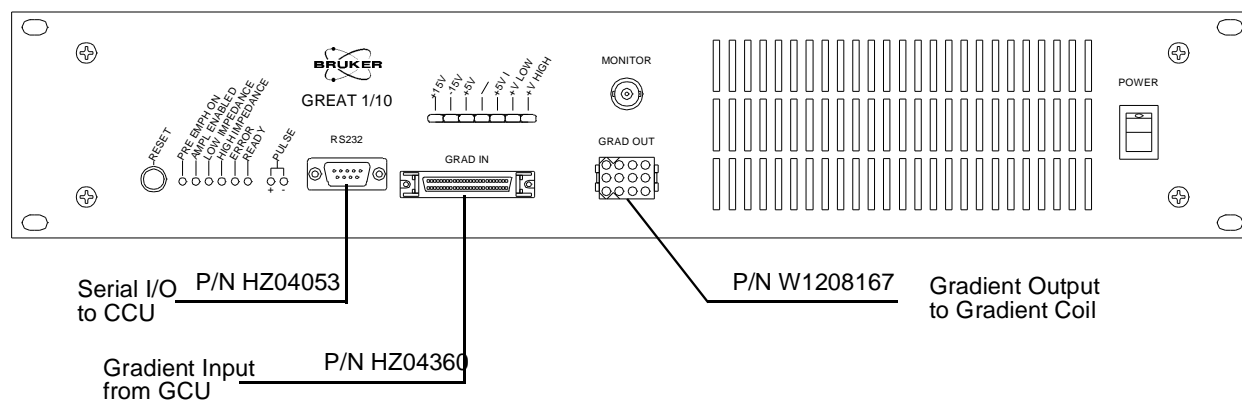
Showing from left to Right: Reset button; LED Pre-emphasis enabled; LED Amplifier enabled; LED Low impedance selected; LED High impedance selected; LED System ready; LED Error protection engaged; RS232 connector to CCU; Gradient input connector to GCU; Gradient output connector to coil; BNC Monitor output; Power on/off switch.

Acustar Connections.   *3.3.2*

Locate the connector **GCU interface** on the rear of the Acustar (figure 3.6) and connect it to the connector **GD** on the GCU using cable H5510. Locate the connector **RS232** and connect it to a free RS232 position on the AQX RS232/485 extension unit using the RS232 cable HZ0548. Note the position chosen (in the case of a CCU, the default position is **TTY06**). Connect one end of the gradient cable 401-117142-02 to the output terminal on the rear of the amplifier and the other end to one side of the RF filter box. Connect one end of the second gradient cable (401-117640-02) to the RF filter box and the other end to the burndy connector on the gradient probe. Finally, connect the Acustar to one of the spectrometer mains sockets by means of the mains cable supplied.

Lock Hold Function   *3.4*

The control pulse for the Lock Hold function is wired from the TCU (**T5 pin T**) either directly to the input **L-HOLD** on the BSMS or via the LAB board in the AQR (**TCU T5 pin T** to **LAB2 pin R** and **LAB2 pin U** to **L-HOLD**). These two methods provide different polarity of the control signal at the BSMS: **active low** for the directly connected signal and **active high** for the path via the LAB board.

On BSMS from ECL04 with firmware version number 940506 and higher, the polarity of the control signal can be selected via the „BSMS" program. When this is available, then the connection should be made directly and **active low** lock hold pulses should be defined. To accomplish this: open a new xterm window and become super user. Start the BSMS program by typing **bsms**. From the main menu select **board functions LCB** (selection B). Select **Lock-Hold Pulse Polarity** (selection A in this submenu) and on the prompt **Enter new level** type **1**. Exit the BSMS program.

Figure 3.5.   Acustar Front Panel
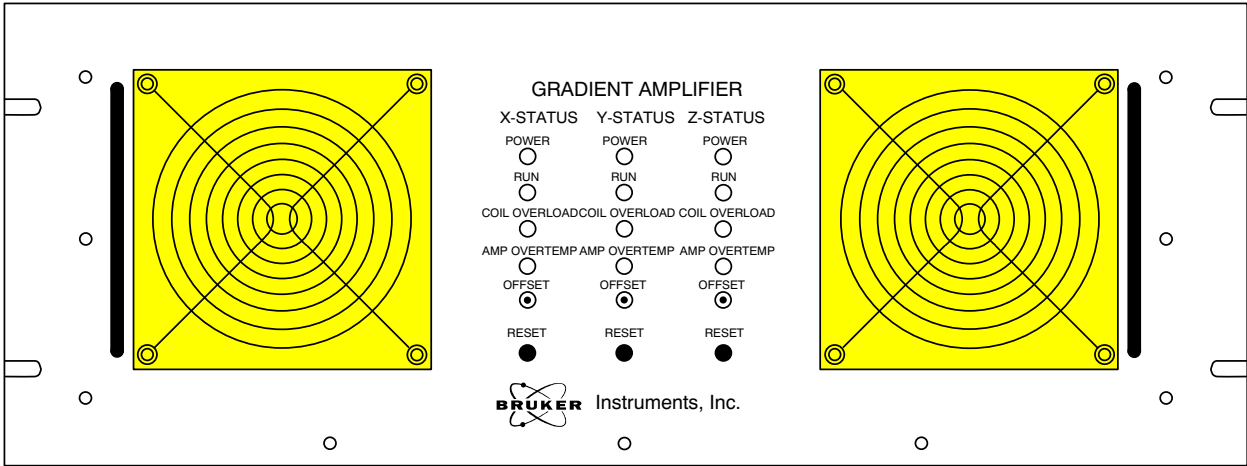
Figure 3.6.   Acustar Rear Panel
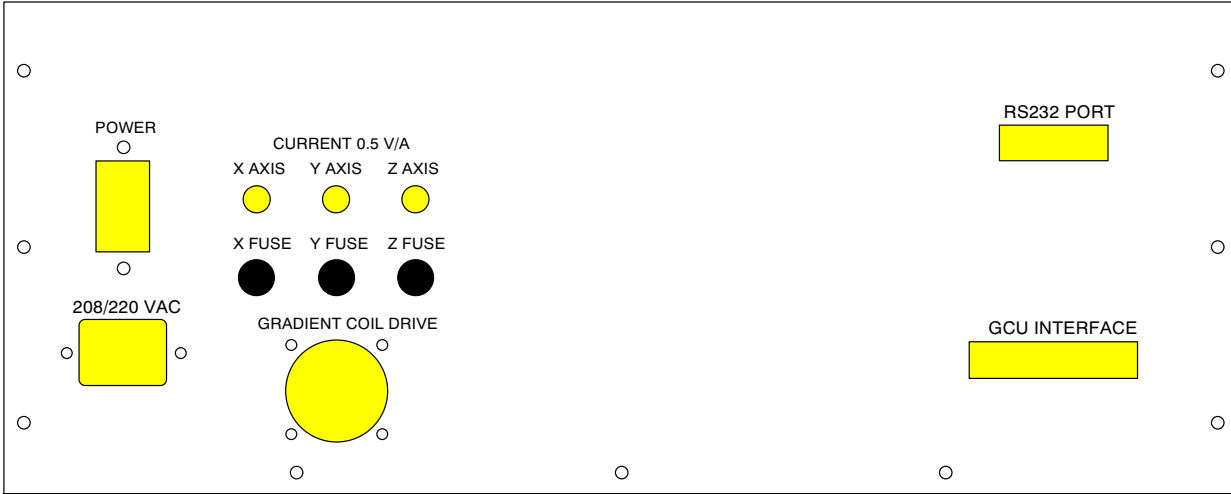
System Software Adaption

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GCU Installation

<div align="right">4.1</div>

In order for the GCU to operate properly it has to be addressed via the AQX bus. Some system files for the CCU (or CPU4) may have to be modified for proper operation.

From the spectrometer nmrstation login on the CCU (typically called "spect") and move to the devices directory:

cd /dev

Verify the existence of the device **gcu** by typing:

ls -l gcu

If it is not found, generate it using the **mknod** command:

mknod gcu c 56 0

Return to the nmrstation by logging out from the CCU. Then, using a text editor, inspect the file **/etc./services** and verify if it contains a line:

aqport6   4006/tcp   #acquisition server

If this is not the case, add it and save the modified file.

Installation of the Serial Link for Pre-emphasis

<div align="right">4.2</div>

XWINNMR needs to be informed of the RS232 channel used for communication with the pre-emphasis control. For this the hardware list needs to be updated. First verify that a file **hardware_list** exists in **/u/conf/instr/<spectrometer>**. For **<spectrometer>** insert the name of the current instrument as used in the **XWINNMR** configuration (typically "spect"). When in doubt, the name can be found in the text file **/u/conf/instr/curinst**. If no hardware list exists, then generate it by the following procedure:
From within **XWINNMR** type:

cf makelist

This generates a file **hardware.exam** in the directory **/u/conf**. Open a new xterm window and become superuser; then type:

cd /u/conf/instr/<spectrometer>
cp ../hardware.exam hardware_list

System Software Adaption

Now edit the file **hardware_list** to conform with the actual spectrometer hardware configuration. In particular ensure it contains one of the following lines:

		H5380				**BGU-II for shielded X-Y-Z-gradients**
		H5530				**BGU-IIZ for shielded Z-gradients**
		BF4050				**Acustar for shielded X-Y-Z-gradients**
		W1207698				**GREAT1/10 for shielded Z-gradients**

Then, reconfigure **XWINNMR** using the command **cf** and answer the question,

Which device is used for Pre-emphasis:

which gives the number of the RS232 channel selected in the hardware installation (section 3.3.1). This completes the software installation of the Gradient accessory.

Installation Adjustments 5

Before the gradient accessory can be operated in a routine fashion, some installation adjustments are necessary to ensure the shortest possible gradient pulse rise and fall times and magnetic field homogeneity recovery. The first adjustment concerns the Amplifier Control Loop in conjunction with the gradient coil used and aims to give fast establishment of a stable current through the gradient coil. The second adjustment is that of the gradient pulse pre-emphasis which, by compensating for residual Eddy currents, will give short Gradient Recovery time. As it is possible to partly compensate for a misadjustment in the amplifier Control Loop by means of pre-emphasis, it is important to execute the two adjustments in the order given and not to attempt pre-emphasis adjustment before the control loop is optimized.

Before starting the installation adjustments, verify that the GRASP accessory has been installed and connected completely and switch on the GREAT1/10 or the ACUSTAR. Load pre-emphasis values by executing the **XWINNMR** command **setpre**. Ensure that the correct operation mode is selected by clicking on the button labelled **Z Gradient**, and that all amplitude parameters are set to zero. If desired save this status in a file, e.g. called **zero** by clicking on **File** and selecting **Save Current Set-up**. Exit via the selection **Quit** in the pull down menu **File**. For more details on the **setpre** command see section 7.3.

Amplifier Control Loop Adjustment (GREAT 1/10 only). 5.1

The GREAT1/10 is delivered with pre-loaded values for the amplifier control loop and these should be correct for the standard Z-gradient coils. The procedure outlined in this section should therefore initially be followed just to check if the gradient pulse edge is well behaved. Only in exceptional cases should an adjustment be necessary.

This adjustment is done by monitoring the edge of a short rectangular gradient pulse. In preparation, connect an oscilloscope to the connector **Monitor** on the front of the GREAT1/10. The signal available at this output is proportional to the current the amplifier delivers: **1V** corresponds to **4A**. Ensure the gradient probe is connected to the amplifier and **is outside the magnet** (see below).

To generate gradient pulses of appropriate duration and duty cycle, first set-up a dataset in **XWINNMR** with normal 1D parameters (e.g. for proton observation). Adjust for short repetition time in order to obtain a stable oscilloscope trace (large SW, small TD, short D1). Set the parameter PULPROG to **preempgp2** and the parameter **GPNAME0** to **rectangle**. Set the length of the gradient pulse **p16=5ms**, its amplitude **GPZ0=100** and the length of the Gradient Recovery delay **d16=100us**. Now, start pulsing by typing **gs**.

On the oscilloscope observe a trace of the current through the gradient coil. Adjust the oscilloscope display to observe the falling edge of the gradient pulse and maximize sensitivity. If the amplifier control loop has not been adjusted properly, then the response will show a slow exponential decay of the gradient current or, alternatively, an oscillating behavior (see figure 5.1).

Figure 5.1.   *Effect of Loop Control on Falling Edge of Gradient Pulse.*
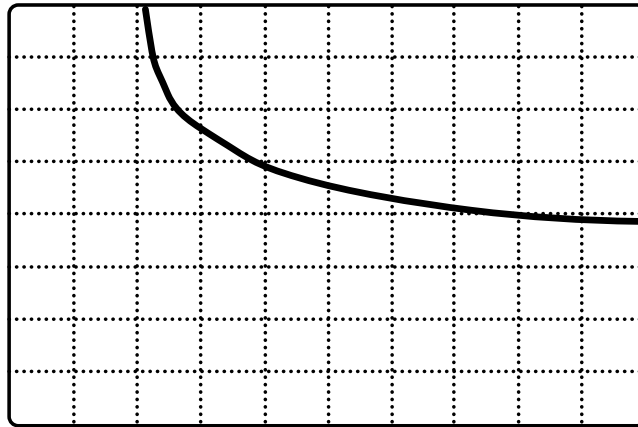
Under compensated
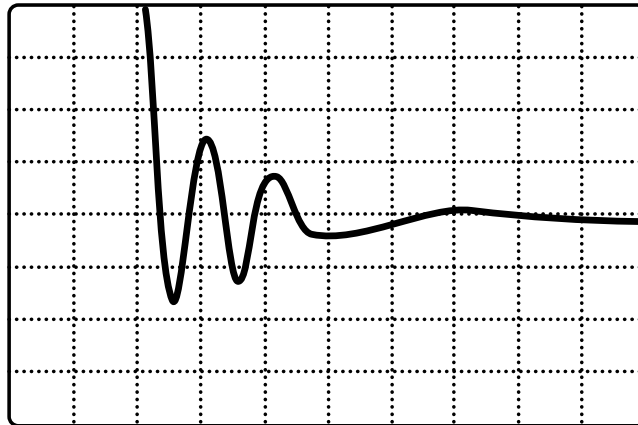
Over compensated
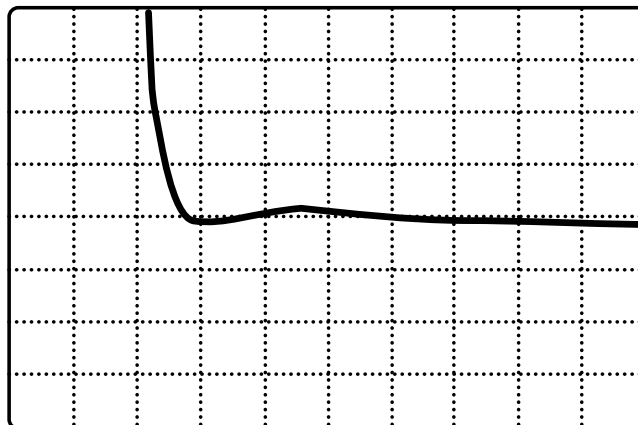
Properly adjusted

Use an oscilloscope adjustment of 100 mV per division vertical and 50 µs per division horizontal and adjust to obtain as square as possible an edge.

Now adjust the resistance and/or the capacitance of the amplifier control loop RC circuitry to obtain the best approximation to a rectangular gradient pulse as possible. The amplifier control loop adjustment is under software control via the selection **Loop Parameters** in the menu **Amplifier Matching** of the **setpre** command (see section 7.3.3).

Once an acceptable pulse shape has been obtained insert the probe into the magnet while applying gradient pulses and observe the pulse shape on the oscilloscope. There should be virtually no difference as compared with running the experiment outside the magnet.

DC offset Correction   5.2

When the DC offsets of the gradient amplifier are not adjusted properly, then a constant current through the shim coils is needed to compensate the DC currents through the gradient coils. This becomes obvious when disconnecting the gradient coils from the amplifier: the lock level will drop and adjustment of the (X, Y and) Z shim is necessary to regain optimum resolution.

In preparation for the DC Adjustment, start by disconnecting the gradient amplifier from the probe. Insert a sample of H_2O/D_2O (if possible doped with 2 mMol $CuSO_4$ to yield a 3 Hz line width) and obtain lock. Now shim to the best resolution. Note the level of the lock signal at the optimal shimming. Adjust to a convenient level (grid line) if desired.

Now re-connect the gradient coils. While observing the lock signal adjust the DC controls of the gradient amplifier (see below) to re-establish the previously obtained lock level. Repeat for each gradient direction (for three axis accessories only).

The DC adjustment for the GREAT1/10 is under software control via the selection **Offset Parameters** in the menu **Amplifier Matching** of the **setpre** command (see section 7.3.3).

On the Acustar, potentiometers for DC offset correction for each of the three gradient directions can be found behind the front panel. They are accessible through three openings above the **Reset** buttons and are labeled: **X (Y, Z) offset** (see figure 3.6).

Pre-emphasis Adjustment   5.3

NOTE: The pre-emphasis adjustment experiment should show the smallest amount of residual Eddy currents and, for this reason, uses a gradient pulse much longer than normally employed in spectroscopy experiments, i.e. 50 ms. Do not attempt to adjust pre-emphasis with a much shorter pulse.

Setup of the Pre-emphasis Experiment   5.3.1

The experiment used to make this adjustment involves acquiring eight FID's following eight gradient pulses with varying delays between the gradient and the RF pulses. The experiment has been designed to display all eight FID's in one dataset in order to obtain a full picture of the Gradient Recovery behavior.

Set-up a dataset in **XWINNMR** with normal 1D parameters (e.g. for proton observation) and adjust for acquisition parameters suitable for shimming on the FID: Acquisition time 500 ms, repetition time 1-2 s, RF pulse flip angle 5-10^0. Adjust O1 to be well off-resonance (by approximately 5 kHz). Verify the set-up by observing the FID in **gs** mode: the FID should be decayed to approximately 50% after about one eighth of the acquisition time (see figure 5.2).

Installation Adjustments

Figure 5.2.   FID suitable for Pre-emphasis Adjustment
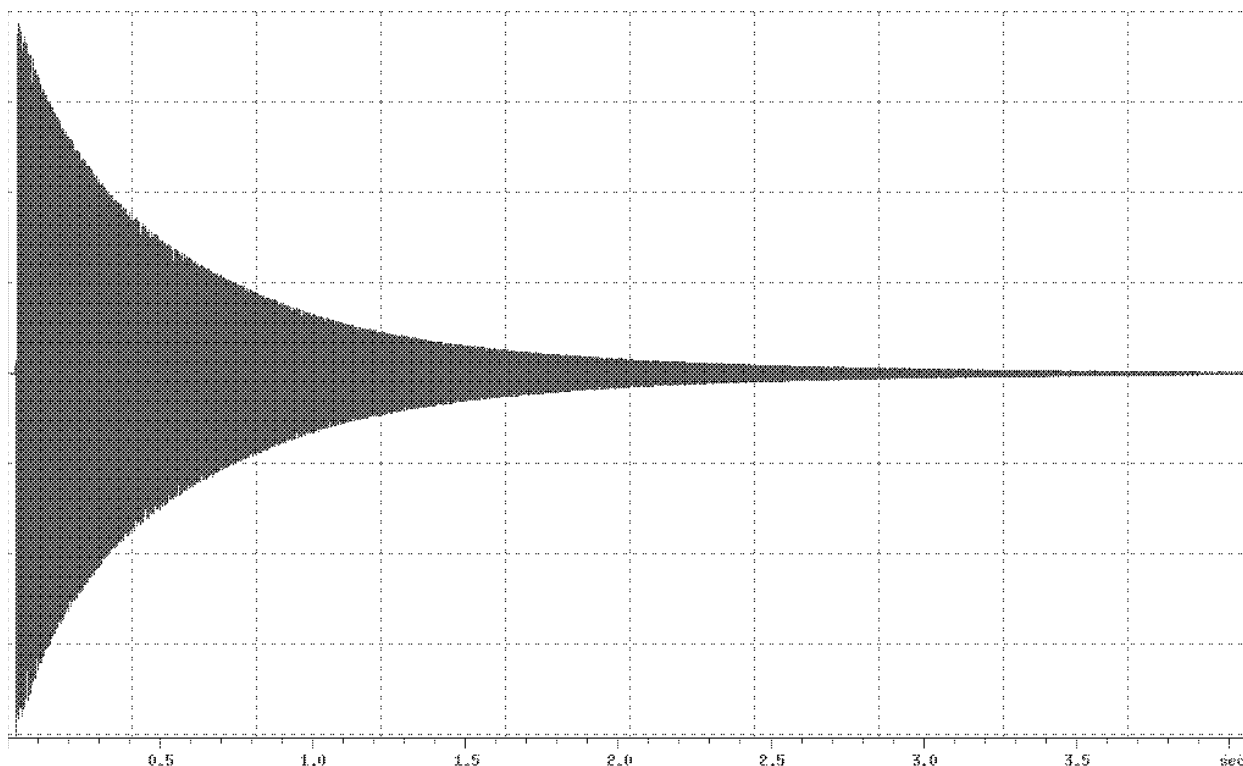
In the pre-emphasis experiment the first 1/8th of the FID (1 1/4 grid division) will be used in each of the eight experiments.

Now set the parameter **PULPROG=preempgp.m** (for DMX) or **PULPROG=preempgp.r** (for DRX/DPX), the parameter **GPNAME0=rectangle** and the parameter **VDLIST=preemp**. Verify that the vd list exists and has the correct values by typing **edlist vd** and selecting the file **preemp**. The values in this file should be: 100u, 300u, 1m, 3m, 10m, 30m, 100m and 300m. For DRX and DPX, set-up for protons on channels F1 and F2 using **edasp** and set **O1=O2**. Set the length of the gradient pulse **p16=50ms** and its strength **GPZ0=20** (for the three axes system, make sure to set **GPX0** and **GPY0** to zero). Set **DS=0** and **NS=1**. Then, start acquisition by typing **gs** and switch to the **XWINNMR** acquisition window by clicking the **acqu** button.

Control of Pre-emphasis Values.   5.3.2

The pre-emphasis adjustment is under control of the **XWINNMR** program by means of the command **set-pre**. This command allows for "on the fly" adjustment of pre-emphasis parameters using a mouse control-led slider mechanism. Time constants, gains and gradient directions are selected by clicking on the appropriate buttons. The result of the adjustment can be stored on disk in pre-emphasis parameter files via the function **Save Current Set-up** in the **File** menu. Alternatively, existing pre-emphasis parameters can be loaded from disk using the function **Open Set-up**. For further information, see section 7.3 and the **Help** menu in the **setpre** window.

Pre-emphasis Adjustment Procedure   *5.3.3*

Adjust the gains and time constants of the available pre-emphasis RC circuits in order to obtain eight FID's showing decay characteristics as similar as possible (see figures 5.3 and 5.4) using the following proce-dure. Start the pre-emphasis adjustment by entering the command **setpre** and selecting the appropriate gradient direction. Set the time base for the **slow**, **mid** and **fast** compensations to e.g. **20ms**, **2ms** and **0.2ms** respectively. Set all gains to zero and all time constants to mid range. Take the last two FID's as reference signals: they should show virtually identical decay behaviour.

Now adjust the gain of the longest time constant to optimize the decay of FID number 6. Meanwhile also observe FID number 5. If FID 6 reaches optimum before FID 5, then proceed to adjust the gain until FID 5 is optimal. Reduce the time constant until FID 6 is undistorted and iterate until both FID's 5 and 6 are simi-lar to the reference FID's. If, on the other hand, FID 5 becomes undistorted before FID 6, then increase the time constant to optimize FID 6 and reduce the gain to improve FID 5. Iterate until both FID's are optimal.

Repeat this procedure for FID's 3 and 4 using the middle time constants and for FID's 1 and 2 using the short ones. In proceeding monitor the later FID's and make minor adjustments to the longer time constants as required. When a satisfactory Eddy current compensation has been reached, stop the set-up mode **gs**. If desired, a permanent record of the Gradient Recovery behaviour can be obtained by executing the expe-riment using **zg**.

For the three axes accessory, repeat the pre-emphasis adjustment for the X and Y gradient directions, using **GPX0=20** and **GPY0=20** respectively (make sure to set the amplitudes for the directions that are not being adjusted to zero).

Figure 5.3.   Pre-emphasis adjustment
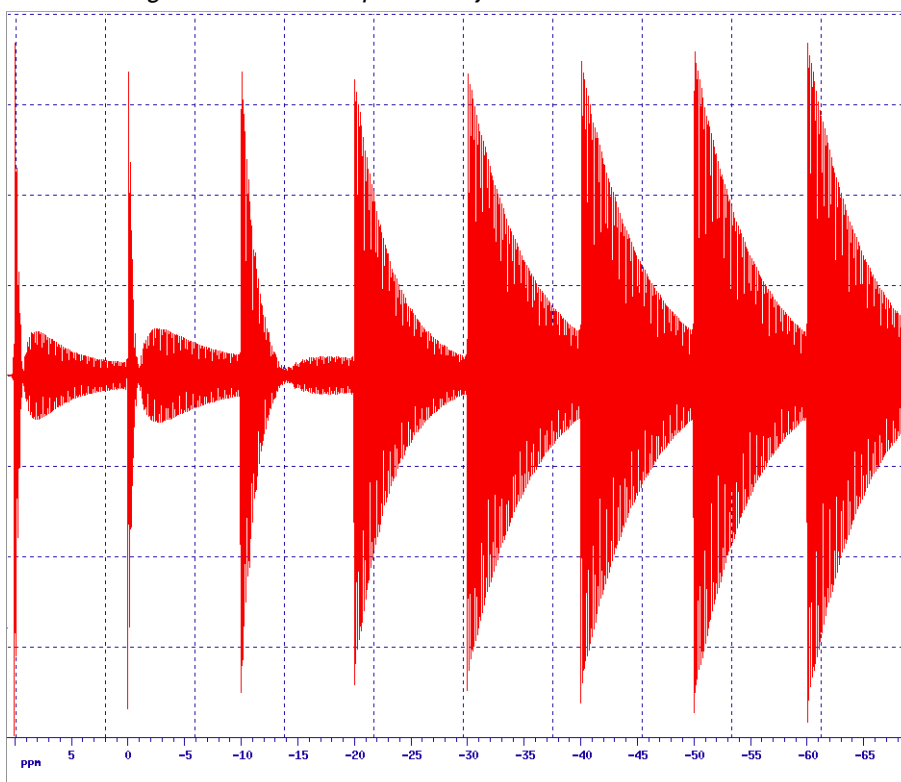
Experiment prior to Eddy current compensation

Figure 5.4.   Pre-emphasis Adjustment
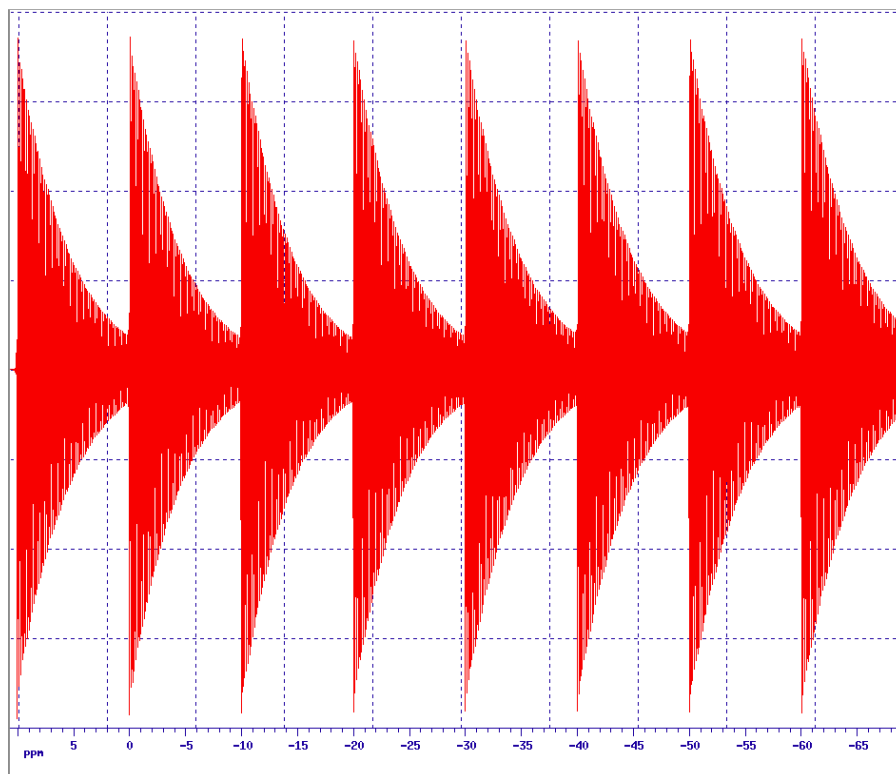
Experiment after Eddy current compensation.

Installation Tests

6

After proper adjustment of the Amplifier Control Loop, the DC offset and the pre-emphasis, the gradient accessory is ready for routine operation. It is important, however, to verify that it is functioning properly by performing the following tests:

Gradient Recovery Test *6.1*

The success of gradient spectroscopy depends strongly on the ability to acquire a high resolution spectrum shortly after a gradient pulse. The recovery test verifies whether the adjustments of the amplifier control loop and the pre-emphasis are satisfactory and checks if no other instabilities are introduced by the gradient pulses.

Using the doped water sample (see section 5), set-up the gradient parameters for pulse program **preempgp2** as described in section **5.1** but set **p16=5ms, d16=1s and** the gradient pulse amplitude **GPZ0=50**. Acquire a spectrum and adjust the phase correction parameters to obtain an absorption line shape. Now acquire a number of spectra for different values of the recovery delay using the AU program paroptlog: **xau paroptlog**. Answer the questions as follows:

Enter parameter to modify: **d16**
Enter initial parameter value: **0.0001**
Enter initial increment: **0.0001**
Enter final parameter value: **1**
Enter # of experiments: **40**

Judge the results by inspecting the initial amplitude and phase in comparison with those at the end of the series (see figure 6.1). If the gradients are working properly, then any differences should be minimal.

For the three axes accessory, repeat for each gradient direction.

Figure 6.1. Gradient Recovery
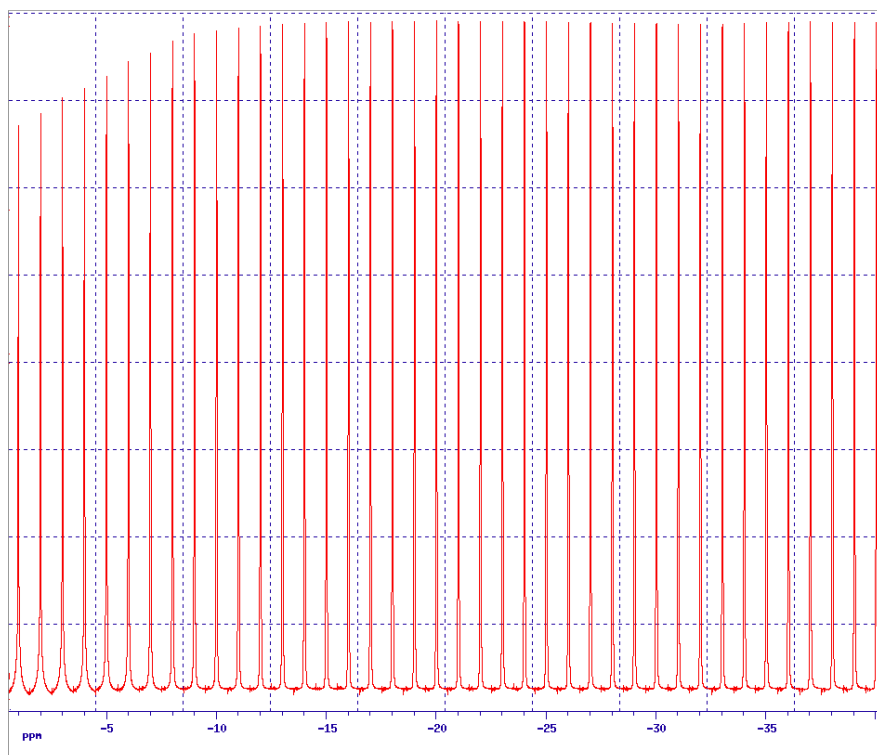
Without pre-emphasis adjustment

Figure 6.2. Gradient Recovery
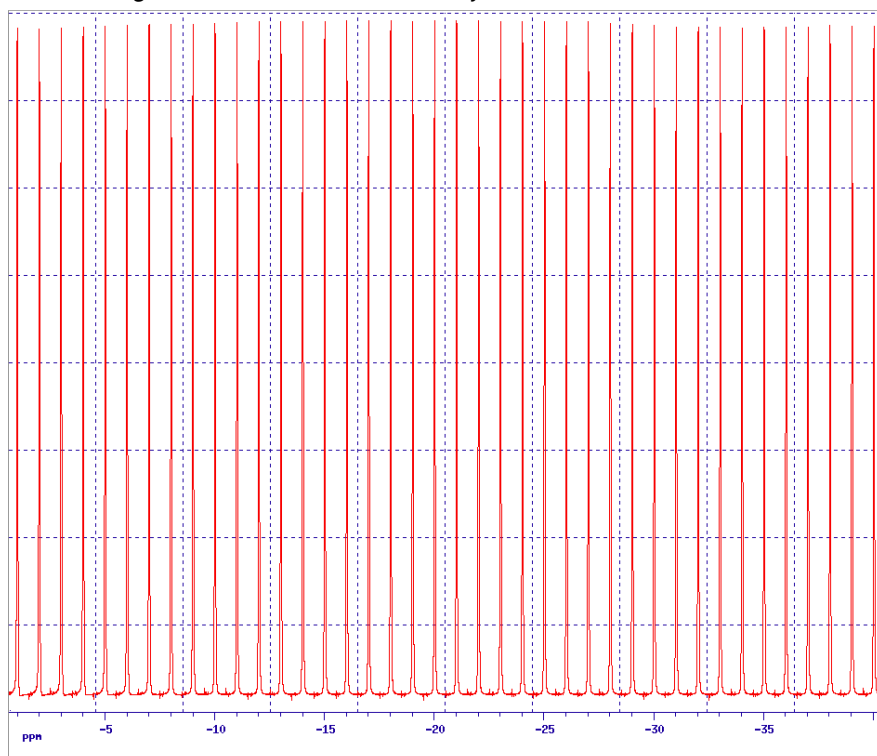
*After pre-emphasis adjustment. See the file **paropt.log** in the processed data directory for a full listing of delay values and intensities*

Calibration of the gradient strength.   *6.2*

The gradient strength is calibrated by measuring the profile of a phantom of known spatial dimensions in the presence of a gradient and noting the spectral width of the profile. For x and y gradient strength calibration the profile of the doped water sample is perfectly adequate.

For z gradient calibration a phantom consisting of a 2 mm plug in a sample tube with a little doped water is used (Gradient calibration sample). It must be positioned with the plug in the centre of the RF coil: Use the sample tube depth gauge and line the phantom up with the mark indicating the centre of the coil, rather than pushing the tube down to the recommended depth for normal samples. Do not attempt to shim this phantom; if it cannot be locked, ensure the field sweep is switched off.

The pulse sequence to measure a profile is a spin echo experiment involving a gradient pulse during the dephasing period and during the rephasing, which is also the acquisition period. The pulse program is **calibgp.m** (for DMX) or **calibgp.r** (for DRX/DPX). Initially use a low gradient strength **GPZ0=10** for the calibration of the Z-gradient, **GPX0=10** for X and **GPY0=10** for Y. Make sure to set the gradient strengths for those directions not being calibrated to zero.

Figure 6.3.   X-profile of Standard 5mm Sample Tube
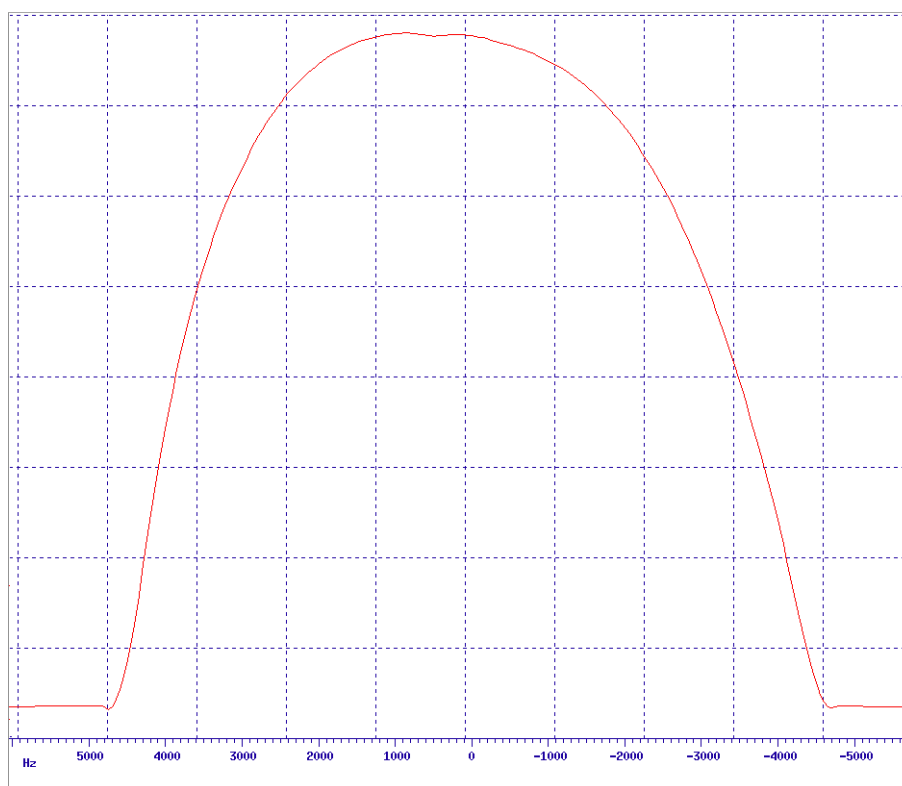
The measured profile width (at 10% gradient strength) of 9.2 kHz for a standard 5mm sample tube (4.2mm I.D.) translates into a gradient strength of 5.14 Gs/cm

Setup acquisition parameters with a very short acquisition time (e.g. 5 ms, using **SWH=50kHz, TD=512**), ensure all relevant pulse program parameters have been set to correct values (**p1=90º, d1=1, NS=2**) and acquire an echo signal. Transform using **fmc**. Measure the width of the profile for x and y gradients (figure 6.2) and the width of the reduced intensity of the profile due to the plug for z gradients (figure 6.3) and calculate the gradient strength using the formula:

$$G = \Delta f / (\Delta r \; x \; 4.258) \; Gs/cm \qquad\qquad (Eq.\ 6.1)$$

where Δf is the measured width in kHz, Δr the inner diameter of the sample tube for x and y gradients (for instance 0.42 cm for Wilmad 507-PP) and the length of the plug (0.2 cm) for z gradients. The gradient strength, extrapolated to 100%, should be approximately 50 Gs/cm.

If desired, repeat the experiment using different values for the gradient strength as is practicable without broadening the profile beyond the selected spectral width, to verify the linearity of the gradient amplifier.

Figure 6.4.   Z-profile of Calibration Phantom
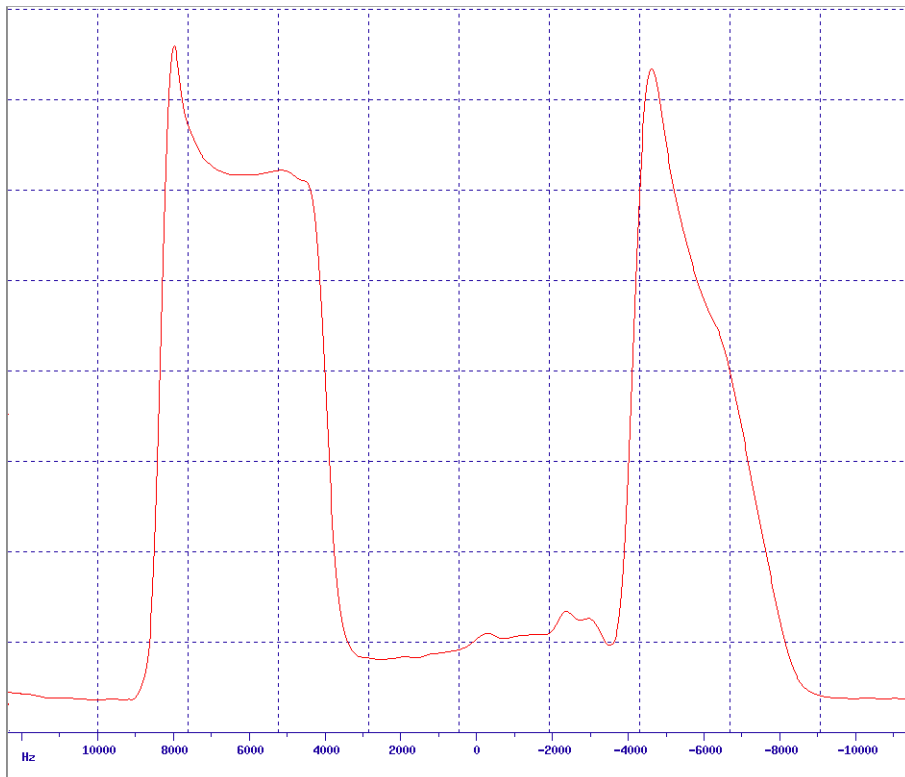
The gap in the profile of a 3.6 mm calibration phantom measures 7.9 mHz. This gives a gradient strength (at 10% amplitude) of 5.15 Gs/cm.

Software for GRASP 7

Gradient spectroscopy is supported in the **XWINNMR** software by a dedicated syntax in the pulse programming language for switching or pulsing of gradients. The relevant parameters for each direction (x, y, z) are: gradient amplitude, pulse duration and shape. The pulse programming syntax and the user interface for the definition of these parameters are similar to that for shaped RF pulses.

Gradient Pulse Programming Syntax 7.1

The most common use of gradients in gradient spectroscopy is the application of gradient pulses. The syntax for this in a pulse program is **pi:gp$j$** where i and j are integer numbers ranging from 0 to 31. The result of this is the application of a gradient pulse of length **pi**, the amplitude and shape of which are taken from the parameter set **GPj** (see below). The assignment of a pulse to more than one set of gradient parameters: e.g. **p16:gp1:gp3** is not allowed.

Shaped gradient pulses are generated by the **GCU** by taking the amplitudes from a file and presenting these to the gradient amplifier system as a concatenated series of short rectangular pulses. The minimum duration of these pulses, called the "gradient dwell time" is approximately 5 μs. Consequently, a minimum duration applies to all shaped gradient pulses, which depends of the digitization of the shape, i.e. the number of data points used for its definition.

For some experiments, it may be desirable to switch the gradients to a predefined value and leave them on until explicitly switched off. This is accomplished by the commands **gron$i$** and **groff**, where **gron$i$** switches on the gradients to an amplitude as defined by the parameter set **GPi** and **groff** switches them off.

Gradient Parameters 7.2

In the menu **eda** a submenu is provided under the parameter name **GP0..31** (see figure 7.1). In this submenu 32 entries are found with headings **GPX**, **GPY, GPZ** and **Filename**. They correspond to the pulse program syntax elements **gp0..31** and **gron0..31**.

Each entry allows for the definition of a gradient amplitude for the single axis accessory by assigning a value to the parameter **GPZi** or a gradient amplitude and direction for the three axes accessory by assigning values to the parameters **GPXi**, **GPYi** and **GPZi**, where i can take any value from 0 to 31. The gradient direction is determined by the vector sum of the three components. The values for the gradient amplitudes are entered as a percentage of the total gradient strength: between -100 and +100. For the syntax elements **gron0..31** the amplitude parameters are the only ones used.

If the syntax elements **gp0..31** are used i.e. in the form **pi:gp$j$** (where both i and j symbolize a number between 0 and 31) then the parameter **GPNAMEj** is also relevant. It contains the name of the shape for a shaped gradient pulse. The length of the shaped gradient pulse is determined by the parameter **Pi**.

The name of the gradient shape in the parameter **GPNAMEj** refers to a file, typically in the directory /u/exp/ stan/nmr/lists/gpwave/, that should contain a legal gradient shape. Three commonly used shapes are deli-

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vered on the **XWINNMR** release tape: **rectangle**, **sine.100** and **sine.20**. The file **rectangle** contains only two data points and, consequently, can be used in a pulse as short as 10 μs (disregarding the amplifier rise time). The file **sine.100** contains a sine function between t=φ and t=π in 100 points. The minimum duration of a pulse using this shape is therefore 500 μs. For shorter sine shaped gradient pulses the file **sine.20** can be used which, with 20 points, can be applied within 100 μs. Other gradient shapes can be calculated using the AU program **gradshapes** or the program **xshape** which should be run under UNIX.

Figure 7.1.   GRASP Parameter Menu GP0-31.

gradient parameters			
Index	GPX	GPY	GPZ	Filename
0	80.0	80.0	80.0	sine.100
1	20.0	20.0	20.0	sine.100
2	−20.0	−20.0	−20.0	sine.100
3	−20.0	−20.0	−20.0	sine.100
4	0.0	0.0	0.0	rectangle
5	0.0	0.0	0.0	rectangle
6	0.0	0.0	0.0	rectangle
7	0.0	0.0	0.0	rectangle
8	0.0	0.0	0.0	rectangle
9	0.0	0.0	0.0	rectangle
10	0.0	0.0	0.0	rectangle
11	0.0	0.0	0.0	rectangle
12	0.0	0.0	0.0	rectangle
13	0.0	0.0	0.0	rectangle
14	0.0	0.0	0.0	rectangle

OK

Figure 7.2.   The "setpre" Window for the GREAT 1/10.
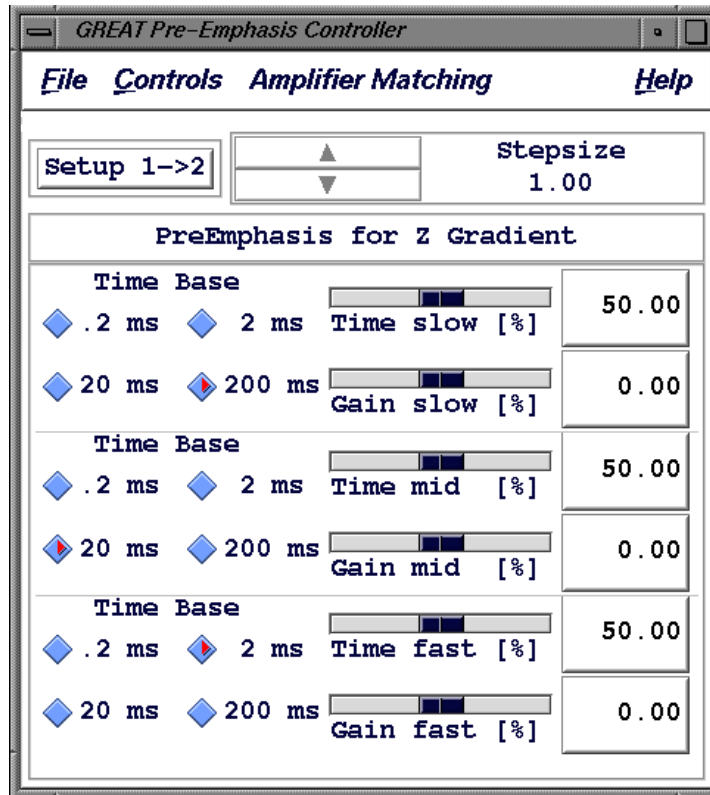
Figure 7.3.   The "setpre" Window for the Acustar.

Amplifier Control *7.3*
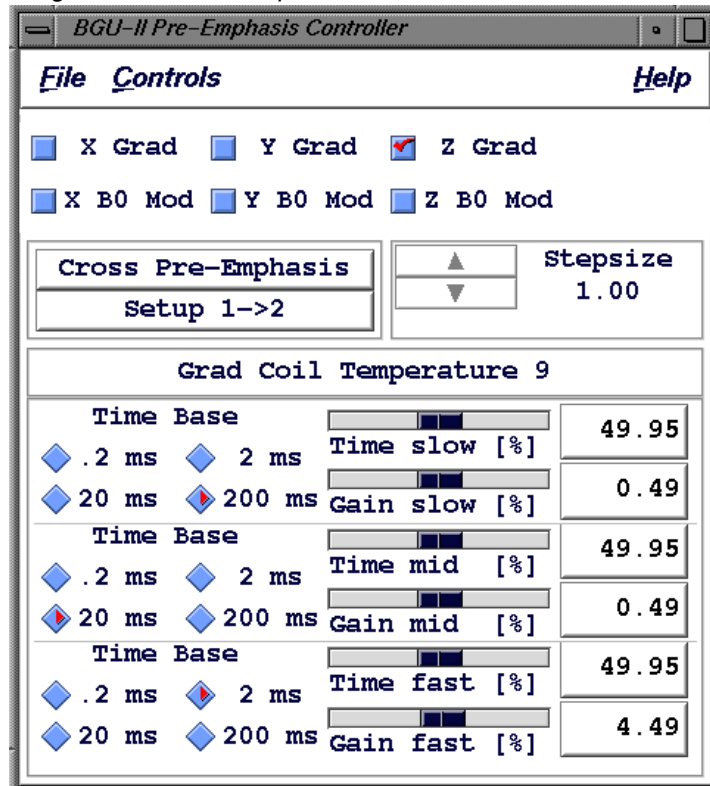
The Gradient amplifier is connected to the CCU via an RS232 link which allows monitoring of the amplifier status and control of various functions such as enabling or disabling of the pre-emphasis, reset from protection mode and adjustment of pre-emphasis, DC offset and the Amplifier Control Loop. **XWINNMR** supports this control by the command **setpre** (see figure 7.2). The routine may be started during e.g. a **gs** run so the effect of the adjustment can be monitored in "real time" (see section 5.3). It operates as a "Windows" application and the menu bar contains pull down menus:

- **File** to enable disk backup of parameters
- **Controls** to switch various functions of the pre-emphasis hardware
- **Help** with an on line description of the **setpre** functions.

When a GREAT1/10 amplifier is being used, then an additional menu appears:

- **Amplifier Matching** for DC offset and loop control

Status Functions *7.3.1*

The amplifier status can be controlled from **setpre** via the pull down menu **Controls** which offers a selection of the following functions:

- **Bypass on/off (Pre Emph on/off)**

  Used to bypass all pre-emphasis by loading zero pre-emphasis parameters into the hardware irrespective of the parameters in **XWINNMR**.

- **Amplifier on/off** (toggle)

  Disables Amplifier end stage (GREAT 1/10 only).

- **B_0 Modify on/off** (not used for GRASP)

- **BGU-II Active/Standby** (toggle)

  Used to disable the input to the amplifier.

- **Status Window**

  Brings up a status window for the GREAT1/10 amplifier

- **High>Low/Low>High Impedance**

  Switches GREAT1/10 amplifier characteristics to match high or low impedance load. Should be set to high (as indicated on the amplifier status lights (see figure 3.4) or in the status window) when used with the standard Z-gradient coil. **WARNING:** Do not operate the amplifier with an incorrect load.

- **Protection Reset**

  Upon detection of some conditions such as too long a gradient pulse or too high a duty cycle, the gradient amplifier enters the protection mode and is disabled. This control allows to reset

the protection mode. It is advisable first to establish the cause of this condition and remove it before activating this button.

Pre-emphasis *7.3.2*

The primary function of the **setpre** module is pre-emphasis adjustment and the main window is completely dedicated to this. The top part of the window contains six buttons to select the gradient direction and type of adjustment. For Gradient Spectroscopy only the function **Gradient** is relevant and **B0 Modify** is not used. The command defaults to **Z Gradient** which is the only one used for the single axis accessory.

The lower half of the window is for the actual control of the pre-emphasis. For three sets of parameters labeled **slow**, **middle** and **fast** the left side of the window contains buttons to select the **Time Base**. Any time base may be selected here, even ones that do not comply with the labels slow, middle and fast, and two sets of parameters may have identical time bases. Typically, **slow** is set to **20 ms**, **middle** to **2 ms** and **fast** to **0.2 ms**.

Adjustment of the pre-emphasis decay rate and amplitude is provided by means of sliders labeled **Time** and **Gain** for each range. The parameters may be modified by "grabbing" a slider with the mouse by moving the mouse over the slider and depressing the left mouse button and then "dragging" it by moving the mouse keeping the left button depressed.

An alternative method is to "click" the mouse to the right or left of the slider to in- or decrease the value of the parameter stepwise. The step size by which the parameters change when using this method can be adjusted by means of the up and down arrows next to the **Step size** in the top half of the window.

Finally, a temporary "back up" of a set of parameters can be made by toggling between two sets "in memory" using the toggle button **Setup 1->2 / 2->1**.

DC offset and Control Loop (GREAT 1/10 only). *7.3.3*

The **GREAT1/10** offers remote control of the DC offset and the Amplifier Control Loop and the functions for this in **setpre** can be found under **Amplifier Matching**. Both types of adjustment provide a slider mechanism for the relevant parameters. Changes can be made by dragging the slider, clicking on either side of it or typing the desired number in the value box. For more details of the adjustment procedures see sections 5.1 and 5.2.

Amplifier Parameters *7.3.4*

The **setpre** offers the possibility to save the gradient amplifier parameters on disk via the pull down menu **File** with the following functions:

   - **Open Setup** to retrieve stored parameters from a file on disk
   - **Save Current Setup** to store the current parameters (i.e. the parameters that are
     visible in the **setpre** window) into a file on disk. Note that the "secondary" set of
     parameters (the ones that could be made current by activating the button **Setup 1>2**)
     are not saved on disk!

XWINNMR offers a second command to inspect and/or modify the amplifier parameters: **edpre** (see figure 7.4). This command brings up a file select table from which a file with pre-emphasis parameters can be chosen. It then enters a parameter editor which contains all parameters for the pre-emphasis unit. The parameter names are encoded in the following manner: **DFTTTTi** where **D** stands for the direction (**X**, **Y**, or

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Z), **F** for the function (**G** for gradient and **B** for B$_0$ compensation; the latter is not used in Gradient Spectroscopy), **TTTT** for the type of parameter: (**GAIN** for gain, **TIME** for time constant, and **BASE** for time base), and **i** for an index running from **1** to **3**. The indices **1**, **2**, and **3** are used to distinguish between parameters for different time bases and correspond to **slow**, **middle**, and **fast** respectively. For example: **ZGGAIN2** controls the pre-emphasis amplitude for the **middle** range in the **Z** direction.

Figure 7.4.   The "edpre" Menu.

Gradient Spectroscopy Experiments

<div style="text-align: right">

8

</div>

Gradient spectroscopy experiments fall broadly into two categories: those where gradient pulses are applied solely to dephase magnetization (spoiler gradient pulses) and those where a sequence of gradient pulses is used to selectively refocus magnetization which has followed a particular coherence pathway (coherence selection experiments).

Whereas for spoiler gradients it is sufficient to decide on the strength and duration of the gradient pulse, for coherence selection it is also necessary to determine the necessary gradient pulse amplitude ratios that will ensure refocussing of the desired magnetization. For optimum results this ratio will have to be fine tuned experimentally in order to maximize the refocussed signal.

In this chapter a few examples of coherence selection experiments and procedures for their optimization will be described in detail.

Coherence Selection   *8.1*

Whenever a gradient pulse is applied the B_0 field is made spatially inhomogeneous and any coherent magnetization will precess with its own local Larmor frequency which will vary in the direction of the gradient. As a result of this, the magnetization will be dephased at the end of the gradient pulse. The phase angle Φ incurred through the application of the gradient pulse is given by:

$$\Phi(r) = \gamma \, p \, G \, r \, t \qquad\qquad \text{(Eq. 8.1)}$$

where γ is the gyromagnetic ratio, p the coherence order of the magnetization, G the gradient strength, r the position in the sample and t the duration of the gradient pulse. If the range of phase angles Φ over the length of the sample is large compared to 2π, then the magnetization will be completely dephased and no measurable magnetization will be left. For example when dephasing transverse magnetization of coherence order 1 (single quantum magnetization), then the residual magnetization is given by:

$$M_x(t) = \text{sinc}(\gamma \, G \, l \, t) \qquad\qquad \text{(Eq. 8.2)}$$

where l is the length of the sample in the direction of the gradient and it is assumed the projection of the sample perpendicular to the gradient direction is a rectangle.

Note that the magnetization can vanish completely for moderate gradient strength (and/or length) by selecting these parameters so that the sinc function passes through zero. Any small variation in either the gradient strength or the duration of the gradient pulse, however, will in this case lead to re-appearance of signal. In order to ensure an effective spoiling of all magnetization which is insensitive to small variations in the gradient parameters the product $G \, l \, t$ is therefore normally made sufficiently large for the sinc function's

oscillations to have damped to very small amplitude.

It is important to realize that, although no signal can be detected after a sufficiently strong gradient pulse, in each infinitesimal thin plane perpendicular to the gradient direction the magnetization is coherent. Consequently, by the application of a second gradient pulse, the amplitude, duration and direction of which are chosen such as to unravel the dephasing effect of the first pulse, it is possible to re-establish a condition of macroscopic coherent magnetization in the sample. The condition for this is:

$$\Phi'(r) + \Phi(r) = 0 \qquad\qquad \text{(Eq. 8.3)}$$

Thus, measurable signal has returned and we have "refocussed" the magnetization. This is the basis upon which the principle of gradient Coherence Selection relies.

As an example, consider the well known spin echo experiment: A 90^0 pulse turns the equilibrium magnetization into the transverse plane where it precesses as a combination of +1 and -1 coherence. A subsequent 180^0 pulse reverses the direction of precession (i.e. it reverses the signs of the coherence orders) and a spin echo occurs. A first gradient pulse, applied in between the 90^0 and 180^0 pulses will introduce a dephasing according to expression (8.1). Suppose a second gradient pulse would now be applied after the 180^0 pulse, causing dephasing according to:

$$\Phi'(r) = \gamma \, p' \, G' \, r \, t' \qquad\qquad \text{(Eq. 8.4)}$$

Because the effect of the 180^0 pulse is that $p' = -p$, it is now possible to satisfy equation (8.3) simply by setting $G' = G$ and $t' = t$.

So two gradient pulses of equal strength and length on either side of a 180^0 pulse work as a refocussing pair. We have seen an example of this in the Gradient Calibration experiment (section 6.2) and another example will be given in the next section in the description of the WATERGATE experiment.

In general, when RF pulses at the Larmor frequency of one or more nuclei are applied in between gradient pulses, they may cause coherence transitions other than from +1 to -1. Indeed, the magnetization may be distributed over a number of different coherence levels and the RF pulses may cause a large number of transitions. Still, when considering two particular coherence levels, one before and one after the RF pulses, it is possible to define a gradient pulse pair that will satisfy equation (8.3) for these two levels. The other coherence levels will in general not be refocussed by the gradient pulses and we have selected one particular pathway.

In the following sections, some specific examples will be given and the gradient amplitudes to obtain the relevant coherence selection will be discussed.

Solvent Suppression Using WATERGATE   *8.2*

WATERGATE[1] is a spin echo sequence using two gradient pulses: one between the 90° and the 180° pulses to dephase all magnetization and one after the 180° pulse to refocus it again (see figure 8.1). As we have seen in the previous section, these gradients pulses select the transition from coherence level +1 to -1 and, consequently, their strengths should be identical.

The experiment derives its solvent suppression characteristics from the nature of the 180° pulse. This pulse is a frequency selective pulse, designed to have a null in its frequency spectrum. If this null is positioned at the chemical shift of the solvent, e.g. water, then its magnetization does not experience a 180° pulse and the second gradient pulse will further dephase rather than refocus it.

Figure 8.1.   The Watergate Pulse Sequence
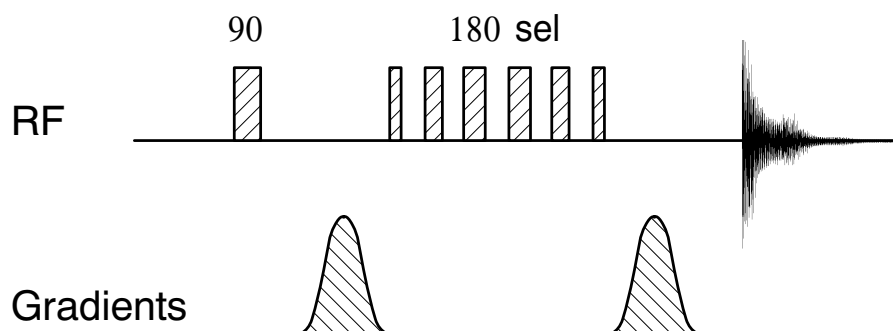
Set-up of the WATERGATE Experiment.   *8.2.1*

In preparation of the WATERGATE experiment, select a suitable sample in H_2O/D_2O (e.g. the sucrose water suppression test sample), set up parameters for acquisition with water on resonance: **SW = 15 ppm, O1P = 4.7 ppm** and determine the 90° pulse length. Acquire a water suppressed reference spectrum using the presaturation pulse program zgpr (see figure 8.2). Now select the appropriate pulse program for WATERGATE by setting **PULPROG = p3919gp**.

Figure 8.2.   1D Proton Spectrum of 2 nMol Sucrose in 90 % H_2O/10% D_2O.
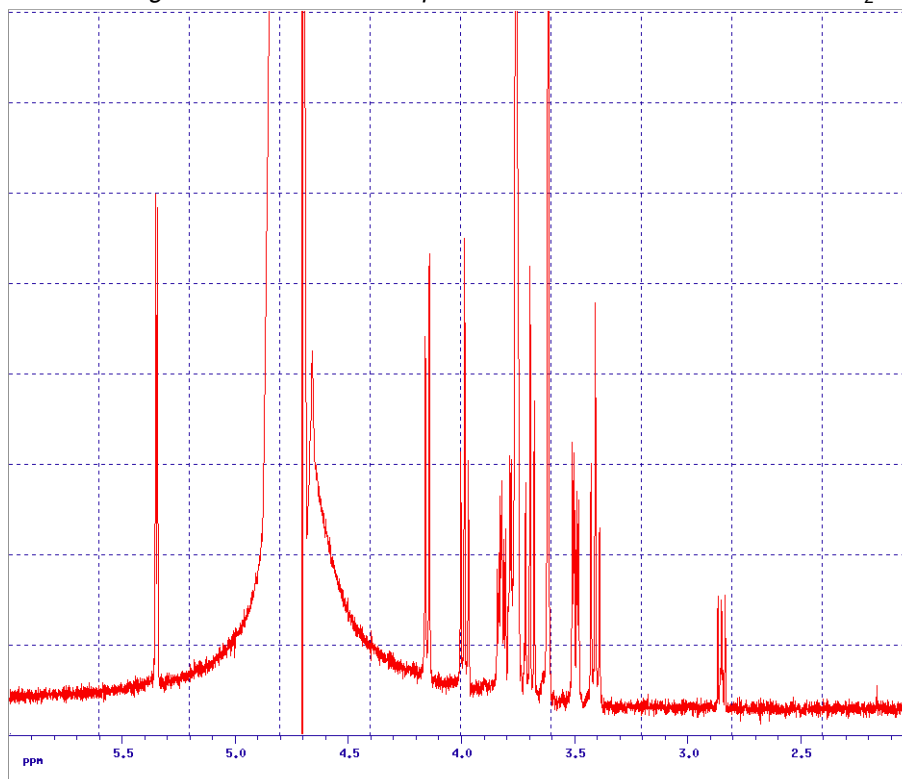
Water suppression by pre-saturation.

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The pulse sequence uses two gradient pulses: **gp0** and **gp1** and the experiment gives optimum results using sine shaped gradient pulses at moderate gradient strength: set **GPNAME0 = GPNAME1 = sine.100** and **GPZ0 = GPZ1 = 25**. (For three axes gradients the directions X and Y may also be used; make sure for each direction the strengths of the first and second pulses are equal). The lengths of both gradient pulses are determined by **P16** and the recovery delay after the gradient pulses by **D16**. Typical values are **1 ms** and **100** μ**s** respectively.

The RF refocussing pulse in the WATERGATE experiment as implemented in the pulse program **p3919gp** is an optimized binomial type pulse sequence ("3-9-19") at reduced power. It uses the parameters **PL18** for its power level and **P28** and **P0** for the lengths of the pulses. **P28** and **P0** should both be set to be a 90° pulse at the chosen power level; **P0** can be fine-adjusted later to optimize the performance of the sequence. As a starting point use a power level **12 dB** below that for the "hard" 90° pulse and set **P28** and **P0** to four times its length.

The "3-9-19" pulse sequence is designed to produce a null on resonance. The width of this null and the region in the spectrum where it produces optimum refocussing depend on the length of the delay in between the elements of the sequence: **D19**. A good indication of the region of refocussing is the position in the spectrum of the next null which is given by **1/(2*D19)**. The shorter **D19** the further will the first null be away from the centre, the wider will be the region of good refocussing, but, also, the wider will be the region of signal suppression. Typically, the first null will be placed at 6 ppm from the water which, for example on a 500 MHz instrument, will yield **D19 = 166** μ**s**.

Optimization of the WATERGATE Experiment.   *8.2.2*

Using the parameters as described in the previous section optimize the receiver gain and acquire a spectrum (see figure 8.3). In comparison with the presaturation spectrum, it should show good water suppression and an adequate recovery of the other signals although some signal loss for resonance's close to the residual water signal should be expected. In order to optimize this spectrum, several parameters are available: the delays and pulse lengths in the "3-9-19" pulse, the gradient pulse length and the gradient amplitude. While fine tuning these parameters consider the following two criteria: The quality of the water suppression and the signal to noise ratio of other resonance's.

Figure 8.3.   1D Proton spectrum of 2 mMol Sucrose in 90% H_2O/10% D_2O.
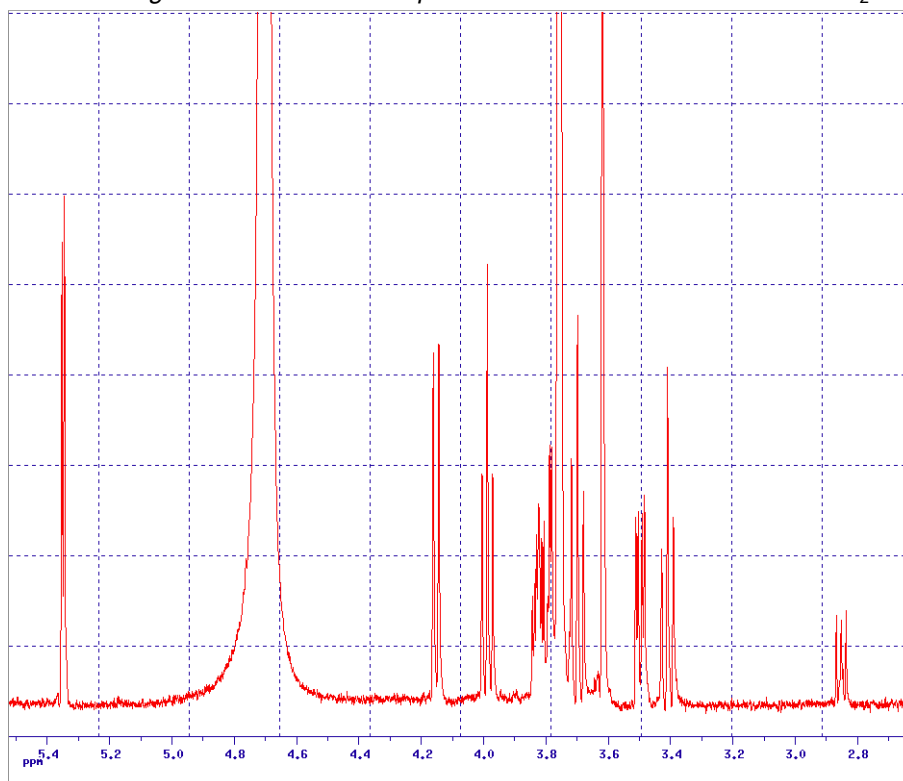
WATERGATE is used to suppress the water signal.

The water suppression largely depends on the overall gradient pulse length and amplitude, and on the ability of the "3-9-19" pulse to produce zero transverse magnetization on resonance. It is not necessarily so that the larger the gradient pulses the better the water suppression and the parameters suggested in the previous section should be close to optimum. The effect of the "3-9-19" pulse can be fine-tuned by means of the parameter **P0**, bearing in mind that this also controls the effectiveness of the refocussing of other signals.

Apart from optimizing the RF refocussing pulse with the object of maximizing the amplitude of the wanted resonance's, it may be well worth while to optimize the gradient refocussing of the signal by fine-adjustment of the gradient pulse amplitude. For this, use the AU program **paroptlin**, varying the amplitude of one of the gradient pulses. e.g. **GPZ0** and monitoring the signal amplitude of some signal of interest (see figure 8.4).

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Figure 8.4.   Optimisation of WATERGATE Gradient Pulses.
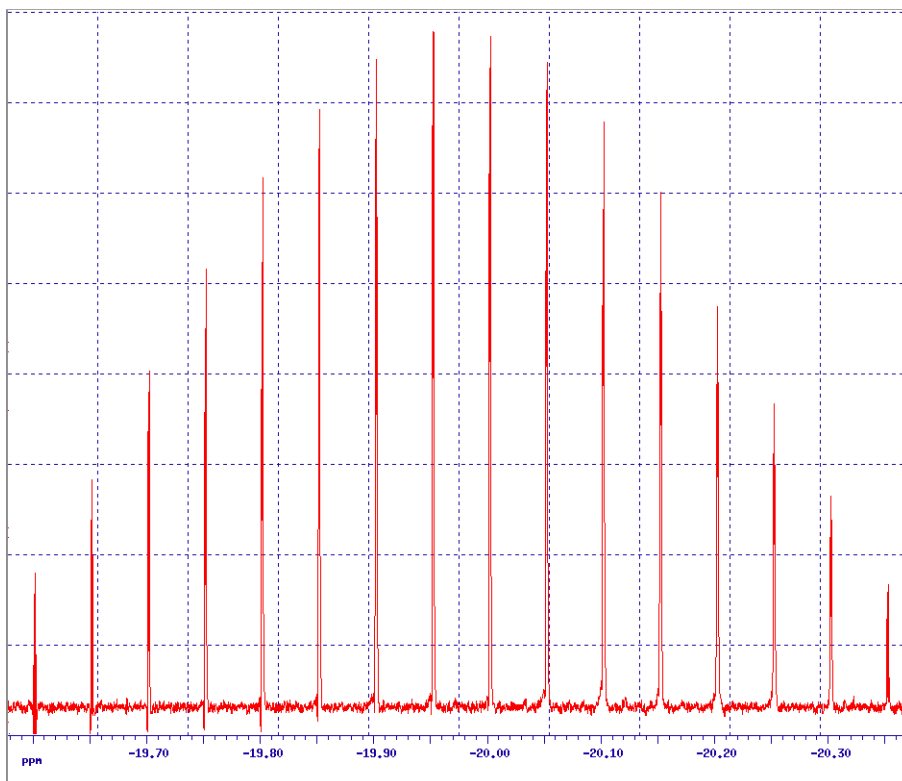
Dependence of the anomeric proton signal on the amplitude of one of the gradient pulses. Variation of the gradient strength from 19.6 to 20.4% in steps of 0.05%.

Phase Sensitive DQF-COSY   *8.3*

Gradient selected double quantum Filtered COSY was first published using an pulse sequence in which a gradient pulse is present during the evolution period[2]. This implicitly destroys the phase information in F1 and, consequently, the 2D spectrum has to be calculated in magnitude mode. (The pulse program for this method is called **cosygpmf** and the corresponding 1D setup program **mqsgp1d2**).

In this section, an alternative technique will be described which will retain the phase information during the evolution period and produce a phase sensitive spectrum with pure absorption lineshapes[3] (see figure 8.5). Gradient pulses are present only during the multiple quantum period and just prior to acquisition (pulse programs **cosygpmftp** and **mqsgp1d)**. Although in this example we will discuss the implementation as a double quantum filter, the quantum order selected depends solely on the ratio of the gradient pulse amplitudes used. The pulse programs do not contain any phase cycling to support a particular coherence selection.

The general approach to determining the correct amplitude ratio for gradient pulses in any coherence selection experiment starts with the convention that states that the measured magnetisation is of coherence level -1. Thus, the refocussing gradient pulse introduces rephasing according to expression (8.1) with $p = -1$. Consequently, if the gradient pulse which is applied during the multiple quantum period is meant to select double quantum coherence (i.e. $p = +/-2$) then its amplitude should be half of that of the refocussing pulse. If both pulses have the same sign, then $p = +2$ is selected, if they have opposite sign, then it will be $p = -2$.

Figure 8.5.   Pulse Sequence for Phase Sensitive Gradient Selected DQF-COSY.
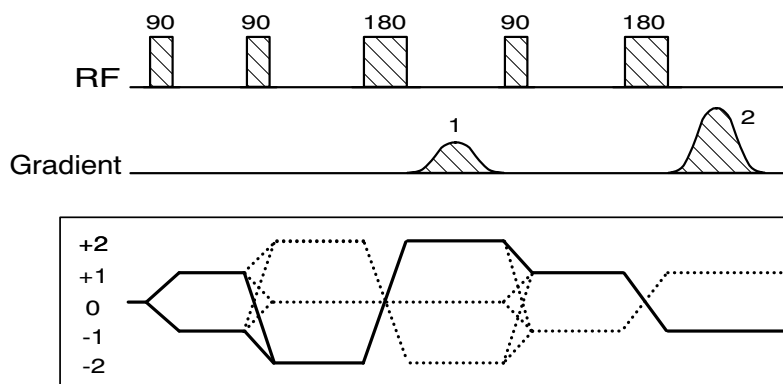
The coherence level diagram shows the pathway selected as solid lines.

Set-up of the 1D DQF-COSY Experiment.   *8.3.1*

Select a suitable sample for DQF-COSY (e.g. the 2D example sample Gramicidin in DMSO, see figure 8.6) and set up appropriate parameters for 1D acquisition. Determine the 90° pulse length and acquire a 1D reference spectrum. Now select the pulse program for 1D multiple quantum filtering **mqsgp1d** and set the following additional parameters: **D4** to the value of 1/4J for a typical J-coupling in the spectrum (preferably of a well isolated doublet resonance), **P2** to the length of a 180° pulse, **P16** to the length of the gradient pulses used (**1 ms** is a good value) and **D16** to the length of the Gradient Recovery delay (say **100** μs).

The gradient pulses used in the multiple quantum filter experiment are placed within the multiple quantum period, for dephasing, and just prior to acquisition, for refocussing. Because dephasing of a quantum order **n** in a gradient is **n** times as fast as for transverse (single quantum) magnetisation, the refocussing gradient needs to be **n** times as strong as the dephasing gradient. For selection of double quantum coherence, consequently, the gradient amplitude ratio needs to be **1:2**.

The gradient pulses in the pulse sequence are called **gp0** and **gp1** and, as in all experiments described in this chapter, we will use sine shaped gradient pulses at moderate gradient strength: set **GPNAME0 = GPNAME1 = sine.100**, **GPZ0 = 25** and **GPZ1 = 50**. (For three axes gradients use can also be made of the directions X and Y as desired; make sure to retain the correct gradient ratio).

Optimisation of the 1D DQF-COSY Experiment.   *8.3.2*

Using the parameters as set up in the previous section, acquire a spectrum, transform and phase correct, bearing in mind, that the resonance used for the determination of the delay **D4** should appear as a pure anti-phase doublet. Now optimize the refocussing by the gradient pulses using the AU program **paroptlin** and varying the value of one of the gradient amplitudes, e.g. **GPZ0**. Because the integral of the anti-phase doublet is zero, the optimisation should be run using **PH_mod = mc**. After determining the optimal gradient strength acquire the double quantum filtered spectrum using this value.

Figure 8.6.   1D Proton Spectrum of Gramicidin-S in DMSO..
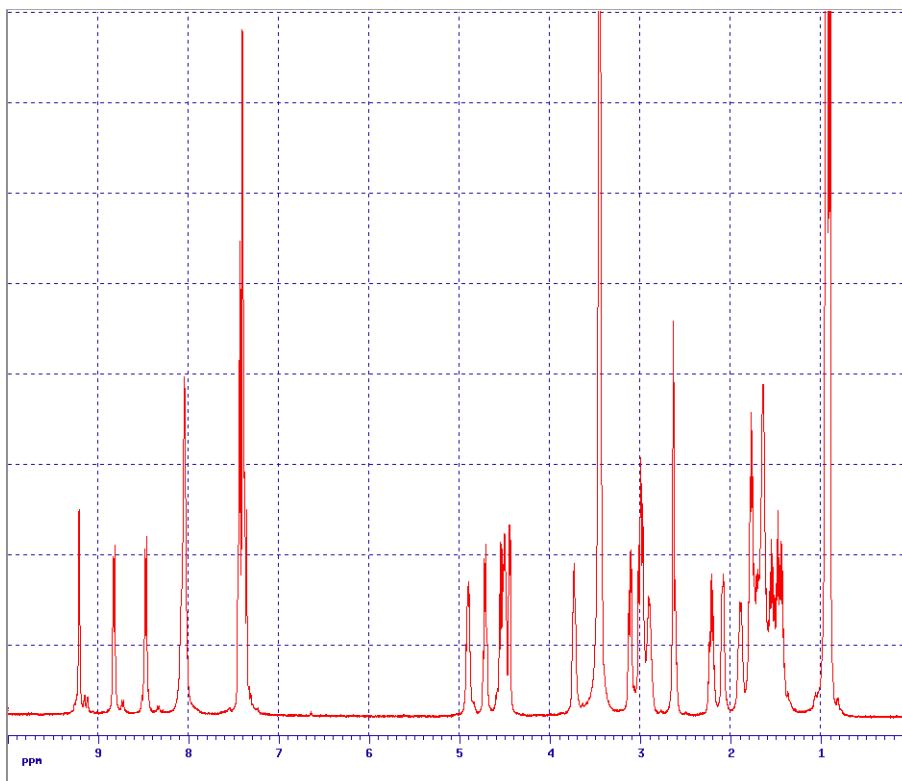
The signal to noise ratio of the resulting spectrum is expected to be lower than in the reference spectrum for several reasons: Firstly, by selecting the double quantum coherence, all singlet signals should be completely suppressed and half of the signal in multiplets is lost. Secondly, by selecting the double quantum coherence using gradients, the sensitivity is decreased by another factor of two. Thirdly, maximum signal recovery in the 1D experiment only occurs for the signals whose J-coupling correspond to the value of **D4**. And finally, some signal loss is to be expected due to T_1 and T_2. In summary, neglecting losses due to relaxation, the signal of the doublet used to set **D4** should be one quarter of the intensity in the reference spectrum.

Set-up of the 2D DQF-COSY Experiment.   *8.3.3*

Using the parameters set up in the previous sections, generate a 2D dataset, set **TD** and **SI** appropriate for a 2D experiment, adjust the parameters for the F1 dimension and set **PULPROG = cosygpmftp**. This completes the setup of the DQF-COSY experiment and a 2D spectrum may now be acquired (see figure 8.7). The optimum gradient strengths determined in the previous section should remain valid as long as the gradient timing is not changed (**P16** and **D16**).

Figure 8.7.   2D Gradient selected DQF-COSY Spectrum of Gramicidin-S in DM-SO..
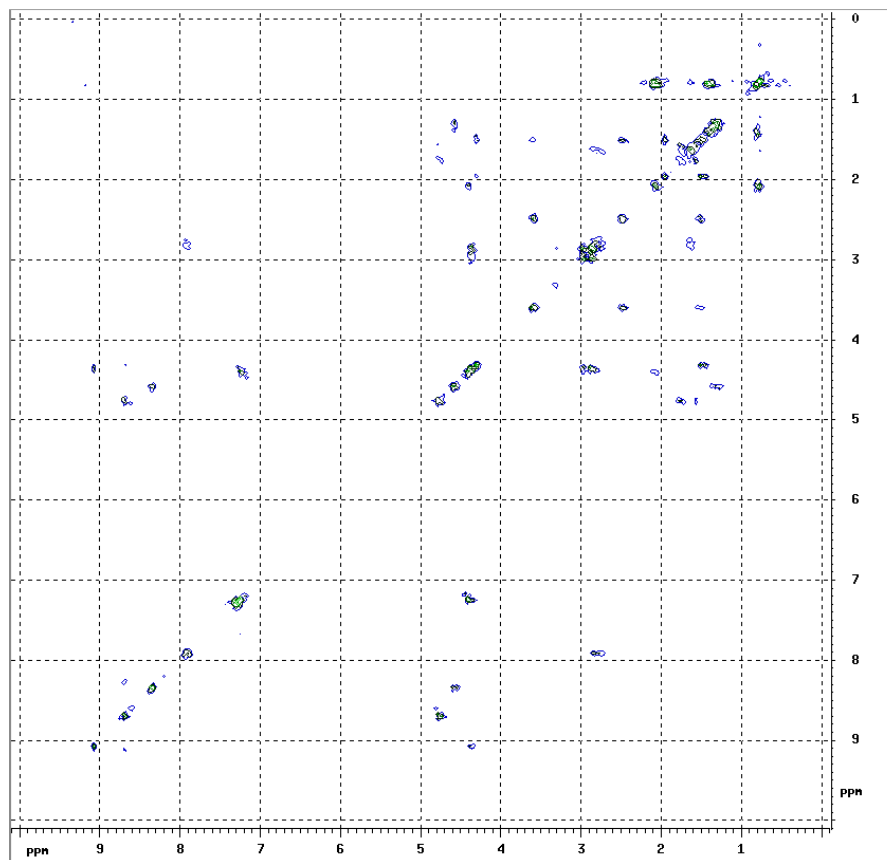
Magnitude HMQC.   *8.4*

Gradient selected Hetronuclear Multiple Quantum Coherence spectroscopy was the first gradient selected inverse detection experiment published[4] (see figure 8.8). When selecting a coherence pathway in which heteronuclear multiple quantum coherence is involved, a new element in our considerations for the selection of gradient amplitudes crops up: a coherence level where nuclei of different gyromagnetic ratio are involved. For the purpose of gradient selection we therefore redefine the coherence level as:

$$p_{XH} = (\gamma_X p_X + \gamma_H p_H) / \gamma_X \qquad\qquad\text{(Eq. 8.5)}$$

where p_{XH} is the level of multiple quantum coherence between nuclei X and H, p_X and p_H the "simple" coherence levels of nuclei X and H and γ_X and γ_H their respective gyromagnetic ratios.

Because the ratio between the gyromagnetic constants of ^1H and ^{13}C is to good approximation 4, for multiple quantum coherence between these two nuclei (8.5) equals one of the values +5, +3, -3 or -5. In the case of ^1H and ^{15}N the ratio is approximately 10 and (8.5) will be +11, +9, -9, -11. Note that within the definition (8.5), just prior to the detection of single quantum ^1H magnetisation when the simple coherence level for ^1H: $p_H = -1$, then in a $^1H/^{13}C$ experiment $p_{XH} = -4$ and for $^1H/^{15}N$ $p_{XH} = -10$.

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Figure 8.8.   Pulse Sequence for magnitude Gradient Selected HMQC:
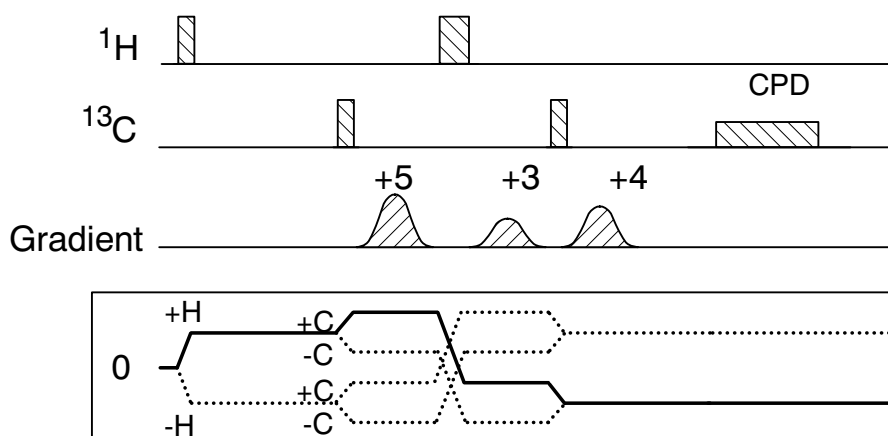
The coherence level diagram shows the pathway selected as solid lines.

As we have seen, the gradient HMQC sequence employs gradient pulses during the multiple quantum period which, at the same time, is the evolution period for encoding of the X-nucleus chemical shift. As a consequence, the experiment yields a magnitude mode spectrum. The pulse program for the 2D experiment is called **inv4gp** and the corresponding 1D setup program **inv4gpnd1d**.

In this section the setup of the HMQC experiment will be illustrated by means of a ^{1}H/^{13}C correlation experiment. Therefore it is necessary that the gradient probe head used is capable of irradiating both protons and carbon. In preparation of the experiment start by setting the spectrometer up for proton observe and carbon decouple by executing the **edsp** command or, alternatively, read in a parameter set with ^{1}H/^{13}C inverse detection parameters using **rpar**.

Set-up of the 1D HMQC Experiment.   8.4.1

Select a suitable sample for HMQC (e.g. the 2D example sample Gramicidin in DMSO) and set up appropriate parameters for 1D acquisition. Determine the ^{1}H 90° pulse length and acquire a 1D reference spectrum. Now select the pulse program for 1D HMQC **inv4gpnd1d** and set the following additional parameters: **D2** to the value of 1/2J for a typical ^{1}H/^{13}C J-coupling in the spectrum (preferably of a well isolated resonance showing clear ^{13}C satellites), **P2** to the length of a ^{1}H 180° pulse. Use estimated values for **P3** and **PL2**: the length and power level for a ^{13}C 90° pulse. Finally, set **P16** to the length of the gradient pulses used (**1 ms** is a good value) and **D16** to the length of the Gradient Recovery delay (say **100** μs). Ensure the length of **P16 + D16** does not exceed the length of **D2**.

The gradient pulses used in the HMQC experiment are placed within the multiple quantum period on both sides of the ^{1}H 180° pulse, for dephasing and just prior to acquisition, for refocussing. The condition for refocussing of the desired signal (the signal from the carbon bound protons) is that the sum of the dephasing effects of the individual gradient pulses is zero. Because three gradient pulses are used in the sequence an infinite number of solutions exist to this condition and a further constraint may be the requirement to minimize unwanted signals. In the pulse program a recommendation is given to use the ratio 5:3:4 for the three gradient pulses. This will select the coherence pathway +^{1}H+^{13}C > -^{1}H+^{13}C > -^{1}H with coherence levels p_{XH} of +5, -3 and -4.

The gradient pulses in the pulse sequence are called **gp0**, **gp1** and **gp2** and for the corresponding para-

meters set **GPNAME0 = GPNAME1 = GPNAME2 = sine.100**, **GPZ0 = 50**, **GPZ1 = 30** and **GPZ2 = 40**. (And corresponding values for the X and Y gradients in case of a three axes system making sure to retain the correct gradient ratio).

Optimisation of the 1D HMQC Experiment. *8.4.2*

Using the parameters as set up in the previous section, acquire a spectrum, transform and phase correct. The spectrum should show minimal or no residual signal from ^{12}C bound protons whereas the ^{13}C satellites will appear as anti-phase doublets. Now optimize the refocussing by the gradient pulses using the AU program **paroptlin** and varying the value of one of the gradient amplitudes, e.g. **GPZ0**. Because the integral of the anti-phase doublet is zero, the optimisation should be run using **PH_mod = mc**.

After determining the optimal gradient strength this same experiment can be used to determine the optimum value for **P3**. When searching for an optimum for this parameter using **paroptlin** bear in mind the signal will go through a maximum when passing a 90° pulse but it will not become negative when going through the 180° condition because the pulse **p3** occurs twice in the sequence.

The intensity of the ^{13}C satellites in the resulting spectrum, neglecting losses due to relaxation, should be approximately one half of their intensity in the reference spectrum.

Set-up of the 2D HMQC Experiment. *8.4.3*

Using the parameters set up in the previous sections, generate a 2D dataset, set **TD** and **SI** appropriate for a 2D inverse experiment, adjust the parameters for the F1 dimension and set **PULPROG = inv4gp**. Set the parameters **CPDPROG**, **P31** and **pl12** to be appropriate for composite pulse decoupling of carbon and acquire the 2D spectrum (see figure 8.9). The optimum gradient strengths determined in the previous section should remain valid as long as the gradient timing is not changed (**P16** and **D16**).

Figure 8.9.   2D Gradient selected HMQC Spectrum of Gramicidin-S in DMSO..
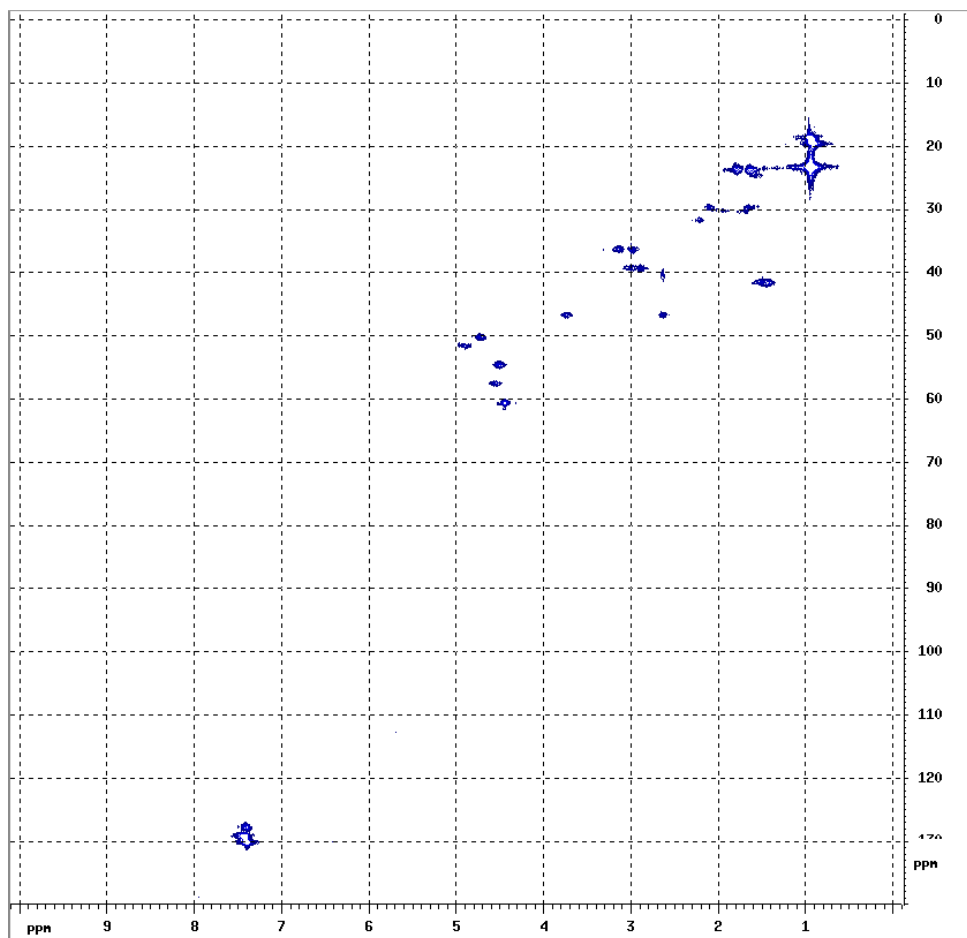
References   *8.5*

[1] Martial Piotto, Vladimir Saudek and Vladimir Sklenar, J. Biomol. NMR **2**, 661 - 666 (1992),
Vladimir Sklenar, Martial Piotto, Raymond Leppik and Vladimir Saudek, J. Magn. Reson.,
A 102, 241 -245 (1993).

[2] Ralph E. Hurd, J. Magn. Reson., **87**, 422, (1990).

[3] Adrian L. Davis, Ernest D. Laue, James Keeler, Detlef Moskau and Joost A. B. Lohman, J.
Magn. Reson, **94**, 637, (1991).

[4] Ralph E. Hurd and Boban K. John, J. Magn, Reson. **91**, 648, (1991).

Appendix: Troubleshooting

Gradient Operation Checks. *A.1*

If, in executing a gradient experiment, it is apparent that no gradient pulses are generated (e.g. by the absence of the periodic "dipping" of the lock signal), then stop the experiment and reset the GCU by executing the command **ii**. First ensure the gradient amplifiers have been switched on and are properly connected (see chapter 3). Verify if the gradient coils have been connected to the gradient amplifiers. Check if the gradient hardware has entered protection status which will be indicated on the amplifier or, alternatively, in the **Status** window of the **setpre** module of **XWINNMR**.

In the pulse program check if the gradient pulses and gradient switching have been programmed properly. In particular make sure commands of the type **gron***i* are matched by corresponding commands **groff** and their occurrence does not lead to unnecessarily long "gradient on" times.

If required, correct the pulse program, make good all connections and reset the gradient hardware by pressing the reset button or activating the **Protection Reset** button in the **Controls** menu of the **setpre** command. Then, check all relevant gradient parameters and ensure no gradient pulses are requested of excessive length and the gradient pulse dutycycle is moderate. If the fault persists seek the help of a Bruker Service Engineer.

Gradient Performance Tests. *A.2*

If the spectrometer appears to generate the correct gradient pulses but the gradient spectroscopy experiments do not perform as expected, e.g. by a sudden increase of artifact signal or reduction of signal to noise ratio, then the following two tests will help to ascertain whether the gradient hardware is operating properly.

Silent Stability Test. *A.2.1*

In order to ascertain the absence of any disturbances to the magnetic field homogeneity by the gradient accessory in standby mode, the stability is tested with the gradient amplifier connected to the gradient coil but not driven by any gradient pulses.

Because the instrument stability depends on many factors, a number of which are external to the instrument, the stability test can only be referenced by preceding or following it immediately by a reference experiment where no gradients are connected. For that reason, proceed as follows:

Using suitable acquisition parameters, acquire a spectrum of the doped water sample (see section 5); process it using the command **trf** and phase it to show the dispersion mode signal (negative and positive going part of equal but opposed amplitude). Using the button **defplot** select a 4 kHz wide part of the spec-

trum containing, in the centre, the measured signal.

Then, having dis-connected or permanently blanked the gradient amplifier(s), execute the AU program paroptlin by typing **xau paroptlin**, answering the questions as follows:

> Enter parameter to modify: **p1**
> Enter initial parameter value: **(value used in setup)**
> Enter parameter increment: **0**
> Enter # of experiments: **100**

Judge the results by inspection (see figure A.1).

Figure A.1.   Reference Stability Test.
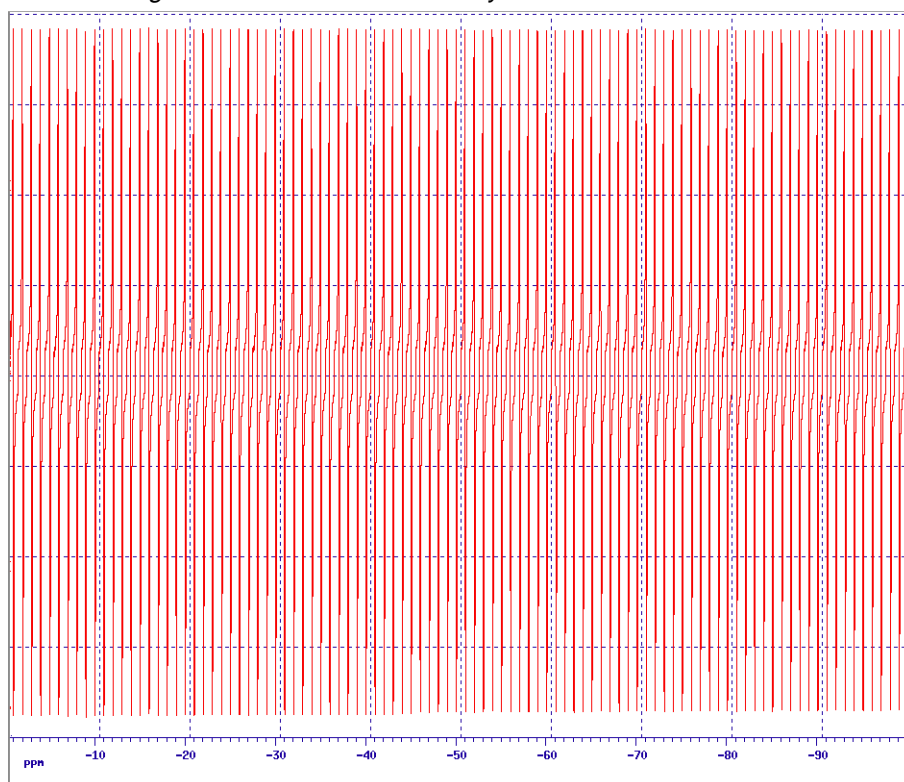
Showing the excellent stability of a DMX500

Now repeat the test with the gradient amplifier(s) connected to the gradient coil. The amplitude instability should not be more than a factor of two larger than that for the reference test (see figure A.2).

Figure A.2.   Silent Stability Test.
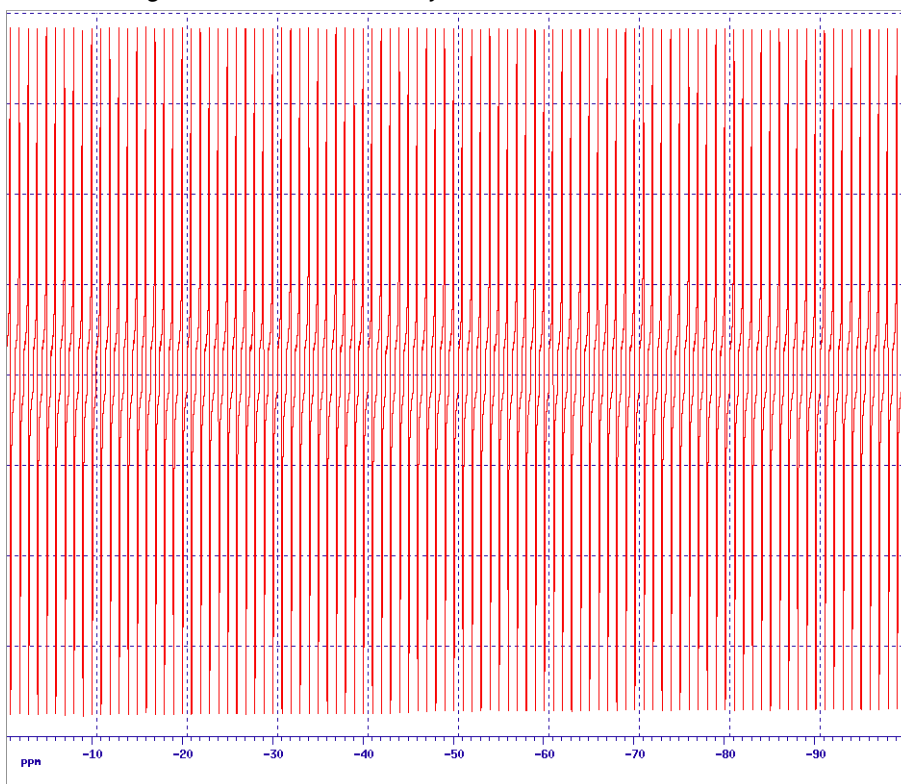
No degradation in the stability is measured after connecting the Acustar 3 axes gradient amplifier.

Refocussing Stability Test.   *A.2.2*

In the majority of gradient spectroscopy experiments it is important that the gradient pulses can be repeated with great accuracy. Any instability in gradient pulse amplitude can give rise to substantial loss of signal. The reproducibility of the gradient pulses can be tested by measuring the stability of signal recovery in a refocussing experiment.

Start off with the same parameters as in the gradient recovery test (section 6.2). Select the pulse program zg and determine the ninety degree pulse. Then, select the pulse program **PULPROG=zggpse** (see appendix B.10) and set the gradient parameters **GPNAME0 = sine.100**, **GPX=0**, **GPY=0** and **GPZ0=50**. Set the gradient recovery delay **d16=100us** and the further spin echo delay **d2=1ms**.

Acquire a spin echo spectrum and adjust the phase correction parameters to obtain a dispersion signal. Then, execute the AU program paroptlin by typing **xau paroptlin**, answering the questions as follows:

> Enter parameter to modify: **p1**
> Enter parameter initial value: **(value used in setup)**
> Enter parameter increment: **0**
> Enter # of experiments: **100**

Judge the results by inspection (see figure A.3). The amplitude instability should not be more than a factor of two larger than that for the silent stability test (see Appendix A.2.1). For the three axes accessory,

repeat for each gradient direction by using **GPX=50**, **GPY=0** and **GPZ=0** (for the X-direction) and **GPX=0**, **GPY=50** and **GPZ=0** (for the Y-direction).

Figure A.3.   Refocussing Stability Test.
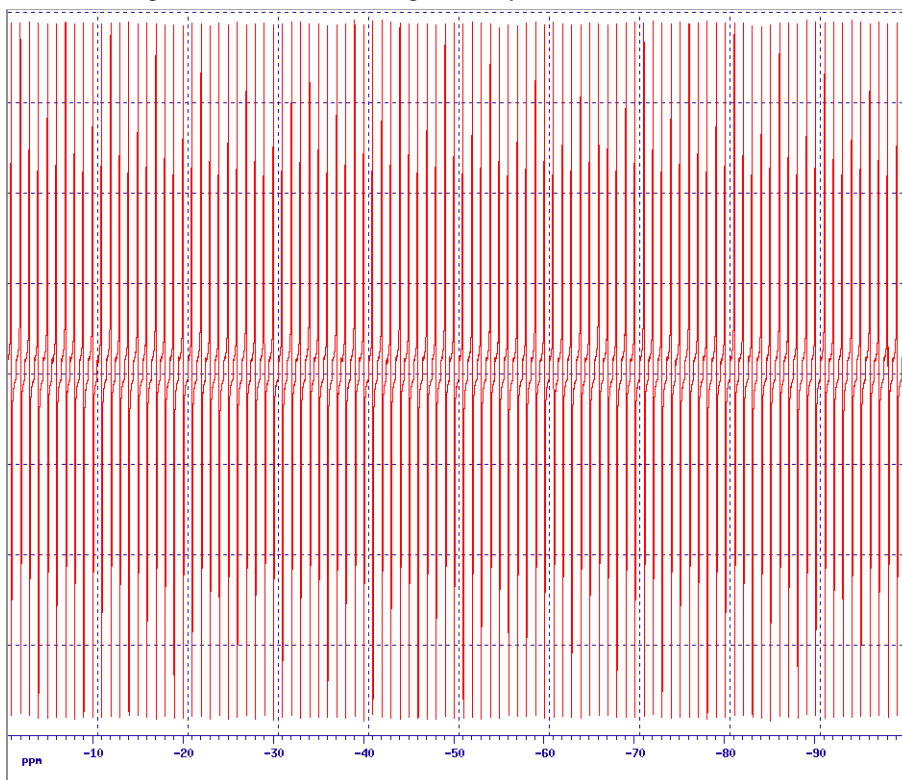
The excellent reproducibility of the gradient pulses is shown in this refocussing experiment.

Diagnostic Tests   *A.3*

A set of diagnostic tests for the GCU have been developed. To run the tests:

1. Log onto „spect"

2. cd /u/syststest

3. ./gcutest

4. auto

This will start an automatic test procedure which takes about 5 minutes to run. More information regarding the tests can be found in the directory /u/systest/doc.

Note that if the hardware ECL of the GCU does not match the software diagnostic test version, than error messages may result. For further information regarding GCU tests contact Mr. Gohlke at (49) 721 51 61 - 448.

Appendix: List of Gradient Pulse Programs

Pulse Program preempgp2.   *B.1*

```
;preempgp2
;avance-version
;pulseprogram for preemphasis adjustment

#include <Avance.incl>
#include <Grad.incl>

;;"d11=30m"

1 ze
2 d1 BLKGRAD
  50u UNBLKGRAD
  p16:gp1
  d16
  p1 ph1
  go=2 ph31
  wr #0
  d11 BLKGRAD
exit

ph1=0
ph31=0
;pl1 : f1 channel - power level for pulse (default)
;p1 : f1 channel -  90 degree high power pulse
;p16: homospoil/gradient pulse
;d1 : relaxation delay; 1-5 * T1
;d11: delay for disk I/O                 [30 msec]
;d16: delay for homospoil/gradient recovery

;use gradient :           gp 1
```

Pulse Program preempgp.m.   *B.2*

```
;preempgp.m
;avance-version - dmx only
;pulseprogram for preemphasis adjustment
;for digital filter only
```

```
#include <Avance.incl>
#include <Grad.incl>

"d11=30m"
"d13=3u"

define pulse advpul
define delay dwell
define loopcounter tdov

"advpul=(dw/decim)"
"dwell=(dw/decim)"
"tdov=td*decim*0.5"

"l4=tdov*0.125"

1 ze
  d13 adc ph31
2 d1 BLKGRAD
3 d11 UNBLKGRAD
  p16:gp1
  vd
  2u:e
  p0:f1:e ph1
  2u:e
  2u
  0.5u ph30:r
4 advpul:x
  dwell
  lo to 4 times l4
  d11 ivd
  lo to 2 times 8
  rcyc=1
  wr #0
  d11 BLKGRAD
exit

ph1=0
ph30=0
ph31=0

;pl1 : f1 channel - power level for pulse (default)
;p0 : f1 channel -  for any flip angle
;p16: homospoil/gradient pulse
;d1 : relaxation delay; 1-5 * T1
;d11: delay for disk I/O                    [30 msec]
;d13: short delay (e.g. to compensate delay line)    [3 usec]
;vd : variable delay, taken from vd-list
;L4:  (td*decim)/8

;use gradient :gp 1

;define VDLIST
;
;example for preemp list:
;                    100u
```

```
;                   300u
;                   1m
;                   3m
;                   10m
;                   30m
;                   100m
;                   500m
```

Pulse Program preempgp.r. *B.3*

```
;preempgp.r
;avance-version - drx only
;pulseprogram for preemphasis adjustment
;for digital filter only

#include <Avance.incl>
#include <Grad.incl>

"d11=30m"

"d13=3u"

define pulse advpul
define delay dwell
define loopcounter tdov

"advpul=(dw/decim)"
"dwell=(dw/decim)"
"tdov=td*decim*0.5"

"l4=tdov*0.125"

1 ze
  d11 syrec
  d13 adc ph31
2 d1 BLKGRAD
3 d11 UNBLKGRAD
  p16:gp1
  vd
  2u:e
  p0:f2:e ph1
  2u:e
  2u
  0.5u ph30:r
4 advpul:x
  dwell
  lo to 4 times l4
  d11 ivd
  lo to 2 times 8
  rcyc=1
  wr #0
  d11 BLKGRAD
exit
```

ph1=0
ph30=0
ph31=0

;pl1 : f1 channel - power level for pulse (default)
;pl2 : f2 channel - power level for pulse (default)
;p0 : f2 channel - for any flip angle
;p16: homospoil/gradient pulse
;d1 : relaxation delay; 1-5 * T1
;d11: delay for disk I/O   [30 msec]
;d13: short delay (e.g. to compensate delay line)   [3 usec]
;vd : variable delay, taken from vd-list
;L4: (td*decim)/8

;define f1 and f2 to be H-1

;use gradient :gp 1

;define VDLIST
;
;example for preemp list:
;   100u
;   300u
;   1m
;   3m
;   10m
;   30m
;   100m
;   500m

Pulse Program calibgp *B.4*

;calibgp
;avance-version
;calibration of gradient strength

#include <Avance.incl>
#include <Grad.incl>

;;"p2=p1*2"

"d28=aq"

1 ze
2 d1 BLKGRAD
 p1 ph1
 50u UNBLKGRAD
 p16:gp1
 d16
 p2 ph2
 50u
 d16
 de1
 de2 adc ph31 syrec

```
  de3 ph30
  d28:gp2
  rcyc=2
  wr #0
exit

ph1=0
ph2=1 3
ph30=0
ph31=0

;pl1 : f1 channel - power level for pulse (default)
;p1 : f1 channel -  90 degree high power pulse
;p2 : f1 channel - 180 degree high power pulse
;p16: homospoil/gradient pulse : ca. aq/2
;d1 : relaxation delay; 1-5 * T1
;d5 : DE/2
;d16: delay for homospoil/gradient recovery
;d28: delay for gradient : d28 = aq
;NS: 2 * n

;use gradient ratio:gp 1 : gp 2
;                    1 :   1

;this program can be used for dmx as well
```

Pulse Program p3919gp *B.5*

```
;p3919gp
;avance-version
;1D sequence
;water suppression using 3-9-19 pulse sequence with gradients
;M. Piotto, V. Saudek & V. Sklenar, J. Biomol. NMR 2, 661 - 666 (1992)
;V. Sklenar, M. Piotto, R. Leppik $ V. Saudek, J. Magn. Reson.,
;   Series A 102, 241 -245 (1993)

#include <Avance.incl>
#include <Grad.incl>

1 ze
2 d1 pl1:f1
  p1 ph1
  50u UNBLKGRAD
  p16:gp1
  d16 pl18:f1
  p28*0.231 ph3
  d19*2
  p28*0.692 ph3
  d19*2
  p28*1.462 ph3
  d19*2
  p28*1.462 ph4
  d19*2
  p28*0.692 ph4
```

```
 d19*2
 p0*0.231 ph4
 46u
 p16:gp2
 d16
 4u BLKGRAD
 go=2 ph31
 wr #0
exit

ph1=0 2
ph3=0 0 1 1 2 2 3 3
ph4=2 2 3 3 0 0 1 1
ph31=0 2 2 0
```

;pl1 : f1 channel - power level for pulse (default)
;pl18: f1 channel - power level for 3-9-19-pulse (watergate)
;p0 : f1 channel - 90 degree pulse at pl18
;   use for fine adjustment
;p1 : f1 channel - 90 degree high power pulse
;p16: homospoil/gradient pulse
;p28: f1 channel - 90 degree pulse at pl18
;d1 : relaxation delay; 1-5 * T1
;d8 : mixing time
;d16: delay for homospoil/gradient recovery
;d19: delay for binomial water suppression
;   d19 = (1/(2*d)), d = distance of next null (in Hz)
;NS: 8 * n
;DS: 4

;use gradient ratio:gp 1 : gp 2
;   20 :   20

Pulse Program mqsgp1d   *B.6*

```
;mqsgp1d
;avance-version
;1D multiple quantum filter
;using gradient pulses for selection
;with multiple quantum filter according to gradient ratio
;use as setup for pulseprogram 'cosygpmftp'

#include <Avance.incl>
#include <Grad.incl>

;;"d13=3u"

"d20=p16+d16"

1 ze
2 d1
3 p1 ph1
 d4
 p2 ph4
```

```
  d4
  p1 ph2
  d13
  d20 UNBLKGRAD
  p2 ph4
  d13
  p16:gp1
  d16
  p1 ph3
  d20
  p2 ph4
  d13
  p16:gp2
  d16
  4u BLKGRAD
  go=2 ph31
  wr #0
exit

ph1=0 2
ph2=0
ph3=0
ph4=0
ph31=0 2


;pl1 : f1 channel - power level for pulse (default)
;p1 : f1 channel -  90 degree high power pulse
;p2 : f1 channel - 180 degree high power pulse
;p16: homospoil/gradient pulse
;d1 : relaxation delay; 1-5 * T1
;d4 : 1/(4J)
;d13: short delay (e.g. to compensate delay line)    [3 usec]
;d16: delay for homospoil/gradient recovery
;d20: p16 + d16
;in0: 1/(2 * SW) = DW
;nd0: 2
;NS: 1 * n
;DS: 16


;use gradient ratio:gp 1 : gp 2
;                    10 :   20   for double quantum filter
;                    10 :   30   for triple quantum filter
```

Pulse Program cosygpmftp. *B.7*

```
;cosygpmftp
;avance-version
;2D homonuclear shift correlation
;phase sensitive using TPPI
;using gradient pulses for selection
;with multiple quantum filter according to gradient ratio
;use pulseprogram 'mqsgp1d' for setup

#include <Avance.incl>
```

```
#include <Grad.incl>

;;"d0=3u"
;;"d13=3u"

"d20=p16+d16+d13"

1 ze
2 d1
3 p1 ph1
  d0
  p1 ph2
  d20 UNBLKGRAD
  p2 ph4
  d13
  p16:gp1
  d16
  p1 ph3
  d20
  p2 ph4
  d13
  p16:gp2
  d16
  4u BLKGRAD
  go=2 ph31
  d1 wr #0 if #0 id0 ip1 zd
  lo to 3 times td1
exit

ph1=0 2
ph2=0
ph3=0
ph4=0
ph31=0 2
```

;pl1 : f1 channel - power level for pulse (default)
;p1 : f1 channel - 90 degree high power pulse
;p2 : f1 channel - 180 degree high power pulse
;p16: homospoil/gradient pulse
;d0 : incremented delay (2D)   [3 usec]
;d1 : relaxation delay; 1-5 * T1
;d13: short delay (e.g. to compensate delay line)   [3 usec]
;d16: delay for homospoil/gradient recovery
;d20: p16 + d16 + d13
;in0: 1/(2 * SW) = DW
;nd0: 2
;NS: 1 * n
;DS: 16
;td1: number of experiments
;MC2: TPPI

;use gradient ratio:gp 1 : gp 1
;   10 : 20   for double quantum filter
;   10 : 30   for triple quantum filter

```
;inv4gpnd1d
;avance-version
;1D H-1/X correlation via heteronuclear zero and double quantum
;   coherence
;using gradient pulses for selection
;use as setup for pulseprogram 'inv4gp'

#include <Avance.incl>
#include <Grad.incl>
;;"p2=p1*2"
;;"d2=1s/(cnst2*2)"
;;"d13=3u"

"d20=d2-p16-d13-4u"

1 ze
2 d1
3 p1 ph1
  d2 UNBLKGRAD
  p3:f2 ph3
  d13
  p16:gp1
  d16
  p2 ph2
  d13
  p16:gp2
  d16
  p3:f2 ph4
  d13
  p16:gp3
  d20
  4u BLKGRAD
  go=2 ph31
  wr #0
exit

ph1=0
ph2=0
ph3=0 2
ph4=0 0 2 2
ph31=0 2 2 0


;pl1 : f1 channel - power level for pulse (default)
;pl2 : f2 channel - power level for pulse (default)
;p1 : f1 channel -  90 degree high power pulse
;p2 : f1 channel - 180 degree high power pulse
;p3 : f2 channel -  90 degree high power pulse
;p16: homospoil/gradient pulse
;d1 : relaxation delay; 1-5 * T1
;d2 : 1/(2J)XH
;d13: short delay (e.g. to compensate delay line)    [3 usec]
;d16: delay for homospoil/gradient recovery
;d20: d2 - p16 - d13 - 4u, but >= d16
;NS: 1 * n
```

;DS: 16

;use gradient ratio:gp 1 : gp 2 : gp 3
;   50 : 30 : 40   for C-13
;   70 : 30 : 50   for N-15

Pulse Program inv4gp. **B.9**

;inv4gp
;avance-version
;2D H-1/X correlation via heteronuclear zero and double quantum
;   coherence
;with decoupling during acquisition
;using gradient pulses for selection
;use pulseprogram 'inv4gpnd1d' for setup

#include <Avance.incl>
#include <Grad.incl>

;;"p2=p1*2"
;;"d0=3u"
;;"d2=1s/(cnst2*2)"
;;"d12=20u"
;;"d13=3u"

"d20=d2-p16-d13-d12"

```
1 ze
2 d1 do:f2
3 p1 ph1
  d2 pl2:f2 UNBLKGRAD
  p3:f2 ph3
  d0
  p16:gp1
  d16
  p2 ph2
  d13
  p16:gp2
  d16
  d0
  p3:f2 ph4
  d13
  p16:gp3
  d20
  d12 pl12:f2 BLKGRAD
  go=2 ph31 cpd2:f2
  d1 do:f2 wr #0 if #0 id0 zd
  lo to 3 times td1
exit

ph1=0
ph2=0
ph3=0 2
ph4=0 0 2 2
```

ph31=0 2 2 0

;pl1 : f1 channel - power level for pulse (default)
;pl2 : f2 channel - power level for pulse (default)
;pl12: f2 channel - power level for CPD/BB decoupling
;p1 : f1 channel - 90 degree high power pulse
;p2 : f1 channel - 180 degree high power pulse
;p3 : f2 channel - 90 degree high power pulse
;p16: homospoil/gradient pulse
;p31: - 90 degree pulse for decoupling sequence
;d0 : incremented delay (2D)   [3 usec]
;d1 : relaxation delay; 1-5 * T1
;d2 : 1/(2J)XH
;d12: delay for power switching   [20 usec]
;d13: short delay (e.g. to compensate delay line)   [3 usec]
;d16: delay for homospoil/gradient recovery
;d20: d2 - p16 - d13 - d12 , but >= d16
;in0: 1/(2 * SW(X)) = DW(X)
;nd0: 2
;NS: 1 * n
;DS: 16
;td1: number of experiments
;MC2: QF
;cpd2: decoupling according to sequence defined by cpdprg2

;use gradient ratio:gp 1 : gp 2 : gp 3
;   50 : 30 :   40   for C-13
;   70 : 30 :   50   for N-15

Pulse Program zggpse.   *B.10*

;zggpse
;avance-version
;1D spin echo with gradients

#include <Avance.incl>
#include <Grad.incl>

;;"p2=p1*2"

1 ze
2 d1
  p1 ph1
  50u UNBLKGRAD
  p16:gp1
  d16
  p2 ph2
  30u
  p16:gp2
  d16
  20u BLKGRAD
  go=2 ph31
  wr #0
exit

ph1=0 0 1 1 2 2 3 3
ph2=1 3
ph31=0 0 1 1 2 2 3 3

;pl1 : f1 channel - power level for pulse (default)
;p1 : f1 channel - 90 degree high power pulse
;p2 : f1 channel - 180 degree high power pulse
;p16: homospoil/gradient pulse
;d1 : relaxation delay; 1-5 * T1
;d16: delay for homospoil/gradient recovery
;NS: 2 * n

;use gradient ratio: gp 1 : gp 2
;   1 :   1

Pulse Program include file Avance.incl.   *B.11*

;Avance.incl
;
;version 95/04/26

#define SPLH1_H setnmr1^7^6|5^4
#define SPLH1_L setnmr1^7^6|5|4

#define H2_LOCK setnmr8^4
#define H2_PULSE setnmr8|4

#define LOCKH_ON setnmr2|0
#define LOCKH_OFF setnmr2^0

#define SWITO_F setnmr2|2
#define SWITO_H setnmr2^2

#define QNP_X setnmr2|3
#define QNP_F setnmr2^3

Pulse Program include file Grad.incl.   *B.12*

;Grad.incl - include file for Gradient Spectroscopy
;avance-version
;version 95/04/26

#define GRADIENT(ampl) p16:ngrad:c34

#define BLKGRAD setnmr2^0 setnmr0^34

#define UNBLKGRAD setnmr2|0 setnmr0|34

#define BLKGRAMP setnmr0^34

#define UNBLKGRAMP setnmr0|34

Appendix: List of Experiment Parameters

Control Loop Adjustments   C.1

F2 - Acquisition Parameters

Date	17/02/95	Time	15.56
INSTRUM	spect	PROBHD	5 mm TXI-Z
PULPROG	preempgp2	AQ_mod	qsim
TD	4096	SOLVENT	D2O
NS	7645	DS	0
SWH	50125.313 Hz	FIDRES	12.237625 Hz
FW	94400.00 Hz	AQ	0.0410100 sec
RG	2	DW	9.975 usec
DWOV	3.325 usec	DECIM	3
DIGTYP	HADC-HRD16	DIGMOD	digital
DR	16	DDR	0
DE	4.50 usec	TE	300.0 K
D1	0.10000000 sec	P16	5000.00 usec
GPX0	0.00 %	GPY0	0.00 %
GPZ0	100.00 %	GPNAM0	block_100
D16	0.00010000 sec	P1	5.30 usec
DE	4.50 usec	SFO1	500.1327007 MHz
NUC1	1H	D11	0.03000000 sec

Pre-emphasis   C.2

F2 - Acquisition Parameters

Date	17/02/95	Time	15.56
INSTRUM	spect	PROBHD	5mm TXI-XYZ
PULPROG	preempgp.m	AQ_mod	qsim
TD	16384	SOLVENT	DMSO
NS	1	DS	0
SWH	40000.000 Hz	FIDRES	2.441406 Hz
FW	100800.00 Hz	AQ	0.1966580 sec
RG	8	DW	12.500 usec
DWOV	3.125 usec	DECIM	4
DIGTYP	HADC-HRD16	DIGMOD	digital
DR	17	DDR	1

DE	4.50 usec	TE	300.0 K
advpul	3.1 usec	dwell	0.0000031 sec
TD	16384	tdov	32768
I4	4096	D13	0.00000300 sec
D1	1.00000000 sec	D11	0.03000000 sec
P16	50000.00 usec	GPX0	0.00 %
GPY0	0.00 %	GPZ0	20.00 %
GPNAM0	block_100	VDLIST	preemp
P1	2.00 usec	SFO1	500.1350000 MHz
NUC1	1H		

Recovery test   C.3

F2 - Acquisition Parameters

Date	17/02/95	Time	15.56
INSTRUM	spect	PROBHD	5 mm TXI-XYZ
PULPROG	preempgp2	AQ_mod	qsim
TD	16384	SOLVENT	D2O
NS	1	DS	0
SWH	2003.205 Hz	FIDRES	0.122266 Hz
FW	121600.00 Hz	AQ	4.0960498 sec
RG	2	DW	249.600 usec
DWOV	2.600 usec	DECIM	96
DIGTYP	HADC-HRD16	DIGMOD	digital
DR	19	DDR	3
DE	4.50 usec	TE	300.0 K
D1	1.00000000 sec	P16	5000.00 usec
GPX0	0.00 %	GPY0	0.00 %
GPZ0	50.00 %	GPNAM0	block_100
D16	1.00000000 sec	P1	5.30 usec
DE	4.50 usec	SFO1	500.1327007 MHz
NUC1	1H	D11	0.03000000 sec

Gradient calibration   C.4

F2 - Acquisition Parameters

Date	17/02/95	Time	15.56
INSTRUM	spect	PROBHD	5 mm TXI-XYZ
PULPROG	calibgp.jab	AQ_mod	qsim
TD	512	SOLVENT	D2O
NS	2	DS	0
SWH	50125.313 Hz	FIDRES	97.901001 Hz
FW	94400.00 Hz	AQ	0.0051700 sec
RG	2	DW	9.975 usec
DWOV	3.325 usec	DECIM	3
DIGTYP	HADC-HRD16	DIGMOD	digital
DR	16	DDR	0
DE	4.50 usec	TE	300.0 K
D1	1.00000000 sec	P1	5.30 usec

P16	2500.00 usec	GPX0	0.00 %
GPY0	0.00 %	GPZ0	10.00 %
GPNAM0	block_100	D16	0.00010000 sec
P2	10.60 usec	D5	0.00000225 sec
SFO1	500.1327007 MHz	NUC1	1H

WATERGATE   C.5

F2 - Acquisition Parameters

Date	17/02/95	Time	15.56
INSTRUM	spect	PROBHD	5 mm TXI-XYZ
PULPROG	p3919gp	AQ_mod	qsim
TD	16384	SOLVENT	D2O
NS	8	DS	4
SWH	6009.615 Hz	FIDRES	0.366798 Hz
FW	121600.00 Hz	AQ	1.3599221 sec
RG	2048	DW	83.200 usec
DWOV	2.600 usec	DECIM	32
DIGTYP	HADC-HRD16	DIGMOD	digital
DR	18	DDR	2
DE	4.50 usec	TE	300.0 K
D1	1.00000000 sec	P1	5.30 usec
P16	1000.00 usec	GPX0	0.00 %
GPY0	0.00 %	GPZ0	24.95 %
GPNAM0	sine.100	D16	0.00050000 sec
P28	21.20 usec	D19	0.00030000 sec
P0	21.20 usec	GPX1	0.00 %
GPY1	0.00 %	GPZ1	25.00 %
GPNAM1	sine.100	DE	4.50 usec
SFO1	500.1323511 MHz	NUC1	1H

DQF-COSY 1D   C.6

F2 - Acquisition Parameters

Date	17/02/95	Time	15.56
INSTRUM	spect	PROBHD	5 mm TXI-XYZ
PULPROG	mqsgp1d	AQ_mod	qsim
TD	16384	SOLVENT	DMSO
NS	16	DS	0
SWH	7507.507 Hz	FIDRES	0.458222 Hz
FW	115200.00 Hz	AQ	1.0977780 sec
RG	2048	DW	66.600 usec
DWOV	2.775 usec	DECIM	24
DIGTYP	HADC-HRD16	DIGMOD	digital
DR	18	DDR	2
DE	4.50 usec	TE	300.0 K
d20	0.00110000 sec	P16	1000.00 usec
D16	0.00010000 sec	D1	1.00000000 sec
P1	5.70 usec	D4	0.02780000 sec

P2	11.40 usec	D13	0.00000300 sec
GPX0	0.00 %	GPY0	0.00 %
GPZ0	25.00 %	GPNAM0	sine.100
GPX1	0.00 %	GPY1	0.00 %
GPZ1	50.05 %	GPNAM1	sine.100
DE	4.50 usec	SFO1	500.1325006 MHz
NUC1	1H		

DQF-COSY 2D   C.7

F2 - Acquisition Parameters

Date	17/02/95	Time	5.56
INSTRUM	spect	PROBHD	5 mm TXI-XYZ
PULPROG	cosygpmftp	AQ_mod	qsim
TD	2048	SOLVENT	DMSO
NS	2	DS	0
SWH	5000.000 Hz	FIDRES	2.441406 Hz
FW	100800.00 Hz	AQ	0.2048500 sec
RG	2048	DW	100.000 usec
DWOV	3.125 usec	DECIM	32
DIGTYP	HADC-HRD16	DIGMOD	digital
DR	18	DDR	2
DE	4.50 usec	TE	300.0 K
d20	0.00110000 sec	P16	1000.00 usec
D16	0.00010000 sec	D13	0.00000300 sec
D1	1.00000000 sec	P1	5.70 usec
D0	0.00000300 sec	P2	11.40 usec
GPX0	0.00 %	GPY0	0.00 %
GPZ0	25.00 %	GPNAM0	sine.100
GPX1	0.00 %	GPY1	0.00 %
GPZ1	50.05 %	GPNAM1	sine.100
DE	4.50 usec	SFO1	500.1325006 MHz
NUC1	1H	TD	2048
IN0	0.00010000 sec		

F1 - Acquisition Parameters

ND0	2	TD	512
SFO1	500.1323 MHz	FIDRES	9.765625 Hz
SW	9.997 ppm	SWH	5000.02 Hz
FOV	20.00 cm		

F2 - Processing Parameters

SI	1024	PPARMOD	2D
SF	500.1299994 MHz	OFFSET	10.000 ppm
SR	-0.65 Hz	HZpPT	.882812 Hz
SW_p	5000.00 Hz	XDIM	128
WDW	EM	SSB	0
LB	0.30 Hz	GB	0

PH_mod	pk	PKNL	TRUE
PHC0	-106.198 degree	PHC1	13.776 degrees
BC_mod	quad	BCFW	1
FT_mod	fqc	FCOR	0.5
ME_mod	no	COROFFS	0
NCOEF	0	LPBIN	0
ABSF1	0.000 ppm	ABSF2	0.000 ppm
ABSG	0	ABSL	0
AZFE	0.100 ppm	AZFW	0.100 ppm
TDeff	2048	TDoff	0
STSR	0	STSI	1024
PSCAL	global	SREGLST	1H.CDCl3
PC	1.00	PSIGN	pos.
MI	0.00 cm	MAXI	10000.00 cm
INTBC	yes	INTSCL	1
ISEN	128	REVERSE	FALSE
AUNMP	proc_1d	SINO	400
NOISF1	3.6	NOISF2	4.6
SIGF1	0	SIGF2	0
ASSFAC	0	ASSFACI	0
ASSFACX	0	ASSWID	0
DATMOD	proc	DC	2
NSP	200	NZP	0
DFILT		TI	
TM1	0	TM2	0
ALPHA	0	GAMMA	0
YMAX_p	75625362	YMIN_p	-56282647
NC_proc	-3	MEAN	0.000
S_DEV	11595.000	AQORDER	3-2-1
NLEV	6	LEV0	0
TOPLEV	0		

F1 - Processing Parameters

SI	1024	MC2	TPPI
SF	500.1298151 MHz	OFFSET	10.000 ppm
SR	-184.91 Hz	HZpPT	4.882835 Hz
SW_p	5000.02 Hz	XDIM	128
WDW	SINE	SSB	0
LB	0.00 Hz	GB	0
TDeff	2048	TDoff	0
STSR	0	STSI	1024
BC_mod	no	PH_mod	pk
PHC0	90.000 degrees	PHC1	0.000 degrees
ABSF1	0.000 ppm	ABSF2	0.000 ppm
ABSG	0	ABSL	0
FT_mod	fsr	REVERSE	FALSE
SYMM	no	TILT	FALSE
TM1	0	TM2	0
ME_mod	LPfr	NCOEF	100
LPBIN	2048		

F2 - Acquisition Parameters

Date	17/02/95	Time	15.56
INSTRUM	spect	PROBHD	5 mm TXI-Z
PULPROG	inv4gpnd1d	AQ_mod	qsim
TD	16384	SOLVENT	DMSO
NS	16	DS	0
SWH	7507.507 Hz	FIDRES	0.458222 Hz
FW	115200.00 Hz	AQ	1.0977780 sec
RG	2048	DW	66.600 usec
DWOV	2.775 usec	DECIM	24
DIGTYP	HADC-HRD16	DIGMOD	digital
DR	18	DDR	2
DE	4.50 usec	TE	300.0 K
d20	0.00247700 sec	D2	0.00350000 sec
P16	1000.00 usec	D13	0.00000300 sec
D1	1.00000000 sec	P1	5.70 usec
P3	10.00 usec	SFO2	125.7665420 MHz
NUC2	13C	GPX0	0.00 %
GPY0	0.00 %	GPZ0	50.00 %
GPNAM0	sine.100	D16	0.00010000 sec
P2	11.40 usec	GPX1	0.00 %
GPY1	0.00 %	GPZ1	30.00 %
GPNAM1	sine.100	GPX2	0.00 %
GPY2	0.00 %	GPZ2	39.90 %
GPNAM2	sine.100	DE	4.50 usec
SFO1	500.1325006 MHz	NUC1	1H

HMQC 2D   *C.9*

F2 - Acquisition Parameters

Date	17/02/95	Time	15.56
INSTRUM	spect	PROBHD	5 mm TXI-Z
PULPROG	inv4gp	AQ_mod	qsim
TD	2048	SOLVENT	DMSO
NS	16	DS	0
SWH	7507.507 Hz	FIDRES	3.665775 Hz
FW	115200.00 Hz	AQ	0.1372660 sec
RG	2048	DW	66.600 usec
DWOV	2.775 usec	DECIM	24
DIGTYP	HADC-HRD16	DIGMOD	digital
DR	18	DDR	2
DE	4.50 usec	TE	300.0 K
d20	0.00247700 sec	D2	0.00350000 sec
P16	1000.00 usec	D13	0.00000300 sec
D12	0.00002000 sec	D1	1.00000000 sec
P1	5.70 usec	P3	10.00 usec
SFO2	125.7665420 MHz	NUC2	13C
D0	0.00000300 sec	GPX0	0.00 %

GPY0	0.00 %	GPZ0	50.00 %
GPNAM0	sine.100	D16	0.00010000 sec
P2	11.40 usec	GPX1	0.00 %
GPY1	0.00 %	GPZ1	30.00 %
GPNAM1	sine.100	GPX2	0.00 %
GPY2	0.00 %	GPZ2	39.90 %
GPNAM2	sine.100	DE	4.50 usec
SFO1	500.1325006 MHz	NUC1	1H
TD	2048	IN0	0.00005301 sec

F1 - Acquisition Parameters

ND0	2	TD	256
SFO1	125.7665 MHz	FIDRES	36.845664 Hz
SW	75.000 ppm	SWH	9432.49 Hz
FOV	20.00 cm		

F2 - Processing Parameters

SI	1024	PPARMOD	2D
SF	500.1299306 MHz	OFFSET	12.644 ppm
SR	-69.36 Hz	HZpPT	7.331550 Hz
SW_p	7507.51 Hz	XDIM	1024
WDW	EM	SSB	0
LB	0.30 Hz	GB	0
PH_mod	pk	PKNL	TRUE
PHC0	-8.000 degrees	PHC1	0.000 degrees
BC_mod	quad	BCFW	1
FT_mod	fqc	FCOR	0.5
ME_mod	no	COROFFS	0
NCOEF	0	LPBIN	0
ABSF1	0.000 ppm	ABSF2	0.000 ppm
ABSG	0	ABSL	0
AZFE	0.100 ppm	AZFW	0.100 ppm
TDeff	2048	TDoff	0
STSR	0	STSI	1024
PSCAL	global	SREGLST	1H.CDCl3
PC	1.00	PSIGN	pos.
MI	0.00 cm	MAXI	10000.00 cm
INTBC	yes	INTSCL	1
ISEN	128	REVERSE	FALSE
AUNMP	proc_1d	SINO	400
NOISF1	3.6	NOISF2	4.6
SIGF1	0	SIGF2	0
ASSFAC	0	ASSFACI	0
ASSFACX	0	ASSWID	0
DATMOD	proc	DC	2
NSP	200	NZP	0
DFILT		TI	
TM1	0	TM2	0
ALPHA	0	GAMMA	0
YMAX_p	312704168	YMIN_p	0
NC_proc	-13	MEAN	0.000
S_DEV	113622400.000	AQORDER	3-2-1
NLEV	6	LEV0	0
TOPLEV	0		

F1 - Processing Parameters

SI	512	MC2	QF
SF	125.7530241 MHz	OFFSET	145.000 ppm
SR	-16975.94 Hz	HZpPT	18.422832 Hz
SW_p	9432.49 Hz	XDIM	512
WDW	SINE	SSB	0
LB	0.00 Hz	GB	0
TDeff	14	TDoff	0
STSR	0	STSI	512
BC_mod	no	PH_mod	mc
PHC0	0.000 degrees	PHC1	0.000 degrees
ABSF1	0.000 ppm	ABSF2	0.000 ppm
ABSG	0	ABSL	0
FT_mod	fqc	REVERSE	FALSE
SYMM	no	TILT	FALSE
TM1	0	TM2	0
ME_mod	no	NCOEF	0
LPBIN	0		

Refocussing stability test   *C.10*

F2 - Acquisition Parameters

Date	17/02/95	Time	5.56
INSTRUM	spect	PROBHD	5 mm TXI-XYZ
PULPROG	zggpse	AQ_mod	qsim
TD	16384	SOLVENT	D2O
NS	1	DS	0
SWH	2003.205 Hz	FIDRES	0.122266 Hz
FW	121600.00 Hz	AQ	4.0960498 sec
RG	2	DW	249.600 usec
DWOV	2.600 usec	DECIM	96
DIGTYP	HADC-HRD16	DIGMOD	digital
DR	19	DDR	3
DE	4.50 usec	TE	300.0 K
D1	1.00000000 sec	P1	5.30 usec
P16	5000.00 usec	GPX0	0.00 %
GPY0	0.00 %	GPZ0	50.00 %
GPNAM0	sine.100	D16	0.00010000 sec
P2	10.60 usec	DE	4.50 usec
SFO1	500.1327007 MHz	NUC1	1H

Figures

Figures

A   Appendix: Troubleshooting   *51*

B   Appendix: List of Gradient Pulse Programs   *55*

C   Appendix: List of Experiment Parameters   *67*

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