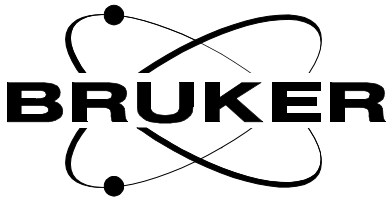

RDCU

Installation Tests Experiments User Manual

Version 001

BRUKER

The information in this manual may be altered without notice.

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General 1

Radiation Damping effects and it's suppression with the electronic feedback approach 1.1

The radiation damping phenomenon and it's suppression 1.1.1

The radiation damping phenomenon exists in all pulsed NMR experiments and is a consequence of the transverse magnetization precessing in the transverse xOy plane. The precessing transverse magnetization induces an e.f.m. in the receiver coil. This e.f.m. generates the radiation damping field B_{rd}.

$$\mathbf{B_{rd} = (2\pi \nu_0 Q f M_0) / 2 \varepsilon_0 c^2} \quad \text{(Eq. 1.1)}$$

where Q is the coil quality factor, ν_0 is the Larmor frequency, f the probehead filling factor.
Radiation damping resembles nutation; both are the rotation of the magnetization and are induced by the coil magnetization interaction. Nutation is induced by the externaly applied rf current and is uniform rotation with the angular velocity described by

$$\mathbf{(d\theta/dt) = \gamma B_1 = \omega_1} \quad \text{(Eq. 1.2)}$$

θ is the angle between the precessing magnetization and the B_0 field. The angular velocity of radiation damping, however, is not uniform. It is a sine function of θ :

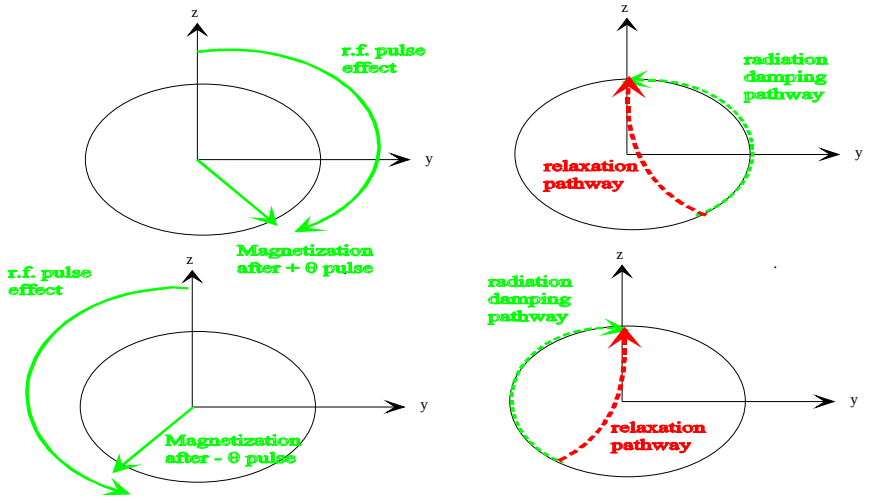
$$\mathbf{(d\theta/dt)_{rd} = \gamma B_{rd} = - \sin \theta / T_{rd}} \quad \text{(Eq. 1.3)}$$

where T_{rd} is the radiation damping time expressed by

$$\mathbf{T_{rd} = 2 \varepsilon_0 c^2 / (\gamma Q f M_0)} \quad \text{(Eq. 1.4)}$$

Nutation is the result of a r.f. shaped pulse (e.g. square, gaussian ...) whereas the radiation damping is the result of transverse magnetization precession. Therefore the radiation damping field is also called the FID field[1]. From ***(Eq. 1.3)***, we can deduce that if the nutation angle θ is between 0 and 180°, the angular velocity is negative and the magnetization rotates counterclockwise toward the Oz direction (***Figure 1.1.***).

Figure 1.1. Effect of the r.f. pulse on z-Magnetization and relaxation and radiation damping relaxation pathway depending on the sense of initial nutation (e.g. the phase of the r.f.pulse)



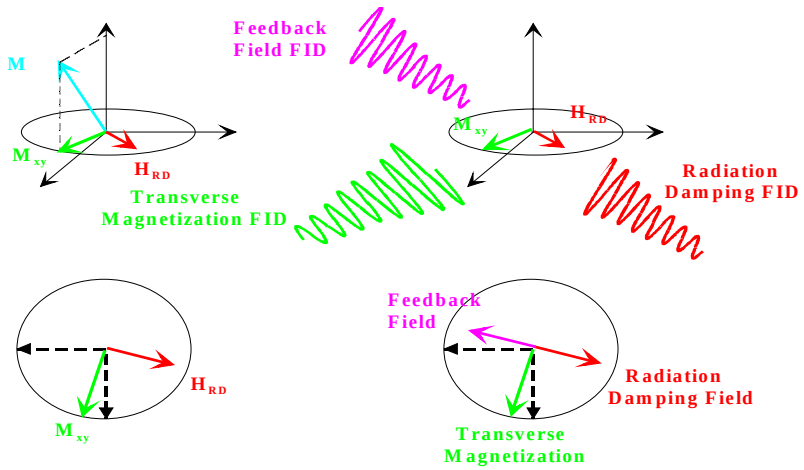
Otherwise if the nutation angle θ is between 0 and -180°, the magnetisation rotates clockwise toward the Oz direction. This explains the reason why, the radiation damping field always rotates the magnetization toward it's equilibrium position. In this way the effect of the radiation damping field can be assimilated to an additionnal relaxation process.

Normaly the radiation damping is present in each sample for each nucleus (as far as RD is generated by the precession of the magnetization) but the most of the time this process can be neglected against the other relaxation processes (field inhomogenety,T_1,T_2 etc..). In the case of very strong magnetization like water in biological samples, the radiation damping effect can no more be neglected. The behaviour of the water magnetization is perturbated by the strong RD effect. Some examples are given in the following manual.

There are some different approaches existing in litterature to suppress the radiation damping phenomenon, with small gradients pulsing [2], probehead Q switching[3] , and suppression using an electronical generated feedback field [4,5]. The last one allows to suppress and/or enhance the RD phenomenon everywhere in a pulse sequence. The ***RDCU is based on the feedback field method***. The way in which the RDCU is working is shown in ***Figure 1.2.***

1. *"(1) Xi-An MAO and Chao-Hui YE in Concepts in Magnetic resonance, 9 (1997), 173-187." on page 61*
2. *"(2) Vladimir. SKLENAR, J. Magn. Reson. Ser. A, 114 (1994), 132-135" on page 61*
3. *"(3) W.E. MAAS, F.H. LAUKIEN and D.G. GORY, J. Magn. Reson. Ser A, 113 (1995), 274-277." on page 61*
4. *"(4) Paul. BROEKAERT and Jean JEENER J. Magn. Reson. Ser A, 113 (1995),60-64." on page 61*
5. *"(5) Alain LOUIS-JOSEPH, Daniel ABERGEL and Jean-Yves LALLEMAND J.Biomol NMR, 5 (1995), 212-216." on page 61*

Figure 1.2. RDCU works using the feedback field method as developped by L'allemand and co workers [1]



In this case we show the particular case for which the phase of the feedback field is set in the manner that the radiation damping is suppressed. As far as we are able to use every phase we want, the phase of the FID sent back to the probehead can be set between 0 and 360° against the initial Magnetization phase.

Installation of the RDCU and hardware requirements 1.1.2

The Radiation Damping Control Unit is inserted in the AQX rack in between the ASU's and the router. A minimum of 3 channels is requested (***This board is not suited for DPX consoles***). Five versions of the RDCU board exist :

- 300-400 MHz (for RX 22 and SE 451 reception systems)
- 500-600 MHz (for RX 22 and SE 451 reception systems)
- 750-800 MHz (only for RX 22 reception systems)

The RDCU board contains an internal 25 dB amplifier. The board is delivered with the needed directional coupler (-27 dB insertion) and the set of cable for connections. The coupler is connected between the output of the HPPR and the probehead. In ***Figure 1.3.*** we show how to connect the input's and output's of the RDCU to the rest of the spectrometer.

The RDCU board can work in radiation damping control mode and can be bypassed in order to use the RDCU driving channel in the normal mode. This function is done by software (I2C Bus) with the RDCU control module. This software module controls also the filter settings for low frequency signal filtration (commutation between the 100 Hz, 1 kHz, 10 kHz and 60 kHz broad band filters).

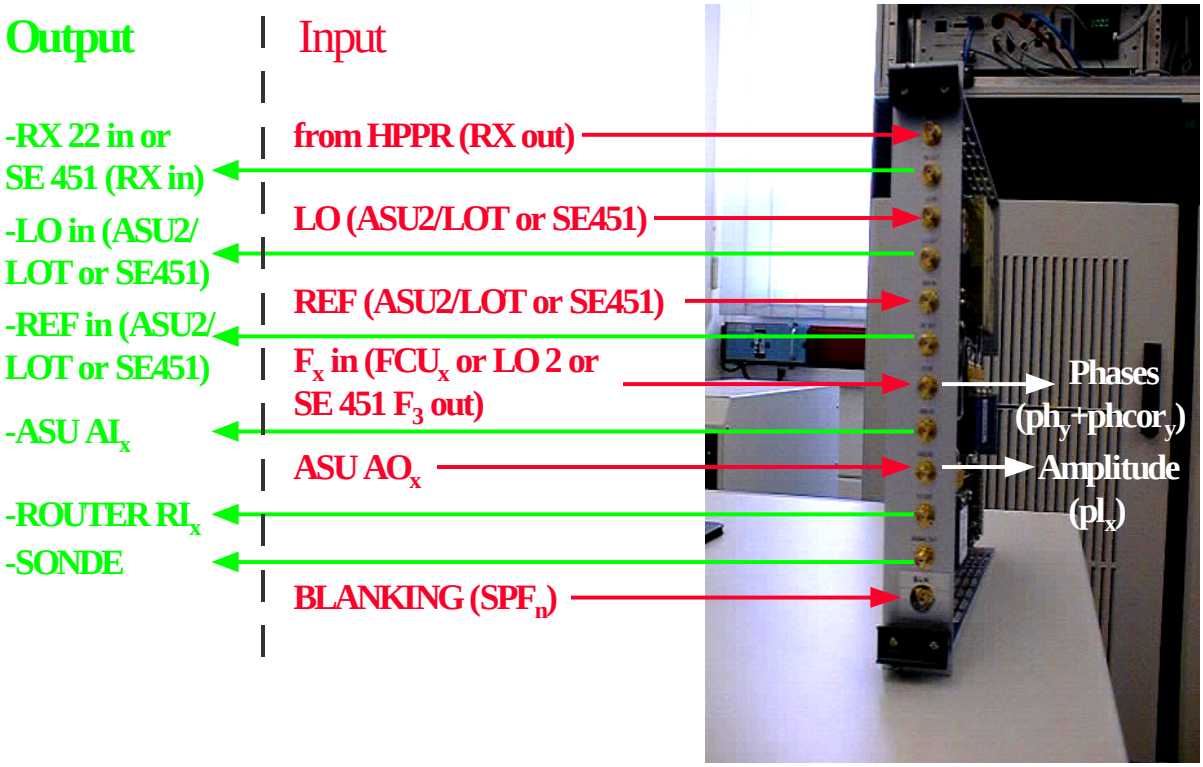
For DMX spectrometers, there are two additional modifications to perform. A ***3 channel SE 451 unit is absolutely necessary***. The SE 451 unit needs containing ***two TFX modules*** (e.g. a perfect phase coherence is necessary between the detection channel and the RDCU pulsing channel). ***A hardware modification of the SE 451 has to be done***. This modification is done by BRUKER France in Wissembourg.

On some spectrometers a upgrate of the FCU's used for detection and Radiation Damping pulsing is necessary. A clean working of the RDCU needs at least EC

1. *"(4) Paul. BROEKAERT and Jean JEENER J. Magn. Reson. Ser A, 113 (1995),60-64." on page 61*

Level 20. The system works on spectrometers equiped with lower EC Level FCU's but with the problem of the phase commutation between the two used channels.

Figure 1.3. Connection scheme of the Radiation Damping Control Unit to the rest of the spectrometer



As can be seen on ***Figure 1.3.*** the phase of the RDCU pulse is set by using the Xwinnmr phase setting commands for the channel connected to the RDCU. The amplitude of the FID sent back to the probehead is driven by the ASU connected to the RDCU board. As a consequence of the hardware conception, ***it is possible to use shape pulses to drive the RDCU board*** (e.g. we can sent back to the probehead a "shaped" FID).

We have used on our DMX system the blanking BLKTR 8 which was free (Word number 0 bit 7). The blanking is absolutely necessary otherwise no pulse comes out from the RDCU board.

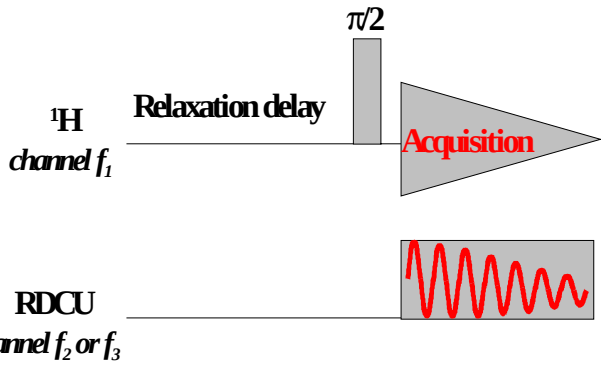
For more details about installation and troubleshouting, please refer to chapter ***"Installation and tests" on page 17***.

Calibration 2

RDCU pulse phase calibration 2.1

The aim of this part is to show how we can calibrate the phase of RDCU pulsing. For this experiment we use a sequence in which the RDCU is pulsing during the acquisition time.

Figure 2.1. RDCU pulsing during acquisition time



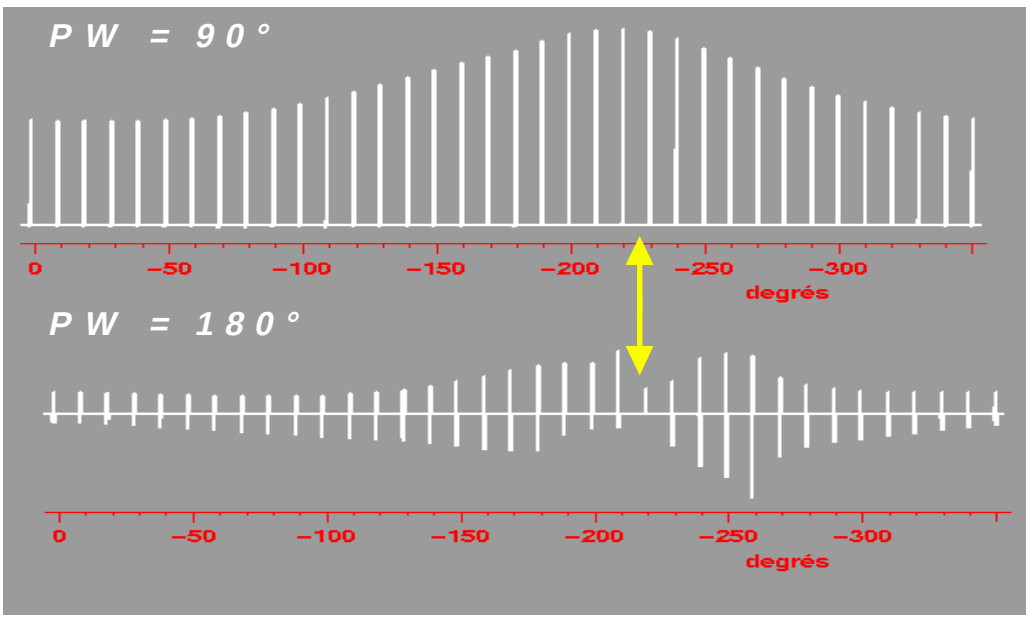
We show here the simplest sequence which can be used for this purpose. This sequence is used for RDCU pulse phase and amplitude calibration

Table 2.1. RDCU pulsing during acquisition time

```
;zg.ru
;AVANCE DMX version
;1D Radiation Damping suppression sequence
;using the RDCU and channels f1 and f3 of the DMX
spectrometer

"p18=aq"
"d12=20u"
"d15=1m"
"d17=4.0125u"

#include <Avance.incl>

1 ze
  d15 reset:f1
  d17 reset:f3
2 d1 do:f3
  d12 pl1:f1
  d12 pl3:f3
  p1:f1 ph1
  10u syrec - DRX version
  2u:f1 ph0
  2u adc ph31
  2u setnmr0|7 ;unblank RDCU transmitter
  p18:f3 ph2:r ;RDCU pulse on channel 3
  2u setnmr0^7 ;blank RDCU transmitter
  rcyc=2
  d15 do:f3
  wr #0
exit


ph1=0 1 2 3
ph31=0 1 2 3
ph2=0
ph0=0



;pl1 : high power level
;pl3 : power for RDCU pulsing
;p1 :  90 degree transmitter high power pulse
;p18 : RDCU pulse lenght
;d1 : relaxation delay; 1-5 * T1
```

The RDCU has to be connected on (in RDCU pulsing mode with the RDCU driving module). The filter width is choosen depending on the band width you choose. For RDCU pulsing on the water peak in a biological sample the best choice is the 1 kHz filter. Set the RDCU pulse power between 20 and 14 ASU dB typically at 400 - 500 MHz, and between 7 and -3 ASU dB at 800 MHz. Use the zg.ru pulse program and start a paropt by changing the phase correction of the RDCU pulse (phcor2) between 0 and 360° in steps of 10°. You will obtain the curve shown in ***Figure 2.2.***. The greatest peak of the curve gives the phase angle for which the feedback field phase has an opposite phase to the radiation damping field. The highest peak is obtained when radiation damping is compensated. Otherwise the smallest peak corresponds to radiation damping enhancement. Normaly you should found a difference of 180° between the highest and the smallest peak. Nevertheless if the RDCU pulse power is high enough in a manner that radiation damping is nearly suppressed or even "oversuppressed", the phase difference between the smallest and highest peak can be less than 180° (the phase curve is no more longer a pure sine function).

Figure 2.2. Profile of the RDCU phase calibration curve



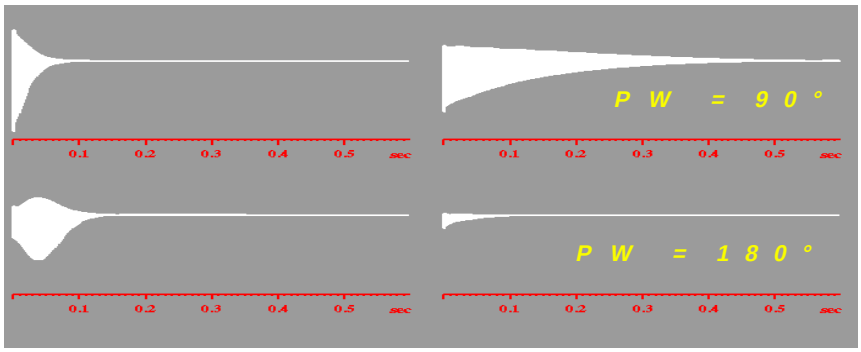
Profile of the RDCU phase calibration curve which should be obtained with the sequence shown in ***Figure 2.1.***, giving the peak intensity variation of the water peak in a 2 mM Sucrose sample (90/10 H2O/D2O) as a function of the phase correction applied to the feedback field. The top curve is obtained with a p/2 preparation pulse and the bottom curve with a p pulse. The yellow arrow marks the RDCU pulse phase correction for which radiation damping is suppressed.

Note : Choose as detection mode qsim or qseq. The DQD mode introduces a spike on the top of the water peak. This calibration can be done on the 2 mM sucrose sample in 90/10 H_2O/D_2O or on your own biological staff.

Fine tuning of the RDCU pulse phase *2.1.1*

The RDCU phase correction values are obtained at ±10° as shown on ***Figure 2.2.***. Sometimes a fine adjustement of the phase value has to be done. We make that in gs mode by using the pulse program of ***Figure 2.1.***. The RDCU phase correction value is manualy changed in steps of 1° and we observe the FID obtained. The phase angle is very well tuned when the FID at the water peak resonance is pure exponential as shown in ***Figure 2.3.***

Figure 2.3. FID obtained by recording proton spectra of 2 mM Sucrose in 90/10 H_2O/D_2O

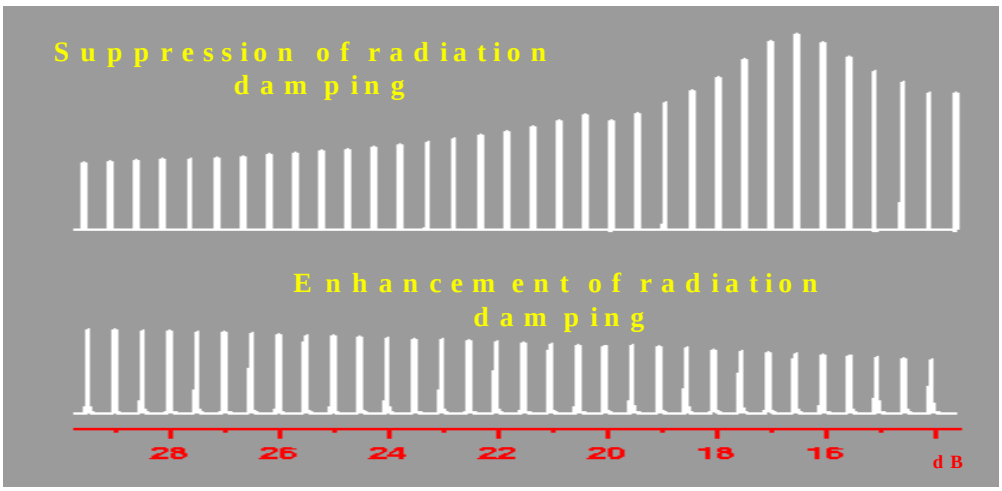


FID obtained by recording proton spectra of 2 mM Sucrose in 90/10 H2O/D2O with the r.f. offset equal to water Larmor frequency. Left : in presence of natural radiation damping - right : with radiation damping suppressed and phase correction properly adjusted after fine tuning.

RDCU pulse amplitude calibration *2.1.2*

The RDCU has to be connected on (in RDCU pulsing mode with the RDCU driving module). The filter width is choosen depending on the band width you choose. For RDCU pulsing on the water peak in a biological sample the best choice is the 1 kHz filter. Set the RDCU pulse phase to the value obtained after the fine tuning of step (see ***"Fine tuning of the RDCU pulse phase" on page 14***). Use the zg.ru pulse program and start a paropt by changing the power level of the RDCU pulse with the command pl3 from 40 to -6 dB. You will obtain a curve like the curve shown in ***Figure 2.4.***. In the same way you can record the power calibration curve for radiation damping enhancement (***Figure 2.4.***) by setting the RDCU pulse phase to the value obtained for the lowest intensity peak obtained in the phase calibration curve (see ***Figure 2.3.***). In the case of radiation damping compensation, the power calibration curve goes through a maximum (radiation damping suppressed) and then falls down. This can be explained by the fact that the feedback field amplitude become greater than the radiation damping field amplitude and we create a "new damping field" whose phase is opposite to the natural one.

Figure 2.4. Profile of the RDCU amplitude calibration curve obtained with the sequence shown in ***Figure 2.1.***



Top : The RDCU phase is set in manner to compensate radiation damping, bottom : The RDCU phase is set in manner to enhance radiation damping.

Note : At higher power levels depending on your spectrometer frequency, you may obtain a water peak superimposed with a high spike. This phenomenon is obtained when the feedback field amplitude is to strong regardless to the radiation damping field amplitude. The compensation system is oscillating. The maximum power which can be used should be taken just before the RDCU oscillates.

Installation and tests

3

The RDCU Package 3.1

The Radiation Damping Control Unit exists in 5 versions for AVANCE spectrometers having more then two channels, depending on the demodulation frequency (22 or 451 MHz) :

- 300-400 MHz for systems working with a 22 MHz intermediary frequency (RX 22)
- 300-400 MHz for systems working with a 451 MHz intermediary frequency (SE 451)
- 500-600 MHz for systems working with a 22 MHz intermediary frequency (RX 22)
- 500-600 MHz for systems working with a 451 MHz intermediary frequency (SE 451)
- 700-800 MHz for systems working with a 22 MHz intermediary frequency (RX 22)

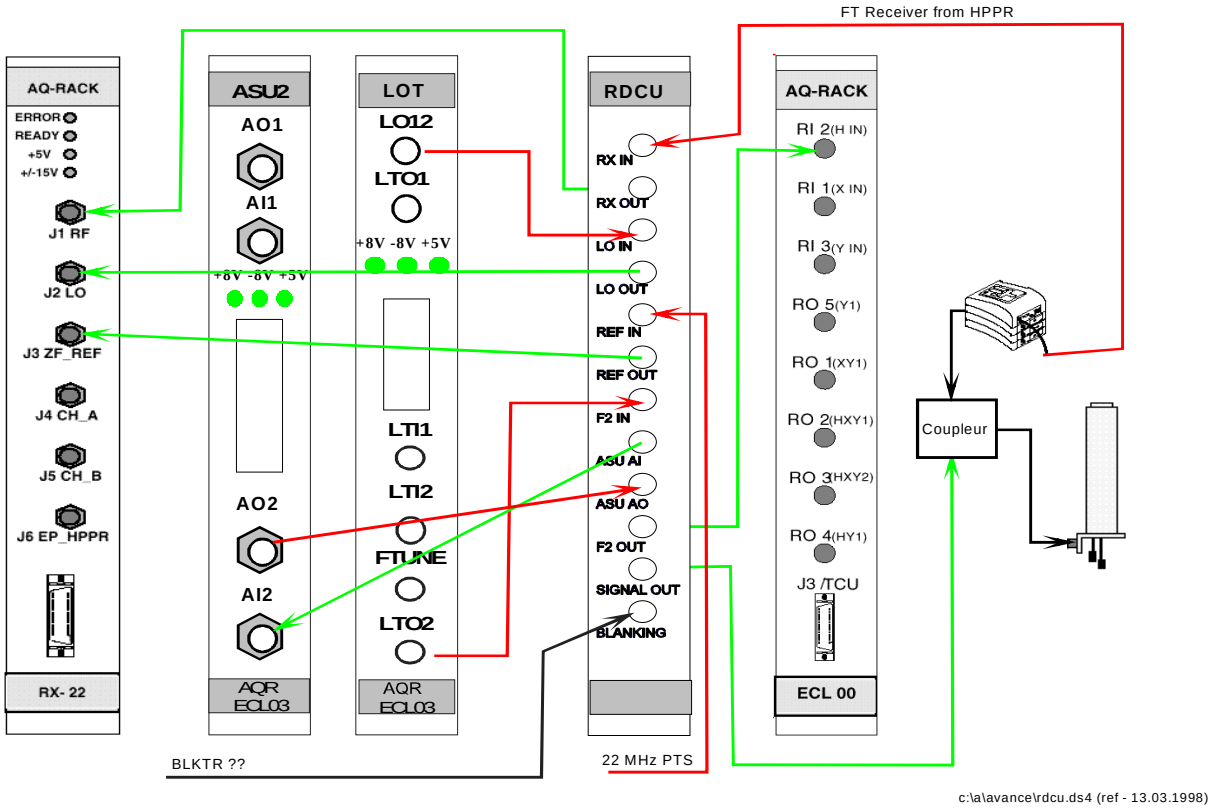
The RDCU package contains the **R**adiation **D**amping **C**ontrol **U**nit (**RDCU**) board, the cables needed for the connections, the directional coupler and the User's Manual. The RDCU board is inserted in the spectrometer in between the ASU's and the Router. For spectrometers working with a ***451 MHz intermediary frequency a three channel SE 451 is necessary***. This SE 451 unit needs containing ***2 TFX and 1 TFH modules***. Additionally ***the SE 451 has to be modified*** by BRUKER France.

Installation of the RDCU on RX22 type spectrometers 3.2

(e.g. : 3 channel DRX)

The connection scheme suited for RX 22 type spectrometers is shown in ***Figure 3.1.***. The following steps have to be executed to ensure a good installation.

Figure 3.1. Installation of the RDCU board on RX 22 type spectrometers



The connections drawn in red show RDCU board inputs whereas the connections drawn in green show RDCU board outputs. Use blanking BLKTR8 (nmr word0 bit 7 to drive the RDCU output blanking)

Step 1 : Put the AQR rack to off, and insert the RDCU board between the last ASU board and the first Router board. Put the AQR rack to on.

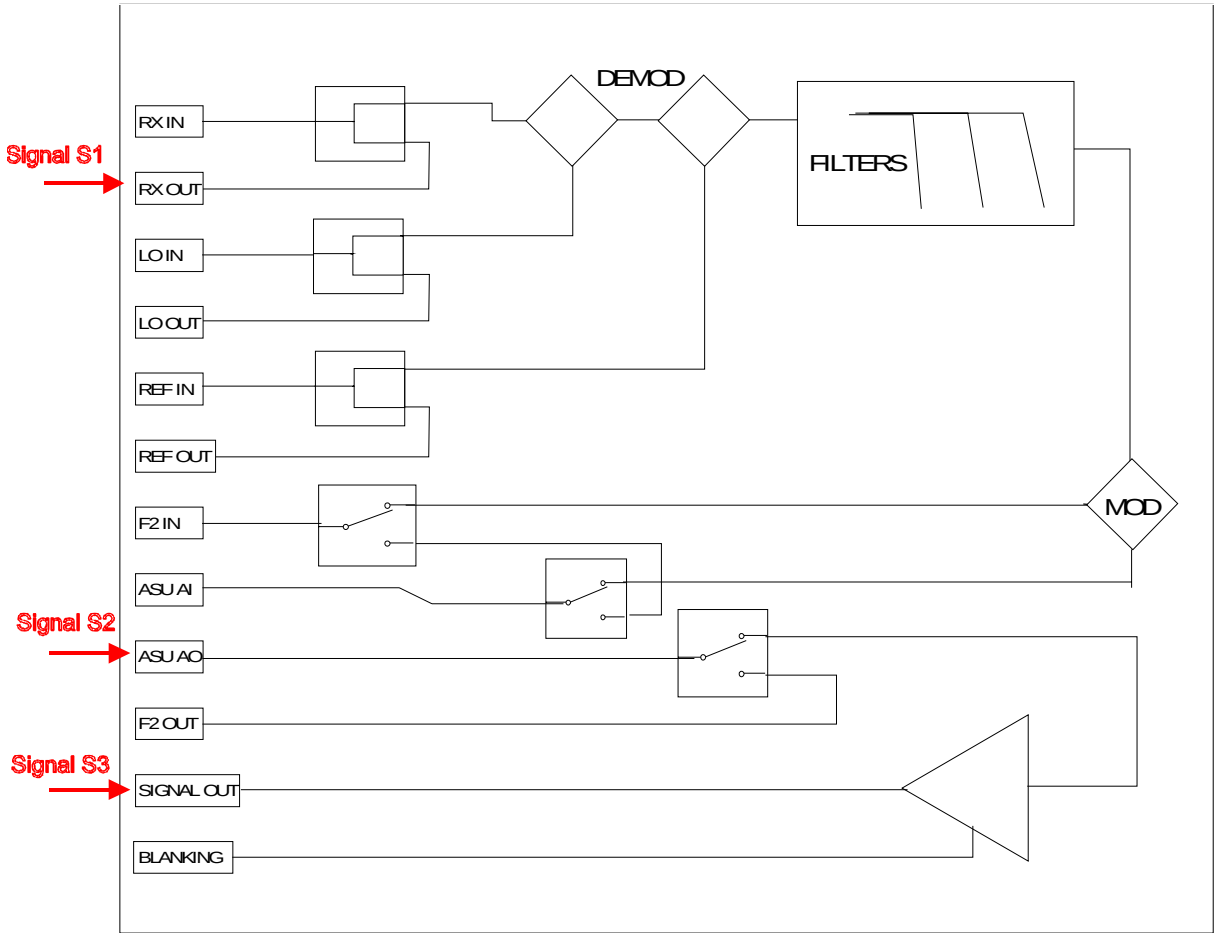
Step 2 : Connect the directional coupler between the preamplifier and the probe-head input.

Step 3 : Connect all the cables of the 5 inputs and the 6 outputs of the RDCU board as shown in ***Figure 3.1.***. Note that this connection scheme is only suitable for AVANCE DRX spectrometers. In the example shown in ***Figure 3.1.*** we use the channel f2 to drive the RDCU board, but it is possible to use all the other channels except channel f1 (default observation channel). For higher fields (e.g. 800 MHz) be careful in the choice of the RDCU driving channel that the frequency synthesizer is able to work at 800.13 MHz.

If the RDCU board is correctly connected to the rest of your spectrometer you can start working with your unit. The RDCU board is able to work in Radiation damping suppression/enhancing mode or in bypassed (on/off command via I2C bus). The on/off command is done by software with the RDCU driving module. In by-

passed mode, the RDCU board is switched off, but the Receiver output, the reference signal and the LO signal go through splitters located inside the RDCU board. Nevertheless the f_X signal is routed directly in the ASU Input (ASU AI_X) and from the ASU Output (ASU AO_X) to the router Input (Router 3/5 ROI_X). Four filters are built in the RDCU board (100 Hz, 1 kHz, 10 kHz and 60 kHz). This filters are used for Low frequency filtering in between signal demodulation and modulation (see ***Figure 3.2.***. The filter value is set by software in the RDCU driving module via the MHzI2C bus.

Figure 3.2. General diagram of the RDCU board.



The signals shown in red are measured in the general RDCU test procedure.

You are now able to start the amplitude and phase calibration procedure using a sample in water as described in chapter ***"Calibration" on page 11***.

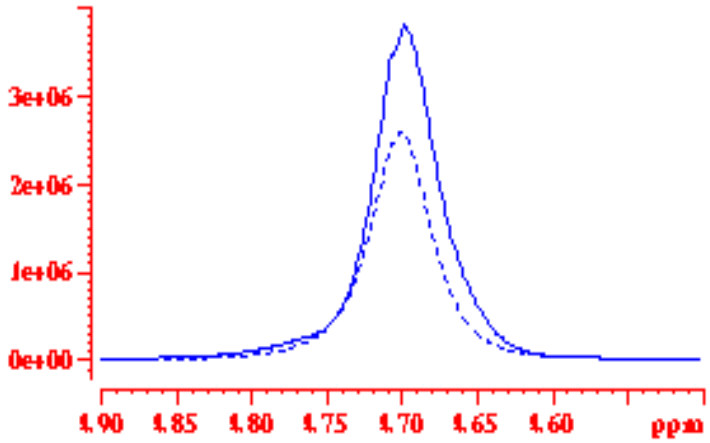
General RDCU test procedure 3.3

Signal to Noise ratio when RDCU enabled/disabled 3.3.1

In this section we describe some characteristics and some tests which can be performed on the RDCU board. This tests allow to control if the board works well and to check the way how the presence of the RDCU affects the general spectrometer performances.

- Test 1 : Checking of the signal obtained at **the RDCU RX output** (Signal S1 on ***Figure 3.2.***) after splitting of the native FID coming from the HPPR. The sample used is the 2 mM Sucrose sample in 90/10 (v/v) H_2O/D_2O. The FID is Fourier transformed and the peak height is compared to the peak height of the same experiment run in the same conditions without the splitter. To perform this test, you need to connect the RDCU RX in and RX out, as well as the RDCU RF in and RF out and the RDCU LO in and LO out together. With this connections you obtain the reference spectrum. Run a 1 scan **zg** experiment. Connect now the RDCU like shown in ***Figure 3.3.*** and repeat the experiment. The intensity ratio between the water peak obtained in both experiments should be $\sqrt{2}$ corresponding to the 3 dB insertion signal loss across the splitter. We found in our experiment a ***ratio of 1.472***.

Figure 3.3. ^1H spectrum of the water peak resonance

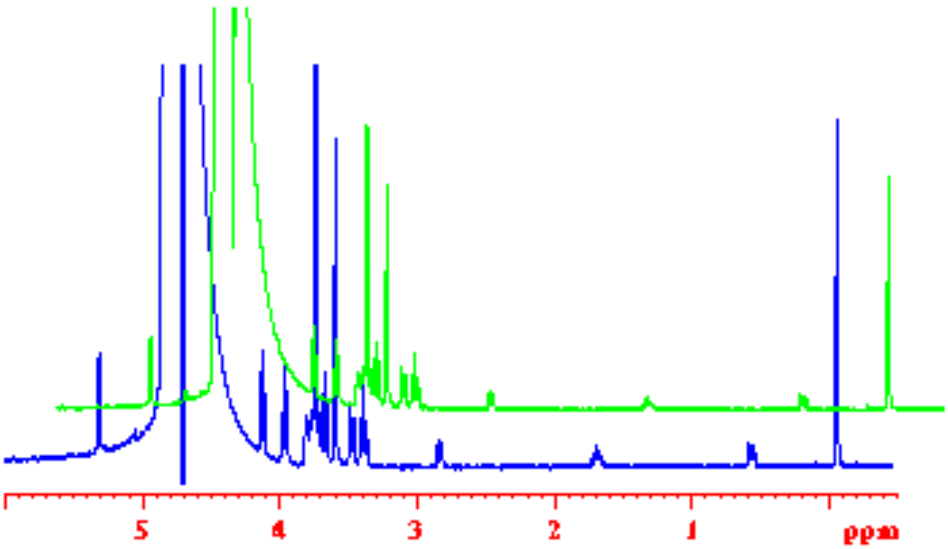


This spectrum was recorded with the single pulse program zg on the 2 mM Sucrose sample in H_2O. The straight line shows the peak obtained when the RX output from the HPPR is directly connected to the RX input of the receiver, the dotted line shows the peak obtained when the RX output signal from the HPPR is split inside of the RDCU before coming into the receiver input.

Test 1 can also be done with the oscilloscope. We measured at the HPPR output when the zg experiment is running on the 2 mM sucrose sample a peak/peak voltage V_{pp} = 320 mV for a noise level of 6.6 mV and at the RX output of the RDCU board (Signal S1) a peak/peak voltage V_{pp} = 212 mV for a noise level of 6.6 mV. This give us a signal ratio of 1.5.

- Test 2 : Checking of the Signal to noise ratio obtained before and after RDCU is inserted in the AQR rack. We make the same kind of experiment as in test 1 but this time we run a presaturation experiment **zgpr** on the 2 mM Sucrose sample in 90/10 (v/v) H_2O/D_2O. The intensity height of some peaks is compared and should be in the same ratio as in test 1. The Signal/Noise ratio should not show a bigger loss then 12 %. As shown in ***Figure 3.4.***, the original Signal/Noise ratio is found again when the receiver gain RG is multiplied by $\sqrt{2}$. As it was the case in test 1 we found the following ratio's : (sucrose anomeric proton ***at 5.3 ppm : 1.37*** and DSS methyl protons ***at 0 ppm : 1.47***). The Signal to Noise ratio drops ***from S/N = 251:1*** when RDCU is bypassed ***to S/N = 222:1*** when the signal is routed inside of the RDCU splitter with the same receiver gain RG = 256. If we now increase the receiver gain to RG = 512 and record the presaturation spectrum of the sucrose with the RX output of the HPPR routed to the receiver RX input across the RDCU splitter, ***the Signal to Noise ratio is again S/N = 252 :1*** even when the RDCU board is commuted on.

Figure 3.4. ^1H presaturation spectra recorded on the 2 mM Sucrose sample in 90/10 (v/v) H_2O/D_2O



The bottom trace (blue) shows the spectrum obtained when the RX output from the HPPR is directly connected to the RX input of the receiver, the top trace (green) shows the spectrum obtained when the RX output signal from the HPPR is split inside of the RDCU before coming into the receiver input.

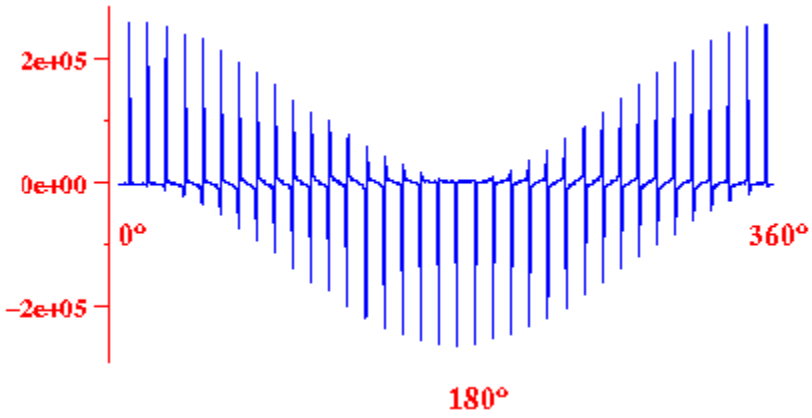
- Test 3 : This test allows to check the RDCU FID construction function, the phase modulation capacity of the RDCU board and if the ASU amplitude modulation works as desired. This test is done by running a single pulse ^{1}H ***zg.rd*** experiment over 1 scan on the 2 mM Sucrose sample in 90/10 (v/v) H_2O/D_2O. The RDCU is pulsing during the whole acquisition time in square pulse mode . The parameters used for this experiment are shown in ***Table 3.1.***. It is important to note that ***the offset of the receiver channel and the channel used for RDCU pulsing should be the same***. The Receiver RX input is connected on the ASU output (of course the ASU used for RDCU pulse amplitude driving) instead of the RDCU RX output or the HPPR RX output. Run a paropt in which the phase (***phcor2***) is changed in 10° steps starting at 0° up to 360° at a given power level pl3 (for example pl3 = 10 dB) but you can use all values between 30 and -6 dB. For higher attenuation (pl3 > 30 dB), the signal intensity became to low to be detected with a good S/N. You should obtain the curve of ***Figure 3.5.***. Run a paropt in which the attenuation (***pl3***) is changed in steps of -2 dB starting at 50 dB up to -6 dB. You should obtain the curve of ***Figure 3.6.***.

Table 3.1. Acquisition parameter table used for running the zg.pr experiment for Test 3

F2 - Acquisition Parameters

Date_	980310	Time	16.58
INSTRUM	spect	PROBHD	5 mm BBI inverse X-Y-Z-Grad
PULPROG	zg.rd	AQ_mod	qsim
TD	16384	SOLVENT	H2O
NS	1	DS	0
SWH	6983.240 Hz	FIDRES	0.426223 Hz
FW	20000.00 Hz	AQ	1.1731443 sec
RG	4	DW	71.600 usec
DWOV	71.600 usec	DECIM	1
DIGTYP	HADC	DIGMOD	analog
DR	16	DDR	0
DE	102.29 usec	TE	300.0 K
p18	1173134.38 usec	d12	0.00002000 sec
d15	0.00100000 sec	D17	0.00000403 sec
D1	2.00000000 sec	PL1	0.00 dB
PL3	12.00 dB	P1	6.60 usec
SFO1	400.1318797 MHz	NUC1	1H
SFO3	400.1318797 MHz	NUC3	1H

Figure 3.5. RDCU pulse phase driving test

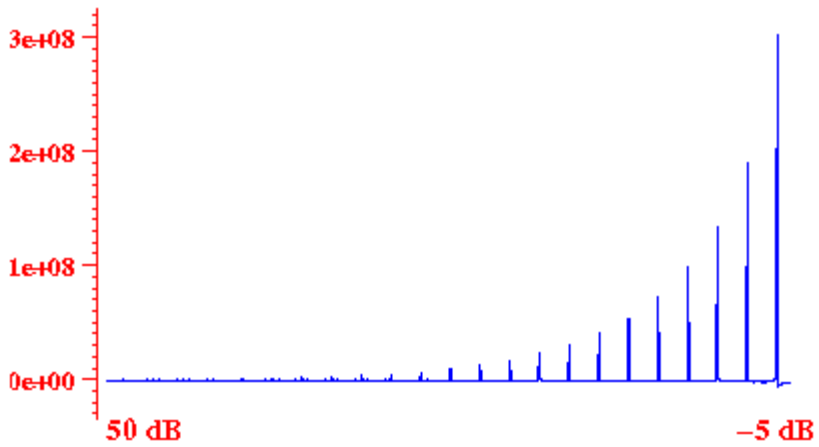


The phase of the Signal measured at the ASU output is a function of the phase of the fx input signal coming from the synthesizer and driven with the command ph2 + ph2cor. The phase ph2 is set to 0 in the zg.rd pulse program. In this experiment phcor2 changes in steps of 10° starting at 0° up to 360°.

If you obtain the data shown in ***Figure 3.5.*** and ***Figure 3.6.***, you may be sure that the demodulation/ modulation, phase driving and attenuation ability of the RDCU board are all working.

This test can also be done ***by measuring the RDCU board Signal2 output voltage*** with the oscilloscope. The peak/peak voltage values found in the case of a 300-400 RDCU board are given as an example in ***Table 3.2.***. In this table we have summarized the measured voltage V_{pp} of the ASU Output voltage (Signal 2) as function of the ASU attenuation pl3.

Figure 3.6. RDCU amplitude modulation test



*The amplitude of the Signal measured at the ASU output is a function of attenuation set with the ASU on the RDCU reconstructed signal. The phase is set to ph2 + phcor2 = 0 whereas the attenuation (**driven with the pl3 command**) changes in steps of -2.5 dB starting at 50 dB down to -5 dB.*

Table 3.2. Peak/peak Voltage measured at the ASU output (RDCU Signal S2) as function of the ASU attenuation set with the command pl3

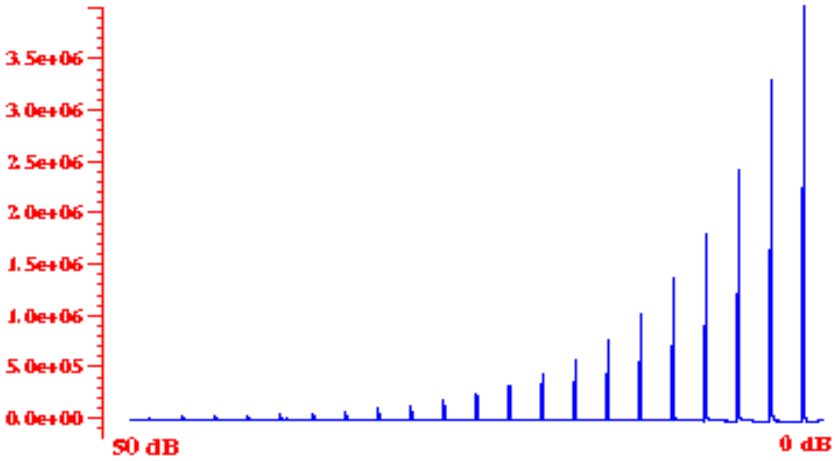
ASU attenuation (pl3)	ASU attenuation (pl3)	ASU attenuation (pl3)	Voltage in mV
-6.0	316.0	12.5	24.4
-5.0	241.6	15.0	18.5
-2.5	152.0	17.5	13.7
0.0	108.1	20.0	10.6
2.5	78.6	22.5	7.7
5.0	58.5	25.0	5.6
7.5	43.5	27.5	4.2
10.0	30.4	30.0	3.1

RDCU board amplifier test *3.3.3*

- Test 4 : This test is the same as the amplitude test described in Test 3. This test is done by running a single ^{1}H ***zg.rd*** experiment over 1 scan on the 2 mM Sucrose sample in 90/10 (v/v) H_2O/D_2O. The RDCU is pulsing during the whole acquisition time in square pulse mode using the parameters given in ***Table 3.1.***. The Receiver RX input is connected on the RDCU output (Signal S3 which is connected to the directional coupler for normal use) instead of the RDCU RX output or the HPPR RX output. Run a paropt in which the ASU attenuation (pl3) changes in steps of -2 dB starting at 50 dB up to -6 dB. You should obtain the curve of ***Figure 3.7.***. The peak/peak voltage values summarized in ***Table 3.3.*** give the oscilloscope measured voltages when the RDCU output is connected to the oscilloscope.

Note that the output signal should be amplified from about 25 dBm against the voltage measured for Signal S2. The S2 Signal discussed in section ***"RDCU board phase and amplitude test" on page 22*** has a peak/peak voltage lower then 1Vpp so the signal can be injected in the Receiver circuit without attenuation (RG = 4 and no attenuator between the ASU output and the receiver RX input). The Signal S3 is much more intense and can be observed only by decreasing the receiver gain (RG = 1 and no attenuator between the ASU output and the receiver RX input). The receiver is saturated for ASU attenuation lower then 0.0 dB. To be able to observe the calibration curve between 50 and -6 dB, a 20 dB attenuator has to be inserted in between the RDCU output and the Receiver RX input.

Figure 3.7. RDCU amplitude modulation test at RDCU output after Signal S2 amplification -> Signal S3



*The amplitude of the Signal measured at the ASU output is a function of attenuation set with the ASU on the RDCU reconstructed signal. The phase is set to ph2 + phcor2 = 0 whereas the attenuation (**driven with the pl3 command**) changes in steps of -2.5 dB starting at 50 dB down to 0 dB.*

This test checks if the RDCU output amplifier is properly working and gives an idea of the voltage sent back to the probehead taken into account that the directional coupler has a 27 dB insertion factor (see ***Table 3.3.***).

Table 3.3. Peak/peak Voltage measured at the RDCU output (RDCU Signal S3) as function of the ASU attenuation set with the command pl3

Att. in dB (pl3)	Voltage (mV)	Amp. (dB)	Att. In dB (pl3)	Voltage (mv)	Amp. (dB)
0.0	1966.0*	18.2	20.0	195.4	25.3
2.5	1445.0*	22.9	22.5	144.1	25.4
5.0	1065.0	25.2	25.0	106.2	25.5
7.5	793.7	25.3	27.5	79.9	25.6
10.0	600.0	25.9	30.0	60.2	25.8
12.5	444.3	25.3	32.5	45.5	-
15.0	341.2	25.3	35.0	34.7	-
17.5	252.2	25.3	37.5	26.2	-

The column Amp (dB) gives the amplification of signal S3 against signal S2 in dB.
(*) Saturation occurs during the measurement of this two values.

- Test 5 : This test allows to check in four single experiments the amplitude behavior of the signal from the RDCU input to the RDCU output. We use the 2 mM Sucrose sample in 90/10 (v/v) H_2O/D_2O. The following four experiments have to be recorded :

  - Experiment 1 : Single pulse (***zg***) experiment on the water peak in 1 scan using the parameters given in ***Table 3.1.***. Connect the HPPR output on the Receiver (RX22 or SE451) input as is the case when no RDCU is installed. The peak intensity gives the reference intensity I_{init} shown in ***Figure 3.8.***

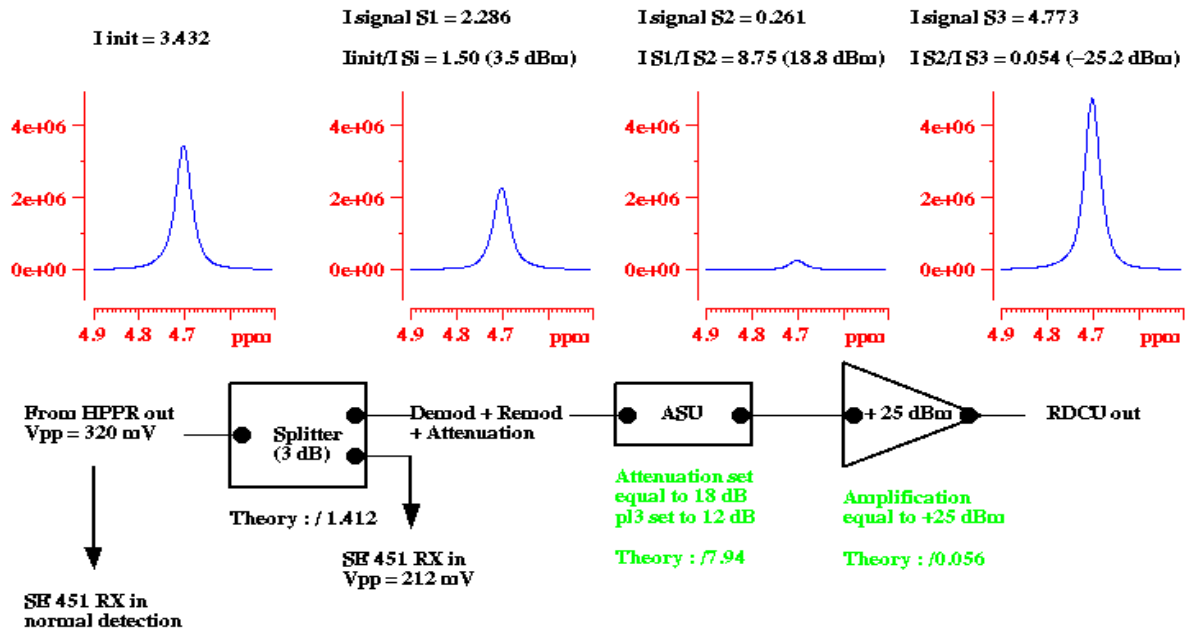
  - Experiment 2 : Single pulse experiment with RDCU pulsing during the acquisition time in square pulse mode (***zg.rd***). Connect the RDCU RX output on the Receiver (RX22 or SE451) input. The peak intensity gives the Signal S1 intensity. You should found $3 \leq I_{init}/I_{S1} \leq 4$ dB.

  - Experiment 3 : Run the same experiment as experiment 2 but this time connect the ASU output on the Receiver (RX22 or SE451) input. The peak intensity gives the Signal S2 intensity. You should found that I_{S1}/I_{S2} = (pl3 ± 1) dB.

  - Experiment 4 : Run the same experiment as experiment 2 but this time connect the RDCU output on the Receiver (RX22 or SE451) input. The peak intensity gives the Signal S3 intensity. You should found that I_{S2}/I_{S3} = (-25 ± 2) dB.

Note that the phase value used when the zg.rd experiment is running has in this test no importance. The test result is independent from the ph2 + phcor2 phase value. The receiver gain has to be set to the maximal RG as determinate with the rga command on experiment 1 and should be the same for all the four experiments. To avoid saturation effects in experiment 4, please use ASU attenuation values in a manner that pl3 > 10 dB.

Figure 3.8. Results obtained when the experiments 1 to 4 are recorded



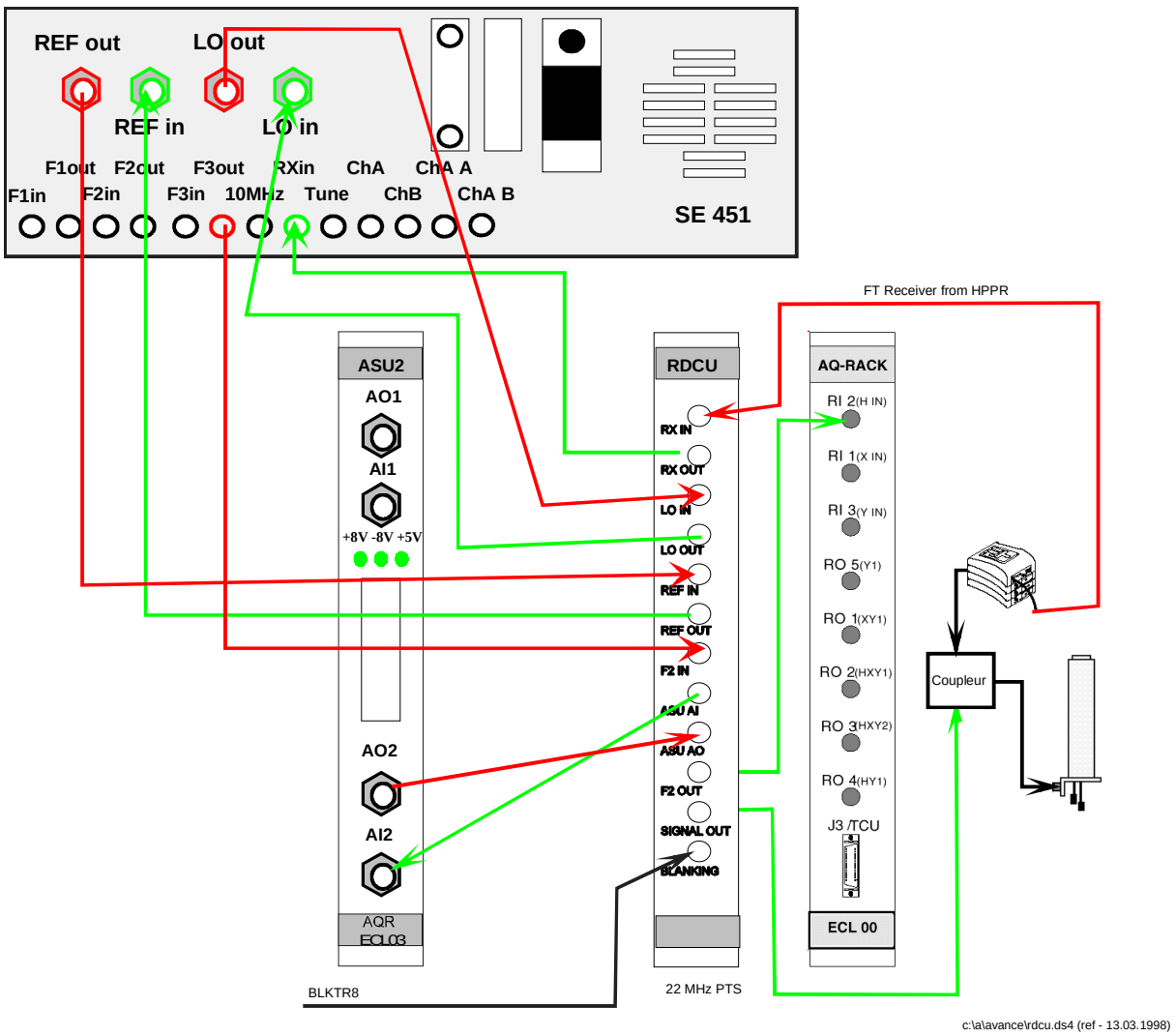
Note that we have used a phase of 0° and an ASU attenuation of 12 dB when the zg.rd pulse program was used. The receiver gain was set to RG = 4 for all experiments. The peak/peak Voltage values have been measured during the experiments 1 and 2 by connecting the HPPR output and the RDCU RX output on the oscilloscope.

Installation of the RDCU on SE451 type spectrometers 3.4

(e.g. : 3 channel DMX)

The connection scheme suited for SE451 type spectrometers is shown in ***Figure 3.9.***. The following steps have to be executed to ensure a good installation.

Figure 3.9. Scheme showing the installation of the RDCU board on SE451 type spectrometers



The connections drawn in red show RDCU board inputs whereas the connections drawn in green show RDCU board outputs. Use blanking BLKTR8 (nmr word0 bit 7 to drive the RDCU output blanking).

RDCU software control 3.5

The Radiation Damping Control Unit pulsing is controlled by the **pl** and **phcor** XWIN-NMR spectrometer control functions as described elsewhere. Two additional features are software driven namely the **RDCU on/off** switch and **the choice of the low frequency filter bandwidth**. This commands are executed via the I2C bus. The RDCU on/off and the RDCU filter bandwidth choice can be software set with **XWINNMR versions starting from version 2.5**. Otherwise this two later functions have to be set directly on the hardware.

RDCU on/off switch on the hardware 3.5.1

Allows to switch the RDCU between the **on** mode (used for RDCU pulsing) and the **off** mode (which allows to use the RDCU driving channel like a normal spectrometer channel). When the RDCU board is switched in off mode, the channel used to drive the RDCU board can be used as normal pulsing channel. We can show that the isolation obtained between the "RDCU out" and the "F2 out" outputs of the RDCU board when the RDCU output switch is set to off, is higher than 50 dB as shown in ***Figure 3.10.***. The switch is driven by **jumper JP 3**. The RDCU is switched to on (byte 1) when the jumper is set. The RDCU is switched to off (byte 0) when the jumper is not set.

RDCU filter setting on the hardware 3.5.2

The choice of the low frequency filter bandwidth can be directly done on the hardware on the **jumpers JP 1 and JP 2**. Four combination are available to select 100 Hz, 1 kHz, 10 kHz or 60 kHz broad band filter widths. The following table shows the correlation between jumper settings and filter bandwidth.

Table 3.4. Correlation between jumper settings and filter bandwidth

JP 1	JP 2	JP 1*	JP 2*	Filter Width
set	set	1	1	100 Hz
set	not set	1	0	1000 Hz
not set	set	0	1	1000 Hz
not set	not set	0	0	broad band

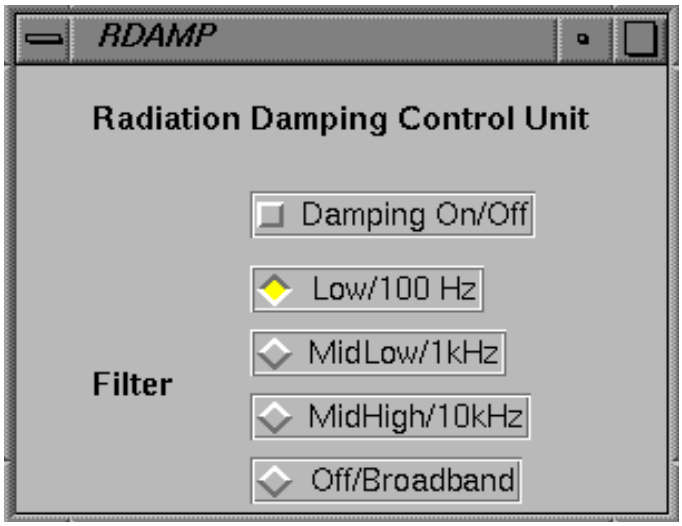
* Binary value associated to the jumper position.

The RDCU board driving software 3.5.3

The RDCU board driving module should be introduced with the **XWINNMR 2.5** software version. The module is started by entering in the XWINNMR window the command **rdcu** on the keyboard. The module (see ***Figure 3.10.***) is displayed on the screen. The RDCU board on/off function is operated with a toggle button as well as the choice of the low frequency filter bandwidth. If an ACB error message is displayed (means that the RDCU board is not recognized by the ACB software),

you can drive the RDCU board in an other way (see ***"Installation of the RDCU on SE451 type spectrometers" on page 28***). Probably the RDCU board BBIS is empty or there is a problem with the information contained in the BBIS eprom.

*Figure 3.10. RDCU driving module window as it is installed on **version 2.5 of XWINNMR**.*



RDCU software trouble shooting *3.5.4*

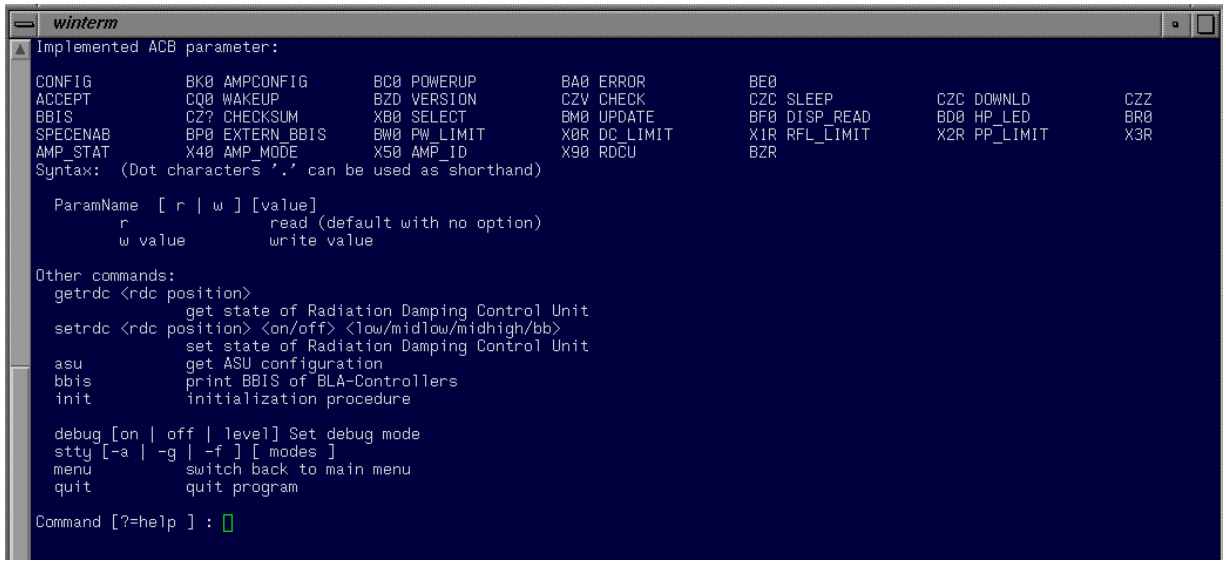
It is possible to drive the RDCU board on/off function as well as the low frequency filter bandwidth outside of the XWINNMR software. The following steps have to be executed in a ***Winterm*** :

step 1: Enter in a ***Winterm*** window,

step 2: Enter the command line ***cd /<XWINNMRHOME>/prog/bin/scripts*** with XWINNMRHOME
= u if your XWINNMR software is installed on the disk u,

step 3: Enter the command line ***rstest*** and select the option ***8*** corresponding to the ACB. You should obtain the display shown in ***Figure 3.11.*** giving all the functions available in this module.

Figure 3.11. Display of the command lines of use for the RDCU board tests and driving



step 4: Three command lines may be used for the RDCU tests and setting :

asu : This command displays a list of all the boards detected in the AQR rack as well as their position counted from right to left starting at the ACB board. The first position at the left of the ACB board is found at the position 0. The RDCU board may be found at positions 4 or 5.

getrdc <rdcu position> : This command displays the actual status of the RDCU board on/off switch as well as the actual value of the low frequency filter band-width. The system displays on the screen the following answer :

correction = off - filter = low if the RDCU board is switched off and the last filter width choice was 100 Hz.

setrdc <rdcu position> <on/off> <low/midlow/midhigh/bb> : This command line allows to switch the RDCU board on/off and to choose the filter band width. For exemple if the RDCU board (located at position 4 in the AQR rack) should be switched on and the low frequency filter bandwidth should be set to 1 kHz you have to introduce on the keyboard the command line :

setrdc 4 on midlow

step 5: Before coming back to XWINNMR enter the command line **quit** to leave the program.

Topics on RDCU pulsing

4

In this part we present how the Radiation Damping Control Unit can be used everywhere in a pulse sequence as well as the different pulse modes that can be used.

RDCU pulsing during non acquisition delays 4.1

Basic pulse program sequence 4.1.1

RDCU pulsing can be done everywhere in a pulse sequence. Nevertheless, we have to consider two cases for the use of the RDCU : pulsing during acquisition delays and elsewhere in the pulse sequence. The programation of the RDCU driving sequence as well as the RDCU phase behaviour is slightly different in each case. The basic pulse program for RDCU pulsing in non acquisition delays is shown in ***Table 4.1.*** for AVANCE DMX and DRX spectrometers. This basic part can be used during every delay in your pulse sequence if you need it. You can find examples of RDCU pulsing in non acquisition periods in part ***"RDCU on aqueous samples" on page 41***.

Table 4.1. Basic pulse program sequence for RDCU pulsing in non acquisition periods written for AVANCE DMX and DRX spectrometers.

"d15=1m" "d17=4.0125u" 1 ze d15 reset:f1 d17 reset:f3 ;necessary for phase coherence	**..10u syrec ; AVANCE DRX** 2u setnmr0|7 ;unblank RDCU transmitter p18:f3:e ph6:r ;RDCU pulse on channel 3 2u setnmr0^7 ;blank RDCU transmitter **10u sytra ; AVANCE DRX**

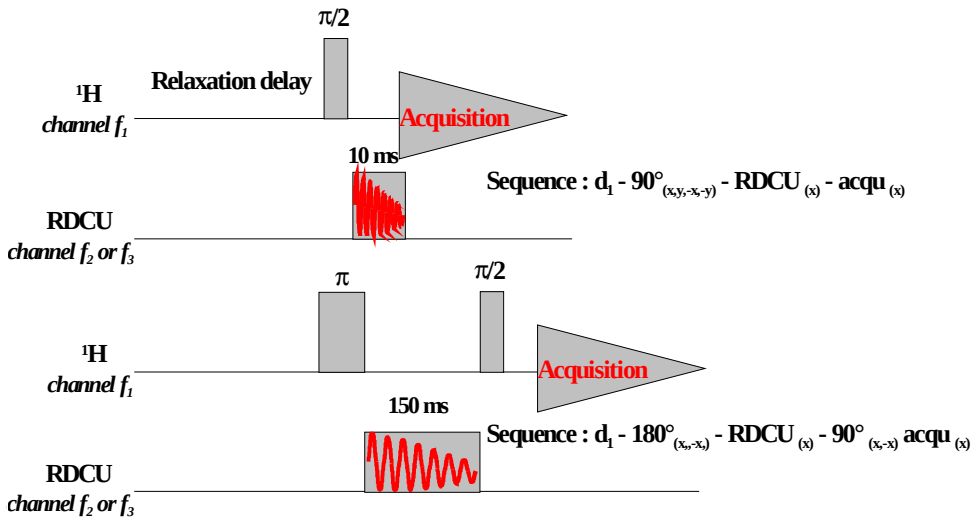
This sequence corresponds to 5 programmation lines for an AVANCE DMX and 3 programmation lines for an AVNACE DMX. The phase cycling is studied in part ***"RDCU pulse phase cycling" on page 34***

It is possible to replace the RDCU square pulse by a shape pulse, by replacing, in the pulse program sequence of ***Table 4.1.***, the line ***(p18:f3:e ph6:r)*** by the line ***(p18:sp1:f3:e ph6:r)*** to avoid sinc effects especially if the RDCU pulsing period is short (< 1 ms). In this way ***the RDCU pulse amplitude calibration has to be done again*** because for non square pulses sp1 > pl3.

RDCU pulse phase cycling *4.1.2*

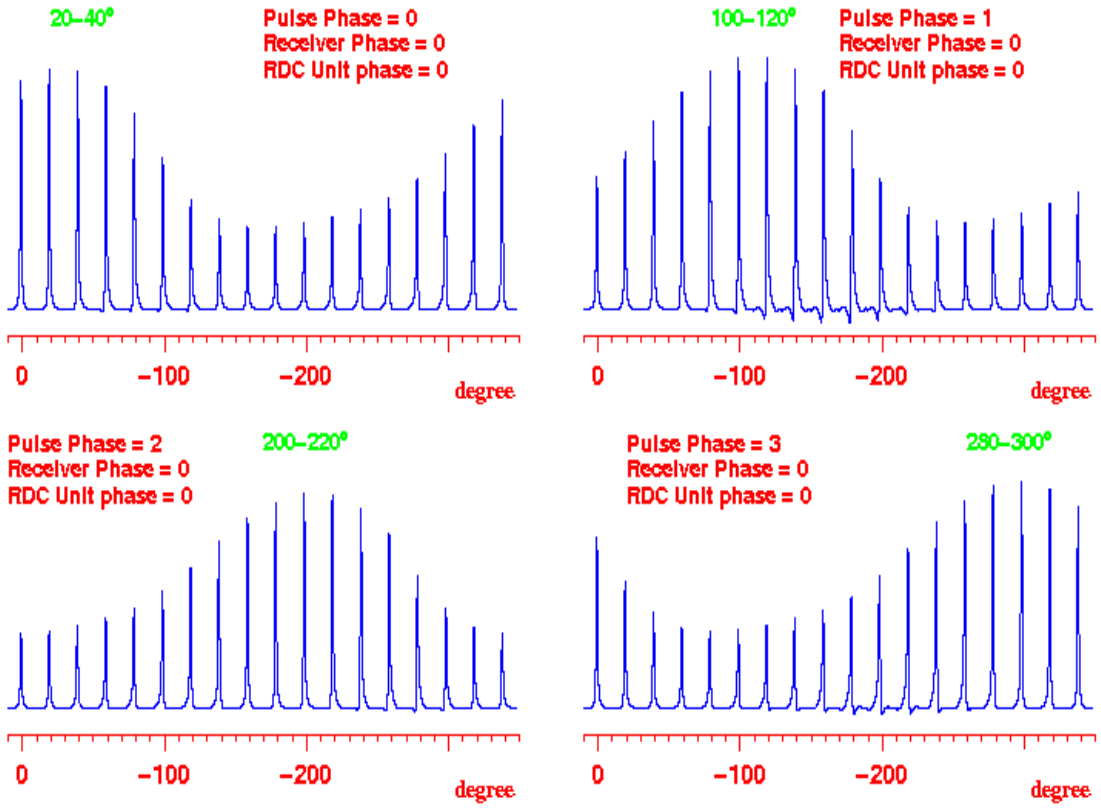
As should be expected for a phenomenon which is generated by precessing magnetization, the RDCU pulse phase (e.g. feedback field phase) is depending on the phase of the preparation pulse. Thus ***the phase cycling of the RDCU pulse should be derived from the phase of the transverse magnetization before RDCU pulsing***. The variation of the RDCU pulse phase against the preparation pulse is shown in ***Figure 4.2.*** and ***Figure 4.3.*** in the case of a π/2 and π pulse applied on the water protons of a sample containing 2 mM Sucrose in 90/10 H_2O/D_2O. The two **figures** below have been obtained with the following pulse sequences **(*Figure 4.1.*)**. In the case of the π/2 pulse the RDCU pulse lenght was 10 ms (to limitate signal loss as a consequence of T_2^* effects) and in the case of the π pulse the RDCU pulse lenght was 150 ms.

Figure 4.1. Pulse sequences used for the RDCU pulse phase cycling test as describen in the text



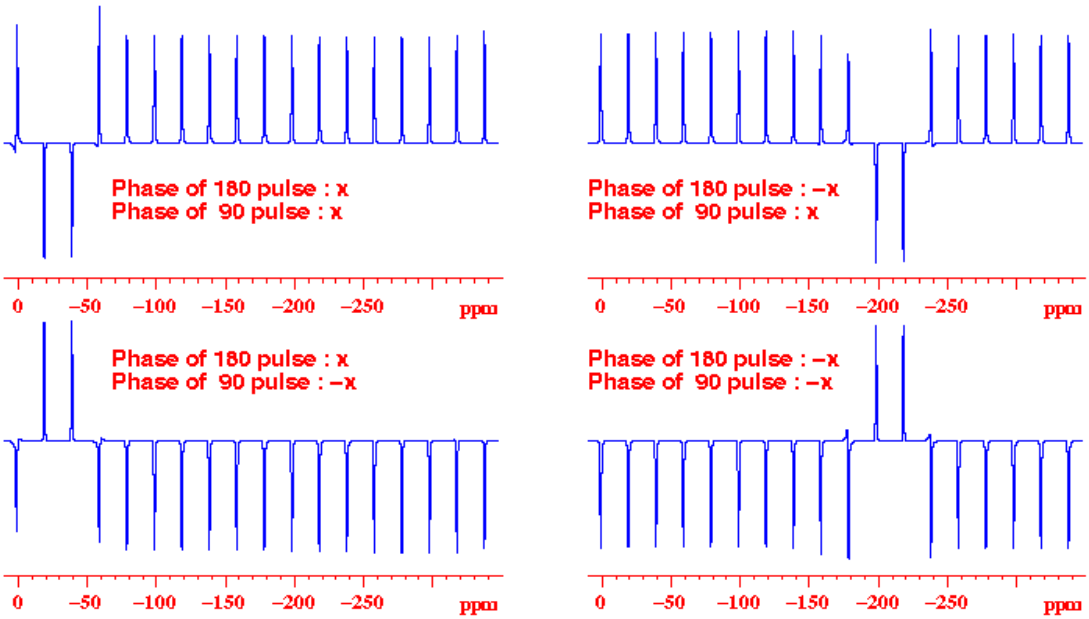
Top : the preparation pulse is a π/2 pulse,
Bottom : the preparation pulse is a π pulse.

Figure 4.2. RDCU pulse phase variation as a function of the phase of the 90° preparation pulse



During this experience, the RDCU and receiver phase are constant whereas the π/2 preparation pulse phase is changed (phase = 0, 1, 2, 3). Note that the phase correction of the RDCU pulse for which radiation damping is suppressed changes with the phase of the π/2 pulse (RDCU phase = 20-40°, 100-120°, 200-220°, 280-300°).

Figure 4.3. RDCU pulse phase variation as a function of the phase of the 180° preparation pulse



During this experience, the RDCU and receiver phase are constant whereas the π preparation pulse phase is changed (phase = 0, 2). Note that the phase correction of the RDCU pulse for which radiation damping is suppressed changes with the phase of the π pulse (RDCU phase = 20-40°, 200-220°).

RDCU pulsing during acquisition delays 4.2

The basic RDCU pulse sequence 4.2.1

The basic sequence with RDCU pulsing during acquisition is shown, discussed and used for RDCU pulse phase and amplitude calibration. This basic sequence, nevertheless, has an owesome drawback. As far as we are pulsing during the acquisition delay, the signal/noise ratio drops down from a factor 3 to 4 againts the signal to noise ratio obtained without RDCU pulsing. Such a sequence can not be used for other topics than RDCU pulse phase and amplitude calibration.

We have tested RDCU square pulsing on the 1,-1 Jump and return water suppression sequence (which is the same kind of sequence as shown in ***Figure 2.1.*** with the preparation $\pi/2$ pulse replaced by $\pi/2_{(x)} - \tau - \pi/2_{(-x)}$) by comparing S/N ratios between the experiment with and without RDCU pulsing during acquisition at power level pl3 = 120 dB (RDCU is pulsing but the feedback filed amplitude = noise). We obtain the values summerized in ***Table 4.2.***

Table 4.2. *S*ignal/Noise ratios as obtained with pulse sequences p11 and p11.ru

Pulse programme	RDCU pulsing	sino H2O	sino (Acétate : 2.91ppm)
p11	no	3987:1	300:1
p11.ru	yes	2012:1	122:1

We use the 2 mM Lysosyme sample in 90/10 H2O/D2O for both experiments.

Time sharing RDCU pulsing 4.2.2

As far as the problem is the same as with the homodecoupling sequence we have tested RDCU pulsing in time sharing mode by changing the pulse program of ***Figure 2.1.*** by the following pulse program (***Figure 4.4.***). The RDCU pulse is applied during 33% of the dwell time in between two acquired data points.

Figure 4.4. Pulse sequence with RDCU square pulsing during acquisition in time sharing mode

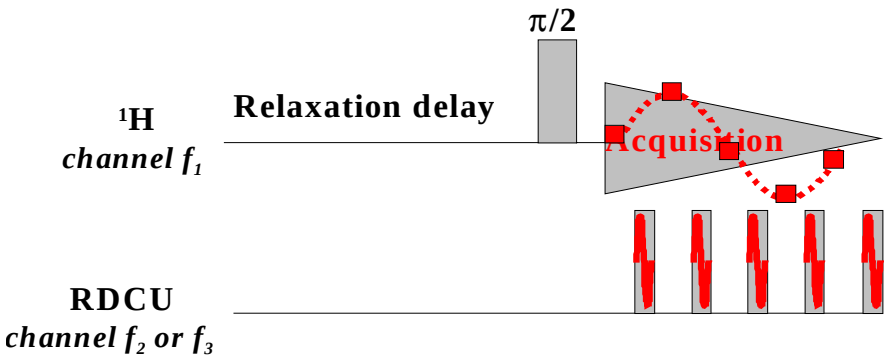


Table 4.3. Pulse sequence with RDCU square pulsing during acquisition in time sharing mode

```
;zgadc.ru
;AVANCE DMX version
;1D RDC Unit pulse phase calibration sequence
;using the RDCU and channels f1 and f3 of the DMX
;spectrometer using time shared RDCU square pulses
;during acquisition
;Ph.LUX (S.a.d.i.s. R&D 971117)

define loopcounter count
"d12=20u"
"d15=1m"
"count=td"
"p0=1u"
"p18=2*dw-d18-d19-8u-p0"
"d17=4.0125u"
"d19=5u"

#include <Avance.incl>

1 ze
  d15 reset:f1
  d17 reset:f3
2 d1 do:f3
  d12 pl1:f1
  d12 pl3:f3                ;RDCU on channel 3 pl3 and
ph2
  p1:f1 ph1
  de1
  de2
  2u:f1 ph0
  10u syrec                 ;AVANCE DRX spectrometers
  2u adc ph31
3 p0:x                      ;external trigger point recording
  d19 setnmr0|7             ;unblank RDCU transmitter
  p18:f3 ph2:r              ;RDCU time shared square pulse (=
                            ;33% of the time between 2 points)
  8u setnmr0^7              ;blank RDCU transmitter
  d18
  lo to 3 times count
  rcyc=2
  d15 do:f3
  wr #0
exit

ph1=0 1 2 3
ph31=0 1 2 3
ph2=0
ph0=0

;pl1: transmitter high power level
;pl3: RDCU power level
;p1 : transmitter high power pulse
;p18: RDC Unit pulse lenght
;d19: short delay for RDCU blanking set
;d18; Time sharing delay to avoid S/N loss
;d1 : relaxation delay; 1-5 * T1
;digmod = analog. and  aqmod = qsim
```

We have tested RDCU square pulsing on the 1,-1 Jump and return water suppression sequence (which is the same kind of sequence as shown in ***Figure 4.4.*** with the preparation $\pi/2$ pulse replaced by $\pi/2_{(x)} - \tau - \pi/2_{(-x)}$) by comparing S/N ratios between the experiment with and without RDCU pulsing during acquisition at power level pl3 = 120 dB (RDCU is pulsing but the feedback filed amplitude = noise). We obtain the values summarized in ***Table 4.4.***

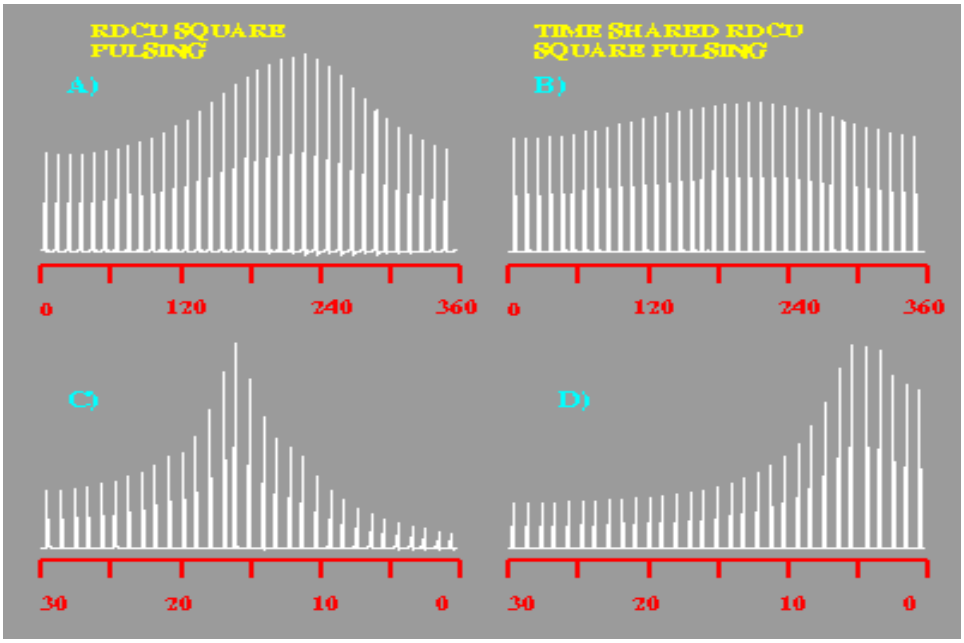
Table 4.4. Signal/Noise ratios as abtained with pulse sequences p11, p11.ru and p11adc.ru

Pulse programme	RDCU pulsing	sino H2O	sino (Acétate : 2.91 ppm)
p11	no	3987:1	300:1
p11.ru	yes	2012:1	122:1
p11adc.ru	yes	3569:1	303:1

We use the 2mM Lysosyme sample in 90/10 H2O/D2O for both experiments

When time sharing RDCU square pulsing mode is used, it is clear that the RDCU pulse correction phase curve is the same as if normal square pulses are used. You will obtain the same curves as shown in ***Figure 2.4.***. Nevertheless the RDCU pulse amplitude should no longer the same. To obtain complete radiation damping suppression, the used ASU gain has to be increased from about 12 dB (at 400 MHz you need to change the power level from 16.0 to 4.0 dB). The RDCU pulse power calibration curves obained with RDCU square and RDCU time shared square pulses are shown in ***Figure 4.5.***

Figure 4.5. RDCU pulse phase and amplitude calibration using square and time shared square RDCU pulsing on an AVANCE 400 MHz



A) RDCU phase calibration using a square pulse at 22 dB, B) RDCU phase calibration using a time shared square pulse at 22 dB, C) RDCU power calibration using a square pulse with the phase set at 220° (e.g. radiation damping compensation) and D) the same as C) with time shared square pulse.

RDCU on aqueous samples

5

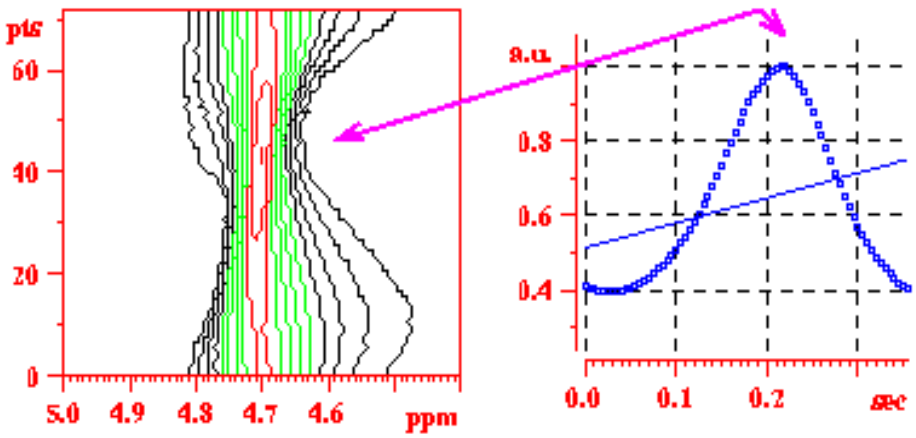
In this part we give some examples of experiments which have been recorded using the BRUKER RDCU board. We hope that this experiments and the rest of this manual gives the reader new ideas for use of the RDCU board. In the following part we try to show that the RDCU can be very helpful in the following experiments :

- Observation of water exchangeable/dipolar coupled protons in biological staff,
- Study of the dynamics of exchangeable protons in presence/absence of radiation damping,
- T_1, T_2 and $T_{1\rho}$ measurements of water protons or water exchangeable protons,
- Water peak narrowing in some cases.

Effect of the RDCU pulsing on the water peak 5.1

Depending on the phase set of the feedback field send back to the probehead (e.g. the phase of the RDCU pulse) we are able either to suppress or to enhance the radiation damping. The principle effect of radiation damping suppression on the water peak in biological staff is to remove the additional "relaxation" process created by natural radiation damping which forces the water magnetization toward it's equilibrium position. This can be best seen on the 2D map shown in ***Figure 5.1.***. The experiment recorded here is the 2D version of the experiment described in part ***"RDCU pulse phase calibration" on page 11*** of this manual. On the 2D version of the basic experiment (see ***Table 5.1.*** for the pulse sequence) we can observe the linewidth and the amplitude behavior of water resonance as a function of the RDCU pulse phase. The maximum of the curve is obtained for point 44 in the f1 dimension. As we have used 5° degree phase steps, we found a phase value of 220° for radiation damping suppression. Point 44 corresponds to the spectrum for which the water line resonance is the narrowest.

Figure 5.1. Water peak linewidth and amplitude variation against the RDCU pulse phase



The 2D version can also be used for phase and amplitude calibration in the same way as the 1D paropt versions. This experiment gives more information if we are studying pulse sequences like water peak suppression with Jump and return, presaturation or watergate techniques.

In ***Table 5.2.*** we have summerized some results obtained on the linewidth and amplitude of the water resonance at 400 and 800 MHz againts the RDCU pulse phase and amplitude. In this table $\Delta\nu_{1/2}$ means the water peak linewidth at half of the amplitude and $\Delta\nu_{1\%}$ means the linewidth at 1% of the amplitude. R is the ratio between the Intensity of the water peak obtained with RDCU pulsing (suppressed or enhanced) and the Intensity of the water peak measured when natural radiation damping occurs. The spectra corresponding to the values given in ***Table 5.2.*** are shown in ***Figure 5.2.***

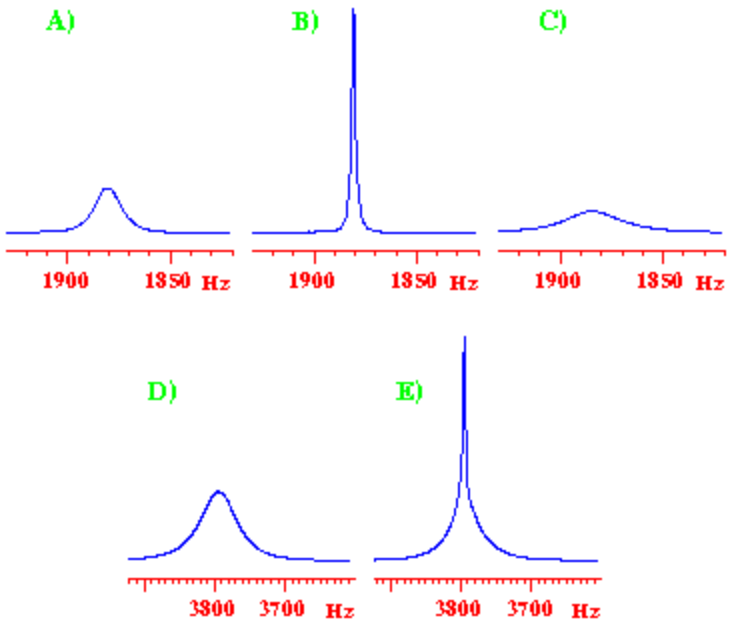
*Table 5.1. 2D version of the zg.ru pulse program for obtention of the 2D map obtained in **Figure 5.1.***

```
;zgadc2d.rd
;AVANCE DMX version
;2D RDC Unit pulse phase calibration sequence
;using the RDCU and channels f1 and f3 of the DMX spectrometer
;using time shared RDCU square pulses during acquisition
;TD1=(360/m) where m is the phcor2 step used in the 1D version
;Ph.LUX (S.a.d.i.s. R&D 971118)

define loopcounter count

"d12=20u"
"d15=1m"
"count=td/2"
"p0=1u"
"p18=2*dw-d18-d19-8u-p0"
"d17=4.0125u"
"d19=5u"



#include <Avance.incl>

1 ze
  d15 reset:f1
  d17 reset:f3
  d1 do:f3
2 d12 pl1:f1
  d12 pl3:f3                ;RDCU on channel 3 pl3 and ph2
  p1:f1 ph1
  de1
  de2
  2u:f1 ph0
  10u syrec                 ;AVANCE DRX version
  2u adc ph31
3 p0:x                      ;external trigger point recording
  d19 setnmr0|7             ;blank RDCU transmitter
  p18:f3 ph2                ;RDCU time shared square pulse (=  ;33% of the time between 2 points)
  8u setnmr0^7              ;unblank RDCU transmitter
  d18
  lo to 3 times count
  rcyc=2
  d15 do:f3
  d1 wr #0 if #0 ip2 zd
  lo to 2 times td1
exit

ph1=0 1 2 3
ph31=0 1 2 3
ph2=(72) 0                  ;m=72 to obtain 360/72 = 5 degree  ;phase steps
ph0=0



;pl1: transmitter high power level
;pl3: RDCU power level
;p1 : transmitter high power pulse
;p18: RDC Unit pulse lenght
;d19: short delay for RDCU blanking set
;d18: Time sharing delay to avoid S/N loss
;d1 : relaxation delay; 1-5 * T1
```

Table 5.2. Linewidth and amplitude of the water peak in a 2 mM Lysosyme sample in 90/10 H2O/D2O obtained in presence of natural radiation damping, when radiation damping is enhanced or suppressed at 400 and 800 MHz

Pulse program	Power (dB)	Linewidth (Hz)	Water S/N	Water Ratio	RD
zg	no RDCU pulse	$\Delta\nu_{1/2}$ = 15.34 $\Delta\nu_{1\%}$ = 74.16	S/N = 26825	Ratio = 1.0 ref 400 MHz	natural
zg.rd	dB_{opt} = 16.0	$\Delta\nu_{1/2}$ = 2.13 $\Delta\nu_{1\%}$ = 19.20	S/N = 78716	Ratio = 2.9	suppressed
zg.rd	dB_{max} = 4.0	$\Delta\nu_{1/2}$ = 46.88 $\Delta\nu_{1\%}$ = 230.60	S/N = 7992	Ratio = 0.3	enhanced
zgadc.rd	dB_{opt} = 5.0	$\Delta\nu_{1/2}$ = 2.13 $\Delta\nu_{1\%}$ = 19.20	S/N = 102944	Ratio = 3.8	suppressed
zg 800 MHz	no RDCU pulse	$\Delta\nu_{1/2}$ = 60.15 $\Delta\nu_{1\%}$ = 323.88	S/N = 39386	Ratio = 1.0 ref 800 MHz	natural
zg 800 MHz	dB_{opt} = -3.0	$\Delta\nu_{1/2}$ = 5.81 $\Delta\nu_{1\%}$ = 192.55	S/N = 114999	Ratio = 2.9	suppressed

Figure 5.2. Water peaks spectra obtained with the 2 mM Lysosyme sample in 90/10 H_2O/D_2O at 300 K



A) in presence of natural radiation damping at 400 MHz, B) with radiation damping suppressed at 400 MHz, C) with radiation damping enhanced at 400 MHz, D) in presence of natural radiation damping at 800 MHz and E) with radiation damping suppressed at 800 MHz.

RDCU pulsing and water peak suppression techniques *5.2*

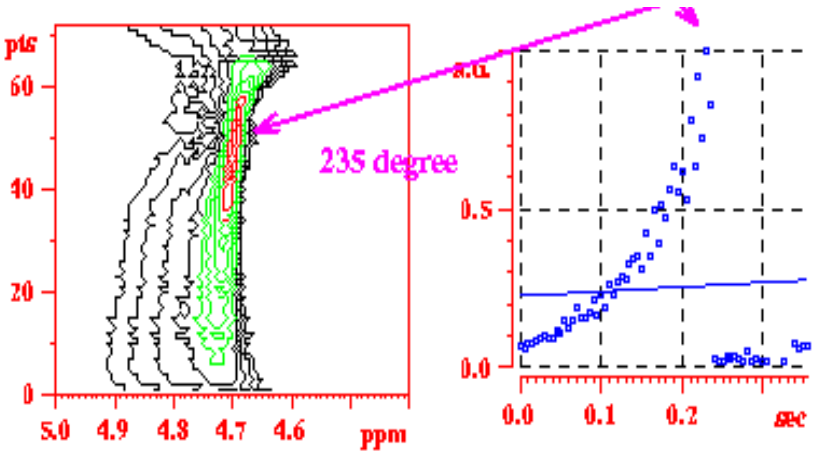
We consider in this part only the case of using the Radiation Damping Control Unit during the acquisition time following a water peak suppression sequence. We want to use the RDCU pulsing for water peak linewidth narrowing. To be sure of the radiation damping suppression efficiency, we have to take into account some physical limits :

- The residual water peak signal must be strong enough to induce an e.f.m. in the receiver coil which should be great enough to make radiation damping efficient. This is ***absolutely not the case at 400 MHz for water suppression sequences like presaturation and watergate***. For this kind of very efficient water suppression sequences at "low" frequencies (400 and probably 500 MHz), the RDCU is not able to decrease the water peak linewidth. Perhaps at higher frequencies and with cryoprobes the RDCU can be useful.
- The broadening of the base of the water peak is due to the inhomogeneity of B_0 and B_1 fields. This depends on how the magnet is and can be shimmed. This depends also on the r.f. homogeneity.
- In the case of less efficient water suppression sequences like the jump and return, or the inversion recovery techniques the Radiation Damping suppression is much more efficient. This can be explained by the fact that for presaturation and watergate the energy levels of the water protons are saturated before the r.f. field is applied. In the case of the jump and return and the inversion recovery techniques, it's the nutation of the r.f. field which causes water peak suppression.

The 1, -1 Jump and return sequence *5.2.1*

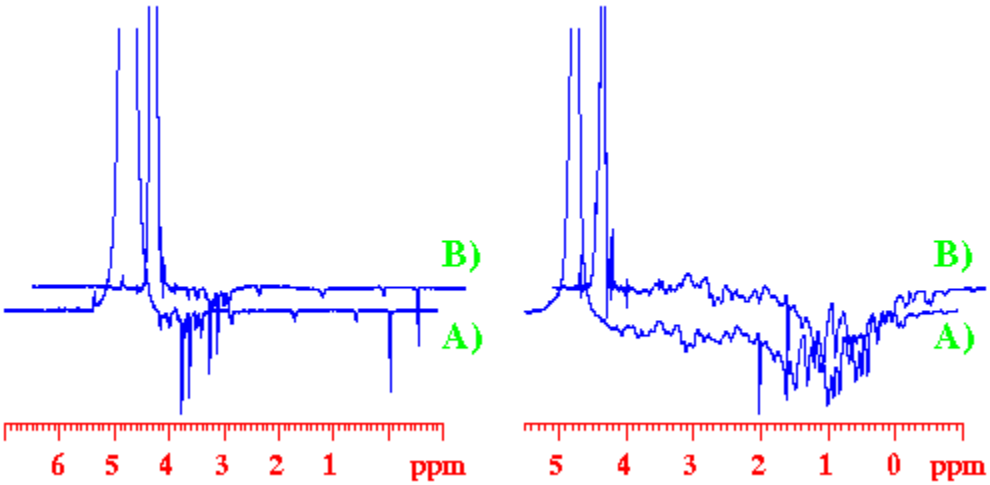
We show here as an example, the Radiation damping suppression effect during the 1,-1 jump and return water suppression experiment. The RDCU pulse phase and amplitude variation 2D map (recorded in the same way as shown in section ***"Effect of the RDCU pulsing on the water peak" on page 42*** by replacing the zgadc2d.ru pulse program by the p11adc2d.ru pulse program) shows that for the phase values corresponding to radiation damping suppression with the basic experiment we observe a water peak line narrowing when we use the 1,-1 Jump and return preparation pulse (***Figure 5.3.***). This line narrowing was observed with the 2 mM sucrose sample in H_2O/D_2O 90/10 and with the 2 mM Lysosyme sample in H_2O/D_2O 90/10 (***Figure 5.4.***). The measured linewidth of the water peak when radiation damping is suppressed against the linewidth obtained when natural radiation damping occurs are summerized in ***Table 5.3.***. The pulse program used is the same as shown in ***Figure 4.4.*** with a ($\pi/2_{(x,y,-x,-y)} - \tau - \pi/2_{(-x,-y,x,y)}$) preparation pulse instide of the $\pi/2$ preparation pulse.

Figure 5.3. 2D map obtained for the calibration of the RDCU pulse phase obtained with the p11adc2d.ru pulse sequence



The RDCU pulse power is the same as obtained in the basic experiment.

Figure 5.4. 1D 1,-1 Jump and return spectra



On <u>left</u> : 2 mM Lysosyme in 90/10 H_2O/D_2O, and <u>right</u> : 2 mM Sucrose in 90/10 H_2O/D_2O. In both figures, the spectra in A) is obtained with radiation damping suppression during the acquisition time and B) is obtained when natural radiation damping occurs during the whole time of the pulse sequence.

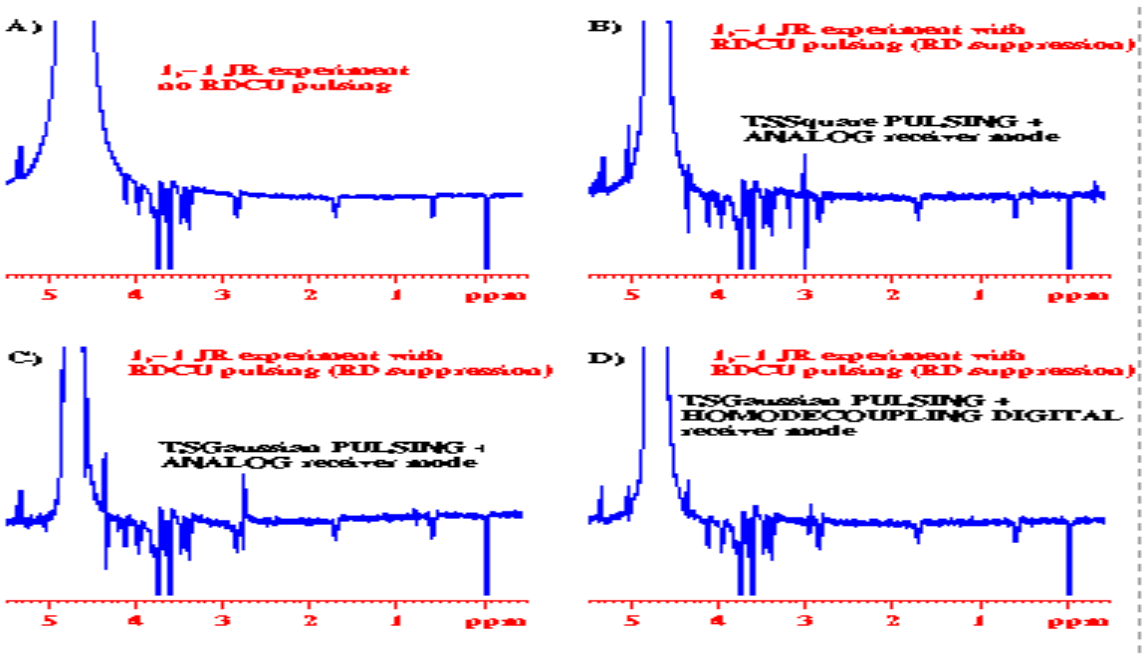
Table 5.3. Linewidth and amplitude of the water peak in a 2 mM Lysosyme sample in 90/10 H_2O/D_2O and in a 2 mM Sucrose sample in 90/10 H_2O/D_2O obtained in presence of natural radiation damping and when radiation damping is suppressed at 400 MHz and 300 K

Sample	RD	$\Delta\nu_{1/2}$ (Hz)	$\Delta\nu_{1\%}$ (Hz)	S/N (H_2O)	S/N (DSS/Ace)
2 mM Sucrose	natural	21.31	176.46	8702.8	64.7
2 mM Sucrose	suppressed	6.39	48.16	52844.2	74.1
2 mM Lysosy	natural	16.62	158.13	4903.0	305.9
2 mM Lysosy	suppressed	3.41	55.84	20229.3	281.9

Note : The experiments on the 2 mM sucrose sample have been recorded with a 5 mm BBI Z-gradient probehead whereas the experiments on the 2 mM Lysosyme sample have been carried out on a 5 mm TBI X,Y,Z gradient probehead. !!!! Calibrations and results are depending on the probehead you are using (see ***(Eq. 1.1)***

We have shown in ***"RDCU pulsing during acquisition delays" on page 37*** that the use of time sharing RDCU pulsing during the acquisition time is a very good alternative to record spectra with avoiding a dramatic Signal to Noise loss. Nevertheless two problems appear as we try to push the RDCU board pulsing towards it's limits. As far as the electronic feedback method used here works as a closed loop, at lower ASU attenuation (higher RDCU pulse power values), the circuit starts to oscillate and spurious spikes appear everywhere in the spectrum **(*Figure 5.5.*)**

Figure 5.5. 1H spectra of 2 mM Sucrose in 90/10 H2O/D2O using the 1,-1 Jump and return sequence for water presaturation



In spectrum A) natural radiation damping occurs. For spectra B)-D) radiation damping is suppressed during the acquisition time.

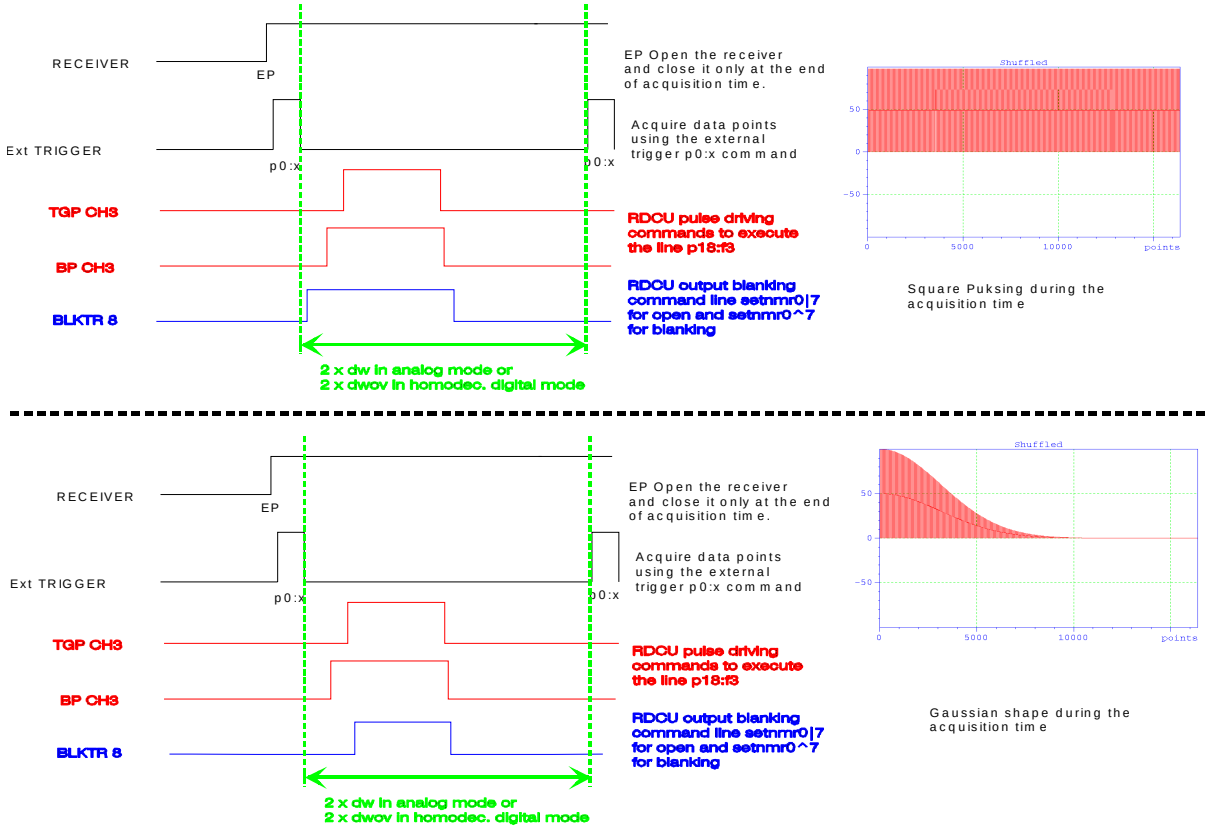
The closed loop circuit starts to oscillate and generates a regular decaying beat even in absence of native water precessing magnetization (e.g. in the second part of the FID where water magnetization should be 0). There is also an additional spike present even if the ASU attenuation pl3 is set to 120 dB (e.g. pulsing at the noise level). This last artifact is a consequence of the way in which time sharing pulsing is done with the pulse sequence shown in ***Figure 4.4.***

The alternative is given in the following section. We have to modify the pulse program given in ***Figure 1.2.*** and the detection manner as following :

- Change the way of pulsing as shown in ***Figure 5.6.***
- Replace the square pulse time sharing RDCU pulsing by a half time reversed gaussian time sharing RDCU pulsing (***Table 5.4.***)
- Change the detection mode from analog to homodecoupling digital mode (e.g. DIGMOD = homodecoupling digital and AQMOD = qsim).

In ***Figure 5.5.*** **B)** a lot of artifacts can be observed as a consequence of the RDCU board oscillation as far as we are using high power level for RDCU pulsing (***pl3 = 2.5 dB***). In ***Figure 5.5.*** **C)** the intensity of artifacts decreases as a consequence of the use of time shared gaussian shape RDCU pulsing (TSGaussian). In ***Figure 5.5.*** **D)** the intensity of the remaining artifacts is decreased as a consequence of using the ***homodecoupling digital acquisition mode instead of the analog mode***. The number of artifacts is decreased by writing the RDCU pulse sequence during the acquisition in the way shown in ***Figure 5.6.*** The pulse program used to record the spectra of ***Figure 5.5.*** **C)** and ***Figure 5.5.*** **D)** is given in ***Table 5.4.***.

*Figure 5.6. Time sharing RDCU pulsing schemes as used in the examples shown in **Figure 5.5.** B) - **Figure 5.5.** D)*



The time shared square (TSSquare) RDCU pulsing fashion is shown on the top scheme, whereas the time shared gaussian (TSGaussian) RDCU pulsing fashion is shown on the bottom scheme.

The use of a gaussian shape for RDCU pulsing eliminates the truncation effect and the the spikes coming from RDCU oscillation. As far as with the chosen gaussian shape pulse the RDCU pulse power is equal to 120 dB for the half of the acquisition time we also reduce the artifacts intensity.

If we use the bottom scheme for time shared pulsing in between two data point sampling (during 2 dw) we eliminate some artifacts. Some artifacts are coming from the channel f3 blanking and transmitter gating pulses. If we use the syntax p18 :f3 ph2 :r in between the two RDCU output blanking commands (2u setnmr0|7 and 2u setnmr0^7) the artifacts coming from the BP CH3 and TGP CH3 pulses are send in the circuit. If we drive now the f3 blanking and transmitter gating pulses separately as shown in the bottom scheme of ***Figure 5.6.***, we are able to blank the RDCU output after sending the BP CH3 and TGP CH3 commands. This operation allows to remove some artifacts.

The pulse program corresponding to the bottom scheme of ***Figure 5.6.***, using the gaussian shape pulse is given in ***Table 5.4.***. ***This pulse program should be used if high performance Radiation damping suppression is wanted during the acquisition time***.

Table 5.4. Pulse program for optimized 1,-1 Jump and return water suppression

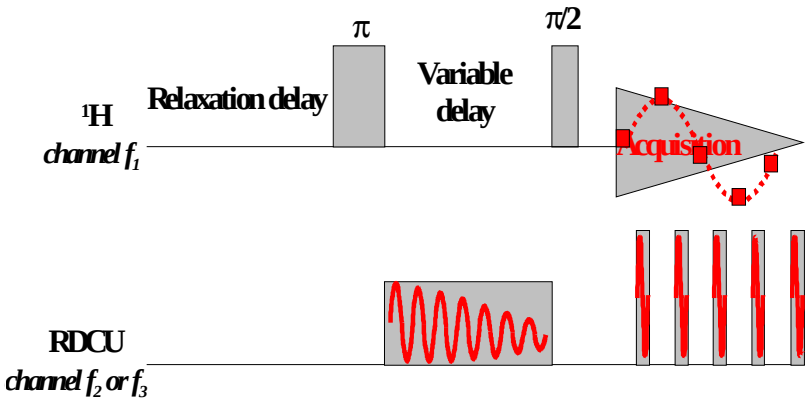
```
;p11adcsel.rd
;AVANCE DMX version
;1D 1-1 Jump and return sequence
;using the RDCU and channels f1 and f3 of the
DMXspectrometer
;using time shared RDCU gaussian pulses during
;acquisition
;Ph.LUX (S.a.d.i.s. R&D 971118)

define loopcounter tdov

"d12=20u"
"d15=1m"
"tdov=td*decim/2"
"p0=0.5u"
"d22=2*dwov/4"
"d18=2*dwov-2*d19-p0-d22-2u"
"d17=4.0125u"
"d19=5u"
"p31=aq"


#include <Avance.incl>

1 ze
; d15 reset:f1
; d17 reset:f3
  d12 pl0:f3
2 d1 do:f3
  d12 pl1:f1
  p1:f1 ph1                ;Jump amd Return sequence
  d20
  p11:f1 ph3
  de1
  de2
  2u:f1 ph0
  2u cpdngs1:f3 ph2:r      ;cpd sequence activation
  5u syrec                 ;for DRX spectrometers
  2u adc ph31
3 p0:x                     ;external trigger point recording
  2u setnmr0 |18|26        ;Open f3 channel
  d19 setnmr0|7            ;Open RDCU out
  d22
  d19 setnmr0 ^7^18^26     ;Close f3 and RDCU out
  d18
  lo to 3 times tdov
  rcyc=2
  d12 do:f3
  wr #0
exit

ph1=0 2 2 0 1 3 3 1
ph31=0 2 2 0 1 3 3 1
ph3=2 0 0 2 3 1 1 3
ph2=0
ph0=0



;pl1: transmitter high power level
;pl3: RDCU power level
;p1 : transmitter high power pulse
;p18: RDC Unit pulse lenght
;d19: short delay for RDCU blanking set
;d18: Time sharing delay to avoid S/N loss
;d1 : relaxation delay; 1-5 * T1
;d19: delay for binomial water suppression
;     d19 = 1/(2*d) where d is the distance of the next null (in
Hz).
;DIGMOD = homodecoupling digital
;AQMOD = qsim
;use cpdprg1 :                1 p31 :sp1 :0 pl=sp1
;                             jump to 1
;use a gaussian shape stored in spnam1.
```

The RDCU pulsing scheme (green characters) during acquisition should be used each time when RDCU pulsing is done during acquisition in presence of power levels inducing oscillation phenomena and generating intense spikes. We use ***a cpd program CPDPRG1*** *which is given below and* ***the gaussian shape*** *shown in* ***Figure 5.6.*** *(spnam1 = RDCU_gauss) whose* ***power level*** *is given this time by the* ***command sp1.***

Water T1 measurement with the RDCU 5.3

As far as one of the most important effect of radiation damping on the water peak is a rapid flip back of the magnetization toward it's equilibrium state, the measurement of the water protons T_1 should be an interesting feature to study. We have performed the inversion recovery experiment traditionally used for T_1 measurements on the 2 mM Lysosyme sample in 90/10 H_2O/D_2O in presence of natural radiation damping and when radiation damping is suppressed by RDCU pulsing using the sequences shown in ***Figure 5.7.***. The experiments have been performed at 400 and 800 MHz. In this example we have studied the behavior of water proton magnetization. It is clear that the here shown sequences can be used to measure T_1 of water exchangeable protons. You have only to take the 1D sequence shown in ***Figure 5.7.*** and convert it in 2D mode by replacing the variable delay vd command by the variable pulse vp command in the non acquisition part of the sequence.

Figure 5.7. Inversion recovery sequences with RDCU pulsing



This sequence is the 1D paropt version but can be easely converted in a 2D version.

Table 5.5. Inversion recovery sequences with RDCU pulsing

```
;t1adc.ru
;AVANCE DMX version
;1D T1 sequence with Radiation Damping suppression sequence
;using the RDCU and channels f1 and f3 of the DMX spectrometer
;Two TFX modules are inserted in the SE451.

define loopcounter count

"d12=20u"
"d15=1m"
"d13=3u"
"p2=p1*2"
"count=td/2"
"p0=1u"
"p19=2*dw-d18*-d19-8u-p0"
"d18=5u"
"d22=p19"
"d17=4.0125u"

#include <Avance.incl>

1 ze
  d15 reset:f1
  d17 reset:f3
2 d1 do:f3
  d12 pl1:f1
  d12 pl3:f3                ;RDCU on channel 3 pl3 and ph2
  p2:f1 ph3                 ;180° preparation pulse
  5u syrec                  ;for AVANCE DRX version
  2u setnmr0|7              ;unblank RDCU transmitter
  p18:f3:e ph2:r            ;p18 may be replaced by vp
  2u setnmr0^7              ;blank RDCU transmitter
  5u sytra                  ;for AVANCE DRX version
  p1:f1 ph1
  de1
  de2
  2u:f1 ph0
  5u syrec                  ;for AVANCE DRX version
  2u adc ph31
3 p0:x
  d19 setnmr0|7             ;unblank RDCU transmitter
  p19:f3 ph4:r              ;Time sharing RDCU pulsing
  8u:e setnmr0^7            ;blank RDCU transmitter
  d18
  lo to 3 times count
  rcyc=2
  30m do:f3
  wr #0
exit

ph1=0 2 2 0 1 3 3 1
ph31=0 2 2 0 1 3 3 1
ph2=0 2 2 0 1 3 3 1
ph3=0
ph4=0
ph0=0


;pl1: transmitter high power level
;pl3: RDCU power level
;p1 :  90 degree transmitter high power pulse
;p2 : 180 degree transmitter high power pulse
;d19 : short delay for RDCU blanking set
;d18 : Time sharing delay to avoid S/N loss
;p18: RDCU pulse lenght for variable delay = vp in ;vptable
;p19: RDCU pulse lenght for time sharing RDCU pulses
;d1 : relaxation delay; 1-5 * T1
```

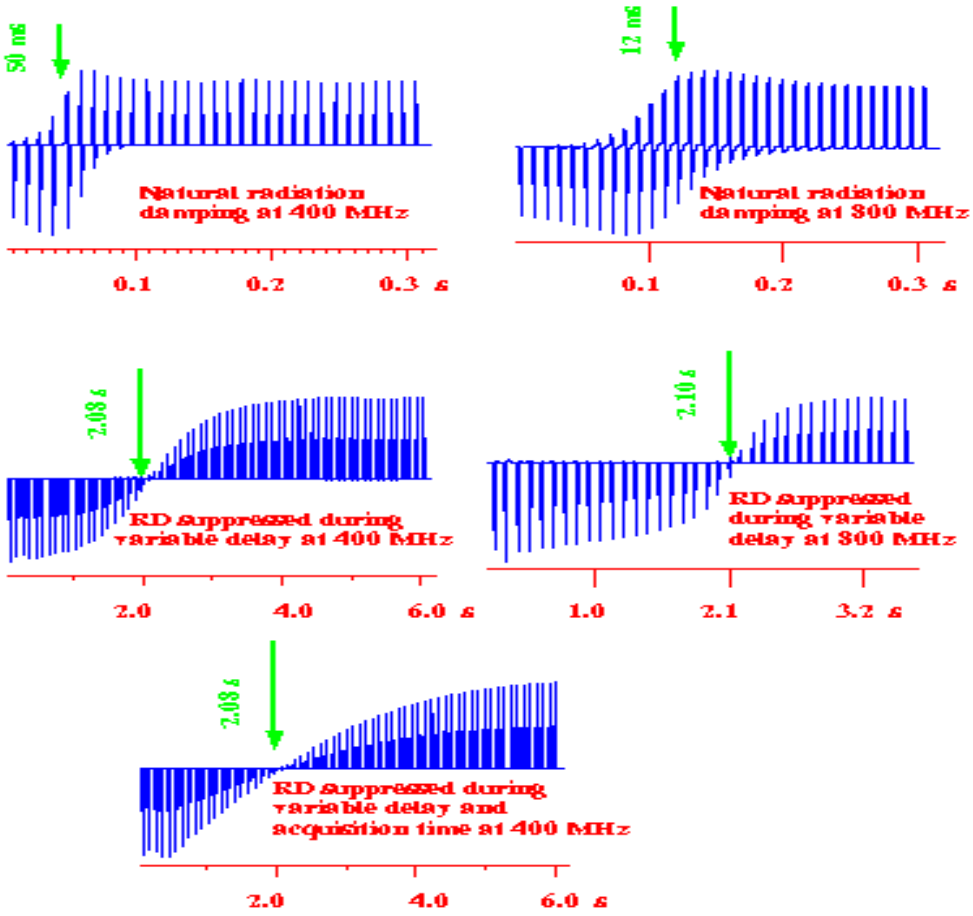
This sequence is the 1D paropt version but can be easely converted in a 2D version.

We have recorded with this sequence T_1 data off the water proton peaks (***Figure 5.8.***) when natural radiation damping occurs (With the known ***invrec*** sequence and with the ***t1adc.ru*** sequence by setting the power levels pl3 and pl4 to 120 dB), when radiation damping is suppressed only during the variable delay inbetween the π and $\pi/2$ pulses, when radiation damping is suppressed during the variable and acquisition delays. This experiments haven been recorded at 400 and 800 MHz. The results are summerized in ***Table 5.6.***.

Table 5.6. Apparent water protons T_1 values (300 K) as deduced from the T_1 curves shown in ***Figure 5.6.***

^{1}H Frequency	Sample	pulse program	RD	M_z=0 in s	$T1_{app}$.in s
400	2 mM Sucrose	invrec	natural	0.035	0.050
800	2 mM Sucrose	invrec	natural	0.012	0.037
400	2 mM Sucrose	t1adc.ru	Supp during delay	2.08	3.00
800	2 mM Sucrose	t1adc.ru	Supp during delay	2.10	3.00
400	2 mM Sucrose	t1adc.ru	Supp during delay	2.08	3.00

Figure 5.8. T_1 water proton longitudinal relaxation curves



This curves are obtained in paropt mode with the t1adc.ru pulse program or with the invrec pulse program for experiments in which natural radiation damping occurs. The two top figures are obtained when natural RD occurs and give and apparent T_1 value of 50 ms and 17 ms respectively at 400 and 800 MHz. The wo figures in the middle are obtained by suppressing the radiation damping during the variable delay. This curves give an apparent T_1 value of 3.0 s at 400 and 800 MHz. The curve in the bottom is recorded with radiation damping suppression

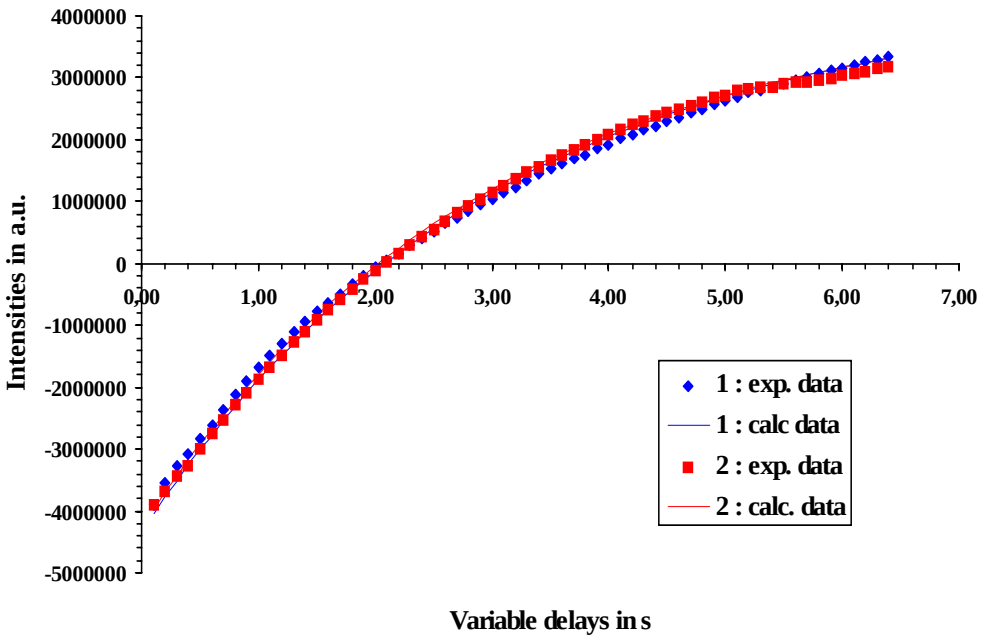
during the variable delay and during the acquisition time at 400 MHz. From this curve we deduce an apparent T_1 value of 3.0 s.

As can be seen on the T_1 curves of ***Figure 5.8.***, we obtain an exponential behaviour of the inversion recovery data points only if the radiation damping is suppressed during the variable delay and during the acquisition time. It is now possible to calculate the T_1 value from this data points (***Figure 5.9.***).

The suppression of the radiation damping allows to determinate the real T_1 of water proton in biological samples. As far as the relaxation behaviour of the water protons influences the relaxation times of the water exchangeable protons of proteins/DNA, it would be interesting to measure the T_1 of the water exchangeables protons in presence of natural radiation damping and when radiation damping is suppressed or enhanced.

As can be seen in ***Figure 5.9.***, the T_1 inversion recovery curves obtained with RD suppressed during the variable delay only and during the variable dalay + the acquisition time have an exponential behaviour. This is normal as far as we have taken the peak ***area's*** for the plot and the calculation. If we plot now the curves of the peak ***intensity variations*** during the inversion recovery experiment, the curve obtained if radiation damping is only suppressed during the variable delay is no longer exponential (***Figure 5.8.***). The same behaviour was observed during the water T_2 experiment recording.

*Figure 5.9. T_1 curves as obtained from the **area** of the water peak paropt inversion recovery curves shown in **Figure 5.8.***



Curves of the experiment for which radiation damping is suppressed during the variable delay and the acquisition time (curve 1) and the curves of the experiment for which radiation damping is only suppressed during the variable delay

From the curves of ***Figure 5.9.***, we calculate the T_1 of water when radiation damping is suppressed during the variable delay and when radiation damping is suppressed during the whole sequence. For both cases we calculate a T_1 value of 3.0 s at 300 K for the 2 mM Sucrose sample in 90/10 H_2O/D_2O.

In the same way as for T_1 measurements, we can record T_2 and $T_{1\rho}$ measurements by replacing in the original sequences respectively the delay t and variable pulse by a RDCU pulse. Additionally it is recommended to perform radiation damping suppression during the acquisition time. We have done the experiments on the water peak, it seems to be clear that radiation damping suppression should be useful for water exchangeable proton relaxation parameter calculation.

Nuclear Overhauser effect in presence of RD : 2D Noesy experiments 5.4

The last example that we present in this user manuel concerns the use of the RDCU during one and two dimensional NOESY expeiments of biological molecules with water exchangeable protons. We have used fop the experiments shown in this section the 2 mM Lysosyme sample in 90/10 H_2O/D_2O as test sample. The principle of the experiment is the same as in the preceeding section, we use the basic 2D NOESY sequence and we replace the mixing time by the RDCU pulse sequence shown in ***Table 4.1.***. Nevertheless we have to be careful by setting the RDCU pulse phase cycling as explained in section ***"RDCU pulse amplitude calibration" on page 15*** of this manual. As example we show in ***Table 5.7.*** the 2D NOESY pulse programs used to record the following experiments. In the following experiments, RDCU pulsing was only performed outside acquisition, but the sequences shown here can be done with time sharing RDCU pulsing during acquisition. At 400 MHz, the RDCU pulsing during the acquisition time of the NOESY experiments was no longer necessary as far as water suppression has be done with the WATERGATE sequence **(see section** ***"RDCU pulsing and water peak suppression techniques" on page 45***).

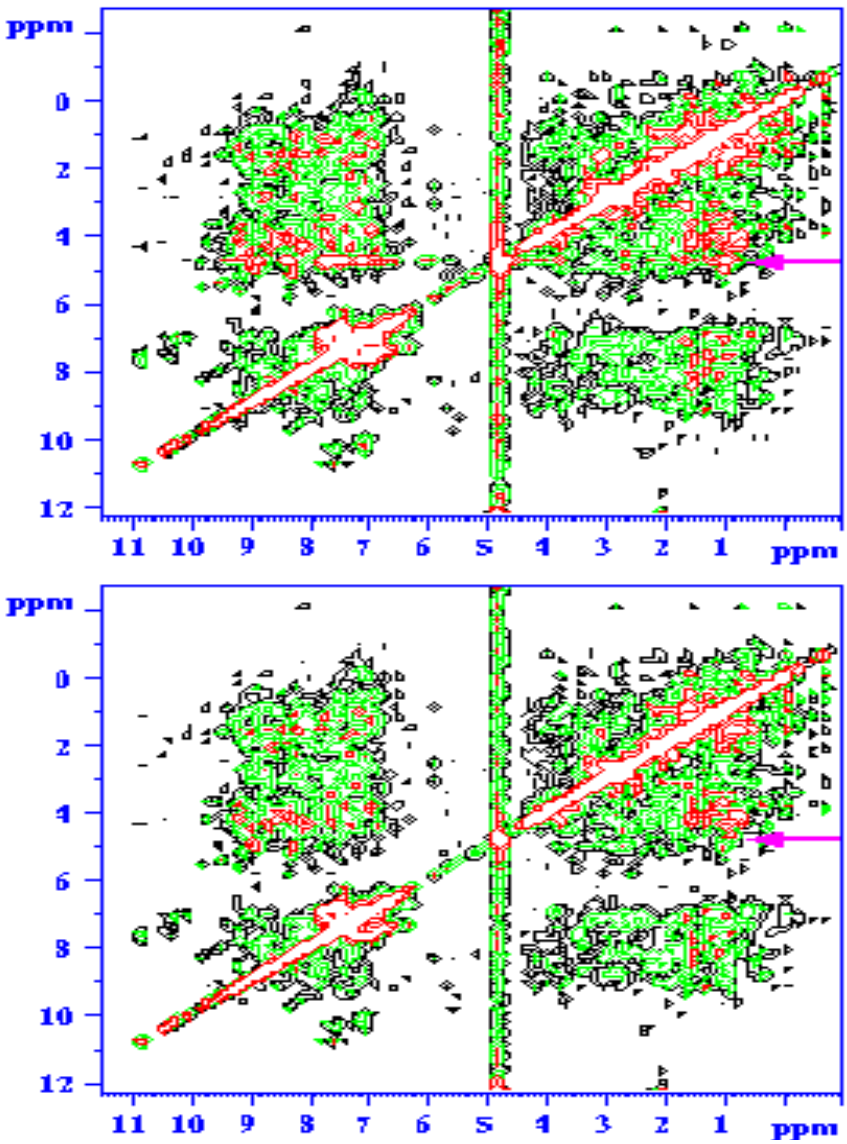
Table 5.7. Pulse sequences for recording of the 2D NOESY experiment with RDCU pulsing during the evolution and mixing times

```
;noesygstp19_rd
;avance-version
;2D homonuclear correlation via dipolar coupling
;dipolar coupling may be due to noe or chemical ;exchange.Phase sensi-
tive using TPPI
;water suppression using 3-9-19 pulse sequence with gradients
;using RDCU pulsing during evolution and mixing times.

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

"d0=6u"
"d12=20u"
"d15=1m"
"d13=4u"
"d17=4.0125u"

1 ze
  d15 reset:f1
  d17 reset:f3
2 d1
3 d12 pl1:f1
  d12 pl3:f3
  p1 ph1
  5u syrec                ;for AVANCE DRX version
  2u setnmr0|7            ;unblank RDCU transmitter
  p19:f3:e ph7:r          ,RDCU pulsing at power pl3
  2u setnmr0^7            ;blank RDCU transmitter
  5u sytra                ;for AVANXE DRX version
  p1 ph2
  5u syrec                ;for AVANCE DRX version
  2u setnmr0|7            :unblank RDCU transmitter
  p18:f3:e ph6:r          ;RDCU pulsing at power pl3
  2u setnmr0^7            ;blank RDCU transmitter.
  5u sytra                ;for AVANCE DRX version
  p1 ph3
  d12 pl18:f1
  50u UNBLKGRAD
  GRADIENT(cnst21)
  d16
  p28*0.231 ph4
  d19
  p28*0.692 ph4
  d19
  p28*1.462 ph4
  d19
  p28*1.462 ph5
  d19
  p28*0.692 ph5
  d19
  p0*0.231 ph5
  46u
  GRADIENT(cnst22)
  d16
  4u BLKGRAD
  go=2 ph31
  d1 wr #0 if #0 ip1 ipu19 zd
  d15 ip6
  d15 ip7
  lo to 3 times td1
exit


ph1=0 2
ph2=0 0 0 0 0 0 0 0
ph3=0 0 2 2 3 3 1 1
ph4=0 2 0 2 0 2 0 2
ph5=2 0 2 0 2 0 2 0
ph6=0 2 0 2 0 2 0 2
ph7=0 2
ph31=0 2 2 0 1 3 3 1


;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
;p2 : f1 channel - 180 degree high power pulse
;p16: homospoil/gradient pulse
;p28: f1 channel -  90 degree pulse at pl18
;p19: variable pulse with increment INP19 = DW
;p18;RDCU pulse during mixing time
;d12: delay for power switching                [20 usec]
;d16: delay for homospoil/gradient recovery
;d19: delay for binomial water suppression
;     d19 = (1/(2*d)), d = distance of next null (in Hz)
;in0: 1/(2 * SW) = DW
;nd0: 2
;NS: 8 * n
;DS: 16
;td1: number of experiments
;MC2: TPPI

;use gradient program (GRDPROG) :2sine


                                ;use gradient ratio:          cnst21
                                : cnst22

                                ;                             20 :
                                20
```

The ***noesygstp19.ru*** pulseprogram is a starting point of all other experiments you want to do. Note that the RDCU pulse is not absolutely necessary during the evolution time as far as the evolution time is the most of the time small against T_{rd}. Nevertheless at higher frequencies (600-800 MHz) and/or in the case of the use of cryoprobes, T_{rd} is very short and it is better to do radiation damping suppression during the evolution time with RDCU pulsing.

We show here the efficiency of radiation damping suppression at 800 MHz for the observation of the water exchangeable protons in the 2 mM lysosyme sample in 90/10 H_2O/D_2O at 300 K. We observe an ovesome intensity increasing of the water exchangeable proton peaks (***Figure 5.10.***). This can be explained by two phsysical phenomena :

Figure 5.10. 2D NOESY maps recorded on the 2 mM Lysosyme sample in 90/10 H_2O/D_2O at 300 K on an AVANCE 800 MHz spectrometer

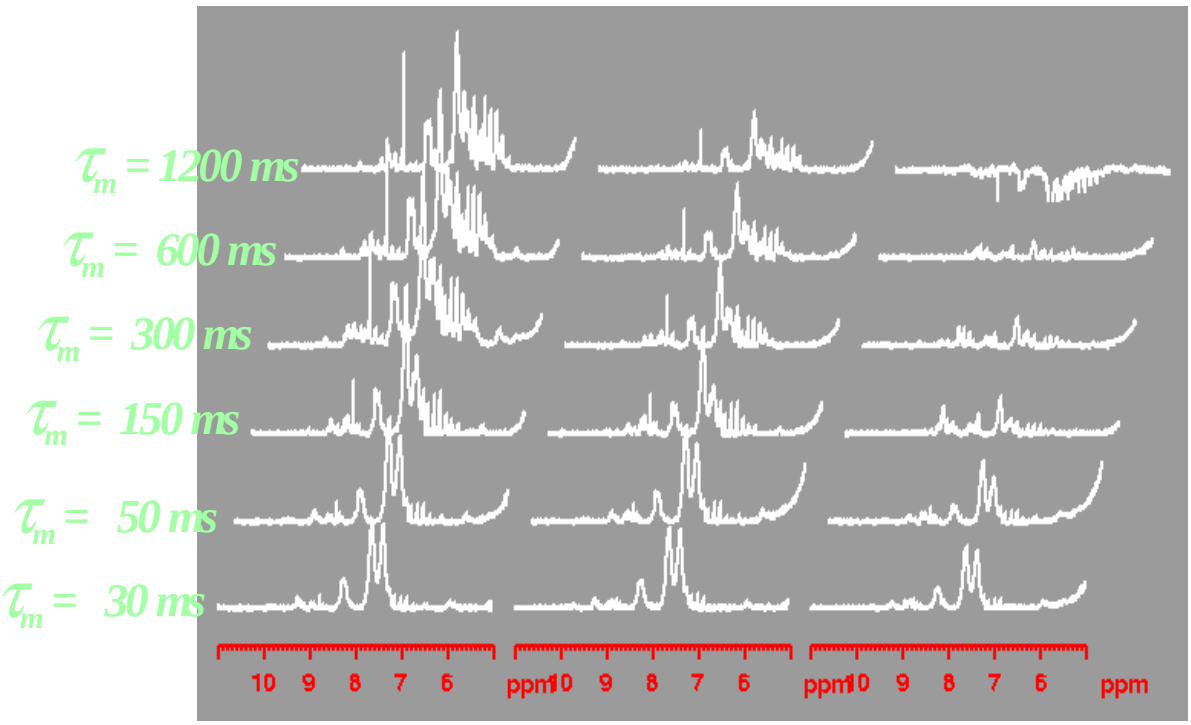


Top : Radiation damping is suppressed during the acquisition and mixing times, bottom : natural radiation damping occurs. The row containing the peaks of the water exchangeable protons is marked by an arrow.

- The radiation damping suppression increases T_1 and T_2 of the water by vanishing the T_{rd} contribution to relaxation. In this way the relaxation behaviour of the water exchangeable protons is no more longer perturbated by the radiation damping phenomenon.
- As far as radiation damping is suppressed, we are able to observe nOe 's between the water protons and the protons of the lysosyme which are dipolar coupled to the water protons. Even at higher mixing times we are able to observe nOe diffusion effects between water protons and the lysosyme protons. At very short mixing times (before the nOe's rise up), we would see only the water exchangeable lysosyme protons (see ***Figure 5.11.***).

The spectra shown in ***Figure 5.11.*** are the rows extracted at the water peak frequency from 2D NOESY maps recorded at 400 MHz and 300 K on the 2 mM Lysosyme sample in 90/10 H_2O/D_2O. Here we show the peak intensity variation of the lysosyme protons which undergoes an exchange and/or a dipolar coupling process with the water protons against the mixing time. The 2D maps have been recorded over a night when naturam radiation damping occurs, when radiation damping is suppressed during the evolution and mixing times and when radiation damping is suppressed during the evolution time but enhanced during the mixing time.

Figure 5.11. Rows extracted at the water peak frequency from 2D NOESY maps recorded on the 2 mM Lysosyme sample in 90/10 H_2O/D_2O

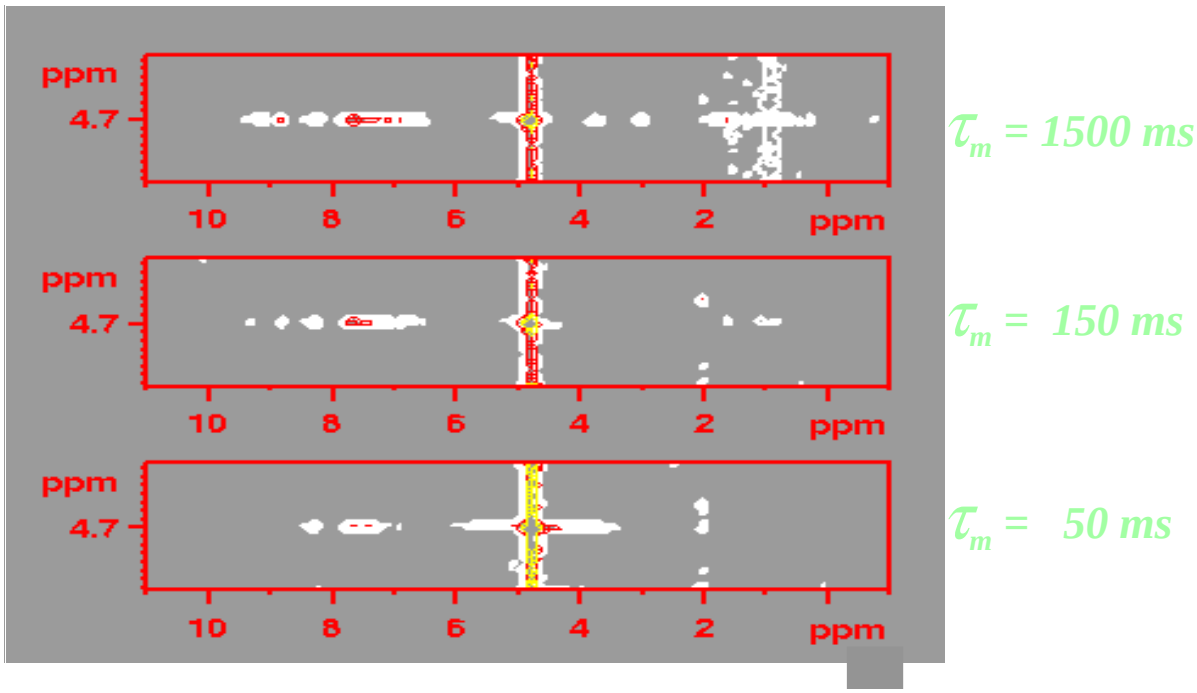


Left : when RD is suppressed during evolution and mixing times, middle : when natural RD occurs and right : when RD is suppressed during the evolution time but enhanced during the mixing time

The last fact that we want to point out is that : as far as with the RDCU we are able to suppress/enhance the radiation damping we can record the 2D NOESY experiment (or directly in 1D mode) by using the sequence of ***Table 5.7.*** in which we change the phase of the RDCU pulse during the mixing time and by holding the

phases of the other pulses and of the receiver constant. Thus we obtain a 2D map (or a 1D spectrum) on which we observe only the lysosyme protons which undergoes an exchange and/or dipolar coupling process with the water protons depending on the used mixing time. The results of this experience is shown in ***Figure 5.12.***

Figure 5.12. "Fast" NOESY 2D maps



"Fast" NOESY 2D maps obtained with the pulse program of ***Figure 5.7.*** *for which radiation damping is suppressed or enhanced every two scans.*

Bibliography 6

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Bibliography

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