TopSpin 4.0 & LC-NMR

Software Manual

Version 20180305

BRUKER

*LC*NMR
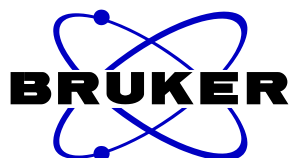
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Content

1. Usage of the Handbook

1.1 Syntax

Any commands which have to be entered in the TOPSPIN command line are underlined. Expressions in <brackets> have to be replaced by a desired/actual value.

rser <row number> <experiment number>

Any names of TOPSPIN elements like pulse programs, AU programs are printed with the font **courier bold-faced**. For example

use pulse program **lc1pnf2** for this experiment.

1.1 Changes TOPSPIN 3.5 → TOPSPIN 4.0

Configuration change

Sercial communication is not more supported by the new AV Neo Consoles. To support automation and data transfer between HyStar and TopSpin and IconNMR the COM port in the Hostcomptuer is used. See details about the configuration. In 3.2 Configure Communication HyStar-TopSpin/IconNMR.

1.2 Changes TOPSPIN 3.2.2-.6 → TOPSPIN 3.5

Flowparameters for FlowProbs and Cryofit edprobe

Flowparameters are now handled differentially, see 3.3 Definition of the FlowProbe or CryoFit.

NMRPT

A new testprocedure for Acceptance tests with FlowNMR systems is introduced that handles the experiments for CryoFit and FlowProbe Shimming. For details check the documentation of NMRPT.

1.3 Changes TOPSPIN 3.2.2 → TOPSPIN 3.2.3

Calibration Procedure for Loop+Cartridge transfer

Experiment LCUVNMR and the used AU programs au_uvnmr + proc_uvnmr now include procedures for determination system times/volumes into empty flow cells with acquisition of 1H and 2H gradient profiles into a 2D file.

1.4 Changes TOPSPIN 3.2.1 → TOPSPIN 3.2.2

Improved Functionality of lczshim – also for HR-MAS

The AU program lczshim hast now dramtically improved functionality. It can not only shim the Z-graident but also other userdefined gradients. It is able to shim several gradients Gradients to shim can be defined as commandline parameters.
In addition the stability of the peaksearch and deconvolution was improved, so tht it is suitable to shim also on small signals (TSP) in the presence of large solvent signal.
Run 'xau lczshim setup-yes' to define the shim routine. Backwards compatible. Starting with 'xau lczshim' behaves as before.

Startup/Shutdown Procedures LC-(SPE)-NMR(/MS) System

In the appendix you now find two comprehensive lists for the startup and shutdown procedure for LC-(SPE)-NMR(/MS) Systems.

Standard LC-NMR Parametersets adjusted

All 1D parametersets now with DIGMOD baseopt, also MULTIPRESAT, LC1D12, LC1DCWPS, LCZG
F1 Sweepwidth of homonuclear 1H correlations changed to 16ppm, HSQCETGPSIWT to 160ppm. Number of Increments changed to 256.

Mixing Time 1D NOESY-presat with selective pulses

Pulsprograms *lc1pncwgpps*, *lc1pncwps*, *lc1pngpps*, *lc1pnps*, *lc2pnps*: For NOESY-presat type pulsprograms the presaturation is maintained during the mixing time. If the presaturation is accomplished with a shape pulse, the length of mixing time **D8** was a multiple of the duration of the shape pulse **P18** used for the presaturation (typically 100msec). Up to now the effective during of the mixing time was rounded up to the next multiple of **P18**.

Now the *exact* mixing time is respected and composed by the maximum possible number of presaturation pulses preceded –where necessary- by a delay without presaturation. A mixing time of **D8**=220msec and a presaturation pulse **P18**=100msec leads to a 20msec delay + 2 shape pulses **P18** of 100msec length.

Receiver gain determination with CryoProbes

AU programs <u>au_lc1d,</u> <u>au_lc2d</u>: For CryoProbes the **DE** was increased to 40usec for RGA. The probe specific setting of **DE** via edprosol makes this procedure obsolete. "Automatic adjustment of DE" in the AU programs now means "keep the original **DE**". Where necessary it is still possible to define a dedicated **DE** value for the **RGA** procedure via the AU program <u>expset</u>.

Bugcorrection

In AU Program lcwetcalc major bug for selection of shapes corrected.

1.5 Changes TOPSPIN 3.1.6, 3.2.0 → TOPSPIN 3.2.1

Standard LC-NMR Parametersets adjusted

Minor changes to BRUKER standard LC-NMR parametersets were applied. If you use those parametersset verify that the new settings are still suitableyou're your applications and consider the effect when comparing data acquired under different versions.

LCZG: Receiver gain (RG) reduce to adapt for usage with CryoProbes. RG changed from 4 to 1 and AUNM changed from au_zg to au_zgonly (without RGA). D1 1sec to 2.4sec

LC1DWTDC: Digitizer (DIGMOD) changed from "digital" to "baseopt" for improved baseline and phasing. For this reason xaua now also performs autophase and pulsecal.

HSQCETGPSIWT, LCML12, LCMLCWPS, MLEVDCPHWT: Sweep width for Proton (SW) set to 16ppm, before 12 – 14ppm.
MULTIPRESAT, MULTIPRESATG: TD and SI changed from 32k to 64k

BUG Corrections

Constants controlling the full automation adjusted. Exclusion of solvent signal satellites was partially not working because XH compling constant (CNST 2) was set to 1 Hz, changed to 136.5Hz.
During full automation with 'xaua' the au program au_lc1d entered the multiple display window. Corrected.

1.6 Changes TOPSPIN 3.0 → TOPSPIN 3.1

New SPE Solvent

New solvent CD3OD_SPE available for elution of SPE cartridges with fully deuterated methanol.

Automatic 13C decoupling position

Region files for the LC-NMR solvents CD3OD_SPE, CD3CN_SPE, CH3CN+D2O and CH3OD+D2O available that allow automatic adjustment of the 13C decoupling position for removal of the 13C-satellites of the solvents.

Baseopt

For the experiments LC1D12, LC1DCWPS the setting **DIGMOD=BASEOPT** and **DIGMOD=DIGITAL** is the default. For usage with xaua the determination of the 90deg pulse and autophase is now also activated. If required, use command xau expset to deactivat this settings.

1.7 Changes TOPSPIN 2.1 → TOPSPIN 3.0

Prosol

The prosol handling has changed. For details check the prosol manual!
Note that during the automatic conversion of prosol settings from TopSpin 2.1 to TopSpin 3.0ff the powerlevel of the 90° observe pulses are directly converted. However all other parameter are set to the *default* values.
For example: The 1H observe pulse is correctly converted, however the persaturation power is set to the standard value of 50Hz and the cpd-decoupling pulses are set to the default length recommended for the field of the spectrometer.
For LC-NMR solvents like CH3CN+D2O (25Hz field) or especially the fully deuterated SPE solvents like CD3CN_SPE (12.5Hz) much weaker fields/power levels were used. Those changes that were defined in the old prosol settings are lost!

Baseopt

The experiments LC1D12, LC1DCWPS can be run with **DIGMOD=BASEOPT**. This provides a flat baseline and easier phasing. To use the feature, set the **DIGMOD=DIGITAL**. And activate either perform a 90deg pulse determination manually or activate the automatic determination in the AU program (xau expset to define parameters)

1.8 Changes TOPSPIN 2.0 → TOPSPIN 2.1

Sino controlled 2D acquisition

New version of AU program **au_lc2d** now also allows reduction of NS in the 2D file if the signal to noise in the 1D is high enough. Maximum number of scans in the 2D is NS (as before) new parameter is L31 which control the minimum number of scans.
If NS and L31 are identical, the program behaves like in the previous version. Just check if signal to noise is sufficient and skip 2D or execute it with the original parameters

Automatic processing for timing experiment LCUVNMR

A processing program **proc_uvnmr** for the experiment for the timing determination LCUVNMR is provided. The user is asked at the end of the acquisition program **au_uvnmr** if the processing should be started.

Off-resonance presaturation

It is recommended to use a 10msec shape pulse for experiments with off-resonance presaturation. In previous versions a 100msec puls was suggested, which was found to be not precise enough.
If you modify the parameters in edprosol and in the parametersets, note that only the pulslength should be changed. The power remains the same.

2. Introduction

2.1 Liquid Chromatography - NMR coupling

For chromatography NMR coupling different kinds of chromatographic separation systems can be coupled to a flow probe of an NMR spectrometer. With the currently available LC-NMR probes and CryoFits™ it is possible to use standard analytical scale chromatography (2.x - 5.xmm i.d. columns).
All Separation techniques like RP (Reversed Phase)-, NP (Normal Phase)- HPLC (High Performance Liquid Chromatography), GPC (Gel Permeation Chromatography) and SEC (Size Exclusion Chromatography) are applicable.
LC-NMR experiments can be performed in four modes.

- On-flow mode
- Direct stopped flow mode
- Loop storage/transfer mode
- Peak Trapping/elution mode

Several levels of automation are possible. The NMR experiment can be run with manual adjustment of all parameters but for each experiment an AU program is provided that can adjusts the parameters and run the experiment automatically. In addition most experiments can be executed in IconNMR in full automation.

On-flow mode

The LC-NMR interface connects the outlet of the LC-detector directly to the NMR probe. While the peaks are eluting, NMR spectra are continuously acquired. The chromatographic system is used to move the samples/peaks through the NMR cell.
Typically the flowrate is reduced to increase the residence time of the peaks in the flowcell to increase the NMR sensitivity by larger NS per spectrum.

Equipment : • Any HPLC system, which delivers a stable pulsation free flow
  • LC-NMR Probe or CryoFit
  • LCNMR interface BSFU-HP or BPSU-36/2
  (or one of the previous versions: BSFU, BPSU-12, BSFU-O, BMSO, BSFU-O+BPSU-36) recommended

With certain restrictions it is also possible to simply connect the probe the the outlet of the chromatography without further hardware.

Direct stopped flow mode

The LC-NMR interface connects the outlet of the LC-detector directly to the NMR probe. The LC-detector (normally UV) is used to detect peaks eluting from the column. When a peak is detected, the flow continues until the peak arrives in the NMR cell. At this time, the chromatography (pump, data acquisition, gradient) stops, a valve in the LC-NMR interface stops the peak in the flowcell and the NMR experiments are performed. Once the NMR experiments are completed, the chromatography resumes until the next peak is found. This process can be repeated several times within one chromatogram.

Equipment : • HPLC system under control BRUKER Chromatography
software HyStar
• LC-NMR Probe or CryoFit™
• LC-NMR Interface BSFU-HP or BPSU-36/2
(or one of the previous versions: BSFU, BPSU-12, BSFU-O,
BMSO, BSFU-O+BPSU-36)
• Controlling station with BRUKER Chromatography software

Loop storage/transfer mode

The LC-NMR interface connects the outlet of the LC-detector to storage loops. The LC-detector (normally UV) is used to detect peaks eluting from the column. A detected peak is moved into one of the storage loops without interrupting the chromatography. When the chromatography is completed the LC-NMR interface transfers the content of the loops by means of the HPLC pump into the NMR flow cell.

Equipment : • HPLC system under control of BRUKER Chromatography
software HyStar
• LC-NMR Probe or CryoFit™
• LC-NMR Interface BPSU-36/2
(or one of the previous versions: BPSU-12, BPSU-36)
• Controlling station with BRUKER Chromatography software.

SPE trapping/elution mode

The LC-SPE-NMR interface connects the outlet of the LC-detector to a clamps with exchangeable SPE cartridges. The LC-detector (normally UV) is used to detect peaks eluting from the column. A detected peak is trapped on a SPE cartridge without interrupting the chromatography. When the chromatography is completed, the LC-SPE-NMR interface washes and dries the cartridges and elutes the compounds with fully deuterated solvents into either an NMR flow cell or NMR tubes located in a Bruker LiquidHandler.

Equipment :   • Any HPLC system under control of BRUKER Chromatography
software HyStar
• LC-NMR Probe, CryoFit™ or a Bruker LiquidHandler for
transfer in NMR tubes.
• Bruker/Spark Prospekt2 SPE system
• Controlling station with BRUKER Chromatography software.

2.2 HyStar Software

The complete liquid handling part of the LC-NMR automation is controlled by a
separate Software **HyStar®**. This software controlles the liquid chromatography
system, performs the on-line data interpretation for peak recognition. In addition
it controls the interfaces which are responsible for intermediate storage/trapping
of peaks as well as the subsequent transfer into an NMR flowprobe.
This software operates completely independent from the NMR software and is
normally installed on a separate computer.

2.3 LC-NMR Automation

The BRUKER chromatography software **HyStar®** and the BRUKER NMR
Software **IconNMR/TopSpin** are able to communicate via a serial interface. In
manual mode this interface is used to import information about the measured
peak from HyStar into the NMR dataset.
In automation mode **HyStar®** can act like a sample changer and transfer peaks
into the NMR spectrometer which are requested by **IconNMR/TopSpin**.
Peak detection in a chromatogramm and positioning into the NMR as well as
transfer of already collected peaks from loops or cartridges can be done by
HyStar® in automation, i.e. without user interaction.
Routines in **IconNMR/TopSpin** are able to perform all tasks for the
acquisition of LC-NMR spectra in full automation without user interaction. This
includes preparation like tuning&matching, shimming and lock and pulse
determination as well as the complex actions for multiple solvent suppression
and signal-to-noise controlled acquisition.
Therefore the combination of **HyStar®** and **TOPSPIN/IconNMR** are able to
run LC-NMR in full automation. **HyStar®** moves samples them into the NMR
probe and the NMR-software performs the acquisition. All information about the
sample (retention time etc.) is automatically documented in the title of the
NMR experiment.

3. General Procedures

3.1 Overview

The following section *generally* describes which actions are necessary to successfully run LC-NMR experiments and gives some background information about those procedures. For a detailed descriptions of the experiments itself check section „Experiments" on page 55.

3.2 Configure Communication HyStar-TopSpin/IconNMR

IconNMR and HyStar are communicating with serial communication. As starting with the AV Neo spectrometer no more TTY/ COM ports are available *in the console*, the communication is now established with the serical port *of the host computer*.

Standard TopSpin computers supplied by BRUKER have one build in COM/TTY port. If is is occupied for other applications, you can add more comports with USB-RS232 converters or interface cards.

- Use the supplied 10meter RS232 cable (Hz10034) to connect a COM port of the HyStar computer to the serial port of the TopSpin computer.
- In the configuration of HyStar define the COM-Port where the cable is connected a port for the NMR.
- Check in the TopSpin computer configuration for the communication port name where the cable is connected.
- In TopSpin run `cf` and define the port where the cable from the HyStar computer is connected.
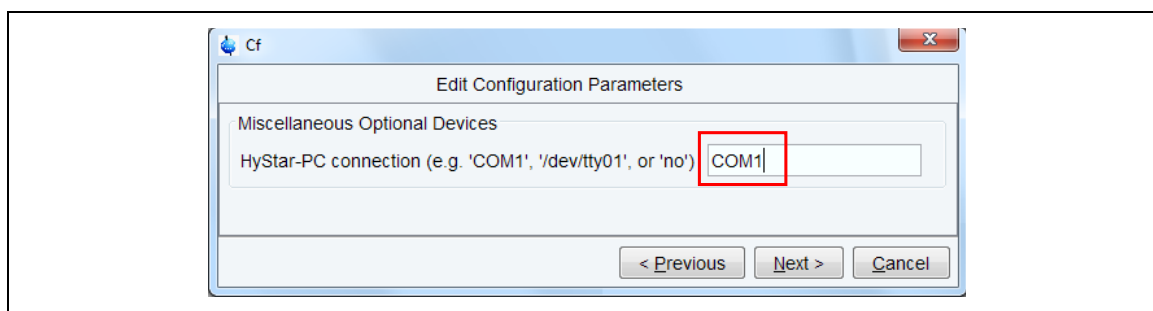
Fig. 3.1:   *Configure the communication port with HyStar.*

With a windows operation system
- The ports are named like 'COM1', 'COM2', …
- If you use an USB-RS232 converter, check in the device manager which COM port is assigned. Often COM2 is internally assigned, so that the first additional port receives COM3

With a linux operating system
- Enter the communication port as '/dev/tty01'
- Typically build in ports are labelled like 'ttyS0' and ports supplied by a USB converters are name 'tty**USB**1'
  Note that ports supplied be USB converters may change the portnumber after a reboot of the computer if multiple converters are used, or the converter is connected to a different USB port of the computer!

Once this is done…
- IconNMR can communicate with HyStar and measure multiple peaks in full automation.
- IconNMR will import information about the measured peask (chromatogramm name and retention time) and enter it in the title.

- The user can manually import the same information with the command 'getinfo'

3.3 Definition of the FlowProbe or CryoFit

For a FlowProbe or a standard Cryoprobe with is equipped with a CryoFit additional parameters which define the flow parameters of the equipment can be defined.

Most relevant is the active cell volume as defined for the used FlowProbe/CryoFit and the outer diameter of the cell. 30µl = typically 2mm (for special applications also 3mm!), 60µl = 3mm and 120µl = 4mm.

Those parameters are interpreted by TopShim. The other volumes are currently only for BEST-NMR system and for documentation.

Dedicated FlowProbe

Start <u>edprobe</u> and navigate to **Flow Parameters**. Most parameters are transferred from PICS and displayed. The cell diameter and the external capillary volume for the peak **PHFAVOL1** have to be defined by the user.
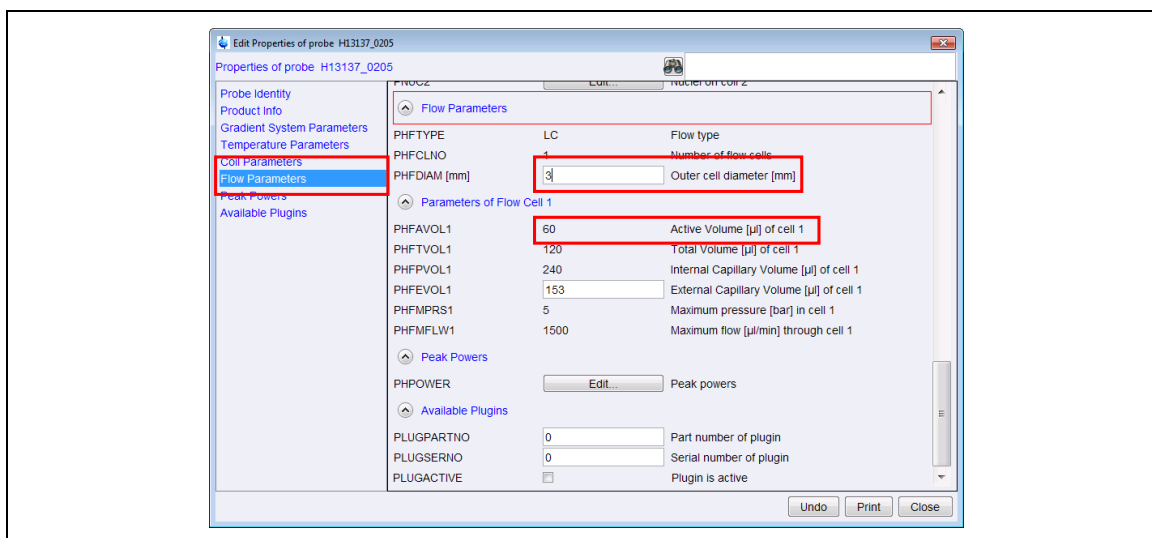
Fig. 3.2   Define the Parameters of a FlowNMR Probe.

CryoProbe with CryoFit

Start edprobe and navigate to **Available Plugins**. Define the part and serial number of the CryoFit and activate the Flag PHPGFL Flowinsert plugin installed to get access to the Flowparameters of the CryoFit.

Here all parameters can be edited by the user, as the CryoFits currently have no electronically stored information. Define the parameters according to the used Cryofit.
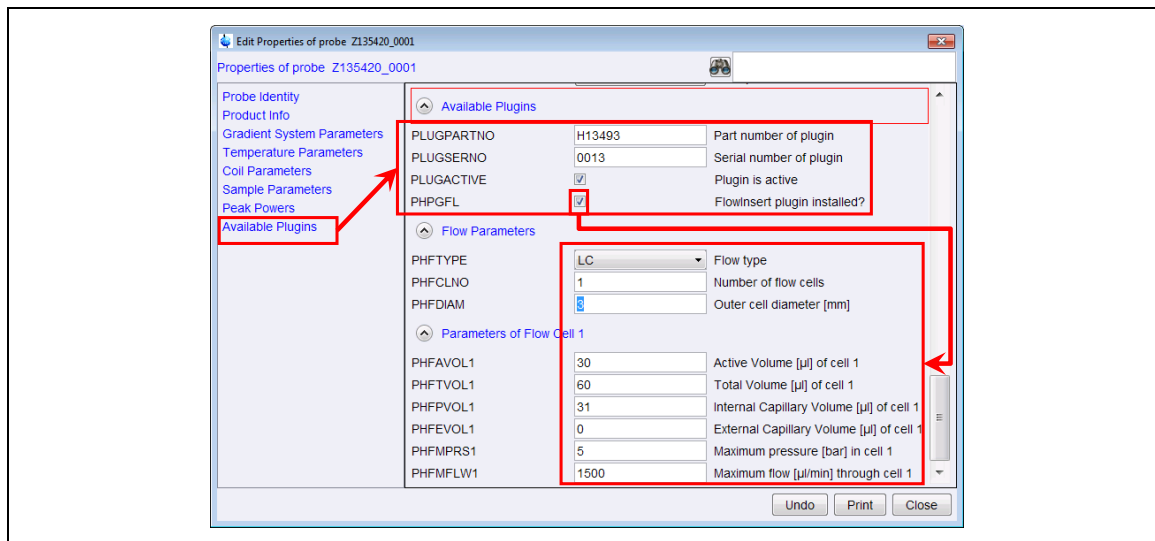
Fig. 3.3   *Define the usage of a CryoFit in a CryoProbe.*

In order to use a CryoFit (i.e. to mark in edprobe that the CryoFit is installed, you have to check both flags **PLUGACTIVE** and **PHPGFL**).

Edprobe remembers the flowparameters, even if the CryoFit is removed and the flags are deactivated. For a later usage it is sufficient to simply activate the flags and the parameters will become visible again.

Only in case you are using two different CryoFits you have to adjust the parameter each time you use a different CryoFit.

3.4 Pulses and Powerlevels

Pulses and powerleves should generally be set by Prosol. For solvent suppression experiments the following specialities apply.

Pulsecal

In LC-NMR the peaks are measured in the HPLC eluent. As typically gradient separations are done, the solvent composition for each peak is different. Therefore it is recommended to determine the 90deg pulse for each peak.
For LC-SPE-NMR all peaks are eluted in the same standard solvent acetonitrile or methanol. Therefore an pulse determination is normally not required.
Howver with pulsecal a fast and efficient procedure is available, that allows the 90deg determination in automation. Therefore I recommend using this feature. The standard AU programs au_lc1d and au_lc2d allow the automatic execution of **pulsecal**. Run the command expset to enable pulsecal for the desired experiments/parametersets.

Baseopt

Best results for solvent suppression spectra regarding phasing and baseline are reached with presatureation program that use the digitizer mode BASEOPT. For the best performance of processing it is required that you exactly determine the 90deg pulse for *each sample*. Therefore the 90deg pulse determination is required for LC-NMR and LC-SPE-NMR experiments.

Presaturation Powerlevels

The presaturation power can be set manually or by **Prosol** (see 3.5 Prosol parameters for LC-NMR). It is recommended to use getprosol to define the presaturation power.
The "power" is given in presaturation "field" so that the values can be easily applied for the actually used probe. Four three different powerlevels are in use.

- Default for presaturation is a 50Hz presatureation field. This powerlevel is adequate for aqueous samples in 5mm NMR tubes and is automatically applied when you setup the prosol parameters. However it is typically to strong for LC-NMR applications.

- For typical LC-NMR applications with acetonitrile (CH_3CN) in small volumes (typical flow cell volume is $30 - 120\mu l$) for the main solvent line the power should be reduced to the typical strong LC-NMR power for a field of 25Hz to minimize the side effects of the presatureation.

- For the "second" solvent signal in LC-NMR solvents -which represents the residual non-deuterated solvent of D_2O- a weak power should be reduced again further. A good default value is 12.5Hz.

- For LC-SPE-NMR only fully deuterated solvents are used. Visible are very weak residual protonated amounts of the solvent and a water signal from ambient humidity. For those solvent a standard presatureation field of 12.5Hz is suitable.

- With solvents of good quality and for freshly opened bottle which contain only small amounts of water, this power typically can be reduced to 5-6Hz. However this should be verified on the actual spectrum.

- For solvent suppression with modulated shape pulses, the overall power level is oriented on the strongest signal and is set to **SPDB6**. For the additional signal the power will be automatically reduced according to the intensity to the signal if the shape is generated automatically (or a manual scaling must be applied if the shape is generated manually).

Type	Field	Example	Parameter used
Default	50 Hz	*H2*O in 5mm tubes	`PLDB 9`
Typical strong	25 Hz	*CH3*CN from HPLC	`PLDB 9` `SPDB6`
		*CH3*OH from HPLC	`PLDB 9` `SPDB6`
		CH3*OH* from HPLC. I.e. the combined signal from D2O and non deuterated CH3OH	`PLDB 21`
weak	12.5 Hz	Water signal in CH3CN+D2O mixtures	`PLDB 21` `SPDB6`
		C(H/D)3CN multiplett from deuterated acetonitrile	`PLDB 9` `SPDB6`
		Water signal (Humidity) in deuterated acetonitrile	`PLDB 21`
Weak optimized	5-6Hz	As above, but verify with actual solvent quality	`PLDB 9` `PLDB 21` `SPDB6`

Tab. 3.1   *Presaturation power for LC-NMR solvents.*

3.5 Prosol parameters for LC-NMR

The procedure **Prosol** handles probehead and solvent dependent parameters (***Pro***behead***sol***vent). This means you can read a BRUKER standard parameterset, define the solvent and the command <u>getprosol</u> will set all pulses and power levels required for this experiment. Fig. 3.4-Fig. 3.9 show the most important and specially adjusted parameters for usage in LC-(SPE)-NMR. required for the setup of the most important parameters. For details read the prosol manual.

Note that an prosol provides automatic settings for most applications, however for the special LC-SPE-NMR solvents some adjustments are recommended as described below.

3.5.1 General Prosol Parameters
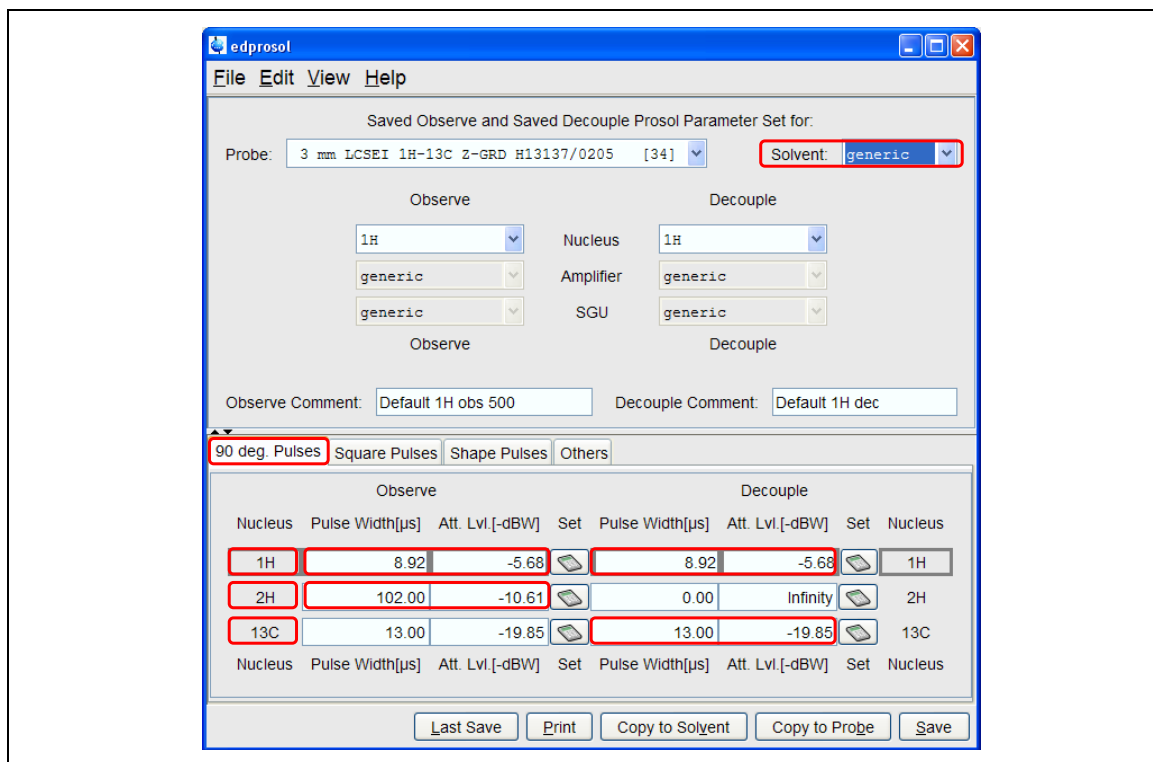
Fig. 3.4   *90deg pulse length definition*

Open the prosol editor with <u>edprosol</u>, select the solvent "generic", and check that at least the following values are defined

- **1H** Observe and Decouple (8 µsec).
- **2H** Observe pulse (required for TopShim)
- **13C** Decouple pulse (for removal of solvent signal satellites).

From the definition of the 90deg pulses ***all other pulses and powerlevels*** are automatically ***calculated***! The default settings give reasonable results, however for LC-NMR and especially LC-SPE-NMR some modifications are recommended. The modifications are shown in the next section.

3.5.2 LC-(SPE)-NMR settings in Prosol for 1H

Open the file for the generic solvent as describe in the previous section "3.5.1 General Prosol Parameters". Do <u>File|Copy</u> to Solvent C**H3CN+D2O**. **CH3CN+D2O**, **CD3CN_SPE**, CD3OD_SPE.
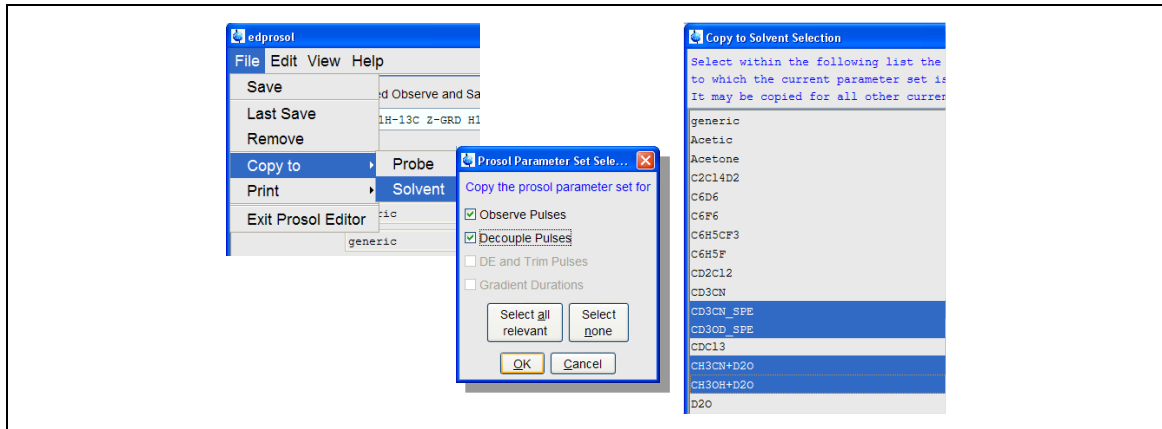
Fig. 3.5   Generate Solvent specific settings

Open edprosol for each of the Solvents. Activate the display of the RF-field with
<u>View|Show RF Fields</u>. Adjust the following pulses and repeat the procedure for
each solvent you want to use.

<u>1H Square Pulses</u>

Change the pulse #3 "Presat (cw irradiation, RF field only …) which is used for
presaturation of solvent signals.

For solvents CH3CN+D2O and CH3OH+D2O choose 25Hz for the Observe
channel (used for the ***first*** strong ***protonated*** solvent, here CH$_3$CN), choose
12.5Hz for the Decouple channel (used for the second less intens solvent, here
the residual HDO and H$_2$O)
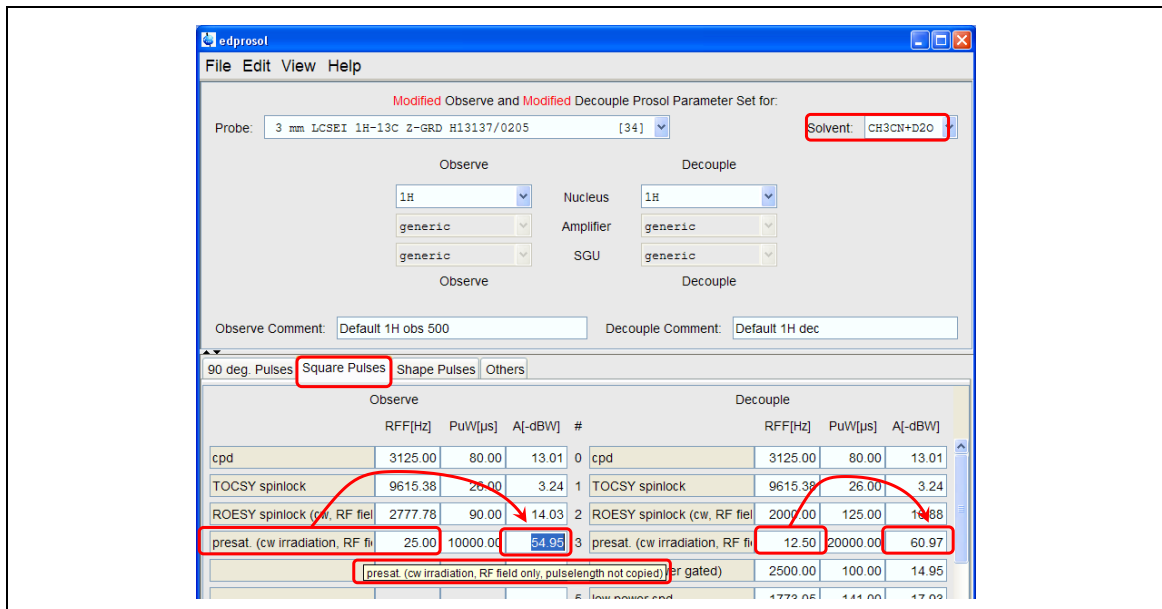
Fig. 3.6   Square pulses for mixtures of a protonated and deuterated solvents like
CH3CN+D2O.

For solvents CD3CN_SPE and CD3OD_SPE choose 12.5Hz for Observe and Decouple channel (all solvent signals are relatively weak, residual C(D/H)3CN and traces of water

Depending on the quality and water content of the deuterated solvents the powerlevels may be reduced further.

Using too high powerlevels for the presaturation (even the default 50 Hz) will not cause any damage, however the area in the spectrum affected by the presaturation will unnecessarily increase.
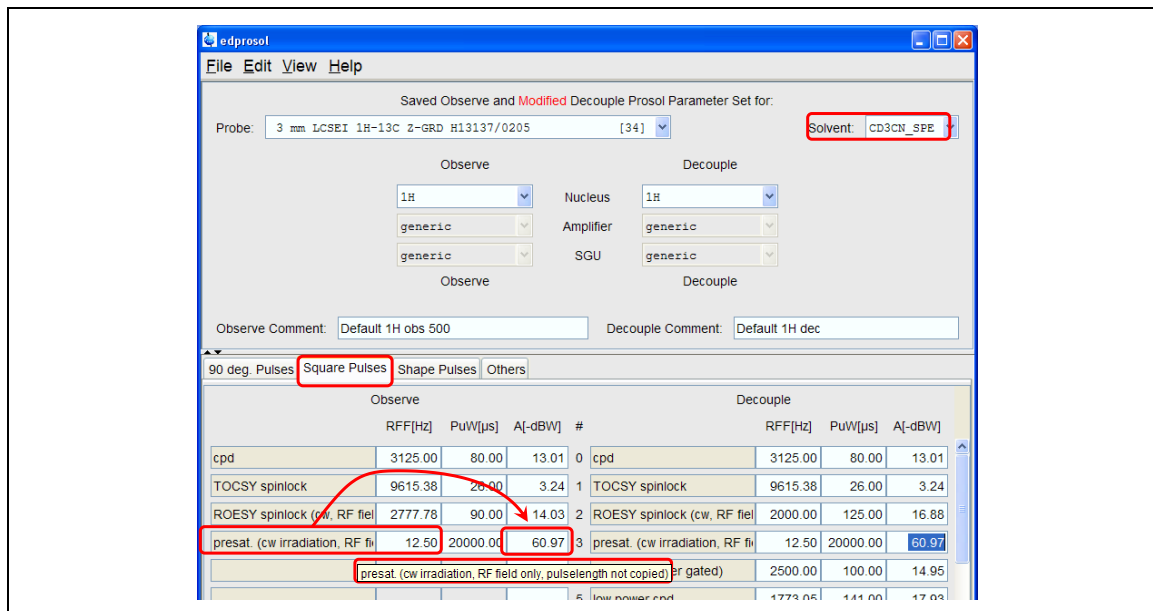
Fig. 3.7   For a fully deuterated solvents like **`CD3CN_SPE`** *.*

Note: Only the calculated power*levels* are relevant for the experiments. (The indicated message appears if you move the mouse cursor over the pulse name) The RF-field allows you to calculate the powerlevel in a convenient way. The displayed pulsewidth must be ignored, ***do not change this value***!

Shape Pulses 1H

Change the pulses #4 "off-resonance presat …" which is used for *multiple-resaturation* experiments like LC1DCWPS.
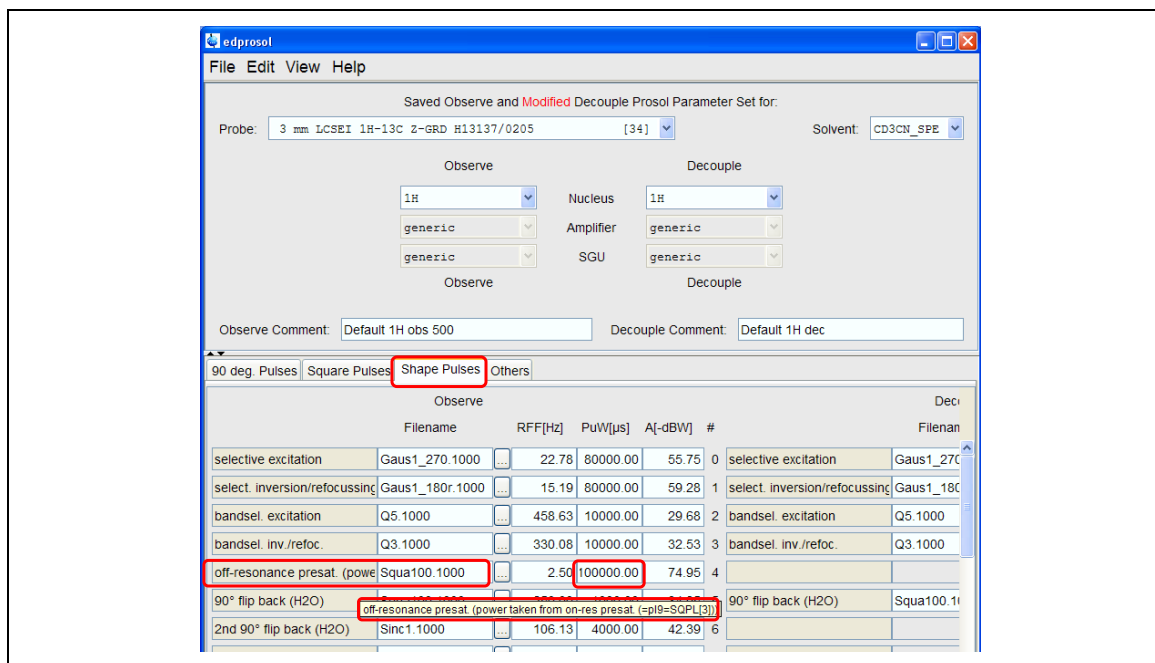
Fig. 3.8   Shape pulses for LC-SPE-NMR applications.

Set the length of the pulse to 100msec to allow precise suppression of multiple solvent signals in a spectrum.

The power and RF field is *not relevant*. It is automatically taken from the 1H Square pulses #3 "Presat (cw irradiation, RF field only …) which was adjusted in the previous section.

Note: Only name and length is relevant! Do *not change* the *powerlevel* or *RF-Field*!

3.5.1 LC-(SPE)-NMR settings in Prosol for 13C

Select 13C for the Decouple channel. The parameters are identical for all solvents, and do not interfere with other experiments. Therefore it is possible to modify the settings for the generic solvent.

90 deg Pulses 13C

Ensure that the 90deg pulses for the decouple channel is defined.
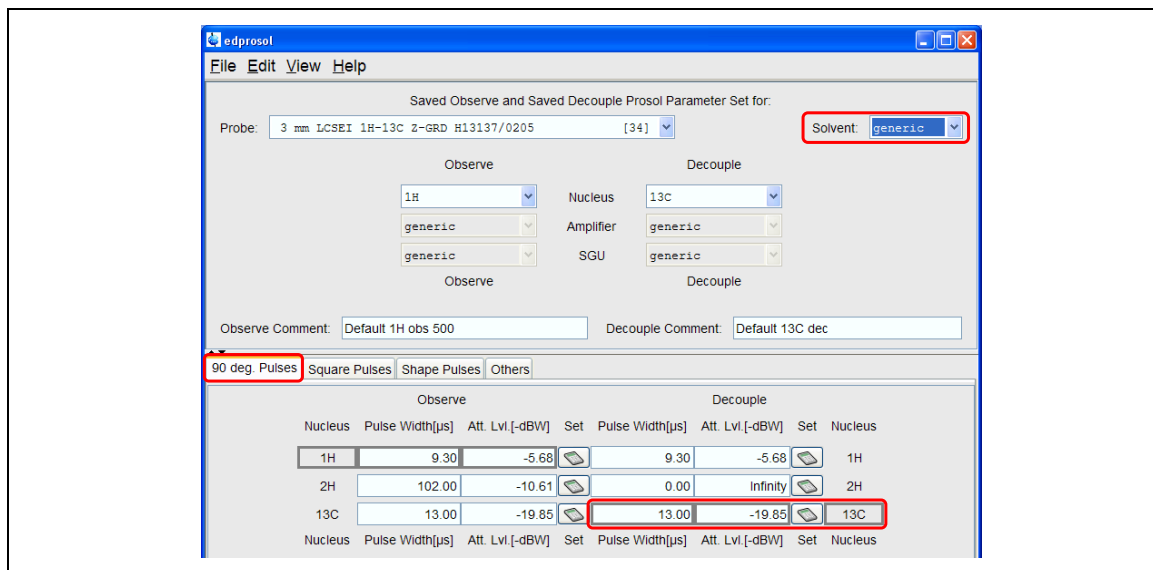
Fig. 3.9   *^{13}C standard pulses.*

In LC-SPE-NMR during one dimensional 1H spectra often a ^{13}C dcoupling is performed, to remove the intens satellites of the solvent lines. For this decoupling which might be >1sec special low decoupler powers are provided.

Pulse #3 presat is used for CW decoupling during 1H experiments (like in LC1DCWPS). The default value of 1000Hz RF filed is reasonable. For fine tuning check that the satellites of the solvent are sufficiently decoupled (the residual solvent is narrow, approx. 40Hz wide) and on the other side the lock signal does not show any disturbance during the acquisition (with too high power it will drop because the sample is heated).

Pulse #5 low power cpd is used for cpd coupling during 1H experiments (like in LC1DWTDC 1H spectrum with WET solvent suppression and 13C decoupling). Fine tuning can be done like for the ***Pulse #3 presat***.

Fig. 3.10   *^{13}C square pulses.*

3.6 Temperature Control

Temperature stabilization is *very* critical. Therefore you need to adjust the conditions so that the displayed temperature shows *no* fluctuations i.e. the instabilities are < 0.1 K.

For a room temperature LC-NMR probe, set the temperature to 293K but at least 10° above the the inlet temperature of the temperature gas. Airflow of 270 to 400 l/h is sufficient. Apply a self tune and store the determined parameters for this probe.

Check with the LC-NMR experiment LC1DCWPS that this conditions apply also *during* the experiment. Presature and ^{13}C decoupling apply considerable RF power and can cause heating of the probe.

For the usage of a CryoFit in a cryoprobes it is recommended to switch the BCU05 off. This is required, because the cooled gas from the BCU gets into contact with the inlet capillary of the CryoFit. This may lead to freezing of the solvent in the capillary and thus a blockage.

For stable temperarture you have to set the sample temperature above the temperature of the incoming gas. Without BCU this is typically 5-°C over room temperature.

Alternatively you can ignore this recommendation and have the BCU05 on. In this case you must be sure, that the solvents entering the probe will show a freezing point above approx.. -10°C. This is possible for LC-SPE-NMR applications which use acetonitrile or methanol. It can be critical with LC-NMR where mixtures of water and organic solvent are used and is definitely not allowed during testing of the CryoFit where aqueous samples (like sucres in D2O) are used.

3.7   Determination of the 90° pulse

For correct pulses you have to tune and match the probe. When using for example a mixture of acetonitrile and water the pulse length will change by only ± 5% when changing from 100% water to 100% acetonitrile, this is negligible for 1D spectra. Therefore it is sufficient to tune and match once with an intermediate solvent composition or even just on the starting eluent.
The situation is of course different when buffers are used and the concentration of buffer changes during the experiment.
Due to the availability of the new efficient routine with xau pulsecal the previously supplied AU program lcp90 is not recommended any more.

3.7.1   Automatic Determination of 90° pulse (LCP90)

<u>Description</u>
For compatibility reasons this AU program is still provided, however the usage is not recommend any more.
It is possible to integrated the 90° pulse determination directly into the setup routine of the experiments. Check the description of au_lc1d and au_lc2d.

3.8 Shimming in LC-NMR experiments

For tube probes the sample container – i.e. the tube – is exchanged each time the sample is changed. Changes in the shape of the sample tube, the positioning, the spinner geometry and the magnetic properties of the sample tubes will affect the homogenity of the magnetic field. A large part of the shimming which is required after a sample change is due to this imperfections in the sample container.

Due to this reason the shimming in a flow probe is in principle much easier. In a flow probe only the sample solution itself is changed. The flow cell remains completely fixed in the probe.

In addition the size of the flow cell is considerably smaller then for standard 5mm tubes. For 60 µl probes the inner diameter of the cell is approx. 2mm. The consequence is, that the shims in the X-Y dimension remain very stable. Shimming in X-Y dimension is often not required once the basic shim was adjusted.

3.8.1 LC-NMR

The sample is typically measured in a solvent mixtures and may be difficult.

If you work under isocratic conditions it is sufficient to shim on the starting conditions the solvent composition will remain the same and will only change very slightly especially if you have a very special sample (e.g. paramagnetic parts, air bubbles, very high sample concentrations).

If you use solvent gradients the composition of the solvent is not fixed and will change from peak to peak. Even within the flow cell the solvent composition will not be unique. First consequence is that you need to shim the probe for each new peak. As a second consequence This leads to a complicated situation which can be overcome easily if certain precautions are kept is can ob observed occasionally that the shim, obtained with the lock signal, leads to a distorted acetonitrile signal -and sample-signals- while the remaining water signal shows a good line shape.

When shimming LC-NMR eluent observe the following advices.

Manual shimming with Tune

- Use the shimming with the locklevel only if you cannot use gradient shimming.

- Ensure that a basic shimming especially was done before the first peak is placed in the flow cell.

- Shim the sample manually or with <u>tune .sx</u> an adjust Z,Y,X, Z2 and Z3 to maximum (other gradients should be shimmed before)

- Run a 1H experiment in `gs`-mode and adjust the Z-gradient to best line width/heigth of the acetonitrile. Ignore the lock, do not turn on the autoshim.

- For automation usage you can also start the AU program **lczshim** after you have optimized the with <u>tune .sx</u>. For details see „Shimming by line deconvolution" page 36.

Automatic shimming with Tune

- Use the shimming with the locklevel only if you cannot use gradient shimming.
- Ensure that a basic shimming especially was done before the first peak is placed in the flow cell.
- Choose tune .sx to adjust Z,Y,X, Z2 and Z3 (other gradients should be shimmed before)
- Start the AU program <u>lczshim</u> this will automatically optimize the lineshape of the acetonitrile. For details see „Shimming by line deconvolution" page 36.

Gradient shimming

- Gradient shimming is the preferabl type of shimming for LC-NMR eluents.
- The lineshape of the sample signals is linked to the lineshape of the acetonitrile. In LC-NMR solvents the acetonitrile is typically non deuterated, therefore you must use 1D 1H shimming (not 1D 2D shimming!).
- Due to the fixed geometry of the setup it is normally sufficient to adjust the $Z - Z^3$ shims, once the other gradients were shimmed with the starting mixture. Therefore a Z-gradient probe is suitable.
- Gradient shimming should be started with the command <u>lctshm</u>. Otherwise you must ensure that the parameters are suitable for the actual composition of the solvent in the probe (especially receiver gain and signal position for selective gradient shimming) For details see "3.8.3 Gradient shimming with TOPSHIM / lctshim"

3.8.2 LC-SPE-NMR

After elution of peaks from the cartridges the solvent present in the NMR flow cell is normally not a mixture but simply a fully deuterated solvent like acetonitrile. Concerning shimming the only difference which is important is the fact that the geometry of the flow cell is different from the geometry of a sample tube.

Manual or automatic shimming with Tune

- No special precautions are required.
- Ensure that the lock parameters are adjusted to the smaller volume i.e. lower intensity of the lock signal.
- Flow probes occasionally react very sensitive on the shims. Adjust the stepsize in the tune-file.

Gradient shimming

- The sample is present in a fully deuterated solvent, therefore you have to use 1D 2D shimming. Do not use 1D 1H shimming.
- Gradient shimming can be run with the standard gradshim or topshim procedure.

3.8.3 Gradient shimming with TOPSHIM / lctshim

Description

With TOPSPIN 2.0 a new gradient shimming routine TOPSHIM is introduced. For the preparation and other details check the TOPSHIM handbook. The handling of shimming with TOPSHIM is highly automated and detects most of the necessary parameters by itself. Especially the differences between the deuterated and non deuterated solvents the the different volumes Handling of different configuration like flowprobes with small volume for partially non deuterated solvents in LC-NMR, LC-SPE-NMR with fully deuterated solvents and standad applications with 5mm tubes probes and the switching between these configurations is much easier to handle then with the previous gradshim routine.

Setup gradient shimming

Typically you will have to perform one procedure with a partially non deuterated sample like H2O+D2O. For details check the TOPSHIM handbook.
Ensure that the prosol parameters for the probe are completely defined, including the 2H pulses.

CryoProbes with CryoFit

For the usage of a CryoFit, it is required to define this in edprobe. You have to activate the *two* flags **PHPGFL** and **PLUGACTIVE.**
TopShim takes the cell geometry from the parameters defined in the flowaprameters. Relevant are the outer cell diameter and the active volume. For details see 3.3 Definition of the FlowProbe or CryoFit.
If this is not done, the performance will be slighty inferior as TopShim will assume the sample size for a normal 5mm tube sample.
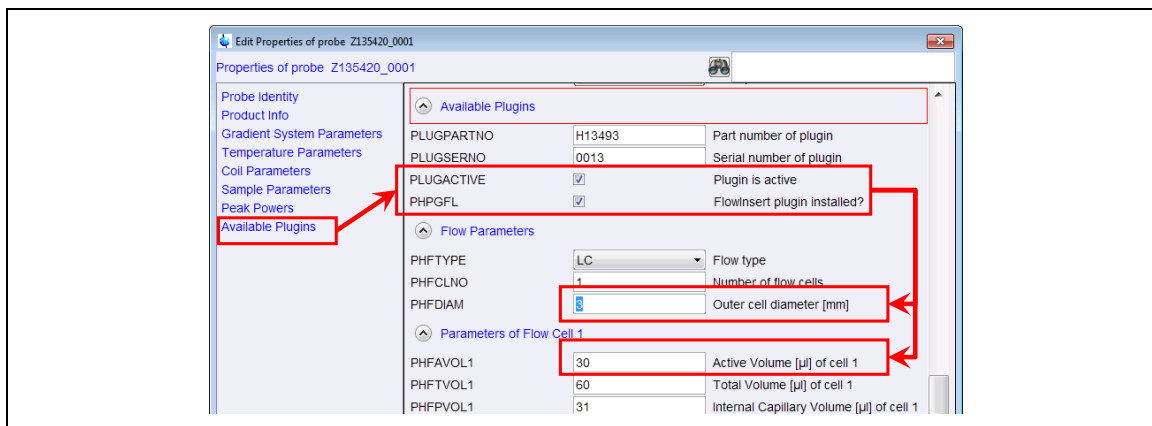
Fig. 3.11   Define the usage of a CryoFit in a CryoProbe.

Gradient shimming with 'topshim' command

You must correctly setup your dataset. I.e. you must use the 'lock' command, because TOPSHIM will determine the shimming method from the current solvent.

Simply start the shimming with the command <u>topshim</u>. From the solvent the the system will decide that 1H shimming is used for solvents like CH3CN+D2O and CH3OH+D2O and 2H shimming for CD3CN in LC-SPE-NMR mode.

For non deuterated LC-NMR solvents like CH3OH+D2O with extreme solvent compositions i.e. 10% CH3CN or 90% CH3CN it might be usefull to start TOPSHIM with <u>topshim rga</u> to compensate for the changing concentration of the solvent signal.

To compensate the shift of the solvent line the solvent position you can determine this position from a test spectrum and define it commandline parameter. For example as <u>topshim rga o1p=3.8</u> for a acetonitril line close to the water.

Gradient shimming with AU program lctshim

An AU program is available which perform the above tasks automatically. Start the shimming with the command <u>lctshim</u>.

For 'normal' solvents (CD3CN, Acetone, …) the AU program it will start <u>topshim</u> and all the standard parameters are used. The only two modifications are

* It will enforce rga. This takes only little time and will compensate for any changes in composition of solvents.

* It will use the parameters of the solvent defined in the starting dataset, rather then the solvent used the last time by lock. This allows you to easily change the used solvent.

For the complex LC-NMR solvents For the special LCNMR solvents CH3CN+D2O and CH2OH+D2O an enhanced procedure is performed.

* A dataset name **LCTSHIM 1 1** on the current **DISK** and for the current **USER** is created.

* The parameterset LCZG together with the solvent from the starting dataset is used and the prosol parameters are read in.

* A 1H spectrum is acquired and the biggest line in the region 4.5 – 1.5ppm for CH3CN+D2O and 4.0 – 3.0 ppm for CH3OH+D2O. is searched.

* For the topshim routine the settings for the solvent of the foreground dataset are used. In addition the optimized O1, a small bandwidth selective exiction and a optimization of the receivergain is defined.

In any case zou can open the user interface with <u>topshim gui</u> and on the report page you can oserver the proceeding of the automation, even if the shimming was not started from the interactive windw.

3.8.4 Gradient shimming (gradshim1d1h)

Gradient shimming with the program gradshim is now replaced with TopShim. Topshim provides better performance and higher degree of automation.

For automatic shimming under LC-NMR conditions with the procedure gradshim the AU program **lcgshim** is still provided, however preferably use the procedure with topshim and refere the the details as described in "3.8.3 Gradient shimming with TOPSHIM / lctshim".

3.8.5 Shimming by line deconvolution (LCZSHIM)

New
The functionality has dramatically improved. Multiple gradients can be shimmed with one function call. Stability of signal search and deconvolution is improved.

This shimroutine is also suitable for HR-MAS probes with biological samples where TopShim is not implemented and the deuterium signal cannot be used for shimming.

Description
The described experiment is designed for configurations where *tune sx* or *topshim* are not working. These are especially complex solvent mixtures where shimming on the lock signal does not provide a good shim quality, probes without gradients or HR-MAS probes where gradient shimming is not implemented.

The linewidth of the biggest signal in a userdefined region is measured by linedeconvolution with *mdcon*. By iteratively changing the defined shim gradient the system tries to find the shims that give the most narrow line. The procedure can be repeated subsequently for multiple shim gradients. The procedure is carried out by the AU program **lczshim**.

A singulett that is isolated by approx.. 50Hz from other signals is required, for example the TSP/TMS signal. Experiment parameters (NS, solvent suppression) can be adjusted so that also weak signals in the presence of strong solvent signals can be used fro shimming.

Run the experiment automatically
All required parameters for this experiment can be predefined in a parameterset, fetched from the starting data set or determined online. The AU program **lczshim** runs the experiment automatically.

The automation is controlled by some special parameters which are not used in the pulse program and will not appear in <u>ased</u>.

Preparation of the experiment

- Start the setup-routine with <u>'xau lczshim <ShimName> setup-yes'</u>. Where <ShimName> is the name of the shim to optimize, vor example <u>'xau lczshim XZ setup-yes'</u>

- Follow the instructions in the interactive setup dialog.

- All requested parameters will be stored in parameterset **LC<ShimName>SHIM_TMP**

Procedure

To start the shim procedure do in

- the TopSpin commandline 'xau lczshim <ShimName>'

- IconNMR enter as Shimcommand 'XAU lczshim <ShimName>'

The AU program lczshim performs the following tasks automatically

- Get solvent and **O1P** from the original data set.

- Change to the data set **LCZSHIM_TMP** and load the parameters with
  rpar LC<ShimName>SHIM. Set parameters **O1**, **SOLVENT**, perform
  getprosol.

- Do one experiment to find the position of the biggest line in the plot region.

- Measure linewidth of biggest line

- Change <ShimName> by userdefinded amount and find the direction where
  the linewidth gets smaller.

- Continue in this direction until the line gets again broader.

- Repeat the procedure with 50% of the step size until the maximum number
  of iterations, minimum stepsize or the lower limit for the line width is
  reached.

- <ShimName> may contain multiple Shims. Theare are treated in the given
  order. For example 'XAU lczshim Z X Y Z XZ YZ Z' is a valid command.

Parameters

Parameter	Default value	Description/Comments
AUNM	**lczshim**	
O1P		From starting dataset. Allows to perform solvent suppression during lczshim if the O1P in the starting dataset is correctly adjusted.
NS	2	Adjust to intensity of shim signl
PULPROG	zg30	For spectra with strong lines avoid truncation with zg30, alternatively ensure correct O1P in the starting dataset and set zgpr or similar.
F1P/F2P	0.25 -0.25	Reagion for the shim signal
CY/MI/MAXI	1000,990,1100	Adjust that pp finds just ONE peak in the plot region
L 28	16	Initial stepsize for gradient variation. This stepsize must show a considerable effect on the line width
L 29	1	Minimum stepsize for gradient. This should be the lower variation that shows an effect on a already well shimmed line.
L31	16	Maximum number of iterations for shimming
CNST 27	1	Lower limit for line width.

Tab. 3.2:   *Special Parameters controlling* **lczshim***'*

Note

If called without commandline parameters the program peforms the shim of the Z gradient as before.

If the parameterset with the userdefined shim parameters (for example LC*XY*SHIM_OPT for the gradient XY) does not exist, the AU program first tries to find the parameterset for the standard shim gradient i.e. LC*Z*SHIM_OPT. If this also does not exist the realese parameterset LCZSHIM is used. Therefore it is possible to shim any gradient without specially preparing parameters for it.

3.9 Acquisition of spectra with solvent suppression

3.9.1 Introduction

Solvents in LC-NMR are often non-deuterated and therefore have intense signals which require solvent suppression. This solvent suppression is necessary to reduce the overall intensity of the FID. Only if the dynamic range of the receiver can be adapted as much as possible to the intensity of the analyte signals they can be digitized.

Water for example has a hydrogen concentration of approx. 100 mol/l (1 g/ml, M=18g/mol). If 1 µg of a sample with a molecular mass of 200g/mol is present in the flow cell volume of 100 µl this results in a concentration of

　　　　　1 µg (Sample) /100 µl (solvent) = 5 nmol/100 µl = 50 µmol/l.

This would result in an relative intensity of sample to solvent signal of $1 : 2.000.000 \approx 1 : 2^{12}$. It is obvious that with a digital resolution of typically 2^{16} - 2^{18} bit the analyte signal are not detectable in presence of the unsuppressed solvent signals. As a consequence the usage of standard parametersets like **PROTON128** or **COSY** is not suitable to detect the sample signals.

Standard parameter sets are available for the suppression of solvent signals. The solvent resonance of water in an H_2O/D_2O mixture is observed at 4.7 ppm. Therefore the solvent suppression for such an aqueous solution can be done by setting the **O1** to 4.7 ppm together with an standard parametersets like **ZGPR** or **COSYPR**.

However, for a *good* solvent suppression which results in minimum baseline distortion, best suppression and minimum interference with nearby analyte signals the determination of the frequencies by ± 0.1-0.5 Hz is necessary.

Furthermore, in contrast to spectra in H_2O/D_2O the LC-NMR solvents are normally mixtures which contain more then one solvent resonance where the exact position of the resonances depend on composition, pH and temperature. In a mixture of acetonitrile and water the resonance of the acetonitrile signal occurs between 2.0 and 4.6 ppm depending on the water content. For a 500 MHz spectrometer this corresponds to a displacement of more then 1000 Hz for 100 % or 10Hz per 1% changement of the composition.

For the acquisition of spectra under those conditions special experiments are provided which are suitable for the suppresion of two or more solvent resonances. However certain parameters, first of all the exact solvent positions but also the receiver gain and others have to be adjusted for each individual sample. To locate the position of the solvent resonance a conventional spectrum (further called **preparation experiment**) without suppression is acquired. From this spectrum the frequencies of the solvents are determined. These frequencies are used as **O1**, **O2** etc. or as frequencies for offset-modulation of shapes in the experiment with solvent suppression (further called **main experiment**).

The following two chapters describe the general procedure for automatic and manual usage of those parametersets. This will be explained at the example of a simple 1D experiment with double presaturation. For a detailed description of

other parametersr which have an effect for certain experiments check chapter „4 Experiments ".

3.9.2 Automatic Procedure

Besides the general procedures like shimming, tuning & matching and temperature control for the preparation the spectrum acquisition with solvent suppression consists of

- Selection of experiment type (presaturation, PFG suppression, ^{13}C-decoupling)
- Adjustment of pulses and powerlevels (values stored in prosol).
- Adjustment of certain general parameters like number and position of solvent lines (to be defined by the user)
- Start of the Acquisition with xaua which automatically performs
    - Acquisition of a solvent search spectrum (Automatic)
    - Identification of solvent lines and adjust of the individual parameters like the exact solvent position and receiver gain (Automatic)
    - Acquisition of main spectrum (Automatic)

The complete procedure for a spectrum acquisition is given below.

Action	TOPSPIN command
Create a new dataset	Edc

- Use the same experiment *name* for all peaks in a chromatogram. Choose the name related to the chromatogram name.
- Choose experiment number 10 for the first peak and 11,12, … for additional experiments this peak. Choose number 20 for the second peak …

Shimming	lctshim

- Shimming must be done for *each* peak.
- Observe the precautions when shimming LC-NMR eluents

Prepare the experiment parameters	rpar

- *Always* read a parameterset. Do *not* use the parameters from the previous dataset which are copied by edc.

Lock the spectrometer	lock

- Use special LC-NMR solvents like CH3CN+D2O or CD3CN_SPE *not* standard solvent D2O / CD3CN

Action	TOPSPIN command
Tune the probe	Atma or wobb

- Tune and match the probe.
- This step is only for the first peak if a isocratic separation is performed or if a solvent gradients without any salt/buffers are used.
- In other cases, for best performance and for 2D experiments perform tuning matching for each peak.

Set pulses and powerlevels	getprosol

- This will enter the default values which might be later changed by the automation.

Start the LC-NMR specific experiment setup program	expset

- This AU program requests special automation parameters. It allows you to conveniently define the most important parameters for solvent search, signal-to-noise control and other automation steps.

To modify other parameters	ased, eda, edp

When changing parameters keep in mind

- The AU-program will change certain parameters which will overwrite the changes you apply here.
- The automation is controlled by a large number of parameters which are not displayed in ased.
- Do not change any processing parameters because this can affect the peak picking and thus the solvent search.

Start the experiment	xaua

- xaua will start the automation program defined in the parameter **AUNM**. Typically this is au_lc1d or au_lc2d. All steps of the automation are done by these programs.
- Do *not* start the experiment with zg! Some important parameters like the frequencies, receivergain need adjustment according to the solvent resonances and *must* be adjusted for each individual sample. This is only done when you start the automation with xaua.

Action	TOPSPIN command
Acquisition of solvent search spectrum	automatic

- The automation will acquire two spectra. First a specrum with modified parameters (decreased NS and RG and increased SW) is acquired which is used to search the solvents.
- When the first spectrum is processed and evaluated the main experiment starts. The first spectrum you see on the screen is only a solvent search spectrum. You will not be able to see any sample signals in this spectrum.
- The same applies for 2D experiments. The solvent search spectrum is acquired as 2D experiment.

Acquisition of main spectrum	automatic

1D experiments
- While the main spectrum is running you can also transfer the acquired NS to the disk with tr and process the spectrum. When you decide that the signal-to-noise is sufficient, terminate the spectrum with stop or halt.

2D experiments
- While the main spectrum is running you can always process the already acquired rows with xfb.

Optimization of parameters	stop, gs, zg

- When you find that the automatic adjustment of the parameters has not worked properly, you can stop the experiment with stop.
- Preferably start the acquisition with gs and modify the desired parameters, for example **RG**, **O1** or the suppression powerlevels,
- To restart the experiment you must use the command zg, otherwise the automation will again overwrite the parameters and cancel you modifications.

Repeat the experiment	rpar, …

- If you want to rerun the experiment either for the same or another sample, do ***not*** run xaua on the this dataset or a copy of this dataset. Once you have executed xaua, you should always reread the original parameterset and restart the procedure from the top of this list.

3.9.3 Manual Procedure

There are two possibilities to acquire the preparation experiment. You can use either use the standard pulse program **zg**, described in the first section. A more complicated preparation experiment which delivers more precise frequencies, is described in second part.

Preparation experiment with pulse program zg
A preparation experiment is acquired, using a simple proton spectrum with one scan and the pulse program **zg**. As explained later, the solvent positions derived

from this experiment will require some fine adjustment in **gs**-mode using the main experiment.

Acquire the preparation experiment

- Create a new data set with <u>edc</u>. Use experiment number 1
- Read the parameter set for the preparation experiment <u>rpar LCZG all</u>.
- Lock the spectrometer with <u>lock <solvent name></u>
  (for example **lock CH3CN+D2O**)
- Set pulses and power levels manually or by **prosol**
  <u>eda\prosol</u> or <u>getprosol.</u>
- Acquire the spectrum with <u>zg</u>
- Transform and phase the spectrum with <u>ef</u> and for example <u>apk</u>

Find the solvents

- Enter the **utilities** menu and set **O1** on the larger and **O2** on the smaller solvent. Note the values you have found for **O1** and **O2**.[1]
- Create the main data set. Preferably to ***not*** overwrite the solvent search spectrum.
- Read in the parameter set for the suppressed spectrum:
  <u>rpar LC1D12 all</u> (for example)
- Lock the spectrometer with <u>lock <solvent name></u>
  (for example **lock CH3CN+D2O**)
- Set pulses and power levels manually or by **prosol**
  <u>eda\prosol</u> or <u>getprosol.</u>
- Enter the values you previously have found for O1 and O2.
- Determine the receiver gain with <u>rga.</u>

Preparation experiment with original pulse program

A problem arises if the frequencies are taken from a preparation experiment as mentioned above. Typically this experiment is acquired with one single scan and the pulse program **zg** whereas the main experiment is later carried out with presaturation and even with carbon decoupling. In other words the situation during the main and the preparation experiment is completely different.

During the presaturation rf-power is applied to the sample which can cause Bloch-Siegert shift or simply signal shift by heating effects. As already mentioned, mixtures like acetonitrile/water are very sensitive for temperature changes. This effects can easily lead to a displacement of several hertz.

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[1] You can also put the cursor on the spectrum and read the displayed frequencies of the solvents directly from the spectrum. Keep in mind that the displayed values in ppm/Hz are O1 + SR! If the spectrum was calibrated (i.e. SR <> 0 Hz) this values are cannot be used for O1.

The situation becomes much worse if carbon decoupling during **AQ** is applied. Here the displacement of the acetonitrile resonance line is in the order of 10 - 30 Hz compared with the position observed in a single pulse spectrum.

Whereas in a simple presaturation experiment a fine adjustment of the frequencies can be easily done in **gs**-mode this is more complicated for all experiments applying shaped pulses or in experiments running under automated conditions. A solution is to acquire the preparation experiment with the original pulse program.

This means a solvent suppression experiment is carried out with the suppression frequencies set off-resonance. As all other conditions (presaturation power & length, decoupling power & length, timings, ...) are the same, the solvent positions correspond exactly to those which will be found later during the main experiment. Thus a fine adjustment of parameters is normally not necessary.

Acquire the preparation experiment

- Create a new data set with <u>edc</u>.

- Read the parameter set for the main experiment <u>rpar LC1D12 all</u>.

- Lock the spectrometer with <u>lock <solvent name></u>
  (for example **lock CH3CN+D2O**)

- Set pulses and power levels manually or by **prosol**
  <u>eda\prosol</u> or <u>getprosol.</u>

- Change **NS** to 4 to shorten the experiment.

- Change the following parameters, so that the solvents are *not* suppressed
  O1, **O2** approx. -2ppm, **RG** = 4.

- Acquire the spectrum with <u>zg</u>

- Transform and phase the spectrum with <u>ef</u> and for example <u>apk</u>

Find the solvents

- Enter the **utilities** menu and set **O1** on the larger and **O2** on the smaller solvent. Note the values you have found for **O1** and **O2**.[2]

- Create the main data set. Preferably to *not* overwrite the solvent search spectrum.

- Read in the parameter set for the suppressed spectrum:
  <u>rpar LC1D12 all</u> (for example)

- Lock the spectrometer with <u>lock <solvent name></u>
  (for example **lock CH3CN+D2O**)

- Set pulses and power levels manually or by **prosol**
  <u>eda\prosol</u> or <u>getprosol.</u>

- Enter the values you previously have found for O1 and O2.

---

[2] You can also put the cursor on the spectrum and read the displayed frequencies of the solvents directly from the spectrum. Keep in mind that the displayed values in ppm/Hz are O1 + SR! If the spectrum was calibrated (i.e. SR <> 0 Hz) this values are cannot be used for O1.

- Determine the receiver gain with <u>rga.</u>

Fine tune the main experiment

The following procedure should be only necessary if the solvent positions where derived from a **zg**-spectrum.

- Go into the acquisition window with <u>acqu</u> and start **gs**-mode with <u>gs</u>

- For the fine adjustment show the separate display of odd and even points with the **ush** button.

- Use the **gs**-control window to adjust **O1** until the FID decays without modulations to zero. A sinusoidal modulation indicates that **O1** is off-resonance. See Fig. 3.12 on page 45.

- For best performance adjust the presaturation power for minimum FID area, after you have optimized **O1**/**O2**.

- Optimize **RG**. When you first enter the acquisition window the vertical size of the window corresponds to the available range of the A/D converter. The FID should fill this window approx. 50 %.
  With 60% acetonitrile an **RG** of 256-512 (500MHz, 4mm probe) should be reached
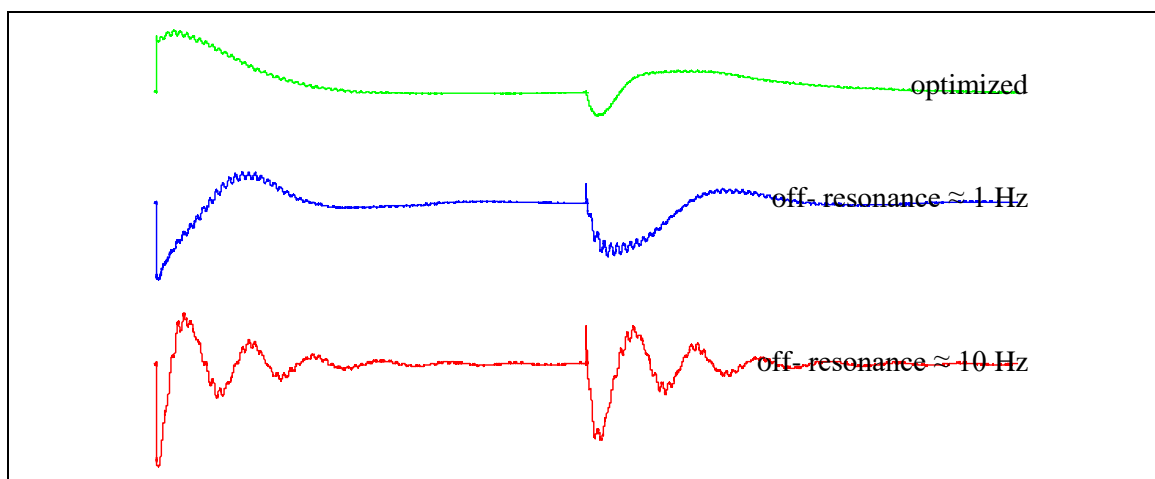
Fig. 3.12:   *Optimization of O1 in* **gs**-*mode.*

Acquisition of the main experiment

After the parameters where optimized as described above, you can start the main experiment. Preferably set it up with a high **NS** and terminated it after the desired signal-to-noise is reached. Otherwise you have to go through the **DS** a second time.

- Stop **gs**-mode with <u>halt</u> and ***immediately*** start the main experiment with <u>zg</u>
  This is especially necessary if you are running an experiment with ^{13}C decoupling. Here it takes 20 - 30 scans to establish a steady state where the signals have reached a stable position. Any delay between **gs** and **zg** will disturb the steady state.

- Check the intensity of the FID. Often the 2nd or 5th scan is more intense than the first one (which cannot be observed in **gs**-mode as there no phase cycling is done). If so it might be necessary to reduce **RG**.

- Do tr to transfer even number of scans to the disk, to observe the resulting spectrum. Go into the acquisition window and do tr when you see **NS** 7 to transfer a spectrum with **NS** 8 to the disk.

3.10 Shaped pulses for multiple offset solvent suppression

For multiple solvent suppression experiments *only one* proton channel is used to apply a pulse which *simultaneously* has an effect at several positions in the spectrum. The standard spectrometers are equipped with 2 channels. Therefore multiple offset solvent suppression is useful for:

- suppressing more than 2 frequencies

- suppressing 2 frequencies while using the second channel for an X nucleus e.g. ^{13}C decoupling

The generation of the shape is described below. After the shape has been generated, an optimization in GS-mode is not possible for the individual solvent frequencies. Keep in mind that if O1 is moved, the other frequencies are moved too. As O1 is normally set to the most intense signal, it can be changed to increase the suppression of this signal at the expense of the slight decrease of the suppression of other (weak) signals.

To change the suppression for the individual frequencies you have to generate a completely new shape.

3.10.1 Shape generation

TOPSPIN allows a convenient calculation of shaped pulses. In TOPSPIN do Help|Application Manuals|Shape Tool to find a detailed description of how to use it.

Acquire the preparation experiment

- Change to a dataset with expno = 1.
- Read the parameter set **LCZG**.
- Set pulses and power levels with getprosol.
- Acquire the spectrum with zg.
- Transform the spectrum with ef and apply phase correction with apk0
- Note the frequency of the spectral lines which should be suppressed.

Create a presaturation shape

- Start the shape tool with stdisp

- Create a new shape with Shape|Basic| Rectangle with 1000 points and 100%

- Choose Manipulate\Phase modulation according to ... and modify the shape with the desired frequencies.

- Pay attention how the shape is centered. Preferably us the option **Reference** as **O1 from current data set** or **first entry in frequency list**. In the later case the frequency list must contain the O1 (as first entry) and the n solvent frequencies so all together n+1 entries for n solvents.

- Choose scaling and decrease the power slightly for smaller peaks.

- Store the shape under a new name and enter this name as **SPNAM6** in the suppression dataset.

- Change O1 according to the reference of the shape.

- Change the powerlevel **SPDB6** according to the number of frequencies which are suppressed. **SPDB6 = PLDB 9** for one frequency, **SPDB6 = PLDB 9** – 6dB for two frequencies etc.

- For further parameters check the experiment description, for example „1D [1]H Spectrum with mult. Presaturation. LC1DCWPS/ LC1DCWGPPS with ^{13}C decoupling" on page 64.

Create a selective excitation shape for WET solvent suppression

- Start the shape tool with <u>stdisp</u>

- Create a new shape with Shape|Clasic|Sinc and choose 1000 points and 1 cycle

- Choose <u>Manipulate\Phase modulation according to ...</u> and modify the shape with the desired frequencies.

- Pay attention how the shape is centered. Preferably us the option **Reference** as **O1 from current data set** or **first entry in frequency list**. In the later case the frequency list must contain the O1 (as first entry) and the n solvent frequencies so all together n+1 entries for n solvents.

- Do ***not*** use scaling, the same powe must be applied for all frequencies.

- Store the shape under a new name and enter this name as **SPNAM7..10** in the suppression dataset.

- Change O1 according to the reference of the shape.

- Change the powerlevel **SPDB7..10** according to the number of frequencies which are suppressed.

- For further parameters check the experiment description, for example „1D [1]H Spectrum with WET solvent suppression and ^{13}C decoupling" on page 70.

3.10.2 Shape pulse suppression in automation

Fully automated suppression with an (nearly) arbitrary number of frequencies is possible. During the automation the required shapes will be automatically generated. For details check the description of the AU-program lcsetsup in „AU-Program **lcsetsup:** Search for solvents" on page 130.

3.11 Processing of LC-NMR Spectra

3.11.1 Processing of 1D LC-NMR Spectra

If the very intense solvent signals do not decay during the acquisition, distortions around those peaks will be observed. If the distortions cannot be eliminated with an em with **LB** = 1.0 use a **shifted square sinebell** (QSINE SSB=2.5) as window function.

If the spectrum shall be processed with smaller **LB** you can also use linear prediction to reduce the effects of zero filling. Therefore switch on linear predictions with

> edp edit processing parameters
> **ME_MOD** lpfr = forward real choose prediction mode
> **LPBIN** 0 number of points to predict, 0 for all points from TD to SI
> **NCOEFF** 128 number of coefficients

Phasing

Phase correction must be done upon analyte signals. Don't try use the residual solvents as reference for the phase, normally they show a strong phase distortion.

Baseline correction of the FID

A remaining phase distortion around the suppressed solvent signals can be further reduced using the baseline correction mode of the FID. Therefore set

> **BC_MOD** qpol

The position of those functions is defined relative to O1 with the parameter

> **COROFFS**

By changing this parameter $\pm$ 1-5 Hz can improve the baseline around the solvent signals.
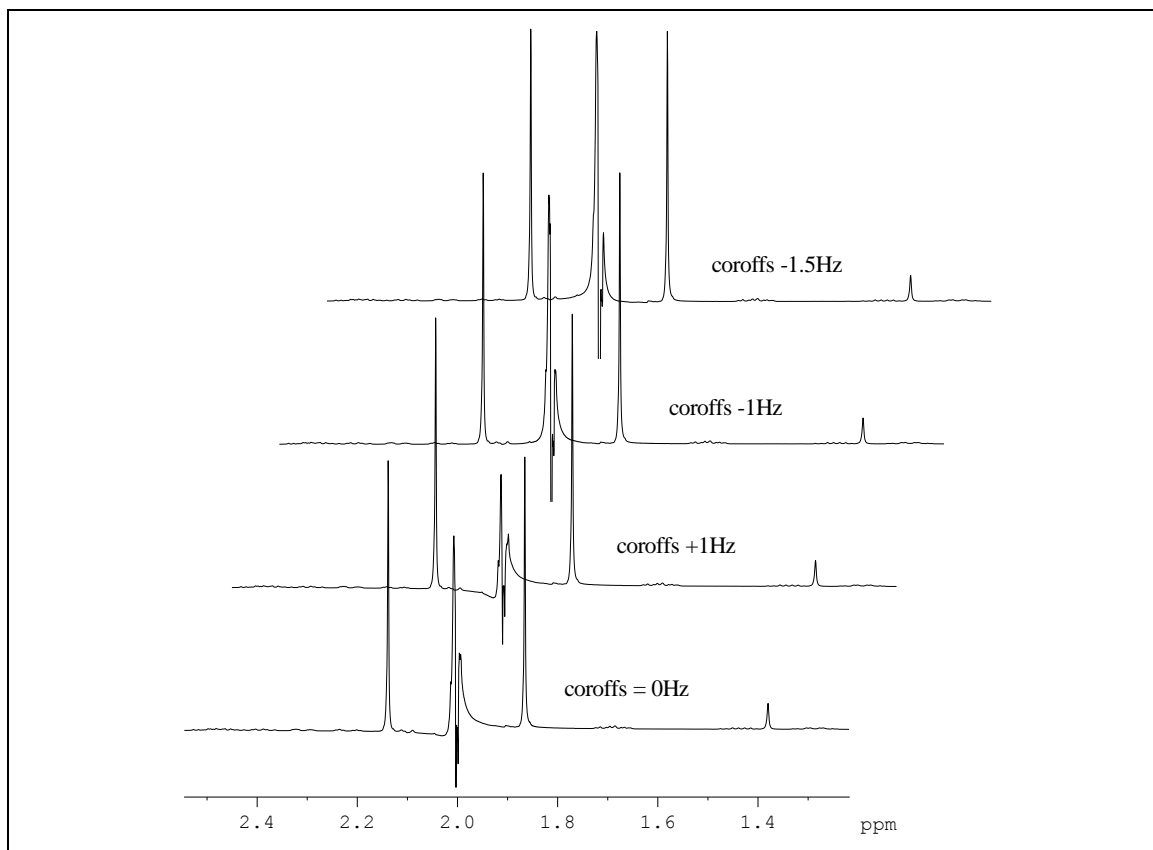
*Fig. 3.13:   Optimize baseline by adjusting **COROFFS**: 0 Hz default, +1 Hz wrong direction, disturbance getting bigger, -1 Hz seems o.k., 1.5 Hz too far.*

Baseline correction of the spectrum

The remaining baseline distortions should be corrected in several steps. Use the function <u>absf</u> and set the limits of the correction as **ABSF1** and **ABSF2**. Reduce the width of the corrected region by excluding the regions where the baseline correction was applied successfully.

Set the left value (**ABSF1**) to the left solvent, set the rigth value (**ABSF2**) to the right border of the spectrum. Execute the baseline correction. Now move the right border towards the solvent. Choose a position where the previous baseline correction still worked, i.e. the baseline is on zero.

This procedure can be done automatically using the AU-program **lcabsf**. For details check page 136 „AU-Program **lcabsf:** Baseline correction".
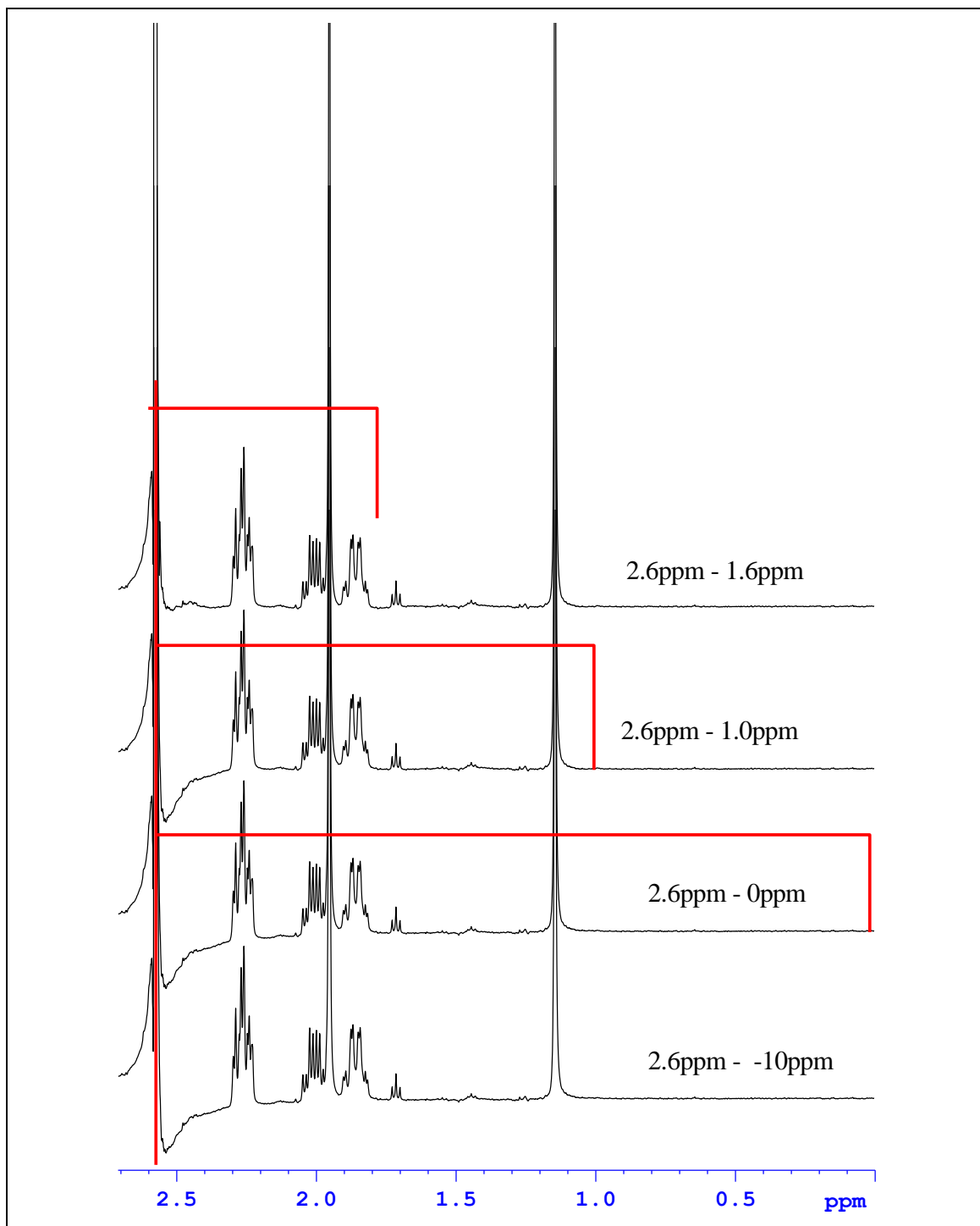
Fig. 3.14:   *Baseline correction with <u>absf</u> in several steps. 1. **ABSF1** 2.6, **ABSF2** -10 then do*
                *<u>absf</u> 2. **ABSF**=0 then do <u>absf</u> again 3. **ABSF**=1.0 then do <u>absf</u> again* ..

3.11.2 Processing of Onflow 2D LC-NMR Spectra

For 2D spectra the same rules apply. Use <u>xf2</u> to transform the spectrum. As the AQ is normally short in pseudo-2D experiments you'll have to use a shifted QSINE window function with SSB 2 or 2.5.

Scaling the f1-axis

The spectra can also be scaled in f1 dimension (HPLC time axis).

- Calculate the total duration with <u>expt</u>
- Divide the result by the number of rows. This is the time resolution in f1 in min/row.

Enter the result into the parameter **IN0** and **1S IN0**. Also set **ND0** and **1S ND0** to 2.

If you set **IN0** = 60 sec with a runtime of 1 h TOPSPIN will display numbers between 0 and 3600. It is more convenient to enter the time in minutes (enter **1S IN0** 1.0 for 60sec = 1.0min). Now the retention time in minutes will be displayed (even so the label of the axis say 'sec')

Phase correction

For phase correction the normal 2D phase correction subroutine can be used.

Baseline correction

The baseline correction is executed with <u>abs2</u> instead of <u>absf</u>. The parameters used are also **ABSF1** and **ABSF2**. Use the same procedure as in the 1D spectra, i.e. perform the correction in steps with decreasing width for <u>abs2</u>.

This procedure can be done automatically using the AU-program **lcabsf**. For details check page 136 „AU-Program **lcabsf:** Baseline correction".

As there is no **undo** function in TOPSPIN available it is useful to save the spectrum after each successful step, (for disk space reasons delete imaginary parts first).

Trapezoidal Baseline correction

For gradient elution, where one signal will change during time, an additional trapezoidal baseline correction command <u>absot2</u> is available. With this command even the shifting water/acetonitrile signals can be compensated. It is controlled by the parameters

    2 ABSF1, **ABSF2**, **SIGF1**, **SIGF2**
    1 ABSF1, **ABSF2**

SIGF1 and **SIGF2** must be entered via the keyboard as they are not available in the **edp** menu. The meaning of the parameters is indicated in the diagram below.
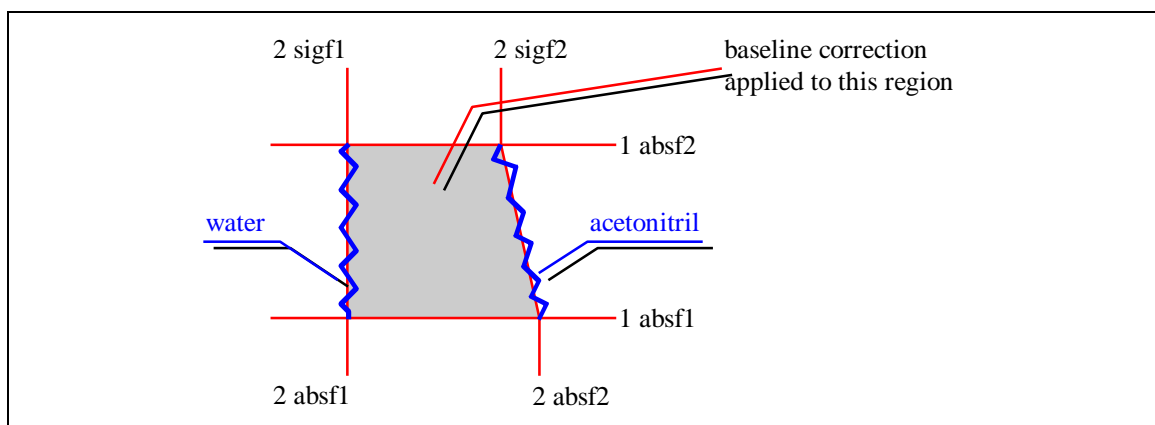
Fig. 3.15:   Parameters used for <u>absot2</u>

4. Experiments

The following section describes the usage of the release LCNMR experiments.
For the general setup of NMR parameters check the AVANCE users guide. In this section only the parameters are listed which are related to the acquisition of solvent suppressed spectra or have a very special meaning in the automation procedures.
For the proper usage of the experiments in automation (i.e. with the AU programs or with ICONNMR) it is necessary to:

- Define pulses and powers for the LC Probe and the solvent with <u>edprosol</u>.

- Define the correct probe head with <u>edprobe|define current</u> to allow correct setting of pulses and power levels.

- Use the **CH3CN+D2O** or **CH3OH+D2O** instead of **D2O** as solvents.

- Lock the spectrometer with <u>lock <solvent></u> rather than with the <u><Lock on/off></u> and <u><Field></u> on the BSMS keyboard.

Each chapter contains the following subsections

Description:	Briefly describes the experiment
Run the experiment automatically:	A step-by-step guide of how to run the experiment with <u>xaua</u>.
Run the experiment manually:	A step-by-step description of how to run the experiment manually.
Parameters:	Details about the experiment. All parameters - automation and manual -
Hints	Trouble shooting or description how to manage special tasks.

4.1 Determination of system times (LCUVNMR)

4.1.1 Description

This is an experiment for the determination of the system parameters during the installation of the probe. Depending on the type of calibration procedure either a series of 1D ^{1}H proton spectrum with solvent suppression of a series of ^{1}H or ^{2}H gradient profiles are acquired. The experiment must be started with the TopSpin command xaua. The AU program **au_uvnmr** will guide the user through the process and will ajust the necessary experiment parameters interactively.

During the process the user has to execute certain commands in the software HyStar controlling the HPLC system and the interfaces. The synchronization of actions in HyStar and tehe NMR spectra is done by the AU program.

Processing is done automationcally with xaup and will produce a series of spectra. The **f2**-axis corresponds to a normal NMR axis and the **f1**-axis corresponds to a time axis. Scanning through the rows shows the status of the flow cell for a certain time.

The required system parameters can be determined from the difference of the time whwn an event was observed in the HyStar software and the visibility of the effect in the flow cell (maximum intensity, completeness of gradient profile).

4.1.2 Run the experiment automatically

The parameter set used is **LCUVNMR.** The required parameters for this experiment are either predefined in the parameter, set by **prosol** or adjusted by the AU program **au_uvnmr**. The AU program is defined in the parameter AUNM and thus can be started by xaua. Manual execution of the experiment is not recommended and therefore not described.

General Procedure
- Typically you are asked to prepare the NMR flowcell for the acquisition. After this is successfully done the NMR data acquisition will start.
- Now you will be asked to initiate a certain process in HyStar and press the OK button in TopSpin, either immediately or when you observe a certain event in HyStar (switching of a valve, appearance of a peak, …)
- The software will check which row is currently acquired and store this number in the dataset.
- After the processing of the NMR spectra the appearance of the event in the NMR spectra can be detected.
- The difference in rows is calculated and together with the known time resolution of the NMR the delay between external event and appearance in the NMR can be calculated.

Run the experiment
- Create a new data set with edc.

- Read the parameter set rpar LCUVNMR
- Lock the spectrometer with the correct lock <solvent>
- Set pulses and power levels with getprosol.
- You *must* start experiment with <u>xaua</u> otherwise the parameters are not correctly defined.
- Follow the instructions of the AU program. The exact procedure depends on the type of calibration.

Processing

- At the end of the acquisition the processing may be started automationcally.
- The AU program **proc_uvnmr** is started.
- Follow the instruction of the AU program. The result will be printed into the title of the experiment.
- At any time you can restart the processing with the command <u>xaup</u>.

Hints

The AU program carries out complex operations, note the following points
- If you change the type of calibration procedure you *must* restart the process with <u>rpar LCUVNMR</u>.
- If you abort the acquisition you also must do <u>rpar LCUVNM</u> before you restart with xaua.

4.1.3 Parameters

General

The parameters are too complex to describe in detail here. Depending on the type of calibration essential parameters will change.

Important parameters

The following parameters have a special meaning.

Parameter	Typical value	Comment
AUNM	**au_uvnmr**	AU program for automation, started with <u>xaua</u>.
AUNMP	**proc_uvnmr**	AU program for automation, started with <u>xaup</u>.
1 TD	180 – 512	Determines the duration of the experiment. Typically a time resolution of 1 sec per row is adjusted which leads to 180 – 512sec
L 27		The automation will store the rownumber in with the relevant event was detected in this parameter.
D1, AQ		The are are restricted to short values as the timeresolution required is typically 1sec. I.e. the sum of D1 and AQ must not exceed 1sec.

Tab. 4.1:   *Automation parameters for LCUVNMR.*

4.1.4 Details - proc_uvnmr

At the end of the acquisition process you will be asked to stop the main experiment. Be sure the the desired process is visible in the NMR flow cell before you do so.

In case you the automatic processing were not succesfull, you can reprocess the file with xf2 and perform further manual operation.

Main task of the AU program proc_uvnmr is the correct scaling of the axis. In case you perform manual processing with xf2 the axis will have an arbitrary scaling. After reading the position of the event you have to calculate the necessary parameters (delays, volumes) manually from the difference of row numbers and the time resolution of the experiment.

4.2 1D ^{1}H Spectrum with double presaturation (LC1D12, LC1D12GP)

4.2.1 Description

The described experiment acquires a 1D proton spectrum with solvent suppression on two channels. The solvent suppression is done by presaturation through two independent channels. The pulse program used is **lc1pnf2/ lc1pngpf2** which is a 1D NOESY sequence providing improved solvent suppression. Presaturation is done during relaxation delay and mixing time.

The Experiment LC1D12GP also includes a spoil gradient for improved solvent suppression and has digitizer mode (DIGMOD) baseopt activated. This is the recommended experiment.

4.2.2 Run the experiment automatically

The parameter set used is **LC1D12, LC1D12GP.** All basic parameters for this experiment are loaded with <u>rpar <parametersetname></u> and <u>getprosol</u>. The AU program **au_lc1d** is able to automatically determine and set all sample dependend parameters.

Prepare the acquisition
- Create a new data set with <u>edc</u>. For peak 1 use expno 10,… peak 2 20,21,…
- Read the parameter set <u>rpar ...</u>.
- Lock the spectrometer with the correct <u>lock <solvent></u>
- Set pulses and power levels with <u>getprosol</u>.
- Presaturation is done on-resonance therefore O1 is set to the most intense solvent. Consider this when reducing SW.
- Solvents must be in the plot region **F1P**, **F2P** for automatic search. However **F1P**, **F2P** may be set outside the spectral window.
- Set the desired **NS**. 512 scans correspond to approx. 30-35 min.

Verify or change Automation parameters
- Change basic and automation parameters with <u>expset</u>. Accept the displayed settings, or enter a setup routine with CANCEL.
- All other parameters can be changed with <u>eda</u>/<u>ased</u>.

Start the acquisition
- Enter <u>xaua</u>. A preparation experiment is acquired.
- Do not change parameters while this experiment is running. (For details about the automation program <u>au_lc1d</u> see page 119).
- After the preparation experiment is processed the main experiment starts.
- During the main experiment you can check the progress with <u>tr</u> and <u>efp</u>, or terminate the experiment with <u>halt</u>.

Manual Optimization

When the experiment has ended or you have stopped it, you can optimize parameters and re-run it.

- Change the parameter(s) in <u>eda</u> or optimize the experiment with <u>gs</u>.

- To re-run the optimized experiment you must start it with <u>zg</u>! If started with <u>xaua</u> the optimized values for RG or O1 will be overwritten by the automation.

Hints

The AU program carries out complex operations, note the following points:

- Do not attempt to process the dataset, during the –first- preparation experiment. Note the parametrs like **SW**, **O1**, **DS**, **NS** are changed during this experiment.

- If you abort the acquisition during the preparation experiment you ***must*** a <u>rpar</u> of the parameter set befor you restart with <u>xaua</u>.

- Preferably do not rerun <u>xaua</u> a second time, do not run xaua on a dataset you have copied with <u>edc</u> or <u>wrpa</u>. Do a <u>rpar</u> before you start <u>xaua</u>.

- If the signal to noise control is active the AU program will do a processing after a certain number of scans. Do not change processing parameters like **LB** or **WDW**. Do not block the data set with you own processing like phase- or baseline correction. Otherwise the signal-to-noise control may fail.

4.2.3 Run the experiment manually

All basic parameters for this experiment are loaded with <u>rpar</u> <u><parametersetname></u> and <u>getprosol</u>. Sample dependent parameters must be determined in a preparation experiment or optimized on the main experiment.

Prepare the acquisition

- Create a new data set with <u>edc</u>. For peak 1 use expno 10,… peak 2 20,21,…
- Read the parameter set <u>rpar ....</u>.
- Lock the spectrometer with the correct <u>lock <solvent></u>
- Set pulses and power levels with <u>getprosol</u>.
- For GP versions determine lockphase with <u>autophase</u> and 90deg pulse with <u>pulsecal</u>.
- Check NS. 512 scans correspond to an experiment time of approx. 30 min.

Acquire the preparation experiment

- Change to a dataset with expno = 1.
- Read the parameter set **LCZG**.
- Set pulses and power levels with <u>getprosol</u>.
- Acquire the spectrum with <u>zg</u>.
- Transform the spectrum with <u>ef</u> and apply phase correction with <u>apk0</u>

Solvent search

- Set O1 to the first (biggest), and O2 to second solvent.
- Note the found values of O1 and O2

Prepare and start the main experiment

- Change back to the main data set.
- Enter **O1** and **O2** as determined in the preparation experiment.
- Go to the acquisition window with <u>acqu</u> and enter <u>gs</u>.
- Change **RG**, so that the FID fills approx. 50% of the Y-scale of the window.
- Do a fine adjustment of **O1** in steps of ± 1 or ± 0.1 Hz steps to minimize the modulations and to get minimum FID area.
- Fine adjustment of the *second* signal is normally not possible. The residuals of the first signal will dominate the FID
- If possible change RG again.
- Store the changed values, stop **gs** and start the main acquisition with <u>zg</u>.

4.2.4 Parameters

General

These are principle parameters that define the experiment. They are loaded with <u>rpar</u> <parametersetname> followed by <u>getprosol</u>.

Parameter	Typical value	Comment
AQMOD		DQD if available.
TD/SW	32k/24ppm	Adjust to give an AQ of approx. 1.5sec.
SI	16k	Normally TD/2.
PULPROG	lc1pnf2	Double presaturation 1D NOESY version.
	lc1pcf2	Double presaturation with bax composite pulse instead of 1D NOESY sequence.
	lc1prf2	Double presaturation only.
NS	≈ 128	Multiple of 8.
DS	8	not less than 8 to establish steady state of solvent suppression before data acquisition starts.
D8	≈ 100msec	Mixing time for NOESY, for large molecules check if shorter delay must be applied to avoid signal loss.
P1/PLDB 1		90° degree proton observe pulse.
PLDB 9	25 - 5Hz	Presaturation power for first solvent, see "Presaturation Powerlevels" page 22

Parameter	Typical value	Comment
PLDB 21	25 - 5Hz	Presaturation power for second solvent. , see "Presaturation Powerlevels" page 22
D1	2.4sec	Relaxation delay approx. 1-3sec. Keep in mind that a 90° pulse is used as observe pulse.
P16/ GP1/2	SMSQ10.100 50/-10% 1msec	Gradient pulse

Tab. 4.2:   *General parameters for LC1D12, LC1D12GP.*

Sample dependent

These are parameters that change for each sample. The user must adjust them individually or start the automation routine with xaua.

Parameter	Typical value	Comment
O1		On-resonance on the larger signal. To minimize **SW** you can also put another signal to **O1** and the larger signal to **O2**. In this case the optimization of **O1** in the **gs**-mode is difficult. Without **DQD** the larger signal should be definitely **O1** to avoid quad-images.
O2		on-resonance to the second solvent line, normally water.
RG		has to be adapted to the sample. With 60% acetonitrile in a 4 mm LC probe at 500 MHz a **RG** or approx. 128 - 512 should be reached.

Tab. 4.3:   *Sample dependent parameters for LC1D12, LC1D12GP.*

Automation

These parameters are relevant if the acquisition is started with xaua or in ICONNMR. As they are not part of the pulsprogram, they do not appear in the **ased** window! Standard values are loaded with rpar. They can be directly changed or using expset. Note that it is strongly recommend to use the expset routine rather than defining parameters manually. Validity of values and exact writing of keywords is only ensured by the automatic setup.

Parameter	Typical value	Comment
AUNM	au_lc1d	AU program for automation, started with xaua.

Parameter	Typical value	Comment
2 USERA1	-	Command line parameters for the AU program. For possible options see description of the AU program. Typical options are :
		pcal-yes: 90° pulse calibration is done
		sref-no: no SREF is done
L25	1	NS for solvent search spectrum.
L28	8	RG during preparation experiment.
L30	2	Number of solvents to search. Must be two for this type of solvent suppression
L31	32	NS after which SINO is checked the first time. For reliable phase correction not less than 32.
CNST 28	2.5	Minimum value of SINO after L31 scans.
CNST 29	0.75	Receiver gain control > 0 : multiplier for **RG** from RGA ≤ 0: use value defind in **RG**
D 31	0	DE during RGA. $= 0$: no change; > 0: value for DE in usec.
CNST 30	0	Exclude region around solvents see page 130
SINO	0	$= 0$ No signal-to-noise control is done > 0 The value is interpreted as desired SINO.
NOISF1, NOISF2	11.0, 10.0	Defines borders of noise region. Solvent positions are automatically exclude.
SIGF1, SIGF2	9.0, 5.5	Defines outer limits of signal region. Solvent positions are automatically exclude.
CNST 2	136.6	110% of this value is exclude around solvent positions during the signal/noise determination.
PSCAL	GLOBAL or PREG	global/preg = in plot region sreg = according to **sreglst**.
F1P, F2P	11.0, -1.0	Defines region for solvent search.

Tab. 4.4:   Automation parameters for LC1D12, LC1D12GP.

4.3 1D ^{1}H Spectrum with mult. Presaturation. LC1DCWPS/ LC1DCWGPPS with ^{13}C decoupling, (MULTIPRESAT/ MULTIPRESATGP without ^{13}C dec.)

4.3.1 Description

The described experiment acquires a 1D proton spectrum with multiple solvent suppression using just one channel. The number of solvent resonance's is arbitrary. The pulse program used is **lc1pncwps/lc1pncwgpps** (**lc1pnps/lc1pngpps**)which is a 1D NOESY sequence providing improved solvent suppression. Presaturation is done during relaxation delay and mixing time.

The multiple presaturation of arbitrary number and position of solvent resonance's is reached with a short rectangular pulse or approx. 10msec. The pulse itself would act like a short presaturation at the applied position (**O1**) By repeating this pulse **D1**/**P18** times a presaturation of **D1** sec is achieved, identical to a conventional presaturation during relaxation delay. If the rectangular shape is modulated by offset frequencies a presaturation is simultaneously done at multiple positions. Thus applying one pulse on one channel simultaneously does a suppression of several solvent resonances.

Decoupling on the ^{13}C channel is applied in **cw**-mode during acquisition which results in a clearance of the position of the ^{13}C satellites for observation of other signals. The version with 13C decoupling is not described separately, those parameters are listed in parentheses.

The experiments LC1DCWPSGP and MULTIPRESATGP also includes a spoil gradient for improved solvent suppression and has digitizer mode (DIGMOD) baseopt activated. These are the recommended experiments.

4.3.2 Run the experiment automatically

The parameter set used is **LC1DCWPS/ LC1DCWGPPS/LC1DCWGPPS** (**MULTIPRESAT/ MULTIPRESATGP**). All basic parameters for this experiment are loaded with rpar <parametersetname> and getprosol. The AU program **au_lc1d** is able to automatically determine and set all sample dependend parameters.

Prepare the acquisition

- Create a new data set with edc. For peak 1 use expno 10,… peak 2 20,21,…
- Read the parameter set rpar ....
- Lock the spectrometer with the correct lock <solvent>
- Set pulses and power levels with getprosol.
- Presaturation is done on-resonance therefore O1 is set to the most intense solvent. Consider this when reducing SW.
- Set the desired **NS**. 512 scans correspond to approx. 40-45 min.

Verify or change Automation parameters

- Change basic and automation parameters with <u>expset</u>. Accept the displayed settings, or enter a setup routine with CANCEL.
- All other parameters can be changed with <u>eda</u>/<u>ased</u>.
- The shape pulse for the multiple presaturation is calculated automatically. Based on the presaturation power **PLDB 9** for a single solvent.

Start the acquisition

- Enter <u>xaua</u>. A preparation experiment is acquired.
- Do not change parameters while this experiment is running. (For details about the automation program <u>au_lc1d</u> see page 119).
- After the preparation experiment is processed the main experiment starts.
- During the main experiment you can check the progress with <u>tr</u> and <u>efp</u>, or terminate the experiment with <u>halt</u>.
- A shape, modulated with the found frequencies is stored under <data set name><expno>p

Manual Optimization

When the experiment has ended or you have stopped it, you can optimize parameters and re-run it.

- Change the parameter(s) in <u>eda</u> or optimize the experiment with <u>gs</u>.
- To re-run the optimized experiment you must start it with <u>zg</u>! If started with <u>xaua</u> the optimized values for RG or O1 will be overwritten by the automation.

4.3.3 Run the experiment manually

All basic parameters for this experiment are loaded with <u>rpar <parametersetname></u> and <u>getprosol</u>. Sample dependent parameters must be determined in a preparation experiment or optimized on the main experiment.

Prepare the acquisition

- Create a new data set with <u>edc</u>. For peak 1 use expno 10,… peak 2 20,21,…
- Read the parameter set <u>rpar ...</u>.
- Lock the spectrometer with the correct <u>lock <solvent></u>
- Set pulses and power levels with <u>getprosol</u>.
- For GP versions determine lockphase with <u>autophase</u> and 90deg pulse with <u>pulsecal</u>.
- Check NS. 512 scans correspond to an experiment time of approx. 45 min.

Acquire the preparation experiment

- Change to a dataset with expno = 1.
- Read the parameter set **LCZG**.
- Set pulses and power levels with <u>getprosol</u>.
- Acquire the spectrum with <u>zg</u>.
- Transform the spectrum with <u>ef</u> and apply phase correction with <u>apk0</u>

Solvent Search

- Go to the Utilities and set O1 to the desired position
- Create a frequency list with the desired solvent positions.
- Start the shape tool and open a Squa100.1000 shape
- Modulate it with the solvent frequencies. As reference take the O1 from the current data set as suppression positions the entries in the frequency list.
- Adapt the power of shape according to the used number of frequencies.
- Store the shape under a new name, do NOT overwrite the shape Squa100.1000.

Prepare and start the main data set

- Change back to the main data set.
- Enter **O1** as used during the shape generation.
- Enter the new generated shape into **P 18 SPDB6** and **SPNAM6**
- Go to the acquisition window with <u>acqu</u> and enter <u>gs</u>.
- Change **RG**, so that the FID fills approx. 50% of the Y-scale of the window.
- Do a fine adjustment of **O1** in steps of ±1 or ±0.1 Hz steps to minimize the modulations and to get minimum FID area. [3])
- If possible change **RG** again.
- Store the changed values, stop **gs** and start the main acquisition with <u>zg</u>.

4.3.4 Parameters

General

These are principle parameters that define the experiment. They are loaded with <u>rpar</u> <parametersetname> followed by <u>getprosol</u>.

Parameter	Typical value	Comment
AQMOD		DQD if available.
TD/SW	16k/20ppm	adjust to give an AQ of below 1sec to avoid excessive sample heating due to the decoupling.
SI	16k	
PULPROG	lc1pncwps (lc1pnps)	multiple presaturation 1D NOESY version.

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[3]) If O1 is moved, the other frequencies are moved too. As O1 is normally the most intense signal, it can be changed to increase the suppression of this signal at the expense of the slight decrease of the suppression of other (weak) signals. To change the frequencies or the power levels in relation to each other you have to generate a completely new shape.

Parameter	Typical value	Comment
NS	≈128	multiple of 8.
DS	24	not less than 24 to establish a steady state of the heating effects caused by the decoupling before data acquisition starts.
D8	≈100msec	mixing time for NOESY, for large molecules check if shorter delay must be applied to avoid signal loss. The delay is filled with the maximum number of presaturation pulses **P18**. The remaining time is filled by an automatically calculated delay. For best suppression **D8** must be a multiple of **P18**.
P1/PLDB 1		90° degree proton observe pulse.
P18	≈ 10msec	Length of shape pulse used for presaturation. It is repeated during d1 and **D8**.
PLDB 21	≈ 20dB	^{13}C decoupling power in **cw**-mode. Power as requird for a 1000Hz field.
O2P		Position for ^{13}C decoupling frequency. Set on resonance to ^{13}C solvent signal for example 2 ppm for acetonitrile. The ^{13}C frequency is relatively independent from the solvent composition, it is not necessary to adjust it for each sample.
D1	3sec	Relaxation delay approx. 1-3sec. Keep in mind that a 90° pulse is used as observe pulse. And that the heating during AQ should be compensated by a longer relaxation delay. D1 is not directly executed, is is used for the calculation of **L 6**.
D1	2.4sec	Relaxation delay approx. 1-3sec. Keep in mind that a 90° pulse is used as observe pulse.
P16/ GP1/2	SMSQ10.100 50/-10% 1msec	Gradient pulse

Tab. 4.5:   *General parameters for LC1DCWPS/ LC1DCWGPPS, MULTIPRESAT/ MULTIPRESATGP.*

Sample dependent

These are parameters that change for each sample. The user must adjust them individually or start the automation routine with xaua.

Parameter	Typical value	Comment
O1		On-resonance on most intens solvent signal. To minimize **SW O1** can be also somewhere and all solvents are suppressed off-resonance. However optimization in **gs**-mode is more difficult..

Parameter	Typical value	Comment
SPDB6	25 – 5 Hz	presaturation power for the solvent suppression. Start with power as set in **PLDB 9** for single, see "Presaturation Powerlevels" page 22 suppression and subtract -6dB for second solvent.
SPNAM6		Name of the shape pulse modulated with the offset frequencies.
RG		has to be adapted to the sample. With 60% acetonitrile in a 4 mm LC probe at 500 MHz a RG or approx. 128 - 512 should be reached.

Tab. 4.6:   *Sample dependent parameters for LC1DCWPS/ LC1DCWGPPS, MULTIPRESAT/ MULTIPRESATGP.*

Automation

These parameters are relevant if the acquisition is started with xaua or in ICONNMR. As they are not part of the pulsprogram, they do not appear in the **ased** window! Standard values are loaded with rpar. They can be directly changed or using expset. Note that it is strongly recommend to use the expset routine rather than defining parameters manually. Validity of values and exact writing of keywords is only ensured by the automatic setup.

Parameter	Typical value	Comment
AUNM	au_lc1d	AU program for automation, started with xaua.
L25	1	NS for solvent search spectrum.
L28	8	RG during preparation experiment.
L30	2	Number of solvents to search.
L31	32	NS after which SINO is checked the first time. For reliable phase correction not less than 32.
CNST 28	2.5	Minimum value of SINO after L31 scans.
CNST 29	0.75	Receiver gain control > 0 : multiplier for **RG** from RGA ≤ 0: use value defind in **RG**
D 31	0	DE during RGA. $= 0$: no change; > 0: value for DE in usec.
2 USERA1		Command line parameters for the AU program. For possible options see description of the AU program. Typical options are : **Offres:** O1 is not changed **pcal-yes:** 90° pulse calibration is done **sref-no**: no SREF is done

Parameter	Typical value	Comment
PLDB 9	25 – 5 Hz	Power for presaturation of ***one frequency***. Reference powerlevel for calculation of the powerlevel **SPDB 6** for multiple presaturation, see "Presaturation Powerlevels" page 22
CNST 30	0	Exclude region around solvents see page 130
2 SINO	0	= 0 No signal-to-noise control is done > 0 The value is interpreted as desired SINO.
NOISF1, NOISF2	11.0, 10.0	Defines borders of noise region. Solvent positions are automatically exclude.
SIGF1, SIGF2	9.0, 5.5	Defines outer limits of signal region. Solvent positions are automatically exclude.
CNST 2	136.6	110% of this value is exclude around solvent positions during the signal/noise determination.
F1P, F2P	11.0, -1.0	Defines region for solvent search.

Tab. 4.7:   *Automation parameters for LC1DCWPS/ LC1DCWGPPS, MULTIPRESAT/ MULTIPRESATGP.*

4.4 1D ^{1}H Spectrum with WET solvent suppression and ^{13}C decoupling (LC1DWTDC)

4.4.1 Description

The described experiment acquires a 1D proton spectrum with multiple solvent suppression using just one channel. The number of solvent resonance's is arbitrary. The solvent suppression is done by WET solvent suppression using a selective excitation shape pulse. The pulse program used is **lc1wetdc** which has a bax composite pulse to improve solvent suppression.

Composite pulse decoupling on the ^{13}C channel is applied during selective excitation and acquisition which leads to a suppression of the satellites and a displacement to the position of the ^{12}C-proton signal.

The WET sequence consists of a selective pulse applied to the solvent frequencies. A dephasing gradient is then used to destroy this unwanted magnetization. This procedure is repeated four times with different flip angles and gradient strength (80:40:20:10).

The suppression process only takes 60-100 msec (4 shape pulses + 4 dephasing gradients). Due to this short duration it is also possible to apply ^{13}C decoupling during the suppression sequence, which means that the ^{13}C satellites are also dramatically reduced in their intensity.

The solvent suppression with WET is relatively tolerant to small deviations from the exact position of the solvent frequencies.

4.4.2 Run the experiment automatically

The parameter set used is **LC1DWTDC.** All basic parameters for this experiment are loaded with rpar <parametersetname> and getprosol. The AU program **au_lc1d** is able to automatically determine and set all sample dependend parameters.

Prepare the acquisition
- Create a new data set with edc. For peak 1 use expno 10,… peak 2 20,21,…
- Read the parameter set rpar ....
- Lock the spectrometer with the correct lock <solvent>
- Set pulses and power levels with getprosol.
- Suppression is done off-resonance, i.e. you can optimize O1 and the spectral window to your sample (they remain unchanged).
- Set the desired **NS**. 512 scans correspond to approx. 40 - 45 min.

Verify or change Automation parameters
- Change basic and automation parameters with expset. Accept the displayed settings, or enter a setup routine with CANCEL.
- All other parameters can be changed with eda/ased.

- The shape pulse used for the WET multiple suppression is calculated automatically from **P11 SPDB2 SPNAM2**.

Start the acquisition

- Enter xaua. A preparation experiment is acquired.
- Do not change parameters while this experiment is running. (For details about the automation program au_lc1d see page 119).
- After the preparation experiment is processed the main experiment starts.
- During the main experiment you can check the progress with tr and efp, or terminate the experiment with halt.
- The automation will generate a shape pulse which is stored under s_<data set name><expno>

Manual Optimization

When the experiment has ended or you have stopped it, you can optimize parameters and re-run it.
- Change the parameter(s) in eda or optimize the experiment with gs.
- To re-run the optimized experiment you must start it with zg! If started with xaua the optimized values for RG or O1 will be overwritten by the automation.

4.4.3   Run the experiment manually

All basic parameters for this experiment are loaded with rpar <parametersetname> and getprosol. Sample dependent parameters must be determined in a preparation experiment or optimized on the main experiment.

Prepare the data set

- Create a new data set with edc.
- Read the parameter set **rpar LC1DWTDC**.
- Set pulses and power levels with eda|prosol->true
- Check NS. 512 scans correspond to an experiment time of approx. 30 min.

Acquire the preparation experiment

- Change to a dataset with expno = 1.
- Read the parameter set **LCZG**.
- Set pulses and power levels with getprosol.
- Acquire the spectrum with zg.
- Transform the spectrum with ef and apply phase correction with apk0

Search the solvent

- Go to the Utilities and set O1 to the desired position
- Create a frequency list with the desired solvent positions.
- Start the shape tool and open the desired selective excitation pulse (for example Sinc1.1000).

- Modulate it with the solvent frequencies. As reference take the O1 from the current data set as suppression positions the entries in the frequency list.
- Do *not* use a scaling for the individual frequencies.
- Adapt the power of shape according to the used number of frequencies.
- Store the shape under a new name, do NOT overwrite the original shape.

Prepare the main data set
- Change back to the main data set.
- Enter **O1** as used during the shape generation.
- Enter the new generated shape pulse as **P11 SPDB1** and **SPNAM1**
- To set the WET pulses calculate the values for SPdB7.

Optimize the parameters
- Go to the acquisition window with <u>acqu</u> and enter <u>gs</u>.
- Change **RG**, so that the FID fills approx. 50% of the Y-scale of the window.
- As we are working with 13C decoupling, wait several scans before you change O1, the heating can move the positions by 10 - 20Hz and will reach it's steady state just after 20 - 30scans.
- Do a fine adjustment of **O1** in steps of ±1 or ±0.1 Hz steps to minimize the modulations and to get minimum FID area. [4])
- Keep in mind that you are moving the center of the shape and thereby all suppression frequencies at the same time.
- For best performance change the power of the last WET pulse **SP 10** in ±0.05dB steps in the order of ±3dB for minimum FID area.
- If possible change **RG** again.
- Store the changed values, stop the **gs**-mode and start the main acquisition with <u>zg</u>.

4.4.4 Parameters

General
These are principle parameters that define the experiment. They are loaded with <u>rpar</u> <parametersetname> followed by <u>getprosol</u>.

Parameter	Typical value	Comment
AQMOD		DQD if available.
TD/SW	16k/20ppm	adjust to give an AQ of below 1sec to avoid excessive sample heating due to the decoupling.

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[4]) If O1 is moved, the other frequencies are moved too. As O1 is normally the most intense signal, it can be changed to increase the suppression of this signal at the expense of the slight decrease of the suppression of other (weak) signals. To change the frequencies or the power levels in relation to each other you have to generate a completely new shape.

Parameter	Typical value	Comment
SI	16k	Normally TD/2.
PULPROG	wetdc	1D spectrum with multiple WET solvent suppression, bax composite pulse and decoupling during WET and acquisition.
NS	≈128	multiple of 8.
DS	24	not less than 24 to establish a steady state of the heating effects caused by the decoupling before data acquisition starts.
P1/PLDB 1		90° degree proton observe pulse.
P11	≈ 10-20msec	Length of shape pulse used for selective excitation.
PLDB 14	≈ 17dB	^{13}C Power level for low power **cpd**-decoupling.
P31	210usec	^{13}C pulse for low power **cpd**-decoupling.
CPDPRG2	garp.p31	carbon decoupling pulse for **cpd**-decoupling.
O2P		Position for ^{13}C decoupling frequency. Set on resonance to ^{13}C solvent signal for example 2 ppm for acetonitrile. The ^{13}C frequency is relatively independent from the solvent composition, it is not necessary to adjust it for each sample.
D1	3sec	relaxation delay approx. 1-3sec. Keep in mind that a 90° pulse is used as observe pulse. And that the heating during AQ should be compensated by a longer relaxation delay.
P16/ GP21…24	SMSQ10.100 80/40/20/10% 2msec	Gradient pulse

Tab. 4.8:   *General parameters for LC1DWTDC.*

Sample dependent

These are parameters that change for each sample. The user must adjust them individually or start the automation routine with <u>xaua</u>.

Parameter	Typical value	Comment
O1		On-resonance on most intens solvent signal. To minimize **SW O1** can be also somewhere and all solvents are suppressed off-resonance. However optimization in **gs**-mode is more difficult..
SPDB7 SPDB8 SPDB9 SPDB10	90° +0.87 90° -1.04 90° +2.27 90° -5.05	selective excitation power for the four WET pulses. The power of the pulses corresponds to four different flip angles in the order of 90°.

Parameter	Typical value	Comment
SPNAM7 – SPNAM10	same shape for all	Name of a shape pulse modulated with the offset frequencies of the solvent signals.
RG	512	has to be adapted to the sample. With 60% acetonitrile in a 4 mm LC probe at 500 MHz a RG or approx. 512 - 1k should be reached.

Tab. 4.9:   Sample dependent parameters for LC1DWTDC.

Automation

These parameters are relevant if the acquisition is started with xaua or in ICONNMR. As they are not part of the pulsprogram, they do not appear in the **ased** window! Standard values are loaded with rpar. They can be directly changed or using expset. Note that it is strongly recommend to use the expset routine rather than defining parameters manually. Validity of values and exact writing of keywords is only ensured by the automatic setup.

Parameter	Typical value	Comment
AUNM	au_lc1d	AU program for automation, started with xaua.
L25	1	NS for solvent search spectrum.
L30	2	Number of solvents to search.
SPDB2	≈ 60dB	Defines the power of a selective 90° excitation pulse for a ***single frequency***. It is taken as starting point for the calculation of the multiple excitation power.
SPNAM2	Sinc1.1000	Selective single excitation pulse. This shape must exist, it will be modified with the offset frequencies.
SPDB15	150	If $-6 \leq$ **SPDB15** ≤ 30 it is used as correction value for the last WET power level (SPdB10).
L31	32	NS after which SINO is checked the first time. For reliable phase correction not less than 32.
CNST 28	2.5	Minimum value of SINO after L31 scans.
CNST 29	0.75	Receiver gain control > 0 : multiplier for **RG** from RGA ≤ 0: use value defind in **RG**
D 31	0	DE during RGA. $= 0$: no change; > 0: value for DE in usec.
CNST 30	0	Exclude region around solvents see page 130
2 USERA1	**offres**	Command line parameters for the AU program. For possible options see description of the AU program. Typical options are : **Offres:** O1 is not changed **pcal-yes:** 90° pulse calibration is done **sref-no**: no SREF is done

Parameter	Typical value	Comment
2 SINO	0	= 0 No signal-to-noise control is done
		> 0 The value is interpreted as desired SINO.
NOISF1, NOISF2	11.0, 10.0	Defines borders of noise region. Solvent positions are automatically exclude.
SIGF1, SIGF2	9.0, 5.5	Defines outer limits of signal region. Solvent positions are automatically exclude.
F1P, F2P	11.0, -1.0	Defines region for solvent search.

Tab. 4.10:   Automation parameters for LC1DWTDC.

Hints

The shape pulse which is used for the multiple solvent suppression is calculated automatically. As starting point of this calculation the following parameters are used.

   P 11, SPDB 2 and **SPNAM2.**

The automation assumes that this parameters contains the correct values for a single excitation 90° selective pulse. On the previous version of **prosol** those parameters were not supported. Therefore make sure that they are enter correctly in **prosol**.

4.5 Time Slicing - Pseudo onflow

4.5.1 Description

For the time-slicing experiment no specific parameter set is provided. The experiment can be done by performing any kind of stop-flow experiments in equidistant intervals on a chromatogram. Possible experiments (for the description check the previous sections) are:

- 1D ^{1}H Spectrum with double presaturation (LC1D12, LC1D12GP).
- 1D ^{1}H Spectrum with mult. Presaturation. LC1DCWPS/ LC1DCWGPPS with ^{13}C decoupling, (MULTIPRESAT/ MULTIPRESATGP without ^{13}C dec.).
- 1D ^{1}H Spectrum with WET solvent suppression and ^{13}C decoupling (LC1DWTDC).

The advantage in comparison with the on-flow experiment (see next section) is, that the spectra are acquired under static conditions with the following effects.

- Absolutely stable conditions.
- Shimming possible for each spectrum / solvent composition.
- Exact solvent search for each spectrum.
- Lower detection limit due to higher NS.

After the experiment, the individual spectra are collated into a 2D file and can be further treated like an real on-flow experiment.

4.5.2 Run the experiment automatically

As the whole experiment takes several hours and a large number of spectra are acquired, the experiment is preferably performed in automation under control of ICONNMR. All types of 1D experiments can be used and the acquisition of the individual experiments is performed by standard automation program **au_lc1d**.

Prepare the acquisition

- Start ICONNMR and prepare one holder with the desired experiment.
- Modify the parameters as described with the selected experiment.
- **RG** optimization and **SINO** control are possible, the differences in intensity will be scaled during the processing.
- **NS** is typically 16 - 64.
- Calculate the number of stops during the chromatogram and create a sufficient number of holder by copying the first holder.

Run the experiment

- In ICONNMR start the automation with GO. Select a short waiting time and select gradient shimming if you not running under isocratic conditions.

- In the chromatography program select time slicing mode and select a slice width which corresponds to flowrate and peak volume.
- Inject the sample, the rest is done automatically

Hints

- Typically a chromatogram is stopped each 15 - 20sec. With a total runtime of 30min for a chromatogram this yields 90 - 120 spectra. With an experiment time of 5 min per experiment the total runtime will be 7 - 10h. Besides the main experiment also a solvent search spectrum and gradient shimming is done, which will take another 2 - 3min. Therefore the total timing of 5 min per spectrum is reached with an NS 32 ≈ 3 min.

4.5.3 Processing

The main task during the processing of such a time slice experiment are

- Collate the 1D spectra into a 2D file for convenient handling
- Remove chemical shift differences which are introduced due to the changes in solvent composition.
- Scale the spectra according to the effectively used RG and NS.

The processing of a time slice experiment is done with the program **proc_onflow**. The AU program will interactively ask for the parameters for the processing.

- Change to the first dataset of the series and start the AU program with <u>xau proc_onflow</u>.
- If you use the processed spectra rather then the FIDs make sure that they all have the same SI as the first spectrum. Only spectra with same SI can be written to a 2D file.
- Make sure that you have enough disk space for this operation, as the data amount is doubled or tripled. 1D data → copy to 2D file → invers FT to second 2D file.

Referencing

In the 2D file the spectra are aligned relatively to the spectral window to each. I.e. the calibration in the 1D spectrum has not effect, as always the middle of all spectra will be aligned. Therefore a special referencing which shifts the spectra with a LS or RS command prior to the WSR might be necessary.

No referencing is necessary if you have selected an experiment where the most intense solvent (acetonitrile) is on-resonance (on O1 = in the middle of the spectrum). This solvent is always in the middle of the spectrum and therefore will appear at the same position in the 2D file for all rows.

If the suppression is done off-resonance, i.e. the O1 remains static for all experiments, the signals shift relatively to the spectral window. Now it is necessary to shift the spectrum with LS or RS prior to the transfer into the 2D file. This shift (referencing) can be done according to the calibration in the spectrum (SR). In this case the signals will be aligned according to their visible ppm-values in the 1D spectrum.

Or the position of the most intense solvent found during the solvent search is used as reference signal (FQ1LIST). This solvent position is taken from a frequency list which is generated during the solvent search. If necessary you can even change the entries in this list to achieve a correct calibration.

SER file

Only the spectra in the 2D file contain the result of the processing (phase, baseline, shift). Once you apply a XF2 command this processing is lost.
Therefore the program gives you an opportunity to do an inverse FT and to create a new SER file (set of FIDs) which already contains all the processing effects. This SERF file then can be processed with XF2. For example

- Expno 10 = first 1D spectrum, here the AU program is started
- Expno 20,30 .... 160 = further 1D spectra
- Expno 8 = 2D file, where the spectrum contains all processing results of the individual 1D files
- Expno 9 = a SER file which can be treated with XF2 without loosing the processing results.

4.6 On-flow Spectrum for isocration LC elution
(LCONFLOW)

4.6.1 Description

The described experiment acquires an on-flow 1D ^{1}H proton spectrum of a HPLC gradient separation with solvent suppression on two channels. The solvent suppression is done by presaturation through two independent channels. The pulse program used is **lc1prf2**.

The pulse program acquires 1D spectra and stores them subsequently into a 2D (SER) file. Processing is done with xf2. Therefore the **f2**-axis corresponds to a normal NMR axis and in the **f1**-axis corresponds to a chromatographic time axis. Scanning through the rows provides NMR spectra for a certain time in the chromatogram, scanning through the columns provides the reconstructed chromatograms for a certain NMR shift.

The number of scans has to be adapted to the chromatographic conditions. Typically 1-2 rows should be acquired during the elution of the halfline width of one chromatographic peak. This may require to slow down the flow rate of the HPLC equipment.

This experiment ist only designed for isocratic HPLC elution where the solvents remain at a relatively stable position.

4.6.2 Run the experiment automatically

The parameter set used is **LCONFLOW.**   The required parameters for this experiment are either predefined in the parameter set or set by **prosol**. The AU program **au_lconflow** is capable to run the experiment automatically.

The automation is controlled by some special parameters which are not used in the pulse program and will not appear in ased.

Prepare the acquisition
- Create a new data set with edc. For peak 1 use expno 10,… peak 2 20,21,…
- Read the parameter set rpar ....
- Lock the spectrometer with the correct lock <solvent>
- Set pulses and power levels with getprosol.

Setup and start of acquisition
- Enter xaua as TOPSPIN command.
- The AU-program au_lconflow will request all further parameters interactively. Summaries for all parts of the automation are displayed. Accept with OK or click CANCEL to enter a dialog to alter the values.
- For the time resolution define a minimum **NS** and a minimum **D1**. Based on this values the **NS** is adapted together with slight modifications of **D1**.
- After the setup the system allows you to create a parameterset with those settings, so that the experiment can be easily repeated.

- The AU program will guide you further through the procedure. A 2D preparation experiment is acquired and from this unsuppressed spectrum the solvents are searched.
- The main experiment is started in **gs**-mode to allow a fine tuning of **RG** and **O1**. The conditions are not as stable as under stop-flow conditions. Set **RG** only 20% of the highest possible value.
- The AU program will finally start the acquisition but go immediately into a pause state. From this state the acquisition can be started without delay whenever the sample is injected.

Processing

- Process the spectrum with xf2.
- Choose a line which shows sample signals for the phasing. Check the general hints in "Processing of Onflow 2D LC-NMR Spectra"on page 52.

Hints

The AU program carries out complex operations, note the following points:

- Do not attempt to process the dataset, during the –first- preparation experiment. Note the parametrs like **SW**, **O1**, **DS**, **NS** are changed during this experiment.
- If you abort the acquisition during the preparation experiment you *must* a rpar of the parameter set befor you restart with xaua.
- Preferably do not rerun xaua a second time, do not run xaua on a dataset you have copied with edc or wrpa. Do a rpar before you start xaua.

4.6.3 Parameters and details

General

These are principle parameters that define the experiment. They are loaded with rpar <parametersetname> followed by getprosol.

Parameter	Typical value	Comment
AQMOD		DQD if available.
TD/SW	8k/20ppm	adjust to give an **AQ** of approx. 0.5sec to allow rapid data acquisition. Good spectral resolution is not necessary in on-flow experiments.
SI	16k	
PULPROG	lc2prf2	Double presaturation only.
NS	≈16	Adjust duration that 2-3 rows are acquired during the elution of on chromatographic peak.
DS	0	First row shows some distortions but calculation of timing is easier to accomplish.
P1/PLDB 1		90° degree proton observe pulse.

Parameter	Typical value	Comment
PLDB 9	25 – 5 Hz	Presaturation power for first solvent, see "Presaturation Powerlevels" page 22
PLDB 21	25 – 5 Hz	Presaturation power for second solvent, see "Presaturation Powerlevels" page 22
D1	0.5sec	Adjust to reach desired time resolution in f1

Tab. 4.11:   General parameters for LCONFLOW.

Sample dependent

These are parameters that change for each sample. The user must adjust them individually or start the automation routine with xaua.

Parameter	Typical value	Comment
O1		On-resonance on the larger signal. To minimize **SW** you can also put another signal to **O1** and the larger signal to **O2**. In this case the optimization of **O1** in the **gs**-mode is difficult. Without **DQD** the larger signal should be definitely **O1** to avoid quad-images.
O2		on-resonance to second solvent line, normally water.
RG		has to be adapted to the sample. With 60% acetonitrile in a 4 mm LC probe at 500 MHz a RG or approx. 64-128 should be reached.
1 TD		Depends on the runtime of the chromatography. Must cover the elution of all peaks of interest in the chromatogram

Tab. 4.12:   Sample dependent parameters for LCONFLOW.

Automation

These parameters are relevant if the acquisition is started with xaua or in ICONNMR. As they are not part of the pulsprogram, they do not appear in the **ased** window! Standard values are loaded with rpar. They can be directly changed or using expset. Note that it is strongly recommend to use the expset routine rather than defining parameters manually. Validity of values and exact writing of keywords is only ensured by the automatic setup.

Parameter	Typical value	Comment
AUNM	au_lconflow	AU program for automation, started with xaua.
L25	1	NS for solvent search spectrum.
L28	8	RG during preparation experiment.
L30	2	Number of solvents to search. Must be two for this type of solvent suppression

Parameter	Typical value	Comment
CNST 29	0.25	Receiver gain control > 0 : multiplier for **RG** from RGA ≤ 0: use value defind in **RG**
D 31	0	DE during RGA. $= 0$: no change; > 0: value for DE in usec.
CNST 30	0	Exclude region around solvents see page 130
2 USERA1		Command line parameters for the AU program. For possible options see description of the AU program. Typical options are : **Offres:** O1 is not changed **pcal-yes:** 90° pulse calibration is done **sref-no**: no SREF is done
F2PHI, F2PLO	11.0, -1.0	Defines region for solvent search

Tab. 4.13:   *Automation parameters for LCONFLOW.*

Interactive setup of parameters

Most of the parameters described above can be set from the dialog.

Write parameters to a parameter set

If desired you can store the parameters defined during the interactive setup into a parameter set of your choice so that you don't have to run the setup for the next experiment again.

Manually optimize parameters

When all parameters for the main experiment are prepared and the receiver gain is determined with RGA, the AU program will then start the acquisition in **gs**-mode and change to the observe FID-window.

Here you have the possibility to optimize the suppression frequency **O1** and the **RG**. When you first enter the acquisition window the vertical size corresponds to the range of the AD-changer. Don't change the vertical scaling of the window (for example with *8 or /8). Adjust the receiver gain so that the FID covers approximately 10-20% of the window. Keep in mind that the conditions are not completely stable and the FID may increase during the experiment. A typical value for the RG will be 64 - 256 even if 512 could be reached.

When you are satisfied with the result stop the **gs**-mode with stop. This starts the main experiment.

Run the experiment

- After the AU program has started the acquisition of the main experiment the acquisition will be suspended until you confirm the sample injection. When you resume the acquisition it starts without any delay.

- While the acquisition is running you can watch the progress either by reading one of the already acquired spectra with <u>rser</u> or by processing the 2D file with <u>xf2</u>.

- You can terminate the acquisition at any time with <u>halt</u> or <u>stop</u>.

4.7 TOCSY with double presaturation (LCML12)

4.7.1 Description

The described experiment acquires a ^{1}H/^{1}H TOCSY spectrum with double solvent suppression on two channels. The solvent suppression is done by presaturation through two independent channels. The pulse program used is **lcmlevf2pctp** which contains a bax composite pulse for improved solvent suppression. Presaturation is done during relaxation delay.

4.7.2 Run the experiment automatically

The parameter set used is **LCML12**. All basic parameters for this experiment are loaded with <u>rpar <parametersetname></u> and <u>getprosol</u>. The AU program **au_lc2d** is able to automatically determine and set all sample dependend parameters.

Prepare the acquisition
- Create a new data set with <u>edc</u>. For peak 1 use expno 10,… peak 2 20,21,…
- Read the parameter set <u>rpar ...</u>.
- Lock the spectrometer with the correct <u>lock <solvent></u>
- Set pulses and power levels with <u>getprosol</u>.
- Presaturation is done on-resonance therefore O1 is set to the most intense solvent. Consider this when reducing SW.
- Set the desired NS. A number of 24 scans corresponds to an experiment time of approx. 2h 45'.

Verify or change Automation parameters
- Change basic and automation parameters with <u>expset</u>. Accept the displayed settings, or enter a setup routine with CANCEL.
- All other parameters can be changed with <u>eda</u>/<u>ased</u>..

Start the acquisition
- Do <u>xaua</u>. The AU program au_lc2d (details see page 123) will run a preparation experiment on the actual 2D data set.
- After the first row is acquired it is processed in the experiment number 999 on the same data set for the solvent search.
- After this the main experiment is started. Once this is running you can check the progress with <u>rser</u> or <u>xfb</u>.
- To terminate the experiment before the defined number of increments simply enter <u>halt</u> or <u>stop</u>)

Hints
The AU program carries out complex operations, note the following points:

- Do not attempt to process the dataset, during the –first- preparation experiment. Note the parametrs like **SW**, **O1**, **DS**, **NS** are changed during this experiment.
- If you abort the acquisition during the preparation experiment you *must* a rpar of the parameter set befor you restart with xaua.
- Preferably do not rerun xaua a second time, do not run xaua on a dataset you have copied with edc or wrpa. Do a rpar before you start xaua.

4.7.3 Run the experiment manually

All basic parameters for this experiment are loaded with rpar <parametersetname> and getprosol. Sample dependent parameters must be determined in a preparation experiment or optimized on the main experiment.

Prepare the acquisition

- Create a new data set with edc. For peak 1 use expno 10,… peak 2 20,21,…
- Read the parameter set rpar ....
- Lock the spectrometer with the correct lock <solvent>
- Set pulses and power levels with getprosol.

Acquire the preparation experiment

- Change to a dataset with expno = 1.
- Read the parameter set **LCZG**.
- Set pulses and power levels with getprosol.
- Acquire the spectrum with zg.
- Transform the spectrum with ef and apply phase correction with apk0

Solvent search

- Set O1 to the first (biggest), and O2 to second solvent.
- Note the found values of O1 and O2

Prepare and start the main experiment

- Change back to the main data set.
- Enter **O1** and **O2** as determined in the preparation experiment.
- Go to the acquisition window with acqu and enter gs.
- Change **RG**, so that the FID fills approx. 50% of the Y-scale of the window.
- Do a fine adjustment of **O1** in steps of ± 1 or ± 0.1 Hz steps to minimize the modulations and to get minimum FID area.
- Fine adjustment of the *second* signal is normally not possible. The residuals of the first signal will dominate the FID
- If possible change RG again.
- Store the changed values, stop **gs** and start the main acquisition with zg.

4.7.4 Parameters

General

These are principle parameters that define the experiment. They are loaded with
<u>rpar</u> <parametersetname> followed by <u>getprosol</u>.

Parameter	Typical value	Comment
AQMOD		DQD if available.
TD/SW	2k/20ppm	adjust to give an AQ of approx. 0.1sec
SI	1k	normally TD/2.
1 TD	256	Preferably use more scans and less increments. Use linear prediction to compensate this.
PULPROG	lcmlevf2pctp	TOCSY with double presaturation and bax composite pulse.
NS	≈32	Multiple of 8. A signal-to-noise of at least 6:1 should be reached in a 1D spectrum with solvent suppression if this value for NS is used.
DS	8	not less than 8 to establish steady state of solvent suppression before data acquisition starts.
D9	>≈ 35msec	Mixing time for TOCSY, adapt to your sample.
P1/PLDB 1		90° degree proton observe pulse.
PLDB 9	25 – 5 Hz	Presaturation power for first solvent, see "Presaturation Powerlevels" page 22
PLDB 21	25 – 5 Hz	Presaturation power for second solvent.
P6/PLDB 10	≈ 25us/16dB	Spinlock pulse.
P 17	≈ 2.5 msec	Trim pulse.
D1	≈ 2,4	Relaxation delay approx. 1-3sec. Keep in mind that a 90° pulse is used as observe pulse.

Tab. 4.14   General parameters for LCML12.

Sample dependent

These are parameters that change for each sample. The user must adjust them
individually or start the automation routine with <u>xaua</u>.

Parameter	Typical value	Comment
O1		On-resonance on the larger signal. To minimize **SW** you can also put another signal to **O1** and the larger signal to **O2**. In this case the optimization of **O1** in the **gs**-mode is difficult. Without **DQD** the larger signal should be definitely **O1** to avoid quad-images.

Parameter	Typical value	Comment
O2		on-resonance to the second solvent line, normally water.
RG		has to be adapted to the sample. With 60% acetonitrile in a 4 mm LC probe at 500 MHz a RG or approx. 256-1k should be reached.

Tab. 4.15   Sample dependent parameters for LCML12.

Automation

These parameters are relevant if the acquisition is started with xaua or in ICONNMR. As they are not part of the pulsprogram, they do not appear in the **ased** window! Standard values are loaded with rpar. They can be directly changed or using expset. Note that it is strongly recommend to use the expset routine rather than defining parameters manually. Validity of values and exact writing of keywords is only ensured by the automatic setup.

Parameter	Typical value	Comment
AUNM	au_lc2d	AU program for automation, started with xaua.
L25	4	NS for solvent search spectrum.
L28	8	RG during preparation experiment.
L20	1	If reference is not defined via edc2 it is calculated as "actual expno - L20"
L30	2	Number of solvents to search. Must be two for this type of solvent suppression
CNST 26	6	Minimum signal-to-noise in the reference spectrum. For a TOCSY 1H/1H a value of 6 is recommended.
NS	32	Max. NS during SINO controlled acquisition.
L 31	8	Min. NS during SINO controlled acquisition.
CNST 29	0.75	Receiver gain control > 0 : multiplier for **RG** from RGA ≤ 0: use value defind in **RG**
D 31	0	DE during RGA. $= 0$: no change; > 0: value for DE in usec.
2 USERA1	-	Command line parameters for the AU program. For possible options see description of the AU program. Typical options are : **pcal-yes:** 90° pulse calibration is done **sref-no**: no SREF is done
CNST 30	0	Exclude region around solvents see page 130
F2PHI, F2PLO	11.0, -1.0	Defines region for solvent search.

Tab. 4.16:   Automation parameters for LCML12.

4.8 TOCSY with mult. presaturation and ^{13}C decoupling (LCMLCWPS)

4.8.1 Description

The described experiment acquires a ^{1}H/^{1}H TOCSY with multiple solvent suppression using just one channel. The number of solvent resonance's is arbitrary. The solvent suppression is done by presaturation using a laminar shifted rectangular pulse. The pulse program used is **lcmlevcwpcpstp** which contains a bax composite pulse for improved solvent suppression. Presaturation is done during relaxation delay.

Decoupling on the ^{13}C channel is applied in **cw**-mode during acquisition which results in a clearance of the position of the ^{13}C satellites for observation of other signals.

The multiple presaturation of arbitrary number and position of solvent resonance's is reached with a modulated rectangular pulse of approx. 10msec. The pulse itself would act like a short presaturation at the applied position (O1) By repeating this pulse D1/P18 times a presaturation of D1 sec is achieved, identical to a conventional presaturation during relaxation delay as described in „TOCSY with double presaturation (LCML12)". If the rectangular shape is modulated by offset frequencies a presaturation is simultaneously done at multiple positions.

4.8.2 Run the experiment automatically

The parameter set used is **LCMLCWPS**. All basic parameters for this experiment are loaded with rpar <parametersetname> and getprosol. The AU program **au_lc2d** is able to automatically determine and set all sample dependend parameters.

Prepare the acquisition

- Create a new data set with edc. For peak 1 use expno 10,… peak 2 20,21,…
- Read the parameter set rpar ....
- Lock the spectrometer with the correct lock <solvent>
- Set pulses and power levels with getprosol.
- Presaturation is done on-resonance therefore O1 is set to the most intense solvent. Consider this when reducing SW.
- Set the desired NS. A number of 24 scans corresponds to an experiment time of approx. 3h 30'.

Verify or change Automation parameters

- Change basic and automation parameters with expset. Accept the displayed settings, or enter a setup routine with CANCEL.
- All other parameters can be changed with eda/ased.

- The shape pulse for the multiple presaturation is calculated automatically. Based on the **PRESATURATION** power **PLDB 9** for a single solvent.

Start the acquisition

- Do xaua. The AU program au_lc2d (details see page 123) will run a preparation experiment on the actual 2D data set.
- After the first row is acquired it is processed in the experiment number 999 on the same data set for the solvent search.
- After this the main experiment is started. Once this is running you can check the progress with rser or xfb.
- To terminate the experiment before the defined number of increments simply enter halt or stop)
- The automation will generate a new shape pulse which is stored under <data set name><expno>p

Hints

The AU program carries out complex operations, note the following points:

- Do not attempt to process the dataset, during the –first- preparation experiment. Note the parametrs like **SW**, **O1**, **DS**, **NS** are changed during this experiment.
- If you abort the acquisition during the preparation experiment you *must* a rpar of the parameter set befor you restart with xaua.
- Preferably do not rerun xaua a second time, do not run xaua on a dataset you have copied with edc or wrpa. Do a rpar before you start xaua.

4.8.3 Run the experiment manually

All basic parameters for this experiment are loaded with rpar <parametersetname> and getprosol. Sample dependent parameters must be determined in a preparation experiment or optimized on the main experiment.

Prepare the acquisition

- Create a new data set with edc. For peak 1 use expno 10,… peak 2 20,21,…
- Read the parameter set rpar ….
- Lock the spectrometer with the correct lock <solvent>
- Set pulses and power levels with getprosol.

Acquire the preparation experiment

- Change to a dataset with expno = 1.
- Read the parameter set **LCZG**.
- Set pulses and power levels with getprosol.
- Acquire the spectrum with zg.
- Transform the spectrum with ef and apply phase correction with apk0

Solvent Search

- Go to the Utilities and set O1 to the desired position

- Create a frequency list with the desired solvent positions.

- Start the shape tool and open a Squa100.1000 shape

- Modulate it with the solvent frequencies. As reference take the O1 from the current data set as suppression positions the entries in the frequency list.

- Adapt the power of shape according to the used number of frequencies.

- Store the shape under a new name, do NOT overwrite the shape Squa100.1000.

Prepare and start the main data set

- Change back to the main data set.

- Enter **O1** as used during the shape generation.

- Enter the new generated shape into **P 18 SPDB6** and **SPNAM6**

- Go to the acquisition window with acqu and enter gs.

- Change **RG**, so that the FID fills approx. 50% of the Y-scale of the window.

- Do a fine adjustment of **O1** in steps of ±1 or ±0.1 Hz steps to minimize the modulations and to get minimum FID area.)

- If possible change **RG** again.

- Store the changed values, stop **gs** and start the main acquisition with zg.

4.8.4 Parameters

General

These are principle parameters that define the experiment. They are loaded with rpar <parametersetname> followed by getprosol.

Parameter	Typical value	Comment
AQMOD		DQD if available.
TD/SW	2k/20ppm	adjust to give an AQ of approx. 0.1sec
SI	1k	normally TD/2.
1 TD	256	Preferably use more scans and less increments. Use linear prediction to compensate this.
PULPROG	lcmlevcwpcpstp	TOCSY with multiple presaturation and bax composite pulse.
NS	≈32	multiple of 8, with the same number of scans a signal-to-noise of at least 6:1 should be reached in a 1D spectrum with solvent suppression.
DS	24	not less than 24 to establish a steady state of the heating effects caused by the decoupling before data acquisition starts.
D9	>≈ 35msec	Mixing time for TOCSY, adapt to your sample.
P1/PLDB 1		90° degree proton observe pulse.

Parameter	Typical value	Comment
P18	$\approx$ 10msec	Length of shape pulse used for presaturation. It is repeated during **D1**.
PLDB 21	$\approx$ 20dB	^{13}C decoupling power in **cw**-mode. Power as requird for a 1000Hz field.
O2P		Position for ^{13}C decoupling frequency. Set on resonance to ^{13}C solvent signal for example 2 ppm for acetonitrile. The ^{13}C frequency is relatively independent from the solvent composition, it is not necessary to adjust it for each sample.
P6/PLDB 1 0	$\approx$ 25us/16dB	Spinlock pulse.
P 17	$\approx$ 2.5 msec	Trim pulse.
D1	$\approx$ 2,4	Relaxation delay approx. 1-3sec. D1 is not directly executed, is used for the calculation of **L 6**.

Tab. 4.17   General parameters for LCMLCWPS.

Sample dependent

These are parameters that change for each sample. The user must adjust them individually or start the automation routine with xaua.

Parameter	Typical value	Comment
O1		On-resonance on most intens solvent signal. To minimize **SW O1** can be also somewhere and all solvents are suppressed off-resonance. However optimization in **gs**-mode is more difficult..
SPDB6	25 – 5 Hz	presaturation power for the solvent suppression. Start with power as set in **PLDB 9** for single suppression and subtract -6dB for second solvent.
SPNAM6		Name of the shape pulse modulated with the offset frequencies.
RG		has to be adapted to the sample. With 60% acetonitrile in a 4 mm LC probe at 500 MHz a RG or approx. 128 - 512 should be reached.

Tab. 4.18   Sample dependent parameters for LCMLCWPS.

Automation

These parameters are relevant if the acquisition is started with xaua or in ICONNMR. As they are not part of the pulsprogram, they do not appear in the **ased** window! Standard values are loaded with rpar. They can be directly changed or using expset. Note that it is strongly recommend to use the expset

routine rather than defining parameters manually. Validity of values and exact writing of keywords is only ensured by the automatic setup.

Parameter	Typical value	Comment
AUNM	au_lc2d	AU program for automation, started with <u>xaua</u>.
L25	4	NS for solvent search spectrum.
L28	8	RG during preparation experiment.
L20	1	If not defined via <u>edc2</u> Reference data set is searched as "actual expno - L20"
L30	2	Number of solvents to search.
CNST 26	6	Minimum signal-to-noise in the reference spectrum. For a TOCSY 1H/1H a value of 6 is recommended.
NS	32	Max. NS during SINO controlled acquisition.
L 31	8	Min. NS during SINO controlled acquisition.
CNST 29	0.75	Receiver gain control > 0 : multiplier for **RG** from RGA ≤ 0: use value defind in **RG**
D 31	0	DE during RGA. $= 0$: no change; > 0: value for DE in usec.
PLDB 9	25 – 5 Hz	Power for presaturation of *one frequency*. Reference powerlevel for calculation of the powerlevel **SPDB 6** for multiple presaturation, see "Presaturation Powerlevels" page 22
2 USERA1	-	Command line parameters for the AU program. For possible options see description of the AU program. Typical options are : **Offres:** O1 is not changed **pcal-yes:** 90° pulse calibration is done **sref-no**: no SREF is done
CNST 30	0	Exclude region around solvents see page 130
F2PHI, F2PLO	11.0, -1.0	Defines region for solvent search.

Tab. 4.19:   Automation parameters for LCMLCWPS.

4.9 TOCSY with mult. WET solvent suppression and ^{13}C decoupling (MLEVDCPHWT)

4.9.1 Description

The described experiment acquires a ^{1}H/^{1}H TOCSY with multiple solvent suppression using just one channel. The number of solvent resonance's is arbitrary. The solvent suppression is done by WET solvent suppression using a selective excitation shape pulse. The pulse program used is **mlevdcphwt** which contains a bax composite pulse for improved solvent suppression.

Composite pulse decoupling on the ^{13}C channel is applied during selective excitation and acquisition which leads to a suppression of the satellites and a displacement to the position of the ^{12}C-proton signal.

The WET sequence consists of a selective pulse applied to the solvent frequencies. A dephasing gradient is then used to destroy this unwanted magnetization. This procedure is repeated four times with different flip angles and gradient strength (80:40:20:10).

The suppression process only takes 60-100 msec (4 shape pulses + 4 dephasing gradients). Due to this short duration it is also possible to apply ^{13}C decoupling during the suppression sequence, which means that the ^{13}C satellites are also dramatically reduced in their intensity.

The solvent suppression with WET is relatively tolerant to small deviations from the exact position of the solvent frequencies.

4.9.2 Run the experiment automatically

The parameter set used is **MLEVDCPHWT**. All basic parameters for this experiment are loaded with rpar <parametersetname> and getprosol. The AU program **au_lc2d** is able to automatically determine and set all sample dependend parameters.

Prepare the acquisition

- Create a new data set with edc. For peak 1 use expno 10,… peak 2 20,21,…
- Read the parameter set rpar ....
- Lock the spectrometer with the correct lock <solvent>
- Set pulses and power levels with getprosol.
- Suppression is done off-resonance, i.e. you can optimize O1 and the spectral window to your sample (they remain unchanged).
- Set the desired NS. A number of 24 scans corresponds to an experiment time of approx. 3h 30'.

Verify or change Automation parameters

- Change basic and automation parameters with expset. Accept the displayed settings, or enter a setup routine with CANCEL.
- All other parameters can be changed with eda/ased.

- The shape pulse used for the WET multiple suppression is calculated automatically from **P11 SPDB2 SPNAM2**.

Start the acquisition

- Do xaua. The AU program au_lc2d (details see page 123) will run a preparation experiment on the actual 2D data set.
- After the first row is acquired it is processed in the experiment number 999 on the same data set for the solvent search.
- After this the main experiment is started. Once this is running you can check the progress with rser or xfb.
- To terminate the experiment before the defined number of increments simply enter halt or stop)
- The automation will generate a new shape pulse which is stored under <data set name><expno>p

Hints

The AU program carries out complex operations, note the following points:

- Do not attempt to process the dataset, during the –first- preparation experiment. Note the parametrs like **SW**, **O1**, **DS**, **NS** are changed during this experiment.

- If you abort the acquisition during the preparation experiment you *must* a rpar of the parameter set befor you restart with xaua.

- Preferably do not rerun xaua a second time, do not run xaua on a dataset you have copied with edc or wrpa. Do a rpar before you start xaua.

4.9.3 Run the experiment manually

All basic parameters for this experiment are loaded with rpar <parametersetname> and getprosol. Sample dependent parameters must be determined in a preparation experiment or optimized on the main experiment.

Prepare the acquisition

- Create a new data set with edc. For peak 1 use expno 10,… peak 2 20,21,…
- Read the parameter set rpar ....
- Lock the spectrometer with the correct lock <solvent>
- Set pulses and power levels with getprosol.

Acquire the preparation experiment

- Change to a dataset with expno = 1.
- Read the parameter set **LCZG**.
- Set pulses and power levels with getprosol.
- Acquire the spectrum with zg.
- Transform the spectrum with ef and apply phase correction with apk0

Search the solvent

- Go to the Utilities and set O1 to the desired position

- Create a frequency list with the desired solvent positions.
- Start the shape tool and open the desired selective excitation pulse (for example Sinc1.1000).
- Modulate it with the solvent frequencies. As reference take the O1 from the current data set as suppression positions the entries in the frequency list.
- Do *not* use a scaling for the individual frequencies.
- Adapt the power of shape according to the used number of frequencies.
- Store the shape under a new name, do NOT overwrite the original shape.

Prepare the main data set

- Change back to the main data set.
- Enter **O1** as used during the shape generation.
- Enter the new generated shape pulse as **P11 SPDB1** and **SPNAM1**
- To set the WET pulses calculate the values for SPdB7.

4.9.4 Parameters

General

These are principle parameters that define the experiment. They are loaded with rpar <parametersetname> followed by getprosol.

Parameter	Typical value	Comment
AQMOD		DQD if available.
TD/SW	2k/12ppm	adjust to give an AQ of approx. 0.1sec
SI	1k	normally TD/2.
1 TD	128	Preferably use more scans and less increments. Use linear prediction to compensate this.
PULPROG	mlevdcphwt	TOCSY with multiple WET solvent suppression and decoupling during WET and acquisition.
NS	≈32	multiple of 8, with the same number of scans a signal-to-noise of at least 6:1 should be reached in a 1D spectrum with solvent suppression.
DS	24	not less than 24 to establish a steady state of the heating effects caused by the decoupling before data acquisition starts.
D9	$> \approx 35$msec	Mixing time for TOCSY, adapt to your sample.
P1/PLDB 1		90° degree proton observe pulse.
P11	$\approx 10\text{-}20$msec	Length of shape pulse used for selective excitation.
PLDB 14	≈ 17dB	carbon decoupling power for **cpd**-decoupling.
CPD2	60usec	carbon decoupling pulse for **cpd**-decoupling.
CPDPRG2	garp.p31	carbon decoupling pulse for **cpd**-decoupling.
O2P		Position for ^{13}C decoupling frequency. Set on resonance to ^{13}C solvent signal for example 2 ppm

Parameter	Typical value	Comment
		for acetonitrile. The ^{13}C frequency is relatively independent from the solvent composition, it is not necessary to adjust it for each sample.
P6/PLDB 10	$\approx$ 25us/16dB	Spinlock pulse.
P 17	$\approx$ 2.5 msec	Trim pulse.
D1	$\approx$ 2,4	Relaxation delay approx. 1-3sec.

Tab. 4.20   *General parameters for MLEVDCPHWT.*

Sample dependent

These are parameters that change for each sample. The user must adjust them individually or start the automation routine with xaua.

Parameter	Typical value	Comment
O1		On-resonance on most intens solvent signal. To minimize **SW O1** can be also somewhere and all solvents are suppressed off-resonance. However optimization in **gs**-mode is more difficult..
SPDB7 **SPDB8** **SPDB9** **SPDB10**	90° +0.87 90° -1.04 90° +2.27 90° -5.05	selective excitation power for the four WET pulses. The power of the pulses corresponds to four different flip angles in the order of 90°.
SPNAM7 – SPNAM10	same shape for all	Name of a shape pulse modulated with the offset frequencies of the solvent signals.
RG	1-4k	has to be adapted to the sample. With 60% acetonitrile in a 4 mm LC probe at 500 MHz a RG or approx. 1-2k should be reached.

Tab. 4.21   *Sample dependent parameters for MLEVDCPHWT.*

Automation

These parameters are relevant if the acquisition is started with xaua or in ICONNMR. As they are not part of the pulsprogram, they do not appear in the **ased** window! Standard values are loaded with rpar. They can be directly changed or using expset. Note that it is strongly recommend to use the expset routine rather than defining parameters manually. Validity of values and exact writing of keywords is only ensured by the automatic setup.

Parameter	Typical value	Comment
AUNM	au_lc2d	AU program for automation, started with xaua.
L25	4	NS for solvent search spectrum.

Parameter	Typical value	Comment
L28	8	RG during preparation experiment.
L20	1	If reference is not defined via <u>edc2</u> it is calculated as "actual expno - L20"
L30	2	Number of solvents to search.
SPDB2	$\approx 60dB$	Defines the power of a selective 90° excitation pulse for a *single frequency*. It is taken as starting point for the calculation of the multiple excitation power.
SPNAM2	Sinc1.1000	Selective single excitation pulse. This shape must exist, it will be modified with the offset frequencies.
SPDB15	150	If $-6 \le$ **SPDB15** ≤ 30 it is used as correction value for the last WET power level (SPdB10).
CNST 26	6	Minimum signal-to-noise in the reference spectrum. For a TOCSY 1H/1H a value of 6 is recommended.
NS	32	Max. NS during SINO controlled acquisition.
L 31	8	Min. NS during SINO controlled acquisition.
CNST 29	0.75	Receiver gain control > 0 : multiplier for **RG** from RGA ≤ 0: use value defind in **RG**
D 31	0	DE during RGA. $= 0$: no change; > 0: value for DE in usec.
2 USERA1	**offres**	Command line parameters for the AU program. For possible options see description of the AU program. Typical options are : **Offres:** O1 is not changed **pcal-yes:** 90° pulse calibration is done **sref-no**: no SREF is done
CNST 30	0	Exclude region around solvents see page 130
F2PHI, F2PLO	11.0, -1.0	Defines region for solvent search.

Tab. 4.22:   Automation parameters for MLEVDCPHWT.

Hints

The shape pulse which is used for the multiple solvent suppression is calculated automatically. As starting point of this calculation the following parameters are used.

P 11, SPDB 2 and **SPNAM2.**

The automation assumes that this parameters contains the correct values for a single excitation 90° selective pulse. On the previous version of **prosol** those parameters were not supported. Therefore make sure that they are enter correctly in **prosol**.

4.10 COSY with mult. presaturation and ^{13}C decoupling (COSYCWPHPS)

4.10.1 Description

The described experiment acquires a ^{1}H/^{1}H COSY with multiple solvent suppression using just one channel. The number of solvent resonance's is arbitrary. The solvent suppression is done by presaturation using a laminar shifted rectangular pulse. The pulse program used is **cosycwphps**. Presaturation is done during relaxation delay.

Decoupling on the ^{13}C channel is applied in **cw**-mode during acquisition which results in a clearance of the position of the ^{13}C satellites for observation of other signals.

The multiple presaturation of arbitrary number and position of solvent resonance's is reached with a short rectangular pulse or approx. 10msec. The pulse itself would act like a short presaturation at the applied position (**O1**) By repeating this pulse **D1**/**P18** times a presaturation of **D1** sec is achieved, identical to a conventional presaturation during relaxation delay. If the rectangular shape is modulated by offset frequencies a presaturation is simultaneously done at multiple positions. Thus applying one pulse on one channel simultaneously does a suppression of several solvent resonances.

Decoupling on the ^{13}C channel is applied in **cw**-mode during acquisition which results in a clearance of the position of the ^{13}C satellites for observation of other signals.

4.10.2 Run the experiment automatically

The parameter set used is **COSYCWPHPS**. All basic parameters for this experiment are loaded with rpar <parametersetname> and getprosol. The AU program **au_lc2d** is able to automatically determine and set all sample dependend parameters.

Prepare the acquisition

- Create a new data set with edc. For peak 1 use expno 10,… peak 2 20,21,…
- Read the parameter set rpar ....
- Lock the spectrometer with the correct lock <solvent>
- Set pulses and power levels with getprosol.
- Presaturation is done on-resonance therefore O1 is set to the most intense solvent. Consider this when reducing SW.
- Set the desired NS. A number of 24 scans corresponds to an experiment time of approx. 3h 30'.

Verify or change Automation parameters

- Change basic and automation parameters with expset. Accept the displayed settings, or enter a setup routine with CANCEL.

- All other parameters can be changed with <u>eda</u>/<u>ased</u>.
- The shape pulse for the multiple presaturation is calculated automatically. Based on the presaturation power **PLDB 9** for a single solvent.

Start the acquisition

- Do <u>xaua</u>. The AU program au_lc2d (details see page 123) will run a preparation experiment on the actual 2D data set.
- After the first row is acquired it is processed in the experiment number 999 on the same data set for the solvent search.
- After this the main experiment is started. Once this is running you can check the progress with <u>rser</u> or <u>xfb</u>.
- To terminate the experiment before the defined number of increments simply enter <u>halt</u> or <u>stop</u>)
- The automation will generate a new shape pulse which is stored under <data set name><expno>p

Hints

The AU program carries out complex operations, note the following points:

- Do not attempt to process the dataset, during the –first- preparation experiment. Note the parametrs like **SW**, **O1**, **DS**, **NS** are changed during this experiment.
- If you abort the acquisition during the preparation experiment you ***must*** a <u>rpar</u> of the parameter set befor you restart with <u>xaua</u>.
- Preferably do not rerun <u>xaua</u> a second time, do not run xaua on a dataset you have copied with <u>edc</u> or <u>wrpa</u>. Do a <u>rpar</u> before you start <u>xaua</u>.

4.10.3 Run the experiment manually

All basic parameters for this experiment are loaded with <u>rpar <parametersetname></u> and <u>getprosol</u>. Sample dependent parameters must be determined in a preparation experiment or optimized on the main experiment.

Prepare the acquisition

- Create a new data set with <u>edc</u>. For peak 1 use expno 10,… peak 2 20,21,…
- Read the parameter set <u>rpar ....</u>.
- Lock the spectrometer with the correct <u>lock <solvent></u>
- Set pulses and power levels with <u>getprosol</u>.

Acquire the preparation experiment

- Change to a dataset with expno = 1.
- Read the parameter set **LCZG**.
- Set pulses and power levels with <u>getprosol</u>.
- Acquire the spectrum with <u>zg</u>.
- Transform the spectrum with <u>ef</u> and apply phase correction with <u>apk0</u>

Solvent Search

- Go to the Utilities and set O1 to the desired position
- Create a frequency list with the desired solvent positions.
- Start the shape tool and open a Squa100.1000 shape
- Modulate it with the solvent frequencies. As reference take the O1 from the current data set as suppression positions the entries in the frequency list.
- Adapt the power of shape according to the used number of frequencies.
- Store the shape under a new name, do NOT overwrite the shape Squa100.1000.

Prepare and start the main data set

- Change back to the main data set.
- Enter **O1** as used during the shape generation.
- Enter the new generated shape into **P 18 SPDB6** and **SPNAM6**
- Go to the acquisition window with <u>acqu</u> and enter <u>gs</u>.
- Change **RG**, so that the FID fills approx. 50% of the Y-scale of the window.
- Do a fine adjustment of **O1** in steps of ±1 or ±0.1 Hz steps to minimize the modulations and to get minimum FID area.)
- If possible change **RG** again.
- Store the changed values, stop **gs** and start the main acquisition with <u>zg</u>.

4.10.4 Parameters

General

These are principle parameters that define the experiment. They are loaded with <u>rpar</u> <parametersetname> followed by <u>getprosol</u>.

Parameter	Typical value	Comment
AQMOD		DQD if available.
TD/SW	2k/12ppm	adjust to give an AQ of approx. 0.1sec
SI	1k	normally TD/2.
1 TD	128	Preferably use more scans and less increments. Use linear prediction to compensate this.
PULPROG	cosycwphps	COSY with multiple WET solvent suppression and decoupling during WET and acquisition.
NS	≈32	Arbitrary. With the same number of scans a signal-to-noise of at least 6:1 should be reached in a 1D spectrum with solvent suppression.
DS	24	not less than 24 to establish a steady state of the heating effects caused by the decoupling before data acquisition starts.

Parameter	Typical value	Comment
P0		20-90° observe pulse
P1/PLDB 1		90° pulse.
P18	$\approx$ 10msec	Length of shape pulse used for presaturation. It is repeated during D1.
PLDB 21	$\approx$ 20dB	^{13}C decoupling power in cw-mode. Power as requird for a 1000Hz field.
O2P		Position for ^{13}C decoupling frequency. Set on resonance to ^{13}C solvent signal for example 2 ppm for acetonitrile. The ^{13}C frequency is relatively independent from the solvent composition, it is not necessary to adjust it for each sample.
D1	$\approx$ 2,4	Relaxation delay approx. 1-3sec.

Tab. 4.23   General parameters for COSYCWPHPS.

Sample dependent

These are parameters that change for each sample. The user must adjust them individually or start the automation routine with xaua.

Parameter	Typical value	Comment
O1		On-resonance on most intens solvent signal. To minimize SW O1 can be also somewhere and all solvents are suppressed off-resonance. However optimization in gs-mode is more difficult..
SPDB6	25 – 5 Hz	presaturation power for the solvent suppression. Start with power as set in PLDB 9 for single suppression and subtract -6dB for second solvent , see "Presaturation Powerlevels" page 22
SPNAM6		Name of the shape pulse modulated with the offset frequencies.
RG	1-4k	has to be adapted to the sample. With 60% acetonitrile in a 4 mm LC probe at 500 MHz a RG or approx. 1-2k should be reached.

Tab. 4.24   Sample dependent parameters for COSYCWPHPS.

Automation

These parameters are relevant if the acquisition is started with xaua or in ICONNMR. As they are not part of the pulsprogram, they do not appear in the ased window! Standard values are loaded with rpar. They can be directly changed or using expset. Note that it is strongly recommend to use the expset routine rather than defining parameters manually. Validity of values and exact writing of keywords is only ensured by the automatic setup.

Parameter	Typical value	Comment
AUNM	au_lc2d	AU program for automation, started with <u>xaua</u>.
L25	4	NS for solvent search spectrum.
L28	8	RG during preparation experiment.
L20	1	If reference is not defined via <u>edc2</u> it is calculated as "actual expno - L20"
L30	2	Number of solvents to search.
CNST 26	10	Minimum signal-to-noise in the reference spectrum. For a COSY ^{1}H/^{1}H a value of 10 is recommended.
NS	32	Max. NS during SINO controlled acquisition.
L 31	8	Min. NS during SINO controlled acquisition.
CNST 29	0.75	Receiver gain control > 0 : multiplier for **RG** from RGA ≤ 0: use value defind in **RG**
D 31	0	DE during RGA. $= 0$: no change; > 0: value for DE in usec.
PLDB 9	25 – 5 Hz	Power for presaturation of *one frequency*. Reference powerlevel for calculation of the powerlevel **SPDB 6** for multiple presaturation, see "Presaturation Powerlevels" page 22
2 USERA1	-	Command line parameters for the AU program. For possible options see description of the AU program. Typical options are : **Offres:** O1 is not changed **pcal-yes:** 90° pulse calibration is done **sref-no**: no SREF is done
CNST 30	0	Exclude region around solvents see page 130
F2PHI, **F2PLO**	11.0, -1.0	Defines region for solvent search.

Tab. 4.25:   Automation parameters for COSYCWPHPS.

4.11 COSY with mult. WET solvent suppression ^{13}C decoupling (COSYDCPHWT)

4.11.1 Description

The described experiment acquires a ^{1}H/^{1}H COSY with multiple solvent suppression using just one channel. The number of solvent resonance's is arbitrary. The solvent suppression is done by WET solvent suppression using a selective excitation shape pulse. The pulse program used is **cosydcphwt**.

Composite pulse decoupling on the ^{13}C channel is applied during selective excitation and acquisition which leads to a suppression of the satellites and a displacement to the position of the ^{12}C-proton signal.

The WET sequence consists of a selective pulse applied to the solvent frequencies. A dephasing gradient is then used to destroy this unwanted magnetization. This procedure is repeated four times with different flip angles and gradient strength (80:40:20:10).

The suppression process only takes 60-100 msec (4 shape pulses + 4 dephasing gradients). Due to this short duration it is also possible to apply ^{13}C decoupling during the suppression sequence, which means that the ^{13}C satellites are also dramatically reduced in their intensity.

The solvent suppression with WET is relatively tolerant to small deviations from the exact position of the solvent frequencies.

4.11.2 Run the experiment automatically

The parameter set used is **COSYDCPHWT**. All basic parameters for this experiment are loaded with rpar <parametersetname> and getprosol. The AU program **au_lc2d** is able to automatically determine and set all sample dependend parameters.

Prepare the acquisition
- Create a new data set with edc. For peak 1 use expno 10,… peak 2 20,21,…
- Read the parameter set rpar ....
- Lock the spectrometer with the correct lock <solvent>
- Set pulses and power levels with getprosol.
- Suppression is done off-resonance, i.e. you can optimize O1 and the spectral window to your sample (they remain unchanged).
- Set the desired NS. A number of 24 scans corresponds to an experiment time of approx. 3h 30'.

Verify or change Automation parameters
- Change basic and automation parameters with expset. Accept the displayed settings, or enter a setup routine with CANCEL.
- All other parameters can be changed with eda/ased.

- The shape pulse used for the WET multiple suppression is calculated automatically from **P11 SPDB2 SPNAM2**.

Start the acquisition

- Do <u>xaua</u>. The AU program au_lc2d (details see page 123) will run a preparation experiment on the actual 2D data set.
- After the first row is acquired it is processed in the experiment number 999 on the same data set for the solvent search.
- After this the main experiment is started. Once this is running you can check the progress with <u>rser</u> or <u>xfb</u>.
- To terminate the experiment before the defined number of increments simply enter <u>halt</u> or <u>stop</u>)
- The automation will generate a new shape pulse which is stored under <data set name><expno>p

Hints

The AU program carries out complex operations, note the following points:

- Do not attempt to process the dataset, during the –first- preparation experiment. Note the parametrs like **SW**, **O1**, **DS**, **NS** are changed during this experiment.
- If you abort the acquisition during the preparation experiment you *must* a <u>rpar</u> of the parameter set befor you restart with <u>xaua</u>.
- Preferably do not rerun <u>xaua</u> a second time, do not run xaua on a dataset you have copied with <u>edc</u> or <u>wrpa</u>. Do a <u>rpar</u> before you start <u>xaua</u>.

4.11.3 Run the experiment manually

All basic parameters for this experiment are loaded with <u>rpar <parametersetname></u> and <u>getprosol</u>. Sample dependent parameters must be determined in a preparation experiment or optimized on the main experiment.

Prepare the acquisition

- Create a new data set with <u>edc</u>. For peak 1 use expno 10,… peak 2 20,21,…
- Read the parameter set <u>rpar ...</u>.
- Lock the spectrometer with the correct <u>lock <solvent></u>
- Set pulses and power levels with <u>getprosol</u>.

Acquire the preparation experiment

- Change to a dataset with expno = 1.
- Read the parameter set **LCZG**.
- Set pulses and power levels with <u>getprosol</u>.
- Acquire the spectrum with <u>zg</u>.
- Transform the spectrum with <u>ef</u> and apply phase correction with <u>apk0</u>

<u>*Search the solvent*</u>

- Go to the Utilities and set O1 to the desired position

- Create a frequency list with the desired solvent positions.
- Start the shape tool and open the desired selective excitation pulse (for example Sinc1.1000).
- Modulate it with the solvent frequencies. As reference take the O1 from the current data set as suppression positions the entries in the frequency list.
- Do *not* use a scaling for the individual frequencies.
- Adapt the power of shape according to the used number of frequencies.
- Store the shape under a new name, do NOT overwrite the original shape.

Prepare the main data set

- Change back to the main data set.
- Enter **O1** as used during the shape generation.
- Enter the new generated shape pulse as **P11 SPDB1** and **SPNAM1**
- To set the WET pulses calculate the values for SPdB7.

4.11.4 Parameters

General

These are principle parameters that define the experiment. They are loaded with rpar <parametersetname> followed by getprosol.

Parameter	Typical value	Comment
AQMOD		DQD if available.
TD/SW	2k/12ppm	adjust to give an AQ of approx. 0.1sec
SI	1k	normally TD/2.
1 TD	128	Preferably use more scans and less increments. Use linear prediction to compensate this.
PULPROG	cosydcphwt	COSY with multiple WET solvent suppression and decoupling during WET and acquisition.
NS	≈32	Arbitrary. With the same number of scans a signal-to-noise of at least 6:1 should be reached in a 1D spectrum with solvent suppression.
DS	24	not less than 24 to establish a steady state of the heating effects caused by the decoupling before data acquisition starts.
P0		20-90° observe pulse
P1/PLDB 1		90° pulse.
P11	≈ 10-20msec	Length of shape pulse used for selective excitation.
PLDB 14	≈ 17dB	carbon decoupling power for **cpd**-decoupling.
P31	210usec	^{13}C pulse for low power **cpd**-decoupling.
CPDPRG2	garp.p31	carbon decoupling pulse for **cpd**-decoupling.

Parameter	Typical value	Comment
O2P		Position for ^{13}C decoupling frequency. Set on resonance to ^{13}C solvent signal for example 2 ppm for acetonitrile. The ^{13}C frequency is relatively independent from the solvent composition, it is not necessary to adjust it for each sample.
D1	≈ 2,4	Relaxation delay approx. 1-3sec.

Tab. 4.26   *General parameters for COSYDCPHWT.*

Sample dependent

These are parameters that change for each sample. The user must adjust them individually or start the automation routine with xaua.

Parameter	Typical value	Comment
O1		On-resonance on most intens solvent signal. To minimize **SW O1** can be also somewhere and all solvents are suppressed off-resonance. However optimization in **gs**-mode is more difficult..
SPDB7 SPDB8 SPDB9 SPDB10	90° +0.87 90° -1.04 90° +2.27 90° -5.05	selective excitation power for the four WET pulses. The power of the pulses corresponds to four different flip angles in the order of 90°.
SPNAM7 – SPNAM10	same shape for all	Name of a shape pulse modulated with the offset frequencies of the solvent signals.
RG	1-4k	has to be adapted to the sample. With 60% acetonitrile in a 4 mm LC probe at 500 MHz a RG or approx. 1-2k should be reached.

Tab. 4.27   *Sample dependent parameters for COSYDCPHWT.*

Automation

These parameters are relevant if the acquisition is started with xaua or in ICONNMR. As they are not part of the pulsprogram, they do not appear in the **ased** window! Standard values are loaded with rpar. They can be directly changed or using expset. Note that it is strongly recommend to use the expset routine rather than defining parameters manually. Validity of values and exact writing of keywords is only ensured by the automatic setup.

Parameter	Typical value	Comment
AUNM	au_lc2d	AU program for automation, started with xaua.
L25	4	NS for solvent search spectrum.

Parameter	Typical value	Comment
L28	8	RG during preparation experiment.
L20	1	If reference is not defined via <u>edc2</u> it is calculated as "actual expno - L20"
L30	2	Number of solvents to search.
SPDB2	≈ 60dB	Defines the power of a selective 90° excitation pulse for a ***single frequency***. It is taken as starting point for the calculation of the multiple excitation power.
SPNAM2	Sinc1.1000	Selective single excitation pulse. This shape must exist, it will be modified with the offset frequencies.
SPDB15	150	If $-6 \leq$ **SPDB15** ≤ 30 it is used as correction value for the last WET power level (SPdB10).
CNST 26	10	Minimum signal-to-noise in the reference spectrum. For a COSY ^{1}H/^{1}H a value of 10 is recommended.
NS	32	Maximum NS during signal-to-noise controlled acquisition.
L 31	8	Minimum NS during signal-to-noise controlled acquisition.
CNST 29	0.75	Receiver gain control > 0 : multiplier for **RG** from RGA ≤ 0: use value defind in **RG**
D 31	0	DE during RGA. $= 0$: no change; > 0: value for DE in usec.
2 USERA1	**offres**	Command line parameters for the AU program. For possible options see description of the AU program. Typical options are : **Offres:** O1 is not changed **pcal-yes:** 90° pulse calibration is done **sref-no**: no SREF is done
CNST 30	0	Exclude region around solvents see page 130
F2PHI, **F2PLO**	11.0, -1.0	Defines region for solvent search.

Tab. 4.28:   *Automation parameters for COSYDCPHWT.*

Hints

The shape pulse which is used for the multiple solvent suppression is calculated automatically. As starting point of this calculation the following parameters are used.

      P 11, SPDB 2 and **SPNAM2.**

The automation assumes that this parameters contains the correct values for a single excitation 90° selective pulse. On the previous version of **prosol** those parameters were not supported. Therefore make sure that they are enter correctly in **prosol**.

4.12 HSQC with WET solvent suppression (HSQCETGPSIWT)

4.12.1 Description

The described experiment acquires a ^{1}H/^{13}C correlation as a 2D spectrum. It is sensitivity enhanced and phase sensitive using echo/antiecho gradient selection.
It also includes WET *multiple* solvent suppression using just one channel. The number of solvent resonance's is arbitrary. However as the HDO resonance is suppressed by the sequence itself, it is normally only necessary to suppress one frequency i.e. the frequency of the acetonitrile/methanol. The solvent suppression is done by WET solvent suppression using a selective excitation shape pulse. The pulse program used is **invietgpsiwt**.
The WET sequence consists of a selective pulse applied to the solvent frequencies. A dephasing gradient is then used to destroy this unwanted magnetization. This procedure is repeated four times with different flip angles and gradient strength (80:40:20:10).
The suppression process only takes 60-100 msec (4 shape pulses + 4 dephasing gradients). Due to this short duration it is also possible to apply ^{13}C decoupling during the suppression sequence, which means that the ^{13}C satellites are also dramatically reduced in their intensity.
The solvent suppression with WET is relatively tolerant to small deviations from the exact position of the solvent frequencies.

4.12.2 Run the experiment automatically

The parameter set used is **HSQCETGPSIWT**. All basic parameters for this experiment are loaded with rpar <parametersetname> and getprosol. The AU program **au_lc2d** is able to automatically determine and set all sample dependend parameters.

Prepare the acquisition

- Create a new data set with edc. For peak 1 use expno 10,… peak 2 20,21,…
- Read the parameter set rpar ....
- Lock the spectrometer with the correct lock <solvent>
- Set pulses and power levels with getprosol. Check decoupling parameters, they may be adjusted for selective decoupling during WET.
- Suppression is done off-resonance, i.e. you can optimize O1 and the spectral window to your sample (they remain unchanged).
- Set the desired NS. A number of 24 scans corresponds to an experiment time of approx. 3h 30'.

Verify or change Automation parameters

- Change basic and automation parameters with expset. Accept the displayed settings, or enter a setup routine with CANCEL.

- All other parameters can be changed with eda/ased.
- The shape pulse used for the WET multiple suppression is calculated automatically from **P11 SPDB2 SPNAM2**.

Start the acquisition

- Do xaua. The AU program au_lc2d (details see page 123) will run a preparation experiment on the actual 2D data set.
- After the first row is acquired it is processed in the experiment number 999 on the same data set for the solvent search.
- After this the main experiment is started. Once this is running you can check the progress with rser or xfb.
- To terminate the experiment before the defined number of increments simply enter halt or stop)
- The automation will generate a new shape pulse which is stored under <data set name><expno>p

Hints

The AU program carries out complex operations, note the following points:

- Do not attempt to process the dataset, during the –first- preparation experiment. Note the parametrs like **SW**, **O1**, **DS**, **NS** are changed during this experiment.
- If you abort the acquisition during the preparation experiment you *must* a rpar of the parameter set befor you restart with xaua.
- Preferably do not rerun xaua a second time, do not run xaua on a dataset you have copied with edc or wrpa. Do a rpar before you start xaua.

4.12.3 Run the experiment manually

All basic parameters for this experiment are loaded with rpar <parametersetname> and getprosol. Sample dependent parameters must be determined in a preparation experiment or optimized on the main experiment.

Prepare the acquisition

- Create a new data set with edc. For peak 1 use expno 10,… peak 2 20,21,…
- Read the parameter set rpar ....
- Lock the spectrometer with the correct lock <solvent>
- Set pulses and power levels with getprosol.

Acquire the preparation experiment

- Change to a dataset with expno = 1.
- Read the parameter set **LCZG**.
- Set pulses and power levels with getprosol.
- Acquire the spectrum with zg.
- Transform the spectrum with ef and apply phase correction with apk0

<u>*Search the solvent*</u>
- Go to the Utilities and set O1 to the desired position
- Create a frequency list with the desired solvent positions.
- Start the shape tool and open the desired selective excitation pulse (for example Sinc1.1000).
- Modulate it with the solvent frequencies. As reference take the O1 from the current data set as suppression positions the entries in the frequency list.
- Do ***not*** use a scaling for the individual frequencies.
- Adapt the power of shape according to the used number of frequencies.
- Store the shape under a new name, do NOT overwrite the original shape.

Prepare the main data set
- Change back to the main data set.
- Enter **O1** as used during the shape generation.
- Enter the new generated shape pulse as **P11 SPDB1** and **SPNAM1**
- To set the WET pulses calculate the values for SPdB7.

4.12.4 Parameters

General
These are principle parameters that define the experiment. They are loaded with <u>rpar</u> <parametersetname> followed by <u>getprosol</u>.

Parameter	Typical value	Comment
AQMOD		DQD if available.
TD/SW	2k/16ppm	adjust to give an AQ of approx. 0.1sec
SI	1k	normally TD/2.
1 TD	256	Preferably use more scans and less increments. Use linear prediction to compensate this.
PULPROG	hsqcetgpsiwt	HSQC echo/anti-echo sensitivity enhanced. With multiple WET solvent suppression and decoupling during WET and acquisition.
NS	96	Arbitrary. With the same NS a signal-to-noise of at least 50:1 should be reached in a 1D spectrum with solvent suppression.
DS	24	not less than 24 to establish a steady state of the heating effects caused by the decoupling before data acquisition starts.
P1/PLDB 1		90° pulse.
P11	$\approx$ 10-20msec	Length of shape pulse used for selective excitation.
PLDB 12	$\approx$ 17dB	carbon decoupling power for **cpd**-decoupling.
CPD2	65usec	carbon decoupling pulse for **cpd**-decoupling of the whole ^{13}C spectrum, not only for the solvents!

Parameter	Typical value	Comment
CPDPRG2	garp	carbon decoupling pulse for **cpd**-decoupling.
O2P		Position for ^{13}C decoupling frequency. Set on resonance to ^{13}C solvent signal for example 2 ppm for acetonitrile. The ^{13}C frequency is relatively independent from the solvent composition, it is not necessary to adjust it for each sample.
D1	$\approx 2,4$	Relaxation delay approx. 1-3sec.

Tab. 4.29　*General parameters for HSQCETGPSIWT.*

Sample dependent

These are parameters that change for each sample. The user must adjust them individually or start the automation routine with xaua.

Parameter	Typical value	Comment
O1		On-resonance on most intens solvent signal. To minimize **SW O1** can be also somewhere and all solvents are suppressed off-resonance. However optimization in **gs**-mode is more difficult..
SPDB7 SPDB8 SPDB9 SPDB10	90° +0.87 90° -1.04 90° +2.27 90° -5.05	selective excitation power for the four WET pulses. The power of the pulses corresponds to four different flip angles in the order of 90°.
SPNAM7 – SPNAM10	same shape for all	Name of a shape pulse modulated with the offset frequencies of the solvent signals.
RG	1-4k	has to be adapted to the sample. With 60% acetonitrile in a 4 mm LC probe at 500 MHz a RG or approx. 1-2k should be reached.

Tab. 4.30　*Sample dependent parameters for HSQCETGPSIWT.*

Automation

These parameters are relevant if the acquisition is started with xaua or in ICONNMR. As they are not part of the pulsprogram, they do not appear in the **ased** window! Standard values are loaded with rpar. They can be directly changed or using expset. Note that it is strongly recommend to use the expset routine rather than defining parameters manually. Validity of values and exact writing of keywords is only ensured by the automatic setup.

Parameter	Typical value	Comment
AUNM	au_lc2d	AU program for automation, started with xaua.
L25	4	NS for solvent search spectrum.

Parameter	Typical value	Comment
L28	8	RG during preparation experiment.
L20	1	If reference is not defined via edc2 it is calculated as "actual expno - L20"
L30	1	Number of solvents to search. Typically only 1 as the HDO signal is suppressed by the sequence itself
SPDB2	$\approx 60dB$	Defines the power of a selective 90° excitation pulse for a *single frequency*. It is taken as starting point for the calculation of the multiple excitation power.
SPNAM2	Sinc1.1000	Selective single excitation pulse. This shape must exist, it will be modified with the offset frequencies.
SPDB15	150	If $-6 \leq$ SPDB15 ≤ 30 it is used as correction value for the last WET power level (SPdB10).
CNST 26	100	Minimum signal-to-noise in the reference spectrum. For a HSQC ^{1}H/^{13}C a value of 50 is recommended.
NS	96	Max. NS during SINO controlled acquisition.
L 31	8	Min. NS during SINO controlled acquisition.
CNST 29	0.75	Receiver gain control > 0 : multiplier for RG from RGA ≤ 0: use value defind in RG
D 31	0	DE during RGA. $= 0$: no change; > 0: value for DE in usec.
2 USERA1	offres	Command line parameters for the AU program. For possible options see description of the AU program. Typical options are : Offres: O1 is not changed pcal-yes: 90° pulse calibration is done sref-no: no SREF is done
CNST 30	0	Exclude region around solvents see page 130
F2PHI, F2PLO	11.0, -1.0	Defines region for solvent search.

Tab. 4.31:   Automation parameters for HSQCETGPSIWT.

Hints

The shape pulse which is used for the multiple solvent suppression is calculated automatically. As starting point of this calculation the following parameters are used.

    P 11, SPDB 2 and SPNAM2.

The automation assumes that this parameters contains the correct values for a single excitation 90° selective pulse. On the previous version of prosol those parameters were not supported. Therefore make sure that they are enter correctly in prosol.

5. AU Programs

5.1 General

As mentioned previously it also is possible to do most of the experiments from „Chapter 4" by execution of AU-programs. The names of those AU programs have already been mentioned. The exact functionality and the parameters which are internally used are described below.

For the proper operation of the AU programs and modules it is necessary to:

- Define the correct probe head with edprobe\define current to allow correct setting of pulses and power levels.

- Define pulses and powers for the LC Probe and the solvent with edprosol.

- Use the solvent **CH3CN+D2O** or **CH3OH+D2O** instead of **D2O** to allow correct calibration of the spectra.
  (With previous XWIN 2.5ff version used solvents **ACND2O** or **MEOHD2O**)

- Lock the spectrometer with lock <solvent> rather than with the <Lock on/off> and <Field> on the BSMS keyboard.

The setup of these special solvents and the prosol table is described in the installation manual.

Only the AU programs which are used in general to acquire NMR spectra are described. The other AU programs which carry out only one special task (for example gradient shimming) are described in the previously in the chapter of the corresponding experiment.

5.2 Debugmessages of AU programs and modules

To get information about the progress of the automation enter as TOPSPIN command xwish debugmod
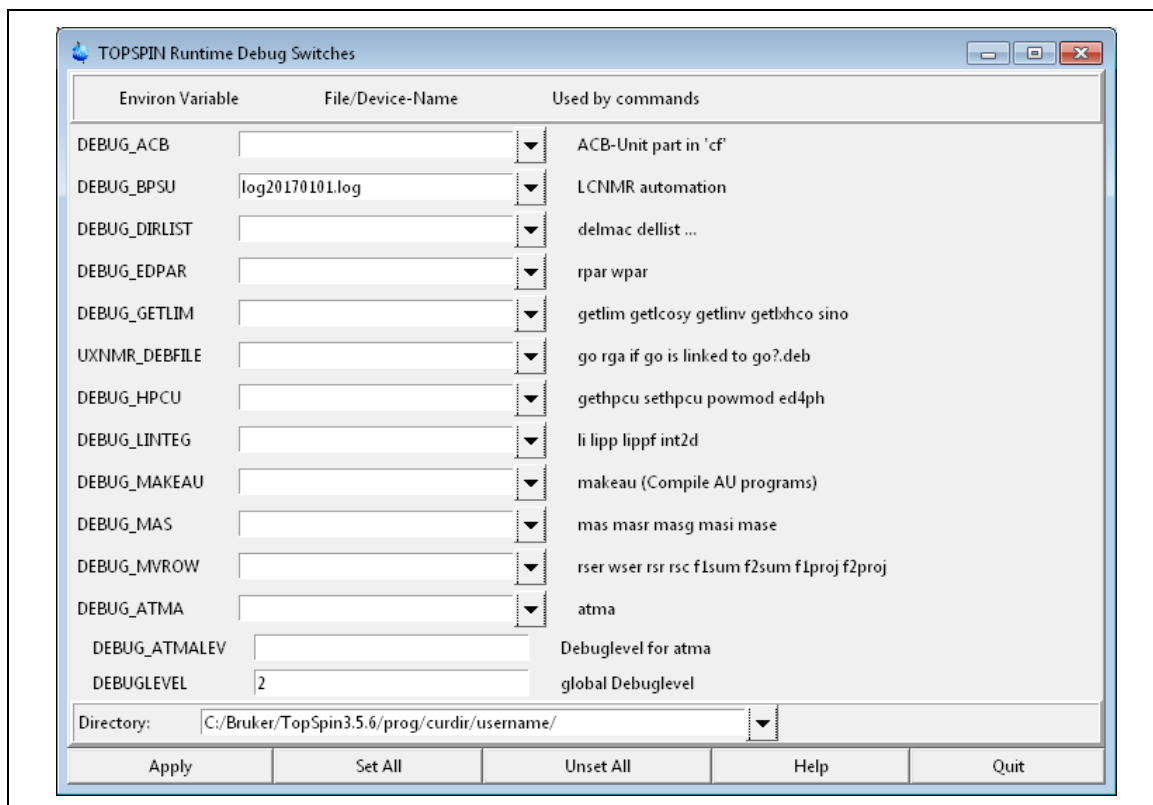
Fig. 5.1   Seeting up debugmessages for AU programs.

Set the variable

DEBUG_BPSU = <filename>

For additional, more detailed information coming from subroutines set the

DEBUGLEVEL = 1 to 3.

Define the Directory where the file shalle be generated in the field

Directory

All special AU programs described in this manual will write detailed messages into the file described by the parameters above. The file is automatically generated if it does not exist, or the information is appended to an existing file.

In case you need assistance from BRUKER:

- Define a NEW (not existing filename)
- Set DEBUGLEVEL to 2
- Run the experiment which causes the problems
- Send the textfile (and preferably the dataset) after the error has appeared.
- Describe what you have done in detail.
    The actions not carried out by the AU program (like rpar, change of parameters, getprosol, ...) are not documented in the logfile.

If the AU program does not produce an error, but does simply not produce the expected results, it might be usefull to inspect the File. The file does not only contain error messages by a detailed listing of the actions taken by the AU programs.

5.3 AU-Program `expset(expsetup)`: Automation setup

The AU program **expset** starts the AU program defined in AUNM in the current dataset with the setup-option. The AU program will then request the parameters which control the automatic spectrum acquisition, when the experiment is started with **xaua**.
Note: the program **expset** replaces the program **expsetup**.

Procedure

- Start the program with xau expset.

- Answer the question of the program.

- The most relevant parameters are request in the first part.

- Typically you can just accept the default values which are proposed.

- All changes are stored *in the parameterset*. The program proposes you to store the modified parameters in a parameterset <original name>_OPT for later usage without expset

- You start the acquisition immediately from within the expset routine or later at any time with **xaua**.

- Currently the programs **au_lc1d**, **au_lc2d** and **lcgshim** are supported.

Details

All changes are stored *in the dataset*, so that the modified parameters can be saved with wpar and reused at a later stage with rpar or within ICONNMR.
It is not the AU program **expsetup** which performs the interactive setup. The Program just calls the program define in AUNM with the commandline parameter **setup**.
In the IconNMR configuration enter **expset** as **userspecific command/parameter**. In the automation window the command is available from the menue **parameters/UserSpecificCommands**
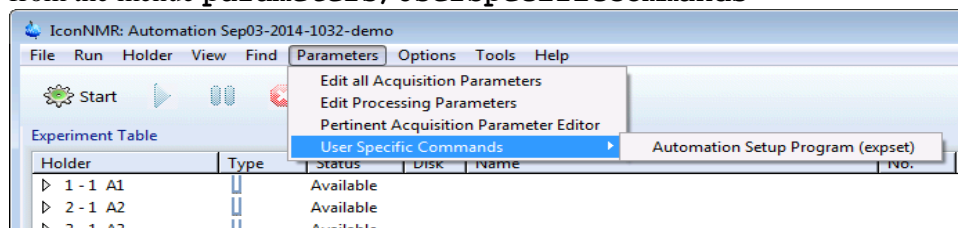
5.4 AU-Program `getinfo`: **Title update**

The TCL script **getinfo** contacts the Hyst PC via a RS232 line, requests the information about the sample and enters this information into the title.
Can be started on any 1D and 2D data set with getinfo.

Procedure

- The Software **HyStar®** is connected via the R232 connection and information about the last transferred peak is requested.

- The information is *appended* to existing title of the actual data set.

Details

- The data connection must be configured with cf

- The chromatography program **HyStar®** running and it must be in a status were it is connected to the RS232 port (i.e. it must be in DoAcquisition or FlowInjection window)

- If the automation software ICONNMR is running in LC-NMR mode, it will block the RS232 connection and getinfo command is not working.

5.5 AU-Program `multicmd`: **Perform multiple commands**

Allows you to perform multiple commands on subsequent data sets. It works similar to the AU program **multizg**, but asks you for the command you want to perform.
Can be started on any 1D and 2D data set with <u>xau multicmd</u>.

Procedure

- Enter <u>xau multicmd</u>

- If the spooler is active choose immediate execution or queuing (details see below(

- Enter the command(s) you want to perform.

- Multiple commands are possible. When entering an empty string the input ends.

- Enter the number of data sets which shall be treated.

Details

- If the Spooler is not running or if you only execute processing commands you can always choose "Normal" mode. In this case each command is executed after ther previous command was finished.

- If the Spooler is active the program multicmd cannot detect if a command was really finished or just entered into the queue (to be executed once it is possible). This will lead to the fact the the processing of dataset #2 will start, while the acquisition command of dataset #2 is still in the queue (waiting that the acquisition of dataset #1 is finished).
  This means you should choose "Spooler" as exec ution, to be sure that all commands are processed in the correct order.
  Only if you perform pure processing you can choose normal mode.

- If the command is **zg** or **xaua** then a experiment time calculation is performed (similar to **multizg**).

- A valid answer is any command you can enter in the commandline of TOPSPIN. Therefore also multiple **EFP**, **APK** and also commands like **NS 128** are possible.

- Major tasks is a multiple **xaua**. If during an overnight run several experiments are conducted, it is useful to perform a solvent search and receivergain adjustment just before the experiment is started. If the experiments are prepared and then a **mulizg** is started any changes in temperature, line shape (and therefore suppression quality) may lead to problems.

5.6 AU-Program `au_lc1d`: **1D spectra with solvent suppression**

The AU program **au_lc1d** controls the acquisition of 1D spectra with all kinds of solvent suppression techniques. The acquisition can be carried out step wise with signal to noise control.
In fact **au_lc1d** use the AU-programs **lcprep** to run a preparation experiment and prepares all parameters for the solvent suppression using the AU-program **lcsetsup**.
The program can be started on any 1D data set with xau au_lc1d or normally with xaua.

Actions carried out by au_lc1d

- Run the AU-program **lcprep** to acquire a 1D solvent search spectrum.

- Run the AU-program **lcsetsup** to find the desired number of solvents and adjust all parameters for solvent suppression on the current data set.

- Determine the RG for the experiment. Either a fixed **RG** can be used, or RGA is executed and the result is correct by **CNST29**.

- The acquisition is always started with the maximum number of scans, determined by **NS** and **TD0**.

- If the signal to noise control is activated the SINO is first checked after **L31** scans and further in iterations of **NS** scans until **TD0** loops or the requested SINO is reached.

- A basic processing is executed after each increment, the signal to noise is determined with AU program lcsino.

Parameters

Further parameters have an effect, as they influence the other AU programs. Check the sections about the AU program **lcprep**, **lcsetsup** and **lcsino**

Parameter	Default value	Description/Comments
CNST 29	0.75	Receiver gain control > 0 : multiplier for **RG** from RGA ≤ 0: use value defind in **RG**
SINO	0	$= 0$ No signal-to-noise control is done > 0 The value is interpreted as desired SINO. The maximum number of scans is determined by NS. Even if the SINO is not reached, the experiment will stop after NS is reached.
L 31	128	NS after which SINO is checked the first time. For reliable phase correction not less than 32.

Parameter	Default value	Description/Comments
CNST 28	3.5	Minimum value of SINO after L31 scans. If it is less then just noise was detected and a further acquisition doesn't make sense.
D 31	0	DE during RGA.
		= 0 : no change; > 0: value for DE in usec.

Tab. 5.1:   *Special Parameters controlling* **au_lc1d**

RG determination

If **CNST 29** is >0 the **RG** is determined with RGA and the result is multiplied with **CNST 29**. By this you have the possibility to correct for overloading or to increase the determined **RG** if the automation is constantly too low.

If **CNST 29** $\leq$ 0 the currently set RG is used. This option is useful, if you want to compare spectra in terms of intensity or signal to noise. Or if timing is very critical, as the RGA may take sometimes up to 30sec.

For special purposes you can define a DE for usage during RGA. The value for DE is stored in D 31. If D 31 is 0 then the original DE is used.

Sino control

The duration of 1D spectra can be controlled with the parameter SINO. Use <u>edp</u> to change the value. If the **SINO** > 0 control is selected then the Signal-to-noise is checked after

- first check after total **NS** = **L31**

- furthers checks each **NS** i.e. after n***NS**, n+1***NS** ... **TD0*****NS**.

The acquisition is stopped if:

> the predefined **SINO** is reached,
> the value is below the minimum defined **SINO** (**CNST 28**),
> the predefined **TD0** are reached

The maximum number of scans is given by **NS*****TD0**. The first check of SINO shouldn't be done before 32 or 64 scans for most reliable phasing.

In previous versions of **au_lc1d** the acquisition was started on expno 999 with the maximum number of scans and the program tried to do a <u>tr</u> after the defined number of scans. Now the acquisition is running on the main experiment and after on loop NS is automatically written to disk..

Processing

For the determination of the signal-to-noise, the program *always* carries out a basic processing. This is even done without SINO control, in case that the data set acts as reference for other data sets. For the signal-to-noise determination the program **lcsino** is started.

Hints

The solvent suppression parameters are adjusted by the AU program **lcsetsup**. Without further command line parameters, all supported solvent suppression techniques are prepared. This means that the AU program **au_lc1d** can be used as **AUNM** for all kinds of suppression parameter sets.

5.7 AU-Program `au_lc2d`: 2D spectra with solvent suppression:

The AU program **au_lc2d** controls the acquisition of 2D spectra with all kinds of solvent suppression techniques. This AU program works similarly to **au_lc1d** and uses the same parameters. During the solvent search the first row of the 2D file is processed in a different data set, i.e. expno 999. With signal to noise control, the SINO achieved in a reference spectrum determines if the acquisition is executed.

In fact **au_lc2d** use the AU-programs **lcprep** to run a preparation experiment and prepares all parameters for the solvent suppression using the AU-program **lcsetsup**.

The program can be started on any 2D data set with xau au_lc2d or normally with xaua.

Procedure

- Evaluation of the Reference spectrum
    - If edc2 is done, this data set is used as reference spectrum
    - If edc2 was not done or the data set is not existing, the expno of the reference data set is calculation from L20 and the actual expno.
- If the reference spectrum was defined, get the SINO achieved in the reference spectrum. Estimate the SINO for the NS of the 2D file. Depending on the result start the continue with the acquisition of the 2D file.
- Run the AU-program **lcprep** to acquire a 2D solvent search spectrum.
- Run the AU-program **lcsetsup** to find the desired number of solvents and adjust all parameters for solvent suppression on the current data set.
- Determine the RG for the experiment. Either a fixed **RG** can be used, or rga is executed and the result is correct by **CNST29**.

Parameters

Parameter	Default value	Description/Comments
CNST 29	0.75	Receiver gain control > 0 : multiplier for **RG** from RGA ≤ 0: use value defind in **RG**
CURDAT2		Reference data set, must contain processed spectrum

Parameter	Default value	Description/Comments
L20	1	If not defined via <u>edc2</u> Reference data set is searched as "actual expno - L20"
CNST 26		= 0 Run 2D without further comparison
		> 0 Use as minimal SINO and compare with reference file
NS	24	Maximal NS during sino controlled acquisition
L 31	0	Minimal NS during sino controlled acquisition.
D 31	0	DE during RGA.
		= 0 : no change; > 0: value for DE in usec.

Tab. 5.2   Special Parameters controlling **au_lc2d**.

Reference data set

The reference data set is defined via the edc2 command. If this is not the case, the program assumes that the reference data set is found under the same name and tries to calculate the experiment number from the actual experiment number and the loop counter L20.

If the actual data set is **test 11 1 u demo** and the loop counter is **L20 = 1** the AU program assumes that the reference is found as

> expno = <actual expno> - L20
> **test 10 1 u demo**

RG determination

If **CNST 29** is >0 the **RG** is determined with RGA and the result is multiplied with **CNST 29**. By this you have the possibility to correct for overloading or to increase the determined **RG** if the automation is constantly too low.

If **CNST 29** $\leq$ 0 the currently set RG is used. This option is useful, if you want to compare spectra in terms of intensity or signal to noise. Or if timing is very critical, as the RGA may take sometimes up to 30sec.

For special purposes you can define a DE for usage during RGA. The value for DE is stored in D 31. If D 31 is 0 then the original DE is used.

Sino control

In contrast to a 1D experiment the NS of a 2D experiment is fixed once the experiment is started. A sino controlled acquisition in this case means that it checks the feasibility of running a 2D by the SINO of the 1D. With the parameter **CNST 26** you can define the minimum signal-to-noise which is necessary for the 2D experiment.

As 1D and 2D file may be acquired with different NS, the SINO for the actual NS of the 2D file is calculated from the value of the reference spectrum is.

- SINO(2D calculated) = SINO(1D) * SQRT{ NS(2D)/ NS(1D)}

Starting with TopSpin 2.0.5 it is not only possible to skip the experiment if the signal to noise in the reference is too small. The program can also reduce NS in the 2D experiment if the signal to noise in the reference is high enough.
The parameters which controll the procedure are:

- NS is the maximum number of scan that will be done.

- L31 defines the minimum number of scans.

- NS will be changed in increments 8 scans (or L31 if L31<8)

The procedure of the signal-to-noise control is:

- If CNST26=0 the experiment is carried out without checking the reference spectrum.

- If CNST26>0 the reference is evaluated. From the signal to noise of the reference it is calculated how many scans are required to reach the signal-to-noise defined in CNST26. The calculated NS is rounded up to the next multiple of 8.

  - If the calculated number for NS is higher then the NS set in the dataset the experiment is skipped.

  - If the calculated number for NS is lower then the NS set in the dataset the NS is reduced to this value. However NS is never set below the minimum value defined.

- As valid increment for NS a stepsize of 8 is assumed to respect the phase cylce. However if the minimum number of NS defined by L31 is <8 the program assume that this is the valid increment.

Hint

Use the command <u>expset</u> to define the parameters of the experiment interactively. In this case you don't have to remember the meaning of the various parameters CNST and L.

5.8 AU-Program `au_lconflow`: **Onflow spectra with solvent suppression**

The AU program **au_lconflow** controls the acquisition of onflow spectra with all kinds of solvent suppression techniques. During an interactive setup all necessary parameters are requested and the user is guided through the procedure. In fact **au_lconflow** uses the AU-programs **lcprep** to run a preparation experiment and prepares all parameters for the solvent suppression using the AU-program **lcsetsup**.

The program should be used together with the Parameterset **LCONFLOW** and can be started with <u>xau au_lconflow</u> or normally with <u>xaua</u>.

Actions carried out by au_lconflow

- Interactive setup of all solvent suppression parameters
- Interactive adjustment of timing
- Run the AU-program **lcprep** to acquire a 1D solvent search spectrum.
- Run the AU-program **lcsetsup** to find the desired number of solvents and adjust all parameters for solvent suppression on the current data set.
- Determine the RG for the experiment. Either a fixed **RG** can be used, or <u>RGA</u> is executed and the result is correct by **CNST29**.
- Start the main acquisition and wait in suspend state to for the sample injection.

Parameters

Further parameters have an effect, as they influence the other AU programs. Check the sections about the AU program **lcprep**, **lcsetsup** and **lcsino**

Timing determination

Minimum value for **NS** and **D1** is requested. The system then asks for the halflinewidth of the chromatographic peak and adjusts the NS accordingly. Fine adjustment is done by slight adjustments of **D1**.

The necessary number of spectra i.e. **1 TD** is calculated from the total runtime of the chromatogram.

RG determination

RG is determined with RGA and the found value is multiplied with **CNST 29**. By this you have the possibility to correct for overloading or to increase the determined **RG** if the automation is constantly too low.

Also a fixed RG can be used by setting **CNST 29** to the corresponding negative value. For example with **CNST 29** = -128 the experiment will be executed with a fixed receiver gain of 128. This option is useful, if you want to compare spectra in terms of intensity or signal to noise. Or if timing is very critical, as the RGA may take sometimes up 30sec.

Start of Acquisition

As the start of the acquisition with <u>zg</u> can take several seconds, the spectrometer is started with zg but a build in suspend command in the pulsprogram pauses the acquisition before the first scan. Only after the start signal from the user, the AU-program will resume the acquisition. The resume works without delay, so that the NMR and LC data acquisition are correctly synchronised.

Hints

The solvent suppression parameters are adjusted by the AU program **lcsetsup**. Without further command line parameters, all supported solvent suppression techniques are prepared. This means that the AU program **au_lconflow** can be used as **AUNM** for all kinds of onflow pulsprograms.

5.9 AU-Program `lcprep`: **Acquire preparation spectrum**

The AU program **lcprep** acquires a preparation experiment under exactly the
same conditions as the main experiment. It will use the same pulse program,
timing and power levels as during the main experiment. Just the RG and the
position of the suppressions are changed. After **lcprep** has finished, the data
set contains a spectrum where **lcsetsup** can be executed for solvent search.
Even for isocratic separation it is useful to determine the exact solvent position
for *every* peak. As the solvent positions depend strongly on pH, temperature,
solvent composition, sometime even the sample concentration.
The program can be started on any 1D or 2D data set with <u>xau lcprep</u>.

Procedure

- Change the parameters for the preparation experiment
    - Reduce RG to L28 or do an RGA
    - Reduce NS to L25 and keep DS
      with command line option "zg" NS 1 and DS 0 and use pulse program
      zg
    - O1p to the left border of the plot region
- Start the acquisition until
    - The 1D spectrum with the reduced NS is finished.
    - The first row of the 2D file is acquired.
- Reset the parameters
- Do Processing for
    - 1D with <u>ef</u>, <u>apk</u> or <u>apkf</u>
    - 2D are not processed, this is done by **lcsetsup**

Parameters

Parameter	Default value	Description/Comments
L 28	8	!=0 value is **RG** for preparation experiment = 0 do **RGA** to find rg for preparation experiment
F1P, F2P	11, 0	**O1** is set to the left spectral border (**F1P**) **SW** is adjusted to contain the region **F1P**-**F2P**
USERA1	keepmix	Command line parameters for the AU program. For possible options see description of the AU program. Typical options are : **D8** is *not* changed during the prep. Experiment Otherwise **D8** is *minimized* to 1ms, the lost time is added to **D1**

Parameter	Default value	Description/Comments
	wrp-no	Do not save solvent search spectra on procno+1 and +2
		By default +1 contains the spectrum without suppression with linebroadening 8Hz and +2 the spectrum with original linebroadening
	zg/-	Pulse program **zg** with **DS**=0 and **NS**=1 is used for the prep. Experiment
		original pulse program, **DS** and **NS**=4 is used
L 25	1	**NS** is set to L25.
D 31	0	DE during RGA.
		= 0 : no change; > 0: value for DE in usec.

Tab. 5.3   *Special Parameters controlling* **lcprep**.

Termination of the program

If the AU-program is aborted before the normal end, the data set rests with changed parameters (reduced NS, O1, ...). Normally the parameters are changed, so that the data set is not suitable for any further data acquisition.

Solvent position

During the preparation experiment the SW enlarged, so that the whole plot region is acquired. By this it is possible to find even solvents which are *outside* the SW of the original spectrum!

Type of spectrum

There are two possibilities of acquiring an preparation spectrum. In the simplest way this is just an one-scan spectrum with pulse program **zg** which will require further manual optimization of **O1**. The other possibility is to use the original data set with original pulse program but with modified parameters in order to receive an non-suppressed spectrum from a suppression sequence. By this procedure the preparation experiment contains the *exact* frequencies of the solvent. This is especially useful for multiple suppression with shape pulse where an individual optimization of the solvent positions is not possible in **gs-**mode. Use **DS** of 32 for ^{13}C decoupled experiments.

Normally the procedure is done in this way. However if speed is an issue and the solvent search can be less precise, you can use the command line option **zg**.

Number of solvents

If the 2D sequence is a multiple quantum sequence it is possible that some of the solvent frequencies (water !) are not visible.

If the concentration of a solvent is very low (HDO) make sure the with the used number of scans it is reliably found. Keep in mind that RG is low.

5.10 AU-Program `lcsetsup`: **Search for solvents**

The AU-program **lcsetsup** carries out a solvent search on an existing 1D spectrum or a 2D data set containing at least 1 acquired row. It searches for the biggest peaks, sets O1 .. O8 on-resonance to those frequencies and produces a shape which can be used for multiple solvent suppression and for wet solvent suppression. The number of solvents which are treated as solvents are defined via the parameters **L30**. Therefore the AU program can be used to setup all parameters for multiple solvent suppression.

Without further command line options, the program will always prepare all parameters (for WET, multiple presat, preaturation through individual channels). Depending on the type of experiment only a subset of this parameters will be used.

Start the program with <u>xau lcsetsup</u> on any 1D or 2D data set which contains a transformed spectrum.

Procedure

- Create a name which is specific for the data set, typically <name><expno>. For example <specificname> = **test10** if the name was **test** and the experiment number **10**.

- If the data set is a 2D file

  - The first row is extracted to expno 999

  - The row is processed with QSIN and LB 4. The magnitude spectrum is generated.

- Baseline correction for the region where the solvents are searched.

- A peak picking is performed with <u>pp</u> according to the actual parameters of **PSCAL** and PP-parameters (**MI**, **MAXI**, **CY** and **PC**).

- The **L30** biggest peaks are used as solvents

  - After the one solvent is found, a region around this peak is exclude for further solvent search. This prevents satellites and 50Hz spikes from being treated as solvents.

- The used frequencies are written in frequency-list format in the directory for the f1-lists. The filename is the <specificname>.
  $TOPSPINHOME/exp/stan/nmr/lists/f1/<specificname>
  A copy of this list is saved data directory under the name
  <disk>/data/<user>/nmr/<name>/<expno>/solvents.f1list

- The used frequencies are written in peaklist-format (as generated by <u>pp</u>) into the acquisition directory in a file called "solvents"
  <disk>/data/<user>/nmr/<name>/<expno>/solvents

- A shape pulse for multiple presaturation is created, starting from the pulse defined in **P18**, **PLDB 9** and **SPNAM6**. The name is **p_automation** or p_<specificname>.

- A shape pulse for multiple excitation is created, starting from the pulse defined in **P11**, **SP2** and **SPNAM2**. The name is **s_automation** or s_<specificname>.

- The shape is transferred into the parameters used for WET solvent suppression **SPDB7**..**10**

- A region file is created from the used frequencies and stored in the processing directory. This file can be used for XWINPLOT scaling <disk>/data/<user>/nmr/<name>/<expno>/pdata/<procno>/reg

- If the second channel is a decoupling channel the position of O2P is calculated as average from all exclusion regions in the file $TOPSPINHOME/exp/stan/nmr/lists/scl/13Cdec.<SOLVENT> or from the regions between 100 and 0 ppm in the file (trying to exclude TSP/TMS and quaternary carbons from the standard region frle) $TOPSPINHOME/exp/stan/nmr/lists/scl/13C.<SOLVENT>

- The found frequencies are written into the title.

Parameter

Parameter	Default value	Description/Comments
F1P,F2P F2PLO,F2PHI	11.0, -1.0	Region for solvent search, must contain solvents, should not contain O1-spike and quad-images. **lcprep** sets O1 on the left side of this region.
L30	2	Number of solvents to search.
PSCAL	preg	global/preg = in plot region sreg = according to **sreglst**
SFO<N>		If SFO2, SFO3 should be changed, they must be within SFO1 ±SWH
CNST 30	0	Exclude region around solvents 0 = 2*CNST 2 for H-solvents 0 = 0,55*CNST 2 for D-solvents >0 = use the value in Hz Exclusion range is +/- the value
CNST 2	136,5	X-H coupling constant. Used for automatic generation of exclusion range.
P18	≈ 10msec	Length of shape pulse used for presaturation. It is repeated during d1 and d8.
USERA1	offres	**O1** is *not* changed during the solvent search. You can optimize O1/SW for the sample. *Attention*, this not possible for all types pulse programs. By default: **O1** is *set to the biggest solvents*. SW must cover the whole spectrum after O1 is moved to the solvent.
	Pcal-yes, pcalf-yes	perform a 90° pulse calibration with AU program pulsecal

Parameter	Default value	Description/Comments
	title-no	Do not append information about solvents into the title
	adjxdecpos-no	Do not change the position of an X-frequence defined on channel 2 or 3.
		By default the position is set into the middle of the solvents (frequencies taken from SCL region file) for effective decoupling
PLDB 9	25 – 5 Hz	Presaturation power for single presaturation, it is taken as starting point for the calculation of the multiple presaturation power. , see "Presaturation Powerlevels" page 22
P11	$\approx$ 10-20msec	Length of shape pulse used for selective excitation.
SPDB2	$\approx$ 60dB	Defines the power of a selective 90° excitation pulse for a ***single frequency***. It is taken as starting point for the calculation of the multiple excitation power.
SPNAM2	Sinc1.1000	Selective single excitation pulse. This shape must exist, it will be modified with the offset frequencies.
SPDB15	1000	If -6 $\leq$ **SPDB15** $\leq$ 30 it is used as correction value for the last WET power level (SPdB10).

Tab. 5.4   Special Parameters controlling `lcsetsup`*.*

Signal selection, Exclusion range

Signals are sorted with descending intensity. Signals which are too near to another are discarded (i.e. the solvent ^{13}C satellites or small signals on the hump of a solvent). The excluded area is defined by CNST 30.
If CNST 30 = 0 the exclusion range is automatically determinded by the type of solvent and the X-H-coupling constant defined in CNST 2.
If the solvent is completely deuterated like "CD3CN" the exclusion range is 0.55 * CNST 2 so that the ^{13}C satellites are excluded. If the solvent name contains a "H" like for CH3CN+D2O the exclusion range is 2 * CNST 2.

```
Range = Signal 1 ± <exclusion range>
```

FREQUENCY   INTENSITY
[PPM]
3.7218   0.35
2.5015   0.39
2.4001   0.47
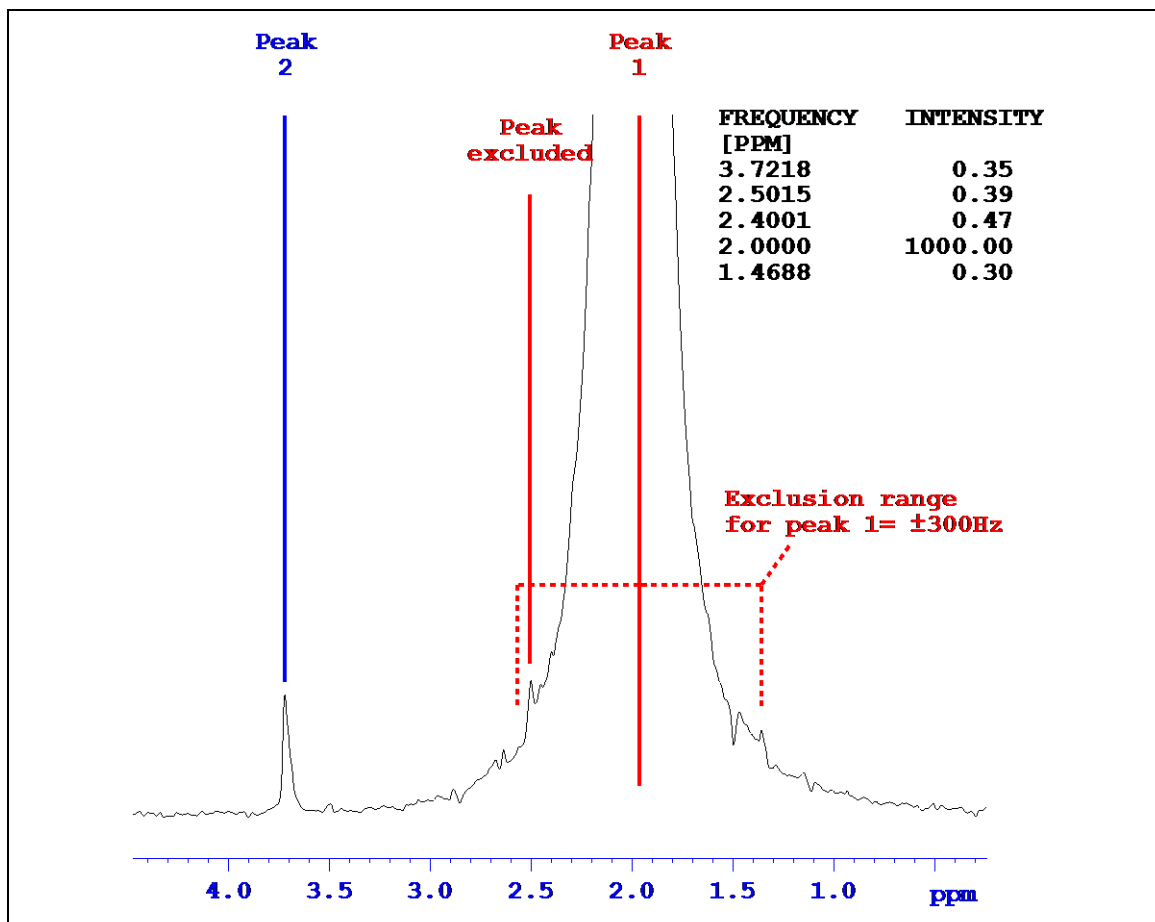2.0000   1000.00
1.4688   0.30

*Fig. 5.2   Solvent search using CNST 30. The signal @ 2.4 ppm (which is not a real
signal) is bigger than the water. Without the exclusion range the second
suppression position would have been at 2.4 ppm*

On resonance suppression

For the suppression the found frequencies are assigned to every spectrometer
channel where the actual SFO is within the range of SFO1 +/- SWH even if the
nucleus is 'off'.
The largest signal in the spectrum is used for **O1**, the second largest for **O2**, and
so on. If not enough signals are found, the remaining channels are set to **O1**.

Shape pulse presaturation

For the generation of a presaturation pulse the shape **Squa100.1000** as
rectangular shape, with 100% truncation level and 1000 points must exist. This
shape is modulated with the found frequencies and is saved. The name depends
on the way how the program lcsetsup is started. In full automation it will use the
default name "p_automation" This is the case when an experiment is started by
ICONNMR or manually with xaua.

Only if lcsetsup is started directly by the user it will use a dataset specific name composed from the label „p_" for „presaturation", the data set name and expno.

> p_<data set name><expno>

Selective excitation

For the generation of a selective excitation shape the shape which was defined with SPNAM2 must exists. This shape is modulated with the desired number of frequencies and is saved. The name depends on the way how the program lcsetsup is started. In full automation it will use the default name "s_automation" This is the case when an experiment is started by ICONNMR or manually with <u>xaua</u>.

Only if lcsetsup is started directly by the user it will use a dataset specific name composed from the label „s_" for „selective", the data set name and expno.

> s_<data set name><expno>

The name of this shape and the power level for the new number of solvents is entered into **SPNAM1** and **SPDB1**. This is a *multiple* excitation selective 90° pulse.

WET parameters

For WET solvent suppression this shape is copied to SPdB7 .. SPdB14 with modified power levels in order to achieve the desired flip angles.

The WET is often optimized by changing the last power level (SPdB10) for minimum FID area.

If -6 ≤ **SPDB15** ≤ 30 then the value of **SP15** is added to the calculated value which is calculated for **SPDB10** (**SPDB10** is *calculated* from **SPDB2**). If **SPDB15** is outside this limits, it is ignored.

In Fig. 5.3 the parameters handling for the WET solvent suppression is displayed.

Shape names

The usage of the shape names has changed from version TOPSPIN 1.3 to 1.6. Shapes generated in full automation (i.e. with xaua or when ICONNMR has started the experiment) will always use and overwrite the shape with the default name "p_automation" and "s_automation". The shapes created for a dataset will only work for this single dataset. Once the sample has left the magnet they are useless. Therefore it is not critical to overwrite the shapes with the parameters from the next sample.

After an experiment which was started xaua has terminated the shape still exists. I.e. you modify certain parameters – like RG or NS – and then restart the experiment with "zg".

If you transfer the next sample into the NMR probe and start the experiment for this sample with xaua it will overwrite the presaturation and selective excitation shape. This is not critical, as the other sample is gone. Even if you bring the same sample back into the probe, slight changes in temperature, shimming etc. will require that you perform a new solvent search, i.e. run the xaua command again, which will again create a new shape.

The reason for the new handling is the fact, that otherwise the wave directory fills up with hundreds or thousands shapes which are of no used any more. The number of shapes generated can create problems with the operating system and makes selection of shapes in the eda editor difficult.
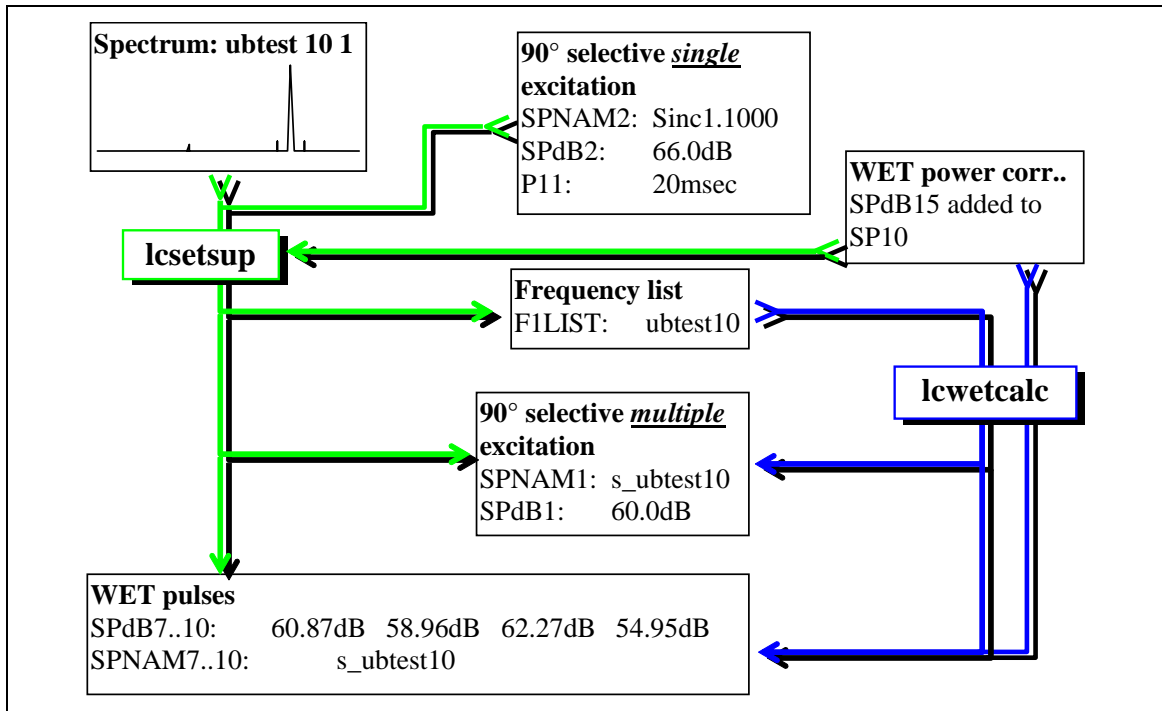

Fig. 5.3:   *Setting parameters for WET with AU programs* `lcsetsup` *and* `lcwetcalc`.

5.11 AU-Program `lcabsf`: **Baseline correction**

The AU program **lcabsf** performs a stepwise baseline corrections for 1D and 2D spectra. The procedure is discussed on page 50 „Baseline correction of the spectrum" in detail.
It tries to localize the solvent positions for the spectrum, so that the baseline correction is carried out in the regions between the solvents. The baseline correction is applied within the plot region.
Start the program on any data set with xau lcabsf

Procedure

- Find the solvent frequencies

- It tries to find the list defined in **FQ1LIST** of the data set.
  This list is generated during the automatic solvent search with the program **lcsetsup**.

- If this list is not defined or existing, the parameter **F1LIST** is evaluated. If the user has generated a frequency list the name will be stored there.

- If this list is also not defined or existing the program looks for a copy of the frequency list in the current data directory. It is generated by the AU-program **lcsetsup** during the solvent search and has the name **solvents.f1list**.

- If this list is not found, the frequencies are take from **O1**, **O2**, ...
  This procedure may fail if a shape pulse sequence was used.

- Make a security copy of the integral file.

- Cut the plot region into regions which are defined by the solvents.

- Carry out a baseline correction for the regions.

- Restore the old integral file, as the **absf** command creates a integral file.

Parameters

Parameter	Default value	Description/Comments
F1P, F2P		Defining the outer limit for the baseline correction
ABSG	5	Degree of polynom used by absf / abs2
FQ1LIST, F1LIST	\<name>\<exp no>	Frequency list with the solvent positions, created by **lcsetsup** during the solvent search. If the list defined in **FQ1LIST** is not found, then the parameter F1list is evaluated. If this list is not existing, then the list in the current directory is searched.
O1 .. O8		If no frequency lists are found, the transmitter positions are evaluated.

Tab. 5.5:   *Parameters controlling* **lcabsf**.

5.12 AU Program `lcsino`: Signal to noise calculation

The AU program **lcsino** performs a baseline correction for the signal and noise region, calculates the signal-to-noise and enters the result into the title.
Similar to the program **lcabsf** it tries to localize the solvent positions for the spectrum. If the signal or noise regions contain a solvent position, they are automatically excluded.

Procedure

- Find the solvent frequencies
- It tries to find the list defined in **FQ1LIST** of the data set.
  This list is generated during the automatic solvent search with the program **lcsetsup**.
- If this list is not defined or existing, the parameter **F1LIST** is evaluated. If the user has generated a frequency list the name will be stored there.
- If this list is also not defined or existing the program looks for a copy of the frequency list in the current data directory. It is generated by the AU-program **lcsetsup** during the solvent search and has the name **solvents.f1list**.
- If this list is not found, the frequencies are take from **O1**, **O2**, ...
  This procedure may fail if a shape pulse sequence was used.
- Adjust or split the signal and noise region, so that they don't contain solvents.
- Carry out a baseline correction for the regions.
- Perform the signal-to-noise determination
- Enter the highest reached value into the title

Parameters

Parameter	Default value	Description/Comments
SIGF1, **SIGF2**		Defines outer limits of signal region. Solvent positions are automatically exclude.
NOISF1, **NOISF2**		Defines borders of noise region. Solvent positions are automatically exclude.
FQ1LIST, **F1LIST**	\<name\>\<exp no\>	Frequency list with the solvent positions, created by **lcsetsup** during the solvent search. If the list defined in **FQ1LIST** is not found, then the parameter F1list is evaluated. If this list is not existing, then the list in the current directory is searched.

Parameter	Default value	Description/Comments
O1 .. O8		If no frequency lists are found, the transmitter positions are evaluated.
CNST2	136.5	Defines the exclusion range around the solvent positions in Hz

Tab. 5.6:   *Parameters controlling* **lcsino**.

5.13 AU Program `proc_onflow`: **1D & 2D processing**

The AU program **proc_onflow** is for processing of on-flow type spectra. The major task is to remove shift induced by solvent composition changes. Also it allows individual phase- and baseline correction of the rows of the 2D file.
In addition it can be also used to collated individual 1D spectra which are for example acquired in timeslice mode. In this case it is also possible to manipulate the spectra to remove effects of different NS and RG.
For details check processing description of the experiments 4.5 Time Slicing - Pseudo onflow on page 77.

5.14 AU Program `rescale`: **Scaling of spectra**

The intensity of spectra which are acquired with different RG/NS and different tuning/matching situation cannot be directly compared because the signal intensity does not correspond to the concentration of the sample.
In LC-NMR the variation of RG and NS (and due to the solvent composition change also of P1) can be large. Major components will be acquired with NS 16 or 64 whereas an impurity will have NS 1024 or more.
In addition the changing solvent composition and solvent suppression efficiency can lead to changing RG values and changes in P1.
The AU program **rescale** will scala a spectrum as if it was acquired with the defined RG/NS (and P1). For a simple comparison you can either scale spectrum A to the conditions of spectrum B. Preferably it is recommended to scale *all* spectra to on common RG and NS (NS 128 and RG 128), then all spectra can be directly compared.

Note: The scaling for the P1 (PULCON) does not convert a spectrum acquired with a 30deg pulse into a spectrum with a 90deg pulse! To use the P1-correction all spectra must be acquired with the same flip angle and the 90deg pulse must have been calibrated (manually or with pulsecal).

Note: The comparison give an *estimation* of the concentration of the sample. For quantitative work many other parameters must be respected.

5.15 AU Program `accept_best`: **Spectra series**

The AU program **accept_best** integrates up to three series of spectra to allow a evaluation of the stability of measurements, a comparison of different sample sets (different sample, different preparation, …) and a comparison against a blank. (Blank and 2nd sample set are optional).
Typical usage is the comparison of a manually prepared/measured sample (Standard/Reference) and an automatically measured sample (BEST-NMR, automatic sample preparation).
Sample A, or if multiple Samples are defined the average of samples A are used as reference.

A series of samples B is compared against this reference and the relative integral (together with min/max deviation of RSD) is reported. This helps to investigate the efficiency and stability of a measurement/preparation procedure against a sample that is considered to be the "gold standard" or simply to compare two different measurement methods..

In a series of Blanks the integral is also evaluated. The average integral together with the min/max value is reported. This allows to evaluate the cross contamination of a sample preparation procedure.

For simple stability measurement it is possible to process only a set of Sample A spectra.

The area which is used for the evaluation must be integrated in the spectrum where the processing is started. All parameters are requested interactively.

5.16 AU Program `lctshim`: Shimming with TOPSHIM

The AU program **lctshim** automates the gradient shimming with TOPSHIM. For details see the description of the gradient shimming procedure in 3.8.3 Gradient shimming with TOPSHIM / lctshim.

5.17 AU Program `lcgshim`: Shimming with gradshimau

The AU program **lcgshim** automates the gradient shimming with the established gradshimau program. In TOPSPIN 2.0 both methods are available. For details see the description of the gradient shimming procedure in 3.8.4 Gradient shimming (gradshim1d1h).

5.18 AU Program `lczshim`: Iterative shimming of the lineshape

The AU program **lczshim** measures the linewidth of a given signal and tries to optimize a gradient for minimal linewidth. This procedure is mainly for systems without gradient or where gradient shimming is not implemented (HR-MAS probes). For details see 3.8.5 Shimming by line deconvolution (LCZSHIM).

6. Full Automation

6.1 Introduction

The program used for automation is ICONNMR, it is also responsible for automation with LCNMR. ICONNMR automation is compatible with all versions of **HyStar®** as well as the previously used software Chromstar/BPSU-12, BSFU.

The chromatography programs will communicate with ICONNMR like a sample changer. I.e. they will send the information that a sample is in the flow cell to the ICONNMR. ICONNMR will start the defined measurements and will request the next sample from chromatography software when the experiments for the actual sample are terminated.

The following text leads you through the set-up of the experiments and the usage of the automation. For specific ICONMR features check the ICONNMR manual.

6.2 Setup ICONNMR

6.2.1 Communication

The full automation with ICONNMR requires that HyStar and IconNMR are communicating. In IconNMR you define NMR experiments for certain holders. Those holder now correspond either to the Loops of a BPSU-36 loop cassette or to the cartridges of an LC-SPE Interface or to a peak eluting directly from the chromatography.

IconNMR and HyStar will communicate and once a sample from the selected source arrives in the flowcell the NMR expermiments defined will be executed. The only specialities you have to respect are linked to the special properties of the sample from LC-NMR and the fact that the sample cannot be ejected and inserted freely.

The properties of the sample requires
- Special shim procedures
- Proper temperature control

- Experiments with solvent suppression and automation programs that can adjust the parameters for the solvent suppression automatically

Once a sample is ejected from the the magnet, you cannot bring it back in an automatic way. Even if you collect the sample and inject it manually the quality will be not the same again, it will be typically diluted. Therefore …

- Ensure that the transfer is working properly
- Normally do not choose "eject last sample"
- Do not use the priority in IconNMR because it may lead to the fact that a sample for an important experiment is inserted, while still not all experiments of the current sample are finished.

Once the system is properly defined you can run LC-NMR experimetns in full automation with IconNMR.

This is especially usefull in the following circumstances

- For screening where you want to measure overview spectra of large number of samples.
- Use the high capacity of an LC-SPE-NMR system which can have up to 192 sample ready for measurement.
- Use timeslicing to step over a chromatogramm to screen for compounds of interest.

6.2.2 Configuration

'ICON-NMR User Manager' ensure that the parameter sets for the LCNMR experiments, are available in the 'Experiment List'. Otherwise click into the 'Experiment List' and select in the 'Entry Box' the desired Parameter sets. Also enter the command **expset** in the user specific commands / parameters. This allows a convenient setup of the automation parameters.

Open the ICONNMR 'configuration' menu. If you are only using LCNMR as automation hardware (no sample changer etc.) go to 'Driver Engine' and select 'Default Automation Module = LC-NMR Module'.

Various parameters control the proceeding of ICONNMR. In the text below the most important paramters for LC-NMR are explained. For general details check the ICONNMR manual.

Before you apply changes, do File|Save. By this you can keep a configuration for a BACS sample changer and another for LC-NMR automation.
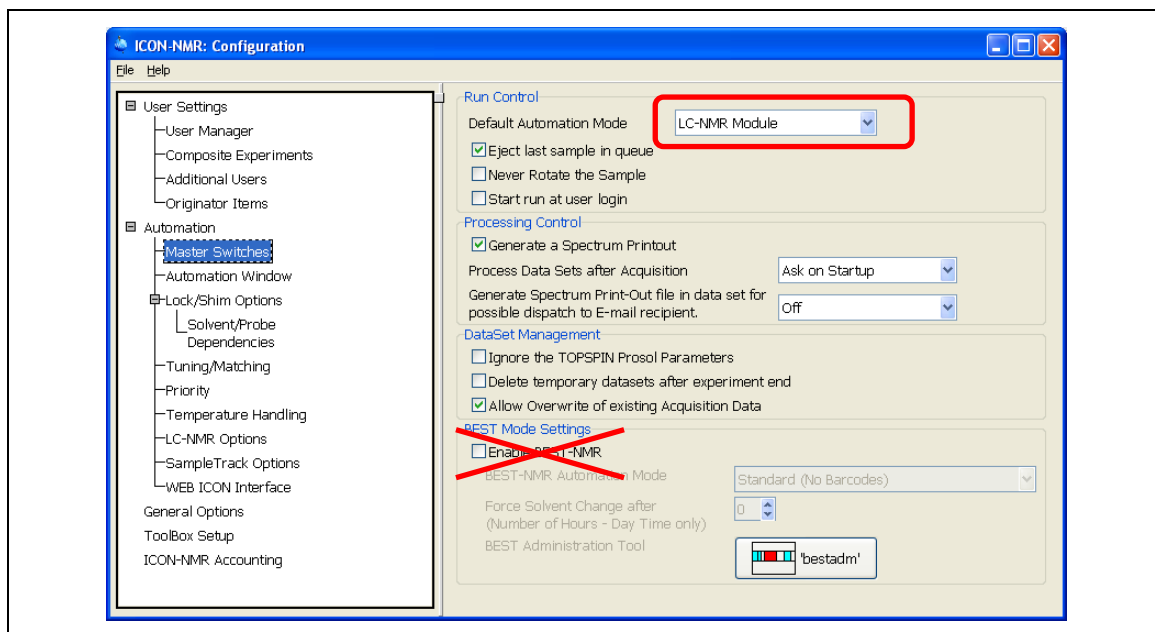
Fig. 6.1:   *Select LC/NMR module as automation mode. Note that you* **must** *disable the BEST NMR automation.*
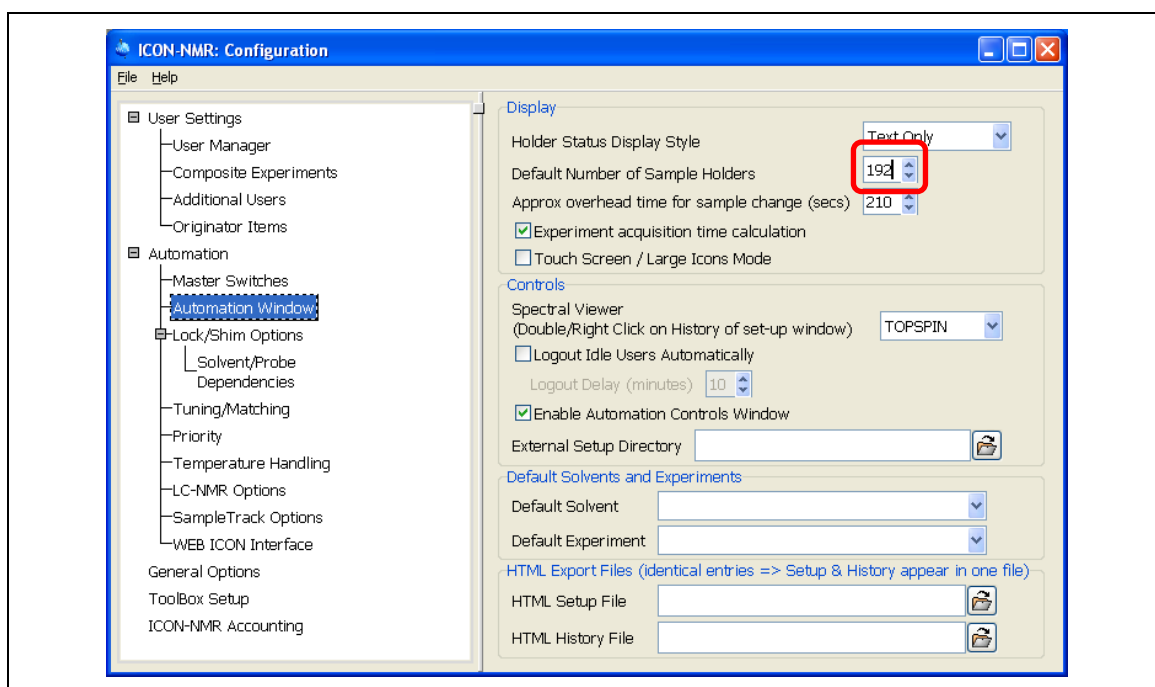

Fig. 6.2:   *In the Automation Window set the number of holders. For LC-SPE-NMR set 192 holders (number of cartridges). For other LC-NMR interfaces set at least 60 holders. Independ of the number of available sample loops (36 for a BPSU-36 or 12 for a BPSU-12) the number of peaks in direct stop-flow mode is in principle not limited.*
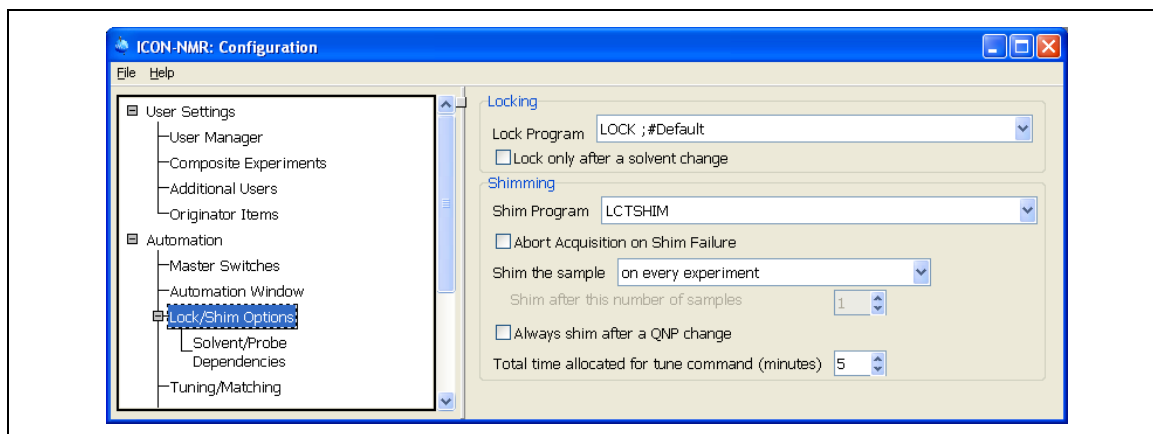
Fig. 6.3:   Define the default values for lock and shim. Those selections can be overwritten in the automation window. Starting with TOPSPIN 2.0 procedures are defined here and not in the specific LC-NMR settings.
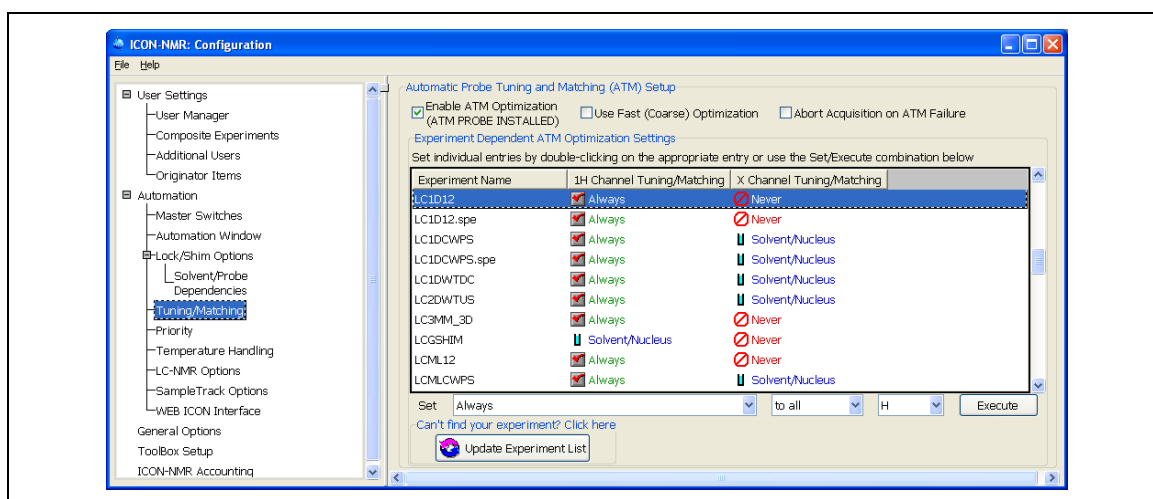

Fig. 6.4:   Typically tuning and matching for ^{1}H should be done for LC-NMR with solvent gradients. Tuning and matching for 13C decoupling is typically only required after a solvent change. Those selections can be overwritten in the automation window.
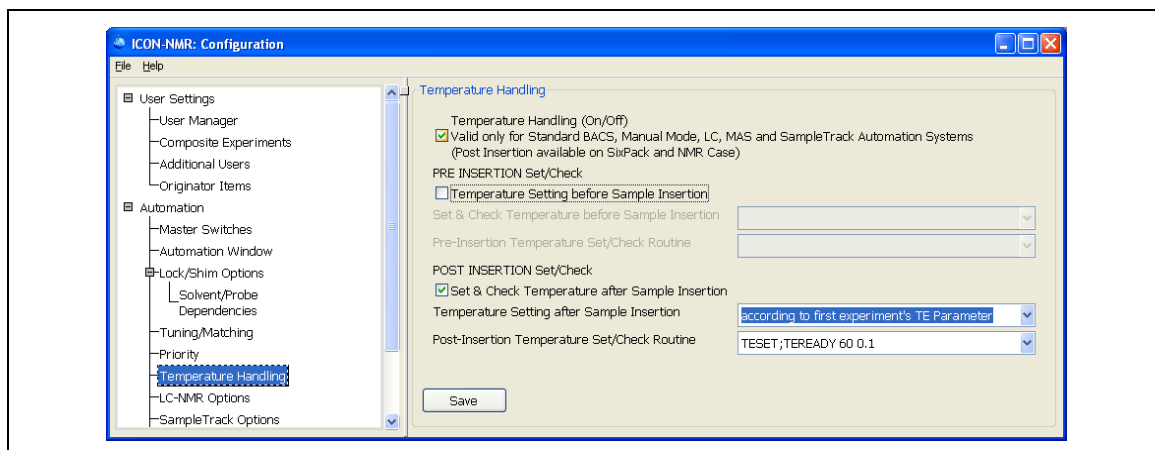
Fig. 6.5:   *Parameters for temperature control. Note that these are general settings which apply for all samples. In LC-NMR all samples are typically measured with the same temperature and temperature control by ICONNMR is not necessary. Starting with TOPSPIN 2.0 those procedures are defined here and not in the specific LC-NMR settings.*
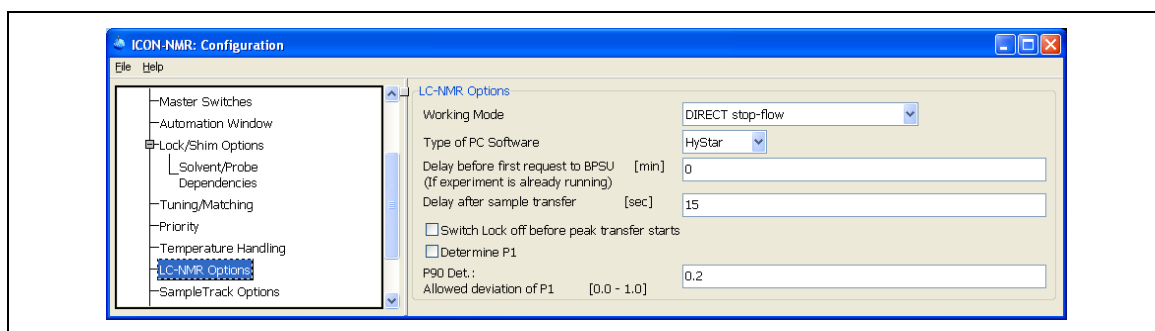

Fig. 6.6:   *Default values for specific LC-NMR settings. This dialog appears when the automation is started in LC-NMR mode and can be changed each time the run is started. In TOPSPIN 2.0 the number of settings is dramatically reduced, most of the parameters concerning lock, shim and temperature are now controlled like in standard automation.*
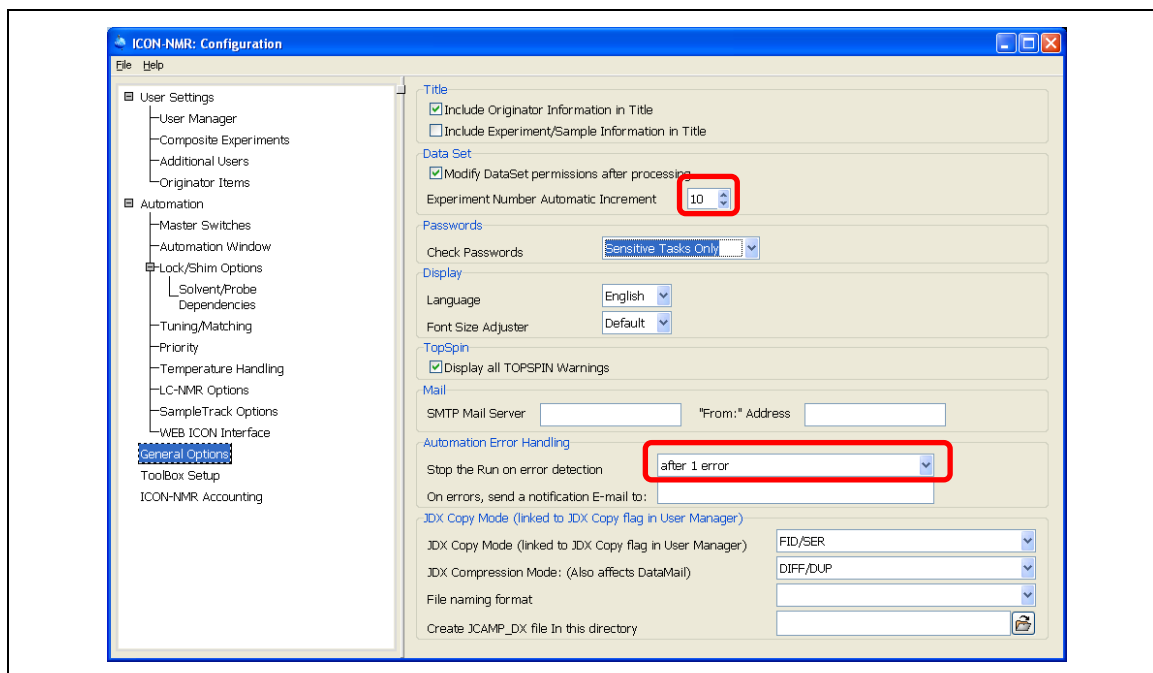
Fig. 6.7:   *For LC-NMR it is recommended to set the Automatic Increment to 10. Samples*
are normally lost after the transfer in the NMR probe. Therefore the automation
should be stopped already after one error.

6.3 Prepare the run

The chromatography program must be started and either the loop transfer or
direct mode.

- Start the ICONNMR module in TOPSPIN with <u>iconnmr</u> and start the
  'automation'. Identify yourself as user.

The holder number corresponds to the loop-number in the BPSU (in loop
transfer mode) or to the peak number in the chromatogram (in direct mode).
Define the experiments for the holders as you do it for the BACS in sample
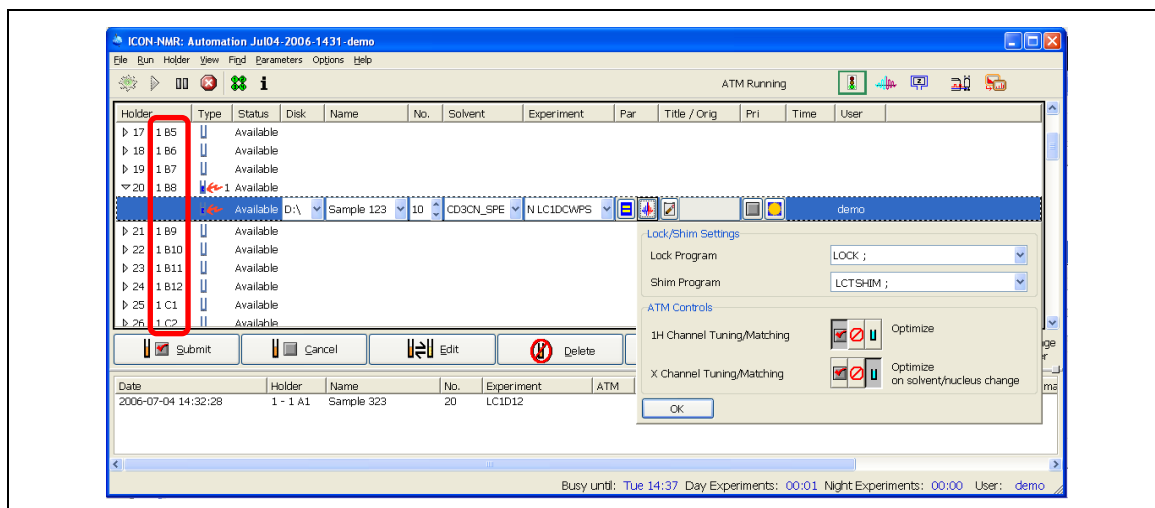changer mode.

Fig. 6.8:   *The example shows the automation window for an LC-SPE-NMR run. If you have set the default automation mode to LC-NMR and the number of holder to 192the holders will alphanumeric labels for the cartridges.*

- Doubleclick on a holder

- Select the **disk**

- Define a name and experiment number

- Select a solvent (Attention LCNMR automation requires normally special solvent (for example **CH3CN+D2O** instead of **D2O**) to work properly.

- Select the experiment. The sample dependent parameters like exact O1,O2 and the receiver gain will be adjusted automatically.

- Enter a title. Essential information like loop number, retention time, chromatogram name etc. are entered *automatically* into the title. It is not necessary to enter "loop 5" or "Peak 3" manually.

- Tuning,matching, lock and shim are preset as define defined in Fig. 6.4 and Fig. 6.3, however you can overwrite this settings here .

With some limitations *all* experiments can be used in automation. As in manual spectra acquisition you have access to all parameters with edit par|eda etc. Nevertheless be careful while altering parameters as they may affect the automation. For each holder also multiple experiments can be selected.
Repeat the procedure for all desired loops (in loop transfer mode) or ensure that enough holders are defined to cover all peaks which are found by the chromatography in direct mode. Use the 'copy' function of ICONMR to create a sufficient number of holders.

6.4 Start the automation

In ICONNMR click GO. Select the 'LC-NMR Module' for the automation. Click again GO this brings up a window with some parameters. For loop/cartridge

transfer the holders correspond to the loop/cartridge numbers. Selec the first holder which shall be measured, for direct mode select holder #1.

Now the window from Fig. 6.6 comes up which allows you to define specific LC-NMR parameters. The most important parameters are:

LCNMR Working Method

Select either 'loop transfer' or 'direct mode'. The working mode for LC-SPE-NMR is identical to loop transfer loop.

  a) DIRECT STOPPED FLOW autodetection:
     Full automation mode. Inject the sample. The peaks are detected by the chromatography software, and moved to the NMR. The predefined NMR experiments are carried out automatically and the chromatography is continued automatically to the next peak. No user interaction necessary.

  b) TRANSFER LOOPS/CARTRIDGES/VIALS:
     The chromatography software should be in the FlowInjection window. The transfer settings must be be set correctly.
     The holder numbers correspond to the loop/cartridge numbers. All submitted holder are subsequently transferred to the NMR and the predefined NMR experiments are carried out automatically.

PC Software

ICONNMR supports **HyStar®** and **Chromstar®** as chromatography software. As the communication with **HyStar®** is slightly enhanced you have to define the type of Software you are using.

Delay before first action:

Enter the delay between the start of the automation and the first request to the BPSU/BSFU/Prospekt2. This is useful in the following example:

A peak is already in the NMR cell. An experiment, which was optimized and started manually, is running. After the end of the experiment the automation shall continue measuring the remaining peaks, loops or cartridges.

Calculate the remaining experiment time of the actual experiment and start the automation. Enter the calculated time as delay.

Delay after sample transfer:

The experiments are not immediately started after the sample transfer. It is useful to wait a short time for temperature-equilibration etc. The time should be in the order of 30 - 240s and can be selected for each run.

Determine 90° pulse:

If the pulse determination is selected, it is carried out after **prosol** has set the pulses and power levels. It modifies the value of P1 in the actual experiment. For more details see page 23 "Prosol parameters for LC-NMR" and page 31 "Automatic Determination of 90° pulse".

Switch lock off before transfer

This avoids that autoshim tryes to shim on a partially empty probe or on an instable situation during sample transfer. This option is recommended for BPSU-36/2 and Prospekt2. Note: if this option is checked, you must define a lock-procedure as described in Fig. 6.3.

6.5 Controlling the automation process

The acquisition itself is carried out by the AU-program defined in 'AUNM' of the selected experiment. It is executed by ICONNMR as you would do it with 'xaua'.

Stop and keep actual peak:

To stop the automation and keep the actual peak in the flow cell press STOP in ICONNMR. Check in the configuration menu if 'stop' will stop the automation immediately and will thereby abort the actual running experiment or if it will attend the end of the actually running experiment.

Stop and go to next peak:

To stop the automation for the actual peak and to go to the next peak in automation enter stop or halt to terminate the actual experiment. ATTENTION be sure to do this only during the MAIN experiment. If you enter 'stop' for example during the acquisition of the preparation experiment only THIS part of the experiment is stopped.

7. Quickstart Guide

7.1 Quickstarts and Checklists

The following sections contains short descriptions in a format of a checklist for typical procedures required for LC-(SPE)-NMR work. You must be familiar with the complete procedures described in this and other relevant manuals for LC-(SPE)-NMR.

Startup procedure LC-(SPE)-NMR(/MS) System

ATTENTION: This is only a _checklist_.
Read the _complete LC-(SPE)-NMR manual_ before using the system!

1. Connect the *solvents* that you intend to use to the HPLC- and makeup pump, Prospekt2 and Gilson215. Connect the *HPLC column* that you intend to use.

2. Insert *cartridge trays/loop cassette* that you intend to use to Prospekt2/BPSU-36/2.

3. Open the *N₂ supply* for Prospekt2 and BPSU-36/2

4. Close the *doors* of BPSU-36/2 loop compartment, auto sampler and Gilson hood.

5. Power on the *computer* and monitor, wait until the computer is on and *login*.

6. Power on the *data interfaces* (Ethernet switch, RS232 converter and ADC) and wait 10sec.

7. Power on *all LC-NMR interfaces* Prospekt2, Bruker LiquidHandler 215[1], BPSU-36/2 BSFU in any order.

8. Power on the BNMI but *don't* connect the *ion source* of the mass spectrometer to the flow path. If it was powered off, check separate documentation of how to power on the MS.

9. Power on all modules of the *HPLC system* in the following order (degasser/organizer, detector, column oven auto sampler, pump, makeup pump, any others).

10. Start HyStar, choose a proper hardware setup, prepare the *sample table* and *chromatography method* for the following analysis and enter the acquisition window.

11. Open the *purge valve* of the HPLC pump, and flush *each channel* with 5ml/min for 2min.

12. Set the *initial conditions incl. flow* for the analysis. Close the purge valve and *flush for 10min*. Check for *stable pressure* and for *leakages*, especially at the column connectors.

13. *Prospekt2:* Enter direct control and *flush both HPD syringes* with 2ml of solvent. Set makeup pump to 5ml/min and flush for 2min. Ensure that the pump delivers solvent.

14. For a *LiquidHandler 215* start PrepGilsonST and perform a *PrimeDilutor-Purge System*.

15. With MS ensure that the *instrument is ready* and nebulizer and dry gas settings are sufficient to keep the high vacuum level, then connect the ion source to the flow.

16. Follow the instructions for the *Insertion of a Flow-NMR* probe or *CryoFit* into the magnet.

17. The instrument is now *ready for usage*.

After a longer downtime it is recommended to run the Bruker standard tests[2] to verify the proper functioning of the equipment. Number and type of the tests depend on the configuration.

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[1] The description covers a Bruker LiquidHandler 215 that is used for Transfer from Prospekt2 to NMR tubes in an LC-SPE-TT setup. For SamplePreparation and BEST-NMR see separate document.

[2] Standard test as have a typical duration of 1 – 2h depending on the application type.

CHECKLIST FOR <u>INSERTION</u> OF A FLOW-PROBE

**ATTENTION: This is only a <u>*checklist*</u>.
Read the <u>*complete manual*</u> before you use a Flow-probe!**

➢ *Eject* any *sample tube / spinner / shuttle* from the magnet.

➢ With a *Sample Changer* power it *off*.

➢ *Save* the actual shim values of the tube-probe with *wsh <probeheadname>.<date>*

➢ For *Room temperature* probes[1])
   - ○ Start *edte* and switch the *heater off*.
   - ○ Remove all *cables* and the *VT gas* tube
   - ○ Remove the *probe* from the magnet.

➢ *Mount* the flow-probe into the magnet, respect the correct X-Y-orientation.

➢ *Connect* all cables and the *VT-gas tube*. If tube-probe and flow-probe use different temperature sensors ensure that the cable(s) are connected at the BVT as well.

➢ Open *edhead* and define the flow-probe as *current probe*.

➢ In *edte* load the flow-probe configuration, switch the *heater on*.

➢ *Read* the last good *shim file* available for the flow-probe with *rsh ...*

➢ It is *recommended* to shim the probe on a standard sample solution *before* you connect it to the flow-system.[2])

➢ *Connect* the *capillaries* of the flow-system to the *in-* and *out-port* of the probe.

➢ *Restrict access* to the space between magnet and flow-system to *protect the capillaries.*

➢ In IconNMR load the *configuration* for the *flow application*.

➢ Start the software of the flow-system and perform a wash procedure for the probe.

➢ The NMR spectrometer is now *ready* for usage of the *flow* application.

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[1]) For CryoProbes, (HR)MAS or other special probes follow the probe manual of how to remove the probe.

[2]) Inject a solution of H_2O/D_2O 90/10 using a syringe. Leave the syringe connected.
Create a 1H dataset, perform lock and atma (or tune manually), do *topshim gui* perform a 3D shim, check for reasonable changes of the shim values and reasonable lineshape.
Inject a humptest solution (make sure to remove H_2O sample completely)
Perform lock and atma (or tune manually), perform a 1D shim with topshim, adjust X, Y, YZ and XZ shims manually, re-run 1D shim with topshim. The probe should be in specifications now.

CHECKLIST FOR <u>INSERTION</u> OF THE CRYOFIT

ATTENTION: This is only a _checklist_.
Read the _complete manual_ before you use the CryoFit!

➢ Check that cell & capillary are _empty_ and _dry_ and the _outside_ of the capillary is _clean_.

➢ Ensure that the capillary is _not bend_.

➢ Switch the cooling unit (BCU) _off_.

➢ _Remove_ any _sample tube / spinner / shuttle_ from the magnet.

➢ With _Sample Changer_
   - _SampleXpress:_ On the _userpanel_ select _remove|cassette_ and remove the cassette.
   - _SampleJet:_ On the _touchpad_ select _manual mode_. Wait that the arm is inside the SampleJet housing, turn the main _power off._ Remove the _rear cover_.
   - _B-ACS:_ Press _reset_, wait until the arm moves away, turn _BACS main power off_.
   - _NMR Case:_ Remove the _carousel_.

➢ Attach _CryoFit Holder_ to the shim upper tube (_Not_ with _SampleJet/SampleXpress_).

➢ _Close_ the _valve_ for the _spinning air_ on top of the magnet.

➢ _Close_ the _Insert Guide_ slot by turning, insert into magnet bore, and switch sample lift off.

➢ _Remove the stopper_ from the T–piece at the _bottom_ of the probe.

➢ _Bend_ the CryoFit and _park it_ into the CryoFit Holder (_Not_ for _SampleJet_).

➢ _Uncoil the capillary_ from capillary holder and insert it into the shim system.
   - A _second person_ must ensure that the capillary can slide out at the bottom of the probe.
   - If the capillary gets stuck, twist it and move it 1-2 cm up and down to find the path. If you can't insert the capillary after 4-5 attempts _remove it completely_ and try again.
   - Resistance after ~1 m (insertion guide), ~1,1m (inside probe) and ~1,4m (T-piece).

➢ Activate the sample lift and _remove_ the _insert guide_. Then switch off the sample lift.

➢ _Insert_ the CryoFit _stepwise_ into the magnet. A second person must ensure that for _each step_ you push the CryoFit in, the capillary _slides out_ at the bottom.

➢ Insert the CryoFit _completely_. The end of the CryoFit must sit _firmly_ in the spinner seat. A _soft_ contact indicates the capillary is coiled up between CryoFit and spinner seat. In this case do not unlock flowcell remove the CryoFit, check the capillary for damages!!!

➢ Turn the _lower part_ of the CryoFit to _unlock_ the flow cell, move the cell down carefully.

➢ _Close_ the _T-piece_ at the bottom of the probe with a _fitting_, do _not tighten_ the fitting.

➢ _Open_ the CryoFit _outlet_ (top) and connect the outlet capillary of the LC/BEST system.

➢ Connect the _inlet_ (bottom) capillary to the BEST/LC system.

➢ In _edhead_ activate the option _Use as flow = yes_, and check the flow parameters.

➢ In Iconnmr load the _configuration_ for the _flow application_ (for example LCNMR.icf, …).

➢ _Restrict access_ to the space between magnet and Flow-system to protect the capillaries.

➢ The CryoProbe is now _ready_ for usage of the _flow_ application.

Quickstart: Manual NMR measurements with LC-SPE

(1) In TopSpin create the test dataset with **new**[1]), and define *all* following parameters
   NAME: **test**, EXPNO=**1**, SOLVENT=**CD3CN_SPE**[2]), EXPERIMENT=**LCZG**

(2) Open TopShim window with **topshim gui** (do *not start* shimming yet!)

(3) Enter pulses and power levels with **getprosol**.

(4) In HyStar enter the flow injection window.

(5) In HyStar check *Transfer/Settings*, select a peak, *start* the transfer and wait that the *cartridge content* is *in the magnet* and the *pump has stopped.*

(6) Go to the *test spectrum* with **re test 1**

(7) Lock the spectrometer with **lock CD3CN_SPE**[2]).

(8) Wait approx. 2min for stabilization of conditions (wait for stable *lock signal*).
   After the end of the transfer it may take 1-2min to stabilize conditions (Temperature, turbulences, ...).

(9) Perform tuning/matching with **atma** or start **wobb** and tune manually.

(10) Shim X & Y manually. Do **topshim gui** and **press start** and check the result page.
   Perform shimming for each peak. Switch autoshim=on!

(11) Acquire a spectrum with **zg; ft; apk** and check the line shape, it must be o.k. now.
   Use the C(DH)₃CN multiplett (splitting better 90%). Do not use the water signal.

(12) Create a dataset for the *main experiment* with **new**.
   NAME: Make a reference to the chromatogram *name* and *number* of HyStar.
   Use EXPNO=**10,11,12**,... for the 1ˢᵗ peak and **20,21**,... for the 2ⁿᵈ peak, ...
   SOLVENT=**CD3CN_SPE**[2]); EXPERIMENT= **LC1DCWPS**[3]), Leave the TITLE empty

(13) Lock the spectrometer with **lock CD3CN_SPE**[2]).

(14) Enter pulses and power levels with **getprosol**.
   *Do **eda** to change simple parameters like NS, D1. Change Automation parameters with **xau expset**.*

(15) Import information about the current peak from HyStar into the title with **getinfo**.

(16) Perform tuning/matching with **atma** or start **wobb** and tune manually.

(17) Start the acquisition with **xaua**.
   *Warning: Do not do stop/halt or perform any processing while the **preparation** experiment is running.*

(18) While the main experiment runs check the result. Do **tr** or **tr 24**[4]) process the spectrum
   with **ef; apk**. *Preferably transfer multiple of 8 scans.*

(19) You can terminate the experiment at any time with **halt, halt 64**[4]).

(20) Process the spectrum with **ef; apk**. Phasing the spectrum with sample signals only, do not phase the suppressed solvent lines.

(21) For *additional* experiments for the *same cartridge*, continue with step (12).

(22) To go to the *next cartridge* continue with step (5).

---

[1] Text formatted **like this** is a command that you must enter in TopSpin.

[2]) If available use special SPE-solvents like **CD3CN_SPE** or **CD3OD_SPE**, otherwise use standard lock solvents like **CDCl3**.

[3]) Standard experiments like PROTON, will work. However due to the typically low sample amounts the LC-NMR like LC1D12, LC1DCWPS, COSYCWPS, ... with suppression are recommended.

[4]) AVII/AVIII spectrometers only, for others enter **tr/halt** when you see 15 scans to get 16 scans on the disk.

Quickstart for manual NMR measurements with stop-flow

(1) In TopSpin create the test dataset with **new**[1]), and define *all* following parameters
     NAME: **test**, EXPNO=**1**, SOLVENT=**CH3CN+D2O**[2]), EXPERIMENT=**LCZG**

(2) Open TopShim window with **topshim gui** (do *not start* shimming yet!)

(3) Enter pulses and power levels with **getprosol**.

(4) In HyStar *inject the sample* and start the chromatography.

---

(5) Wait that a peak is found or select a peak manually, the *peak is* positioned *in the magnet* and the chromatography has stopped.

(6) Go to the *test spectrum* with **re test 1**

(7) Lock the spectrometer with **lock CH3CN+D2O**[2]).

(8) Wait approx. 2min for stabilization of conditions (wait for stable *lock signal*).
     After the end of the transfer it may take 1-2min to stabilize conditions (Temperature, turbulences, …).

(9) Perform tuning/matching with **atma** or start **wobb** and tune manually.

(10) Start shimming with **xau lctshim,** change to the topshim window to see the results.
     Perform shimming for each peak. Do not shim on the locklevel. Do not switch autoshim=on!

(11) Acquire a spectrum with **zg; ft; apk** and check the line shape, it must be o.k. now.
     Use the CH₃CN satellites or a sample signal(linewidth <1.xHz). Do not use the CH₃CN center line or the water signal.

---

(12) Create a dataset for the *main experiment* with **new**.

     NAME: Make a reference to the chromatogram *name* and *number* of HyStar.

     Use EXPNO=**10,11,12**,… for the 1ˢᵗ peak and **20,21**,… for the 2ⁿᵈ peak, …

     SOLVENT=**CH3CN+D2O**[2]); EXPERIMENT= **LC1DCWPS**[3]), Leave the TITLE empty

(13) Lock the spectrometer with **lock CH3CN+D2O**[2]).

(14) Enter pulses and power levels with **getprosol**.
     *Do **eda** to change simple parameters like NS, D1. Change Automation parameters with **xau expset**.*

(15) Import information about the current peak from HyStar into the title with **getinfo**.

(16) Perform tuning/matching with **atma** or start **wobb** and tune manually.

(17) Start the acquisition with **xaua**.
     *Warning: Do not do stop/halt or perform any processing while the **preparation** experiment is running.*

(18) While the main experiment runs check the result. Do **tr** or **tr 24**[4]) process the spectrum with **ef; apk**. *Preferably transfer multiple of 8 scans.*

(19) You can terminate the experiment at any time with **halt, halt 64**[4]).

(20) Process the spectrum with **ef; apk**. Phasing the spectrum with sample signals only, do not phase the suppressed solvent lines.

---

(21) For *additional* experiments for the *same peak*, continue with step (12)

(22) To go to the *next peak* in HyStar press *continue*, and continue with step (5).

---

[1]) Text formatted **like this** is a command that you must enter in TopSpin.

[2]) Use special solvents (**CH3CN+D2O, CH3OH+D2O**) not **D2O**!

[3]) Typical 1D experiments for LC-NMR are LC1D12, LC1DCWPS or MULTIPRESAT.

[4]) AVII and AVIII spectrometers only, for others enter **tr** when you see 15 scans to get 16 scans on the disk..

Quickstart Manual NMR measurement with Loop Transfer

(1) In TopSpin create the test dataset with **new**[1]), and define *all* following parameters
     NAME: **test**, EXPNO=**1**, SOLVENT=**CH3CN+D2O**[2]), EXPERIMENT=**LCZG**

(2) Open TopShim window with **topshim gui** (do *not start* shimming yet!)

(3) Enter pulses and power levels with **getprosol**.

(4) In HyStar enter the flow injection window.

(5) In HyStar check *Transfer/Settings*, select a peak, *start* the transfer and wait that the *loop content* is *in the magnet* and the *pump has stopped.*

(6) Go to the *test spectrum* with **re test 1**

(7) Lock the spectrometer with **lock CH3CN+D2O**[2]).

(8) Wait approx. 2min for stabilization of conditions (wait for stable *lock signal*).
     After the end of the transfer it may take 1-2min to stabilize conditions (Temperature, turbulences, ...).

(9) Perform tuning/matching with **atma** or start **wobb** and tune manually.

(10) Start shimming with **xau lctshim,** change to the topshim window to see the results.
     Perform shimming for each peak. Do not shim on the locklevel. Do not switch autoshim=on!

(11) Acquire a spectrum with **zg; ft; apk** and check the line shape, it must be o.k. now.
     Use the CH₃CN satellites or a sample signal(linewidth <1.xHz). Do not use the CH₃CN center line or the water signal.

(12) Create a dataset for the *main experiment* with **new**.
     NAME: Make a reference to the chromatogram *name* and *number* of HyStar.
     Use EXPNO=**10,11,12**,... for the 1st peak and **20,21**,... for the 2nd peak, ...
     SOLVENT=**CH3CN+D2O**[2]); EXPERIMENT= **LC1DCWPS**[3]), Leave the TITLE empty

(13) Lock the spectrometer with **lock CH3CN+D2O**[2]).

(14) Enter pulses and power levels with **getprosol**.
     *Do **eda** to change simple parameters like NS, D1. Change Automation parameters with* **xau expset**.

(15) Import information about the current peak from HyStar into the title with **getinfo**.

(16) Perform tuning/matching with **atma** or start **wobb** and tune manually.

(17) Start the acquisition with **xaua**.
     *Warning: Do not do stop/halt or perform any processing while the **preparation** experiment is running.*

(18) While the main experiment runs check the result. Do **tr** or **tr 24**[4]) process the spectrum with **ef; apk**. *Preferably transfer multiple of 8 scans.*

(19) You can terminate the experiment at any time with **halt, halt 64**[4]).

(20) Process the spectrum with **ef; apk**. Phasing the spectrum with sample signals only, do not phase the suppressed solvent lines.

(21) For *additional* experiments for the *same loop*, continue with step (12).

(22) To go to the *next loop* continue with step (5).

---

[1] Text formatted **like this** is a command that you must enter in TopSpin.

[2]) Use special solvents (**CH3CN+D2O, CH3OH+D2O**) not **D2O**!

[3]) Typical 1D experiments for LC-NMR are LC1D12, LC1DCWPS or MULTIPRESAT.

[4]) AVII and AVIII spectrometers only, for others enter **tr** when you see 15 scans to get 16 scans on the disk..

Quickstart for time-slice stop-flow measurements

(1) In HyStar™ open the method with the chromatographic conditions and define the fraction treatment. Typically use the settings below.

    o First timewindow should contain the empty part of the chromatogram without peaks of interest. Set the target to NMR but detection mode manual or on-flow.

    o Second timewindow should start approx. 0.5-0.25min before the first peak of interest. Set detection mode "time-slice", and a slice width adjusted to the exchange rate of the flowcell, typically 15sec for a 120ul, 7sec for a 60ul and 4sec for a 30ul probe.[1])

    o Load the method in the acquisition window. Define the solvent composition for the last stop of the timeslice experiment. Start the pump switch the interface (BSFU, BPSU) to direct mode and wait for stable conditions. Stop the pump.

(2) In TopSpin create the test dataset with **new**[2]), and define *all* following parameters

(3) **NAME**: **test**, **EXPNO=1**, **SOLVENT=CH3CN+D2O**, **EXPERIMENT=LCZG**, lock the spectrometer with **lock CH3CN+D2O**, enter pulses and power levels with **getprosol**.

(4) Open TopShim window with **topshim gui** and start a 1D shimming.

(5) In HyStar *inject the sample* and start the chromatography.

---

(6) Wait that a peak is found or select a peak manually, the *peak is* positioned *in the magnet* and the chromatography has stopped.

(7) Go to the *test spectrum* with **re test 1**

(8) Lock the spectrometer with **lock CH3CN+D2O**.

(9) Wait approx. 2min for stabilization of conditions (wait for stable *lock signal*).
After the end of the transfer it may take 1-2min to stabilize conditions (Temperature, turbulences, …).

(10) Perform tuning/matching with **atma** or start **wobb** and tune manually.

(11) Start shimming with **xau lctshim,** change to the topshim window to see the results.
Perform shimming for ***each*** *peak. Do* ***not*** *shim on the locklevel. Do* ***not*** *switch* ***autoshim=on*** *!*

(12) Acquire a spectrum with **zg; ft; apk** and check the line shape, it must be o.k. now.
Use the CH$_3$CN **satellites** *or a sample signal(linewidth <1.xHz). Do* **not** *use the CH$_3$CN* **center** *line or the water signal.*

---

(13) Create a dataset for the *main experiment* with **new**.

    NAME: Make a reference to the chromatogram *name* and *number* of HyStar.

    Use **EXPNO=10,11,12,**… for the 1st peak and **20,21,…** for the 2nd peak, …

    SOLVENT=CH3CN+D2O; **EXPERIMENT= LC1DCWPS**, Leave the **TITLE** empty

(14) Lock the spectrometer with **lock CH3CN+D2O**.

(15) Enter pulses and power levels with **getprosol**.
Do **eda** *to change simple parameters like NS, D1. Change Automation parameters with* **xau expset**.

(16) Import information about the current peak from HyStar into the title with **getinfo**.

(17) Perform tuning/matching with **atma** or start **wobb** and tune manually.

(18) Start the acquisition with **xaua**.
Warning: Do not do stop/halt or perform any processing while the ***preparation*** *experiment is running.*

---

[1]) Flow cell 60ul -> total volume approx. 120ul, flowrate 1ml/min --> takes a minimum 120/1000 = 0,12min or 7sec to exchange the flow cell volume.

[2] Text formatted **like this** is a command that you must enter in TopSpin.

(19) While the main experiment runs check the result. Do **tr** or **tr 24**[1]) process the spectrum with **ef; apk**. *Preferably transfer multiple of 8 scans.*

(20) You can terminate the experiment at any time with **halt, halt 64**[4]).

(21) Process the spectrum with **ef; apk**. Phasing the spectrum with sample signals only, do not phase the suppressed solvent lines.

(22) For *additional* experiments for the *same peak*, continue with step (12)

(23) To go to the *next peak* in HyStar press *continue*, and continue with step (5).

---

[1]) AVII and AVIII spectrometers only, for others enter **tr** when you see 15 scans to get 16 scans on the disk..

CHECKLIST FOR <u>REMOVAL</u> OF THE CRYOFIT

ATTENTION: This is only a <u>*checklist*</u>.
Read the <u>*complete manual*</u> before you use the CryoFit!

➢ *Wash cell and capillaries* with salt free liquid, empty *and dry* it*!*

➢ *Disconnect* the CryoFit *inlet capillary* (bottom) from the BEST or LC system.

➢ *Disconnect* the CryoFit *outlet capillary* (top) and close with *a stopper*.

➢ *Remove the fitting* from the T-piece at the bottom of the probe.

➢ *Clean the outside* of the inlet capillary especially *if it was in contact with the floor*.

➢ Carefully pull the flow cell into the Cryo Fit and *lock it* by turning the *lower part*.

➢ *Pull* the CryoFit and capillary out of the magnet. A *second person* must ensure that the capillary slides into the magnet as you pull the CryoFit out on the top.

➢ *Close the T-piece* with a plug.

➢ Attach the *capillary holder* to the CryoFit, (slide on, *not snap on!*)

➢ *Coil up* the capillary, but leave the *last 50cm* straight.

➢ *Open the valve* for the *spinning air* on top of the magnet.

➢ Switch the *BCU on*.

➢ *Disconnect* the *CryoFit holder* (*Not* with *SampleJet/SampleXpress*).

➢ With *Sample Changer*
  ○ *SampleXpress:* Insert the *cassette* and wait that it is *recognized/initialized*.
  ○ *B-ACS: Power on* the BACS
  ○ *SampleJet:* Mount the *rear cover*. switch the main *Power on*. Select the desired *working mode* at the *touchpad*,
  ○ *NMR Case:* Install the *carousel*.

➢ In *edhead de*activate the option *Use as flow = no*.

➢ In Iconnmr load the *configuration* for the *standard application* (for example SampleXpress.icf, …).

➢ The CryoProbe is now *ready* again for using NMR *sample tubes*.

CHECKLIST FOR <u>REMOVAL</u> OF A FLOW-PROBE

ATTENTION: This is only a <u>*checklist*</u>.
Read the <u>*complete manual*</u> before you use the flow-probe!

➢ *Save* the actual shim values of the tube-probe with *wsh <flowprobeheadname>.<date>*

➢ In the software of the flow-system perform a wash procedure for the probe with salt free organic liquid! If this is not possible, use a syringe to flush and fill the probe with acetonitrile or methanol.

➢ Start *edte* and switch the *heater off*.

➢ *Disconnect* the *capillary* from the probe *inlet* and close it with a *stopper*.

➢ *Disconnect* the *capillary* from the probe *outlet* and close it with a *stopper*.

➢ Remove all *cables* and the *VT-gas* tube.

➢ Remove the *probe* from the magnet and store it. The probe typically should remain filled with the cleaning liquid.

➢ *Mount* the normal tube-probe[1] into the magnet, respect the correct X-Y-orientation.

➢ Connect all *cables* and the *VT-gas* tube. If flow-probe and tube-probe use different temperature sensors ensure that the right cable is connected at the BVT.

➢ Open *edhead* and define the normal tube-probe as current probe.

➢ In *edte* load the tube-probe configuration and switch the *heater on*.

➢ *Read* the last good *shim file* available for the tube-probe with *rsh ...*

➢ With *Sample Changer* power the sample changer *on*

  ○ *SampleJet:* Select the desired *working mode* at the *touchpad*.

➢ It is *recommended* to shim the probe on a standard sample solution *before* you work with your samples.[2]

➢ In IconNMR load the *configuration* for the *standard application* (BACS, ...).

➢ The NMR spectrometer is now *ready* for using NMR *sample tubes*.

---

[1] For CryoProbes, (HR)MAS or other special probes follow the probe manual of how to mount the probe.

[2] Use the water suppression sample 2mM sucrose in H_2O/D_2O 90/10. Create a 1H dataset, perform lock and atma (or tune manually), do *topshim gui* perform a 3D shim, check for reasonable changes of the shim values and reasonable lineshape.
Insert the humptest sample. Perform lock and atma (or tune manually), perform a 1D shim with topshim, adjust X, Y, YZ and XZ shims manually, re-run 1D shim with topshim. The probe should be in specifications now.

Shutdown procedure LC-(SPE)-NMR(/MS) System

ATTENTION: This is only a _checklist_.
Read the _complete LC-NMR manual_ before using the system!

1. For a downtime >1 week check if **solvents** with **non-volatile** additives1 are connected to the HPLC-, makeup pump, HPD or Gilson215. In this case exchange with similar solvents. Replace deuterated solvents only with deuterated solvents. Aqueous solvents should contain a small amount of acid (0.1-0.01% acetic or formic acid2).

2. In HyStar enter the **acquisition window**.

3. If solvents were changed, open the purge valve and **flush each changed channel** for 2min with 5ml/min. If **makeup pump** solvent was changed, set the flow to 5ml/min and switch the pump on for 2min.

4. Set the composition to **80% organic/20% water** and flush system incl. column with 0.25 - 1ml/min3 for **10min**.

5. **Prospekt2**: Do **condition** (500ul) and **equilibration** (500ul) of **all loaded**4 cartridges. However treat at **least 4 cartridges**, if necessary it's OK to treat used, equilibrated or new.

6. **BPSU-36/2**: Set the composition to 80% organic and 20% water and flow to 1ml/min and perform a wash of all loops6 for 30sec. Open the door of the loop compartment.

7. **BSFU**: no special procedure required.

8. **Bruker LiquidHandler 215**5 connected to a Prospekt2: Perform a wash needle and a dry-wash-dry probehead with 300ul of Solvent and 3min drying.

9. **Flow-NMR probe** or **CryoFit:** Follow the separate instructions for the **removal**.

10. **MS:** close the **instrument control** and switch the MS to **standby**. For a downtime >1 week check separate documentation of how to power off the MS completely.

11. Close **HyStar**

12. Power off **all modules of the HPLC system** in any order.

13. Power off the **interfaces** Prospekt2, Bruker LiquidHandler 2156), BPSU-36/2 and/or BSFU in any order

14. For Prospekt2/BPSU-36/2 **close the N2** supply. Do not close **the N2 supply for the MS**.

15. Power off **the data interfaces** like RS232 converter, Ethernet switch and ADC converter.

16. Power off the **computer** and **monitor**

17. The LC-(SPE)-NMR(/MS) System **is off** can be left unattended for longer time.

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[1]) The solvents must not contain salts like ammonium formate, phosphate buffer etc.
[2]) This is to avoid growth of microorganisms in the water. Check the compatibility with column.
[3]) Select a flow rate suitable for the column.
[4]) Before your wash loops/cartridges, ensure that the contained samples are no more required.
[5]) The description covers a Bruker LiquidHandler 215 that is used for Transfer from Prospekt2 to NMR tubes in an LC-SPE-TT setup. For SamplePreparation and BEST-NMR see separate document.
[6]) The description covers a Bruker LiquidHandler 215 in a SPE-TT system, i.e. directly connected to a Prospekt2.

8. Tips and Tricks

8.1 General Remarks, Troubleshooting

Preparation of the sample

Always measure the original sample before starting the LCNMR measurements. If possible, dissolve the product in the eluent to be used for the chromatography. As there may be considerable changes due to the acetonitrile/D_2O as solvent (shift of signals, loss of signals due to deuterium exchange). This spectrum will show where to expect signals.

Additionally, from the intensity of the NMR signals you can estimate how much sample you'll need to inject on the column. Therefore prepare your original solution such that the injection volume is diluted to the NMR cell volume.

```
Injection volume 20 µl
NMR volume 120 µl
```

Prepare a sample by using 20µl of solution which is injected in the chromatographic system and dissolve it in 120µl.

```
⇒ mix 200µl with 1200µl
```

If you manage to get the whole chromatographic peak you will have the same concentrations during the LC-NMR measurement.

Exchangable protons

Normally exchangeable protons can not be seen when using D_2O as the eluent except when using H_2O or for example $CDCl_3$ (in GPC). When solvent suppression of the water (water traces in $CDCl_3$) is done, all protons in this exchange process are also suppressed. If you want to observe those protons you can use different techniques like binomial sequences, WATERGATE or WET for the suppression.

Which peak should be at O1

Normally the largest Peak should be on O1, this is commonly the acetonitrile. If you do not use DQD than check where the quad-images will appear.

It might be suitable to have acetonitrile on resonance even if the water signal is bigger. Otherwise you will find a quadimage around 7.2ppm.

How often can we stop the chromatography?

In isocratic separations the total run time should not be more than 3-5h. Depending upon the acquisition time for one experiment, the number of possible stops can be calculated.

For gradient separations up to 20 h total runtime can be allowed without significant loss of separation for the later peaks.

Can gradient runs be performed in automation?

Yes! The solvent frequencies are optimized for *each* peak/loop. Therefore no limitation about the gradient exist except that at least 3-5 % of deuterated solvent must always be present. If buffer gradients are used the determination of the 90° pulse for each peak might be necessary.

Solvent suppression with modified NOESY sequence

If you use the pulse programs with the modified NOESY sequence for presaturation two things are important

- Use a 90º pulse for p1

- Scans - To avoid phasing problems always store scans in multiples of 8 on disk.

- Check what mixing time is suitable for your samples. Commonly 80 msec are used but this may be too much for larger molecules.

No NMR signal of the sample

If no NMR signal appears or the sensitivity seems to be very poor, check the following:

- Do you use the correct system times in the chromatography program?

- Has anything changed in the setup (Capillaries, UV detector), which may lead to differences in the timings?

- Is the eluent *really* going through the probe? Click 'continue' or 'transfer' and check the outlet of the probe. If no solvent is going through check for leaks in the system at the over pressure valve, fittings, ...etc.

- Check if the concentration is high enough to see the sample? Often the concentration of the sample in the injection solution is much less than expected (due to decomposition, adsorption ...)

- Is the flow rate correct?
  For direct mode load the used method and start the pump. Measure the delivered volume.
  For storage mode: Switch to wash sample loop, choose a washing time of 10min, set the flow rate to 1.0 ml or whatever is used for the transfer. Check if the pump runs stable with this low flow rate (observe the pressure) and check the delivered volume.