1 Introduction

This manual gives an introduction into basic one- and two-dimensional nuclear magnetic resonance (NMR) spectroscopy. After a short introduction the acquisition of basic 1D ^{1}H and ^{13}C NMR spectra is described in the Chapters 3 to 7. Homonuclear 2D [^{1}H,^{1}H] correlation spectra are described in Chapter 8 (COSY), 9 (TOCSY), 10 (ROESY) and 11 (NOESY). Heteronuclear 2D [^{13}C,^{1}H] correlation experiments are described in Chapter 12 (XHCORR), 13 (COLOC), 14 (HMQC) and 15 (HMBC). The Chapter 16 contains the description of inverse 2D [^{13}C,^{1}H] correlation experiments using pulsed field gradients, and some special NMR experiments are described in chapters 17 to 20. A brief introduction to NMR automation with the IconNMR program is given in chapter 21.

1.1 An Important Note on Power Levels

Several times throughout this manual, the user is asked to set the power levels **pl**1, **pl**3, etc. to the "high power" level for the corresponding channel (f1 or f2). ***In order to avoid damaging the probehead or other hardware components***, the user is advised to use only the power levels indicated in Table 1 below, if no other information (e.g. final acceptance tests) is available.

Note that these "power levels" are really attenuation levels, and so a higher value corresponds to a lower power. Also note that these power levels pertain ***only*** to the specific spectrometers and amplifiers listed below, which correspond to the AVANCE instruments as of July 2000. It is assumed that no correction tables (CORTAB) are existing.

Table 1: Suggested "Proton and Carbon High Power" Levels for Avance Instruments

Nucleus	Spectrometer	Amplifier	Power Level
^{1}H	Avance	BLA2BB	≥ + 3dB
		BLARH100	≥ + 3dB
		BLAXH300/50	≥ 0dB
	Avance DPX	BLAXH20	= - 6dB
		BLAXH40	= - 3dB
		BLAXH100/50	≥ 0dB
	Avance DRX	BLAXH150/50	≥ 0dB
		BLAXH300/50	≥ 0dB
	Avance DMX	BLARH100	≥ + 3dB

Nucleus	Spectrometer	Amplifier	Power Level
^{13}C	Avance	BLA2BB	≥ + 6dB
		BLAX300/50	≥ + 6dB
		BLAX300	≥ + 6dB
		BLAX500	≥ + 9dB
	Avance DPX	BLAXH20	= - 6dB
		BLAXH40	= - 6dB
		BLAXH100/50	≥ - 3dB
	Avance DRX	BLAXH40	≥ - 3dB
		BLAXH150/50	≥ 0dB
		BLAXH300/50	≥ 6dB
	Avance DMX	BLAX300	≥ + 6dB
		BLAX500	≥ + 9dB

1.2 NMR Spectrometer

The NMR spectrometer consists of three major components: (1) The superconducting magnet with the probe, which contains the sample to be measured; (2) The console, which contains all the electronics used for transmission and reception of radio frequency (rf) pulses through the pre-amplifier to the probe; (3) The computer, from where the operator runs the experiments and processes the acquired NMR data.

1.3 Classical Description of NMR

A more complete theoretical description of NMR is given in chapter 22.

Among the various atomic nuclei, about a hundred isotopes possess an intrinsic angular momentum, called spin and written $\hbar I$. They also possess a magnetic moment m which is proportional to their angular momentum:

$$m = g\hbar I$$

where g is the gyromagnetic ratio.

The Larmor theorem states that the motion of a magnetic moment in a magnetic field B_0 is a precession around that field, where the precession frequency is given by:

$$w_0 = -gB_0 \quad \text{Larmor frequency}$$

By convention, the external static field (B_0) is assumed to be along the z-axis and the transmitter/receiver coil along either the x- or y-axis. After the sample has been inserted into the magnetic field it shows a magnetization vector $\vec{M}$ along the z-axis. In this state, no NMR signal is observed, as we have no tranverse rotating magnetization.

By application of an additional rotating magnetic field B_1 in the x-y-plane, the orientation of $\vec{M}$ can be tilted into the x-y plane where it precesses around the total magnetic field, e.g. the vector sum of B_0 and B_1. Such a rotating magnetic field is obtained by applying rf-pulses, and the components of $\vec{M}$ are described by the Bloch equations:

$$\frac{d}{dt}M_x^r = 0$$

$$\frac{d}{dt}M_y^r = gB_1M_z$$

$$\frac{d}{dt}M_z = -M_y^r gB_1$$

Assuming the magnetization at time 0 to be along the z-axis with amplitude M_0, we find the following solution to the above equation:

$$M_y^r(t) = M_0 \sin(gB_1t)$$

$$M_z(t) = M_0 \cos(gB_1t)$$

The magnetization vector is precessing around the B_1 axis which is aligned with the x-axis of the reference system. If we choose the time t of suitable duration, we obtain:

$$b = gB_1t = \frac{p}{2}$$

which is defined as the 90 degree pulse creating maximum y-magnetization, which in turn yields maximal signal intensity.

1.4 Spin Operators of a One-Spin System

All NMR experiments start from the thermal equilibrium. In thermal equilibrium, the classical description gives a magnetic moment parallel to the static field, M_z. In the Spin Operator formalism, this is described by:

$$s_{eq} = I_z$$

where σ_{eq} is the equilibrium density matrix representing the state of the spin system under investigation.

Now there are only two basic types of evolutions: (1) An external perturbation, e.g. a rf-pulse, or (2) an unperturbed evolution which will eventually bring the system back to the thermal equilibrium.

1.4.1 Effect of rf-Pulses

The effect of an rf-pulse is that of a rotation along the pulse axes according to the following calculus rules:

$$I_z \xrightarrow{b_x} I_z \cos b - I_y \sin b$$
$$I_z \xrightarrow{b_y} I_z \cos b + I_x \sin b$$
$$I_x \xrightarrow{b_x} I_x$$
$$I_y \xrightarrow{b_y} I_y$$
$$I_x \xrightarrow{b_y} I_x \cos b - I_z \sin b$$
$$I_y \xrightarrow{b_x} I_y \cos b + I_z \sin b$$

If the flip angle β = 90° then:

$$\mathrm{I_z} \xrightarrow{90_{y,x}} \pm \mathrm{I_{x,y}}$$
$$\mathrm{I_{x,y}} \xrightarrow{90_{y,x}} \mp \mathrm{I_z}$$

We find the expected result, that a 90° pulse will generate transverse magnetization. The rest of this chapter will be concerned with finding out about the fate of this transverse magnetization in time.

We introduced tacitly the arrow notation, where we find on the left side the system before and on the right side after the specific evolution under the operator noted above the arrow. This notation is simple, very convenient and not only limited to the description of rf-pulses. We will discuss this notation in more details in the next section.

1.4.2 Effect of Chemical Shift Evolution

The so-called chemical shift Hamiltonian is given by:

$$\mathrm{H} = d \cdot I_z$$

where d is the chemical shift of the corresponding nucleus in the NMR spectrum ($d = w_0 - w$ where w_0 is the Larmor frequency of the spin and ω the carrier frequency of the interaction frame).

The calculus rules for the chemical shift evolution are the following:

$$\mathrm{I_z} \xrightarrow{\delta \cdot \mathrm{I_z} \cdot \mathrm{t}} \mathrm{I_z}$$
$$\mathrm{I_x} \xrightarrow{\delta \cdot \mathrm{I_z} \cdot \mathrm{t}} \mathrm{I_x} \cos(\delta\,\mathrm{t}) + \mathrm{I_y} \sin(\delta\,\mathrm{t})$$
$$\mathrm{I_y} \xrightarrow{\delta \cdot \mathrm{I_z} \cdot \mathrm{t}} \mathrm{I_y} \cos(\delta\,\mathrm{t}) - \mathrm{I_x} \sin(\delta\,\mathrm{t})$$

The time *t* is the period, during which the Hamiltonian is valid. The Hamiltonian of a spin system can change with time, for example if the experimental setup prescribes first a rf-pulse and then a period of unperturbed evolution. For the calculus rules it is mandatory, that each Hamiltonian is time independent during the time *t*.

What's the general idea? The whole NMR experiment is divided into time intervals, during which the Hamiltonian can be made time independent by

choice of a suitable interaction frame. Typical experiments are divided in pulse intervals and free evolution times.

During the pulses, the chemical shift and scalar coupling interaction is ignored. Only the applied B_1 field is considered. This approach is justified for pulses with $t_{pulse} \ll T_1, T_2$.

1.4.3 Effect of Scalar Coupling

Apart from the chemical shift, there is a second very import interaction between spins, the scalar coupling. The scalar depends on the mediation of electrons, which are confined in orbitals around both nuclei. The scalar coupling is expressed in Hz and noted as J. The operator expression for the scalar coupling is:

$$2\pi J_{12}\, I_{1z} I_{2z}$$

The above Hamiltonian expresses the scalar coupling between spin 1 and spin 2 with a coupling constant J_{12}. The evolution Hamiltonian for this spin system is then:

$$H = \delta_1 I_{1z} + \delta_2 I_{2z} + 2\pi J_{12}\, I_{1z} I_{2z}$$

To calculate the effect of this Hamiltonian, it is divided into 3 parts:

$$\delta_1 I_{1z}$$
$$\delta_2 I_{2z}$$
$$2\pi J_{12}\, I_{1z} I_{2z}$$

which are applied in sequence, where this sequence is arbitrary. After a 90° pulse has been applied to the two spins, we first calculate the two chemical shift terms:

$$\begin{aligned}\sigma_{eq} = I_{1z} + I_{2z} &\xrightarrow{\delta_1 \cdot I_{1z} \cdot t} I_{1x}\cos(\delta_1 t) + I_{1y}\sin(\delta_1 t) + I_{2z} \\ &\xrightarrow{\delta_2 \cdot I_{2z} \cdot t} I_{1x}\cos(\delta_1 t) + I_{1y}\sin(\delta_1 t) \\ &\quad + I_{2x}\cos(\delta_2 t) + I_{2y}\sin(\delta_2 t) \Rightarrow \sigma_1\end{aligned}$$

The next step will be to compute the evolution under the scalar coupling.

The scalar coupling term can be evaluated with a simple set of rules:

$$\begin{aligned}I_{1z} &\xrightarrow{2\pi J_{12} I_{1z} I_{2z} t} I_{1z} \\ I_{1x} &\xrightarrow{2\pi J_{12} I_{1z} I_{2z} t} I_{1x}\cos(\pi J_{12} t) + 2 I_{1y} I_{2z}\sin(\pi J_{12} t) \\ I_{1y} &\xrightarrow{2\pi J_{12} I_{1z} I_{2z} t} I_{1y}\cos(\pi J_{12} t) - 2 I_{1x} I_{2z}\sin(\pi J_{12} t) \\ 2 \cdot I_{1x} I_{2z} &\xrightarrow{2\pi J_{12} I_{1z} I_{2z} t} 2 I_{1x} I_{2z}\cos(\pi J_{12} t) + I_{1y}\sin(\pi J_{12} t) \\ 2 \cdot I_{1y} I_{2z} &\xrightarrow{2\pi J_{12} I_{1z} I_{2z} t} 2 I_{1y} I_{2z}\cos(\pi J_{12} t) - I_{1x}\sin(\pi J_{12} t) \\ 2 \cdot I_{1x} I_{2y} &\xrightarrow{2\pi J_{12} I_{1z} I_{2z} t} 2 I_{1x} I_{2y}\end{aligned}$$

which can then be applied to the various terms of s_1 above:

$$\sigma_1 \xrightarrow{2\pi J_{12} I_{1z} I_{2z} t} \{I_{1x}\cos(\pi J_{12}t) + 2 I_{1y} I_{2z}\sin(\pi J_{12}t)\}\cdot\cos(\delta_1 t)$$
$$+ \{I_{1y}\cos(\pi J_{12}t) - 2 I_{1x} I_{2z}\sin(\pi J_{12}t)\}\cdot\sin(\delta_1 t)$$
$$+ \{I_{2x}\cos(\pi J_{12}t) + 2 I_{1z} I_{2y}\sin(\pi J_{12}t)\}\cdot\cos(\delta_2 t)$$
$$+ \{I_{2y}\cos(\pi J_{12}t) - 2 I_{1z} I_{2x}\sin(\pi J_{12}t)\}\cdot\sin(\delta_2 t)$$
$$= \sigma_2$$

References: O. W. Sørensen, G.W. Eich, M. H. Levitt, G. Bodenhausen, R. R. Ernst, *Progres in NMR Spectroscopy*, **16**, 163 (1983).

1.5 Sensitivity of NMR Experiments

The sensitivity of NMR experiments is given by the signal to noise ratio:

$$S/N = \frac{N g_{exc} T_2 \left(g_{det} B_0\right)^{3/2} \sqrt{ns}}{T}$$

S/N	=	signal to noise ratio
N	=	number of spins in the system (sample concentration)
g_{exc}	=	gyromagnetic ratio of the excited nucleus
g_{det}	=	gyromagnetic ratio of the detected nucleus
ns	=	number of scans
B_0	=	external magnetic field
T_2	=	transverse relaxation time (determines the line width)
T	=	sample temperature

(Comment: here we can already see that it might be useful for a better signal to noise ratio to excite one kind of nuclei and detect another kind with a better gyromagnetic ratio in the same experiment. This is done in inverse experiments which are described in sections 14 to 16).

1.6 Useful Coupling Constants

Many NMR constants such as chemical shift ranges, sensitivities, common NMR solvent properties etc. can be found in the Bruker Almanac. Here we added the values of some common coupling constants that are used more often as parameters (**`cnst1`** – **`cnst5`**) in some pulse programs.

1.6.1 Coupling Constants: $^nJ_{CH}$

As a rule of thumb it is possible to estimate the $^1J_{CH}$ coupling constant from the following equation: $^1J_{CH}$ ~ 500*(fractional CH s character). That is: 125Hz < $^1J_{CH}$ < 250Hz, so that $^1J_{CH}$ = 145Hz is a good approximation in most cases.

The values of $^2J_{CH}$ coupling constants increase with increasing HCαCβ angles and with the electronegativity of the Cβ substituent. They vary between –5 and 50Hz.

The $^3J_{CH}$ coupling constants are mostly positive and are maximal at CCCH angles of 0° and 180°. The values for *trans* couplings are larger as for *cis* couplings (Karplus relation).

Table 2: Useful CH Coupling Constants

Compound	**$^1J_{CH}$ in Hz**
Ethane	124.9
Acetonitrile	136.0
Ethene	156
Benzene	159
Dichloromethane	178.0
Chloroform	209.0
Formaldehyde	222.0

System	**$^2J_{CH}$ in Hz**
$C(sp^3)C(sp^3)H$	-10 to +6
$C(sp^3)C(sp^2)H$	0 to +30
$C(sp^2)C(sp^3)H$	-7 to -4
$C(sp^2)C(sp^2)H$	-4 to +14

System	**$^3J_{CH}$ in Hz**
$C(sp^3)C(sp^3)C(sp^3)H$	0 to 8
$C(sp^3)C(sp^2)$ $C(sp^2)H$	0 to 20
$C(sp^2)C(sp^2)$ $C(sp^3)H$	0 to 20
	$J_{trans} > J_{cis}$

References: H.-O. Kalinowski, S. Berger, S. Braun; *^{13}C-NMR-Spektroskopie;* Georg Thieme Verlag; Stuttgart, New York.

1.6.2 Coupling Constants of Hydrocarbons: $^nJ_{HH}$

Usually $^2J_{HH}$ coupling constants are negative and vary in a range between -0.5Hz and -15Hz in hydrocarbons. $^3J_{HH}$ coupling constants are mostly positive and usually range from 2 up to 18Hz. The $^{n>3}J_{HH}$ coupling is positive or negative with smaller absolute values, that range from 0 to 3Hz. The Karplus relation is also valid: $J_{trans} > J_{cis}$.

Table 3: Useful HH Coupling Constants

System	**$^2J_{HH}$ in Hz**
$HC(sp^3)H$	-12 to -15
$HC(sp^2)H$	-0.5 to -3

System	**$^3J_{HH}$ in Hz**
$HC(sp^3)C(sp^3)H$	2 to 9
$HC(sp^3)C(sp^2)H$	4 to 10
$HC(sp^2)C(sp^2)H$	6 to 18
$HC(sp^3)CHO$	1 to 3
$HC(sp^2)CHO$	2 to 4

System	**$^4J_{HH}$ (abs. value) in Hz**
$HC(sp^3)C(sp^3)C(sp^3)H$	0
$HC(sp^3)C(sp^2)C(sp^2)H$	0 to 3
$HC(sp)C(sp)C(sp^3)H$	2 to 3

Heteroatoms with considerable I or M effect can shift the J values dramatically.

2 Preparing for Acquisition

2.1 Sample Preparation

The sample quality can have a significant impact on the quality of the NMR spectrum. The following is a brief list of suggestions to ensure high sample quality:

- Always use clean and dry sample tubes to avoid contamination of the sample.
- Always use high quality sample tubes to avoid difficulties with shimming.
- Filter the sample solution.
- Always use the same sample volume or solution height (recommended values: 0.6 ml or 4 cm of solution for 5 mm sample tubes, 4.0 ml or 4 cm of solution for 10 mm sample tubes). This minimizes the shimming that needs to be done between sample changes.
- Use the depth gauge to position the sample tube in the spinner. This is discussed further in Chapter 5 ‘Sample Positioning' of the BSMS User's Manual.
- Check that the sample tube is held tightly in the spinner so that it does not slip during an experiment.
- Wipe the sample tube clean before inserting it into the magnet.
- For experiments using sample spinning, be sure that the spinner, especially the reflectors, are clean. This is important for maintaining the correct spinning rate.

2.2 Bruker NMR software

There are three major tasks that are controlled by the NMR software: acquisition, processing and plotting. The XWinNMR program is the user interface for all of these tasks. The commands can either be called up by selecting the menu items or by typing the appropriate command in the command line followed by RETURN. There are many parameters that are important for each job and they can be accessed and edited by the user. These parameters and the measured data as well as the processed spectra are stored in datasets which are specified by names, experiment numbers (expno) and processing numbers (procno).

Each parameter can be accessed directly by entering it's name in the command line followed by RETURN or in the **eda**, **edp** or **edg** window for acquisition-, processing- or plotting parameters respectively. Since these panels contain all possible parameters and are rather large, it is often more convenient to use somewhat more reduced parameter editor interfaces. The **ased** command opens the panel for the acquisition parameters that are of importance only for the selected pulse program. Here the parameters are also commented on.

2.2.1 Predefined Parameter Sets

The XWinNMR philosophy is to work with predefined parameter sets that are suitable for most of the NMR tasks and experiments you are facing. These parameter sets include the pulse program, acquisition and processing AU programs as well as all other necessary parameters except spectrometer specific values for pulse lengths and power levels. These standard parameter sets usually have the same base name as the corresponding pulse program. Each parameter set can be called up into a dataset of your choice by the command **rpar**. You can modify the parameters and save the new parameter set by the command **wpar**. Bruker predefined parameter sets are written in capital letters, and we recommend that you do not change them but rather create new ones that you can use just as well.

Therefore the most simple way to run a certain experiment is to create a new dataset with a specific name, using the command **edc**. Then you would read the corresponding parameter set by **rpar** (i.e. **rpar PROTON all**), set the pulse lengths and power levels by **getprosol** and type **xaua** to start the acquisition. (It is assumed that the sample is shimmed and the probe is matched and tuned for the specific nuclei). If you are using the Bruker predefined parameter sets, you can always process the data by typing **xaup**.

The following list is a short summary of the most commonly used experiments and the corresponding parameter sets. The emphasis is on the spectroscopic information that you will get from the experiments rather than on the type of experiment. (For the experiments in this table, it is always recommended to use the gradient version of the experiment if you have the required z-gradient hardware. These experiments usually require less time than the ones without gradients).

Table 4: Short List of Typical Experiments, Parameter Sets and What They Do

Atom / Group	Information (1D Experiments)	a.k.a.	Parameter Set
H	^{1}H chemical shift and coupling	1D^{1}H	PROTON
C	^{13}C chemical shift, ^{1}H decoupled (signal enhancement, integration not possible)	1D^{13}C	C13CPD
C	^{13}C chemical shift, ^{1}H coupled (signal enhancement, integration not possible)	1D^{13}C	C13GD
CH, CH_2, CH_3	^{13}C chemical shift, select CH, CH_2 and CH_3 signals only (same phase)	DEPT45	C13DEPT45
CH	^{13}C chemical shift, select CH signals only	DEPT90	C13DEPT90
CH, CH_3	^{13}C chemical shift, select CH and CH_3 signals only (opposite phase)	DEPT135	C13DEPT135

Correlation	Information (2D Experiments)	a.k.a.	Parameter Set
H–H	^{1}H/^{1}H nearest neighbor, through bond chemical shift correlation	COSY	COSYGPSW[1] COSY45SW
H–H	^{1}H/^{1}H nearest neighbor, through bond chemical shift correlation plus coupling constants	DQF-COSY	COSYGPDFPHSW[1] COSYDQFPHSW
H–(–)$_n$H	^{1}H/^{1}H total spin system through bond chemical shift correlation	TOCSY	MLEVPHSW
C–H	Sensitive ^{1}H/^{13}C directly bound chemical shift correlation (one bond), lower resolution in ^{13}C dimension	HSQC HMQC	HSQCGPPH[1] HMQC
C–H	Sensitive ^{1}H/^{13}C directly bound chemical shift correlation (one bond), lower resolution in ^{13}C dimension (small molecules, solemnly select ^{13}C/^{1}H not ^{12}C/^{1}H)	BIRD-HMQC	HMQCBI
C–H	Insensitive ^{1}H/^{13}C directly bound chemical shift correlation (one bond), high resolution in ^{13}C dimension	HETCOR	HCCOSW
C–(–)$_n$H	Sensitive ^{1}H/^{13}C long range chemical shift correlation (more than one bond), lower resolution in ^{13}C dimension	HMBC	HMBCGPLPND[1] HMBCLPND
C–(–)$_n$H	Insensitive ^{1}H/^{13}C long range chemical shift correlation (one and more bonds), high resolution in ^{13}C dimension	COLOC	HCCOLOCSW
H···H	^{1}H/^{1}H non bound structural neighbor, through space chemical shift correlation (small molecules, low fields)	ROESY	ROESYPHSW
H···H	^{1}H/^{1}H non bound structural neighbor, through space chemical shift correlation (large molecules, proteins)	NOESY	NOESYPHSW

In most of the 2D parameter sets there is a spectral width optimization implemented (*PULSEPROGRAM*SW). So if you acquire the corresponding 1D experiments in the previous experiment number the spectral width for the 2D will be optimized according to the 1D information.

A complete list of parameter sets can be called up by typing **`rpar`** without a following name. The nomenclature of the parameter sets follows the rules for

[1] z-gradient hardware required

the nomenclature of the pulse programs. They can be found in the file: $XWinNMRHome/exp/stan/nmr/lists/pp/Pulprog.info

However in this manual, we focus on the manual setup of the experiments from scratch and the optimization of the corresponding parameters. therefore the **rpar** command will not be used throughout this text.

2.2.2 XWinNMR parameters and commands

A list of commonly used acquisition and processing commands and parameter names as well as a description of the corresponding command or parameter is given in short in the tables below.

Table 5: General Commands and AU Programs

setres	customize the XwinNMR interface
edmac	edit or create an XWinNMR macro
edau	edit or create an XWinNMR AU program
edpul	edit or create an XWinNMR pulse program
xau listall_au	create a file called “listall” in your home directory with a list of all available AU programs including short descriptions
edcpul	edit the current pulse program

Table 6: Data Set Related Commands

edc, new	create a new data set, experiment number or processing number
xau iexpno	copy the current experiment number including all parameters to the consecutive experiment number
wrpa	copy of the current data set including the spectra
re	move to a specific experiment number within the data set
rep	move to a processing number within the experiment number
browse	browse the data set directories
search	find a specific data set
wpar	save the current parameters
rpar	select and read a predefined parameter set

Table 7: Acquisition Parameters

ns	number of scans
ds	number of dummy scans
td	Time domain, number of acquired data points
sw	sweep width in ppm
aq	acquisition time
o1p	transmitter frequency of f1 channel in ppm
o2p	transmitter frequency of f2 channel in ppm
rg	receiver gain
pulprog	definition of the pulse program
aunmp	definition of the acquisition AU program

Table 8: Acquisition and Pre-acquisition Commands

edhead	define the current probehead
edprosol	define probehead specific pulse lengths and power levels
getprosol	use probehead specific pulse lengths and power levels in the

	current pulse program
xau pulse	calculate the power level from pulse lengths and vice versa
edsp, edasp	configure the routing of the spectrometer
edcpul	open the current pulse program in a text editor window
eda	edit all acquisition parameters
ased, as	edit the acquisition parameters that are relevant for the current pulse program
ppg	graphical display of the current pulse program
spdisp	open the graphical pulse program editor
dpa	display all status parameters for the acquisition

wbchan	select the wobbling channel for tuning and matching
wobb	tuning and matching the probe
atma	automatic tune and match the ATM probe
atmm	manually tune and match the ATM probe
edsolv	define solvent parameters
edlock	define lock parameters for probhead and solvent
lock	Automatically lock on solvent (parameters defined in edlock)
lockdisp	open the lock display window
rsh	select and read shim values
gradshim	start the gradient shimming subprogram
wsh	save the current shim values

edte	open the temperature control window
edau	select or edit AU programs
stdisp	open the shape tool

expt	estimate the experiment time
rga	Automatically adjust the receiver gain
zg	start acquisition
xaua	start the acquisition AU program (this also starts the acquisition)
gs	Interactive adjustment of acquisition parameters
tr	data transfer during acquisition

halt, stop	stop the acquisition
kill	kill a specific process

Table 9: Processing Parameters

si	size of the real spectrum
phc0, phc1	Parameters for zero order and first order phase corrections
lb	line broadening factor for **em**
aunmp	definition of the processing AU program

Table 10: Processing Commands

edp	edit all processing parameters
dpp	display all status parameters for processing
ft	Fourier transform the current data
em	apply exponential window function
ef	combined command of **ft** and **em**
phase	set the phase correction defined by **phc0** and **phc1**
apk	Automatically phase correct the spectrum
abs	Automatically baseline correct and integrate the spectrum
efp	combined command of **ft**, **em** and **phase**

sr	spectral referencing
sref	Automatically calibrate the spectrum

edc2	select a second and a third data processing number
dual	invoke the dual display

edo	select an output device
edg	edit all graphics and plotting parameters
view	plot preview
xwinplot	start the plot program

Table 11: Pulse Program Specific Parameters

pl1	f1 channel – power level for pulse (default)
pl2	f2 channel – power level for pulse (default)
pl9	f1 channel – power level for presaturation
pl10	f1 channel – power level for TOCSY-spinlock
pl11	f1 channel – power level for ROESY-spinlock
pl12	f2 channel – power level for CPD/BB decoupling
pl14	f2 channel – power level for cw saturation
pl15	f2 channel – power level for TOCSY-spinlock

sp1	f1 channel – shaped pulse for selective excitation or f1 channel - shaped pulse for water flipback
sp2	f1 channel – shaped pulse 180 degree or f2 channel - shaped pulse 90 degree (on resonance)
sp7	f2 channel – shaped pulse 180 degree (off resonance2) or f2 channel – shaped pulse 180 degree (adiabatic) or f1 channel - shaped pulse for wet

p0	for different applications i.e. f1 channel - variable flip angle high power pulse in DEPT
p1	f1 channel - 90 degree high power pulse
p2	f1 channel – 180 degree high power pulse
p3	f2 channel - 90 degree high power pulse
p4	f2 channel – 180 degree high power pulse
p6	f1 channel - 90 degree low power pulse
p11	f1 channel - 90 degree shaped pulse (selective excitation or water flipback/watergate or wet)
p15	f1 channel – pulse for ROESY spinlock
p16	homospoil/gradient pulse
p17	f1 channel – trim pulse at pl10 or pl15
p18	f1 channel – shaped pulse (off resonance presaturation)

d0	incremented delay (2D) [3 usec]
d1	relaxation delay 1-5 * T1
d2	1/(2J)
d3	1/(3J)
d4	1/(4J)
d6	delay for evolution of long range couplings
d7	delay for inversion recovery
d8	NOESY mixing time
d9	TOCSY mixing time
d11	delay for disk I/O [30 msec]
d12	delay for power switching [20 usec]
d14	delay for evolution after shaped pulse

d16	delay for homospoil/gradient recovery
d17	delay for DANTE pulse-train
d18	delay for evolution of long range couplings
d19	delay for binomial water suppression
d20	for different applications

cnst0	for different applications
cnst1	J (HH)
cnst2	J (XH)
cnst3	J (XX)
cnst4	J (YH)
cnst5	J (XY)
cnst11	for multiplicity selection
cnst12	for multiplicity selection

vc	variable loop counter, taken from vc-list
vd	variable delay, taken from vd-list

l1	loop for MLEV cycle (((p6*64) + p5) * l1) + (p17*2) = mixing time
l2	loop for GARP cycle l2 * 31.75 * 4 * p9 => AQ
l3	loop for phase sensitive 2D or 3D using States et al. or States-TPPI method l3 = td1/2
l4	for different applications i.e. noediff

Note that the default units for pulses are microseconds (u), the units for delays are seconds (s), but one can always enter a value combined with a unit to define a time slot in XWinNMR. The nomenclature here is: **s** = seconds, **m** = milliseconds and **u** = microseconds. For example To set the value of **d1** to **500m** would define **d1** to last for half a second.

The complete information on the nomenclature and default usage of the pulse program parameters can be found in:
$XWinNMRHome/exp/stan/nmr/lists/pp/Param.info

The nomenclature and description of the standard pulse programs and predefined parameter sets can be found in:
$XWinNMRHome/exp/stan/nmr/lists/pp/Pulprog.info

Acquisition, processing and plotting commands can be given either in the XWinNMR command line or via menu selection. Examples are **zg**, which starts the acquisition, **ft** which performs a Fourier transformation on the current data or **apk** which invokes the automatic phase correction.

Another possibility to manage different task in XWinNMR are AU programs. They handle many routine jobs an can be selected or edited by the **edau** command. AU programs have to be compiled before first usage. Compile and start AU Programs by entering **xau** followed by the program name.

XWinNMR also offers extensive online documentation, which can be accessed via the help menu in the XWinNMR menu bar.

2.2.3 Changes for XWinNMR 3.5

XWinNMR version 3.5 is shipped with new systems now. There are some new commands and the handling of some pulse programs have changed from the software version 3.1.

- In XWinNMR 3.5 the names of pulse program and parameter files have been adjusted to the general NMR nomenclature. For recording HSQC, HMQC and HMBC spectra pulse program and parameter files starting with the 4 letter code hsqc, hmqc, and hmbc, respectively, have to be given in the **`pulprog`** line in the **`eda`** table.
- A new parameter **`TD0`** is now available in the **`eda`** table. This parameter brings about a storage of your 1D data after recording ns/TD0 scans. This is especially useful for very long 1D experiments.

For more information on general changes, please refer to the release letter of your software packet. Information for pulse program specific changes can be found in: $XWinNMRHome/exp/stan/nmr/lists/pp/Update.info

2.3 Tuning and Matching the Probe

In a probehead there are resonant circuits for each nucleus indicated on the probehead label (e.g., one for ^{1}H and one for ^{13}C in a dual $^{1}H/^{13}C$ probehead; one for ^{1}H and one for a wide range of nuclei in BBO or BBI probeheads). There is also a resonant circuit for the lock nucleus, but the standard user will never need to adjust this, so we will ignore it in the following. Each of the circuits has a frequency at which it is most sensitive (the resonance frequency). Once the sample is inserted, the probehead should be tuned and matched for these individual frequencies.

Tuning is the process of adjusting this frequency until it coincides with the frequency of the pulses transmitted to the circuit. For example, the frequency at which the ^{1}H resonant circuit is most sensitive must be set to the carrier frequency of the ^{1}H pulses (which is **`sfo1`** if the ^{1}H circuit is connected to the f1 channel, **`sfo2`** if it is connected to the f2 channel, etc.). Matching is the process of adjusting the impedance of the resonant circuit until it corresponds with the impedance of the transmission line connected to it. This impedance is 50 Ω. Correct matching maximizes the power that is transmitted to the coil. A probehead is said to be tuned and matched when all of its resonant circuits are tuned and matched. Once a probehead has been tuned and matched, it is not necessary to retune or rematch it after slight adjustments of the carrier frequency, since the probehead is generally tuned and matched over a range of a couple of hundred kHz. On the other hand, large adjustments to the carrier frequency, necessary when changing nuclei, warrant retuning and rematching of the probehead. Thus, a broadband probe needs to be retuned and rematched each time the heteronucleus is changed.

If you have an ATM probe, enter **edsp** and set the spectrometer parameters for the channels that should be matched and tuned. For 1H on channel F1 and 13C on channel F2 enter the following values:

NUC1	1H
NUC2	13C
NUC3	OFF

This automatically sets **sfo1** to a frequency appropriate for ^{1}H and **sfo2** to the corresponding ^{13}C frequency for tuning and matching. Exit **edsp** by clicking **SAVE**.

Type **atma**. This will invoke the automatic match and tune program for all nuclei that were selected previously in **edsp**. Therefore it is not necessary to tune and match manually.

2.4 Tuning and Matching ^{1}H (non ATM Probes)

When the NMR experiments to be performed are ^{1}H homonuclear experiments (e.g., ^{1}H 1D spectroscopy, COSY, NOESY, or TOCSY), only the ^{1}H circuit of the probehead has to be tuned and matched.

Make sure that the sample is in the magnet, and the probehead is connected for standard ^{1}H acquisition. Note that there is no special configuration for tuning and matching. Also, it is recommended to tune and match *without* sample spinning.

2.4.1 Set the Parameters

In XWIN-NMR, enter **edsp** and set the following spectrometer parameters:

NUC1	1H
NUC2	OFF
NUC3	OFF .

This automatically sets **sfo1** to a frequency appropriate for ^{1}H tuning and matching. There is no need to adjust **sfo1** carefully now. Exit **edsp** by clicking **SAVE**.

2.4.2 Start Wobbling

Tuning and matching are carried out simultaneously using XWIN-NMR. During wobbling, a low power signal is transmitted to the probehead. This signal is swept over a frequency range determined by the parameter **wbsw** (the default value is 4 MHz) centered around the carrier frequency (i.e., **sfo1**, **sfo2**, etc., depending on which nucleus is being tuned/matched). Within the preamplifier (High Performance Preamplifier Assembly or HPPR), the impedance of the probe over this frequency range is compared to the impedance of a 50 Ω resistor. The results are shown both on the LED display of the HPPR and in the acquisition submenu of XWIN-NMR. Both displays show the reflected power of the probehead versus the frequency of the

signal. The user observes either one or both of these displays while tuning and matching the probehead.

Before starting the wobbling procedure, ensure that no acquisition is in progress, e.g., enter **stop**.

Enter **acqu** to switch to the acquisition window of XWIN-NMR, if it is desired to use this to monitor the tuning and matching. Notice that being in the acquisition window slows down the wobbling procedure, so if the HPPR LED display will be used to monitor tuning and matching, it is best to remain in the main XWIN-NMR window and not to switch to the acquisition window.

Start the frequency sweep by typing **wobb**. The curve that appears in the acquisition window is the reflected power as a function of frequency. Unless the probehead is quite far from the correct tuning and matching, there will be a noticeable dip in the curve. When the ^{1}H circuit is properly tuned, the dip will be in the center of the window, denoted by the vertical marker; and when the circuit is properly matched, the dip will extend all the way down to the *x* axis. Similar information is conveyed by the LED display on the HPPR. The horizontal row of LED's indicates tuning and the vertical row matching. When the circuit is properly tuned and matched, the number of LEDs is minimized. This usually means that only green LED, are lit in both the horizontal and vertical displays.

2.4.3 Tune and Match

Adjust the tuning and matching screws (labeled T and M) at the base of the probehead. Note that the screws are color coded and those for the ^{1}H circuit are usually yellow. *Also note that the screws have a limited range and attempting to turn them beyond this range will damage the probehead*.

Since there is an interplay between tuning and matching, it is generally useful to adjust the T and M screws in an iterative fashion. Turn the M screw until the dip is well matched at some frequency (the dip extends to the *x* axis and the number of LEDs lit in the vertical HPPR display is minimized). Most likely this will ***not*** be the desired frequency. Adjust the T screw slightly to move the dip toward the center of the window, or equivalently, to reduce the number of LEDs lit in the horizontal HPPR display. Rematch the dip by adjusting the M screw again. Note that it is possible to run out of range on the M screw. If this happens, return M to the middle of its range, adjust T to get a well matched dip at some frequency, and walk the dip towards the correct frequency as described above.

As mentioned above, ideal tuning and matching is when the dip is centered in the window and extends to y = 0 (the *x* axis) on the acquisition window, or equivalently, when the number of LED's lit on the preamplifier is minimized in both the vertical and horizontal display.

When the ^{1}H circuit is tuned and matched, exit the wobble routine by typing **stop**. Click on **return** to exit the acquisition window and return to the main window.

2.5 Tuning and Matching ^{13}C (non ATM Probes)

Since most ^{13}C experiments make use of ^{1}H decoupling, besides ^{13}C the ^{1}H should be tuned and matched as well. When tuning and matching a probehead with multiple resonant circuits, it is best to tune and match the lowest frequency circuit first. Thus, when tuning and matching a probehead for both ^{1}H and ^{13}C, first do the ^{13}C and then the ^{1}H adjustments.

Make sure that the sample is in the magnet, and the probehead is connected for the appropriate experiment. Also, it is recommended to tune and match *without* sample spinning.

2.5.1 Set the Parameters

In XWIN-NMR, enter **edsp** and set the following spectrometer parameters:

NUC1	13C
NUC2	OFF
NUC3	OFF .

This automatically sets **sfo1** to a frequency appropriate for ^{13}C tuning and matching. Exit **edsp** by clicking **SAVE**.

2.5.2 Start Wobbling, Tune and Match

Ensure that no acquisition is in progress, enter **stop**.

Enter **acqu** to switch to the acquisition window, if this will be used to monitor the tuning and matching.

Start the frequency sweep by typing **wobb**. The curve that appears in the acquisition window is for ^{13}C. Adjust the tuning and matching following the guidelines given above for ^{1}H. Notice that some probeheads (e.g., broadband probeheads) have sliding bars instead of screws, one set labeled tuning and another labeled matching. Set the tuning and matching sliding bars to the values indicated for ^{13}C on the menu. Adjust the tuning and matching bars until the dip is well tuned and matched at some frequency as described above for ^{1}H.

Once the ^{13}C circuit is tuned and matched, the ^{13}C wobbling must be stopped before the ^{1}H wobbling. Exit the wobble routine by typing **stop**. Enter **edsp**, change NUC1 to 1H, and exit by clicking **SAVE**. Start the ^{1}H frequency sweep by typing **wobb**. After a few seconds the ^{1}H curve appears in the acquisition window and the ^{1}H circuit can be tuned and matched as described above.

Alternatively, if the user already has a data set in which NUC1 = 1H and NUC2 = OFF, there is no need to redo **edsp** for the current data set. The user may simply read in the ^{1}H data set and then type **wobb**.

Once the probehead is tuned and matched for ^{13}C and ^{1}H, exit the wobble routine by typing **stop**.

Click on return to exit the acquisition window and return to the main window.

2.6 Locking and Shimming

Before running an NMR experiment, it is necessary to lock and shim the magnetic field.

2.6.1 Locking

To display the lock signal enter **lockdisp**. This opens a window in which the lock trace appears.

The most convenient way to lock is to use the XWIN-NMR command **lock**. To start the lock-in procedure, enter **lock** and select the appropriate solvent from the menu. Alternatively, enter the solvent name together with the lock command, e.g., **lock cdcl3**. During lock-in, several parameters such as the lock power, the field value, and the frequency shift for the solvent are set according to the values in the lock table. This table can be edited using the command **edlock**. Note that the lock power listed in this table is the level used once lock-in has been achieved. The field-shift mode is then selected and autolock is activated. Once lock-in is achieved, the lock gain is set so that the lock signal is visible in the lock window. At this point the message "lock: finished" appears in the status line at the bottom of the window.

The lock-in procedure outlined above sets the frequency shift to the exact frequency shift value for the given solvent as listed in the **edlock** table. It also sets the field value to the value listed in the **edlock** table and then adjusts it slightly to achieve lock-in (the absolute frequency corresponding to a given ppm value no longer depends on the lock solvent). Following this lock-in procedure, the **solvent** parameter in the **eda** table is set automatically, which is important if you wish to use the automatic calibration command **sref** (see "Spectrum Calibration and Optimization").

The lock-phase adjustment by monitoring the sweep wiggles (i.e., while the field is not locked but is being swept) is recommended each time the probehead is changed, because autolock may fail. If the original phase is reasonably close to the correct value, lock-in can be achieved and the phase can be adjusted using autophase. Note that the lock phase for each probehead is stored in the **edlock** table. In some cases, the lock power level listed in the **edlock** table is set too high leading to a saturation of the lock signal. Usually, lock-in can be achieved, but the signal oscillates due to saturation. A quick fix is simply to reduce the lock power manually after lock-in. However, it is better to change the power level in the **edlock** table. Note that the appropriate lock power level depends on the lock solvent, the field value, and the probehead.

2.6.2 Shimming

If the sample has been changed, the first step after locking is shimming the magnetic field. Enter **rsh** and select an appropriate shim file from the menu. Usually, only the Z and Z^2 shims (and probably the X and Y) must be adjusted while observing the lock signal. The best shim values correspond to the highest lock level (height of the lock signal in the window). For further

discussion of shimming see Chapter 6 ‘Shim Operation' of the BSMS User's Manual.

If you have a gradient probe, you can also use the gradient shimming tool, which can be started by the command **`gradshim`**. For more Information, please refer to the gradient shimming installation and users guide which is available online in the XWinNMR help menu.

2.6.3 Optimize lock settings (optional)

Once the magnetic field has been locked and shimmed, the user may wish to optimize the lock settings as described below. It is strongly recommended to follow this procedure before running any experiment requiring optimal stability (e.g., NOE difference experiments).

After the field is locked and shimmed, start the auto-power routine from the BSMS keyboard (see Chapter 2 ‘Key Description' of the BSMS User's Manual). For lock solvents with long T_1 relaxation times (e.g., $CDCl_3$), however, auto power may take an unacceptably long time and the lock power should be optimized manually. Simply increase the lock power level until the signal begins to oscillate (i.e., until saturation), and then reduce the power level slightly (approximately 3 dB). For example, if the lock signal begins to oscillate at a power of –15 dB, the optimal magnetic field stability can be expected when a level of approximately –18 dB (or even –20 dB) is used. The field stability will be significantly worse if a power level of, say, –35 dB is used instead.

When the lock power is optimized, start the auto-phase routine, and finally the auto-gain routine. Take note of the gain value determined by auto gain. Using this value, select the appropriate values for the loop filter, loop gain, and loop time as shown below in Table 12.

Table 12: Lock Parameters (BSMS Firmware Version 980930)

Lock RX Gain (after auto gain) [dB]	**Loop Filter [Hz]**	**Loop Gain [dB]**	**Loop Time [sec]**
120	20	–17.9	0.681
	30	–14.3	0.589
110	50	–9.4	0.464
	70	–6.6	0.384
	100	–3.7	0.306
	160	0.3	0.220
	250	3.9	0.158
	400	7.1	0.111
90	600	9.9	0.083
	1000	13.2	0.059
	1500	15.2	0.047
	2000	16.8	0.041

So, for example if auto gain determines a lock gain of 100 dB, the user should set the loop filter to 160 Hz, the loop gain to 0.3 dB, and the loop time to 0.220 sec (see Chapter 4 ‘Menu Description' of the BSMS User's Manual for how to set these parameters from the BSMS keyboard).

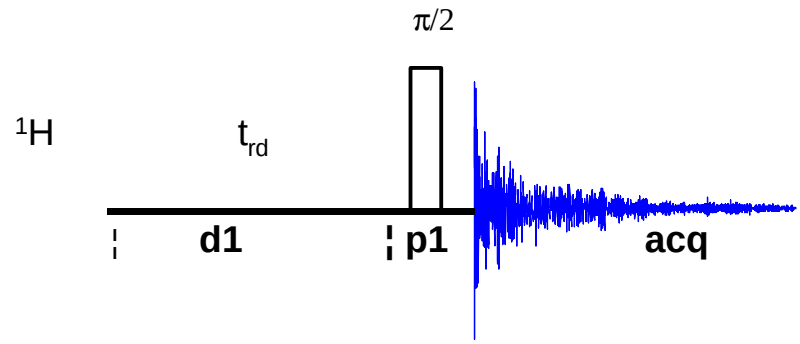
3 Basic ^{1}H Acquisition and Processing

3.1 Introduction

This chapter describes the acquisition and processing of a 1D ^{1}H NMR spectrum using the simple one-pulse NMR experiment shown in Figure 1. The pulse sequence consists of the recycling delay, t_{rd}, the radio-frequency (RF) pulse, and the acquisition time during which the signal is recorded. The pulse angle is shown to be $\pi/2$, although, in practice, it is often chosen less. The two parameters, **d1** and **p1**, correspond to the length of the recycle delay, t_{rd}, and the length of the RF pulse, respectively.

Note that the time intervals depicted in the pulse sequence diagrams are not drawn to scale. For example, **d1** is typically a few seconds while **p1** is typically a few microseconds in length.

Figure 1: 1D ^{1}H NMR One-Pulse Sequence



3.1.1 Sample

The sample used for demonstrating the basic 1D ^{1}H experiment is 100 mg Cholesterylacetate in $CDCl_3$ with 0.5 % TMS.

3.1.2 Preparation

Make sure that you have done the following steps (see also Chapter 2 'Preparing for Acquisition'):

- Insert a suitable probehead
- Read in the corresponding shim file
- Insert the sample
- Lock the spectrometer

- Optimize the Z and Z^2 (and probably X and Y) shims
- Tune and match the probehead for ^1H

3.2 Spectrometer and Acquisition Parameters

Before the acquisition of a spectrum a new data set must be created. All the spectrometer and acquisition parameters are entered within the new data set.

The spectrometer parameters are responsible for the hardware settings necessary for configuring the spectrometer for a particular experiment. The command **edsp** calls up a window in which the spectrometer parameters for the observe and the decoupler channel(s) are set.

The acquisition parameters include all pulse sequence parameters, the number of data points, number of scans, receiver gain, and many others. These may be displayed and edited by entering **eda**. Notice that the spectrometer parameters are also listed in the **eda** table. It is important to set the spectrometer parameters before setting the acquisition parameters, because the values from **edsp** automatically overwrite the corresponding ones from the **eda** table.

3.3 Create a New File Directory for the Data Set

To create a new data set, type **edc** in the command line of the XWIN-NMR window. This calls up a small window entitled “Current Data Parameters”. Enter a data set name (NAME), an experiment number (EXPNO), a processed data number (PROCNO), the disk unit (DU) where the data is stored, the user id (USER), and the data type (TYPE). Change the parameters as follows:

NAME	proton
EXPNO	1
PROCNO	1

Click on **SAVE**. This exits **edc** and creates the data set proton/1/1. The message “NEW DATA SET” should now appear on the screen.

3.4 Set Up the Spectrometer Parameters

Enter **edsp** and set the following spectrometer parameters:

NUC1	1H
NUC2	off
NUC3	off

Since there is no decoupling, the only relevant spectrometer parameters are SFO1. Click on **SAVE** to save the spectrometer parameters and return to the main window. The spectrometer is now prepared to pulse and detect at the ^{1}H frequency.

3.5 Set Up the Acquisition Parameters

Enter **eda** and set the acquisition parameters as shown in Table 13, where only the relevant parameters are listed. Note that the parameters **d1**, **p1**, and **pl1** are included in the parameter arrays D, P and PL in the **eda** table, respectively. These parameters can be edited within **eda**, by clicking the ' **Array**-button next to the corresponding parameter

Table 13: Basic ^{1}H Spectrum Acquisition Parameters

Parameter	Value	Comments
PULPROG	zg	see Figure 1 for the pulse sequence diagram
AQ_mod	DQD	If DQD is not available, use qsim
TD	32 k	32 k is a standard value for a high-resolution 1D spectrum
PARMODE	1D	One-dimensional experiment
NS	1	one scan is recorded for parameter optimization
DS	0	no dummy scans are recorded
D1	2	the default unit for delays is seconds; entering "2" sets a delay of 2 seconds (click the D**Array** button)
P1	3	the default unit for pulse lengths is microseconds; entering "3* sets a pulse length of 3 microseconds (µs) (click the P**Array** button)
PL1	PL1 =	power level for the p1 pulse see also "An Important Note on Power Levels" on page 3 (click the P**Array** button)
SW	50	for the first spectrum of an unknown sample use a large spectral width; when you enter "50" the registered value is slightly different
RG	64	suggested receiver gain
NUC1	1H	observe nucleus
O1P	15	position of the carrier frequency is 15 ppm

Click on **SAVE** to save the acquisition parameters and return to the main window. Click on **DONE** to save the changes and return to the **eda** table. As with most acquisition parameters, however, **d1**, **p1**, and **pl1** can also be edited by typing them in the command line of the main XWIN-NMR window. As mentioned before, most of the acquisition parameters for the current pulse program can also be entered in the **ased** table.

3.6 Acquisition

Enter **acqu** to switch to the acquisition window. While it is possible to acquire a spectrum from the main window, the buildup of the FID can only be observed in the acquisition window.

Enter the command **rga** which performs several acquisitions and sets a suitable value for the receiver gain (**rg**). Enter **zg**, which deletes any previous data (zero') and starts the experiment (go'). The message **scan 1/1** indicates that the spectrometer is performing the first scan and that only one scan will be performed.

If, at any time, a submenu is entered accidentally, click on the return button located on the button bar and then enter **acqu** to switch back to the acquisition window.

If, at some point the message “DATA OUT OF WINDOW” appears, or if the scaling is unsuitably large or small, click on the and buttons located on the button bar.

3.7 Processing

After the FID has been acquired the next step is to process the acquired data. The processing parameters are displayed and edited by entering **edp**. First, Fourier transformation is performed by entering the command **ft**. The number of points used to resolve the resulting spectrum is determined by the processing parameter **si** (size). The spectrum consists of **si** real points and **si** imaginary points, and thus the default setting of **si** is **td**/2, where **td** is the acquisition parameter indicating the number of time domain data points. In general, **td**/2 and **si** are numbers described by powers of 2 (2, 4, 8, 16, 32, 64, 128, ...). If **si** < **td**/2 not all the time domain data is used for the Fourier transformation, and if **si** > **td**/2 the time domain data is zero-filled with 2(**si**) before the Fourier transformation. In 1D spectroscopy, it is often recommended to zero-fill once, i.e., to set **si** = **td**.

Check the value of **si**. Enter **si** and when prompted enter 32k (appropriate for **td** = 32 k). Enter **ft**: The display automatically switches from the acquisition window to the main window and displays the. The FID can still be viewed by returning to the acquisition window. If the *x* axis of the Fourier transformed spectrum is displayed in Hz, click on Hz/ppm to convert into a ppm scale. If necessary, use the buttons as described above to scale the spectrum.

You can zoom into a part of the spectrum by defining the appropriate 1D plot range. Move the cursor into the display window and press the left mouse button to tie the cursor to the spectrum. Move the cursor to one side of the desired zoom region and click the middle mouse button to define it. Move the cursor to the other side of the desired plot region and click the middle mouse button again to zoom into this region. To display the whole spectrum push the ⏭ button.

3.8 Phase Correction

Once the spectrum is Fourier transformed it must be phase corrected. Click on phase enter the phase correction submenu. Click on biggest for setting the reference for the 0th-order phase correction to the position of the biggest peak in the spectrum and adjusts its phase. To adjust the 0th-order phase manually, place the cursor on PH0 and hold down the left mouse button. Move the mouse until the reference peak is positive and the baseline on either side is as flat as possible.

Most likely, the peaks on either side of the reference peak are not yet phased correctly and require a 1st-order phase correction. To adjust the 1st-order phase correction, place the cursor on PH1 and hold down the left mouse button, and move the mouse until the peaks far from the reference point are also in-phase.

Note that it is advisable to select the reference peak for the 0th-order phase correction near one edge of the spectrum. However, for some samples the biggest peak will be located in the middle of the spectrum. In such cases, click on cursor and define the reference peak by moving the cursor onto the desired peak and clicking with the middle mouse button.

Once the spectrum is phased correctly, click on **return** to exit the submenu and save the phase corrections by selecting **Save & return**. The 0th- and 1st-order phase correction values are stored as processing parameters **`phc0`** and **`phc1`**, respectively. To quit the phase correction submenu without saving the corrections, simply click on **return** and select **return**. In either case, the display returns to the main menu and the spectrum appears on the screen.

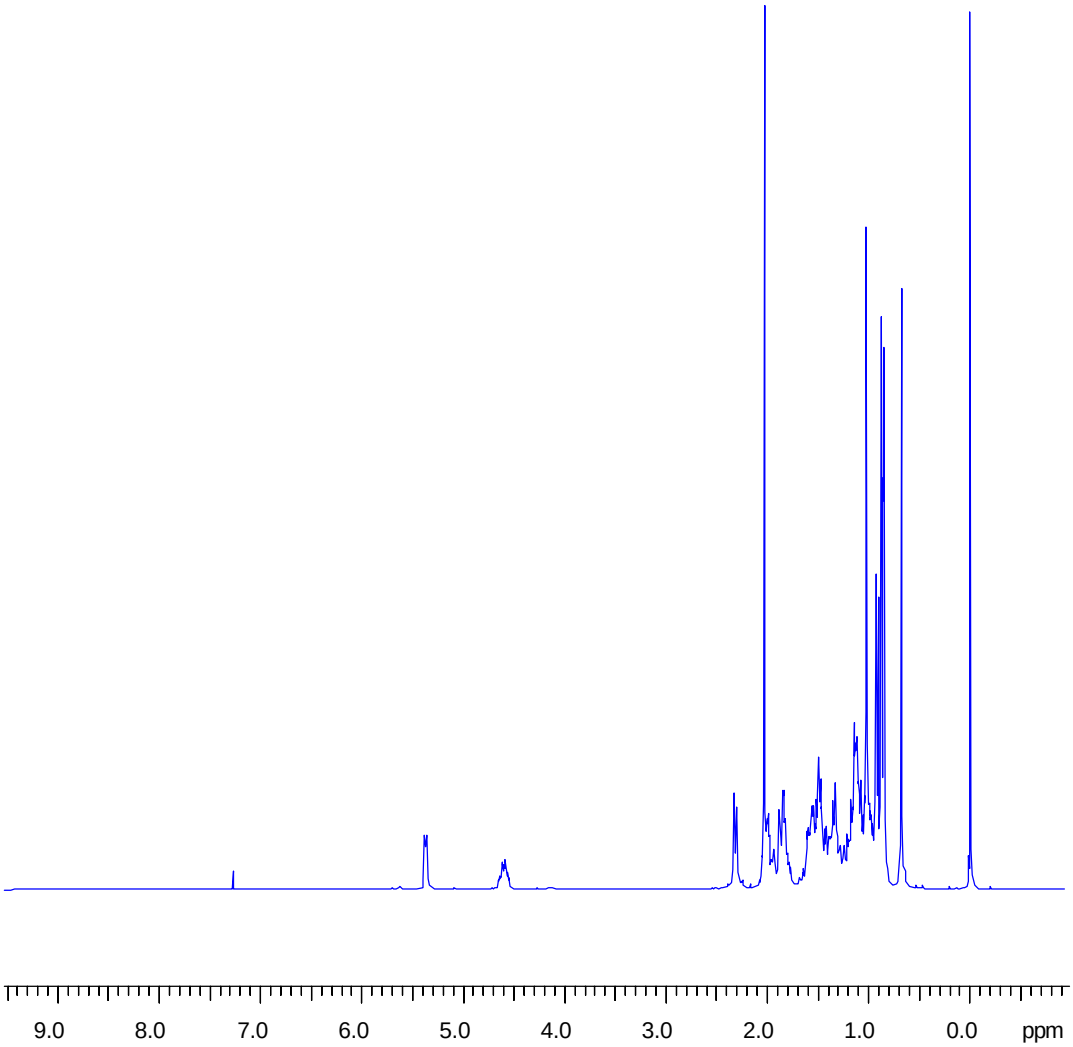
Note that once suitable values of **`phc0`** and **`phc1`** have been stored it is possible to use them for phase correcting subsequent spectra by typing the command **`pk`**. In addition, the Fourier transformation (**`ft`**) and the phase correction (**`pk`**) can be performed within one step using the command **`fp.`**

3.9 Windowing

Before the Fourier transformation is performed, it is common to apply a window (or filter) function to the time domain data. The main reason for this is the improvement of either signal-to-noise, or resolution. Usually, for a simple 1D spectrum as described here, the signal-to-noise ratio is improved by multiplying the FID with a simple exponential function achieved by the command **`em`**.

The decay rate of the exponential function determines the amount of line broadening. This rate is determined by the processing parameter **lb** (in Hz). Enter **lb** and set the value to 0.3, which corresponds to an appropriate line broadening for high-resolution ^{1}H spectra. Enter **em** to perform the multiplication, and then enter **fp** to Fourier transform and phase correct the filtered data. You can also use the combined command **efp**, which performs the windowing, Fourier transformation and the phasing with the previously determined phase correction. The final spectrum should look like the one shown in Figure 2.

Figure 2: ^{1}H 1D Spectrum of 100 mg Cholesterylacetate in CDCl3



3.10 Integration

To quantitatively analyze an observed signal, the integrated intensity of the peaks are compared within each other.

Click integrate to enter the integration submenu. To integrate a peak, first move the cursor into the spectral window and click the left mouse button. Next, click the middle mouse button once at each end of the range of interest; the integral appears automatically. Click the left mouse button again to release the cursor from the spectrum. An asterisk or a vertical arrow appears next to the right end of the integral (if not, select the integral with the left mouse button). Correct the baseline of the integral with the slope bias buttons. Integrate the other areas or peaks in the same way.

For the calibration, select an integral (asterisk/arrow) and click on calibrate Enter 100 to calibrate this integral to 100%. Upon return select **Save & store 'intrng'** to save the integral and normalization constant and return to the main 1D processing window.

It is also possible to compare integral values of spectra located in different data sets: Integrate both spectra and calibrate the integral(s) in one of them, e.g. to 100 as described above. Enter the integration mode in the second spectrum, select the corresponding integral (asterisk/arrow) and click on the lastscal button to display the integral value compared to the calibrated 100% of the other signal.

4 Pulse Calibration: Protons

4.1 Introduction

This chapter describes pulse calibration procedures for ^{1}H and ^{13}C. It is assumed that the user is already familiar with acquisition and processing of simple 1D NMR spectra. Appendix A (Data Sets and Selected Parameters), which lists all data sets generated throughout this course, and Appendix B (Pulse Calibration Results), which provides all the pulse lengths and power levels determined during this course, maybe useful in this context.

4.2 Proton Observe 90° Pulse

For the calibration of a ^{1}H 90° pulse on the observe channel (F1), the one-pulse sequence described in Chapter 3 is used. The carrier frequency (**o1p**) is set onto the resonance frequency of a peak in the ^{1}H spectrum of an appropriate sample. This peak is monitored while the length (**p1**) and/or the strength (**pl1**) of the RF pulse is adjusted to determine the exact conditions for a 90° pulse.

A common sample used for the ^{1}H pulse calibration is 0.1% Ethylbenzene in $CDCl_3$. Ethylbenzene shows a simple ^{1}H spectrum with well-separated signals, which facilitates the selection of a single resonance line. However, due to the relatively long spin-lattice *or* longitudinal relaxation time (T_1) of Ethylbenzene, a long recycle delay time must be used.

4.2.1 Preparation

Insert the sample and lock the spectrometer (**lock**). Readjust the Z and Z^2 shims until the lock level is maximal (use **lockdisp**). Tune and match the probehead for ^{1}H observation (*see* Chapter 2.3).

First, create a new data set. Since this will be a ^{1}H observe experiment, it is best to start out from a previous ^{1}H data set, e.g., proton/1/1: Enter **re proton 1 1**, then enter **edc** and change the following parameters:

NAME	test1h
EXPNO	1
PROCNO	1

Click on **SAVE** to create the data set test1h/1/1.

Enter **eda** and set the acquisition parameter values as shown in Table 14.

Table 14: 1D ^{1}H one-pulse Acquisition Parameters

Parameter	Value	Comments
PULPROG	zg	see Figure 1 for the pulse sequence diagram.
TD	4 k	
NS	1	number of scans
DS	0	no dummy scans
D1	10	interscan delay (10s, because of long T_1)
P1	3	start with 3µs, which should correspond to less than a 90° pulse
PL1		power level for the p1 pulse see "An Important Note on Power Levels" on page 3
SW	20	start with a large spectral width of 20ppm; which will be optimized lateron
o1p	5	will be optimized lateron

Enter **rga** to perform an automatic receiver gain adjustment, then enter **zg** to acquire the FID, and **edp** to set the processing parameters as shown in Table 15.

Table 15: 1D ^{1}H one-pulse Processing Parameters

Parameter	Value	Comments
SI	2 k	
LB	1 Hz	
PSCAL	global	

Fourier transform the spectrum with the command **ef** and phase the spectrum according to Chapter 3.8. Type **sref** to calibrate the spectrum and confirm the message "no peak found in 'sref' default calibration done".

4.2.2 Optimize the Carrier Frequency and the Spectral Width

The carrier position (**o1p**) should now be set to the signal used for monitoring the 90° pulse calibration, which is the quartet signal of the Ethylbenzene ^{1}H spectrum. Expand the spectrum so that only the quartet at 2.6 ppm is displayed. Click on utilities to enter the calibration submenu. Click on o1 with the left mouse button, move the cursor to the center of the quartet and click the middle mouse button to assign **o1p** to this frequency. Click on return to exit the calibration submenu and return to the main window. Reduce the spectral width by entering **swh** and change the value to 1000 (Hz).

Enter **zg** to acquire a new FID using the new values for **o1p** and **swh** and process the spectrum using the command **ef**.

4.2.3 Define the Phase Correction and the Plot Region

The phase correction and the spectral region plotted in the output file must be optimized before the automation program for the pulse calibration is executed. Phase correct the spectrum according to Chapter 3.8 in a way that the quartet signal is positive. Expand the spectrum so that the quartet covers approximately the central quarter of the screen. Click on dp1 with the left mouse button and hit return for the following three prompts, or answer them as follows:

F1	2.8 ppm
F2	2.4 ppm
change y-scaling on display according to PSCAL?	y

The preparations are now completed and the calibration experiment can be executed as described in the next section.

4.2.4 Calibration: High Power

For the 90° pulse calibration an automation program called **paropt** is used. (Since the execution of this automation is time consuming, it is not the best choice if the correct pulse times and power levels are already known approximately. In such cases, the correct values are usually just checked by acquiring 1D spectra with different pulse widths to check for maximal signal.)

The automation program is started by typing **xau paropt** and answering the appearing questions as follows:

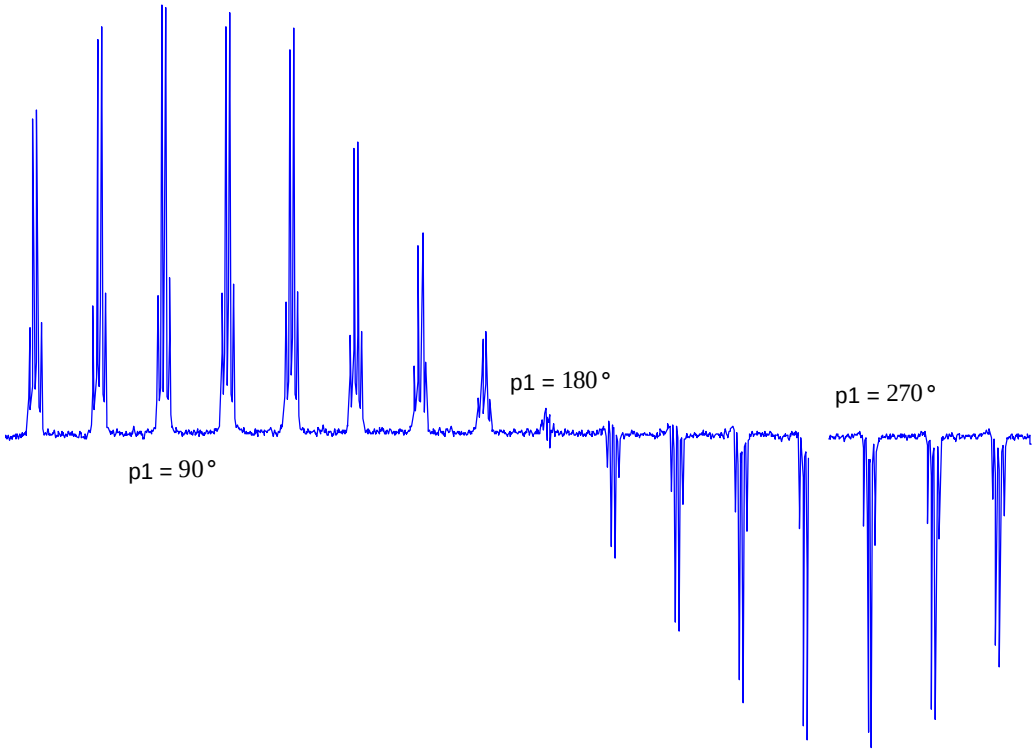
Enter parameter to modify:	p1
Enter initial parameter value:	2
Enter parameter increment:	2
Enter # of experiments:	16

The spectrometer acquires and processes 16 spectra with incrementing the parameter **p1** from 2 µsec by 2 µsec to a final value of 32 µsec. For each of the 16 spectra, only the spectral region defined above is plotted, and all the spectra are plotted side-by-side in the file test1h/1/999 as shown in Figure 3. At the end of the experiment, the message “paropt finished” and a value for the parameter **p1** is displayed, which corresponds to the 90° pulse length of the ^{1}H transmitter with the power level as defined by **pl1**. Write this value down and follow the procedure described below to obtain a more accurate 90° pulse measurement.

Return to the data set test1h/1/1 by entering **re 1 1**. Type **p1** and enter a value which corresponds to a 360° pulse (i.e., four times the 90° value determined by paropt before). Acquire and process a new spectrum by typing **zg** and **efp** (*see* Chapter 3.9) respectively. Change **p1** slightly and acquire and process a spectrum again, until the quartet undergoes a zero crossing as expected for an exact 360° pulse. Note that the quartet signal is negative for pulse angles slightly less than 360° and positive when the pulse angle is slightly more than 360°.

The 360° pulse length divided by four yields the accurate ^{1}H 90° transmitter pulse length for the actual power level **pl1**.

Figure 3: Paropt Results for 1H 90° Pulse Calibration



4.2.5 Calibration: Low Power for MLEV Pulse Train (TOCSY)

The ^{1}H 90° pulse for the MLEV pulse train used during the spinlock period of a TOCSY sequence is between 30 µsec to 40 µsec. The procedure outlined below uses the paropt routine to determine the corresponding power level. However, the power level can be estimated roughly by using a rule of thumb: The pulse length doubles for an additional 6 dB increase of the power level. For example, the 90° pulse length (**p1**) was determined 8 µsec for **pl1** = 0 dB. Thus, the **p1** = 16 µsec for **pl1** = 6 dB, or the **p1** = 32 µsec for **pl1** = 12 dB.

For performing the exact determination of the low power pulse, return to the file test1h/1/1 (**re 1 1**). Enter **p1** and change the value to 35 (µsec), type **xau paropt** and answer the questions as follows:

Enter parameter to modify:	pl1
Enter initial parameter value:	0
Enter parameter increment:	1
Enter # of experiments:	16 .

Again, the 16 spectra will be displayed in the file test1h/1/999 and at the end of the experiment, the message "paropt finished" and a value for **pl1** is displayed. This value corresponds to the ^{1}H transmitter power level for a 90° pulse length of 35 µsec. Write down this value and follow the procedure described below to obtain a more accurate 90° pulse measurement.

Return to test1h/1/1 (**re 1 1**), type **p1** and change the value to 140 µsec (= 360° pulse). Acquire and process a spectrum (**zg**, **efp**) by using the power level **pl1** determined by paropt above. Change **pl1** slightly until the quartet undergoes a zero-crossing indicating the accurate 360° pulse. Divide this 360° pulse time by four to get the 90° pulse length.

Note that the parameters used by the TOCSY sequence are **p6** for the 90° pulse length and **pl10** for the power level, rather than **p1** and **pl1**.

4.2.6 Calibration: Low Power for ROESY Spinlock

The power level required for the cw spinlock pulse used with ROESY experiments corresponds to a 90° pulse length of 100 µsec to 120 µsec. As described for the 90° pulse determination of the MLEV pulse above in Chapter 4.2.5, the power level can again be estimated using the rule of thumb, or measured using the paropt automation.

When using paropt, return to the file test1h/1/1 (**re 1 1**), enter **p1** and change the value to 110 (µsec), and type **xau paropt**. Answer the questions as follows:

Enter parameter to modify:	pl1
Enter initial parameter value:	10
Enter parameter increment:	1
Enter # of experiments:	16 .

The results are displayed in the file test1h/1/999, and at the end of the experiment, the message "paropt finished" and a value for **pl1** corresponding to the ^{1}H transmitter power level for a 90° pulse length of 110 µsec are displayed. Follow the same procedure as described in Chapters 4.2.4 and 4.2.5 for a more accurate determination of the power level.

Note that since ROESY uses cw spinlock, only the power level determination is important here, but not the actual 90° pulse length. The power level parameter used with the ROESY sequence is **pl11**, rather than **pl1**.