AVANCE

SGU Based Frequency Generation

Beginners Guide
English Version

Version 002

The information in this manual may be altered without notice.

BRUKER accepts no responsibility for actions taken as a result of use of this manual. BRUKER accepts no liability for any mistakes contained in the manual, leading to coincidental damage, whether during installation or operation of the instrument. Unauthorized reproduction of manual contents, without written permission from the publishers, or translation into another language, either in full or in part, is forbidden.

This manual was written by:

Eamonn Butler

This manual was desktop published by:

Stanley J. Niles

© May 7, 2003: Bruker Biospin GmbH

Rheinstetten, Germany

P/N: Z31633E
DWG-Nr: 1344002

Contents

Contents iii

1 Introduction 7

1.1 Sources of Hazards 7
Software Version and Command Syntax 8

2 Safety 9

2.1 Introduction 9
2.2 Magnetic Safety 9
Safety Precautions within the Inner Zone 10
Safety precautions within the outer zone 11
2.3 Cryogenic Safety 11
2.4 Electrical Safety 11
2.5 Chemical Safety 12
2.6 CE Certification 12

3 Introductory Theory and Terminology 13

3.1 Introduction 13
3.2 NMR Analysis of Chloroform 16
3.3 Reference Compounds, Hertz, ppm 17
3.4 Proton NMR - Chemical shift 19
3.5 Proton Spectrum of Benzene 20
3.6 Proton Spectrum of Benzylacetate 21
3.7 Proton Spectrum of Ethylbenzene with Spin/Spin Coupling . 22
3.8 Decoupling 25
3.9 FID and Spectrum 26

4 System Description 29

4.1 Introduction 29
4.2 Operator Console and Connections 30
4.3 Console 30
4.4 Link Between the Host Computer and the AQS 31
4.5 Magnet, Shim System, HPPR and Probe 31
4.6 The Magnet and Magnet Dewar 34
Room Temperature Bore 35
Helium Tank 35
Nitrogen Tank 36
4.7 Introduction to the Lock System 37
4.8 Probes 37
Dual 13CI1H probe 39
Changing a Probe 41

5 The NMR Sample 43

5.1 Introduction 43
5.2 Solvent Selection 43
5.3 Sample Tube 44
5.4 Sample Handling 45

6 Basic procedures 47

6.1 Introduction 47
6.2 BSMS Keyboard 47
Storing a Set of Shim Values (Write Shim Command) 48
Reading a Stored Shim File (Read Shim Command) 49
BSMS Functions 49
Sample Control Functions 49
Manual Lock Functions 49
Manual Shim Functions 50
Helium Level Functions 51
6.3 Inserting the Sample into the Spinner 51
6.4 Inserting the Sample Plus Spinner into the Magnet 52
6.5 Spinning the Sample 53
6.6 Tuning and Matching the Probe 53
Tuning and Matching Using the Wobble Curve 54
Tuning and Matching Using the HPPR LEDs 56
Tuning and Matching More than One Nucleus 57
6.7 Locking the Sample 58
Procedure to Lock the Sample 59
6.8 Shimming 61
Initial Shimming 62
Routine Shimming 62

7 Preparing for Acquisition, Data Sets, edasp/eda Commands 65

7.1 Introduction 65
7.2 Data Sets 65
7.3 Creating a Data Set 67
7.4 Spectrometer Parameters edasp 68
Layout of the edasp Window 69
Some Features of the edasp Window 72
7.5 Basic Acquisition Parameters: The “eda” Table 72
Numerical Explanation of Transmitted, Basic and Offset Frequencies 79

8 Pulse Programs/Commands as/ased 83

8.1 The Pulse Programs “zg” and “zg30” 83
8.2 Details of the “zg30” Program 83
8.3 The Commands “as” and “ased” 86

9 Proton Spectrum 87

9.1 Introduction 87
9.2 Creating a New Data Set 87

9.3 Reading in the Standard Parameter Set 88
The “getprosol” Command 88
9.4 Setting the Receiver Gain 89
9.5 Starting the acquisition 90
9.6 Fourier Transform and phase correct the spectrum 91
9.7 Basic Processing: Fourier Transformation 92
9.8 Phase Correction 93
9.9 Calibration of Spectrum 94
Procedure to Expand the Spectrum Horizontally 95
Calibration Procedure 95
9.10 Adjusting the Spectral Width with the SW-SFO1 function ... 96
Adjusting the SW for Cholesterylacetate Spectrum 97
9.11 Increasing the number of scans 98

10 13C Spectrum without decoupling 101

10.1 Introduction 101
10.2 Procedure 101

11 13C spectrum with decoupling.....105

11.1 Introduction 105
11.2 Procedure 105
11.3 Establishing the Decoupling Frequency 106
11.4 Adjusting the Decoupling Parameters 108
11.5 The pulse program zgpg30 108

12 Basic Troubleshooting 111

12.1 Introduction 111
12.2 Switching the Spectrometer On and Off 111
12.3 Switching on the Spectrometer 112
12.4 Switching off the Spectrometer 112
Starting XWIN-NMR for Windows Systems 113
Starting XWIN-NMR for SGI IRIX Systems 113

Figures 115

Tables 117

Index 119

Contents

Introduction 1

The goal of this manual is to enable a relatively inexperienced user to carry out a series of basic 1-D High Resolution (HR) NMR experiments. Cholesterylacetate was chosen as an example. Both proton observe and carbon observe (with and without proton decoupling) will be described. To assist the user standard parameter sets delivered with every XWIN-NMR software package will be used. However, rather than simply reading in standard parameter sets, a genuine effort has been made to help the user understand the relevance of the various parameters. In particular, this manual will concentrate on describing the acquisition procedure, to some extent, at the expense of processing the acquired data. This emphasis minimizes the time spent on the actual spectrometer itself, particularly in the case where relatively large numbers of undergraduate students are being trained. In this scenario processing can be easily carried out on a separate workstation using the tutorials delivered with the spectrometer documentation.

For the purpose of instruction within this manual it is assumed that the user has:

1. A basic knowledge of the XWIN-NMR software package;
2. A probe or probes capable of observing proton, observing carbon and observing carbon while decoupling protons;
3. A basic knowledge of how to operate the BSMS keyboard or the BSMS control panel.

While every effort has been made to genuinely provide a step by step description, new users will invariably have some questions, and as such will require occasional assistance from a more experienced user. The goal of this manual is, where practicable, to enable users to work independently and acquire a basic understanding of how to operate the system. It is hoped that the time taken to train new users will be significantly reduced with the use of this manual.

Sources of Hazards 1.1

The following chapter will deal with safety in detail, but for now it is worth highlighting potential hazards that arise when operating an NMR spectrometer at this introductory stage. For such a sophisticated system there are surprisingly few opportunities for inexperienced users to damage the equipment, so few that the most important ones are worth itemizing. With normal operation the most likely causes of damage are:

1. Removing a sample from the magnet with the bore still plugged;
2. Inserting a sample into the magnet without the presence of the supporting air cushion;
3. Emitting RF pulses that are either too long, too powerful or both;
4. Transmitting RF power along unconnected cables or poorly matched probes.

New users are advised to acquaint themselves with these potential hazards before commencing. System managers are advised to ensure that any new users understand the issues mentioned above.

Software Version and Command Syntax *1.1.1*

This manual has been written for XWIN-NMR version 3.1. Throughout the manual procedures to enter various commands will be outlined. The command to be typed will be in single quotes using magenta colored text. For example,

enter 'eda'

means type the command 'eda' at the command line and press the "Return“ or "Enter“ button.

Safety 2

Introduction 2.1

In terms of safety the presence of a relatively strong magnet is what differentiates NMR spectrometers from most other laboratory equipment. When designing an NMR laboratory, or training personnel who will work in or around the laboratory, no other feature is of greater significance. As long as correct procedures are adhered to, working in the vicinity of superconducting magnets is completely safe and has no known harmful medical side effects. Negligence however can result in serious accidents. It is important that people working in the vicinity of the magnet fully understand the potential hazards. **Of critical importance is that people fitted with cardiac pacemakers or metallic implants should never be allowed near the magnet.**

The magnet is potentially hazardous due to:

1. The large attractive force it exerts on ferromagnetic objects.
2. The large content of liquid nitrogen and helium.

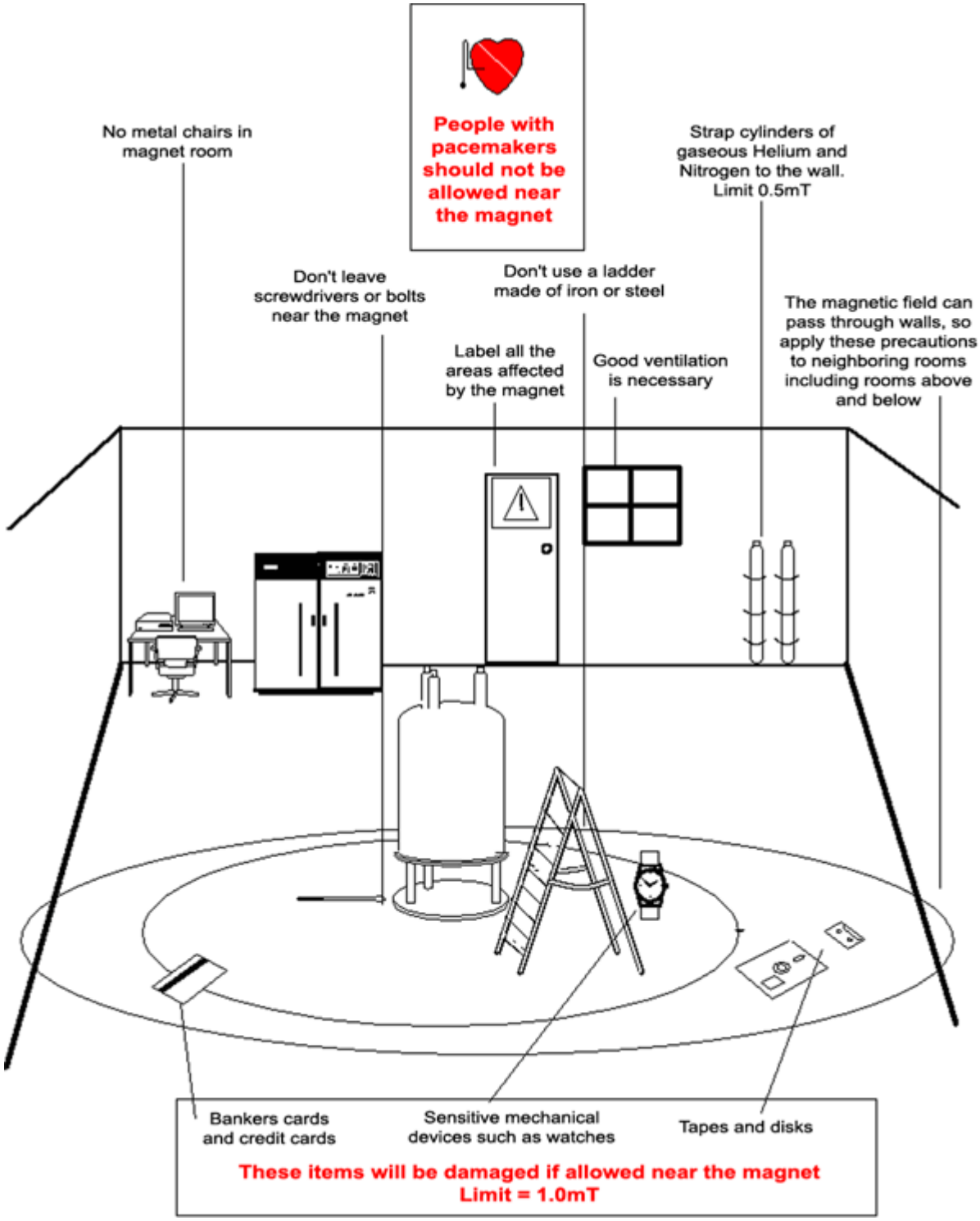
Magnetic Safety 2.2

A magnetic field surrounds the magnet in all directions. This field (known as the stray field) is invisible and hence the need to post warning signs at appropriate locations. Objects made of ferromagnetic materials, e.g. iron, steel etc. will be attracted to the magnet. If a ferromagnetic object is brought too close, it may suddenly be drawn into the magnet with surprising force. This may damage the magnet, or cause personal injury to anybody in the way!

Because the strength of the stray field drops significantly as one moves away from the magnet, it is useful to discuss safety in terms of two broadly defined regions, the inner and outer zone. In terms of organizing a laboratory as well as defining good work practices, the concept of an inner and outer zone is particularly useful.

The physical extent of these two zones will depend on the size of the magnet. The bigger the magnet, the stronger the stray magnetic fields and hence the larger the extent of the two zones. ***Figure 2.1.*** shows the concept of the two zones (not drawn to scale). Details of stray fields for various magnets can be found in the Site Planning Guides delivered with the BASH CD.

Figure 2.1. Safety Precautions within the Inner and Outer Zones



Safety Precautions within the Inner Zone *2.2.1*

The inner zone extends from the magnet center to the 1mT (10 Gauss) line. Within this region objects may suddenly be drawn towards the magnet center. The attractive force of the magnet can change from barely noticeable to uncontrollable

within a very short distance. **Under no circumstances should heavy ferromagnetic objects be located or moved within this zone**.

Any ladders used when working on the magnet should be made of non-magnetic material such as aluminum. Helium and nitrogen dewars which are used to top up the liquid levels inside the magnet must be made of non-magnetic material.

Do not allow small steel objects (screwdrivers, bolts etc.) to lie on the floor near the magnet. These could cause serious damage if drawn into the magnet bore, especially when no probe is inserted in the magnet.

Mechanical watches may be damaged if worn within the inner zone. Digital watches can be worn safely. Of course, the precautions for the outer zone which will now be discussed must also be adhered to within the inner zone.

Safety precautions within the outer zone *2.2.2*

The outer zone extends from the 1mT line to the 0.3mT line. The magnet's stray field does not get blocked by walls, floors or ceilings and the outer zone may well encompass adjoining rooms. The stray field may erase information stored on magnetic tapes or discs. Bank cards, security passes or any devices containing a magnetic strip may be damaged. CD's will not be damaged, although CD drives may contain magnetic parts. When using pressurized gas cylinders made of steel, they should be located well beyond the outer zone (preferably outside the magnet room) and must always be properly fixed to the wall. The color display of computer monitors may suffer some distortion when located too close to the magnet, although permanent damage is unlikely. Once beyond the outer zone any special precautions on account of the magnet stray field are no longer necessary.

Cryogenic Safety *2.3*

The magnet contains relatively large quantities of liquid helium and nitrogen. These liquids, referred to as cryogens, serve to keep the magnet core at a very low temperature.

Because of the very low temperatures involved, **gloves**, a **long sleeved shirt or lab coat** and **safety goggles** should always be worn when handling cryogens. Direct contact with these liquids can cause frostbite. The system manager should regularly check and make sure that evaporating gases are free to escape from the magnet, i.e. the release valves must not be blocked. Do not attempt to refill the magnet with helium or nitrogen unless you have been trained in the correct procedure.

Helium and nitrogen are non-toxic gases. However, because of a possible **magnet quench**, whereupon the room may suddenly fill with evaporated gases, adequate ventilation must always be provided.

Electrical Safety *2.4*

The spectrometer hardware is no more or less hazardous than any typical electronic or pneumatic hardware and should be treated accordingly. Do not remove any of the protective panels from the various units. They are fitted to protect you

and should be opened by qualified service personnel only. The main panel at the rear of the console is designed to be removed using two quick release screws, but again, this should only be done by trained personnel. Please note that, unless disconnected, cooling fans on the rear panel will continue to run even with the panel removed.

Chemical Safety *2.5*

Users should be fully aware of any hazards associated with the samples they are working with. Organic compounds may be highly flammable, corrosive, carcinogenic etc.

CE Certification *2.6*

All major hardware units housed in the AVANCE with SGU consoles as well as peripheral units such as the HPPR, shim systems, probe and BSMS keyboards comply with the CE Declaration of Conformity. This includes the level of any stray electromagnetic radiation that might be emitted as well as standard electrical hazards. Note that to minimize electromagnetic radiation leakage, the doors of the console should be closed and the rear paneling mounted.

Introductory Theory and Terminology

3

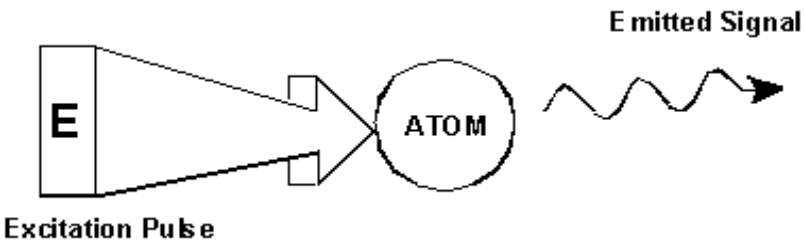
Introduction 3.1

NMR is a technique used to analyze the structure of many chemical molecules, primarily organic compounds. A typical compound might consist of carbon, hydrogen and oxygen atoms.

In its simplest form, an NMR experiment consists of three steps:

1. Place the sample in a static magnetic field.
2. Excite nuclei in the sample with a radio frequency pulse.
3. Measure the frequency of the signals emitted by the sample.

Figure 3.1. Excitation and Response



From the emitted frequencies analysts can deduce information about the bonding and arrangement of the atoms in the sample. The NMR active nuclei in the sample resonate at different frequencies which are called "**resonance frequencies**". These are the frequencies emitted by the nuclei when they are excited by the incoming radio frequency pulse. The value of a resonance frequency depends on two factors:

a) Type of Nucleus:

Every isotope has a particular combination of protons and neutrons in its nucleus. The nuclear structure largely determines the value of the resonance frequency. Thus every isotope displays a “basic resonance frequency”. ^{13}C nuclei will have a different basic resonance frequency compared to that of ^{1}H etc. Note the large variation in basic resonance frequencies between different isotopes as listed in ***Table 3.1.***

Table 3.1. Table of Data for Various Isotopes.

Frequencies quoted for an 11.7T magnet

Nucleus	NMR active	Basic resonance frequency (approx.) [MHz]	Natural Abundance [%]
^{1}H	yes	500	99.98
^{2}H	yes	77	0.015
^{3}H	yes	533	0.005
^{12}C	no	---	98.89
^{13}C	yes	126	1.11
^{35}Cl	yes	49	75.53
^{37}Cl	yes	41	24.47

b) Local Atomic Environment:

Superimposed on the basic resonance frequency is an effect due to the local atomic environment in which an isotope is situated. The precise value of the resonance frequency of a ^{1}H nucleus in a particular compound will depend upon the atoms it is bonded to and surrounded by. The nucleus is surrounded by electrons which may be viewed as moving electrical charges with associated magnetic fields. These electrons act as a source of magnetic shielding for the nucleus. The extent of the shielding will depend on the precise local atomic environment. The size of typical local field variations (which will result in a frequency variation) will depend on the isotope and the strength of the magnetic field in which the sample is placed. The table below shows the typical frequency variation for two of the most widely used NMR nuclei, ^{1}H and ^{13}C. It is clear that the local atomic environment has a relatively small effect on the basic resonance frequency.

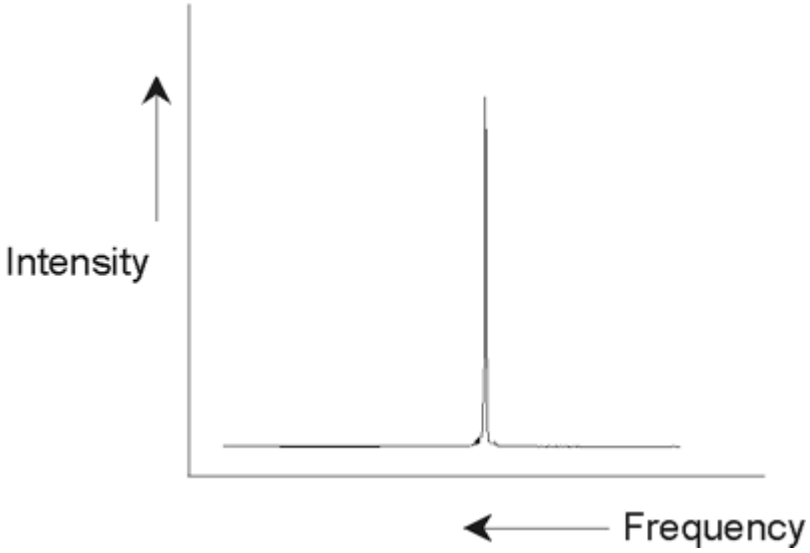
Table 3.2. Frequency Variations

(quoted for an 11.7 T magnet)

Nucleus	Typical variation in basic resonance frequency due to local atomic environment.
^{1}H	6 kHz
^{13}C	100 kHz

NMR signals are usually plotted as spectra and analyzed with respect to two features, frequency and intensity. It is conventional in NMR to plot frequency on the horizontal axis and increasing towards the left.

Figure 3.2. NMR Spectrum



As mentioned above, the frequency yields qualitative information regarding the local atomic environment. The **integrated intensity** of a signal is a **measure of signal strength** and is determined by integrating the area under the signal peak. The integral will be directly proportional to the number of nuclei contributing to a signal at a particular frequency (if all nuclei are equally excited) and hence will provide quantitative information regarding chemical structure.

To excite a given nucleus in an NMR experiment, the frequency of the excitation pulse should closely match the resonance frequency of the nucleus. This frequency is referred to as the **carrier frequency**. Thus, if experiments are carried out using a 11.7 T magnet, the ^{1}H nuclei would require a carrier frequency of approximately 500 MHz, whereas ^{13}C nuclei would require a carrier frequency close to 126 MHz. The carrier frequency is specified by the parameter SFO1. The nucleus that is excited by this carrier frequency is referred to as the **observe nucleus**.

Note that there are experiments in which more than one nucleus gets excited, e.g. during polarization transfer or decoupling. In these cases one has more than one carrier frequency but still only one observe frequency.

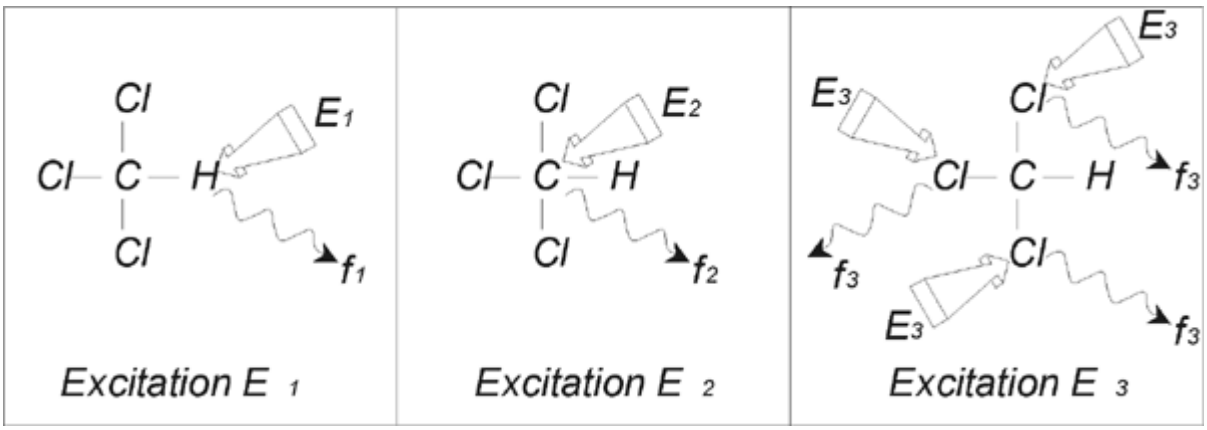
Not all **isotopes** will respond to radio frequency pulses, i.e. not all are **NMR active**. Three isotopes of the element hydrogen are found in nature: ^{1}H (hydrogen), ^{2}H (deuterium), and ^{3}H (tritium, radioactive!). The natural abundance of these isotopes are 99.98%, 0.015%, and 0.005% respectively. All three are NMR active, although as can be seen in ***Table 3.1.***, they all display a large variation in resonance frequency. To analyze a sample for hydrogen, the ^{1}H isotope is excited, as this isotope is by far the most abundant. Of the carbon isotopes found in nature, only one is NMR active. By far the most common isotope, ^{12}C (98.89% natural abundance) is inactive. Hence, NMR analysis of organic compounds for carbon rely on the signals emitted by the ^{13}C isotope, which has a natural abundance of only 1.11%. Obviously, NMR analysis for carbon is more difficult than that of, for example, ^{1}H. (There are other factors which affect sensitivity. These will be discussed in the next section of this chapter).

Using the brief introduction to NMR outlined above, it is a good exercise to consider how the technique could be used to analyze the composition of chloroform ($CHCl_3$).

NMR Analysis of Chloroform 3.2

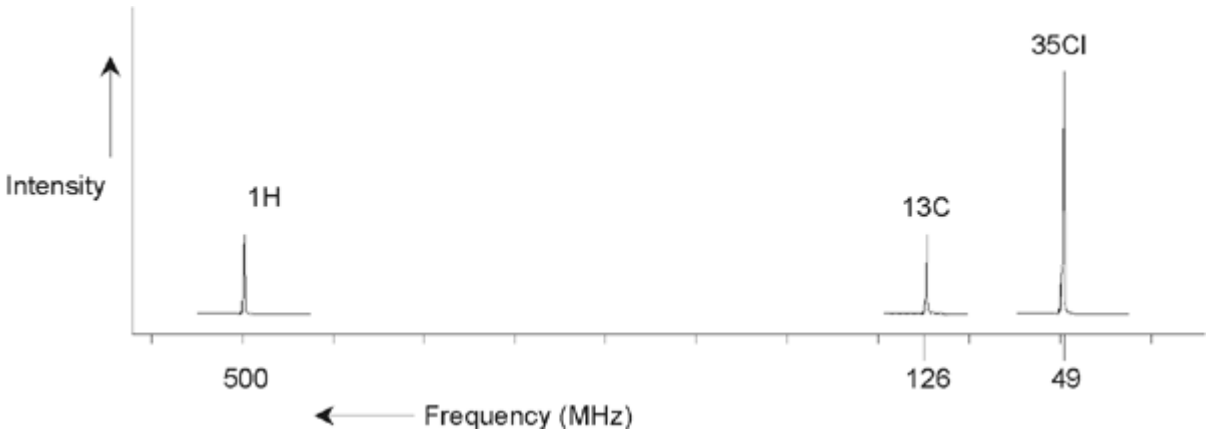
Three separate experiments, as outlined in the figure below, could be performed corresponding to the three possible observe nuclei ^{1}H, ^{13}C and ^{35}Cl.

Figure 3.3. NMR Analysis of $CHCl_3$



Three excitation pulses (E_1, E_2, E_3) at appropriate carrier frequencies would be directed at the sample. E_1 would correspond to the ^{1}H resonance frequency, E_2 to the ^{13}C frequency and E_3 to the ^{35}Cl frequency. Assuming the three isotopes were successfully excited, the sample will emit signals at three frequencies f_1, f_2 and f_3 which would be recorded on three separate spectra. If the emitted signals would be displayed in a single plot, the user can expect a spectrum similar to that of figure 3.4. (Note that the signal frequencies illustrated are for a 11.7 T magnet and that all signals have been plotted as singlets i.e. single peaks.)

Figure 3.4. NMR Signals Emitted by $CHCl_3$



This artificial spectrum shows three peaks corresponding to the three isotopes. Taking the relative numbers of the three isotopes into account, one might expect that the intensity of the chlorine, hydrogen and carbon peaks would be in the ratio of 3:1:1. However, the natural abundance of the three isotopes (see ***Table 3.1.***) must also be accounted for, resulting in a ratio of 227:100:1. The user would find that the experimentally determined peak intensity ratios would not agree with

these values. The reason is that every isotope has an inherent sensitivity to the NMR technique. The ^{1}H is 63 times more sensitive to NMR than ^{13}C. Thus, even if a sample would contain exactly the same number of ^{1}H nuclei as ^{13}C, the intensity of the ^{1}H signals would be 63 times greater than the ^{13}C signals.

With a plot such as that in ***Figure 3.4.***, any detailed information would be lost and precise determination of a particular frequency would be impossible. The spectrum would be said to display very poor resolution. (The horizontal resolution of a spectrum is a measure of how well the spectrum differentiates between two signals that are close in frequency).

A further complication is the huge range in vertical scaling. The variation in inherent sensitivity to NMR, coupled with the variations in natural abundance, would often make the plotting of signals from different isotopes onto a single plot unfeasible. In fact, the vertical resolution of the spectrum would be very poor. (The vertical resolution, i.e. the signal to noise ratio of a spectrum is a measure of sensitivity)

If our analysis of chloroform is proving rather complicated, it is because we are attempting to compare the signals from three different observe nuclei on a single spectrum. (We are ignoring here any hardware/electronic restrictions). Therefore, in practice, NMR experiments are performed with a single observe nucleus. Although more than one isotope may be excited simultaneously, by using more than one carrier frequency (e.g. decoupling experiments), we only ever observe the signals from a single isotope. This greatly simplifies spectrum analysis.

It was mentioned earlier that variations in the basic resonance frequency due to the local atomic environment tend to be relatively small. Thus, large spectral ranges will not be encountered. Furthermore natural abundance and inherent sensitivity will always be the same for a particular isotope. Hence, the relative intensity of say two signals emitted from ^{1}H isotopes on a single spectrum will depend only on the number of atoms contributing to the signal. This greatly simplifies analysis of spectra for quantitative information. Before proceeding further with a more detailed description of NMR the reader should become familiar with the concept of measuring signals in ppm (parts per million) with respect to a reference signal.

Reference Compounds, Hertz, ppm *3.3*

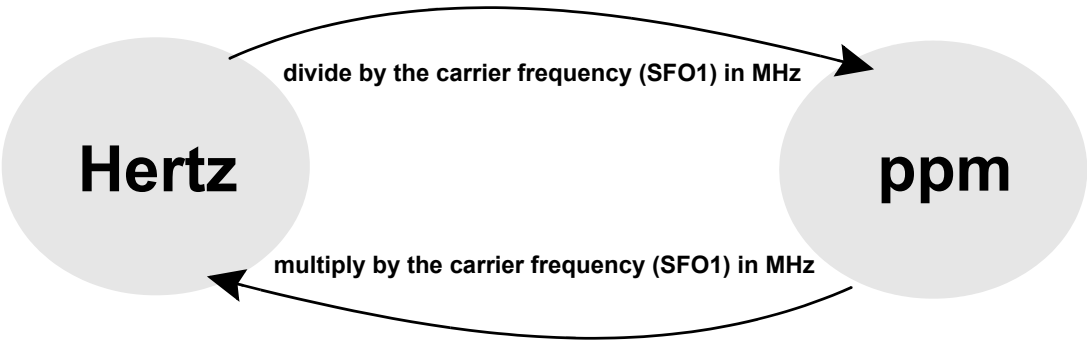
It has already been stated that NMR signals are analyzed with respect to two characteristics, intensity and frequency. Absolute frequencies are measured in Hertz (Hz - cycles per second) or Megahertz (MHz). Reporting on measured signals is simplified if all frequency measurements are made with respect to a reference. For ^{1}H NMR the recommended reference is a chemical called tetramethylsilane (TMS). When a ^{1}H or a ^{13}C spectrum, is acquired, the presence of TMS gives rise to a single, easily identifiable peak. This peak is referenced to zero and the frequencies of all other peaks are given in terms of their frequency relative to the TMS frequency. Thus, we can talk of a signal being 2.5 kHz "above“ the TMS peak. This is preferable to quoting the absolute frequency of the signal which might look like 500.1325 MHz.

Referencing signals to the TMS peak clearly cuts down the number of digits needed to describe the frequency of a signal. However, this can be even more simplified if the ppm unit is used instead of Hertz. The ppm unit represents frequencies as a fraction of the absolute resonance frequency which will depend on the strength of the magnet. The advantage of the ppm unit is that frequency measure-

ments are independent of magnet strength. This greatly simplifies the comparison of spectra acquired on different spectrometers.

The conversion factors to be used when changing from Hertz to ppm and vice versa are shown in the diagram below.

Figure 3.5. Converting Hertz and ppm



The advantages of using the ppm unit is best illustrated with a practical example.

Suppose a ^{1}H signal was observed at 2.5 kHz above TMS using a carrier frequency (SF01) of 500 MHz. The frequency of any emitted NMR signal is directly proportional to the strength of the magnet. The same signal would appear at 3.0 kHz above TMS on a 600 MHz spectrometer and at 2.0 kHz above TMS on a 400 MHz machine. A single conversion may not be a great inconvenience but it must be done for every peak for each system. Now consider the same signal but quoted in the ppm unit.

Frequency in Hertz divided by SFO1 = Frequency in ppm

Examples:

2500 Hz / 500 MHz = 5 ppm

3000 Hz / 600 MHz = 5 ppm

2000 Hz / 400 MHz = 5 ppm

The ^{1}H signal may now be described as being located at 5 ppm "above“, i.e. "downfield“, the TMS peak regardless of the spectrometer frequency.

Experienced users will always deal in ppm, and spectra reproduced in scientific journals will have the horizontal scale graduated in ppm and not in Hertz.

The reader should be aware of some simplifications that have been made in the above example. The value of the ^{1}H carrier frequency on a 500 MHz spectrometer will not be exactly 500 MHz. The carrier frequency to be used in a ppm calculation should be the precise value assigned to the parameter SF01. Similarly for the 600 MHz and 400 MHz spectrometers, quoted above the ^{1}H carrier frequency, will not be exactly 600 MHz and 400 MHz, respectively.

Note also that a positive ppm value denotes a frequency higher TMS and is defined as "downfield“ relative to TMS.

Proton NMR - Chemical shift *3.4*

Since ^1H is the most commonly observed isotope in NMR experiments, this shall now be dealt with in greater detail. A ^1H nucleus contains a single proton and spectra in which ^1H is the observe nucleus are normally referred to as proton spectra.

It was stated previously that a proton in an 11.7 T magnet will display a basic resonance frequency of 500 MHz approximately but that the precise resonance frequency will depend upon the local atomic environment. A proton in a molecule of chloroform resonates at a slightly different frequency compared to a proton in benzene (C_6H_6). Therefore, the emitted frequency acts as a label which gives analysts qualitative information on the local atomic neighborhood in which a proton is located. This is the basis of NMR.

The variation in precise resonance frequency is referred to as the “chemical shift”. The resonance frequency is shifted by the effect of neighboring atoms and in particular the extent of magnetic shielding from local electrons discussed earlier. The size of the shift is normally measured in ppm relative to the TMS peak which is referenced to 0 ppm.

Most protons, regardless of the organic compound to which they are bonded, display chemical shifts within 14 ppm of TMS.

Figure 3.6. ^1H Chemical Shifts in Organic Compounds

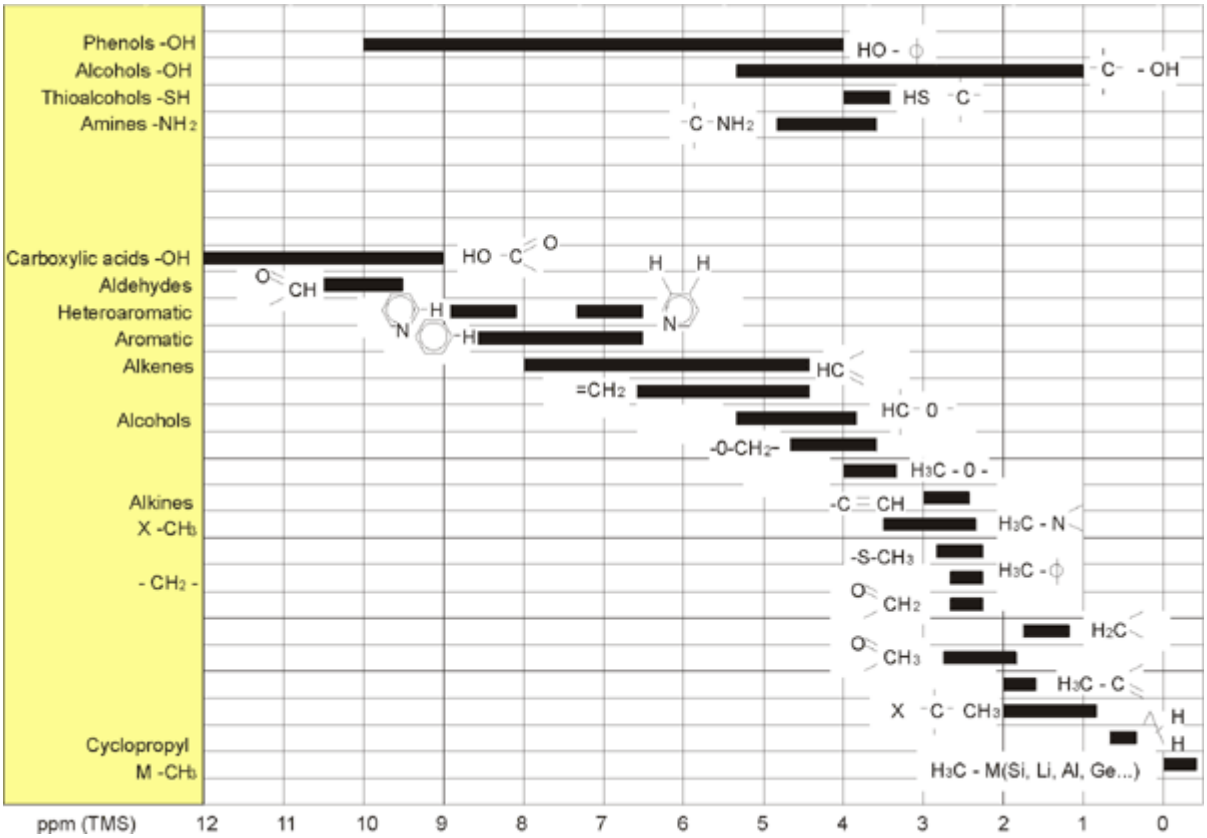
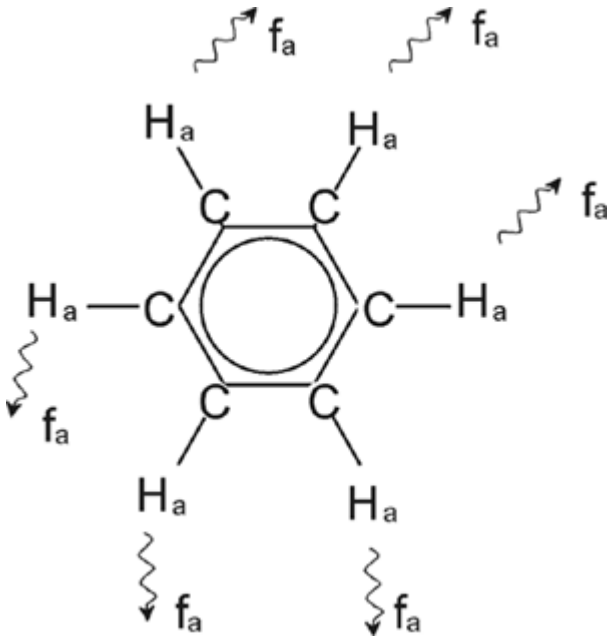


Figure 3.6 is an illustration of typical proton chemical shifts in organic compounds.

Proton Spectrum of Benzene 3.5

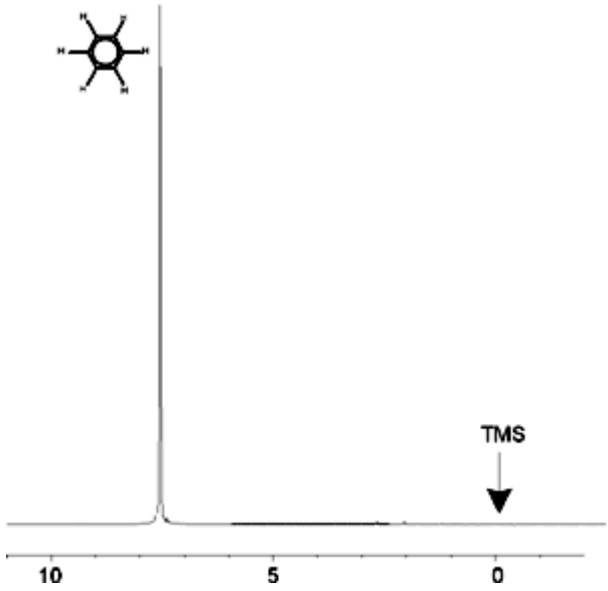
The structure of the benzene ring is illustrated in the following figure:

Figure 3.7. Benzene Ring



All six protons (labeled H_a) may be deemed identical. They are each single-bonded to one carbon atom. Each carbon atom in turn forms two aromatic bonds with its nearest carbon neighbors. Hence each of the six protons is in an identical chemical environment and is said to be “chemically equivalent”, and in this case even "magnetically equivalent“. They will all resonate at precisely the same frequency f_1 without coupling to each other. Therefore, for pure benzene we can expect a single signal. The figure below is a spectrum of benzene in acetone - d_6 and shows that the signal is located at 7.5 ppm.

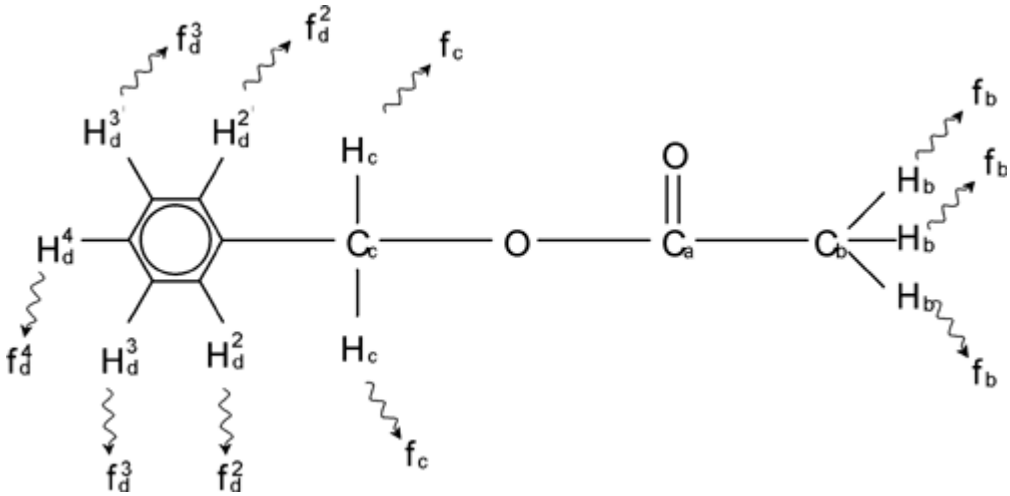
Figure 3.8. Spectrum of Benzene



Proton Spectrum of Benzylacetate *3.6*

Benzylacetate (C_6H_5 - CH_2 - O - CO - CH_3) is a more complicated organic molecule whose structure is illustrated in the following figure:

Figure 3.9. Benzylacetate



We can now distinguish between three different groups of protons that have been labeled accordingly. For example, the three protons labeled H_b clearly find themselves in a different atomic environment to that of the two protons labeled H_c.

The three H_b protons are bonded to the carbon C_b which is single-bonded to another carbon atom C_a. The two H_c protons are bonded to the carbon C_c which itself is single-bonded to the benzene ring and an oxygen atom. The third group of protons consists of the five protons H_d of the benzene ring itself. ***Figure 3.10.*** is a

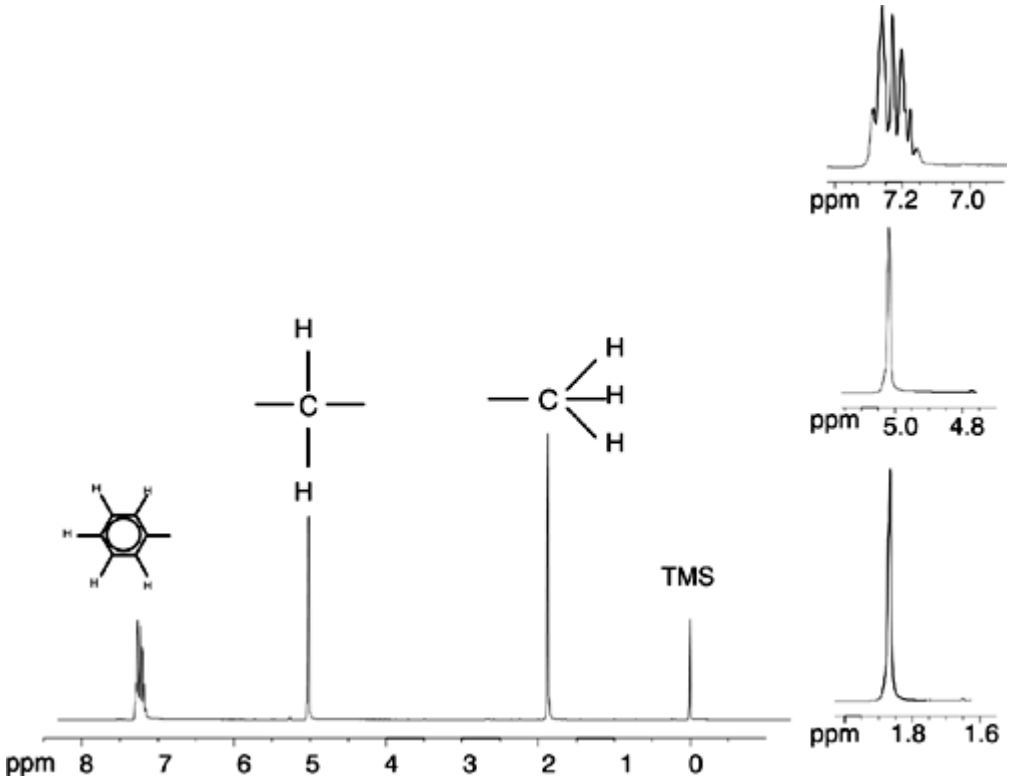
proton spectrum of benzylacetate in acetone - d_6. In this spectrum we expect three signals corresponding to the three groups of protons.

Note that the position of signals from the benzene ring protons has been shifted slightly from 7.5 ppm (***Figure 3.8.***) to approximately 7.2 ppm (***Figure 3.10.***).

The benzene ring protons are no longer magnetically equivalent, are to some extent not even chemically equivalent, and have been labeled accordingly. It is clear from ***Figure 3.10.*** that the signal arising from the H_d protons is a multiplet, but such details will be omitted until the next section.The three proton peaks shown in this figure have clearly different intensities.

Quantitative analysis of the spectrum is relatively simple, since all the signals are emitted by the same ^1H isotope i.e. natural abundance and inherent sensitivity to the NMR technique is the same for each peak. Therefore the area under the benzene, CH_2 and CH_3 peaks should be in the ratio of 5:2:3 respectively corresponding to the contributing number of protons.

Figure 3.10. Proton Spectrum of Benzylacetate



Proton Spectrum of Ethylbenzene with Spin/Spin Coupling *3.7*

The description of proton NMR spectra thus far has been greatly simplified by the fact that all signals, with the exception of those from the benzene ring in benzylacetate, have been singlets. The structure of the organic compound ethylbenzene and the corresponding proton spectrum are illustrated in ***Figure 3.11.***and ***Figure 3.12.*** respectively. As before, the protons have been labelled as three distinct groups corresponding to three basic atomic environments.

The most obvious difference between the signals in this spectrum and those of benzylacetate is the splitting into **multiplets**. The signal emitted by the CH_3 protons is a **triplet** and the signal from the CH_2 protons is a **quartet**. Note also that the signal positions do not coincide. The CH_3 protons in benzylacetate emit a signal at 1.85 ppm, while the corresponding CH_3 protons in ethylbenzene emit the triplet signal at 1.25 ppm. This is hardly surprising, because the two CH_3 groups are in different chemical environments.

The cause of the multiplet splitting is due to an effect known as spin-spin coupling. A full account of this effect is beyond the scope of this manual and the reader should refer to a standard NMR text for details. For our purpose a brief outline of the spin-spin coupling should suffice.

Figure 3.11. Ethylbenzene

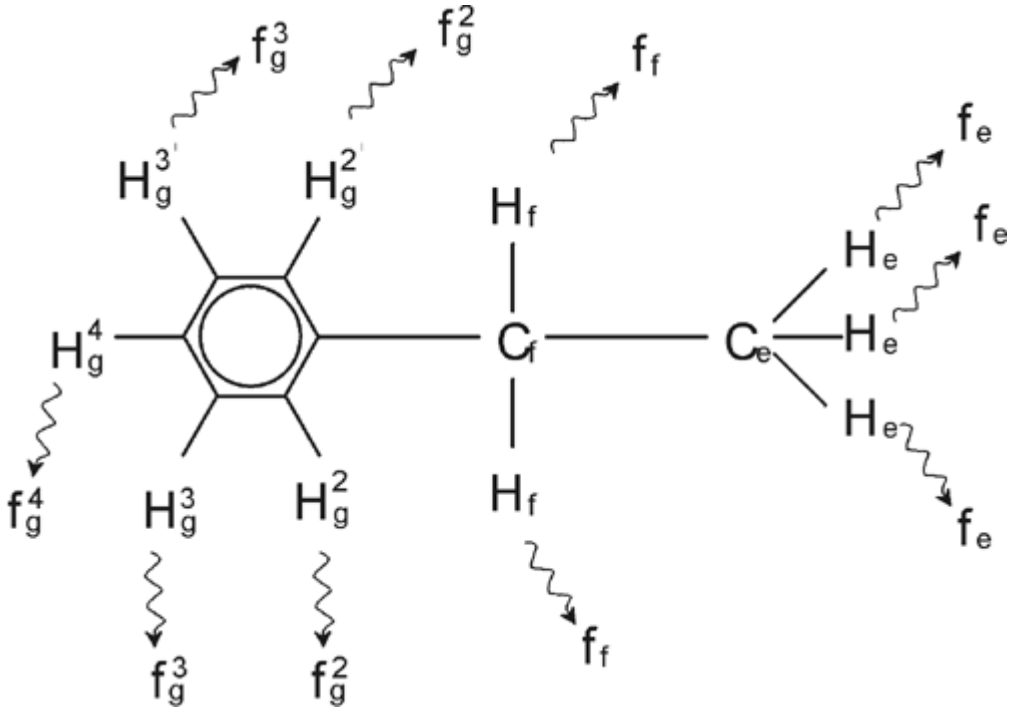
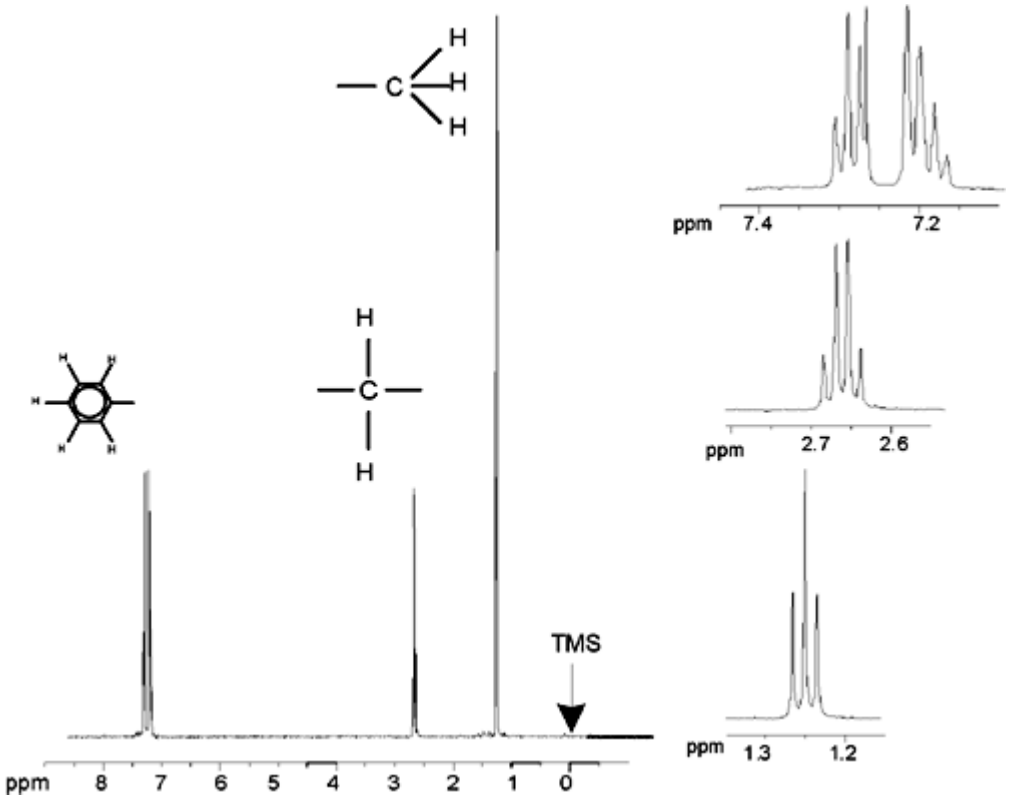


Figure 3.12. Ethylbenzene Spectrum



The splitting of NMR signals in ***Figure 3.11.*** results from a magnetic interaction between neighboring protons. The two H_f protons are magnetically equivalent and do not interact with each other. Similarly, the three H_e protons are magnetically equivalent and do not affect each other. However, the two H_f protons and the three H_e protons are in different local environments and are “coupled” to each other via their bonding electrons. The net result of this coupling is that the two groups of protons interact with each other and cause the splitting of the NMR signals.

The two H_f protons can combine to exist in three possible magnetic states. (This is a result of spin orientation and hence the term spin-spin coupling). As a result of coupling, the NMR signals emitted by the H_e protons resonate at three possible frequencies and a triplet is observed.

Similarly, the effect of the H_e protons is to split the H_f signals. The three H_e protons can combine to exist in four possible magnetic states. Consequently the H_f protons resonate at four possible frequencies, so the signal is split into a quartet.

The signals from the benzene protons have also been split as a result of magnetic non-equivalence and resulting spin-spin coupling. The question arises why the CH_2 and CH_3 protons of ethylbenzene interact with each other whereas the two comparable groups of protons in benzylacetate do not. The answer lies in the number of bonds separating the two groups. In ethylbenzene (***Figure 3.11.***) the two proton groups are attached to adjacent carbon atoms and may be expected to interact sufficiently with each other. In benzylacetate however (***Figure 3.9.***), the two carbon atoms C_c and C_b are connected across two extra bonds between oxygen and another carbon atom. As a result the proton groups are too far away from each other to display significant spin-spin coupling.

Decoupling *3.8*

The effect of spin-spin coupling can be removed by a technique called "decoupling“. The effect of decoupling is to mask the presence of a particular proton group, e.g. the H_e protons in ***Figure 3.11.***. A spectrum is acquired as if the H_e protons were absent! This is achieved by transmitting a decoupling pulse sequence at the H_e resonance frequency f_e and thereby permanently changing the spin orientation of these protons. For the spectrum illustrated in ***Figure 3.12.*** the decoupling frequency would be 1.25 ppm above the TMS peak.

Decoupling pulses tend to be longer and of lower power than excitation pulses. ***Figure 3.13.*** is a representation of a decoupling experiment, while ***Figure 3.14.*** shows the decoupled spectrum. The CH_2 quartet has now become a **singlet**. Spectroscopists speak of the quartet collapsing to a singlet. Furthermore, the area under the singlet should be equal to that of the original quartet. (Compare the relative heights of the CH_2 and benzene ring peaks in the two figures) The signal from the CH_3 group at 1.25 ppm is missing from the decoupled spectrum, because the decoupling pulses effectively removes the effects of the presence of the CH_3 protons.

Figure 3.13. Decoupling Experiment

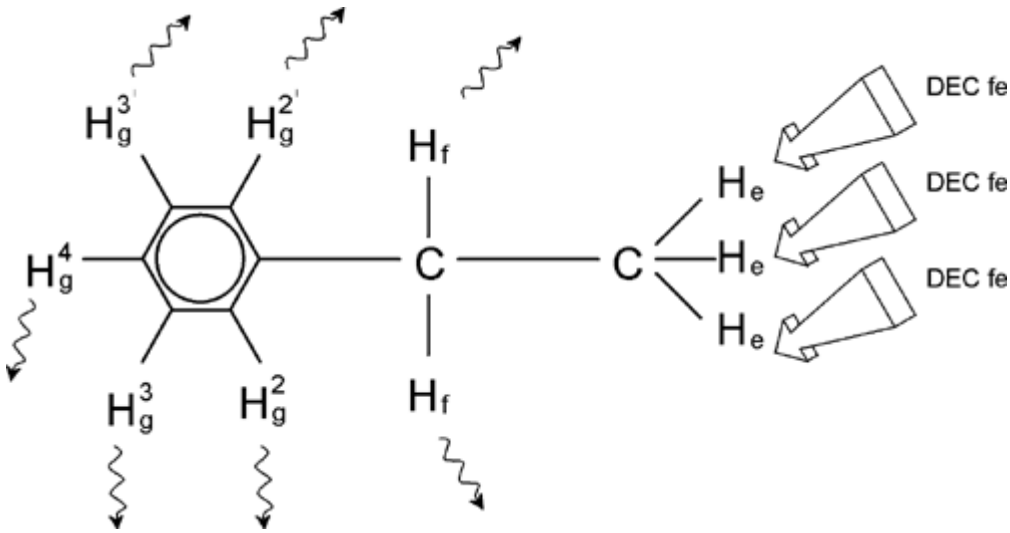
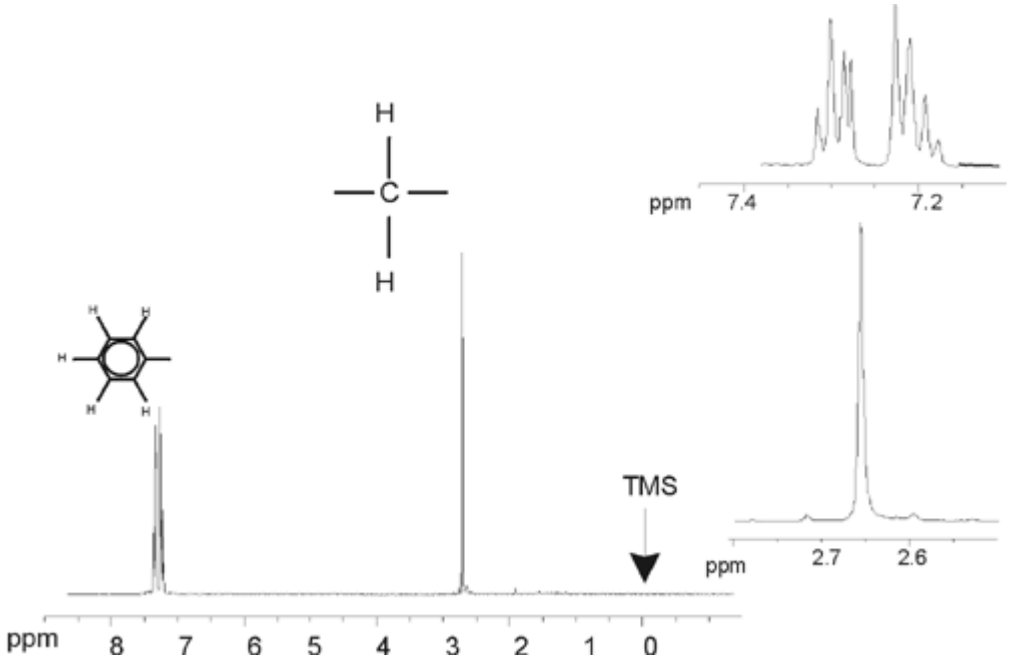


Figure 3.14. Ethylbenzene Spectrum with Homodecoupling



The experiment described above is an example of homodecoupling in that the same isotope, namely ^{1}H, is observed and decoupled. Heterodecoupling is where the isotope to be observed is different to that which is decoupled. In the chapter ***"13C spectrum with decoupling"*** of this manual you will perform a heterodecoupling experiment where you observe ^{13}C and decouple ^{1}H.

AVANCE with SGU spectrometers have the ability to perform very complicated types of experiments depending upon the number of channels installed. A four channel spectrometer can be used to observe one nucleus and decouple three other nuclei. With up to eight independent channels the possible range of experiments is quite amazing. The user should be aware that currently the limiting factor is not in the generation of RF excitation and decoupling pulses but in transmitting these pulses to the sample via the probes and to some extent the preamplifiers.

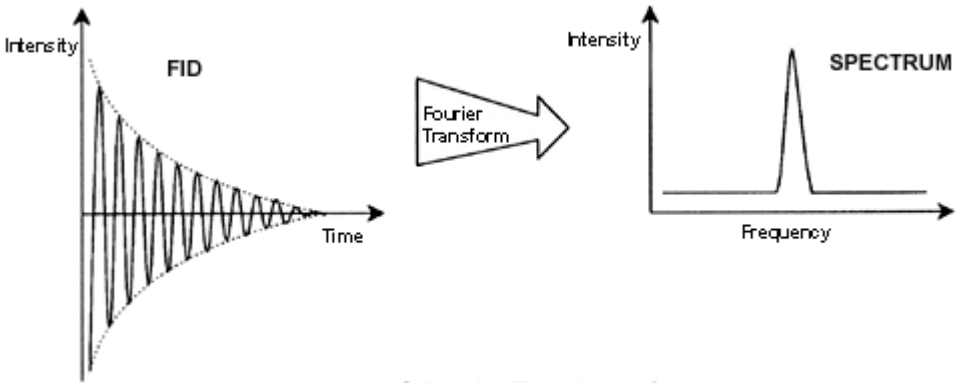
The signal routing of the experiment to be carried out is set up using the "edasp“ menu which will be discussed in the section ***"Spectrometer Parameters edasp" on page 68***.

FID and Spectrum *3.9*

The signals emitted by the excited atoms in the sample are received and Fourier transformed by the spectrometer. The process of receiving the NMR signals is called an acquisition. Data is said to be acquired. A distinction should be made between the two terms; “FID” (time domain) and it’s associated “spectrum” (frequency domain).

When an acquisition is carried out, “raw” data is acquired and the received signal is called an FID (Free Induction Decay). A typical FID is illustrated in the figure below.

Figure 3.15. Fourier Transformation



Before an FID can be usefully analyzed it must first be transformed to the frequency domain. This is achieved by applying a Fourier transformation using the command 'ft'. A **Fourier transformation** is a mathematical operation which converts the FID into a frequency spectrum. An **FID** is a signal whose intensity varies with time whereas a spectrum displays how intensity varies with frequency. Fourier transformation is the most important one of several processing operations that are normally carried out on raw data.

System Description 4

Introduction 4.1

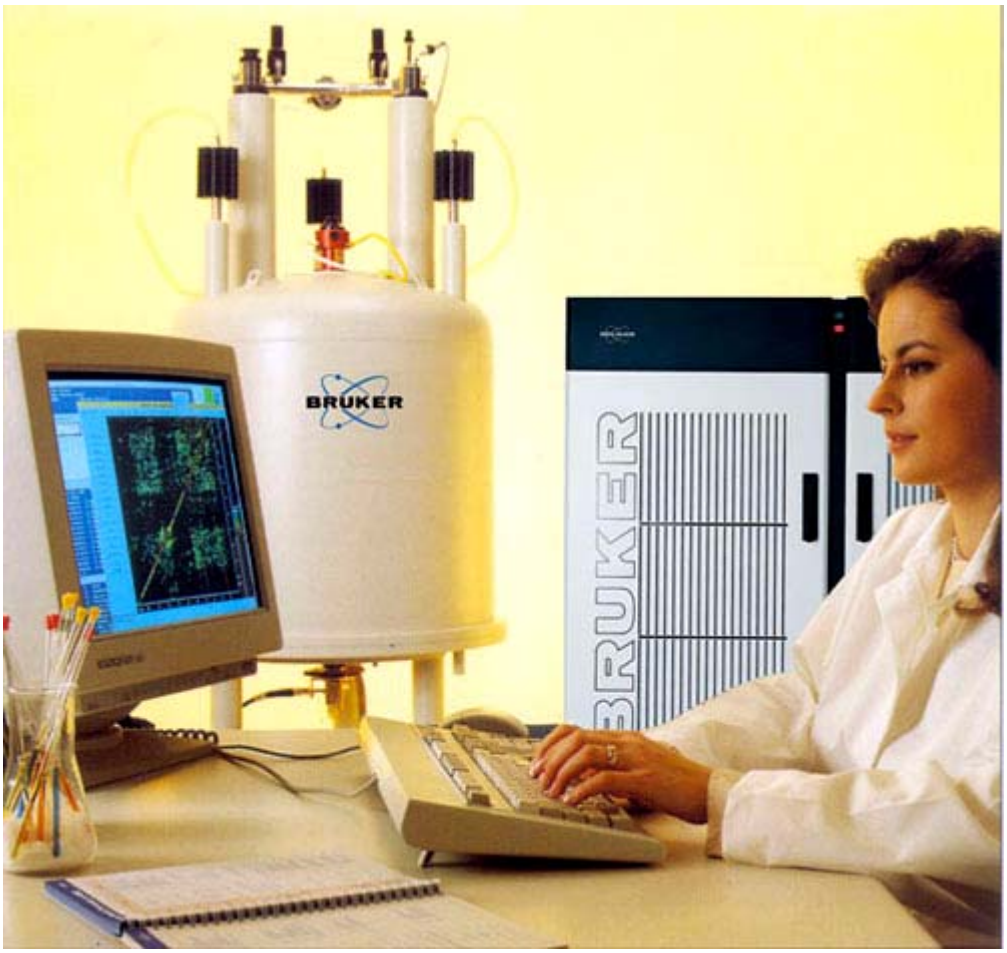
The spectrometer consists of the following subunits:

Operator console including the host computer, monitor, keyboard, and BSMS keyboard[1].

Console containing the electronic hardware.

Magnet system including the shim system, preamplifiers (HPPR) and probe.

Figure 4.1. Operator console, console and magnet



1. The BSMS keyboard is optional with some systems.

Operator Console and Connections 4.2

All aspects of the spectrometer operation are controlled from the operator console. The design and implementation of experiments as well as the analysis of data are controlled by commands entered by the operator at the console. The sub components of the operator console are as follows:

Host computer: This can be either a PC or a Silicon Graphics O2. The host computer runs the XWIN-NMR program and handles all the data analysis and storage. All operations relevant to data acquisition are controlled by a second computing system called the Acquisition Control System (AQS) housed within the console itself.

Ethernet connection from host computer to AQS: This is used to transfer data and instructions between the host computer and the Acquisition Control System.

RS232 connection from host computer to AQS: This serial interface is an alternative (to the ethernet) link to the host computer. Note that this RS232 link is not actually necessary for operation, since all the information is transferred through the much faster ethernet link. However, it is particularly useful for troubleshooting should the ethernet link become inoperative.

BSMS keyboard: This (optional) device enables the user to control the lock and shim system as well as basic operations such as inserting, spinning and removing the NMR sample.

BSMS keyboard connection to BSMS CPU: The keyboard is attached to the BSMS CPU housed in the console. If the BSMS is switched off, the connection must be reestablished. As this is proceeding, the messages "booting“ and "connecting“ will appear on the keyboard display. The absence of any error messages will confirm that the connection has been successfully reestablished.

Console 4.3

This unit may be single bay or double bay depending upon the system and houses most of the electronic hardware associated with a modern digital spectrometer. The principal units are the **AQS** (Acquisition Control System), the **BSMS** (Bruker Smart Magnet System), the **VTU** (Variable Temperature Unit) as well as various amplifiers.

AQS: The various units within the AQS generate the radio frequency pulses used to excite the sample and receive, amplify and digitize the NMR signals emitted by the sample. Once the data is received and digitized, the information is transferred to the host computer for further processing and storage. The main link with the host computer is via the **CCU** (Communications Control Unit). It is important to emphasize that the Acquisition Control System has total control over spectrometer operation within the duration of an experiment. This is to ensure uninterrupted operation and so guarantee the integrity of the acquisition.The rack contains a set of digital and analogue slot-in type boards that prepare the signal to be transmitted and also receive, amplify and digitize the NMR signal. A detailed description of these boards is beyond the scope of this manual.

BSMS: This system is controlled via the BSMS keyboard or software (‚bsmsdisp‘ command) and is used to operate the lock and shim system as well as controlling the sample lift and spin.

VTU: Depending on the model the VTU may be a separate standalone unit or may be incorporated into the BSMS. Its function is to vary the sample temperature in a controlled manner or maintain it at a constant value.

Amplifiers (also known as transmitters). Signals of relatively large amplitude are often required to excite the NMR sample and hence the need for amplifiers. Amplifiers can be internal (incorporated into the AQS rack) or external (separate standalone units). Cables running directly from the amplifier outputs to the HPPR carry the RF signal to the sample. Although there is a wide range of available amplifiers (including solid amplifiers) the two main categories are:

> **Selective amplifiers** (also known as ^{1}H or proton amplifiers) are specifically designed to amplify the higher frequencies associated with ^{1}H and ^{19}F.
>
> **Broadband amplifiers** (also known as X amplifiers) are designed to amplify a wide range of frequencies (excluding ^{1}H and ^{19}F).

The RF power enters the amplifier via the SMA type connector labeled "RF in“. This is a weak signal with maximum amplitude of $1V_{pp}$. However the quality of this signal is critical as it has the frequency, timing, shape and phase of the final signal. More experienced users may wish to monitor this signal on an oscilloscope. The function of the amplifier is to take the input signal and apply a fixed amount of amplification. Any amplitude control via the parameters pl0-pl31 is implemented prior to the amplifier in the SGU. The RF signal leaving the amplifier can be of the order of several hundred volts and is not recommended to be viewed on the scope without attenuation.

Link Between the Host Computer and the AQS *4.4*

Although during a typical XWIN-NMR session this link is permanently in operation and effectively invisible to the user, the link is broken anytime either the host computer or the AQS is switched off and must be re-established when they are switched back on.

From the user point of view it is perhaps easiest to consider the AQS as a second computer. This computer is usually referred to as "spect“ since it is most closely associated with the spectrometer hardware.

After rebooting the AQS it is possible to **log onto spect**. This is physically logging onto the CCU of the AQS and is usually done with the RS232 connection to tty00. The screen interface is called up by clicking on the CCU Hyperterminal icon of the host computer desktop (for PC only!) or via a Unix shell for systems fitted with an O2. Note that you can also use the command "telnet spect“ to access via the ethernet link. Once you can successfully log onto "spect“ you can be sure that the AQS has booted and that the link between the host computer and the AQS is functioning correctly.

In the chapter ***"Basic Troubleshooting" on page 111*** procedures to switch on and off the various elements of the spectrometer will be described.

Magnet, Shim System, HPPR and Probe *4.5*

The **magnet** generates the magnetic field required to induce NMR transitions. To maintain a superconducting system the magnet core is cooled to very low temper-

atures using liquid nitrogen and helium (for more details see the section ***"The Magnet and Magnet Dewar" on page 34***).

The room temperature **shim system,** mounted into the lower end of the magnet, is a set of current carrying coils (known as shims) used to maximize field homogeneity by offsetting any existing inhomogeneities. The currents in these room temperature shims (so called as they are not cooled by being immersed in a bath of liquid helium) are controlled by the BSMS and may be adjusted from the BSMS keyboard to optimize the NMR signal. This has a major effect on signal resolution and sensitivity. This action of adjusting the currents in the room temperature shims is referred to as **shimming** the magnet.

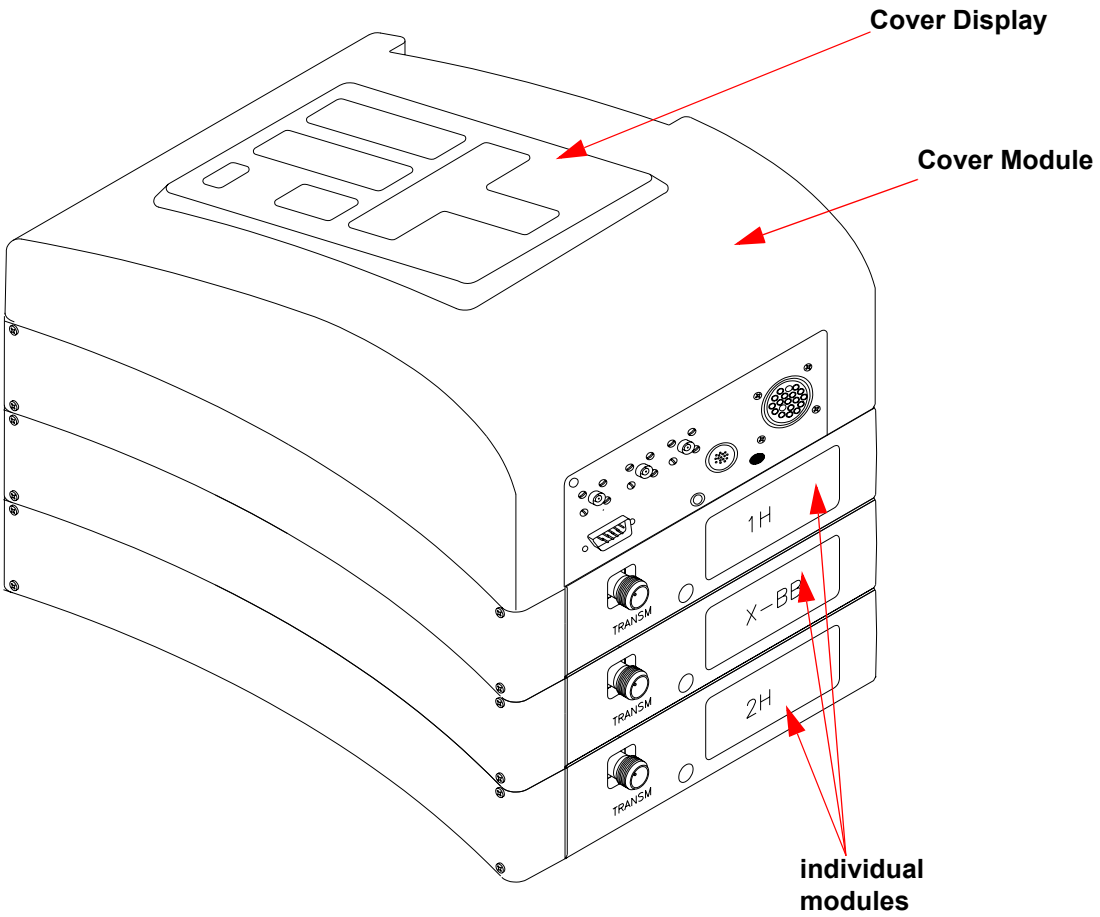
Figure 4.2. Photo of Magnet, Shim System, Probe and HPPR

Although the **HPPR** (High Performance Preamplifier) carries the transmitted signal ***to*** the sample it is primarily concerned with magnifying the relatively weak signals emitted ***from*** the sample. It is located at the base of the magnet to amplify the NMR signal at the earliest possible opportunity and thus minimize losses along the cable. Once the signal has been amplified with the HPPR any subsequent losses in cabling are less critical. The HPPR also transmits and receives the deuterium (or fluorine) lock signals and is used in the wobble routine.

Up to 5 (HPPR) or 8 (HPPR/2) individual modules (excluding the cover module which is always present) may be configured and will automatically be displayed in the "edasp“ window. A very common configuration consisting of three individual modules, a proton, X-BB and ^{2}H along with a cover module, is shown in ***Figure***

4.3. The ^{2}H module is used to transmit and receive the lock signal. How to connect the HPPR modules to the probe and amplifiers is shown in ***Figure 4.6.*** Much of the HPPR technology is concerned with what is known as the **Transmit / Receive switching**. Effectively the signal going to the probe is transmitted without any action. Once this signal has elapsed the signal path is altered so as to amplify the received signal by typically 30dB. The trick is to make this switch as fast as possible and suppress leakage so that the transmitted signal does not swamp the received signal.

Figure 4.3. HPPR with three Modules and Cover Module



The probe is inserted into the shim system at the base of the magnet and essentially consists of various coils used to transmit the excitation pulses to the sample as well as receive the emitted signal. The probe also transmits and receives the lock signal.

The Magnet and Magnet Dewar *4.6*

A range of magnets are available with different strengths. The **strength of the magnet** is graded according to the frequency of the NMR signals emitted by hydrogen atoms. The stronger the magnet the higher this hydrogen frequency. For example, with a 500 MHz magnet (11.7 T), this means that when a chemical sample is placed in the magnet for analysis, the ^{1}H atoms in the sample will emit signals with a frequency very close to 500 MHz. Bruker magnets are available in the range of 200-900 MHz.

Superconducting magnets are **electromagnets**, and as such make use of the fact that an electric current produces a magnetic field. The **magnet core** consists of a large coil of current carrying wire in the shape of a solenoid. At the center of the coil a very intense static magnetic field exists. The sample to be analyzed is placed inside this magnetic field.

At very low temperatures certain materials show the remarkable property of superconductivity. A superconducting wire carries electricity without the need for any driving energy (i.e. battery or mains supply). Once a current is started in a superconducting loop it will continue forever. Bruker magnets consist of such a superconducting loop. When the magnet is first installed, a current is started in the main coil. This is referred to as "charging" the magnet. Once charged, the magnet should continue to operate for many years as the current, once started, will never stop! The only maintenance required by the magnet is to ensure that the superconducting coil is kept at a sufficiently low temperature.

A temperature of 4K (-269°C) is the boiling point of helium. Under normal conditions, at temperatures above 4K, it is a gas. At temperatures below 4K it is a liquid. Thus, if the coil is kept immersed in liquid helium we can be sure that the temperature is at 4K or below. This is sufficiently cold to ensure that the magnet coil remains superconducting. However, should enough of the liquid helium evaporate, such that a large part of the superconducting coil is no longer covered with liquid He, then the temperature of the superconducting coil will increase and at some stage become non-superconducting. The resistance in the coil would then result in a sudden breakdown of the magnetic field accompanied with the generation of heat which would very quickly lead to the evaporation of large quantities of liquid helium and nitrogen. The magnet room may suddenly become filled with evaporated gas and the magnet is said to have "**quenched**". This may or may not permanently damage the magnet. In any case, recharging the magnet is time and money consuming and should be avoided under any circumstance.

Much of the magnet's technology is involved with the difficult task of ensuring that as little as possible of the liquid helium that covers the magnet coil evaporates. This is achieved by minimizing the flow of heat from the NMR lab (room temperature) to the magnet core (4K). The magnet consists of several sections. The outer casing of the magnet is evacuated and inner surfaces are silvered. (This is the same principle as the Thermos flask except we are trying to prevent heat getting in instead of out.) Next comes a bath of nitrogen which reduces the temperature to 77.35K (-195.8°C). Finally a tank of helium within which the superconducting coil is immersed, and which is thermally isolated against the nitrogen bath by a second evacuated section (see the figure ***"Superconducting Magnet" on page 36***).

Room Temperature Bore *4.6.1*

The helium and nitrogen tanks are wrapped around a central column known as the magnet bore. A metal plug normally closes off the top of the bore. Magnets are available with either standard bore or wide bore. Samples to be analyzed are introduced into the magnet via the top of the bore. Probes, which hold the sample and carry signals to and from the sample, are inserted from the bottom.

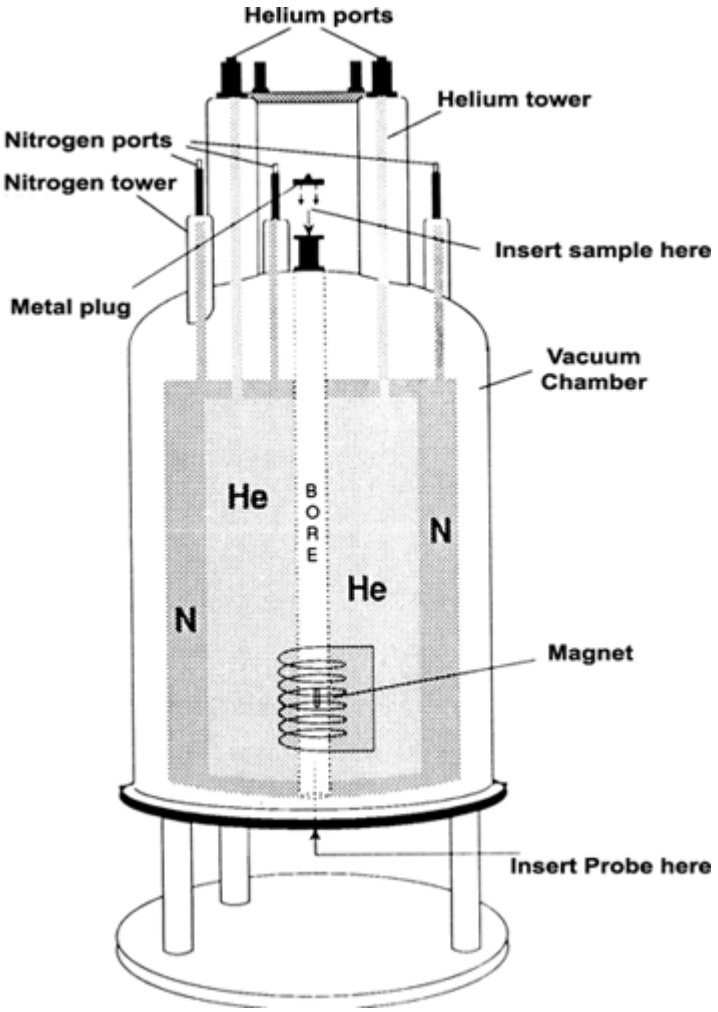
Helium Tank *4.6.2*

In a standard magnet the helium tank is suspended from two necks which extend high above the magnet. Access to the helium tank can be made via two ports. One of these ports permits refilling of the liquid helium and is also the entry for a **helium level sensor**. The other port is used only when the magnet is being charged or discharged. The helium necks may support several valves which control the release of the small quantities of helium that will inevitably evaporate. The system manager should check that these valves are working properly, i.e. are not blocked by ice.

⇨ ***It is important that the helium ports are not left open for extended periods of time (e.g. >30 seconds).***

The helium level can be checked either manually or electronically. Manual checking involves inserting a long dipstick into the helium tank via one of the access ports (experienced users only!). Electronic monitoring of the helium level is carried out using the BSMS keyboard and will be discussed in the section ***"BSMS Functions" on page 49***.

Figure 4.4. Superconducting Magnet



Nitrogen Tank *4.6.3*

The three shorter necks extending above the magnet allow access to the nitrogen tank. Any one of the three ports can be used to check the nitrogen level with a suitable dipstick (experienced users only!!!) and top-up the level if required. Again the system manager should regularly check that any valves inserted onto the nitrogen ports are not blocked with ice.

As already mentioned, the magnets are designed to minimize liquid evaporation. During normal operation a small amount of nitrogen will evaporate per day. This is perfectly normal and indeed the absence of **nitrogen boil-off** indicates that the nitrogen ports have become blocked. How often the levels of nitrogen and helium need to be topped-up depends on the magnet size and design. It is good practice to check and top-up the nitrogen level every week. It is also good practice to check the helium level at the same time and keep a record of it. Although the **helium boil-off** is much less than that of nitrogen, which results in much longer intervals before one has to refill the helium (3-6 months), the regular check will ensure that the helium boil-off has not changed.

Introduction to the Lock System 4.7

This section has been included to give the user a basic understanding of the principles of the lock system. Practical aspects such as how to actually lock the sample will be dealt with in section ***"Locking the Sample" on page 58***.

The aim of the lock system is to ensure that the strength of the magnetic field surrounding the sample does not change during an experiment or that the field is not modulated by external disturbances. NMR analysis involves measuring the precise frequency of signals that are emitted by the sample. The frequencies of these signals are directly proportional to the magnetic field strength, i.e. if the field strength varies, so does the emitted frequency. Therefore, the user must be confident that the magnetic field strength is always maintained at precisely the same strength, which is referred to as "**locking**" the sample. The **lock system** is essentially a separate spectrometer designed to observe deuterium. It should be mentioned that the signals emitted by deuterium are normally far removed from frequencies of interest. However, if the deuterium frequency is unsuitable, then a **Fluorine (^{19}F) lock** may be used. Since it is by far the most popular, only the deuterium lock will be dealt with here, but the reader should note that the principle of the deuterium and the fluorine lock are identical.

In AVANCE with SGU systems the BSMS provides the hardware required to implement the lock and a separate deuterium module in the HPPR transmits and receives the lock signals. Some deuterium must of course be introduced into samples that are under analysis. This can most easily be done by dissolving the sample in a deuterated solvent. A deuterated solvent is one in which a large percentage of the hydrogen atoms have been replaced by deuterium. Commonly used deuterated solvents are acetone-d_6, benzene-d_6 and chloroform-d, although many other solvents are available. The sample which will be used to illustrate some basic NMR techniques in this manual is cholesterylacetate in chloroform-d.

The frequency of the signals emitted by deuterium for a particular size of magnet is precisely known. Therefore, if the magnetic field strength is correct, any deuterium nucleus in the sample should emit this exact frequency. If the magnet strength varies so will the deuterium frequency. The lock system uses a receiver (housed in the BSMS rack) to monitor this deuterium frequency and makes adjustments to the magnetic field strength accordingly.

The receiver in the lock system is designed so that when the field strength is correct (i.e. the correct deuterium frequency is detected), no adjustments are made to the field. However, should the field strength vary (drift), a current is changed in a special coil (the H_0 coil) located within the shim system of the magnet, which has the effect of returning the field strength to the correct value. The deuterium frequency is measured several thousand times per second. Hence, as long as the system is locked, the user can be confident that the field is maintained at a constant strength during acquisition.

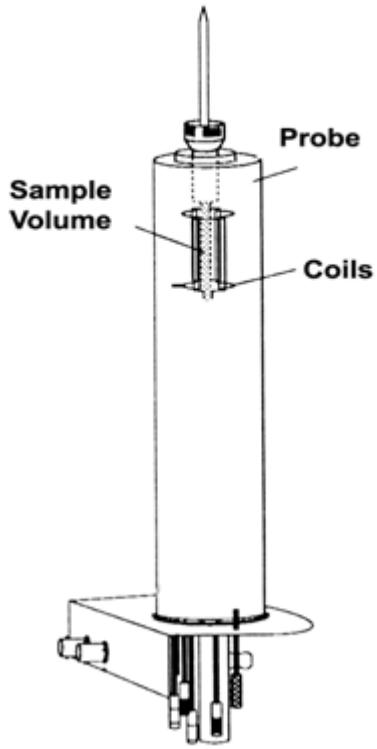
Probes 4.8

The probe is designed to hold the sample, transmit radio frequency signals which excite the sample and receive the emitted response. The transmission and reception is achieved by using specially designed RF coils.

The probe is inserted into the bottom of the magnet and sits inside the room temperature shims. Coaxial cables carry the excitation signals from the console amplifiers to the probe and the NMR signal back from the sample to the receiver. The

cables are routed through a set of preamplifiers (the HPPR) which are situated next to the base of the magnet. The **preamplifiers** are needed to boost the NMR signals which are typically very weak.

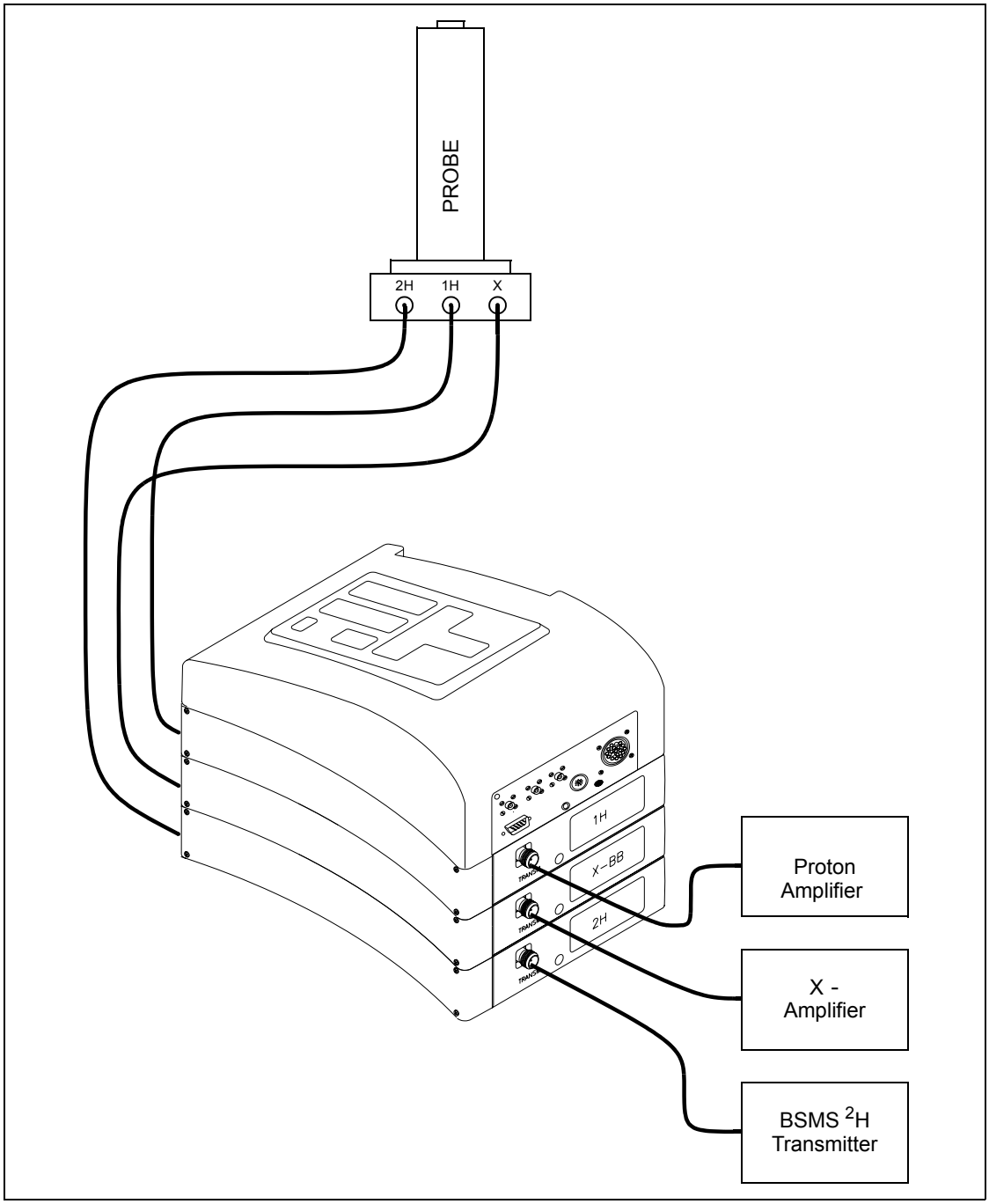
Figure 4.5. Sample in Probe



Probes come in different sizes and types. The size of the probe is given in terms of the sample tube sizes it can hold, with 5mm and 10mm sample tube diameters the most popular. Different types of probes are used depending upon the type of experiment. **Selective probes** are specially designed to observe specific nuclei, e.g. ^{13}C, while **multinuclear** (X-BB or broadband) **probes** may be used to analyze a wide range of nuclei. The number and design of the internal coils are what physically distinguishes one type of probe from another. In addition, the outer diameter and length of the probe is built to the specifications of the various magnets (wide bore or standard bore; different length from the bottom to the center of the magnetic field for magnets with different field strength).

Signals enter and leave the coils of the probe via connectors which are clearly labelled and located at the base of the probe.The same cable is used to carry the signal to and from the probe. Each probe has a inner coil (the **observe coil**). This coil is located closest to the sample volume to maximize sensitivity. The color coding of the inner coil BNC follows a simple rule. It always has the same color as the rectangular strip located directly above the BNC connectors. The following figure shows the labeling of a multinuclear probe. In this case the broadband coil is the inner coil.

Figure 4.6. Typical HPPR Cabling



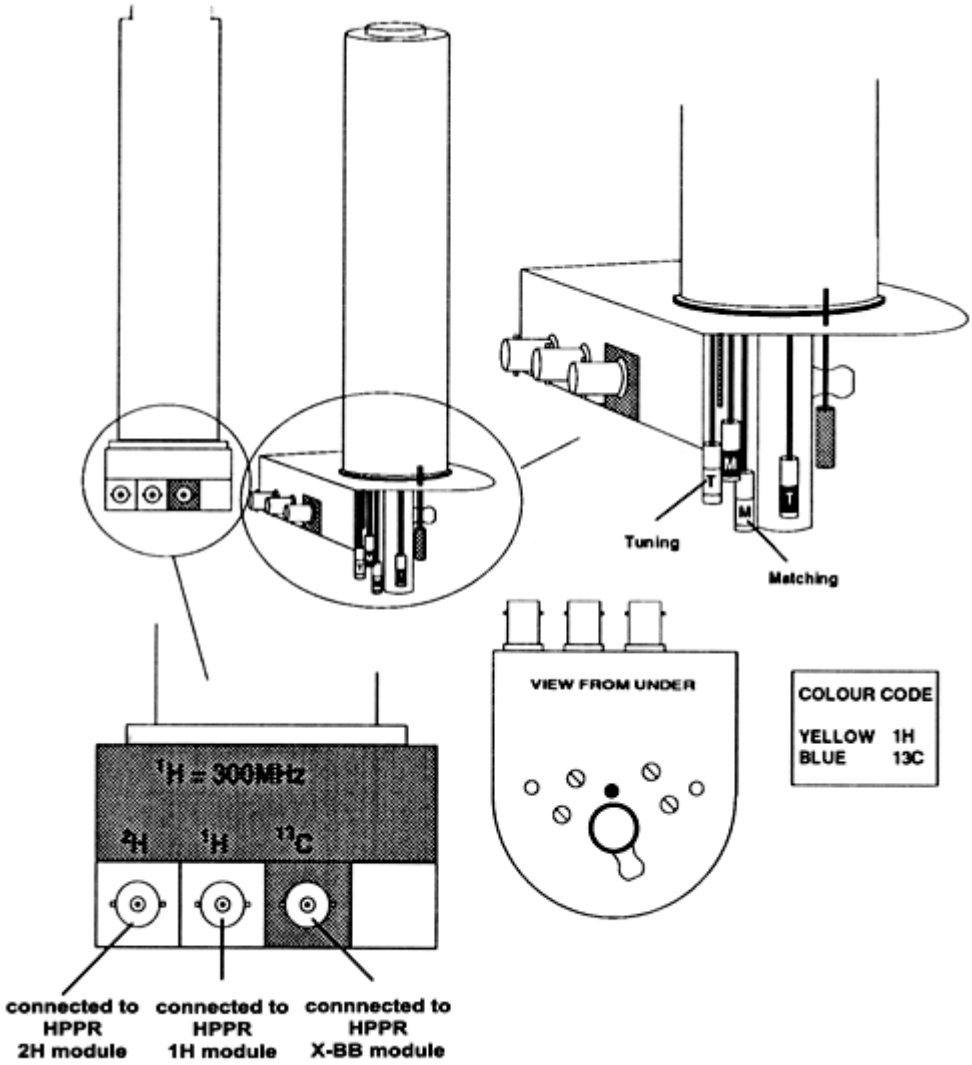
Dual ^{13}C/^{1}H probe *4.8.1*

One type of probe, which will be described here as an example of a typical probe is the **Dual ^{13}C/^{1}H probe**. As its name suggests this probe is designed for users who are specifically interested in analyzing samples for carbon and hydrogen.

The left BNC is marked ^{2}H (see ***Figure 4.7.***) and is used to carry the lock signal. The other two connections are for the ^{1}H and ^{13}C signals and are labeled accord-

ingly. The cables leaving the ^{1}H, ^{13}C and ^{2}H BNCs are connected to the HPPR situated at the base of the magnet

Figure 4.7. Dual $^{13}C/^{1}H$ probe



In modern NMR, probes are designed to enable the temperature of the NMR sample to be controlled. A heater may be inserted and used in conjunction with an air/ **N_2 transfer line** to control the sample temperature. A **thermocouple** serves as a thermometer to monitor the sample temperature. All these devices are attached to the base of the probe and are easily accessible.The Variable Temperature Unit (**VTU**) constantly monitors the thermocouple reading and makes adjustments in the heater power to maintain the required temperature.

Finally, several adjustable screws labeled either **T** (tuning) or **M** (matching) can be seen on the base of the probe. These are used to make fine adjustments to the probe in order to optimize the performance. When a compound is analyzed it is excited with signals of a definite frequency (the resonance frequency). Different nuclei will be excited by different frequencies and tuning involves adjusting circuitry within the probe so that it is at its most sensitive for the frequency of interest. The probe is also matched to ensure that as little as possible of the excitation sig-

nals and the FID are reflected (i.e. wasted). Both tuning and matching are interactive in that they can not be adjusted independently of each other.

For routine work in organic solvents, it is probably sufficient to check the setting on a weekly or indeed monthly basis, once the probe has been initially tuned and matched. However, for higher level research in which the spectrometer performance must be optimized the probe should be tuned and matched every time the sample in the magnet is changed. Each coil in the probe is tuned and matched separately using the "wobble“ routine which is described in ***"Tuning and Matching the Probe" on page 53***. For the dual probe in ***Figure 4.7.*** there are separate color-coded tune and match screws for ^{1}H and ^{13}C.

Changing a probe will require reconnecting the probe to the HPPR, however the cabling connecting the HPPR to the console electronics is usually not altered.

Changing a Probe 4.8.2

If the probe is to be changed, the procedure below should be followed. Probes are fragile and expensive and you should check with the system manager before attempting to change the probe. The magnet, and in particular eddy currents, greatly affect the mechanical motion of the probe. As you remove the probe from the magnet expect it to accelerate suddenly as it reaches the lower end of the magnet bore. Also be prepared for some resistance when you place the probe into the magnet.

Procedure for Changing a Probe:

1. Ensure that no acquisition is running by entering stop or halt on the command line of the main XWIN-NMR window.
2. Turn off any **heating or cooling** from the system. Use the ’edte’ command to switch off the heater and set the air flow to zero. Allow the probe to settle to room temperature. Do not shut off the system itself!
3. If an air/N_2 flow is attached to the base of the probe, remove it.
4. After making sure that the magnet bore is not plugged, remove any sample that may be present in the magnet by activating the LIFT key on the BSMS keyboard.
5. Turn off the LIFT.
6. Disconnect all BNC cables from the probe base.
7. Disconnect the thermocouple, and heater if fitted, and any other gradient or PICS connection.
8. Loosen the two milled screws (gold colored) which secure the probe in the magnet.
9. Lower the probe straight down and out of the magnet.
10. Insert the new probe and secure using the two screws (gold colored).
11. Reconnect the coaxial cables, thermocouple, heater or cooling lines and any other connections as appropriate.
12. Turn the heater back on.

The NMR Sample 5

Introduction 5.1

When a **solid** is investigated using the NMR technique, signals tend to be broad and the fine structure, which is of most interest to scientists, cannot be resolved. Consequently, solid samples are usually dissolved in a suitable solvent prior to acquisition. The same goes for **liquid samples**. In organic solvents, small amount of a reference compound may be added. However, to achieve best results the sample should be as pure as possible. Signals from **impurities** will at best make the spectrum unnecessarily complicated and at worst mask genuine signals. Particular care should be taken to ensure that the sample is free of **magnetic impurities** as these can distort the magnetic field and hence degrade the spectrometer resolution. Solid impurities can be most easily removed by filtering. For samples in organic solvents, dissolved water can be removed as far as possible by thoroughly drying the sample prior to dissolution.

Solvent Selection 5.2

Once the sample has been sufficiently purified and dried, the next step is to choose a suitable solvent. Since deuterium is by far the most popular lock nucleus the sample is usually dissolved in a deuterated solvent (a deuterated solvent is one in which a large proportion, typically more than 99%, of the hydrogen atoms have been replaced by deuterium). Commonly used **deuterated solvents** are acetone-d_6, benzene-d_6 and chloroform-d though many other solvents are available. Factors to be considered when choosing a solvent are:

1. Solubility:

Clearly the more soluble the sample is in the solvent the better. This maximizes the amount of sample within the sensitive volume which increases the sensitivity of the experiment. High solubility is particularly important if only small quantities of the sample are available.

2. Interference of solvent signals with the sample spectrum:

The solvent itself will inevitably produce NMR signals which will obscure regions of the spectrum. These ‘residual solvent peaks’ should not overlap with signals from the sample.

3. Temperature dependence:

For experiments above or below room temperature the solvent‘s melting and boiling points are also important factors. Furthermore the solubility of the sample is likely to vary with temperature.

4. Viscosity:

The lower the solvent viscosity, the better the resolution of the experiment.

5. Cost:

Clearly for routine NMR where many samples need to be measured, the solvent's cost is an important consideration. As a rule of thumb, the price increases with the number of deuterated atoms.

6. Water content:

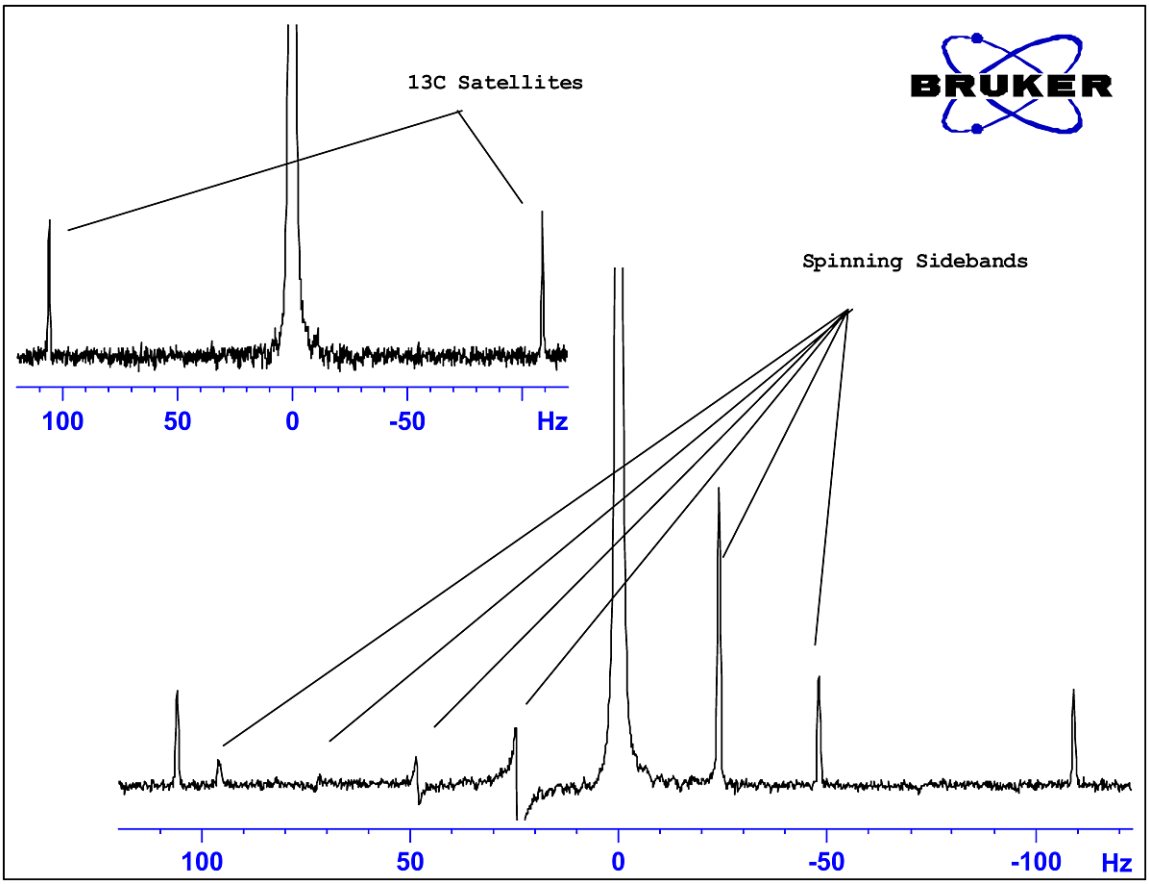
Almost all NMR solvents contain water traces. Also many are hygroscopic (they absorb water from the atmosphere) and hence the longer they are stored the more water they contain. The presence of a water (HDO) peak will only serve to degrade the quality of NMR spectra. The water level in the solvent can be greatly reduced by filtration through a drying agent or by storing the solvent over molecular sieves.

The choice of solvent for a particular sample will be the best compromise between the various advantages and disadvantages of each. For precise details of specific solvents the reader should refer to a standard NMR text.

Sample Tube *5.3*

When the sample is being analyzed it may be rotated, depending on the type of probe or experiment. **Spinning** the sample has the effect of cancelling out field inhomogeneities in the X and Y direction and consequently improves the spectral resolution. A disadvantage of spinning is that may lead to the presence of **spinning sidebands**. These are spurious signals (i.e. peaks) that result from the modulation of the magnetic field at the spinning frequency. The peaks always appear on either side of any large genuine peak at a separation equal to the spinning rate. The intensity of these sidebands will be proportional to the intensity of the genuine peak. Thus if the spin rate is 20 revolutions/second (= 20 Hz), you would look for spinning side bands at frequencies 20 Hz above and below the resonance frequencies of genuine signals.

Figure 5.1. Spectrum Showing Spinning Sidebands



While the presence of spinning sidebands may be inevitable, their size often depends on the sample tube quality. Ideally, the sample tube should have perfect cylindrical symmetry. Unusually large sidebands might suggest that the **tube symmetry** is inadequate and might warrant using tubes with higher specifications (and of course greater cost).

Sample tubes must always be kept clean and free from dust and scratches. Do not scrub the tubes with test tube brushes. Be aware that new NMR tubes cannot be assumed to be clean. The tubes may be cleaned by washing in Acetone or distilled water. Liquid detergent may be used as long as it is rinsed out within a few minutes to prevent etching of the tube. The tubes may also be cleaned ultrasonically in an appropriate solution. If all the above measures fail, the tubes should be soaked in AQUA REGIA for up to two days and then rinsed thoroughly before being dried. NMR tubes can be oven dried but should not be heated above 100°C as they can become distorted and subsequently fail to spin as required. Drying is best achieved by passing filtered nitrogen through the tube.

Sample Handling *5.4*

It is good practice to filter NMR solutions directly into the sample tube to keep the solution free from dust and other contamination. Possible suitable filters are cotton wool, glass fiber[1] or celite. Note the sample tube should always be held by the top!

1. Note some problems may occur when using glass fiber, especially when you want to measure T_1.

A typical procedure might be as follows:

1. For a solid sample using a 5mm tube dissolve up to 20 mg of the sample in about 0.6 cm^3 of the chosen solvent (for 10mm tubes dissolve 80 mg in 2.5 cm^3). Typically for a liquid sample, and when observing protons, dissolve 20% sample in 80% deuterated solvent.
2. Add a small amount (~0.1%) of reference compound Tetramethylsilane (TMS). Make sure the TMS signal is smaller than the most intense sample or solvent signal (otherwise the signal-to-noise ratio is wasted because of low receiver gain).
3. Filter the solution into the sample tube through a Pasteur pipette containing an oven dried glass wool plug.
4. Filter 0.2cm^3 of solvent through the filter into the tube. The resulting solution should have a depth of three to four centimeters.
5. Close the tube with a cap, seal the top with parafilm to reduce evaporation and label the tube near the top. Be careful to ensure that the cap, parafilm and label are concentric or otherwise they will adversely affect sample spinning.

Insertion of the sample into the spinner will be discussed in the section ***"Inserting the Sample into the Spinner" on page 51***.

Basic procedures 6

Introduction 6.1

This chapter will outline basic operations which are used every time spectra are acquired. They include BSMS keyboard operation, inserting and spinning the sample, tuning and matching the probe and finally shimming. The reader may skip this chapter if they are already familiar with these operations.

Note: Some systems do not have a BSMS keyboard, in which case BSMS operation is software controlled ("bsmsdisp“ command).

BSMS Keyboard 6.2

A comprehensive description of all the BSMS keyboard functions and its handling/ operation is available in the BSMS Users Manual which is on the BASH CD. For the purposes of this manual it will be assumed that the user has a basic knowledge of how to operate the BSMS keyboard. For your assistance a list of the most important BSMS keyboard functions is given in section ***"BSMS Functions" on page 49***

In principle, the BSMS keyboard is used to:

1. Operate the lock system
2. Control the sample spinning and lift
3. Adjust the currents in the room temperature shim system (called "**shimming**“)
4. Monitor the helium level inside the magnet

Several versions of the keyboard are available but their distinction is not relevant here (see figure ***"BSMS Keyboard (BOSS Version)" on page 48***. for one example).

Warning: Before you start, be aware of two keys that should be handled with caution.

1. The HELIUM MEASURE key is best left untouched for reasons outlined later (see section "Helium Level Functions" on page 51).

2. Ensure that the magnet bore is not plugged before activating the LIFT key. If you are placing a sample onto the top of the magnet bore ensure that the airflow is on by first activating the LIFT button.

Before you start working with the BSMS keyboard, you might want to store the values of some important parameters called "**shim values**“ set by the BSMS unit (see the section ***"Storing a Set of Shim Values (Write Shim Command)" on page 48***). In this way any adjustments that you make may be easily undone at a later stage. The shim values are adjusted using the algebraically labeled keys (e.g. X, Y, XZ, X^3 etc.) in the bottom three rows of the BSMS keyboard.

Figure 6.1. BSMS Keyboard (BOSS Version)

Storing a Set of Shim Values (Write Shim Command) *6.2.1*

To store the current shim values, write a shim file using the command "wsh“ (write shim file).

1. Enter ’wsh’ on the XWIN-NMR command line. A new display will appear which is a list of shim files that have previously been stored.
2. Click on TYPE NEW NAME
3. Enter any name you wish, e.g. monday.

The current shim values are now stored in a file called "monday“.

You may now manipulate the shim values with the BSMS keyboard in the knowledge that the old values may be easily restored by simply reading back the values stored in the shim file entitled "monday“.

Reading a Stored Shim File (Read Shim Command) 6.2.2

1. Enter 'rsh' (read shim file) on the XWIN-NMR command line. A list of shim file titles will appear.
2. Click on "monday" (or your file name). (You may have to scroll through the list of shim file titles).

The BSMS keyboard will flash for a few seconds. A message "rsh:finished" at the bottom of the screen will appear when the operation is complete. The old shim values are now restored.

The user should become familiar with the concept of reading and writing different types of files, as this is very important when it comes to parameter sets. If you wish to store a set of parameters you write them to disk. The values of the various parameters are stored in a file. The file has a unique name (of the user's choice) so that different parameter sets may be distinguished from each other. If at some later stage you wish to use these parameters, read in the appropriate file from disk.

BSMS Functions 6.2.3

Only the most relevant functions are described below. For a more comprehensive list see Chapter 2 of the BSMS Users Manual delivered on the BASH CD.

Broadly speaking the functions can be grouped into four categories: sample control functions, lock functions, shim functions and helium level functions

Sample Control Functions 6.2.4

LIFT ON-OFF: LIFT ON will raise the sample out of the magnet bore. LIFT OFF will drop the sample gently back into the magnet bore where it will be positioned at the top of the probe.

⇨ ***Never operate the LIFT ON function if the top of the magnet bore is plugged.***

Always ensure that if you are placing a sample onto the top of the magnet bore, that you make sure that the airflow is on by first activating the LIFT button.

SPIN ON-OFF: Starts/stops the spinning of the sample. Sample spinning (if activated) will automatically start 20 seconds after LIFT OFF has been activated.

SPIN RATE: Allows the rate of spinning to be adjusted. Measured in Hz.

SPIN MEASURE: Shows the actual spin rate on the right side of the display and the set spin rate on the left side. The two values should be equal.

Manual Lock Functions 6.2.5

The function of the lock system was discussed in the section ***"Introduction to the Lock System" on page 37***.The spectrometer locks onto the NMR signals emitted by the deuterium in the sample. This can be achieved by sweeping (increasing

and decreasing) the field within the magnet. At a certain field strength the deuterium atoms are excited at their resonance frequency and a signal is observed on the lock display.

There are two steps in the operation. First, the field surrounding the sample is "swept“ and then the sample is "locked“. The keys on the BSMS keyboard controlling the sweeping action as well as the lock will now be described.

SWEEP RATE: The speed at which the field is swept may be varied.

SWEEP AMPLITUDE: The magnetic field strength is continuously swept within a certain range. The size of this range may be controlled with this key.

SWEEP: Switches the magnetic field sweep on and off. This key will automatically be deactivated whenever the system is locked.

LOCK ON-OFF: When activated the system attempts to lock the sample. The green LED on the key will flash for a few seconds and then light permanently if and as soon as the system is locked. Under normal circumstances data should only be acquired when the spectrometer is locked.

LOCK POWER: Regulates the power of the signal used to excite the deuterium in the sample.

LOCK GAIN: Regulates the gain (sensitivity) of the lock signal receiver.

When searching for a lock signal two methods may be used. Either the lock frequency is held constant and the magnetic field strength is varied. This is called the "**field mode**“. Alternatively the magnetic field strength is held constant and the frequency is varied. This is called the "**shift mode**“ (frequency locking).

FIELD: During the lock routine the magnetic field can be swept above and below a central field strength by varying the current in the H_0 coil of the shim system. This central field strength can be varied using the **FIELD** key. The function is operational when the BSMS is set to "FIELD“ mode and this is the default mode of operation. However, if the BSMS is set to "SHIFT“ mode, the value of the FIELD will be displayed on the keyboard but may not be altered.

LOCK SHIFT: During the lock routine the lock frequency can be swept above and below a certain central value by alterations in the electronic circuitry in the BSMS. This central value can be varied using the **SHIFT** key. The function is only operational when the BSMS is set to "SHIFT“ mode. If not in this mode the value of the SHIFT in ppm will be displayed on the keyboard but may not be altered.

AUTOLOCK: When pressed, the spectrometer attempts to lock automatically. This function is useful for automation. The value of the "FIELD“ will be adjusted automatically during the AUTOLOCK routine to center the lock signal. Note that pressing the "Autolock“ key will not take account of solvent dependent FIELD shifts listed in the "edlock“ table.

Manual Shim Functions *6.2.6*

Shim values

The shim settings are may be adjusted using the algebraic keys located below the central alphanumeric display. These keys are used to control the value of shim currents inside the magnet as discussed in ***"Magnet, Shim System, HPPR and Probe" on page 31***. The settings for the shim currents are very important for good spectrometer performance and their adjustment will be described in the sec-

tion ***"Shimming" on page 61***. A specific set of shim current values is stored whenever a shim file is written to disk.

AUTO SHIM: Can be used to adjust the shim settings automatically to obtain the highest lock level. The shims selected for the autoshim routine are those shims whose shim amplitude is non zero. The AUTO SHIM function is particularly useful for automation. Note that autoshim is only effective when minor adjustments to the shim values are required.

Helium Level Functions *6.2.7*

HELIUM LEVEL: The spectrometer automatically measures the level of helium in the magnet once every 24 hours and records the result in the predefined log. By default the log is located in the following directories:

Windows NT/2000 - C:\Bruker\Xwin-nmr.

LINUX - /opt/xwinnmr

SGI - /u

The level is displayed as a percentage (i.e. 100 = full). When the key is pressed the last two recorded levels are displayed for five seconds (the most recent level on the right). No new measurement is made.

When the He level sinks below a certain level (magnet type dependent) a warning message will appear and liquid helium refill should not be delayed any further.

HELIUM MEASURE: Starts a measurement which takes about 12 seconds. The user should note that the process of measuring the helium level will in itself lead to some boil-off. Therefore the HELIUM MEASURE function should not be used too frequently.

Inserting the Sample into the Spinner *6.3*

The glass tube containing the sample to be analyzed is held in a plastic spinner. A **sample depth gauge** is provided to control the depth of the sample tube in the spinner. This is to ensure that the sample is correctly aligned with the coils inside the probe. The depth gauge has a graduated scale which can be used to set the depth of the sample.

1. Using the graduated scale set the top of the white plastic base between 18mm and 20mm below the center line (0mm line) for 5mm probes. For 10 mm probes set the top of the white plastic base to 20mm below the center line.

   These are only recommended sample depths and you should consult with the system manager as to the appropriate depth for a particular magnet and probe. Once you have established the correct sample depth you should always use that depth to minimize the need for reshimming whenever the sample is changed.

2. Holding the sample by the top, place the sample tube in the spinner and the spinner in the depth gauge.

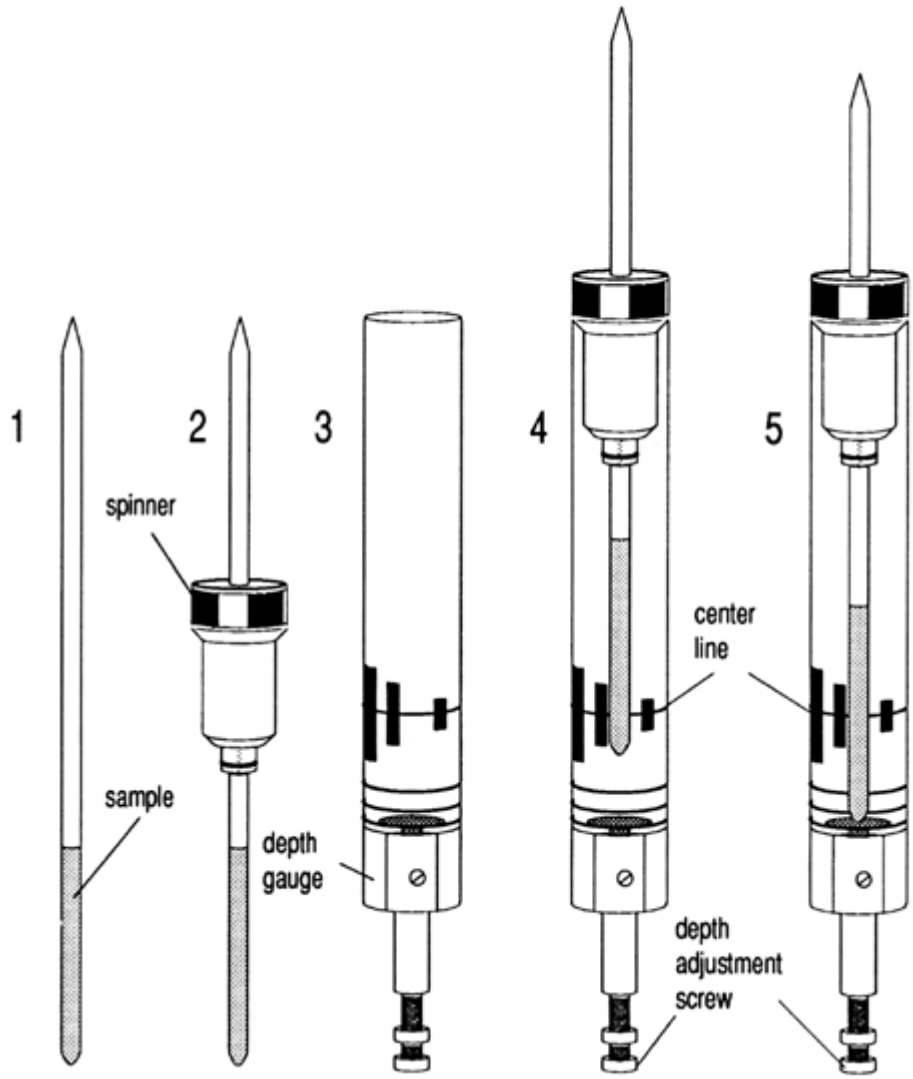
3. Gently push the sample tube down so that the depth of the sample above and below the center line is equal. If there is enough sample volume then push the sample down so that it just touches the white plastic base.

4. **Remove the depth gauge before inserting the sample and spinner into the magnet.**

Inserting the Sample Plus Spinner into the Magnet *6.4*

The raising and lowering of the sample is controlled by a stream of pressurized air. Be careful never to lift the sample with the plug still inserted at the top of the magnet bore. Newer BOSS-2 shim systems are designed not to enable the LIFT if the magnet bore is still plugged. Furthermore, make sure that the air flow is present (it is quite audible) before placing a sample onto the top of the bore.

Figure 6.2. Inserting the Sample into the Spinner



To insert the sample plus spinner into the magnet use the following procedure:

1. If present, remove the plug from the top of the magnet bore

2. Activate the LIFT button on the BSMS keyboard. A flow of air will be heard and if a sample is already in the magnet it will be raised and suspended on a cushion of air at the top of the magnet bore.
3. Remove the old sample and place the new sample onto the air cushion
4. Press the LIFT key again. The sample will gently drop into the magnet and will settle at a precise position within the probe.
5. Replace the plug on top of the magnet bore (this is important as it helps prevent metallic particles from getting into the magnet).

Spinning the Sample *6.5*

A second function of pressurized air is to enable the sample to rotate. The spinning of the sample serves to "even-out" some of the inhomogeneities that may exist in the magnetic field at the center of the magnet. Note that samples to be investigated using inverse probes are normally not rotated.

Set the spin rate using the following procedure:

1. Activate the SPIN RATE key on the BSMS keyboard.
2. Use the black knob to set the rate.
3. To begin spinning activate the SPIN key.

Suggested spin rates are:

20 Hz for a 5 mm probe

12 Hz for a 10 mm probe

Tuning and Matching the Probe *6.6*

The sensitivity of any probe will vary with the frequency of the signal transmitted to it and there exists a frequency at which the probe is most sensitive. Furthermore this frequency may be adjusted over a certain range using tuning capacitors built into the probe circuitry. **Tuning** involves adjusting the probe circuitry so that the frequency at which it is most sensitive is the relevant transmission frequency (SFO1, SFO2 etc.) Each coil in the probe will be tuned (and matched) separately.

If the probe has been changed or the transmission frequency altered significantly, it may be necessary to retune the probe. For routine work in organic solvents with selective probes, the value of the transmitted frequencies are unlikely to vary greatly. Hence, once the probe has been initially tuned, slight variations in frequency will not warrant retuning. Typically the transmitted frequency would need to be altered by at least 100kHz to warrant retuning. However for broadband probes the frequencies transmitted will vary greatly from nucleus to nucleus and so the probe will need to be tuned each time the selected nucleus is altered.

Whenever a probe is tuned it should also be matched. **Matching** involves ensuring that the maximum amount of the power arriving at the probe base is transmitted up to the coil which lies towards the top of the probe. This ensures that the minimum amount of the power arriving at the probe base is reflected back towards the amplifiers (and consequently wasted). All Bruker BioSpin amplifiers are designed to have an output impedance of 50 Ohms. Therefore optimal matching occurs when the probe impedance along with all cables is also 50 Ohms.

Tuning and matching can be carried out by observing either:

The **wobble curve** in the XWIN-NMR ACQU window

or

The green and red LED's on the HPPR cover display.

Both methods will now be described, but unless it is not possible to observe the monitor screen, the first method is more convenient and thus will be described first.

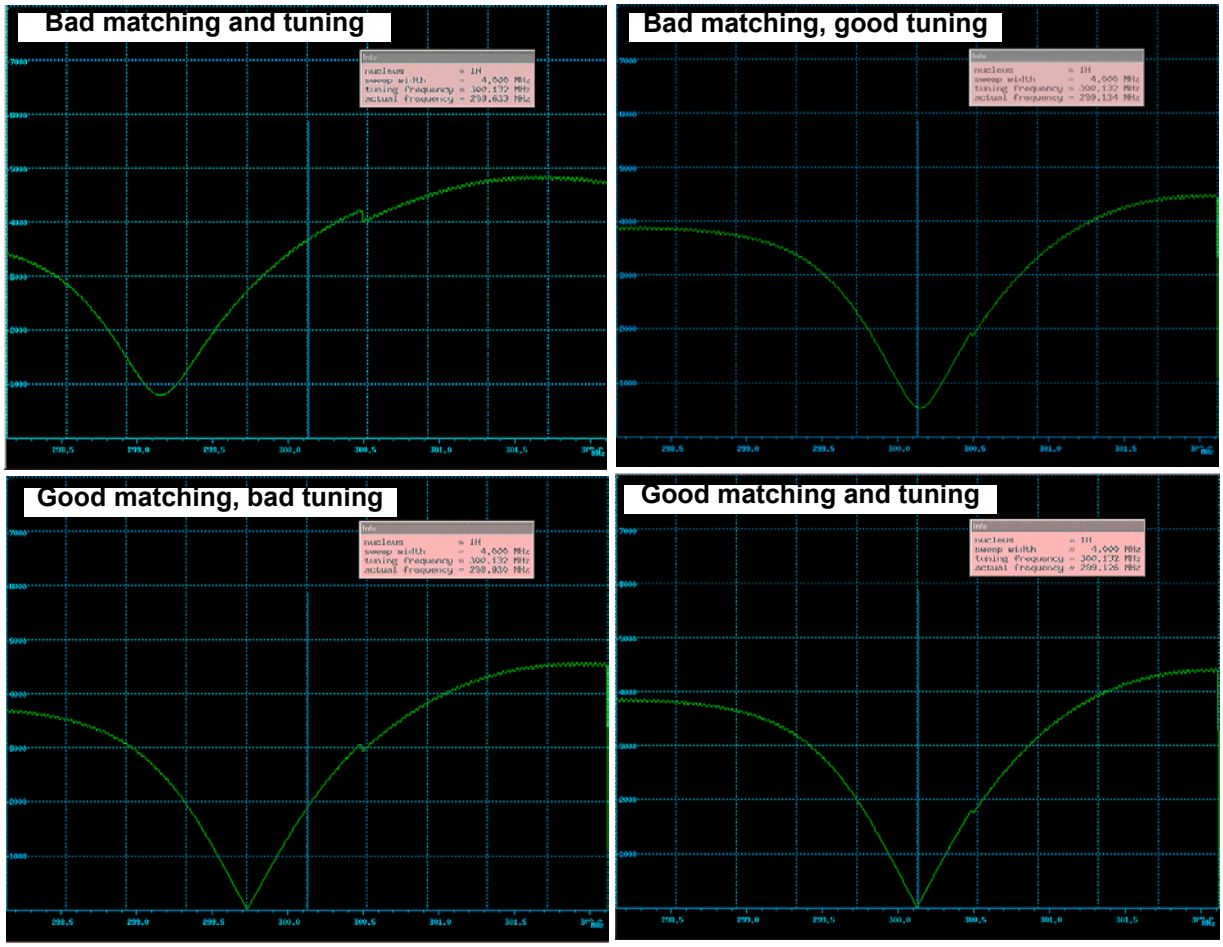
Warning: The tuning and matching screws which are described below have a limited amount of play, therefore turning them beyond this will damage the probe.

Tuning and Matching Using the Wobble Curve *6.6.1*

The wobble routine works by transmitting a weak signal to the probe and comparing the probe and cable impedance with a 50 Ohm reference within the HPPR. The transmitted frequency is centered on SFO1, SFO2 etc. but "swept“ over a range determined by the size of the parameter WBSW (see below). The resultant curve is the familiar response curve of a resonance circuit and is simply a measure of the amplitude of the reflected signal (vertical axis) against frequency (horizontal axis).

Matching simply involves adjusting the probe so that the minimum of the wobble curve is at the base of the display (i.e. touching the horizontal frequency axis). This represents minimum reflection of the transmitted signal. **Tuning** involves ensuring that this occurs at the frequency of transmission which will be at the center of the screen's horizontal scale. The adjustment is made using two screws labelled T and M at the base of the probe with the special tool provided. The screws are color coded with the front label of the probe for identification, with a separate pair for each coil in the probe. Matching will generally alter the vertical position of the wobble curve minimum while tuning will alter the horizontal position. It will be seen however that the tune and match adjustments interact with each other and must be adjusted in tandem. When the wobble curve minimum is centered and at the base of the screen the probe has been optimally tuned and matched.

Figure 6.3. Examples of Wobble Curves with Different Tuning and Matching



The wobble routine is initiated using the "wobb“ command.When carrying out the wobble routine two parameters may be modified:

WBST (number of wobble steps):

1K is the default value though the value is not critical.

WBSW wobble sweep width in MHz:

4 MHz is default. The closer you are to optimal tuning the smaller this can be made. If you suspect however that the probe is incorrectly tuned or mismatched, or if you have difficulty observing the typical resonance curve, then start with a large value of WBSW (e.g. 32, 64 MHz) and reduce this to 8 or 4 MHz as the optimal tuning and matching is reached.

Procedure:

1. Ensure that SFO1 is set close to the final transmission frequency. Click on ACQU.
2. Enter ’wobb’.
3. Observe the wobble curve and adjust the tune and match screws accordingly.
4. When completed enter or click on "**stop**“.

If required you may set the parameter WBST to 1K and WBSW to 4MHz if not set already. This can be done via the "eda“ menu or typed in directly on the command line.

Tuning and Matching Using the HPPR LEDs *6.6.2*

This method is only required if the location of the monitor is such that it may not be viewed while adjusting the tune and match screws. The only difference with this method is that the adjustments are made based on green and red LEDs on the top panel of the HPPR cover display module.

The horizontal row of LED's represent tuning and the vertical row matching.The adjustments are made in the direction that reduces the number of LED's that are illuminated in both directions. Just as with the wobble routine, the tune and match screws must be adjusted in tandem. A well tuned and matched probe should have only one or two LED's lighting in either direction (note that one LED represents optimal tuning and matching.)

Note: To maximize the refresh rate when using this method, the main XWIN-NMR window and not the acquisition window should be open.

Procedure:

1. If required click on "**return**" to enter the main XWIN-NMR window.
2. Set the parameter WBST to 1K and WBSW to 4MHz.
3. Enter 'wobb'.
4. Observe the HPPR LEDs and adjust the tune and match screws accordingly.
5. When completed enter or click on "**stop**".

Figure 6.4. HPPR/2 Cover Display

Tuning and Matching More than One Nucleus *6.6.3*

The software allows the user to change the nucleus (i.e frequency) that is selected without stopping the wobble routine. This is particularly useful for decoupling experiments where the probe must be tuned and matched for more than one nucleus and frequency. Obviously a different probe circuit is adjusted for each nucleus. The nuclei and corresponding frequencies should first be set up using the "edasp“ command as this is where the wobble routine gets its information.

To alter nucleus and frequency during the wobb routine either

Click on "**wobb-SW**“ in the ACQU window or enter ’wbchan’ on the main XWIN-NMR command line

or, if using the HPPR cover display,

Click on CHANNEL SELECT for the HPPR1

Use the cover display submenu for the HPPR2

In each case the nuclei are selected in order of increasing frequency.

Locking the Sample *6.7*

All aspects of the lock may be adjusted manually from the BSMS keyboard, but for relatively new users it is recommended to use a lock routine initiated by entering ‘lock’ in the XWIN-NMR command line. This will automatically adjust certain lock parameters to optimize the lock-in routine.

As explained in the section ***"Introduction to the Lock System" on page 37***, deuterated solvents are used to generate the signal to be detected and monitored by the lock system. The frequency and strength of this signal will depend on the solvent used. The main feature of the XWIN-NMR lock routine is that it sets parameters such as the lock power, gain and frequency to a value appropriate to the solvent. With these default values set close to that which would be expected for that solvent, the BSMS can quickly locate and "lock“ onto the solvent signal by sweeping through a range of frequencies or magnetic field values. The solvent dependent parameters are taken from the "edlock“ table.

Table 6.1. The Default edlock Table

Solvent	LPower	LGain	LTime	LFilt	LPhase	Nucl.	Distance	Ref.	Width	RShift
Acetic	-40.0	-10.0	0.100	100	-1.0	2H	2.0300	0.0000	0.500C	0.0000
Acetone	-40.0	-10.0	0.100	100	-1.0	2H	2.0400	0.0000	0.500C	0.0000
CDCl3	-25.0	-15.0	0.200	100	-1.0	2H	7.2400	0.0000	0.500C	0.0000
CD2Cl2	-30.0	-15.0	0.100	100	-1.0	2H	5.3200	0.0000	0.500C	0.0000
CD3CN	-40.0	-10.0	0.100	100	-1.0	2H	1.9300	0.0000	0.500C	0.0000
C6D6	-26.0	-15.0	0.200	100	-1.0	2H	7.2800	0.0000	0.500C	0.0000
D2O	-20.0	-5.0	0.200	200	-1.0	2H	4.7000	0.0000	0.500C	0.0000
H2O+D2O	-23.0	-15.0	0.200	100	-1.0	2H	4.7000	0.0000	0.500C	0.0000
DEE	-30.0	-15.0	0.200	100	-1.0	2H	1.0700	0.0000	0.500C	0.0000
DME	-35.0	-10.0	0.100	100	-1.0	2H	3.3000	0.0000	0.500C	0.0000
DMF	-25.0	-8.0	0.200	200	-1.0	2H	2.9100	0.0000	0.500C	0.0000
DMSO	-25.0	-8.0	0.200	200	-1.0	2H	2.4900	0.0000	0.500C	0.0000

Note that certain values need to be optimized by the administrator.

The BSMS can use two methods when searching for the solvent signal.

In "**Field**“ mode the magnetic field surrounding the sample (and hence the deuterium resonance frequency) may be adjusted so that regardless of the solvent the lock frequency is constant. The edlock table provides the information regarding the solvent dependent adjustment of the field strength. **This is the default mode of operation**.

In "**Shift**“ mode the lock frequency itself may be adjusted to take account of the variation with solvent. If "Shift“ mode is chosen then it must be selected explicitly from within a BSMS menu.

The user has three methods available to lock the sample:

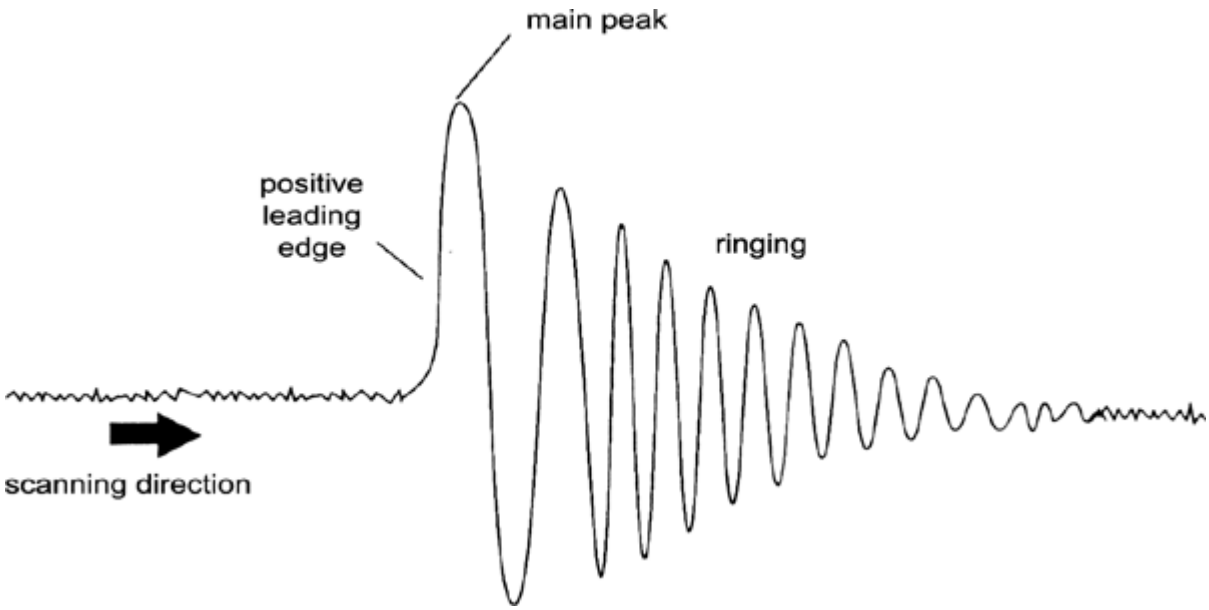
1. Enter ’lock’ in the XWIN-NMR command line as described above and click on the appropriate solvent. This will first alter the value of the magnetic FIELD to a value determined in advance by the system administrator which is equal for all solvents. When the system has been locked, the message ‘lock: finished‘ will be displayed.
2. Press AUTOLOCK on the BSMS keyboard. This does not take into account the solvent, but will alter the value of the magnetic FIELD so that the detected lock signal is centered within the sweep, thus aiding in achieving lock.
3. Press LOCK-ON on the BSMS keyboard. This will not alter the FIELD value, therefore will only lock the system if a strong enough lock signal already lies within the swept range. This method is only recommended if a sample has been replaced with one using the same solvent, or, if it is already known that the various lock parameters are suitable.

Procedure to Lock the Sample *6.7.1*

The lock signal can be viewed by entering the command ’lockdisp’.

Assuming lock has not been achieved, the signal on the screen represents two overlapping continuous wave spectra of deuterium; one scanning left to right, the other right to left. The speed of scanning can be adjusted using the SWEEP RATE function of the BSMS keyboard. A good quality CW spectrum is illustrated in
Figure 6.5..

Figure 6.5. A Typical Lock Signal



If lock has already been achieved, the signal should be of the form of a horizontal line with some associated noise or ripples (see the figure ***"Lock Display After Locking the Sample" on page 61***). The height of this line is called the lock level.

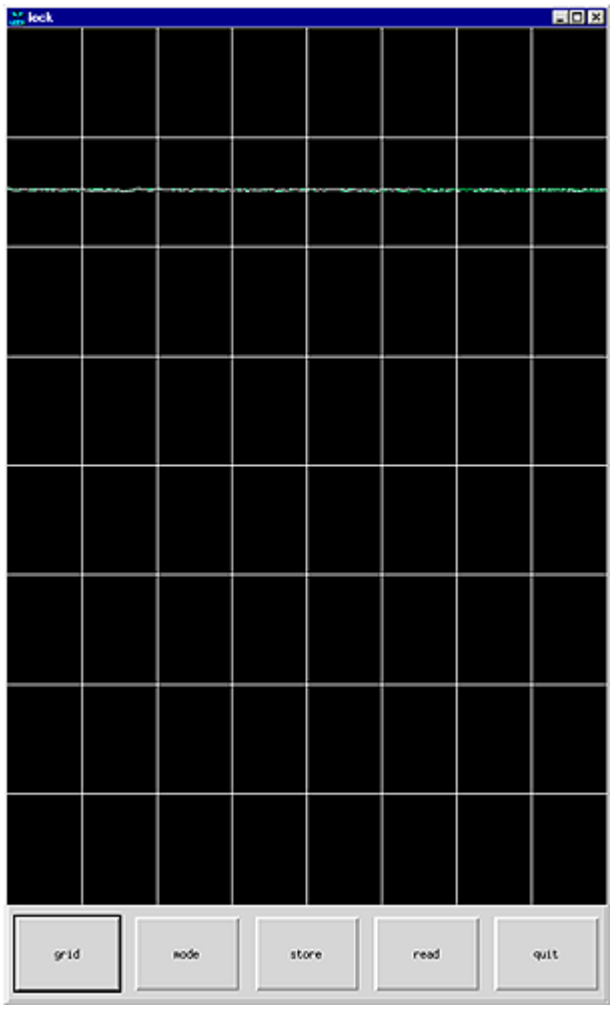
Procedure:

1. Enter 'lockdisp' to view the lock signal.
2. Enter 'lock'.
3. Select the appropriate solvent from the menu (for the sample used for experiments described in this manual select $CDCl_3$).

The autolock LED of the BSMS keyboard will begin to flash until lock has been achieved. When this happens the message "lock: finished“ will appear on the screen and the Lock On/Off LED will light.

4. If the lock level is unstable (due to saturation) it may be necessary to adjust the Lock Power manually using the BSMS keyboard. (To prevent the problem from reoccurring, a better solution is to adjust the power setting in the edlock table for that solvent. Indeed it is worth consulting with the system manager to check that the three edlock parameters "LPower“, "LGain“ and "LTime“ are optimized)
5. If necessary adjust the Lock Phase to maximize the lock level.

Figure 6.6. Lock Display After Locking the Sample



Shimming *6.8*

Shimming is a process in which minor adjustments are made to the magnetic field until the field homogeneity (uniformity) is optimized. Improving the homogeneity will result in better spectral resolution. It will be necessary to reshim each time a probe or sample is changed. Storing appropriate shim values (in so called shim files) for each probe will greatly reduce the shimming time required whenever a probe is changed (see the section ***"Storing a Set of Shim Values (Write Shim Command)" on page 48***).

Shimming is controlled by a set of shim coils, in the room temperature shim system, inserted into the bore of the magnet. The current in the coils, and hence the field inside the magnet, can be adjusted using the BSMS keyboard. The number of coils will depend on the type of BSMS system. They all have algebraic names Z, Z^2, X, Y, etc. and the corresponding keys may be found at the lower section of the BSMS keyboard. The magnetic field is three dimensional and the naming of the shims tries to reflect the corresponding algebraic functions in the XYZ coordinate system.

One measure of the field homogeneity is the height of the lock signal on the screen (the lock level) at constant lock power and gain. The higher the lock level, the greater the field homogeneity. One method of shimming therefore involves observing the lock display and adjusting the shim currents until no further improvement in the height of the signal can be achieved. (Note: the lock level is not the only measure of the field homogeneity that can be used to shim. The shape and area of the FID may also be used).

The following sections discuss the two broad categories of shimming that can be identified.

Initial Shimming *6.8.1*

When a magnet is charged for the first time it must be shimmed from scratch by the installation engineer. The size of the current in each individual shim coil is optimised. The procedure is complicated by the fact that many of the shims are interdependent and adjusting one may require readjusting several others. The magnet must be shimmed for each different probe that is used. The shim values for a particular probe can be stored in a shim file so that if the probe inside the magnet is changed, the corresponding optimal shim values can be loaded easily. Consult with the system manager as to which shim file should be used for a particular probe.

Routine Shimming *6.8.2*

Once the initial shimming has been completed the operation is greatly simplified and becomes a matter of making fine adjustments to the more important shims as long as the filling height of the sample tube is constant. This is routine shimming and should be carried out at the beginning of every NMR session, and whenever the sample in the magnet is changed. Routine shimming involves making fine adjustments to the Z and Z^2 shims (and possibly to X and Y). The procedure is outlined as follows:

1. If the probe has been changed you should first read the correct shim file into the BSMS unit. This sets the shim values to suit the probe. Enter 'rsh' and a list of stored shim files will be displayed. Click on the appropriate file and within a few seconds the following message will appear on the screen.

   rsh: finished

2. Ensure that the LOCK key on the BSMS keyboard is activated, i.e. the sample is locked. If the sample is to be rotated, set the spinning rate to 20 Hz for 5mm probes, and 12 Hz for 10 mm probes).
3. Adjust the LOCK PHASE to maximize the lock level.
4. Adjust the LOCK POWER, if necessary, so that it has a value of 6 dB below saturation. The onset of saturation is that level of LOCK POWER at which the lock level will be become unstable and tends to "**wander**".
5. Activate the Z key ("on-axis" + "Z1") of the BSMS. Use the black knob to adjust the value of the Z shim so that the lock level is maximized.
6. Activate the Z^2 key. Adjust the Z^2 shim to maximize the lock level. (You may have to reduce the LOCK GAIN to keep the signal on the screen).
7. Repeat steps 5 and 6 until no further improvement can be achieved.

8. Finally adjust the LOCK PHASE to maximize the LOCK LEVEL.
9. Turn the sample spin off and adjust the X and Y shims ("X“/“Y" + "Z0“) to maximize the lock level.

Note: Over a period of time conditions inside and outside the magnet may change slightly and for optimum performance higher order shims such as Z^3, Z^4 and Z^5, may need to be adjusted. Furthermore for samples in H_2O/D_2O more extensive shimming may be required to achieve optimal water suppression.

The lock level is not the best criterion for evaluation of magnet homogeneity. Although a description is beyond the scope of this manual, the lineshape (width and symmetry) of a signal is in fact a better indication of field homogeneity. As you gain more experience, you might discuss with the system manager the need for optimizing the higher order shims using signal lineshape.

Please note that there is a procedure called "**gradshim**“ (gradient shimming) which can be used for protons and deuterium. If this option is available, you should consult with the system manager, as it greatly simplifies the shimming procedure.

A more detailed description of shimming is given in Chapter 6 of the BSMS User Manual delivered on the BASH CD.

Preparing for Acquisition, Data Sets, edasp/eda Commands

7

Introduction 7.1

This chapter will explain two of the most important groups of parameters invoked by the commands “eda“ and “edasp“. Although there is a third command “edsp“, which is very similar to “edasp“, only “edasp“ will be described here as it is more versatile. Before assigning values to various parameters, it is important that the user understands the concept of data sets, as groups of parameters are inextricably linked to these.

Data Sets 7.2

Regular use of a spectrometer will soon result in the accumulation of large amounts of data. Users will want to store data in conveniently named files so that later they can easily access their data. This is particularly important in a multi-user environment. When a set of data is acquired, it can be stored in what is called a **data set**. Each data set must have a unique descriptor (identifier) so that different data sets can be distinguished. A full descriptor for a data set requires the use of five parameters: DU, USER, NAME, EXPNO, and PROCNO. These parameters are explained below.

1. **DU** (Disk Unit): On systems that have a very large amount of data, more than one hard disk may be required. This possibility is recorded by using the parameter DU, which is the disk partition where the data set is located.
2. **USER**: Different users are likely to have access to the same spectrometer, therefore the log-in ID of the user who acquired the data should form part of the data set descriptor. Every data set descriptor must include a USER. Typically, only users who belong to the same group can manipulate or delete that group‘s data sets.
3. **NAME**: Even if only a single user acquires data, it is likely that many different samples will be analyzed. To distinguish between different samples, the parameter NAME is used in defining the data set descriptor. The user can freely choose any NAME, but normally they should use one that is associated with the sample. For example, data sets belonging to a sample of gramicidin might be assigned the NAME "gram“, whereas, data sets acquired from a sample of cholesterylacetate might be given the NAME "cholac“.

Before explaining the next two data set parameters, a distinction must be made between the terms **raw data** and **processed data**.

If a sample is inserted into the magnet and an acquisition carried out, then the data set that is acquired is called a **raw data set**. This is either one FID for 1D experiments or a series of FIDS for 2D experiments. It is "raw“ data in the sense that it has not yet been processed.

The FID’s are processed by applying a Fourier transform which converts the data from the time domain to the frequency domain and usually by measures including window functions and phase correction. The resulting data set is termed a **processed data set**.

A single raw data set may be processed in many different ways and data set descriptors must take account of this. Two parameters are used for this distinction: EXPNO and PROCNO. Unlike the parameters USER and NAME, the parameters EXPNO and PROCNO are assigned numerical values.

4. **EXPNO** (Experiment Number): Each different **raw** data set is given a unique EXPNO. For example, a single chemical sample might be analyzed several times using different acquisition parameters. To distinguish between the different raw data sets, a unique EXPNO is assigned to each.
5. **PROCNO** (Processing Number): The PROCNO is used to distinguish between different **processed** data sets belonging to the same raw data sets.

The correct use of data set parameters may best be illustrated using an example. However, no reference will be made to the parameters USER or DU as these will probably not change during an NMR session.

Example:

Suppose two samples of gramicidin are to be analyzed. We can differentiate between the two samples by creating two different data set NAMES called "grama“ and "gramb“. You might decide to analyze and acquire FIDs (raw data) from each sample under different conditions, e.g. you might wish to analyze each sample at three different temperatures Tx, Ty, and Tz. Accordingly, you can distinguish between each experiment by assigning each a unique EXPNO.

This will lead to a total of six raw data sets, as listed in the following table:

Table 7.1. Data Sets with Differing NAMEs and EXPNOs

NAME	EXPNO	COMMENT
grama	1	Tx
grama	2	Ty
grama	3	Tz
gramb	1	Tx
gramb	2	Ty
gramb	3	Tz

At this stage each raw data set may be uniquely identified using the parameters NAME and EXPNO.

The user may now decide to process each raw data set in two different ways, e.g. with and without exponential multiplication. To distinguish between the two processing methods and the corresponding spectra, the parameter PROCNO should be used. Each raw data set is processed twice, therefore two PROCNOs must be used. Now there should be a total of twelve processed data sets, which could be represented as shown in the table below.

Table 7.2. Data Sets with Differing NAME's, EXPNO's and PROCNO's

NAME	EXPNO	PROCNO	COMMENT
grama	1	1	Temperature Tx without exponential multiplication
grama	1	2	Temperature Tx with exponential multiplication
grama	2	1	Temperature Ty without exponential multiplication
grama	2	2	Temperature Ty with exponential multiplication
grama	3	1	Temperature Tz without exponential multiplication
grama	3	2	Temperature Tz with exponential multiplication
gramb	1	1	Temperature Tx without exponential multiplication
gramb	1	2	Temperature Tx with exponential multiplication
gramb	2	1	Temperature Ty without exponential multiplication
gramb	2	2	Temperature Ty with exponential multiplication
gramb	3	1	Temperature Tz without exponential multiplication
gramb	3	2	Temperature Tz with exponential multiplication

Note: By using the parameters NAME, EXPNO and PROCNO no two data sets have the same descriptor, i.e. they are all uniquely defined.

Creating a Data Set *7.3*

Any data set acquired with the spectrometer is automatically stored in what is called the "**current**" data set. Details of the current data set are always found at the top left hand corner of the XWIN-NMR display.

Before you acquire any new data, you should always create a new data set in which it can be stored. This will prevent you from overwriting existing data.

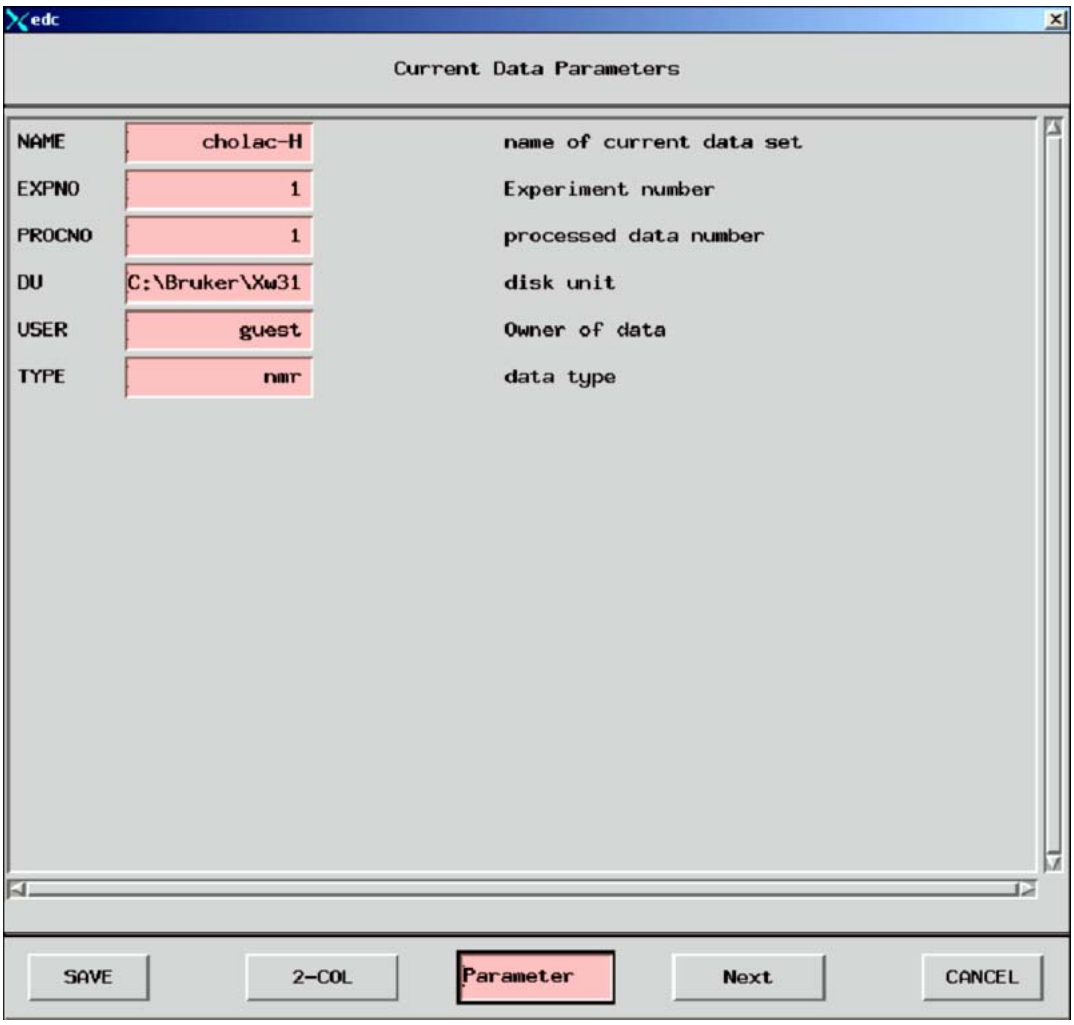
The term "creating a new data set" may be a little misleading. Up to this point you have not yet acquired any data, however, by creating a new data set the computer prepares a set of files in which your data will be stored.

To create a new data set enter 'edc'. This will display details of the current data set as illustrated in ***Figure 7.1.***, where the current data set is /guest/default/2/3

You can now create your own data set. For NAME you may enter a string of up to 13 characters. For both EXPNO and PROCNO enter '1'. Note that your newly created data set is now the current data set and details of the descriptor parameters will appear at the top left hand corner of the screen.

A final word regarding the various parameters assigned to the new data set. Parameters such as acquisition parameters, which were set for the example XWinNMRHome/guest/default/2/3 are carried over to the new data set. These values can now be changed.

Figure 7.1. The edc Display



Spectrometer Parameters edasp *7.4*

The command “edasp“ is short for **edit acquisition spectrometer parameters**. These spectrometer parameters serve to configure the spectrometer for a particular experiment. For example, when they are set, they prepare devices such as the router to deliver the correct signal to the appropriate amplifier. The “edasp“ command allows the user to design an experiment in terms of the various nuclei that will be investigated, as well as, selecting the appropriate amplifiers, amplifier outputs and HPPR modules.

When preparing an experiment the first steps are reading in a parameter set with the "rpar“ command and editing the “edasp“ window. It is important that the spec-

trometer parameters are set up correctly **before** using the “eda“ command to set the acquisition parameters. The user should note that:

1. Any adjustments made in the spectrometer parameter set (via the “edasp” window) will always be carried over to the acquisition parameter set (“eda“ table).
2. Any changes in the acquisition parameter set (e.g. frequency offsets) will only be carried over to the spectrometer parameter set, if “edasp“ (but not “edsp“) is subsequently entered. This is assuming that the changes made in the “eda“ table have already been saved.
3. If after making adjustments in the acquisition parameters, the “edsp“ (but not “edasp“) command is entered, the changes will be lost and the previous spectrometer parameters will be reinstated.

The user should note that when a standard parameter set is loaded, both the acquisition and spectrometer parameters are set.

As already mention, the “edasp“ command is more versatile than “edsp“ and the user is recommended to become familiar with its operation. Note that “edasp“ and not “edsp“ is called up when clicking on NUCLEI in the “eda“ table.

Assuming that the hardware (FCU’s, Routers, SGU’s etc.) has been wired and configured correctly, users can design whatever experiment they wish as long as it is available in the edasp window. The software will take account of the hard wiring between the FCUs and the amplifiers as well as any required internal switching within the routers or amplifiers (see ***Figure 7.2.***). For example, if protons are selected as the observe nucleus (Channel 1), then the second FCU2 will be selected as default, as this is consistent with the hard wiring. Note however, that the software can not control the connections between any given amplifier output and the HPPR. Although the connection to the HPPR is shown in the edasp window, making alterations to the HPPR connection will have no physical effect on the hardware. The user must ensure that the corresponding cable connections are present. (Note: The HPPR modules are displayed to enable the software to detect the ultimate destination of the F1 channel as this module will be selected as the OBS module).

The user should feel at ease familiarizing themselves with the “edasp” window. Making changes in the “edasp” window has absolutely no effect on any spectrometer hardware until the command "ii“ (initialize interfaces) is given. Even after initializing all interfaces, actual pulse transmission will not start until commands such as "zg“, "gs“, or "go“ are entered.

If you have altered the spectrometer parameters you can easily restore the standard settings by loading a standard parameter set. For example

enter ’rpar PROTON’ (click on acqu)

to reset spectrometer parameters for observing ^{1}H

enter ’rpar C13CPD’ (click on acqu)

to reset spectrometer parameters for observing ^{13}C with CPD decoupling.

Layout of the edasp Window *7.4.1*

The AVANCE with SGU spectrometer software is designed in such a way that the spectrometer configuration will automatically customize the “edasp” window. In this way, the user sees only that hardware which is actually installed in their par-

ticular spectrometer. The “edasp” window is organized into various vertical columns which are described below.

1. Frequency

The frequencies of the transmitted signals on channels one, two and three etc. are given by SFO1, SFO2 and SFO3, etc., respectively. However, these frequencies may not be set directly (you will notice that they can not be highlighted using the mouse).

The transmitted frequencies are controlled by setting offsets to basic frequencies BF1, BF2 and BF3 etc.

For the observe channel:

SFO1 = BF + offset

transmitted frequency	automatically set	set by the user

Similarly, for the next two decoupling channels

SFO2	=	BF2	+	offset
SFO3	=	BF3	+	offset

The offsets are labeled OFSH1, OFSX1, OFSF1 etc. according to the naming conventions listed in the section ***"Some Features of the edasp Window" on page 72***. Note that these parameters cannot be retrieved from the command line.

When a specific nucleus is selected, the appropriate basic frequency is automatically set. After reading a standard parameter set, the basic frequency will be set correctly and only the offset values need to be adjusted.

Remember that SFOX is the most relevant parameter in that it is the frequency that is actually transmitted to the sample. Note also that the offsets can be set to zero in which case SFOX = BFX. A more detailed description is given in section ***"Numerical Explanation of Transmitted, Basic and Offset Frequencies" on page 79***.

2. Logical Channel

In this column the nuclei under investigation are selected. Logical channel F1 or NUC1 is always the observe nucleus. If a channel is not in use, it should be switched off. The list of nuclei that may be selected is taken from the file:

XWinNMRHome/conf/instr/<instrument name>/nuclei

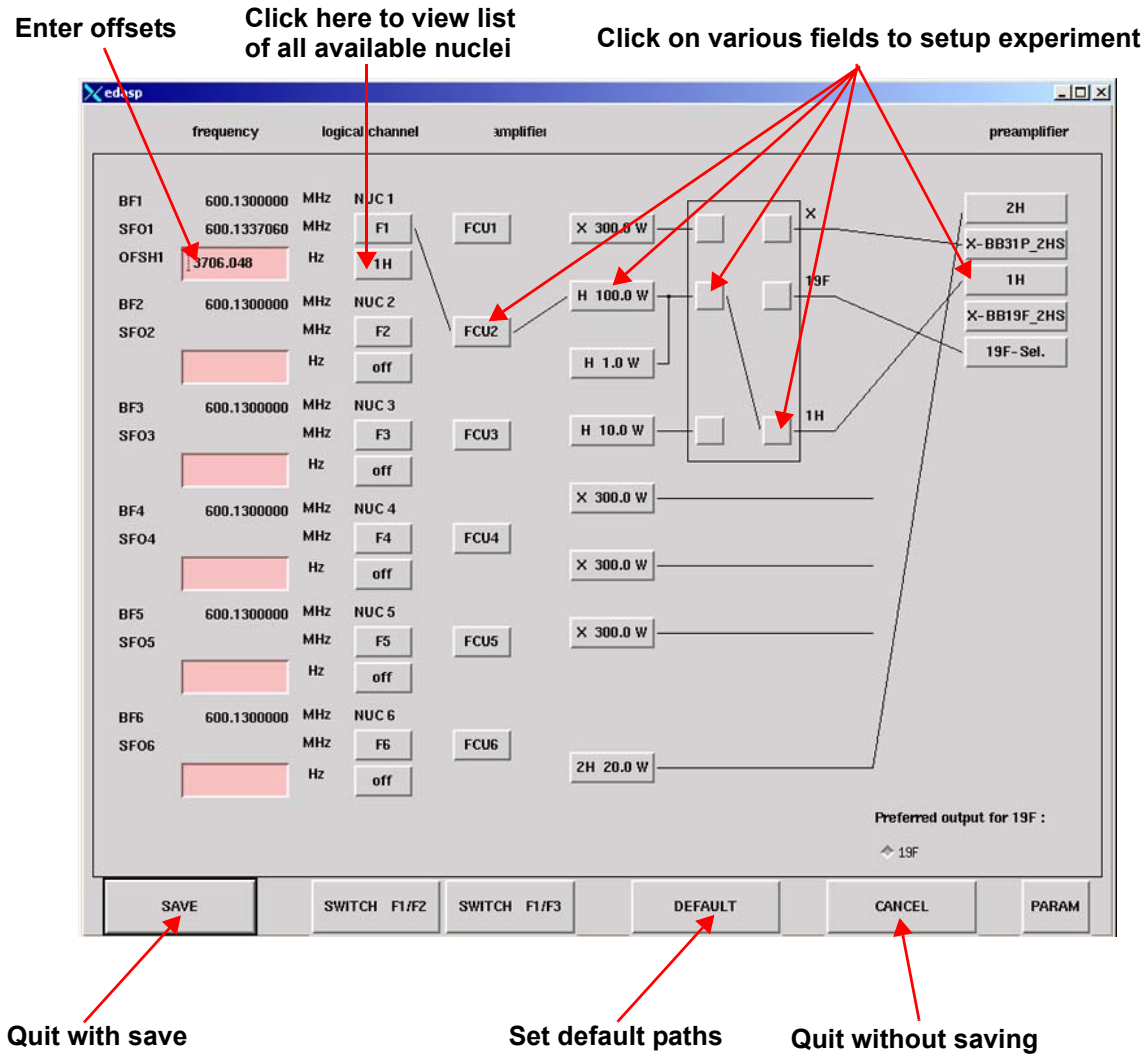
More experienced users may wish to edit this file to customize the list of available nuclei.

Note that one immediate effect of changing the nucleus type in the logical channel is that the basic frequency in the frequency column is automatically adjusted.

3. FCU’s

The connections to the appropriate FCU’s are automatically made and inexperienced users are advised not to alter these default connections.

Figure 7.2. The “edasp” Menu
(after loading the standard ’PROTON’ parameter set)



4. Amplifier

Again, the connections to the appropriate amplifier are made automatically. The amplifier outputs are easily identified as they are located on the right hand side of the amplifier and labelled “X”, “19F” and “1H” etc. The “edasp” display will vary depending on the types of amplifiers installed.

For Systems with Internal Amplifiers:

With the BLA2BB, the ^{1}H output is connected to the ^{1}H HPPR, and the X output connected to the X-BB HPPR module. These connections are unambiguous and there is no possibility for error. If the system also contains an internal BLAX300, there are two possible X amplifiers. You must only ensure, regardless of which X amplifier is selected on the observe channel, that a cable connects the output of this amplifier to the HPPR X-BB module.

For Systems with External Amplifiers:

There are a variety of possible amplifier configurations, some amplifiers have three possible outputs usually labeled: "X“, "19F“ and "^{1}H“. Regardless of the configuration, the same basic principle applies. When an amplifier is chosen in the

“edasp” window, make sure that the selected output of that amplifier is either physically connected to an appropriate HPPR module, or directly connected to the probe.

5. Preamplifier

Depending on the system up to 5 HPPR modules may be configured. In many systems there is a one to one correspondence between the amplifier outputs and the HPPR modules. Whichever amplifier output that is used to transmit the observe pulses must be connected via a HPPR module. Decoupling channels are usually connected via an HPPR module, although they can also be connected directly to the probe.

Some Features of the edasp Window *7.4.2*

When using the “edasp” window, the following should be noted:

1. Logical Channel F1 is always the OBS (observe) channel, all other channels (F2, F3 etc.) are decoupling channels.
2. The software takes into account various standard hardwire configurations, e.g., if protons are to be observed, the default is FCU**2**. This is because router output **2** (not shown in the “edasp” display) is connected to the proton amplifier input.
3. Regarding the grouping of nuclei, the following conventions apply:

   H = hydrogen nucleus i.e. ^{1}H

   F = ^{3}H, ^{19}F,

   X = All other nuclei.

Basic Acquisition Parameters: The “eda” Table *7.5*

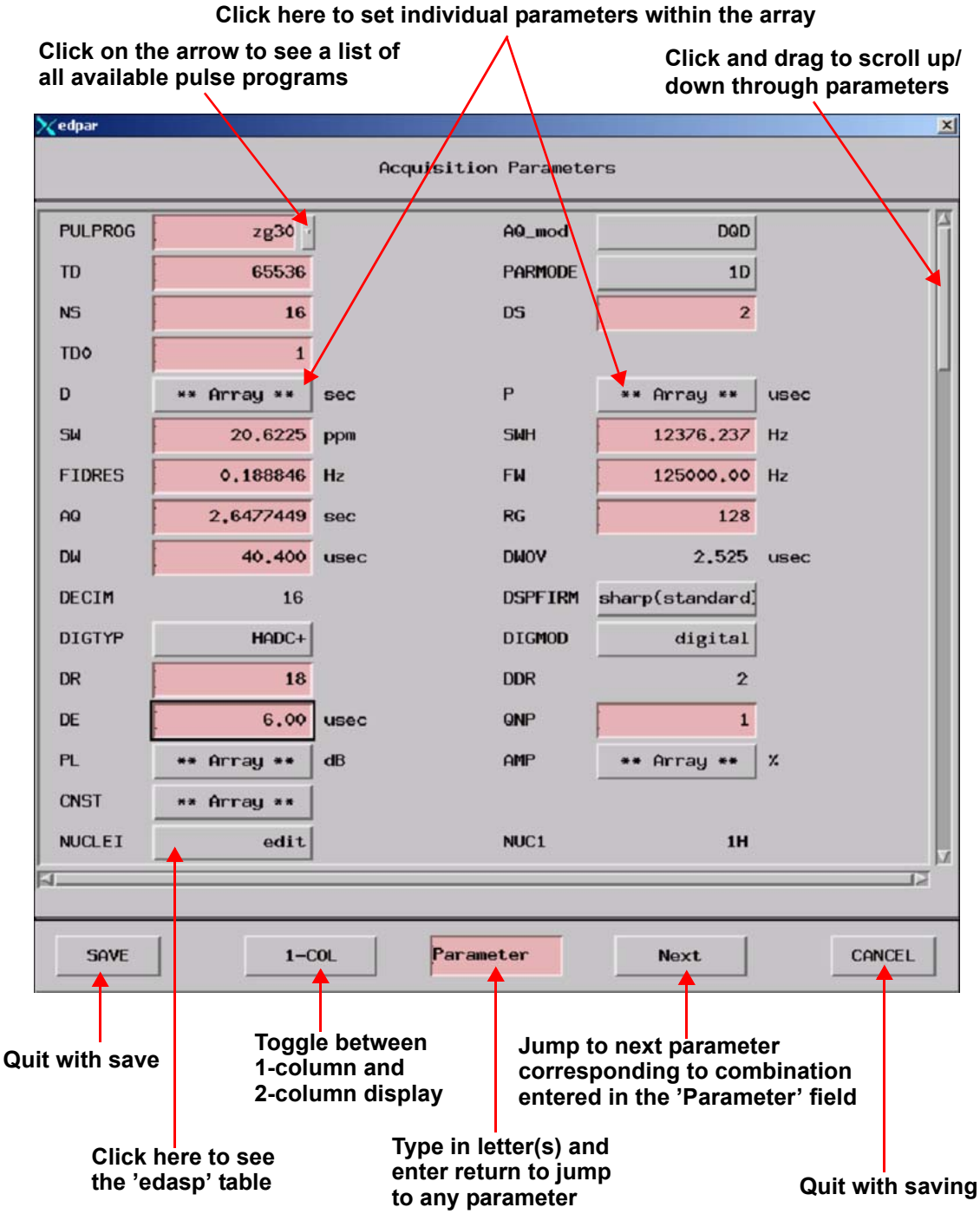
Along with the spectrometer parameters, the set of parameters called up with the “eda” (**ed**it **a**cquisition parameters) command are most important when preparing an acquisition. The following section is a brief summary of the most important relevant parameters. A new user should become familiar with these. Additional details of these and other parameters, are available in the "XWIN-NMR Acquisition Reference Manual“ or in the "XWIN-NMR Processing Reference Manual“. Sufficient details for use with this manual are given below. If the user is only concerned with producing a spectrum, they may decide to skip forward to the chapter ***"Proton Spectrum" on page 87***, and use the standard parameter set entitled “PROTON”. This will automatically set suitable acquisition parameters. The disadvantage of this method, is that a genuine understanding of the basic processes will not be acquired, thus the user will not gain the knowledge required to make appropriate modifications.

The parameters here are presented in the order as they appear in the 2nd column display mode of the “eda” table.

PULPROG: The **pul**se **prog**ram used for the acquisition. It is executed when using any of the following commands: “zg”, “rga”, “gs”, “go”.

Figure 7.3. First Page of “eda“ Parameters

(After loading the standard "PROTON“ parameter set) Note that only those fields highlighted in pink or displayed as an **Array may be adjusted by the user.**



An NMR experiment involves exciting the sample with a precisely defined sequence of RF pulses. The sequence is defined by a pulse program and is typically a set of pulses interspersed with delays. To distinguish between different programs, each one is given a unique name. The name is entered for the PULPROG parameter. The pulse program “**zg30**” which will be used in this manual is often used for simple 1D NMR experiments if signal accumulation is required.

AQ_mod: Acq**uisition Mod**e.

Determines how the acquired data is shared among two receiving channels. Select DQD for AVANCE with SGU systems.

TD: Time **D**omain Data Size.

The signal emitted by the NMR sample is digitized before any processing is carried out. The value assigned to TD is the number of points to be sampled and digitized to form the FID (see ***Figure 7.4.***). Typically TD is set to 16, 32 or 64K for standard 1D FID's. Although increasing the size of TD will improve the FID resolution, it does so at the expense of a longer acquisition time.

PARMODE: Parameter Set **Mode**

Can be set to 1D, 2D or 3D depending on the type of NMR experiment being carried out. Select 1D for any acquisitions described in this manual.

NS: Number of **S**cans.

In NMR the accumulation of a number of transients (scans) is often carried out to improve the quality of the spectrum. (The time taken to carry out the experiment is of course increased.) The number of acquisitions is specified with the parameter NS. NS should usually be a multiple of 8 to accommodate standard phase cycling which is incorporated into pulse programs. When setting up an experiment for the first time however, it saves time to set NS = 1 and work on optimizing various other parameters before setting NS to a higher value.

DS: Number of **D**ummy **S**cans

Several sets of pulses which are identical to those used for acquisition are sometimes transmitted to the sample before any signals emitted by the sample are allowed to enter the receiver. This is to allow the sample to reach a steady state or equilibrium. The pulse trains are called "**dummy scans**" as no data is acquired. The number of such dummy scans depends on the sample relaxation time and susceptibility to heating, and is set with DS. Typical values for standard experiments are 4 or 8.

Darray**: D**elay D0 - D31.

Clicking on this parameter in the "eda" table will yield an array of 32 delays labelled D0 to D31. Each of these 32 delays can be set separately and incorporated into a pulse program. Note that if a delay is entered as a number without any units, then it defaults to seconds. However this may be changed by typing "*u*" for microseconds, or "*m*" for milliseconds, before pressing "*enter*". (If you only observe 16 delays in the array then you should change to the 2-column display). The delay "D1" is almost always used as the relaxation delay. It is usually quicker to set the delay explicitly by typing it in on the command line as opposed to using the array of the "eda" table. Although delays appear in the "eda" table with upper case letters, when entered into a pulse program or typed explicitly on the XWIN-NMR command line, lower case letters must be used.

Parray**: P**ulse P0 - P31

An array of 32 possible pulse widths which can be incorporated into a pulse program. Default unit is micro-seconds (type "u" for "µ"). Typing "*m*" or "*s*" will change the units to milliseconds or seconds respectively. The pulse width "P1"is almost always used as the standard 90 degree excitation pulse. Although pulse widths appear in the "eda" table with upper case letters, when entered into a pulse program, or typed explicitly on the XWIN-NMR command line, lower case letters must be used.

When setting pulse widths, the user should be aware that a too long pulse may cause the probe to arc or overheat, particularly if the power level is not attenuated sufficiently. Typical observe pulses (as opposed to decoupling) are 6-15 µs in duration.

SW: Spectral **W**idth.

Sometimes referred to as **sweep width**, this is a measure of the width of the frequency spectrum to be analyzed. Typically, a sample will emit a range of frequencies rather than a single frequency, all of which should lie within the spectral width.

Signals whose frequency fall within the spectral width will be detected, whereas signals outside this range will be filtered out. If you have a good idea of your sample‘s resonance frequencies, you can set a small SW (there are some advantages to this). On the other hand, when analyzing an unknown sample, start by using a large SW. The SW, set in ppm, can be thought of as the width of the window though which you will observe the spectrum. For proton spectra, a SW of 20 ppm will be large enough to capture any signal, as long as the transmission frequency around which the SW is centered (SFO1) is correct. The standard parameter set "PROTON“, which is used throughout this manual, has a SW of 20.6ppm.

A useful function when adjusting the SW is the SW - SF01 function which can be found in the utilities submenu. It adjusts the SW so that it has the same value as the region displayed on the screen and sets SFO1 in the center.

SWH: Spectral Width in **H**ertz.

Same as SW, but measured in Hertz as opposed to ppm (see the section ***"Reference Compounds, Hertz, ppm" on page 17***). Changing the value assigned to SW will automatically change SWH and vice versa. When an arbitrary value of SWH or SW is entered, the software will automatically adjust it slightly to ensure that the digitizer dwell time has a discrete value. The maximum value that may be assigned to SWH depends on the digitizer type.

FIDRES: FID Resolution in Hertz (per point)

This is set automatically according to the values assigned to SWH and TD. It has the value of SWH/TD. The spectrum that appears on the screen is in reality a set of points joined by straight lines. Each point thus represents a frequency range and is called the FID resolution. The smaller the value of FIDRES, the more accurate the representation of the spectrum.

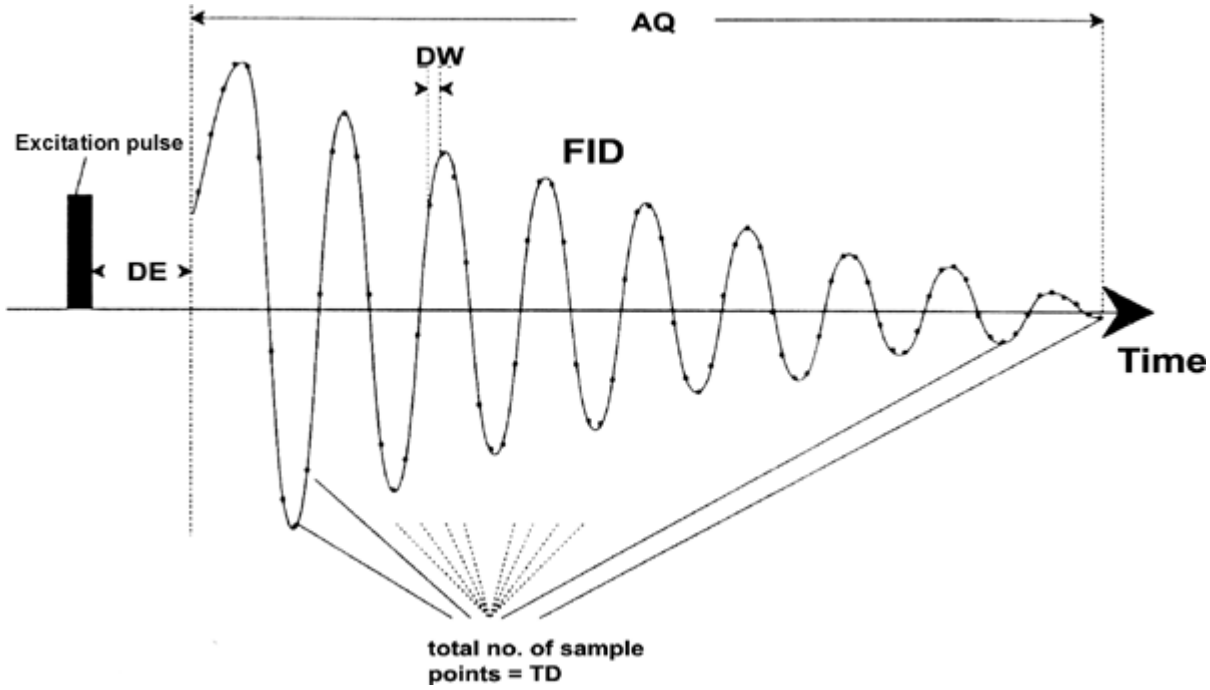
FW: Filter **W**idth.

Generally the analog filter is set automatically depending on the value of SW and SWH. It is set to filter out any signals received outside the spectral width. Since digital filtering is now standard, only coarse analogue filters are required. Depending on the digitizer, you can expect to see a limited number of possible values in the “eda” table, such as 20 KHz, 90 KHz, 125 KHz, 625 KHz, etc.

AQ: Ac**q**uisition Time.

The acquisition time is the time (in seconds) needed to acquire one scan. It is set automatically and is based on the values assigned to TD and SW. However, it can be set manually and will cause a corresponding adjustment of the value assigned to TD.

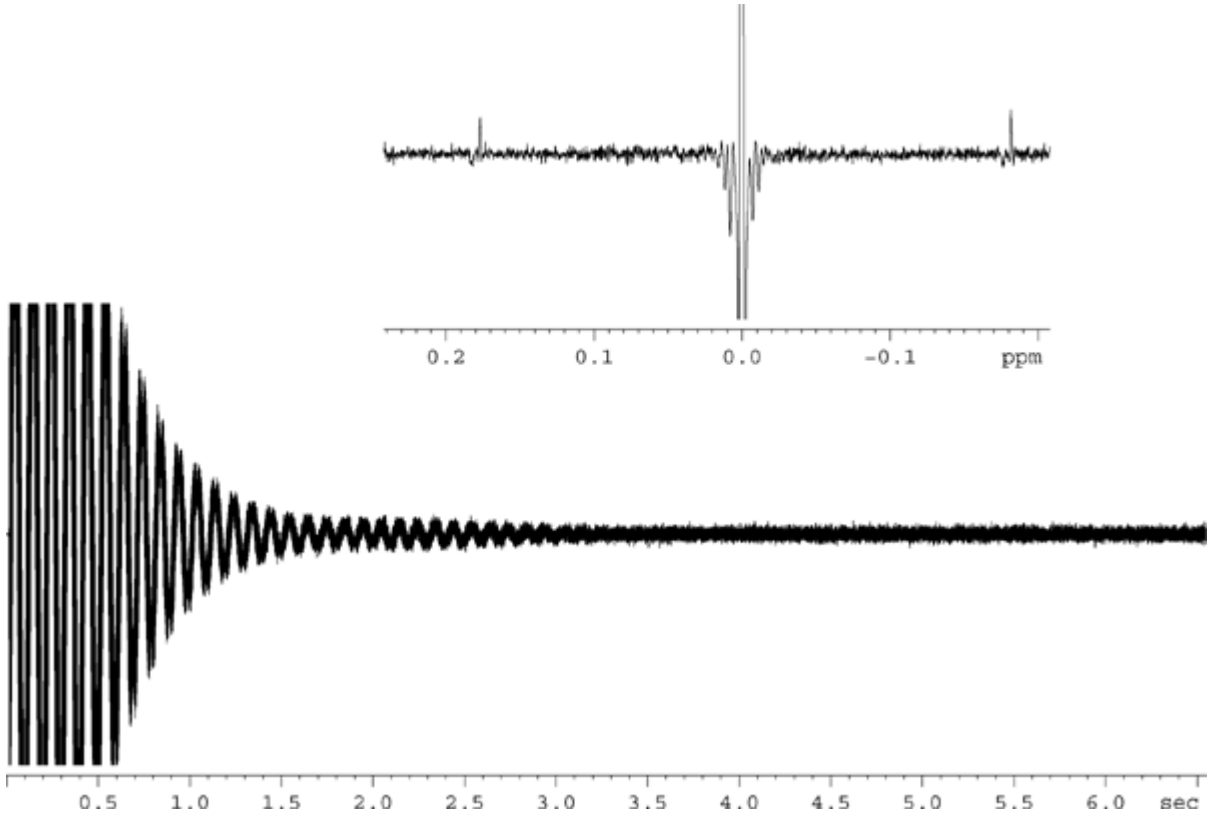
Figure 7.4. Graphical Representation of Some Acquisition Parameters



RG: Receiver **G**ain.

The receiver gain is a very important parameter that is used to match the amplitude of the FID to the dynamic range of the digitizer. The correct setting of the gain may be determined by inspecting the FID. If the FID is “clipped” at the top or the bottom of the screen display, then the gain should be reduced. On the other hand, if the gain is too low and not utilizing a suitable (25-50%) part of the dynamic range of the digitizer, it should be increased. When viewing the FID amplitude on the screen ensure that the vertical scaling is at the default value. The gain may be set automatically with the “rga” command, which is highly recommended for new users.

Figure 7.5. Clipped FID as a Result of RG Set too High



DW: Dwell Time.

Since the advent of digital filtering and oversampling, this parameter is of less significance. It represents the theoretical time interval in microseconds between the digitalization of two sampled points of the FID to satisfy the Nyquist criterion. Set automatically, it is calculated in microseconds using the formula DW = 10^6/ 2*SWH.

DWOV: Oversampling **Dw**ell Time.

This is the actual DW time used by the digitizer assuming digital filtering and oversampling has been enabled by setting the parameter DIGMOD (see below) to either “digital” or “homodecoupling-digital”. The value of DWOV is calculated automatically.

DECIMATION: Decimation Rate of Digital Filter

This is the ratio of DW to DWOV and represents the factor by which the FID is oversampled. It is set automatically using the formula DECIMATION=DW/DWOV.

DSPFIRM: Digital **S**ignal **P**rocessing **Firm**ware.

Set to "sharp (standard)“

DIGTYP: **Typ**e of **Dig**itizer.

The type of digitizer used in the spectrometer. This may be SADC, HADC+, FADC etc. This parameter is made available for systems fitted with more than one digitizer in mind. Only those digitizers actually available on a specific spectrometer will be offered for selection in the “eda” table. If you have selected the DIGTYP during the “expinstall” routine, this will be automatically set whenever a standard parameter set is loaded.

DIGMOD: Digitizer **Mod**e:

For AVANCE with SGU systems "digital“ mode should be selected. If a homodecoupling experiment is to be performed, the mode “homodecoupling-digital” should be used. If “analog” mode is used, digital filtering will not be enabled.

DE: Prescan **De**lay.

The Prescan delay is the time delay prior to the onset of data acquisition. This delay, automatically set in microseconds, ensures that the excitation pulse has completely elapsed before the onset of data acquisition. During the “expinstall” routine, the user is prompted to enter a value for DE. Reading in the standard parameter set “PROTON” will set this value automatically to 6µs, which is suitable for all experiments described in this manual.

PL **array: P**ower **L**evel in dB

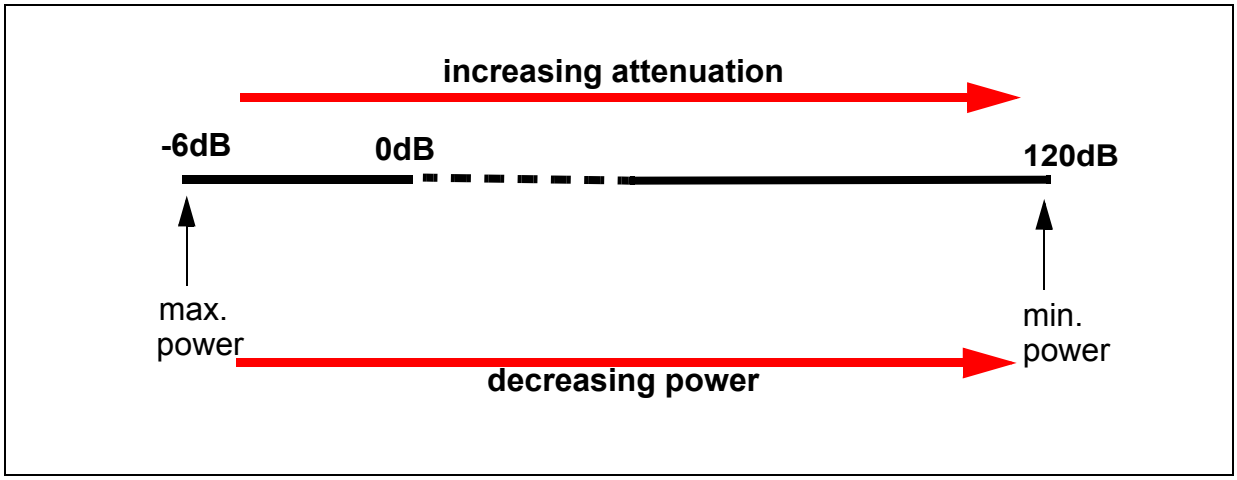
Clicking here will yield an array of 32 power levels, labeled PL0 - PL31. Each of the 32 levels can be set separately and incorporated into a pulse program. The greater the value assigned to a PL parameter, the greater the attenuation, and thus the weaker the signal. The effect of any given power level depends on the type of amplifier used, so the same PL value will have very different effects if applied to two different amplifier types.

Power levels can be explicitly assigned to any of the eight channels within a pulse program. If a power level is not explicitly assigned, PLx will be assigned as default to channel “Fx”. For the purposes of this manual the principal power level is “pl1” which is used to set the attenuation on the F1 (observe) channel.

As with all arrays it is often more convenient to enter the PL value explicitly on the XWIN-NMR command line as opposed to entering the array in the “eda” table. Again power levels appear in the “eda” table with upper case letters, when entered into a pulse program or typed explicitly on the XWIN-NMR command line lower case letters must be used.

The maximum power (minimum attenuation) on any channel is -6db for historical reasons. This convention has been maintained to minimize changes for experienced users working with newer spectrometers. Note that reduced power levels are achieved by actually increasing the attenuation level (measured in dB) applied to the excitation signal.

Figure 7.6. Relationship Between Power and Attenuation



Users should be aware that the simplest way to damage a probe is to use a power level that is too high. For this reason the default levels assigned for standard parameter sets is always 120 dB which represents maximum attenuation, i.e. zero power. Users should check with the system manager as to what power levels are appropriate for various probes, or use the “getprosol” routine. The “getprosol” routine will be described in the section ***"The “getprosol” Command" on page 88***.

NUCLEI: Switch to “edasp” Menu.

This enables the user to make quick changes in the spectrometer setup. Since the “eda“ table has not been saved, changes in offsets made in the “eda“ menu will not be incorporated into the spectrometer parameters. If offsets in the “eda” table are to be incorporated into the spectrometer setup, first save the “eda” table before entering "edasp“.

LOCNUC: Name of **Loc**k **Nuc**leus

Normally this is ^{2}H (deuterium), although sometimes ^{19}F (fluorine) is used in cases where deuterium signals would interfere with the “genuine” sample signals. The ^{19}F lock requires special hardware.

NUC1: Name of Observe **Nuc**leus

The name of the nucleus to be observed. The observe nucleus is normally set using a different menu (edasp) which will overwrite the “eda“ entry. For user information, the observe nucleus is listed in the “eda“ menu table.

O1: Transmitter Frequency **O**ffset for Channel F**1** in Hz.

SFO1: Transmitter frequency.

This is the frequency used to excite the observe nucleus (logical channel F1) and will be at the center of the spectrum. It may be thought of as the central frequency in the window through which the spectrum is observed.

O1P: Transmitter frequency offset for channel F1 in ppm.

BF1: Basic transmitter frequency for channel F1 in Hertz.

This frequency is set automatically depending on which nucleus (e.g. ^{13}C, ^{1}H) is to be observed.

The correct use of the three parameters SFO1, BF1 and O1 will now be explained. The reader should note that it is conventional in NMR spectroscopy to have frequency on the horizontal axis and increasing towards the left.

The three parameters SFO1, BF1 and O1 are related by:

SFO1 = BF1 + O1.

For up to eight logical channels there exist the corresponding parameters such that for channel **X** then the chosen nucleus is NUC**X** and:

SFOX = BFX + OX

The user must understand the relevance of these parameters and they shall now be discussed using practical examples.

Numerical Explanation of Transmitted, Basic and Offset Frequencies *7.5.1*

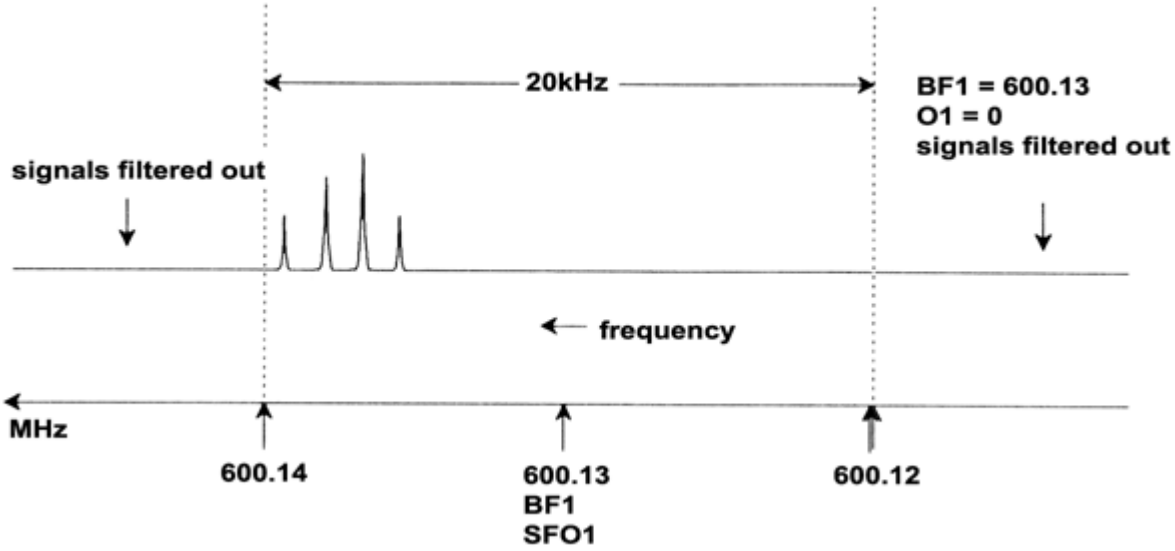
Consider a 600 MHz spectrometer used to observe hydrogen. The spectrometer is configured to have a BF1 of 600.13 MHz. (A 500 MHz spectrometer normally has a BF1 of 500.13 MHz, a 400 MHz a BF1 of 400.13 etc.)

If O1 is set to zero then:

SFO1 = 600.13 + 0 = 600.13 MHz

Thus the spectrum center would lie at 600.13 MHz. If SWH was set to 20 kHz, the spectrum might look like the one below.

Figure 7.7. Spectrum with BF1=600.13 MHz, 01=0Hz



It is clear from our hypothetical spectrum that the NMR signals all lie towards the high frequency end of the spectral width. Furthermore it is possible that some signals may lie above 600.14 MHz and since these signals lie outside the spectral window they have been filtered out and are not observed. To check for the presence of such signals two options are available:

a) The spectral width could be increased to encompass any missing signal. This however has disadvantages such as increasing the FID resolution (the lower the value of FIDRES, the better the resolution).

b) The preferred option would be to leave the spectral width unchanged, but assign a value to O1 to shift the center of the window.

In our example, the detected signals all lie in the region of 600.138 MHz and we wish to center the spectrum at this frequency.

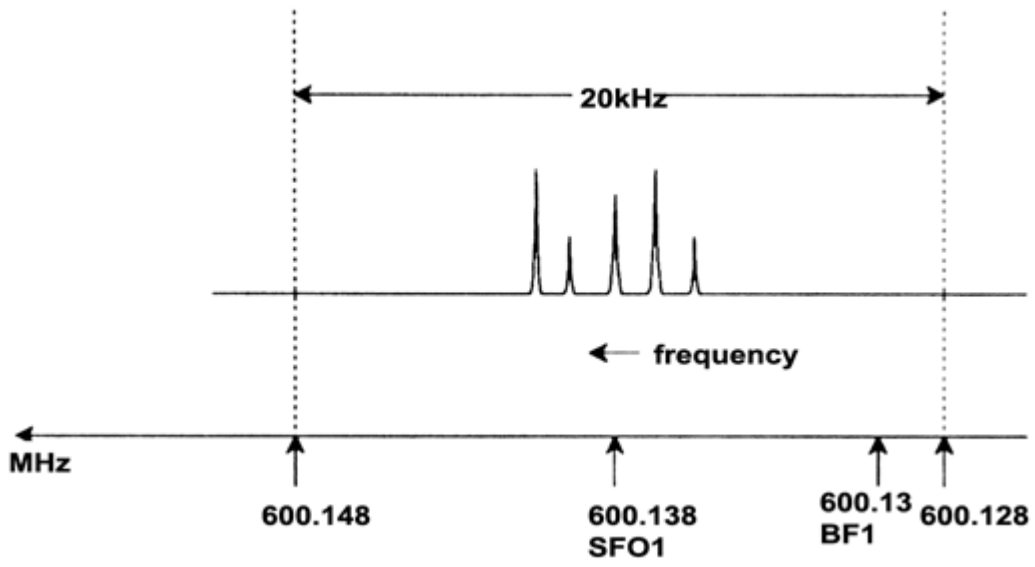
=> SFO1 = 600.138 = BF1 + O1

=> 600.138 = 600.13 + O1

=> O1 = 0.008 MHz = 8 kHz

Hence if O1, the offset frequency, is set to 8 kHz, the window is shifted to look like that in ***Figure 7.8.***.

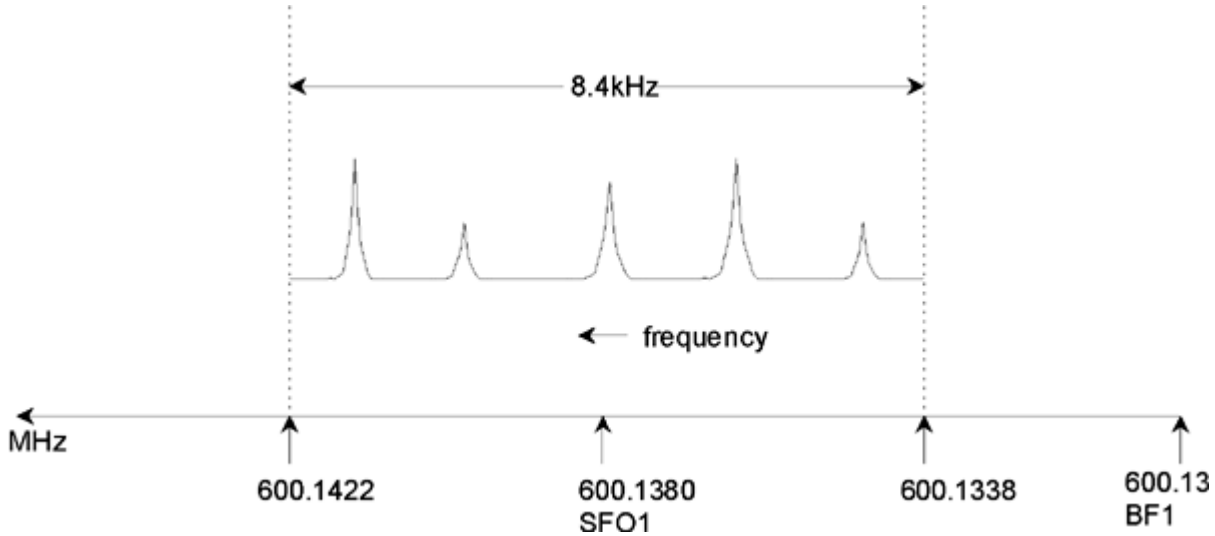
Figure 7.8. Spectrum with BF1 =600.13 MHz, 01= 8 kHz



Finally it is clear from the figure above that the NMR signals emitted by the protons in our hypothetical sample occupy only part of the spectral width. Therefore the spectral width could be reduced without any loss of relevant data. One advantage of decreasing the SW is that the spectral resolution is improved.(One disadvantage is that the time taken to acquire the data is proportionally increased).

In Chapter 3, ***"Introductory Theory and Terminology"***, it was stated that the chemical shifts of protons rarely exceed 14 ppm.This corresponds to 8.4 kHz on a 600 MHz spectrometer. ***Figure 7.9.*** shows the hypothetical spectrum redrawn with the value assigned to SWH reduced from 20 kHz to 8.4 kHz.

Figure 7.9. Spectrum with BF1=600.13 MHz, 01=8 kHz, SWH = 8.4kHz

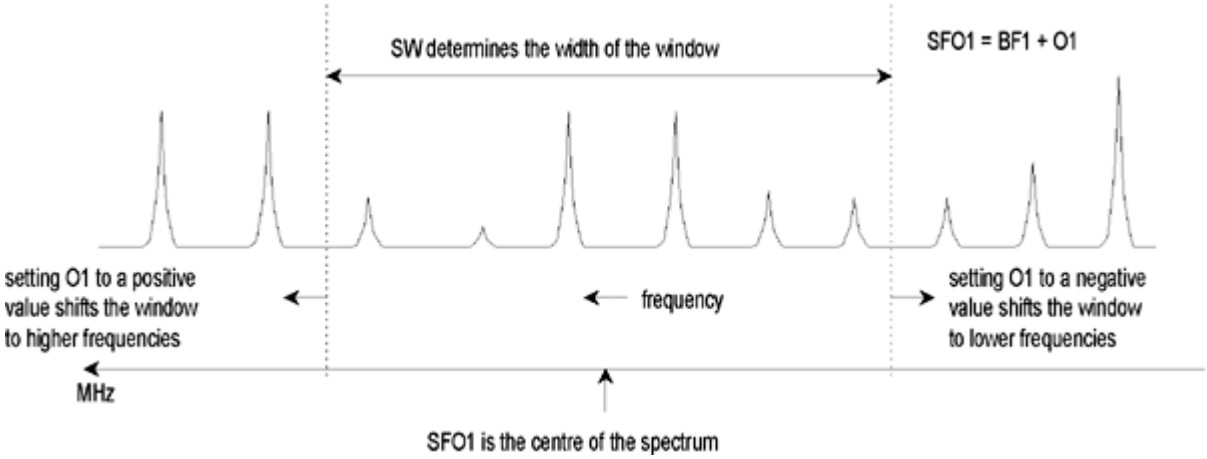


It should be noted that the value of SWH used in any given experiment is determined only by the sample under analysis and the required spectral resolution. The value of 14 ppm for hydrogen spectra will ensure that most proton signals are de-

tected. For a detailed study of a particular signal, however, much smaller values of SWH are used.

The figure below illustrates the general principles of how SFO1, BF1 and O1 interact (here shown with a new sample).

Figure 7.10. Interaction of SFO1, BF1 and O1.



To assist in setting suitable spectral (sweep) widths, a special function SW-SFO1 has been incorporated into the software under the utilities submenu. For more details see ***"Adjusting the Spectral Width with the SW-SFO1 function" on page 96***.

Pulse Programs/ Commands as/ased 8

The Pulse Programs “zg” and “zg30” 8.1

As mentioned in the section on acquisition parameters (***"Basic Acquisition Parameters: The “eda” Table" on page 72***), pulse programs are used to define the pulse sequence with which the sample is to be excited. A newly delivered AVANCE with SGU spectrometer will already have a “library” of standard pulse programs installed. The list of pulse programs may be viewed by entering the command ’edpul’. Scroll through the list until the program “zg30” is found. Click on “zg30” and the program, reproduced in ***Figure 8.1.***, will be displayed.

A detailed description of pulse programming is beyond the scope of this manual, although a few comments on the program entitled “zg30” should help the user understand which parameters are important and why. A standard 1-D experiment would use a 90 degree pulse to excite the NMR signal to maximize the emitted signal. However, this also maximizes the duration that must elapse between successive pulses in order for the sample to relax. It can be shown that in the case of repeated excitation of a sample, a more efficient method is to excite the sample with a 30 degree pulse and reduce the relaxation delay accordingly. Although each individual emitted signal is weaker, the faster accumulation of data that the 30 degree pulse allows results in an overall increase in sensitivity. The two standard programs are "zg“ for a 90 degree excitation pulse and "zg30” for a 30 degree excitation pulse.

For more information on the conventional nomenclature used for power levels, pulses, delays and loop counters throughout the Bruker series of pulse programs, refer to the text file **$XWinNMRHome/exp/stan/nmr/lists/pp/Param.info**. For information on the conventional nomenclature used in the names of Bruker pulse programs refer to **$XWinNMRHome/exp/stan/nmr/lists/pp/Pulprog.info**. Finally, a useful file for checking changes in the latest software release is **$XWinNMRHome/exp/stan/nmr/lists/pp/update.info**.

$XWinNMRHome stands for the name of the directory in which the XWIN-NMR program was installed (typically /u under IRIX, C:\Bruker\XWIN-NMR under Windows and /opt/XWINNMR under Linux).

Details of the “zg30” Program 8.2

Any entries in a pulse program that are preceded by a semicolon are simply comments which are included to assist the user. The program compiler will ignore the contents of any line that begins with a semicolon. For the purposes of this description each line of the “zg30” program has been assigned a number which however will not appear on the XWIN-NMR screen whenever the pulse program is displayed.

The first four lines are examples of comments. Line 1 simply gives the title of the pulse program and line 2 the version. Line 3 suggests that this pulse program is suitable for a 1-D NMR experiment and line 4 reminds the user that instead of a 90 degree excitation pulse the program is designed to use a 30 degree pulse (and hence the name of the program).

Figure 8.1. The Pulse Program “zg30 “

```
1.  ;zg30
2.  ;avance-version (00/02/07)
3.  ;1D sequence
4.  ;using 30 degree flip angle

5.  #include <Avance.incl>

6.  1 ze
7.  2 d1
8.   p1*0.33 ph1
9.   go=2 ph31
10.   wr #0
11. exit

12. ph1=0 2 2 0 1 3 3 1
13. ph31=0 2 2 0 1 3 3 1

14. ;pl1: f1 channel - power level for pulse (default)
15. ;p1: f1 channel - 90 degree high power pulse
16. ;d1: relaxation delay; 1-5 * T1
```

comments

actual pulse program

comments

Line 5 is not a comment, but rather a standard inclusion dealing with pulse programming text defined in separate files.

Line 6: 1 ze - This line is not preceded by a semicolon and is actually the first line of the program proper. Any line within a program can be numbered to facilitate loops and this one is naturally numbered as line 1. The command "ze" (zero) is a command that causes any data currently in memory to be replaced by data acquired during the first scan. Data acquired during subsequent scans will then be added in the memory. The command "ze" effectively clears, or zeroes, the memory in preparation for data that will be acquired during an experiment.

Line 7: 2 d1 - This line is a delay command. The delay is called "d1“. A delay is simply a pause during which no pulses are transmitted. The length of d1 can be specified by entering a suitable value in the delay array of the "eda“ menu or entering it explicitly in the XWIN-NMR command line.

Line 8: p1*0.33 ph1 - The command p1*0.33 instructs the spectrometer to emit a pulse with length p1 multiplied by 0.33. This is the 30 degree excitation pulse, since conventionally p1 is the 90 degree pulse. Any pulse will have three principle characteristics: 1) length, 2) amplitude, and 3) phase.

The **length** or duration of the pulse may be set by entering a suitable value of p1 in the pulse array of the eda menu or explicitly in the XWIN-NMR command line.

The **amplitude** of the pulse is often referred to as its power level. In this case, no power setting is specified, so the spectrometer defaults to the value assigned to pl1. The value of “pl1” may be set by entering a suitable value in the power level array of the “eda” menu, or explicitly in the XWIN-NMR command line.

It should also be mentioned, that when no channel is specified for a particular pulse, the pulse is automatically sent to the observe channel (i.e. channel F1). Line 8 also assigns the **phase** to the pulse with the command “ph1”.

Line 9: go=2 ph31 - This single command starts a train of processes including the opening of the receiver and digitalization of NMR signals. When the acquisition has been completed, i.e. after TD points have been digitized, the program loops back to the line beginning with "2" which is Line 7. The entire process is repeated NS times (assuming that DS=0). If NS=8 then lines 7, 8, and 9 will be looped through 8 times. Line 9 also sets the phase of the receiver with the command ph31.

The need for the delay “d1” in Line 7 should now be more apparent. Exciting an NMR sample with a series of pulses and insufficient relaxation delay causes it to become saturated. In other words it absorbs more energy than it can release. To give the sample time to release more energy, the delay “d1” precedes each pulse. This is referred to as allowing the sample sufficient time to relax.

Line 10: wr #0 - This command instructs the computer to store the acquired data on the hard disk. The data is stored in the current data set as defined by the parameters NAME, PROCNO, EXPNO, etc.

Line 11: Exit - This command tells the computer that the end of the pulse program has been reached.

Line 12 and 13: These two lines define the phase cycling sequence for both the transmitter (ph1)and receiver (ph31).

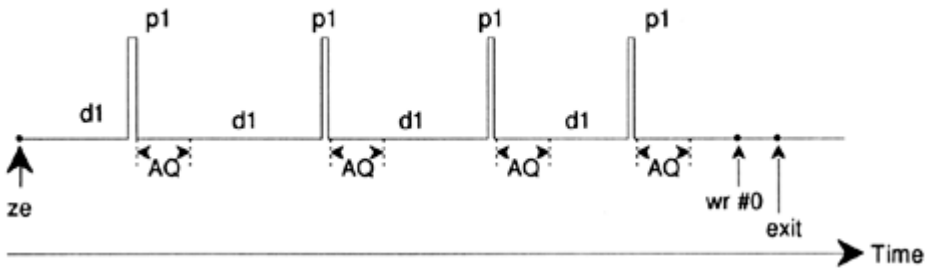
The above description has been included to give the reader some idea of the parameters that must be set when running the pulse program “zg30”, namely “d1”, “p1”, and “pl1”. Note that the last three lines of the pulse program are comments used to explain the significance of these three parameters to the user. The precise value assigned to “p1”, “d1”, and “pl1” will depend on the sample to be analyzed and the type of probe to be used.

The common pulse program entitled “zg” is identical to “zg30” except that a 90 degree pulse is transmitted rather than a 30 degree pulse.

Figure 8.1. is a pictorial representation of the pulse program “zg”, with NS=4 and DS = 0.

At this point, the reader should have gained a basic understanding of the main elements involved in acquisition. The rest of this manual will be concerned with step by step accounts of how to actually acquire spectra.

Figure 8.2. The Pulse Program “zg” where NS=4 and DS=0.



The Commands “as” and “ased” *8.3*

Now that you are more familiar with the acquisition software, and some basic pulse programs, it might be opportune to introduce two further commands: “as” and “ased”. Both commands fulfill the same purpose and differ only in presentation. The commands may be used to set the acquisition parameters for a particular pulse program, e.g. pulses, delays, etc.

The two commands refer to the current pulse program i.e. the program entered for PULPROG in the “eda” table. Having ensured that “zg30” is the current pulse program enter ’ased’.

The display changes to an “eda” type table. Note, however, that the “eda” table presents the complete delay and pulse arrays, and that the “ased” table simply contains “p1” and “d1”. This is because the pulse program “zg30” uses no other pulses or delays. The parameters in the “ased” table may now be modified to suit the experiment. Any changes will be carried over into the “eda” table. Click on SAVE to return to the MAIN MENU. Thus, “ased” is a useful command in that it concentrates on all relevant parameters for a specific experiment.

Whereas “ased” displays all relevant parameters at once, the command “as” will display them sequentially.

1. Enter ’as’.

The screen will display the present setting of PULPROG as “zg30”.

2. Press the "**enter**“ key. The pulse program “zg30” is displayed on the screen. This enables the user to view the current pulse program.

The user is now prompted to assign values to the various acquisition parameters relevant to “zg30”. The current value of the parameter is displayed and to leave this value unchanged press the ENTER key. To assign a new value simply enter the new number.

When all parameters are set the message “**as: finished**” will be displayed at the base of the screen:

Finally, do not forget that any individual parameters may be adjusted directly from the XWIN-NMR command line. This is particularly useful when a “fine tuning” of an experiment is required.

Proton Spectrum 9

Introduction 9.1

This section describes the procedure to acquire a proton spectrum. The sample to be used is 100 mg of cholesterylacetate in chloroform-d with 0.5% TMS. The principal steps are:

1. Insert the sample and start spinning if desired.
2. Lock the spectrometer (refer to the section ***"Locking the Sample" on page 58***).
3. Tune and match if required.
4. Optimize the Z and Z^2 shims.
5. Create a new data set, e.g. hydrogen 1 1.
6. Read in a standard parameter set and adjust the parameters “p1” and “pl1”.
7. Set the receiver gain with "rg“ or “rga”.
8. Reduce NS from 16 to 1 if you wish to acquire a single scan spectrum only (optional).
9. Enter ’zg’ to start the acquisition.
10. Enter ’ft’ and ’apk’ to transform and automatically phase correct the spectrum.

Various other processing techniques, such as manual phase correction and calibration, will be described.

Steps 1, 2, 3 and 4 have already been described in earlier sections, so we will begin at step 5.

Creating a New Data Set 9.2

Before setting the appropriate acquisition parameters, we recommend you to create a new data set. In the following description, data sets with specific NAMES are created, although you may choose any NAME you wish. The user is always advised to create a new data set before adjusting the acquisition parameters. In this way the effect of the modification can always be observed by comparing the two data sets. The easiest way to define a new data set is to simply increment the parameter EXPNO.

Create a new data set.

Enter ’edc’ and create the following data set:

NAME	hydrogen
EXPNO	1
PROCNO	1

Click on 'Save'.

Reading in the Standard Parameter Set 9.3

Load the standard parameter set entitled "PROTON“ by entering:

'rpar proton'

When prompted click on “acqu” and “proc”, and then “copy“.

Regardless of any previous settings in "edasp”, it will now be set to "observe proton” once the parameter set entitled "PROTON“ has been loaded. You can easily check this by entering 'edasp'. All other nuclei will be set to "off”.

The “getprosol” Command 9.3.1

The “prosol” table, which may be viewed with the command “edprosol”, is a list of spectrometer and probe specific parameters. Parameters, such as the 90 degree or decoupling pulse length with associated power levels, can be stored for any specified nucleus on any available channel. If the “prosol” file has been set up correctly, the user should be able to easily access these values. These values are automatically loaded using the command “getprosol“. The software will recognize which nucleus has been selected for which channel and load the appropriate values. It is important to note that the “prosol” routine will only work correctly, if the “edhead” command has been used to define the current probe to match the probe in the magnet. You will notice in the table below that the “getprosol” command has been suggested as a simple way to set values to parameters such as “p1” and “pl1”.

Enter 'eda' and observe the acquisition parameters that have been set as listed in the table below. Only the most relevant parameters will be discussed here. You should note that several parameters are largely dependent on the particular system, and therefore there is no "ideal“ value possible in a standard parameter set. You can use either the "as” or "ased” commands to conveniently check whether the parameters have been set correctly.

Table 9.1. The “eda” Parameters after Loading Standard Parameter Set “Proton”

Parameter	Value	Comment
PULPROG	zg30	For a description of this pulse program see section **8.2**
TD	65536	Not critical. 64K is rather large. You may reduce this to 16K points if you wish to save time as long as the FID decays sufficiently quickly or if “d1” is sufficiently long.
NS	16	Until other parameters have been optimized, there is little point in investing time on a large number of scans. You may reduce this to 1 if you wish to save time initially.
DS	2	Two dummy scans is fairly standard.

Table 9.1. The “eda” Parameters after Loading Standard Parameter Set “Proton”

Parameter	Value	Comment
SW	20.6 ppm	For proton spectra this is rather large. However, a large spectral width is best as we are analyzing the sample for the first time. Note it is easier to deal with the parameter SW as opposed to SWH.
NUC1	1H	This parameter merely provides the user with information.
SFO1		See O1P
O1P	6.175 ppm	This will determine the value of the transmission frequency and hence the center of the spectrum in ppm. With the center frequency of 6.175 ppm and a spectral width of 20.6 ppm, a chemical shift range between -4.125 ppm and +16.475 ppm is covered. The greater the SW, the less critical the value of the offset.
BF1	system dependent	Set automatically
D*array*	d1=1-2 s	The default unit for delays is seconds. So simply entering ’2’ will set a delay of 2 seconds. In the pulse program “zg30”, d1 is the only delay that is used. Its purpose is to allow the sample enough time to relax and its value is not critical as long as it is not too short. With the present sample, 1 or 2 seconds is fine.
P *array*	p1= 10 µs	The parameter “p1” is the value of a 90 degree excitation pulse and a pulse of duration p1*0.33 is the only pulse used in the “zg30” program. You should consult with the system manager on how to set “p1”. The simplest method is to use the “getprosol” command. The 10 µs value set by the standard parameter set is only a typical value. Remember that if you set 10 µs then the pulse actually transmitted will be 3.33 µs. If you are familiar with the “paropt” procedure you might wish to use it to optimize “p1”. Finally, the experiment should still work if “p1” is not optimized, however sensitivity will be lost.
PL *array*	pl1 = 120 dB	Again you may wish to consult with the system manager on how to set “pl1”. As before, the simplest method is to use the “getprosol” command. The optimum value for “pl1” will depend on the particular system. Remember that “p1” and “pl1” are linked. To protect the probe, the value of “pl1” in a standard parameter set is set to 120 dB. This is way too high and will need to be reduced.
RG	4	The optimal value will depend on the particular system, although 4 is rather low. Use “rga” to automatically optimize the receiver gain.

Setting the Receiver Gain *9.4*

Enter ’rga’.

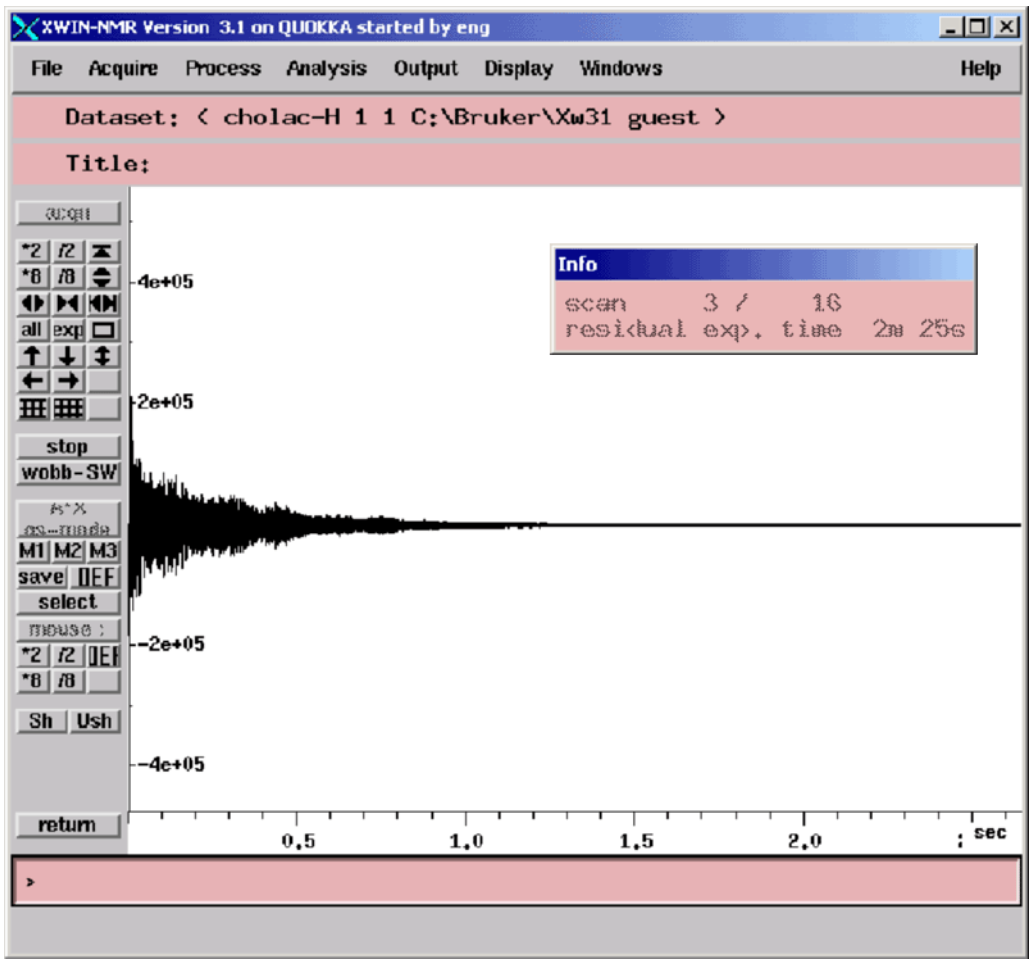
The spectrometer will carry out several acquisitions in order to find the optimal gain. The value of RG will be automatically loaded into the “eda” table and used for any further acquisitions.

Starting the acquisition 9.5

To start the acquisition, enter 'zg'.

During acquisition you can observe the buildup of the FID in the acquisition window by entering 'acqu' on the command line. If NS>1, you can observe the accumulation of successive FID's. Note the information in the acquisition window which is a real-time update of the status of the scan counter and the example below is to illustrate its operation (refer to the figure below).

Figure 9.1. Acquisition Window - Status of the Scan Counter



Scan 3/16 would mean that the spectrometer has completed three scans and is currently performing the fourth of 16 scans.

On the BSMS keyboard you will also observe a real-time display of the

- Pulse transmission
- Forward and reflected power
- ADC switching on and off

Each scan takes place in a matter of seconds. You can repeat it as often as you wish to get familiar with the various displays. Each time simply enter 'zg' to acquire an FID. In each case, the new FID(s) overwrites the previous one(s) (assuming that you have not incremented the EXPNO).

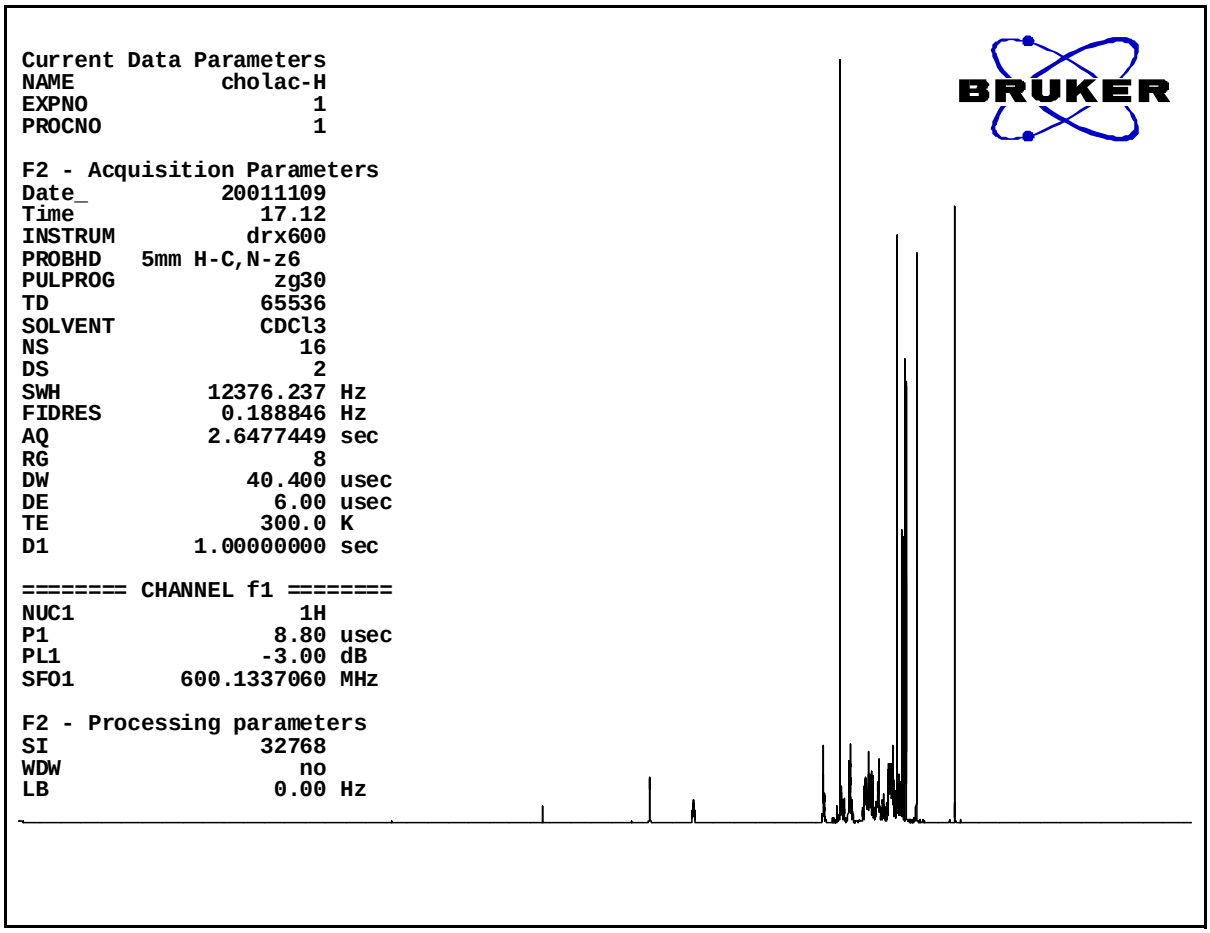
Fourier Transform and phase correct the spectrum 9.6

Enter 'ft', followed by 'apk'.

This carries out a Fourier transformation, followed by an automatic phase correction.

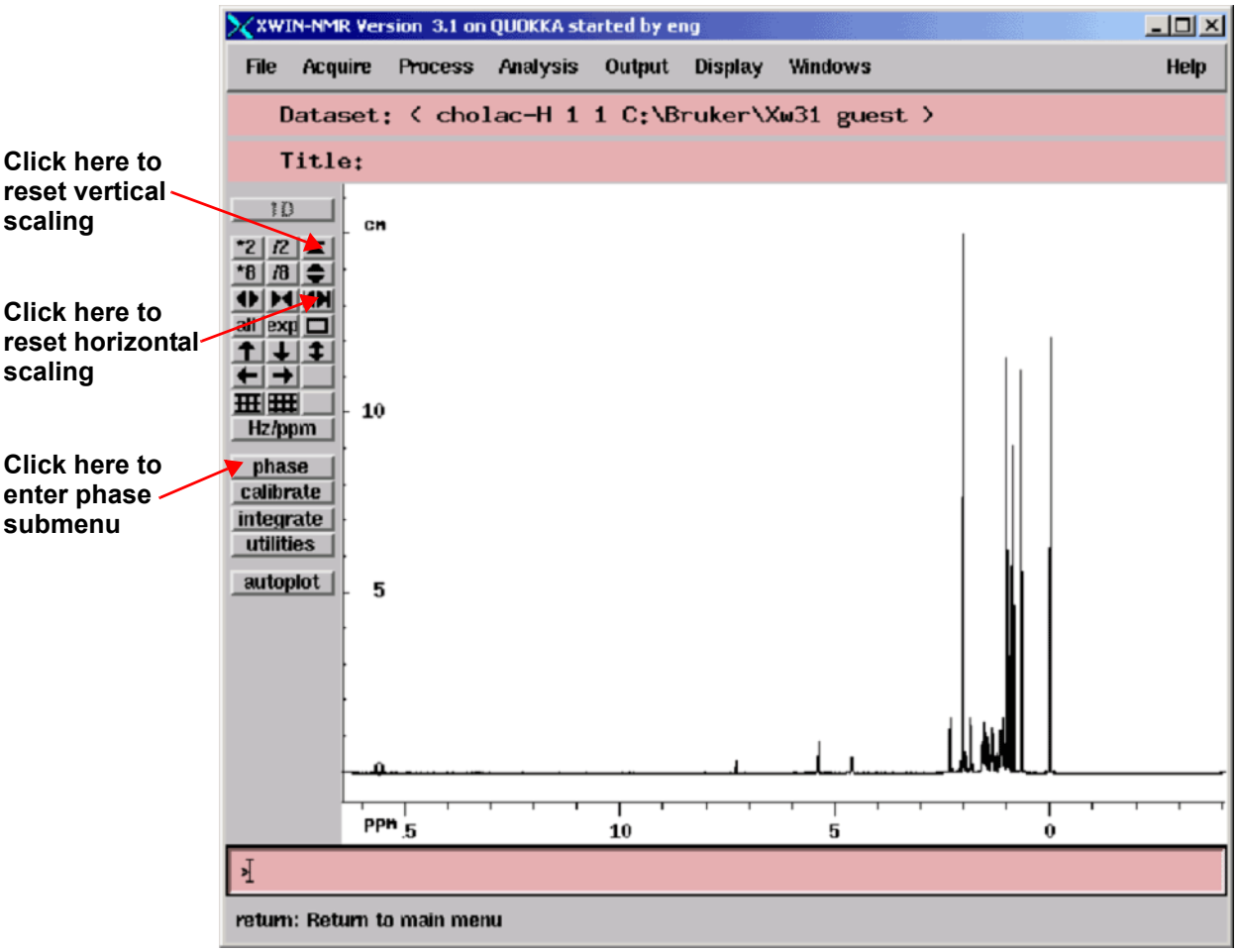
The resulting spectrum should be more or less the same as the one shown below (the parameter list has been added for convenience):

Figure 9.2. Proton Spectrum of Cholesterylacetate. Sixteen scans. SW = 20.66



Before proceeding to the next section it is instructive to describe some basic processing techniques.

Figure 9.3. Some Useful Features of the Main XWN-NMR Window



Basic Processing: Fourier Transformation *9.7*

A Fourier Transformation is used to convert the FID to a frequency spectrum. This is implemented using the "ft“ command. The number of points used to form the resulting spectrum is determined by the parameter SI (size). The FID is transformed into a spectrum consisting of SI data points in the real part and SI data points in the imaginary part. The normal setting for SI is SI = TD/2. If you have loaded the parameter set "PROTON“ you can easily verify that TD = 64K and Si = 32K.

Upon entering the command ’ft’, the display automatically returns to the main window. If you wish to reenter the acquisition window, enter ’acqu’.

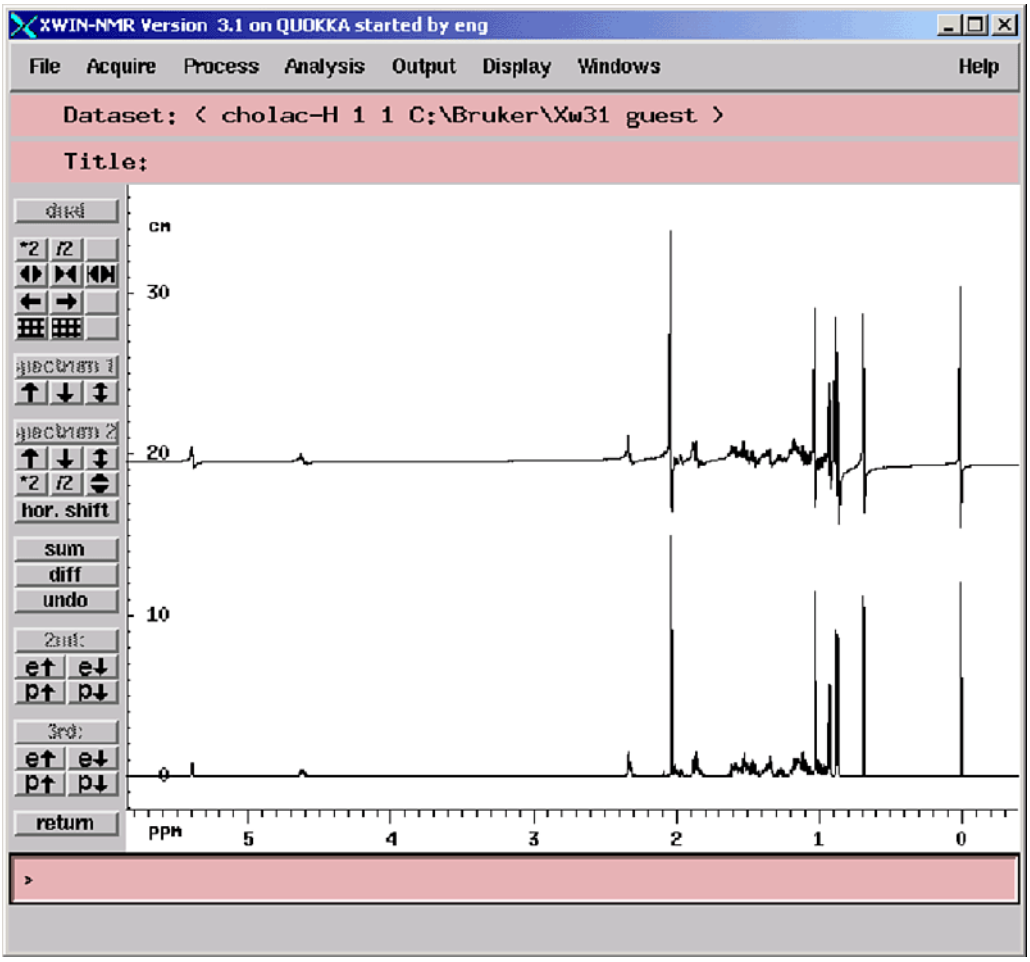
If at some stage the data disappears from view, click on the scaling functions demonstrated in ***Figure 9.3.*** to restore the data to the screen window.

Note that the spectrum may appear distorted after the “ft” command, but this can be overcome using phase correction techniques described below.

Phase Correction 9.8

Phase shifts of either the transmitted or received signal within the spectrometer hardware are inevitable and must be initially corrected for. Once this correction has been made and assuming that no acquisition conditions or parameters have been changed, the phase correction should remain constant and thus is easily stored and reapplied. The figure below is an example of the effects of phase correction.

Figure 9.4. Example of Spectrum with Phase Correction (below) and without (above)



The user needs to understand the distinction between zero and first order phase corrections.The numerical values of these two corrections are stored using the parameters phc0 and phc1, respectively. The value of these two parameters can be inspected by entering them at the command line or by calling up a table of processing parameters with the “edp” command

Zero order phase correction applies the same phase correction to the entire spectrum. This is to account for any phase shifts that may have occurred independently of the signal frequency.

First order phase correction applies a phase correction proportional to the signal frequency.This is because phase shifts usually contain a frequency dependent component.

To phase a spectrum, it is usually necessary to apply both a zero and a first order phase correction. To do this, there are several options available. The simplest procedure is automatic phase correction which is implemented using the command “apk”.

The following table summarizes some useful phase correction procedures. The user can easily experiment with the various options. The raw data is not affected by whatever processing is applied.

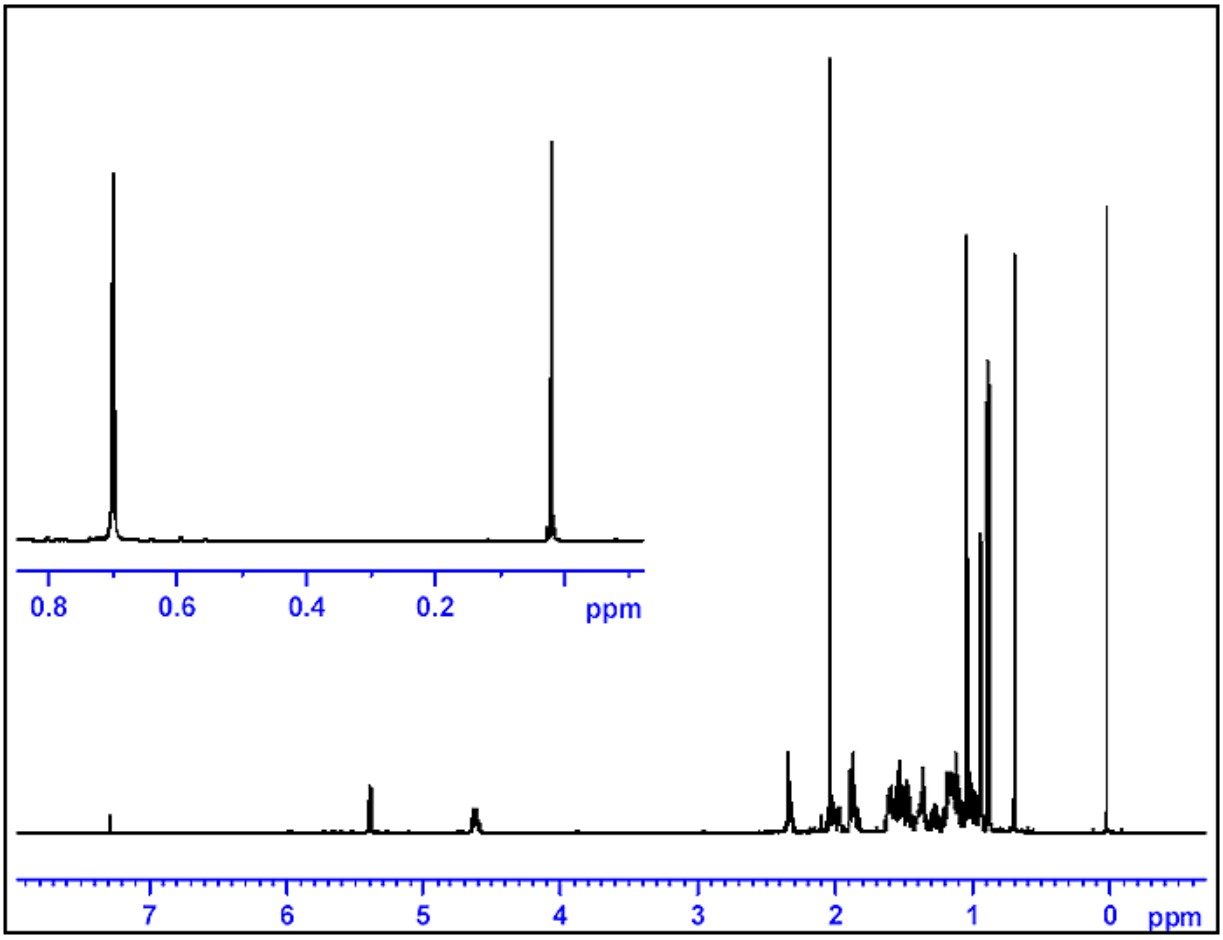
Table 9.2. Phase Correction Methods

Window	Method	Result
Main	Enter command ’apk’	Phase correction is carried out automatically.
Main	Enter command ’pk’	Apply phase correction based on the last phase values stored.
Main	Enter command ’fp’	Combines a Fourier Transform and phase correction based on last stored phase correction values.
Phase Submenu	Click on biggest	The spectrum is phase corrected by adjusting the phase of the most intense peak. The same zero order phase correction is automatically applied to the entire spectrum.
Phase Submenu	Click on ’ph0’	Allows the user to apply a manual zero order phase correction using the mouse.
Phase Submenu	Click on ’ph1’	Allows the user to apply a manual first order phase correction using the mouse.
Phase Submenu	Click on cursor	The user may choose the reference peak based on which zero order phase correction is applied to the entire spectrum. This is an alternative to using the most intense peak.

Calibration of Spectrum *9.9*

It is conventional in NMR spectroscopy to calibrate the spectrum by setting the TMS peak to 0 ppm. Prior to calibration it may be useful to expand the spectrum horizontally in the region on either side of the TMS signal itself, as this will aid in pinpointing the exact position of the peak. The TMS signal can be identified as the lowest frequency (right most) signal in the spectrum.

Figure 9.5. Plot Identifying Location of TMS Peak as right most Peak in Spectrum



Procedure to Expand the Spectrum Horizontally *9.9.1*

1. Ensure that the main XWIN-NMR window is displayed.
2. Using the mouse position the cursor within the spectral window and click the left mouse button. It will automatically be tied to the spectrum.
3. Using the mouse position the cursor on one side of the region of interest and click the middle button.
4. Using the mouse position the cursor on the other side of the region of interest and click the middle button. The defined region will be automatically expanded to fill the entire screen. Click the left mouse button to release the cursor.
5. To reset the horizontal spectrum and view the entire spectrum, click on the scaling button as demonstrated in ***Figure 9.3.***.

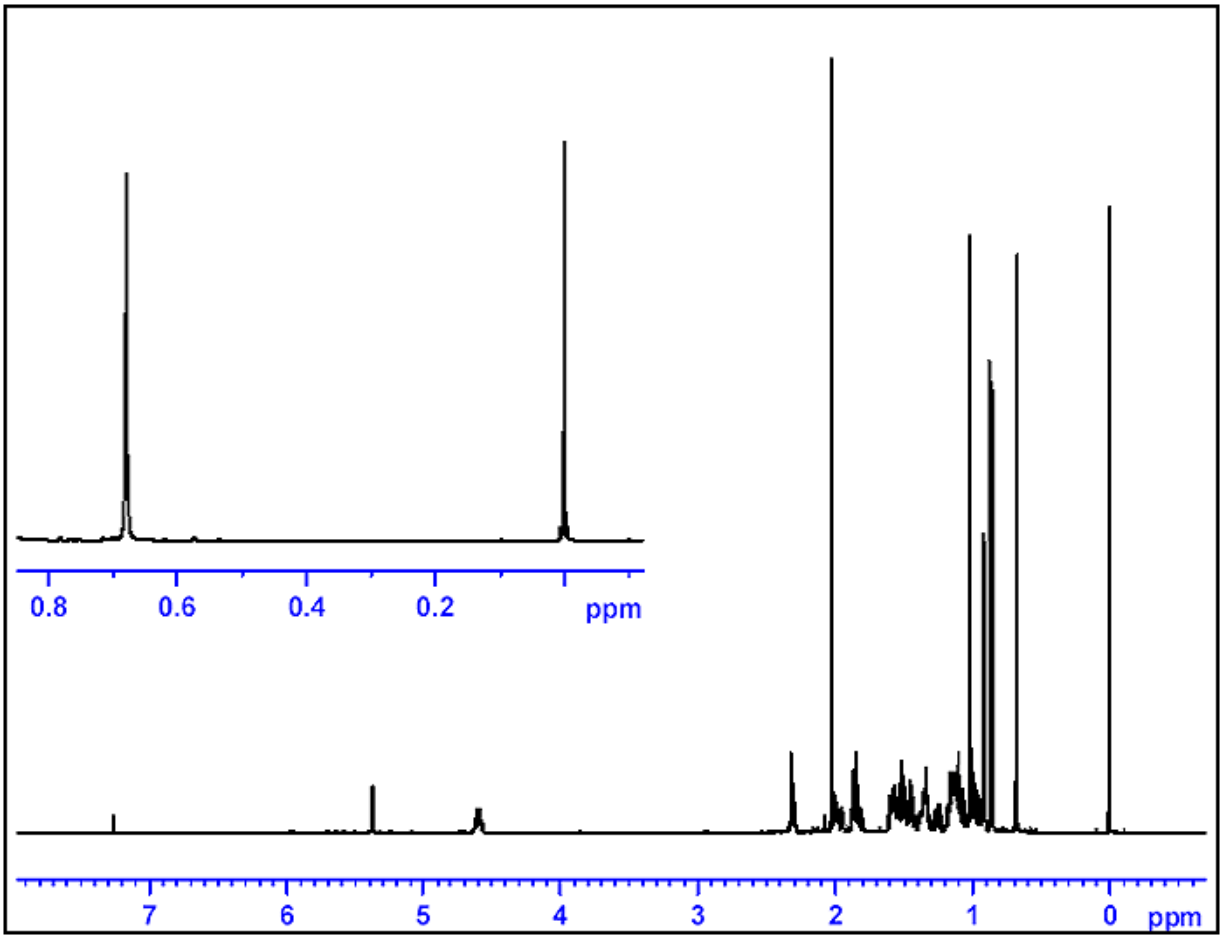
Calibration Procedure *9.9.2*

1. If required, click on the “**Hz/ppm**” button so that the horizontal axis is displayed as ppm.

2. Click on “**calibrate**”. The cursor will be automatically tied to the spectrum. Move the mouse until the cursor is positioned on the TMS peak.
3. Click the middle mouse button, and when prompted for the cursor frequency in ppm, enter zero.

The horizontal scale of the spectrum will be adjusted displaying the TMS peak at 0 ppm. The spectrum will appear as in ***Figure 9.7.***

Figure 9.6. Proton Spectrum of Cholesterylacetate



Sixteen scans. SW = 20.66 pm, TMS peak calibrated to 0ppm.

An automatic calibration of the spectrum may also be achieved by entering the command ’sref’. This starts a procedure in which the software searches for a signal in the region of 0 ppm and automatically sets its value to exactly 0 ppm. For the “sref” procedure to work, the “edlock” table must be set correctly and the appropriate solvent chosen in the lock routine.

Adjusting the Spectral Width with the SW-SFO1 function *9.10*

It is apparent from ***Figure 9.7.*** that all the proton NMR signals from cholesterylacetate lie in the 0-8 ppm region and thus there is no advantage in using the SW of

20.66 ppm as set by the standard parameter set “PROTON”. (The 20.66 ppm SW is set in the standard parameter set to encompass a large enough range of proton signals regardless of the sample or solvent). A useful technique in setting the appropriate SW is to expand the spectrum horizontally so that only the region of interest is displayed. Clicking on the SW-SFO1 button, which can be found in the “utilities” submenu, will then have two effects:

1. The SW will automatically be set to encompass only the displayed region.
2. The observe frequency (SFO1) will be set to the middle of the displayed region, i.e. the observe frequency (SFO1) will be at this point.

Adjusting the SW for Cholesterylacetate Spectrum *9.10.1*

As this will change basic acquisition parameters you may decide to create a new data set. The easiest way to define a new data set is to simply increment the parameter EXPNO.

Procedure

1. Enter ’edc’ or ’new’ and create the data set as below:

NAME hydrogen

EXPNO 2

PROCNO 1

This creates a new data set “hydrogen 2 1”. A quick check in the “eda” table will show that the acquisition parameters set for “hydrogen 1 1” will be carried over to the new data set. The parameters and data in “hydrogen 1 1” will now not be overwritten.

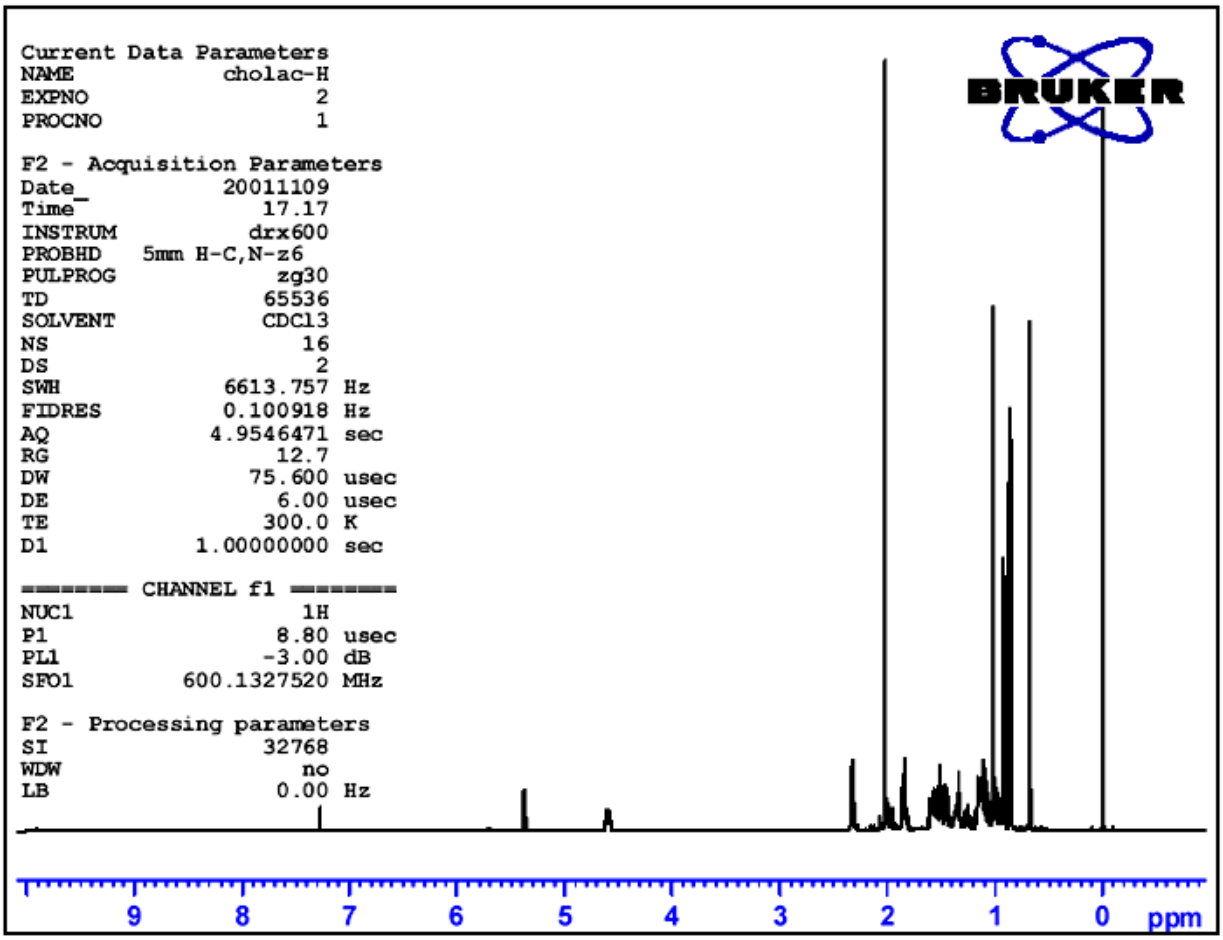
2. Reduce NS to 1, acquire an FID and use the command *fp* to transform and phase correct. Note that the stored phase correction is carried over as well as the calibration of the TMS peak to 0 ppm.
3. Using the mouse, position the cursor within the spectral window. Click the left mouse button and it will automatically be tied to the spectrum.
4. Position the cursor at 10 ppm (approx.) and click the middle mouse button.
5. Position the cursor at -1 ppm (approx.) and click the middle mouse button.
6. Click the left mouse button to release the mouse from the spectrum.
7. The spectral region from -1 to 10 ppm is now expanded to encompass the entire screen width.
8. Enter the utilities submenu and click on “SW-SFO1” while the expanded region is displayed.

   The SW will automatically be set to encompass only the displayed region and the observe frequency (SFO1) will be set to the middle of this displayed region. You can check this in the eda table where new values of SW (11 ppm approx.), 01P and SFO1 will have been be set.
9. Acquire an FID, Fourier transform and phase correct.

Note that the use of "fp“ will most likely not yield the correct phase correction. This is a consequence of the new SW and SFO1. You can use the "apk” command or adjust the phase manually as described in section ***9.8***.

10. Increase the number of scans to 16, acquire a spectrum, transform and phase correct. The spectrum should now appear as in the figure below.

Figure 9.7. Proton Spectrum of Cholesterylacetate



Sixteen scans. SW = 11pm TMS peak calibrated to 0ppm.

Increasing the number of scans *9.11*

The signal/noise ratio of a spectrum may be improved by signal accumulation. The quantitative improvement is proportional to the square root of the number of scans, e.g. 64 scans will increase the sensitivity by a factor of 8 compared to a single scan at the cost of an increased acquisition time. The number of scans to be carried out is set by the value assigned to NS.

1. Enter ’edc’.
2. Assign the value of “3” to EXPNO.The current data set is now "hydrogen 3 1“.
3. Enter ’eda’ and set NS to 64

   (alternatively enter ’ns’ at the command line and you will be prompted for a value of NS).
4. Enter ’acqu’ to view the acquisition window

5. Enter ’zg’

Note that the acquisition window now displays the progress of the 64 scans in real-time. Also displayed is the residual experimental time. The BSMS keyboard display will flash as each excitation pulse is transmitted and the ADC LED will be observed to flash on and off.

6. When the acquisition is complete, Fourier transform and phase correct. The spectrum should display an improved (eight fold) signal/noise ratio.

At this stage you may wish to explore the SINO program which is very useful for automatic calculation of the signal to noise ratio. Although a description of SINO is beyond the scope of this manual, it is described in the XWIN-NMR Processing Reference Manual available in the help menu.

Comparing various spectra is best achieved using the Dual Display function. This is also described in the XWIN-NMR Processing Reference Manual. You may wish to explore this function at this stage.

^{13}C Spectrum without decoupling

10

Introduction 10.1

This chapter will describe the analysis of a sample of cholesterylacetate for carbon. To begin with, the pulse program “zg30”, which does not use decoupling, will be used. The next chapter will describe a carbon observe with proton decoupling experiment which is more suitable. However, for beginners it is worth performing the acquisition without decoupling to help understand the benefits of decoupling. If you only wish to produce a spectrum, then skip to the next chapter (after performing steps 1-4 below). If a probe unsuited to observing carbon is in the magnet, it will be necessary to change the probe. The procedure to change a probe was outlined in the section ***"Changing a Probe" on page 41***, but you should consult with the system manager beforehand.

Procedure 10.2

1. Replace the 100 mg cholesterylacetate in $CDCl_3$ sample used in the previous section with a 1 g sample in the same solvent. NMR is less sensitive to carbon than to proton, thus the use of a more concentrated sample is advisable.
2. Insertion of the new sample will result in a loss of the lock. Lock the new sample as outlined in the section ***"Procedure to Lock the Sample" on page 59***.
3. Readjust the Z and Z^2 shims until the lock level is optimized (see the section ***"Routine Shimming" on page 62***).
4. If it has not already been done tune and match the probe for ^{13}C observation as outlined in the section ***"Tuning and Matching the Probe" on page 53***.
5. Use the “edc” command to create a new data set, e.g. carbon 1/1.
6. Read parameter set C13CPD with the command "rpar C13CPD all“.
7. Enter ’edasp’, set Channel F1 to ^{13}C, and set all the other channels to "off“.

8. Enter 'eda' and set the parameter values as shown in the table below. You can use either the "as" or "ased" commands to conveniently check whether the parameters have been set correctly.

Table 10.1. The "eda" Parameter Values

Parameter	Value	Comment
Pulprog	zg30	Comparable to previous proton spectra
TD	16k	
NS	16	
DS	0	
d1	2s	
SW	250ppm	Carbon spectra cover a wider range than ^{1}H spectra
O1P	100ppm	This is a suggested value which can be optimized later. 100 ppm is the typical shift used in carbon standard parameter sets
RG	8k	Alternatively you can use the "rga" command to set the receiver again
P1	-	The parameter "p1" is the value of a 90 degree excitation pulse and a pulse of duration P1*0.33 is the only pulse used in the "zg30" program. You should consult with the system manager on how to optimize "p1" or use the "getprosol" command. If you are familiar with the "paropt" procedure you might wish to use this to optimize "p1". Finally, the experiment should still work if "p1" is not optimized, but sensitivity will not be optimum.
pl1	-	Again you may wish to consult with the system manager as to how to set "pl1" or use the getprosol command. The optimum value for "pl1" will depend on the particular system and in particular the probe. Remember that "p1" and "pl1" are linked.

9. Enter 'rga' to set an appropriate receiver gain (RG).
10. Acquire the FID by entering 'zg'.

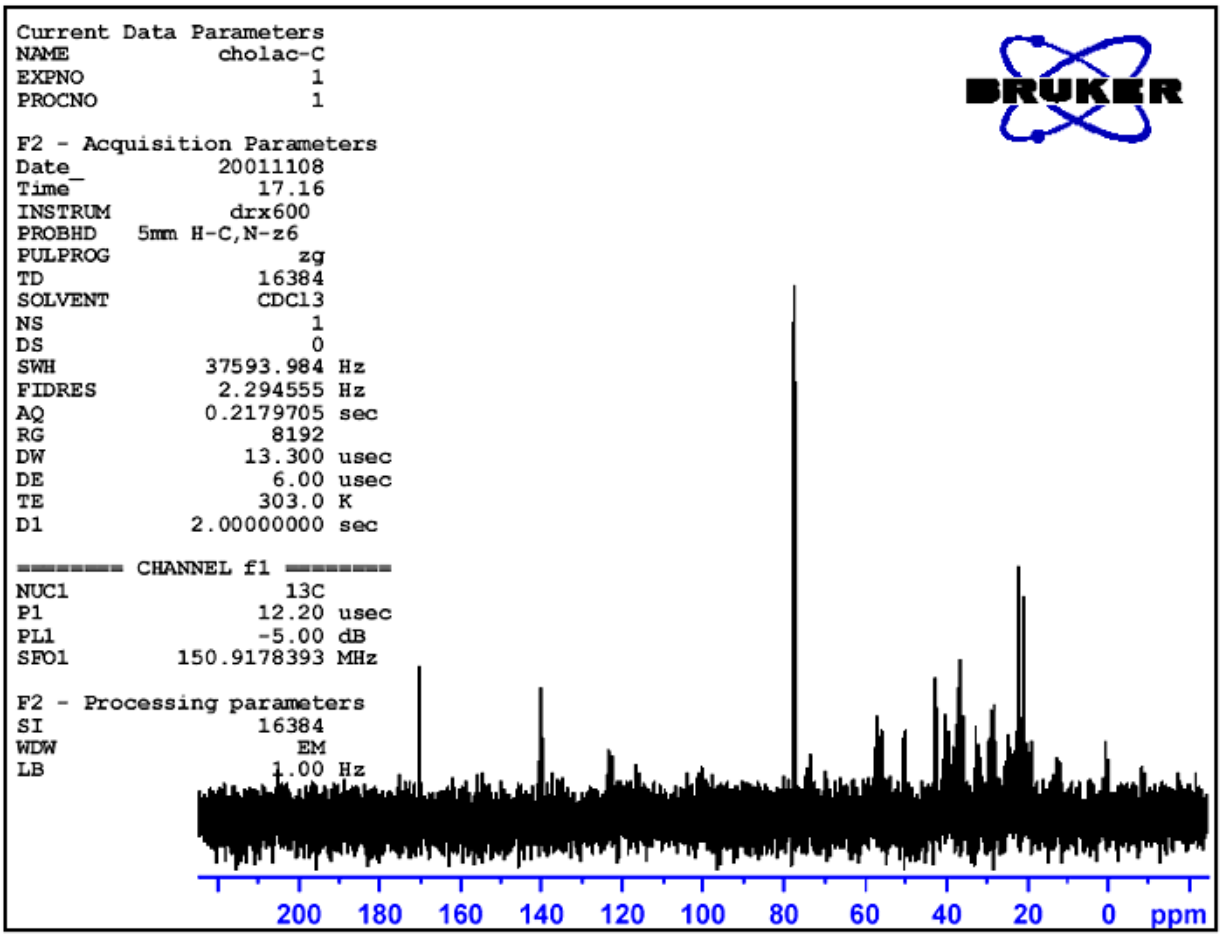
Note that the row of LED's for an X type nucleus of the BSMS keyboard display will flash.

11. Enter 'si', and when prompted enter a value of 8k.
12. Enter 'ft'.
13. Phase correct the spectrum using the mouse and store the correction. Note that the phase correction stored for the proton spectrum should not be used for the carbon spectrum. However, once the ^{13}C spectrum has been phase corrected, then this phase correction may be used for subsequent ^{13}C spectra. Alternatively, enter 'apk' to automatically phase correct.

The resulting spectrum is most likely to be noisy and possibly display only one significant peak. This largest peak arises from the chloroform solvent. Expand the spectrum around this peak and you will see that the chloroform signal is actually a triplet due to coupling to deuterium (Unlike protons, which has a spin of 1/2, Deuterium has a spin of 1, which leads to different splitting).

14. Calibrate the central peak of the triplet to 77 ppm. This is in fact equivalent to calibrating the TMS peak to 0 ppm, but the TMS peak is not yet visible.

Figure 10.1. 13C Spectrum of Cholesterylacetate. Single Scan. No Decoupling

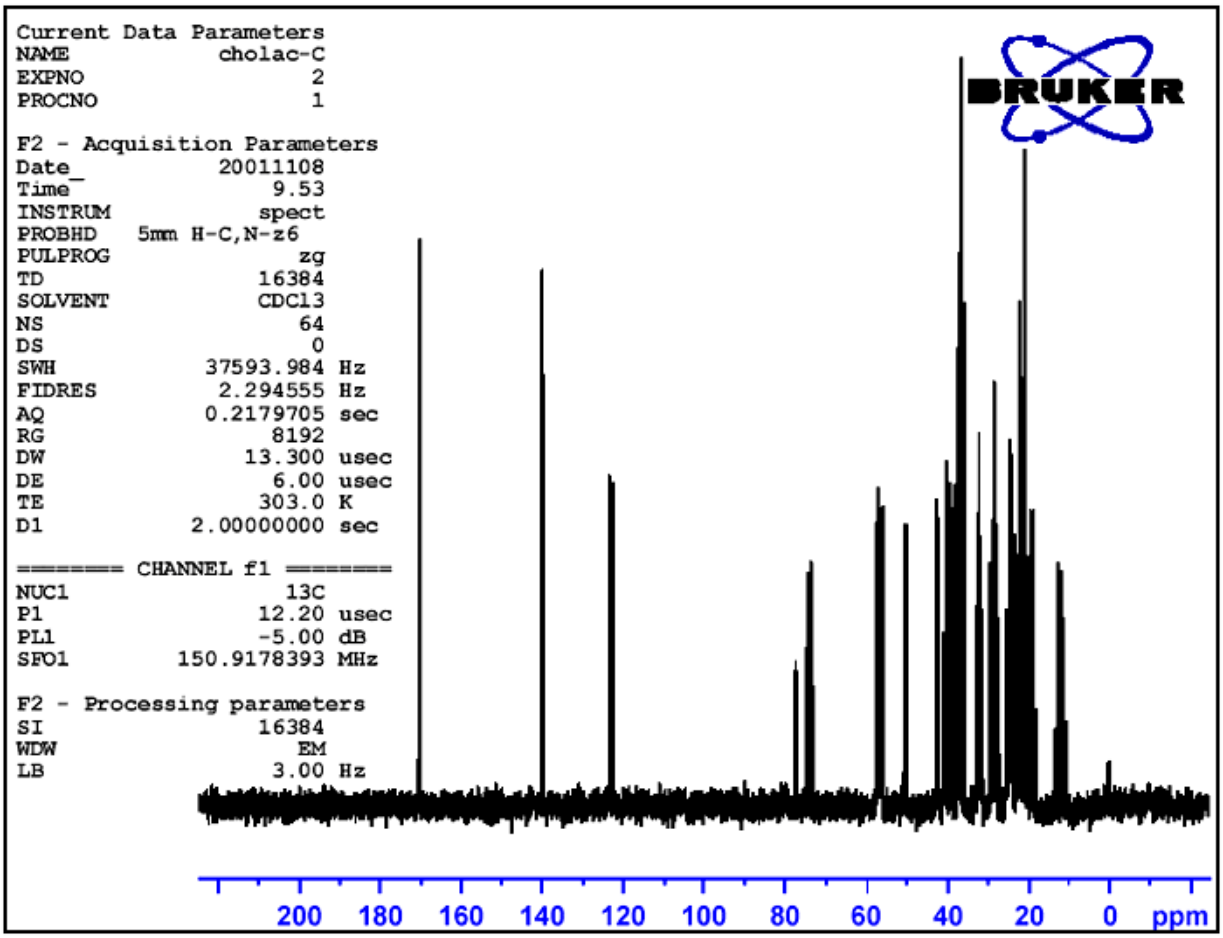


The user may now decide that the noise in the spectrum might be significantly reduced by increasing the number of scans.

15. Create the data set “carbon/2/1”
16. Set NS to 8, 16, 32 or 64.
17. Acquire the FID, Fourier transform and phase correct.

The following figure is a typical ^{13}C Spectrum of cholesterylacetate after 64 scans.

Figure 10.2. 13C Spectrum of Cholesterylacetate. 64 Scans. No Decoupling



The repetitive scanning has certainly dramatically increased the number of significant peaks, but the spectrum still contains too much noise. The user could also now optimize the SW and observe frequency utilizing the SW-SFO1 function described in the section ***"Adjusting the Spectral Width with the SW-SFO1 function" on page 96***.

The next step is not to increase the number of scans, but rather to decouple the ^{13}C signals from the protons. This will be described in the next chapter.

^{13}C spectrum with decoupling

11

Introduction 11.1

The following description will make use of the standard parameter set entitled C13CPD which is specifically designed for observing carbon while decoupling protons using a CPD (Composite Pulse Decoupling) sequence. Channel 1 will be used to transmit a carbon observe pulse while proton decoupling will be transmitted on channel 2 before during and after the acquisition. One feature of the pulse program is that the decoupling power will be adjusted immediately prior to the observe pulse. The details are incorporated into the pulse program “zgpg30” which will be described in the section ***"The pulse program zgpg30" on page 108***

The principal steps to be taken are:

1. Establish a new data set.
2. Read in the standard parameter set “C13CPD”.
3. Establish and set the decoupling frequency.
4. Set parameters “pl12”, “pl13”, and “pcpd2”.

Procedure 11.2

1. Starting from the data set “carbon 2/1” create a new data set “carbon /3/1”. This will ensure that the 01 offset frequency of “carbon 2/1” is maintained. If the value of 01P is not 100 ppm, then you should note its value as the standard parameter set C13CPD will automatically set the offset to 100 ppm. If you are not availing of the "prosol“ routine, then note also the value of “pl1” and “p1” as these will also be overwritten.
2. Enter ’rpar C13CPD all’ and when prompted click on "**acqu**”.

The principal features of the standard parameter set C13CPD is that edasp will be set to:

Channel F1 = ^{13}C

Channel F2 =^{1}H

All other channels set to off.

The pulse program zgpg30 will also be loaded as part of C13CPD. This is explained in the section ***"The pulse program zgpg30" on page 108***. The explanation has been deferred to the chapter end so as not to break the flow of the procedure. If you are not familiar with the program you should refer to it now.

The main parameters to be set are “p1” and “pl1” for the observation pulse along with the decoupling frequency SF02, the decoupling power levels “pl12” and

"pl13" and the pulse duration pcpd2. The values of "p1" and "pl1" will have been established in the experiment described in the previous chapter. How to establish the value of SFO2 will be described in the following section.

Establishing the Decoupling Frequency *11.3*

1. Enter 're hydrogen 2 1'.

   This will call up the data set "hydrogen/2/1" which will be similar to the spectrum in ***Figure 9.7.*** If we ignore the peaks resulting from TMS and the chloroform solvent, it is clear that all the proton signals lie in the 0.5 - 5.5 ppm region. Therefore you might decide to center the decoupling frequency at 3 ppm. One should note that the nature of CPD decoupling is that a range of proton signals are decoupled and hence it is sufficient to center the decoupling frequency in the correct region as opposed to a precise frequency. The C13CPD standard parameter set will set "O2" to 4 ppm. You should note that the ppm values will only be correct if you have correctly calibrated the proton spectrum by assigning the TMS peak the value of 0ppm as described in the section ***"Calibration of Spectrum" on page 94***

2. Enter 're carbon 3 1' and set "O2P" to 3 ppm.

3. Enter 'eda' and set the parameters as in the table below.

Table 11.1. The eda parameters after loading standard parameter set C13CPD

Parameter	C13CPD Parameter Set Value	New Value	Comment
PULPROG	zgpg30	zgpg30	For a description of this pulse program see sec. ***11.5***
TD	65536	64k	Not critical. 64K is rather large.
NS	1K	8	1K is too large. Reduce it to 8
DS	2	2	
SW	238 ppm	238 ppm	Carbon covers a wide spectral range and there is little point in reducing this at this stage
O1P	100 ppm	-	This frequency should correspond to the SFO1 of carbon2/1 and may need to be adjusted
O2P	4 ppm	3 ppm	This is not critical as CPD decouples over a certain region.
p1	14 µ	-	The parameter "p1" is the value of a 90 degree excitation pulse and a pulse of duration P1*0.33 is used to excite the carbon in the zgpg30 program. Use the value used previously in "carbon 2/1" or enter 'getprosol'
pl1	120 db	-	Set the value used previously in "carbon 2/1" or enter 'getprosol'
pl12	120 db	-	Consult with the system manager as to how to set "pl12" or use the getprosol command. See also the section ***"Adjusting the Decoupling Parameters"***.

Table 11.1. The eda parameters after loading standard parameter set C13CPD

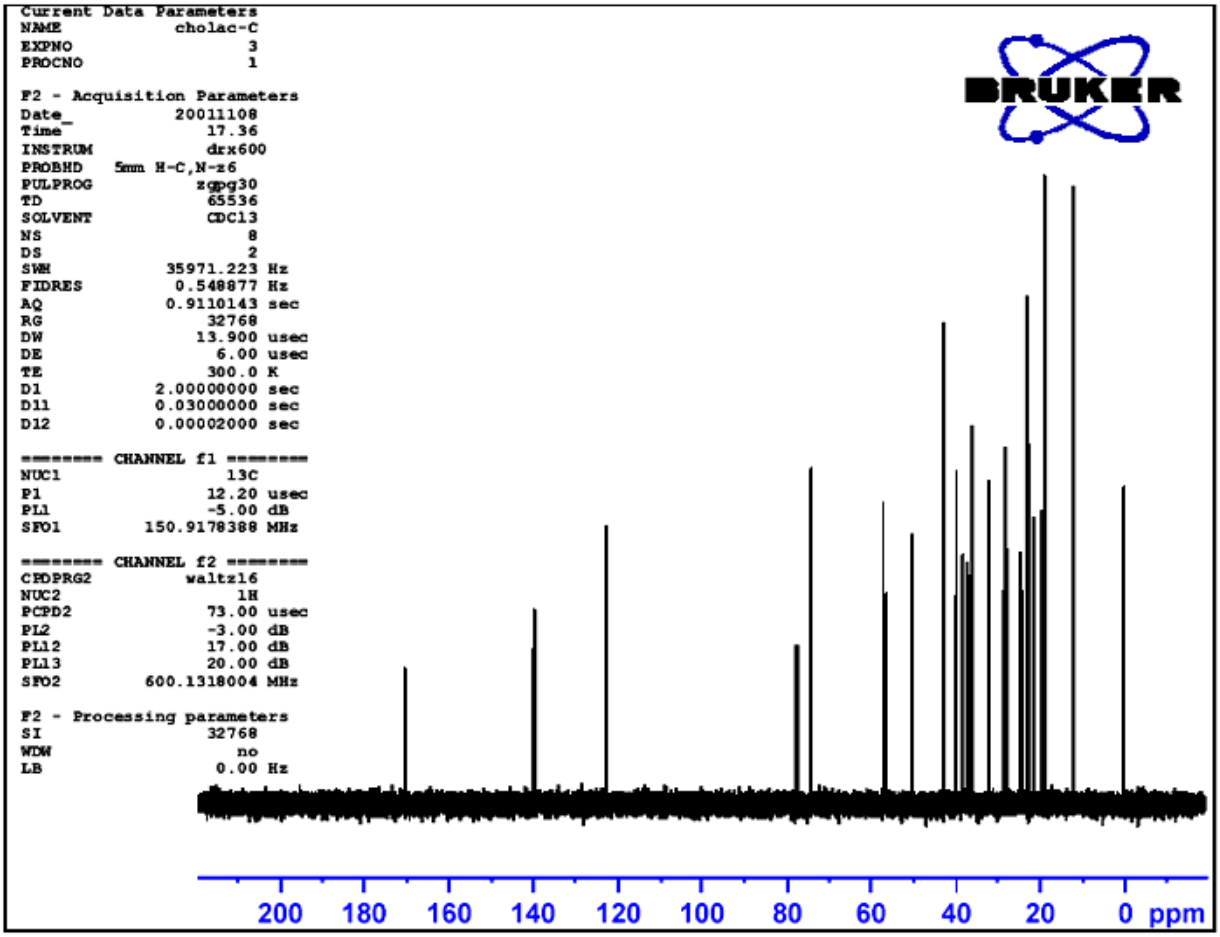
Parameter	C13CPD Parameter Set Value	New Value	Comment
pl13	120 db	-	Consult with the system manager as to how to set pl12 or use the getprosol command. See also the section ***"Adjusting the Decoupling Parameters"***.
d11	30 ms	30 ms	
D*array*	d1=2	2 s	Not critical.

You can use either the “as” or “ased” commands to conveniently check that the parameters have been set correctly.

4. Enter ’zg’ to start the acquisition.
5. Transform and phase correct.

The figure below is an example of the decoupled spectrum. Note that the TMS peak at 0 ppm is now visible.

Figure 11.1. 13C Spectrum of Cholesterylacetate. 8 Scans with Decoupling



Note also the large increase in signal/noise ratio. The effect of decoupling is so dramatic that experienced users rarely waste time running ^{13}C spectra without decoupling.

Adjusting the Decoupling Parameters 11.4

The quality of the decoupling will depend on two factors:

1. The decoupling frequency as set by SF02.
2. The power of the decoupling sequence as set by pl12 and pl13.

You might now wish to explore the effects of altering the values assigned to these two parameters. For example, set the decoupling frequency to values such as 3, 8, 13 ppm above TMS. Observe also the effect of setting “pl12” and “pl13” to various values between 10 and 20 dB. Typically “pl13” is set 3 dB above “pl12” to maintain NOE. Consult with an experienced user before setting “pl12” or “pl13” to a value below 5 dB, because this could overheat the probe.

The pulse program zgpg30 11.5

A copy of the pulse program (shown in ***Figure 11.2.***) can be viewed on the screen by entering the command ’edcpul’. For the purposes of this description each line of the program has been assigned a number which however will not appear on the XWIN-NMR screen whenever the pulse program is displayed.

The first four lines are examples of comments. Line 1 simply gives the name of the pulse program and line 2 the version. Line 3 suggests that this pulse program is suitable for a 1-D NMR decoupling experiment and line 4 reminds the user that instead of a 90 degree excitation pulse the program is designed to use a 30 degree pulse (and hence the name of the program).

Line 5

This line is not a comment but rather a standard inclusion dealing with pulse programming text defined in separate files.

Lines 6 and 7

Two delays “d11” and “d12” which are explained at the end of the pulse program are set here to values of 30 m and 20 µ respectively. The standard parameter set C13CPD will set identical values.

Line 8: 1 ze

This line is not preceded by a semicolon and is actually the first line of the program proper. Any line in a program can be numbered to facilitate loops and this one is naturally numbered as line 1. The command "ze" is a command that causes any data currently in memory to be replaced by data acquired during the first scan. Data acquired during subsequent scans will then be added to that already in memory. The command "ze" effectively clears the memory in preparation for data that will be acquired during an experiment.

Line 9: d12 pl13:f2

This sets the power level for decoupling on the F2 channel to the value assigned to “pl13”. The delay “d12” ensures that there is sufficient time to set the power before decoupling commences.

Figure 11.2. The Pulse Program "zgpg30"

```
1. ;zgpg30
2.;avance-version (00/02/07)
3.;1D sequence with power-gated decoupling
4.;using 30 degree flip angle

5.#include <Avance.incl>

6."d11=30m"

7."d12=20µ"

8.      1 ze
9.2 d12 pl13:f2
10. d1 cpd2:f2
11. d12 pl12:f2
12. p1*0.33 ph1
13. go=2 ph31
14.   wr #0
15. d11 do:f2
16.      exit

17.ph1=0 2 2 0 1 3 3 1
18.ph31=0 2 2 0 1 3 3 1

19.;pl1: f1 channel - power level for pulse (default)
20.;pl12: f2 channel - power level for CPD/BB decoupling
21.;pl13: f2 channel - power level for second CPD/BB decoupling
22.;p1: f1 channel - 90 degree high power pulse
23.;d1: relaxation delay; 1-5 * T1
24.;d11: delay for disk I/O                    [30 msec]
25.;d12: delay for power switching             [20 µsec]
26.;cpd2: decoupling according to sequence defined by cpdprg2
27.;pcpd2: f2 channel - 90 degree pulse for decoupling sequence
```

Line 10:d1 cpd2:f2

This starts the decoupling sequence defined by the program “cpdprg2” in the eda table. A quick check in the eda table will reveal that the standard parameter set has set cpdprg2 to waltz16. The decoupling will continue with power level “pl13” for the duration of “d1”.

Line 11: d12 pl12:f2

To prepare for decoupling during the excitation pulse and acquisition the power level for the F2 channel is set to “pl12”. Again a delay of 20 µ (d12) is included to facilitate the power level setting.

Line 12: p1*0.33 ph1

Line 12 This line is a standard 30 degree excitation pulse and was explained in ***"Details of the “zg30” Program" on page 83***. The difference now is that the pulse is at the carbon frequency not the proton frequency. The duration of the pulse will accordingly be different.

Line 13: go=2 ph31

Again this standard line was explained in the section ***"Details of the “zg30” Program" on page 83***.

Line 14: wr #0

See the section ***"Details of the “zg30” Program" on page 83***.

Line 15: d11 do:f2

The "do“ stands for "decoupling off”.

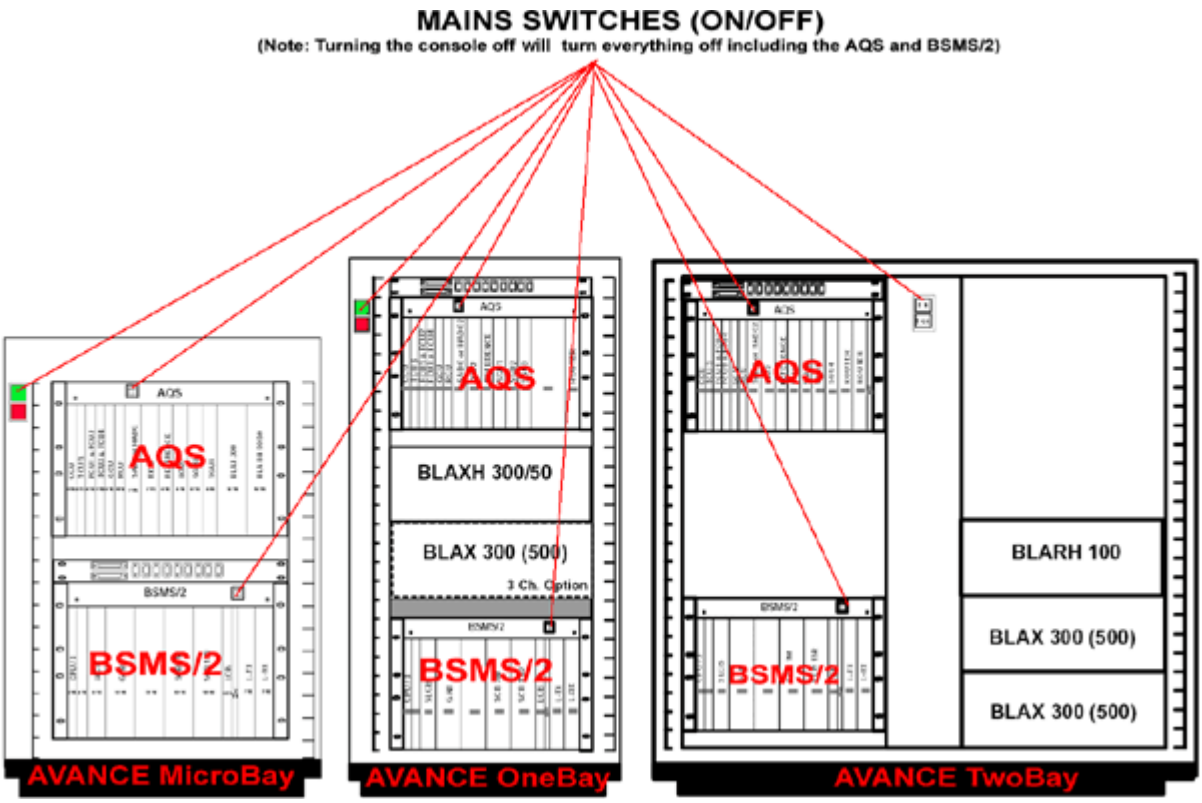
Basic Troubleshooting

12

Introduction 12.1

For a beginner's guide it is unfeasible to describe any advanced level of trouble-shooting. However, all users should at least be able to execute a complete power on and off of the system.

Figure 12.1. Location of the Mains Switches for the Cabinet, AQS and BSMS



Switching the Spectrometer On and Off 12.2

With normal operation, the cabinet mains, subunits, and the host computer are left switched on, even if no experiments are running. If individual units within the AQS or BSMS racks are to be exchanged, it is sufficient to switch off the AQS rack or the BSMS rack as appropriate. It is not necessary to execute a power down of the complete system.

The user should note that:

1. The CCU10 automatically boots up (powers down) whenever the AQS rack is switched on (off).
2. The BSMS CPU and keyboard automatically boot up (power down) whenever the BSMS rack is switched on (off).

Switching on the Spectrometer 12.3

Procedure:

To execute a complete "**power on**“ of the entire spectrometer, carry out the following steps:

1. Switch on the host computer, either a PC or a Silicon Graphics (SGI) O2. Log on, but do not start XWIN-NMR.
2. Switch on the cabinet mains using the green switch on the front panel.
3. Switch on any internal units such as the AQS rack, amplifiers, BSMS, VTU etc.
4. Switching on the AQS will cause the CCU10 to reboot. Observe the booting procedure by "logging onto spect“ using the Hyperterminal icon (for systems running on a PC) or by opening a Linux/UNIX shell (for systems running on Linux or an SGI O2). The "logging onto spect“ is physically logging onto the CCU10, and is usually done with the RS232 connection to tty00. The message "system is ready“ will signify that the booting is complete.
5. Switching on the BSMS causes the BSMS CPU to reboot. Observe this on the BSMS keyboard where the word "booting“ and "connecting“ will be displayed.
6. Start XWIN-NMR from the host computer.

After rebooting the CCU10 it is customary to "log onto spect“ again.

Note that you can also use "telnet spect“ to "log onto spect“ via the ethernet link.

Switching off the Spectrometer 12.4

To execute a complete "**power off**“ of the entire spectrometer carry out the following steps

1. Exit XWIN-NMR
2. Shut down the CCU10 with the "init 5“ command and switch off the AQS. On a system running with a PC host, click on the Hyperterminal icon to enter the "init“ command. For a system running on a SGI O2 host, open a Unix shell to enter the "init“ command.
3. Switch off any internal units such as the BSMS, amplifiers, VTU etc.
4. Switch off the cabinet mains using the red switch on the front panel.

Starting XWIN-NMR for Windows Systems *12.4.1*

1. Ensure that the spectrometer ("spect“) is booted. This can be done by clicking on the CCU Hyperterminal icon of the host computer desktop. Note that you can also use "telnet spect“ to access via the ethernet link.
2. Click the icon XWIN-NMR on the desktop.

Starting XWIN-NMR for SGI IRIX Systems *12.4.2*

1. Ensure that the spectrometer ("spect“) is booted. This can be done by opening a unix shell, and entering ‘telnet spect’ to access via the ethernet link.
2. Open a UNIX shell
3. Enter ’xwinnmr’.

Figures

1 Introduction 7

2 Safety 9

Figure 2.1. Safety Precautions within the Inner and Outer Zones 10

3 Introductory Theory and Terminology 13

Figure 3.1. Excitation and Response 13
Figure 3.2. NMR Spectrum 15
Figure 3.3. NMR Analysis of CHCl3 16
Figure 3.4. NMR Signals Emitted by $CHCl_3$ 16
Figure 3.5. Converting Hertz and ppm 18
Figure 3.6. 1H Chemical Shifts in Organic Compounds 19
Figure 3.7. Benzene Ring 20
Figure 3.8. Spectrum of Benzene 21
Figure 3.9. Benzylacetate 21
Figure 3.10. Proton Spectrum of Benzylacetate 22
Figure 3.11. Ethylbenzene 23
Figure 3.12. Ethylbenzene Spectrum 24
Figure 3.13. Decoupling Experiment 25
Figure 3.14. Ethylbenzene Spectrum with Homodecoupling 26
Figure 3.15. Fourier Transformation 27

4 System Description 29

Figure 4.1. Operator console, console and magnet 29
Figure 4.2. Photo of Magnet, Shim System, Probe and HPPR 32
Figure 4.3. HPPR with three Modules and Cover Module 33
Figure 4.4. Superconducting Magnet 36
Figure 4.5. Sample in Probe 38
Figure 4.6. Typical HPPR Cabling 39
Figure 4.7. Dual 13C/1H probe 40

5 The NMR Sample 43

Figure 5.1. Spectrum Showing Spinning Sidebands 45

6 Basic procedures 47

Figure 6.1. BSMS Keyboard (BOSS Version) 48
Figure 6.2. Inserting the Sample into the Spinner 52
Figure 6.3. Examples of Wobble Curves with Different Tuning and Matching 55
Figure 6.4. HPPR/2 Cover Display 57
Figure 6.5. A Typical Lock Signal 60
Figure 6.6. Lock Display After Locking the Sample 61

7 Preparing for Acquisition, Data Sets, edasp/eda Commands 65

Figure 7.1. The edc Display 68
Figure 7.2. The “edasp” Menu 71
Figure 7.3. First Page of “eda“ Parameters 73
Figure 7.4. Graphical Representation of Some Acquisition Parameters 76
Figure 7.5. Clipped FID as a Result of RG Set too High 77
Figure 7.6. Relationship Between Power and Attenuation 78
Figure 7.7. Spectrum with BF1=600.13 MHz, 01=0Hz 80
Figure 7.8. Spectrum with BF1 =600.13 MHz, 01= 8 kHz 81
Figure 7.9. Spectrum with BF1=600.13 MHz, 01=8 kHz, SWH = 8.4kHz 81
Figure 7.10. Interaction of SFO1, BF1 and O1. 82

8 Pulse Programs/Commands as/ased 83

Figure 8.1. The Pulse Program “zg30 “ 84
Figure 8.2. The Pulse Program “zg” where NS=4 and DS=0. 86

9 Proton Spectrum 87

Figure 9.1. Acquisition Window - Status of the Scan Counter 90
Figure 9.2. Proton Spectrum of Cholesterylacetate. Sixteen scans. SW = 20.66 91
Figure 9.3. Some Useful Features of the Main XWN-NMR Window 92
Figure 9.4. Example of Spectrum with Phase Correction (below) and without (above) 93
Figure 9.5. Plot Identifying Location of TMS Peak as right most Peak in Spectrum 95
Figure 9.6. Proton Spectrum of Cholesterylacetate 96
Figure 9.7. Proton Spectrum of Cholesterylacetate 98

10 13C Spectrum without decoupling 101

Figure 10.1. 13C Spectrum of Cholesterylacetate. Single Scan. No Decoupling 103
Figure 10.2. 13C Spectrum of Cholesterylacetate. 64 Scans. No Decoupling 104

11 13C spectrum with decoupling 105

Figure 11.1. 13C Spectrum of Cholesterylacetate. 8 Scans with Decoupling 107
Figure 11.2. The Pulse Program “zgpg30” 109

12 Basic Troubleshooting 111

Figure 12.1. Location of the Mains Switches for the Cabinet, AQS and BSMS 111

Tables

1 Introduction 7

2 Safety 9

3 Introductory Theory and Terminology 13

Table 3.1. Table of Data for Various Isotopes. 14
Table 3.2. Frequency Variations 14

4 System Description 29

5 The NMR Sample 43

6 Basic procedures 47

Table 6.1. The Default edlock Table 58

7 Preparing for Acquisition, Data Sets, edasp/eda Commands 65

Table 7.1. Data Sets with Differing NAMEs and EXPNOs 66
Table 7.2. Data Sets with Differing NAME's, EXPNO's and PROCNO's 67

8 Pulse Programs/Commands as/ased 83

9 Proton Spectrum 87

Table 9.1. The "eda" Parameters after Loading Standard Parameter Set "Proton" 88
Table 9.2. Phase Correction Methods 94

10 13C Spectrum without decoupling 101

Table 10.1. The "eda" Parameter Values 102

11 13C spectrum with decoupling 105

Table 11.1. The eda parameters after loading standard parameter set C13CPD 106

12 Basic Troubleshooting 111

Index

A

Absolute frequencies 17
Acquisition 90
Acquisition Control System 30
Acquisition parameters 68
Acquisition window 90
ADC switching on and off 90
Amplifier 71
Amplifiers 31
    Broadband 31
    External 71
    Internal 71
    Selective 31
AQ 75
AQ_mod 74
AQS 30
Auto Shim 51
AUTOLOCK 59
Autolock 50

B

Benzene 20
Benzylacetate 21
BF 70
BF1 79
BOSS-2 shim systems 52
Broadband coil 38
Broadband probes
    Tuning 53
Bruker Smart Magnet System 30
BSMS 30
BSMS keyboard 30, 47
    When not present 47

C

Cardiac pacemakers 9
Carrier frequency 15
CCU 30
CE Declaration of Conformity 12
Chemical Safety 12
Chemical shift 19
Chemically equivalent 20
Commands
    "edlock" table 58

"ft“ ... 92
"go“ ... 69
"gs“ ... 69
"rpar“ ... 68
"wsh" ... 48
"zg“ ... 69
“as” ... 86
“ased” ... 86
“eda“ ... 65, 69
“eda” ... 72
“edasp“ ... 65, 68
“edasp” Menu ... 79
“edasp” window ... 70, 72
“edhead” ... 88
“edsp“ ... 65
“getprosol” ... 88
“go” ... 72
“gs” ... 72
“rga” ... 72
“zg” ... 72
’acqu’ ... 90
’apk’ ... 91
’edc’ ... 67
’ft’ ... 91
’lock’ ... 58 – 59
’lockdisp’ ... 59
’rga’ ... 89
’rsh’ ... 49
’wbchan’ ... 57
Commands "ze" ... 84
Composite Pulse Decoupling ... 105
Connections ... 30
Console ... 29
CPD ... 105
Creating a new data set ... 67
Cryogenic Safety ... 11

D

D**array** ... 74
d1 ... 84
Data set ... 65
Creating a new data set ... 67
Processed data set ... 66
Date set
Raw data set ... 66
DE ... 78
Decimation ... 77
Declaration of Conformity ... 12
Decoupling ... 25
Decoupling frequency ... 106, 108
Decoupling pulses ... 25
Decoupling sequence
Power of the ... 108
Deuterated solvent ... 37

Deuterated solvents 43
DIGMOD 78
DIGTYP 77
DS 74
DSPFIRM 77
DU 65
DW 77
DWOV 77

E

Electrical Safety 11
Electromagnets 34
Ethernet connection 30
Excitation pulse 13
EXPNO 65

F

F1 70
FADC 77
FCU's 70
FID 26
    Definition 27
FIDRES 75
Field 50
Field homogeneity 61
Field mode 59
First order phase correction 93
Fluorine lock 37
Forward and reflected power 90
Fourier Transformation 27, 92
Frequencies
    Transmitted, Basic and Offset 79
FW 75

G

Gradient shimming 63
gradshim 63

H

HADC 77
Helium
    boiling point 34
    level 47
    measure key 47
Helium boil-off 36
Helium Level 51
Helium level 47
Helium level sensor 35
Helium Measure 51
Helium ports 35

Hertz 17
conversion 18
Heterodecoupling 26
Host Computer
Link between host and AQS 31
Host computer 30
HPPR 32
Module connection 33
Typical cabling 39
HPPR/2 32

I

Impurities 43
Intensity
of a signal 15
Isotopes 13, 15
Isotype
Basic resonance frequency 13

L

LGain 60
Lift On-off 49
Lineshape 63
Liquid samples 43
Lock Gain 50
Lock On-off 50
Lock Phase 60
Lock Power 50
Lock Shift 50
Lock System
Description 37
Receiver 37
Lock system
Purpose 37
Locking the sample 37
LOCK-ON 59
LOCNUC 79
Logical Channel 70
Logical channel
F1 70
NUC1 70
LPower 60
LTime 60

M

Magnet
quench 34
Safety 9
Stray fields 11
System description 34
Magnet core 34

Magnet Dewar 34
Magnet quench 11
Magnet system 29
Magnetic impurities 43
Magnetic shielding 14
Magnetically equivalent 20
Matching ..... 40, 53 – 54
Metallic implants 9
Multinuclear probes 38

N

N2 transfer line 40
NAME 65
Natural abundance 16
Nitrogen
    Liquid Nitrogen 11
Nitrogen boil-off 36
NMR active 15
NS 74, 98
NUC1 ..... 70, 79
NUCLEI 79
Nucleus
    Types of 13

O

O1 79
O1P 79
Observe coil 38
Observe nucleus 15
OFSF1 70
OFSH1 70
OFSX1 70
Operator Console 30
Operator console 29
Organic solvents 43

P

P**array** 74
Parameters
    DU 65
    EXPNO 66
    NAME 65
    PROCNO 66
    USER 65
PARMODE 74
Phase correction 93
    First order 93
    Method ’apk’ 94
    Method ’fp’ 94
    Method ’ph0’ 94
    Method ’ph1’ 94

Method 'pk' 94
Zero order 93
Phase shifts 93
PL **array** 78
ppm 17
conversion 18
Preamplifier 72
Preamplifiers 38
Probes 37
Broadband coil 38
Changing a probe 41
Dual 1H/13C probe 39
Heater 40
Multinuclear probes 38
Observe coil 38
Selective probes 38
X-BB 38
PROCNO 65
Proton spectrum 87
PULPROG 72
Pulse
Amplitude 85
Length or duration 85
Phase 85
Pulse program
"zg30" 73, 83
"zgpg30" 105
PULPROG 72
Pulse transmission 90

Q

Quench 34

R

Reference Compounds 17
Resonance frequencies 13
RG 76
RS232 connection 30

S

SADC 77
Safety
Cardiac pacemakers 9
Chemical 12
Cryogenic 11
Electrical 11
Inner zone precautions 10
Introduction 9
magnet quench 34
Magnets 9
Outer zone precautions 11

Pressurized gas cylinders ... 11
Stray fields ... 9
Safety Precautions ... 10
Sample depth gauge ... 51
Sample lift ... 47
Sample spinning ... 47
Sample temperature ... 40
Sample tubes ... 45
Selective probes ... 38
Sensitivity ... 17
SFO1 ... 70, 79
Shift mode ... 59
Shim system ... 32, 47, 61
Shim Values
Storing ... 48
Shim values ... 47
Storage of ... 48
Shimming ... 47, 61
Definition ... 32
Intial shimming ... 62
Routine ... 62
SI data points ... 92
Sidebands
Intensity of sidebands ... 44
Signal accumulation ... 98
Signal strength
measure of ... 15
Signal/noise ratio ... 98
Singlet ... 25
SINO program ... 99
Site Planning Guide ... 9
Solids ... 43
Solvent
Factors in selecting ... 43
Solvents
Solubility ... 43
Viscosity ... 43
Water content ... 44
Sovents
Temperature dependence ... 43
Spectral resolution ... 61
Spectrum ... 26
Spin Measure ... 49
Spin On-off ... 49
Spin Rate ... 49
Spinner
Inserting sample in spinner ... 51
Spinning ... 44
Spinning sidebands ... 44
Spin-spin coupling
The effects of ... 25
Standard settings ... 69
Stray fields ... 9
Superconducting magnets ... 34
SW ... 75
Sweep ... 50

Sweep Amplitude 50
Sweep Rate 50
SWH 75
SW-SFO1 97

T

TD 74, 92
Thermocouple 40
Transmit / Receive switching 33
Transmitters 31
Tube symmetry 45
Tuning ..... 40, 53 – 54

U

USER 65

V

Variable Temperature Unit 30, 40
VTU 30, 40

W

Water content 44
WBST 55
WBSW 55
Wobble curve 54
Wobb-SW 57

Z

ze 84
Zero order phase correction 93

LAST PAGE OF DOCUMENT