1334 – Ressonância Magnética Nuclear

Equipamento

Espectrômetro NMR	41727.8E	41727.8E	1

Este item é composto por um conjunto para o estudo do fenômeno da Ressonância Magnética Nuclear.
Este conjunto vai acompanhado de detalhado manual de instalação e operação.
As fotos seguintes se referem à instalação e operação do equipamento na UFES – Vitória, com o Prof. Dr. Jair Freitas.

Fig. 1: montagem e processo de configuração da interface p/ computador.

Fig. 2: o conjunto está em operação e o monitor do PC mostra o início da varredura.
Ao fundo e à direita, temos o conjunto para estudo das propriedades dos Raios X, também instalado na UFES.

1334 – Ressonância Magnética Nuclear

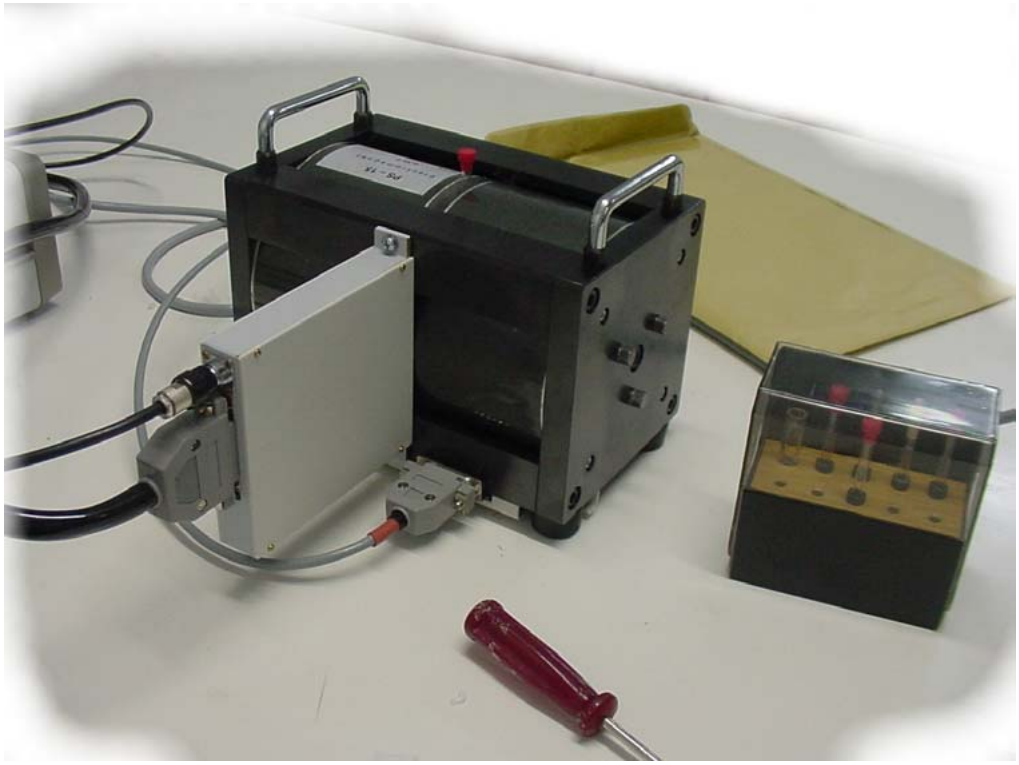


Fig. 3: módulo em que se aplicam os campos magnéticos. A extremidade do tubo que contém a amostra se destaca pela pequena tampa vermelha. À direita, o conjunto de portadores de amostras.

Fig. 4: destaque p/ a tela do PC mostrando o início da varredura.

1334 – Ressonância Magnética Nuclear

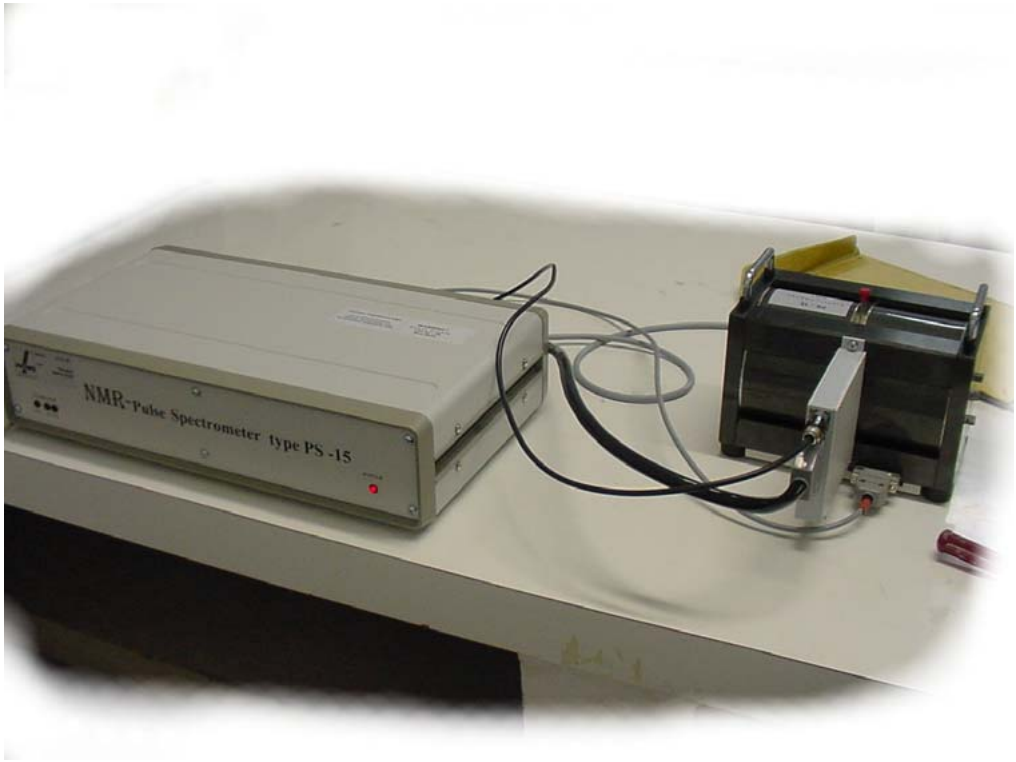


Fig. 5: ligação do amplificador ao conjunto portador da amostra p/ estudo da RMN.

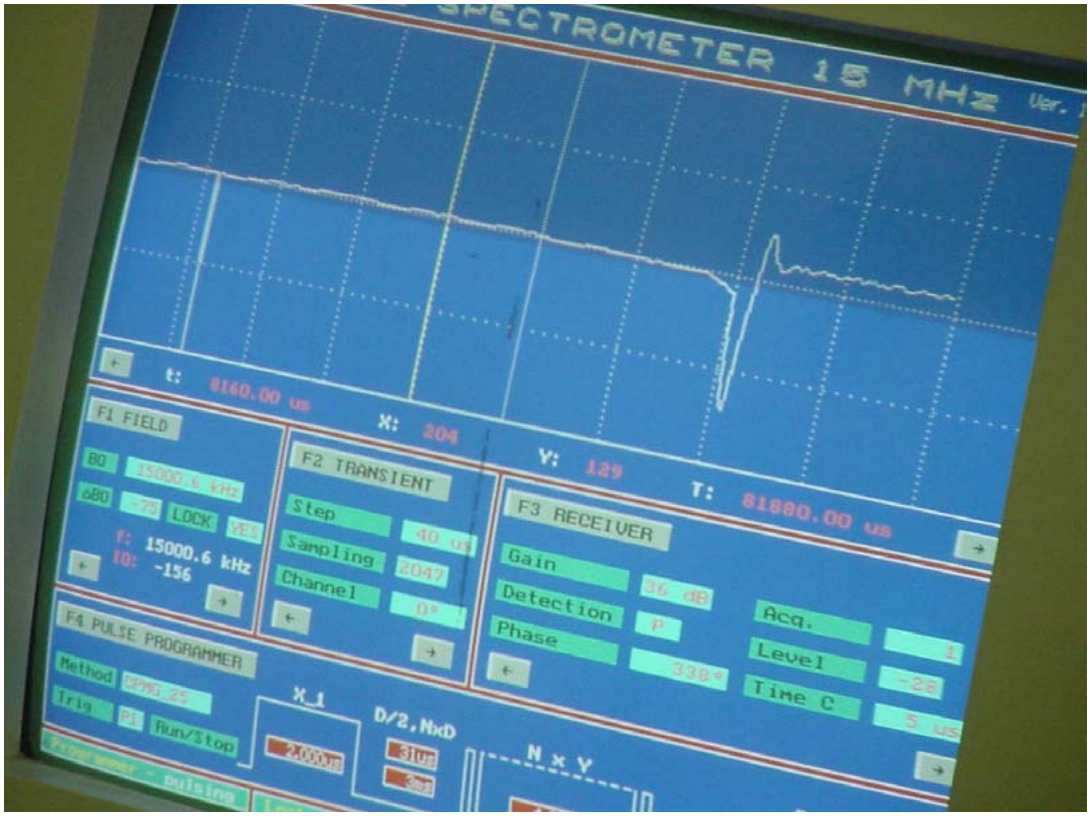


Fig. 6: tela do PC. Com a varredura identifica-se o ponto da RMN.

Getting Started

Pulsed NMR Spectrometer PS-15

Introduction to NMR

Quick Start

1334 – Ressonância Magnética Nuclear

Contents

1334 – Ressonância Magnética Nuclear

Introduction

Since the very first Nuclear Magnetic Resonance were performed in the late 1940s, the technique of NMR spectroscopy has become one of the most important analytical tools in research areas from solid state physics, through the whole of chemical science, to biology and medicine.

The PS-15, a pulsed spectrometer, with an operational frequency of 15 MHz is designed specifically for teaching. It provides physics, chemistry, biology, geology, and other science students with the hands-on approach to learning about magnetic resonance phenomenon, instrumental analysis and methods of recording week NMR signals.

The student can perform his own experiments and learn about NMR spectroscopy in a straight-forward and easy manner simply by following pictorial descriptions found in this manual or in other publications that pertain to NMR spectroscopy. Designed as an entry-level system the PS-15 is designed to help teach the basic principles of pulsed NMR. The emphasis is on how information is obtained.

The new PS-15 spectrometer was designed by a team of physicists and engineers working in the research level of NMR and MRI sciences. Sophisticated technology has been incorporated into the system. Quadrature phase detection (QPD) of the NMR signal and NMR lock field stabilization are examples of this technology. It is fully computer controlled. The software allows the user to have complete control of the system including pulse shaping, phase shifting, signal detection, data acquisition and data processing. Although the system offers a greatly improved user interface and state-of-the-art electronics, its primary objective is to be a teaching aid for the advanced students labs.

1334 – Ressonância Magnética Nuclear

1334 – Ressonância Magnética Nuclear

NMR - Basic Principles

The following chapter provides a brief tutorial on NMR. This will help a novice in the subject to get started

NUCLEAR RESONANCE

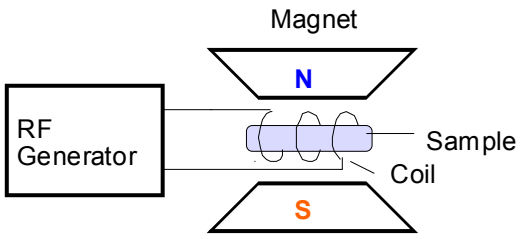


FIG.1

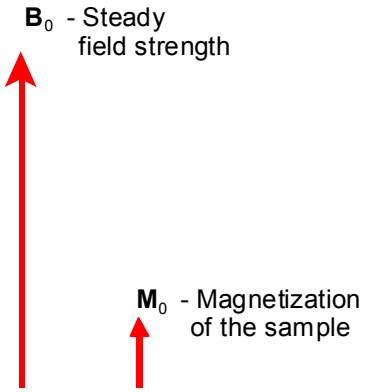
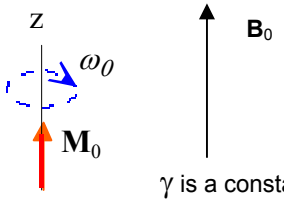


FIG.2

Fig.1 is a diagram showing the essential features of an NMR spectrometer. The sample is placed inside a coil connected to the RF generator. Because the coil is between the poles of the magnet the sample becomes magnetized along the direction of the steady field $\mathbf{B}_0$. Each sample will become magnetized at an exponential rate if placed in a magnetic field. Liquids may need a few seconds and solids or high viscosity samples require less time to become fully magnetized. The sample gains a small macroscopic magnetization that is represented by the vector $\mathbf{M}_0$ along the z-direction which is the direction of the magnetic field (See Fig.2).

The magnitude of $\mathbf{M}_0$ will depend on the value of $\mathbf{B}_0$ and on the properties of the sample. For proton resonance, $\mathbf{M}_0$ is proportional to the total number of hydrogen nuclei in the sample. These nuclei have a motion called spin. A consequence of the nuclear spin is that $\mathbf{M}_0$ is, in fact, precessing about $\mathbf{B}_0$ field at a rate given by the formula (and called Larmor frequency)



$$\omega_0 = \gamma B_0$$

γ is a constant called the gyromagnetic ratio. Different species of nuclei have different values of γ . For protons γ = 267628 radian per second per mT. It is more usual to think in terms of frequency, that is, in cycles per second rather than radians per second. In these terms the frequency of the spin of the magnetization is:

$$\nu_0 = \gamma B_0 / 2\pi$$

γ /2π has the value 0.042577 MHz / mT for protons. The PS-15 uses a field of ~ 350 mT and a frequency of 15 MHz. *(0.042577MHz / mT x 352 mT = 15 MHz)*

So far we have considered only the effect of the steady field $\mathbf{B}_0$. If the RF generator is turned on this produces an alternating magnetic field, $\mathbf{B}_1$, inside the coil ($\mathbf{B}_1 << \mathbf{B}_0$).

1334 – Ressonância Magnética Nuclear

Nuclear magnetic resonance takes place when a steady and an alternating magnetic field are applied simultaneously to the sample and when the frequency of the alternating field and the strength of the steady field are in the ratio given by the formula: $\nu_0 = \gamma B_0 / 2\pi$

i.e., when the generator frequency is made equal to the frequency of the precession of M_0. The frequency v_0 is called the resonance frequency when it reaches Larmor frequency.

It is possible to design a coil that produces a rotating field. Most NMR instruments, however, use a normal coil such as a solenoid. This type of coil is much easier to make but it is half as efficient as the rotating-field coil in producing the NMR effect. In fact, the alternating field directed along the axis of the solenoid, alternating between $+2\mathbf{B}_1$ and $-2\mathbf{B}_1$ each cycle, is exactly the same as would be produced by two rotating fields, each of magnitude B_1, one rotating clockwise and one rotating counter-clockwise, each with the rotational frequency equal to the frequency of the alternating field. NMR occurs when the frequency of the alternating field is made equal to the rotational frequency of $\mathbf{M}_0$. It is this $\mathbf{B}_1$ field which causes the resonance effect. The other $\mathbf{B}_1$ field does not produce any observable effect on $\mathbf{M}_0$ and can be ignored.

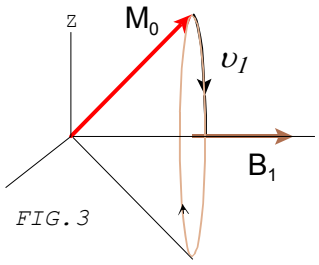


FIG. 3

$\mathbf{M}_0$ rotates about $\mathbf{B}_0$ when the sample is in the steady field. If the $\mathbf{B}_1$ generator is switched on, then $\mathbf{M}_0$ continues to rotate about $\mathbf{B}_0$ but it also rotates about the effective $\mathbf{B}_1$ field produced by generator the along the x-axis (Fig.3). As far as the observer who is unaware of the rotation about $\mathbf{B}_0$ is concerned, $\mathbf{B}_1$ appears to be a steady field always along the y-axis all the time the generator is switched on. $\mathbf{M}_0$ immediately starts to rotate about $\mathbf{B}_1$ in the z-y plane, Fig.5. The frequency at which $\mathbf{M}_0$ rotates about $\mathbf{B}_1$ depends upon the magnitude of $\mathbf{B}_1$ in the same way as the frequency of rotation about $\mathbf{B}_0$ depends upon the magnitude of $\mathbf{B}_0$, hence;

$$\nu_1 = \gamma B_1 / 2\pi$$

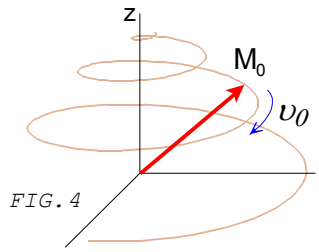


FIG. 4

these two simultaneous rotations produce a complex motion of $\mathbf{M}_0$ (Fig.4). It is easier to picture what is happening if we can think of the rotation of $\mathbf{M}_0$ about the effective $\mathbf{B}_1$ in isolation. We would not then be aware of the rotation of $\mathbf{M}_0$ about $\mathbf{B}_0$ but only of the rotation of $\mathbf{M}_0$ about $\mathbf{B}_1$. The effect of the static field can be cancelled by working in a coordinate system rotating about the static field, $\mathbf{B}_0$, at the applied RF, $\mathbf{B}_1$. Such transformation to this so-called **rotating frame** of reference simplifies the understanding of NMR experiments, Fig.5.

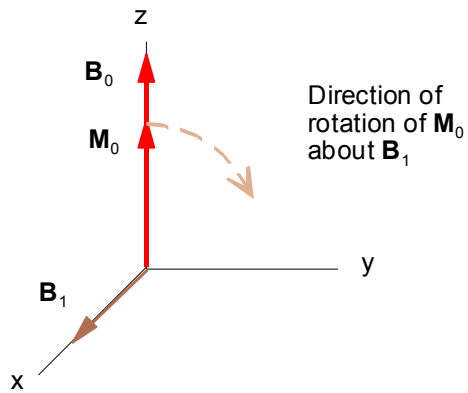


FIG. 5

$\mathbf{M}_0$ is along the z-axis before the generator is switched on. When the generator is switched on this produces a field $\mathbf{B}_1$ along the x-axis. $\mathbf{M}_0$ immediately starts to rotate about $\mathbf{B}_1$ in the zy-plane.

It is normal to apply the resonance condition for only a short time. In pulsed NMR this is done by switching the generator on for only a short time (the pulse). The generator is usually switched on for a time that gives only a fraction of one cycle of the rotation about $\mathbf{B}_1$. One quarter of one cycle is often used as this gives the largest signal from the sample after the pulse. This

length of pulse is called a **90° pulse** because it rotates $\mathbf{M}_0$ through 90°, from along the z-axis to along the y-axis.

We can rotate $\mathbf{M}_0$ to any direction that we desire. The angle θ, through which the magnetization precesses around $\mathbf{B}_1$ is given by the formula: $\theta = \gamma B_1 t$. The angle θ is directly proportional to both the strength (B_1) and length (t) of the pulse. The strength of the pulse is kept constant in many spectrometers (the same applies to the PS-15) therefore the angle θ can be changed by changing the duration (t) of the pulse. The magnitude of $\mathbf{B}_1$ required to produce a 90^0 pulse in a certain time is independent of the value of $\mathbf{B}_0$ used for magnetizing the sample. But the frequency of the pulse generator, ν_0, does depend on the value of $\mathbf{B}_0$ as given by the formula $\nu_0 = \gamma B_0 / 2\pi$

Fig.6 illustrates the effect of different pulses on $\mathbf{M}_0$ in the rotating frame of reference. The rotating magnetic field $\mathbf{B}_1$ appears fixed in the xy plane. (Some authors designate the rotary frame axes as x', y', z')

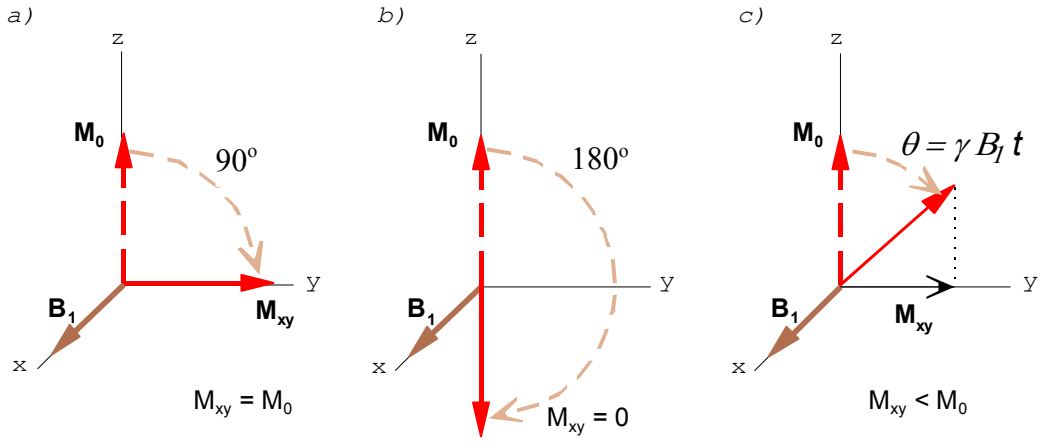


FIG. 6

The part of the FID immediately following the pulse cannot be measured accurately as it takes some time for the instrument to recover from the high-powered pulse. It is usual to switch off the receiver during this period so this interval is known as the dead time of the instrument. The shape of the rest of the FID depends upon the sample being analyzed and on the characteristics of the instrument

NMR SIGNALS

The reason for rotating $\mathbf{M}_0$ away from the direction of $\mathbf{B}_0$ is that $\mathbf{M}_0$ produces no signal while it is directed along $\mathbf{B}_0$. It is only when $\mathbf{M}_0$ is away from the $\mathbf{B}_0$ direction that a signal may be measured. The closer the deflection of $\mathbf{M}_0$ to the x-y plane, the bigger the signal. The signal is proportional to the projection of $\mathbf{M}_0$ onto the xy plane.

The 90° deflection produces the largest signal and is normally used in pulsed NMR for this reason.

1334 – Ressonância Magnética Nuclear

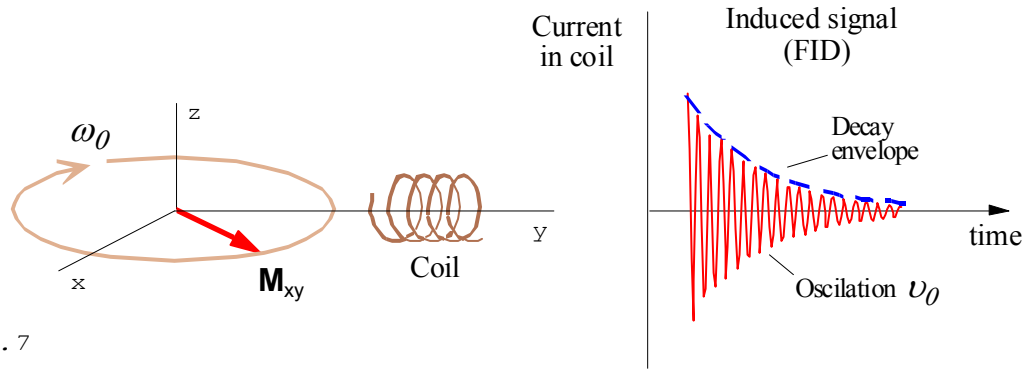
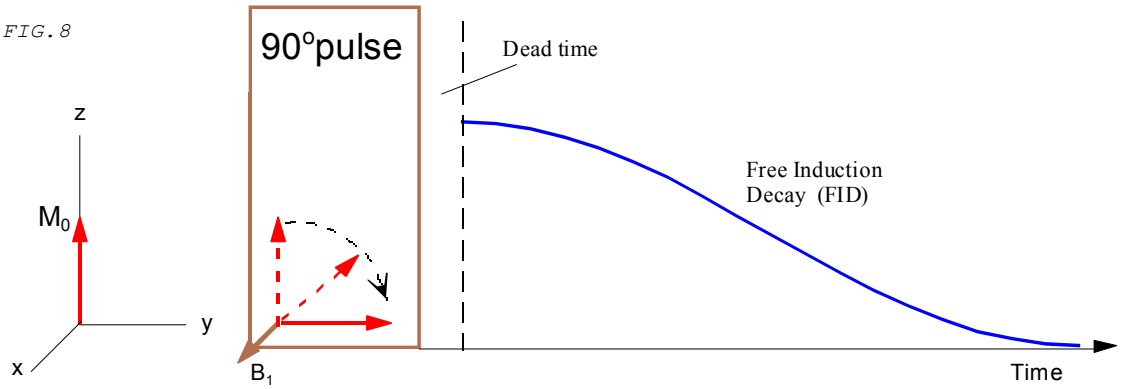


FIG. 7

The magnetization $\mathbf{M}_0$ after a 90° pulse will precess in the xy plane of the stationary (laboratory) frame of reference (Fig.7). A receiver coil with its axis in the xy plane will pick up a signal from this precessing magnetization. The electric current induced in the coil will oscillate at the Larmor frequency ν_0 ; in the PS-15 this frequency is ~15MHz. The amplitude of the signal will decrease with time. This oscillating signal in the coil is called the **free induction decay** or **FID**. "Free" refers to the fact that the magnetization is freely precessing and no longer experiencing the torque produced by the RF pulse. "Induction" indicates that the current was produced using the principle that a changing magnetic field (produced by the changing magnetization of the sample) within a coil will induce an electric current in the coil. Note particularly that we measure only the xy component of magnetization of $\mathbf{M}_0$, and not the absorption or emission of energy by the protons.

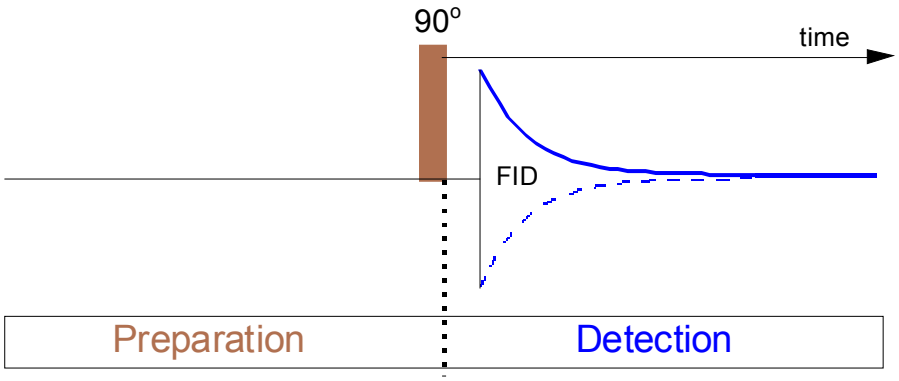
If the coil is connected to a suitable receiver the signal produced by the sample can be measured. This signal is a sine wave oscillation at a frequency f_0 . It starts immediately after the pulse at maximum amplitude dying away to zero in a time that depends on the nature of the sample and on the characteristics of the instrument. The alternating signal (See Fig. 7) is amplified and then detected to provide information on the sample. Fig.8, shows the output from the detector obtained after a 90° pulse. The decay envelope is displayed as a signal. Hence the NMR signal reflects rather the situation as seen in the rotating frame of reference. It is common to use the term FID to refer only to the decay envelope of the oscillating signal.

FIG. 8



NMR MEASUREMENTS

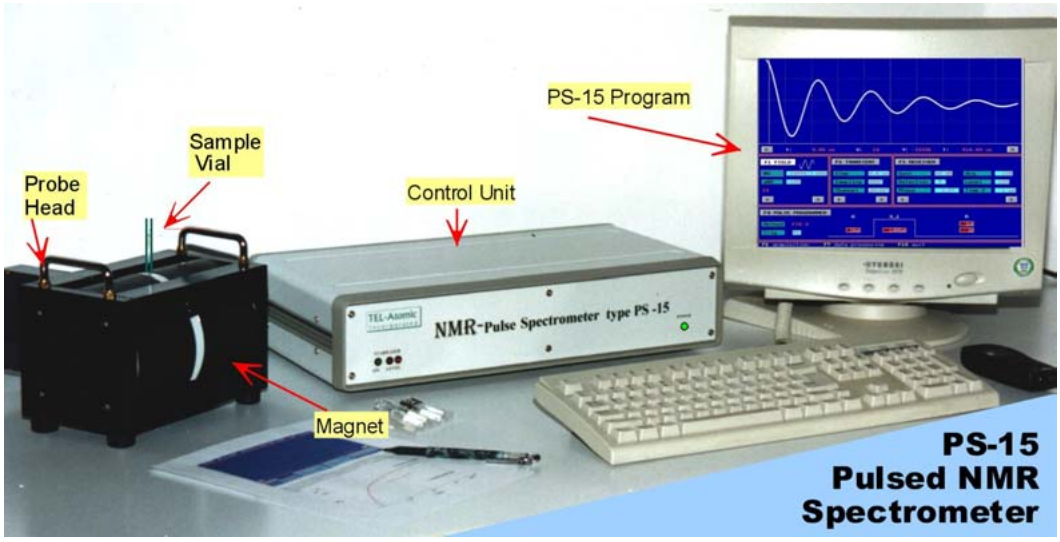
Each NMR experiment consists of two sections: preparation and detection.



Preparation includes of instrument setting and sample. During preparation the spin system of the sample is set to a define state. The latter is determined by a delay between repeat scans (Recycle delay) to allow relaxation; that is, the return of the macroscopic magnetization M_0 to the *z*-axis after the previous pulse and before the next pulse is applied.

The instrumental preparation involves adjusting the steady **magnetic field** and setting the duration of a **pulse** (the "flip" angle), **recycle delay**, the length of other **pulse delays in the sequence** if applicable and the **acquisition parameters**.

STARTING NMR WITH PS-15



PS-15 is not only easy to use, it is easy to understand since each module has its own clearly defined function in the spectrometer and is accessible to individual examination with the computer keyboard.
The PS-15 program supplied with each instrument provides the user with control over the system.

SYSTEM SETUP

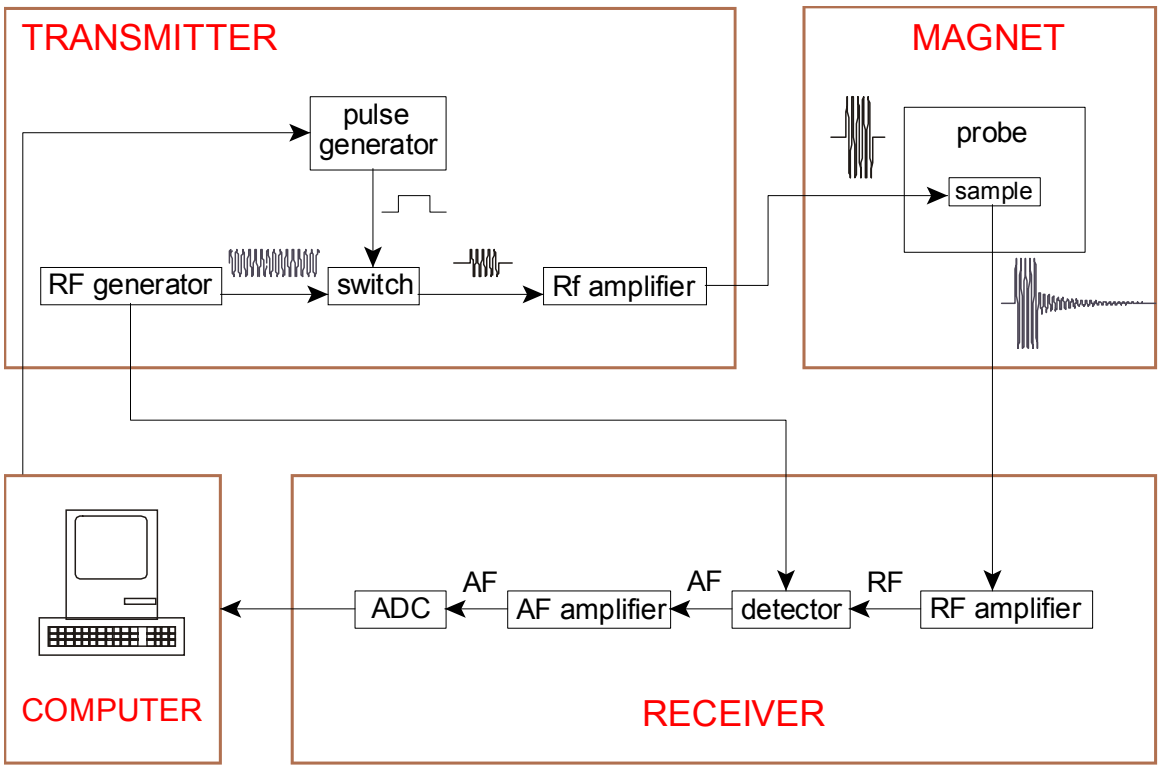
1) Connect the control unit with the rest of the components that is, with the probe head, magnet and computer (COM2 or COM1). The cables and slots on the controller are clearly labelled.
2) Connect the control unit to an electrical outlet. It is recommended to use the same outlet for both the computer and control unit.

Measurements of Pulsed NMR with the PS-15 spectrometer

This manual is intended to familiarise the novice user with the PS-15 spectrometer and how to use it to make pulsed NMR experiments. No prerequisite background is required regarding the operation of this spectrometer and of NMR theory. However, a familiarity with the latter would be helpful to better

understand the observed phenomena and would be necessary to take advantage of the capability of the spectrometer to further explore the exciting and important applications of pulsed NMR.

Block diagram of the PS-15 Spectrometer



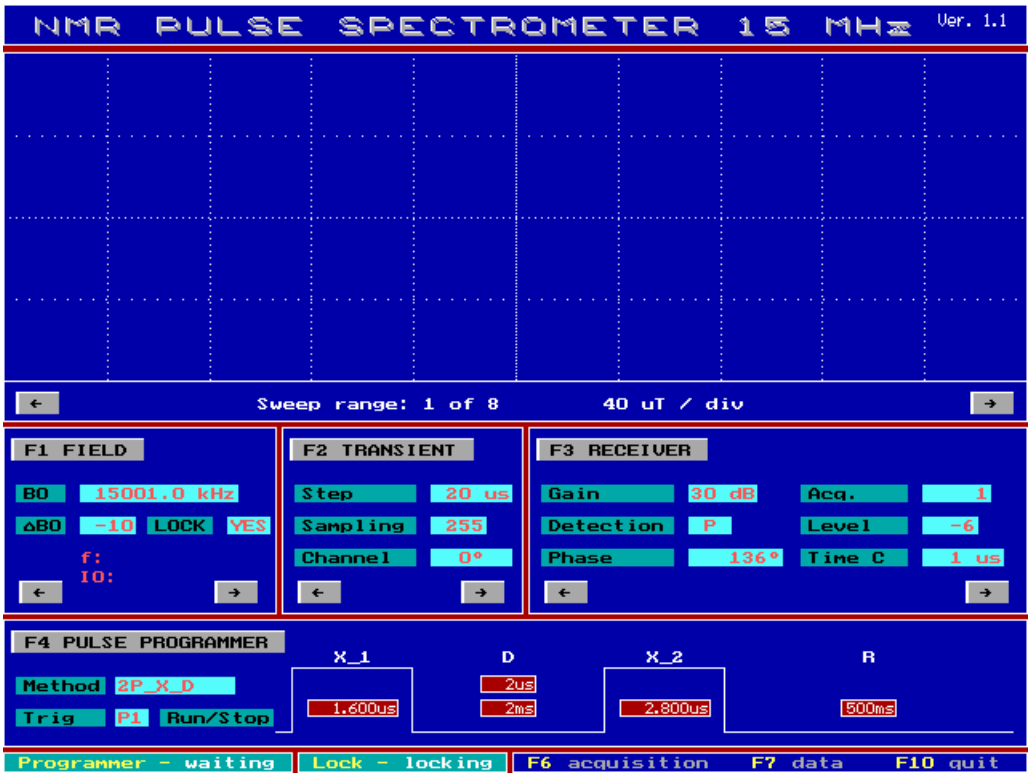
DC pulses for gating the RF signal are supplied by the pulse generator. The pulses are amplified and sent to the sample coil and irradiate the sample. The probe holds the sample, couples the RF field to the spins, and picks up the ensuring NMR signal. Its crucial component is a coil of wire around the sample, to which the transmitter pulses are applied; the alternating current in the coil generates a magnetic field coherently with the transmitter. The precessing magnetization excited by this RF field induces in the coil an oscillating voltage, the NMR signal, which is passed to the receiver.

After amplification, the NMR signal from the probe is mixed with a reference voltage, of the same frequency as the pulses used to excite the spins. This mixing subtracts the reference frequency from the NMR signal to produce an audiofrequency (AF) voltage, which is amplified further, digitized, stored and processed in the computer.

About the PS-15 Program

The program environment is divided into three major sections: Setup, Acquisition and Data processing. A given section can be selected by pressing the corresponding F-key.

F 5 - SETUP
F 6 - ACQUISITION
F 7 - DATA PROCESSING.
F 10 – Exit the program



The program opens the Setup screen (F5) and starts the NMR measurements after the completion of the automatically adjusting and stabilizing the magnetic field B_0 .

SETUP (F 5)

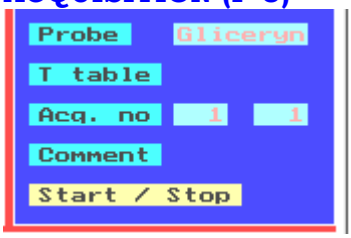
This section is used to select the settings for measurements**.** Trial measurements can be viewed on the upper half of the screen. The lower half of the Setup screen is divided into different windows labelled as F1, F2, F3 and F4.

With these windows the user can control the instrument settings and perform the NMR experiments. By pressing the function **F**-key shown in the window and then using the **Arrow**-keys the user can select and find the optimum conditions for acquiring the NMR signal for the sample under investigation. The <**F1** FIELD> tunes the magnetic field strength $\mathbf{B}_0$ to the resonance condition. The spectrometer can be set to perform either "on-resonance" or "off-resonance" experiments. The <**F2** TRANSIENT> window allows the setting of the data acquisition parameters and the <**F3** RECEIVER> sets the electronic parameters of the acquired NMR signal (receiver amplifier gain, detection method, phase, number of acquisitions, DC level offset, RC filter time constant).

The <**F4** PULSE PROGRAMMER> is an intuitive pulse sequence programmer. The user needs only to adjust the length of the rf pulses and time delays between the pulses. These settings are made in the displays that are placed on a sequence diagram where pulses and time delays are denoted by the rectangles and base line segments respectively. The user has the option of choosing a sequence pattern from the list in the "Method" menu. The list of pre-programmed sequences includes many frequently used pulse methods.

Run/Stop command allows to start or to halt the pulse programmer during pulsing. The same function is available from any place of the SPI program by pressing +/- keys. Current status of the programmer (**Pulsing/Waiting**) is shown in the bottom left corner of the SETUP window.

ACQUISITION (F 6)



This section is used for acquiring and storing the desired measurements. Once the settings have been chosen in "SETUP" this section allows the recording of the signal for the sample under investigation. When running the Acquisition mode the user performs a real experiment with signal storage in a binary file. The screen is divided into two viewing windows. The two-display mode is used for presentation of the real-time run NMR signal and to display the amplitude of the signals during T_1 and T_2 measurements, for a certain time delay.

DATA PROCESSING (F 7)

This section is designed for basic data handling. The user can display the chosen data file content on the screen. The binary data can be saved and exported in ASCII format for further processing, like relaxation times calculation and Fourier transformation.

Quick Start

*This section will familiarise the user with the three major modules of the system**: Setup, Acquisition and Data Processing**. More detailed information on the available adjustments and their practical application will be introduced as we proceed through the T_1 and T_2 experiments.*

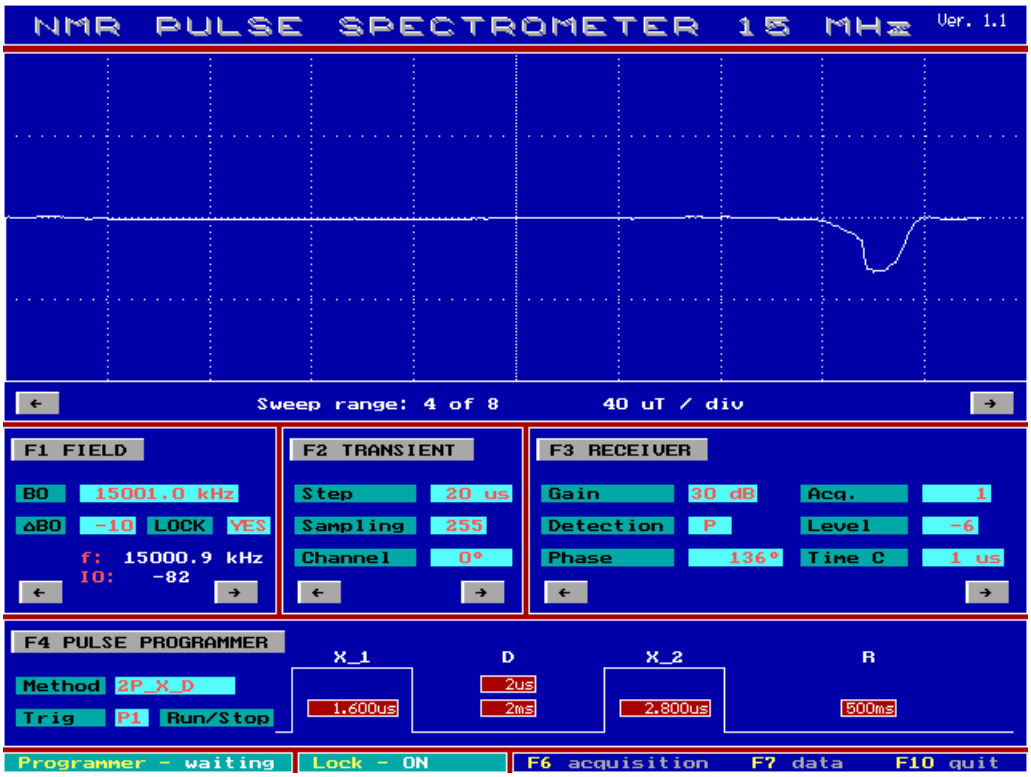
1) Insert a vial with the sample into the probehead cavity between the poles of the magnet.
2) Turn on the control unit with the switch at the rear panel. It turns on the power indicator.
3) **Run the PS15 program "spi.exe"**.

Note

Any sample can be used. However, it is recommended to start with the glycerine sample, which gives strong signal and has reasonable relaxation times. The glycerine standard sample and factory test settings are supplied with each spectrometer. In addition, we will refer to the signals from glycerine in the examples.

The sequence of the steps 2) and 3) is not important. If the spectrometer is not turned on then the message "Device is not ready" will appear on the screen. Press Enter when the system is on to re-establish communication. Press < Shift, 5 > to work off-line. If the sample is not inserted into the measurement probe head only the horizontal line (baseline) modulated by noise will be seen in the display.

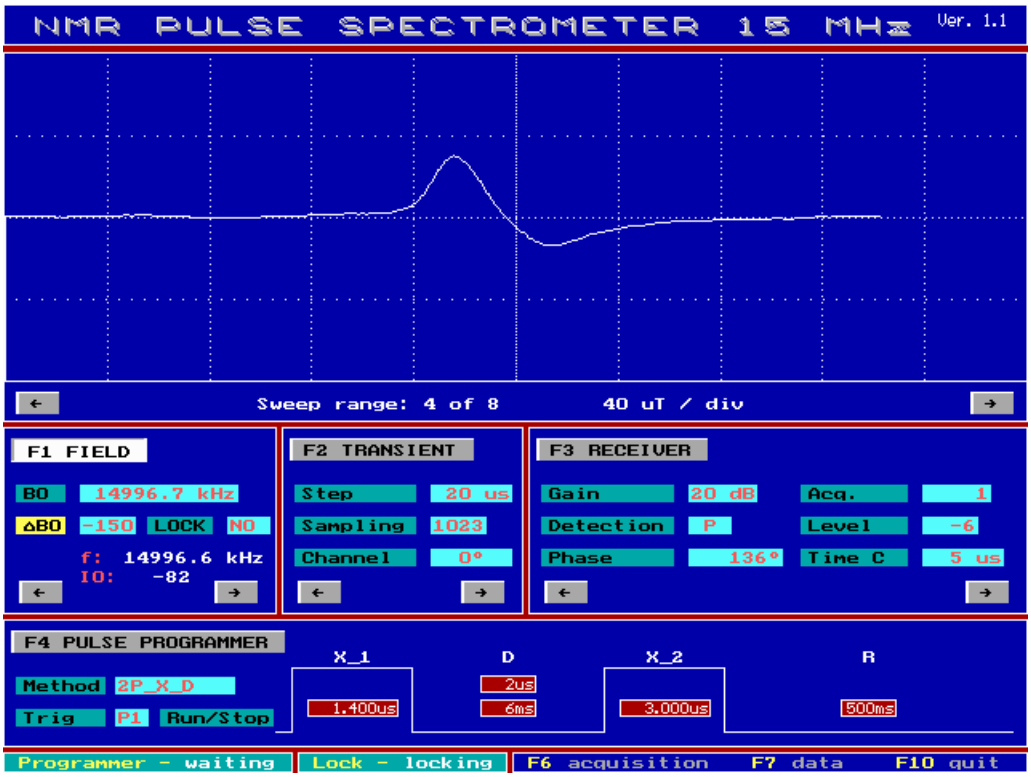
The program opens the Setup screen (F5) and starts automatically adjusting and then stabilizes the magnetic field B_0. A CW NMR signal from the built-in reference sample is visible on the screen. Note slight disorientation on the CW signal due to the change of the sweep speed from fast to slow.When locked the **lock status line** on the screen bottom shows **Lock-ON**.

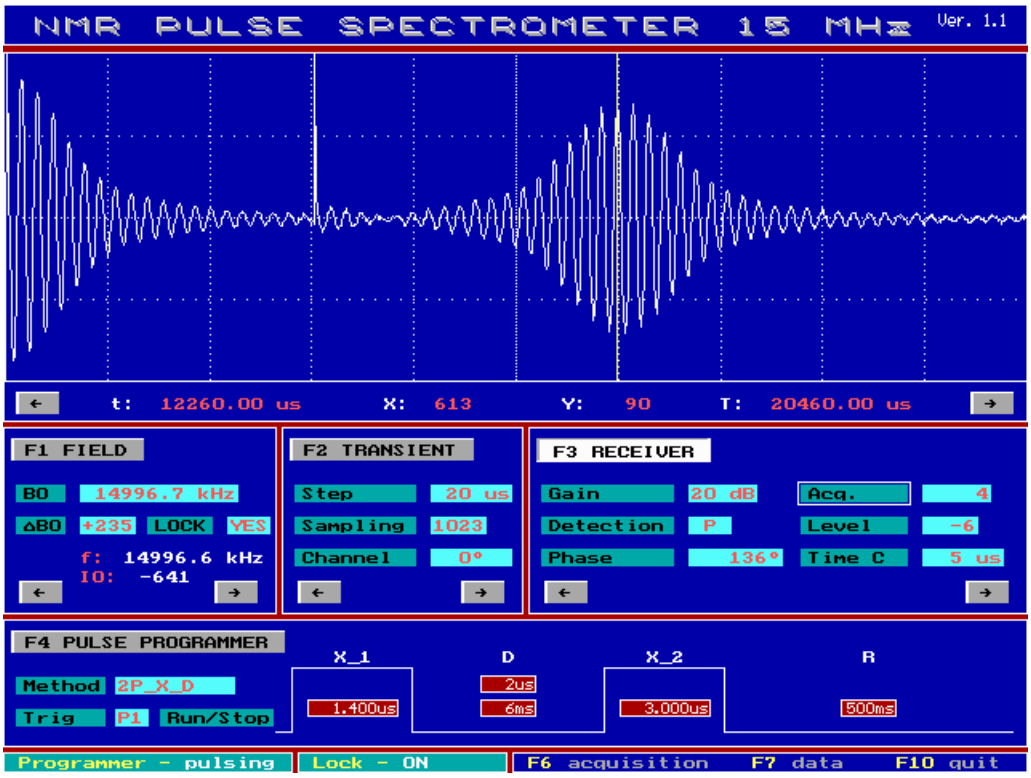


The **LOCK YES/NO** switch in the F1 Field window indicates an option to disable the stop of final lock during the sweep. When **LOCK NO** program works continously in a loop showing priodically the first derivative of ^{19}F NMR absorption signal from the lock sample as illustrated below. This option is dedicated to check the magnet homogeneity and the lock performance. During the sweep the lock status line shows **Lock-locking**. To skip this option select **LOCK YES** and wait for the final lock indicated by the lock status as **Lock-ON**, which takes 2-3 minutes

(More about the stabilization process can be found in the "*Flux Stabilizer and FFL"* section).

1334 – Ressonância magnética nuclear

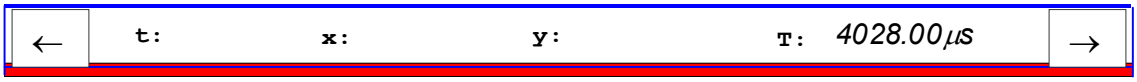




The system starts operating with the last run settings. The pulse or sequence method selected in the F4 window is applied to the sample and the NMR signal is shown on the screen. The repetition of pulse (or pulse sequence) occurs with the frequency shown in the display under the letter "R" (Recycle delay) in the last column of the pulse sequence pattern in the F4 window. (If there is a column of two settings the recycle delay is shown in the lower display (in sec)). The signal is displayed with each scan or after the number of repetitions (the resultant signal) that depends on the number selected with the command <Acq.> in the <F3 Receiver> window. Select Acq. = 1 to observe the signal after each run.

The user may now proceed to the step no.4 or start practicing with the available settings and observe the effect on the acquired signals. *The factory test settings supplied with each spectrometer can be repeated for the purpose of this section.* The "*Reference Guide"* (Page 18) section will assist the user to understand the function of the available settings and adjustments.

The NMR signals are displayed in a time domain. The user should be familiar with the display settings while adjusting the magnetic field or performing the experiments. The total time of observation T (in µs) is shown at the bottom of the display.



The other settings in this line describe the signal with respect to the end of the pulse at the location of the pointer (vertical line); t – time, x – sampling point number, y – amplitude. (Press the Esc key twice to activate the pointer and use the arrow keys to move the pointer into desired location).

The period of acquiring data can be changed with the <F2 Transient> window. The user is encouraged to practice with the settings.

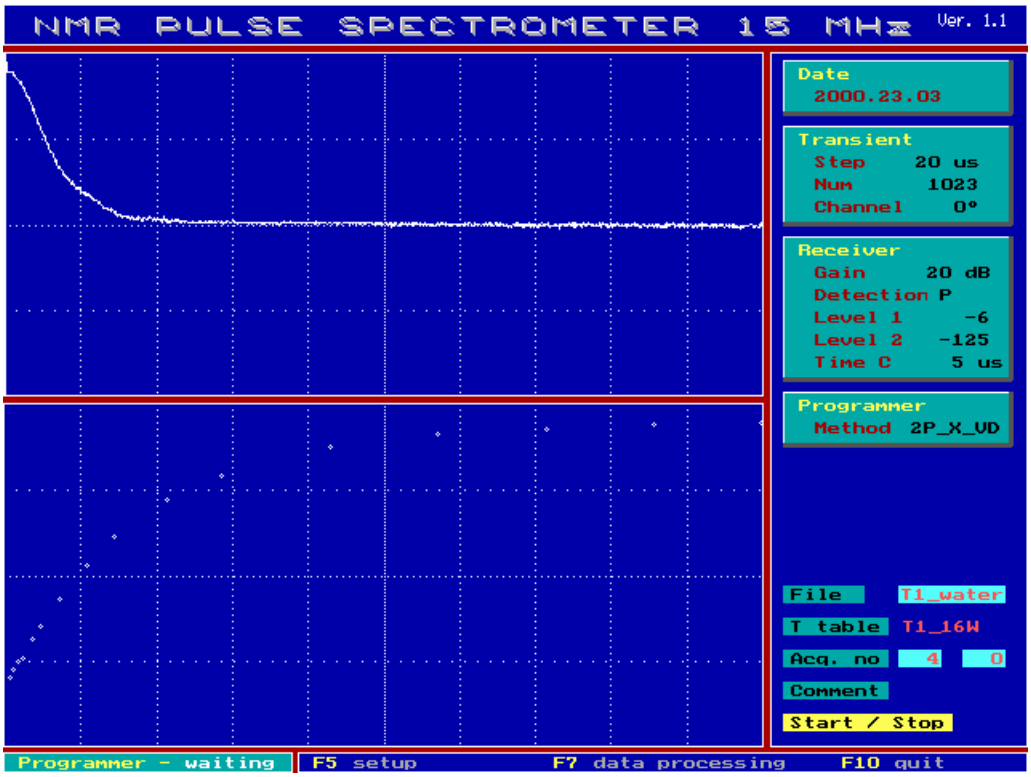
`Step` - Dwell Time: the time between data points; defines the sampling time interval, **τ**, i.e., the time increment between data points, τ range; / 0.4 - 400 / µs.

`Sampling` - Data Size: defines the total number of data points per scan, **N**. N range; / 256 - 8192 / points.

The total time of acquisition, $T = \tau \cdot N$

For example, 40 µs · 1024 = 4028 µs (~ 4 ms). Combination of both (Step & Sampling) allows changing the acquisition period and resolution.

4) **Press the F6 key to start acquiring the measurements.**



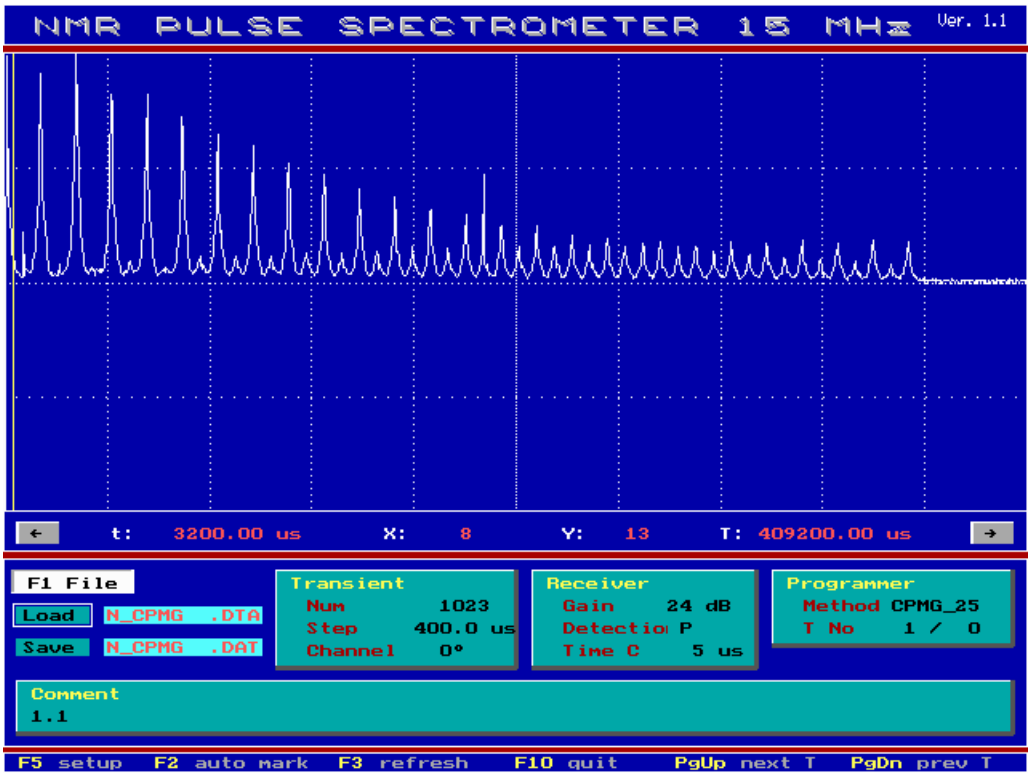
When running the Acquisition mode the user performs an experiment with signal storage in a binary file. The screen is divided into two viewing windows. The upper window is used for presentation of the real-time run NMR signals and the bottom window to show signals current amplitdes versus delay acording to Variable Delay (VD) table. This is useful function during T_1 and T_2 measurements (See Practical Considerations in *T_1* and *T_2 experiment*).

The user can name the data file (**File**), select the **VD** table (if applicable) and number of runs (**Acq. no**), and make notes (**Comment**).

The system will acquire the spectrum. The acquired spectrum and data can be saved in the DATA folder. The user can stop the process at any time and continue or return to the Setup mode (by pressing F5) to change settings and acquire new data or overwrite the existing data.

The user can open any file saved in the DATA directory with the Data Processing section

5) **Press the F7 key to open data processing section.**



To download the file press the <F1 File>, Press Enter twice, highlight the desire file on the list with the arrow keys and click Enter to view the file. To save the file for exporting, press F1, highlight the save button, click Enter and name your file, click Enter. The file is saved in ASCII format in the DATAOUT folder. To explore the other processing capabilities see the "*Viewing and saving acquired data with F7 - data processing"* section in the *T_1 and T_2 experiments(Page 35 and 50).*

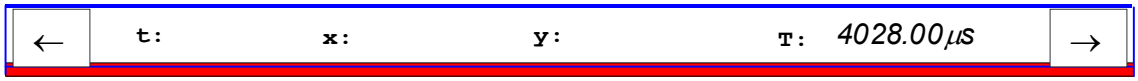
Reference Guide

F1 FIELD

Measurements start with adjusting the magnetic field $\mathbf{B}_0$ and frequency to fulfil the resonance condition. The steady magnetic field, $\mathbf{B}_0$, can be tuned to the desired resonance condition with the ΔB_0 adjustment in the <F1 Field> window (For more detailed description see the following sections *"**FID signals <F1 Field>**" (Page 21)* and *"**Flux Stabilizer and FFL**"*) (Page 27).

F2 TRANSIENT

The NMR signals are displayed in a time domain. The total time of observation T (in µs) is shown at the bottom of the display.



The other settings in this line describe the signal with respect to the end of the pulse at the location of the pointer (vertical line); t – time, x – sampling point number, y – amplitude.

The period of acquiring data can be changed with the Step and Sampling commands.

Step Dwell Time; the time between data points; defines the sampling time interval, **τ**, i.e., the time increment between data points, (0.4 – 400 µs).

Sampling determines the total number of data points taken per one run, N. / 225 ÷ 8191 / points

The total time of acquisition, T, seen on the display equals $T = \vartheta \times N$.

For example, 4 µs × 2047 = 8,188 µs (~ 8.2 ms)

VERTICAL POINTER on the display can be activated by clicking the <Esc> button and then can be moved to the desired location with the Arrow keys ← , → and/or < Ctrl, Arrow key >. The position of the cursor is described at the bottom of the display (t, x, y).

Channel 0^o ; 90^o ; $0^o + 90^o$; $\sqrt{0^o + 90^o}$

The system employs the detection method called *quadrature phase detection* (QPD). The output from the quadrature detector, S(t), is divided into two signals of equal intensity, S_1 and S_2 , which are fed along two separate channels called “real” and “imaginary” *(labeled as 0^o and 90^o ; the designation as real or imaginary is entirely arbitrary).* Each of them is subjected to different phase-sensitive detectors, whose reference phases differ by 90^o .

The channel selections display as follows:

- < 0^o > or < 90^o > signal S_1 or S_2
- < $0^o + 90^o$ > both signals simultaneously
- < $\sqrt{0^o + 90^o}$ > magnitude of both signals computed as the square root of the sum of squares of the real and imaginary data: $Mag = \sqrt{(\mathrm{Re})^2 + (\mathrm{Im})^2}$

The program displays and stores data according to the selected option.

F3 RECEIVER

Gain 0 - 60 dB

Typical setting 30 – 50 dB.

This should be adjusted so that the input voltage fits the digitizer operating voltage in order to get a strong and visible signal that is not "cut off" or truncated.

Detection P (Phase detection), A (Amplitude detection)

Typical setting P (Amplitude detection, A, is used mostly for testing the instrument

Phase 0^o - 360^o, resolution 2^o

If < 0^o > or < 90^o > is selected, corrects phase to reach maximum signal amplitude (either positive or negative.)

Acq. 1 - 128

Setting depends on signal/noise ratio and desired resultant output signal.

Level ±125

Sets the vertical positioning of the recorded signal on the display.

Time C 1, 5, 30 µs (Time constant of the receiver RC filter)

Typical setting

1 µs and 5 µs - solids, semi-solids and high viscosity liquids

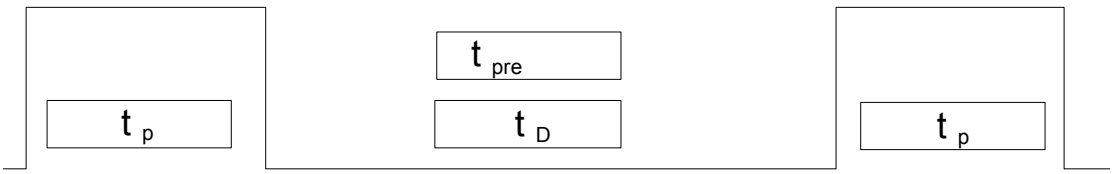
5 µs and 30 µs – liquids (e.g. water).

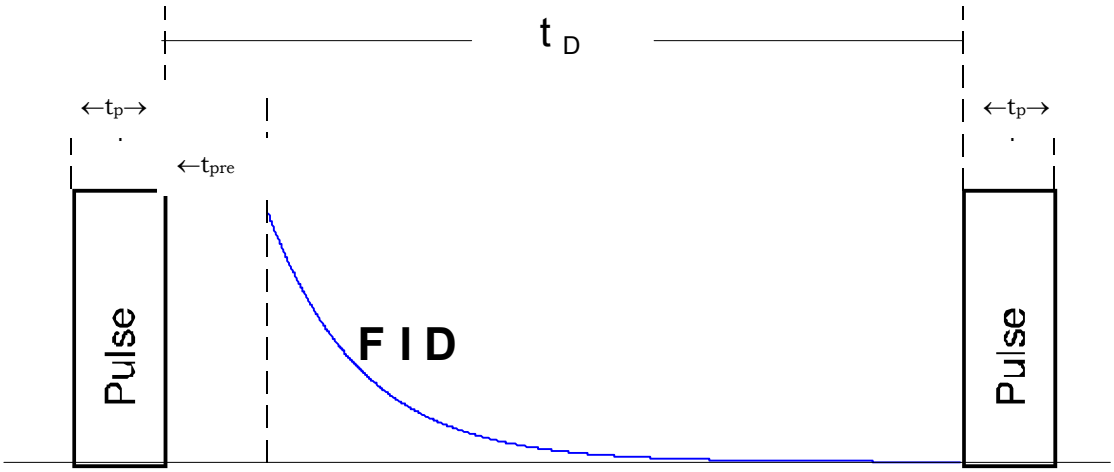
F4 PULSE PROGRAMMER

The following figures illustrate the setting of the pulse sequence employed in the "method" diagram.

The list of pre-programmed sequences is enclosed in Appendix A (Page 54).

An application of some pulse sequences can be found in the *T_1 and T_2 experiments*.





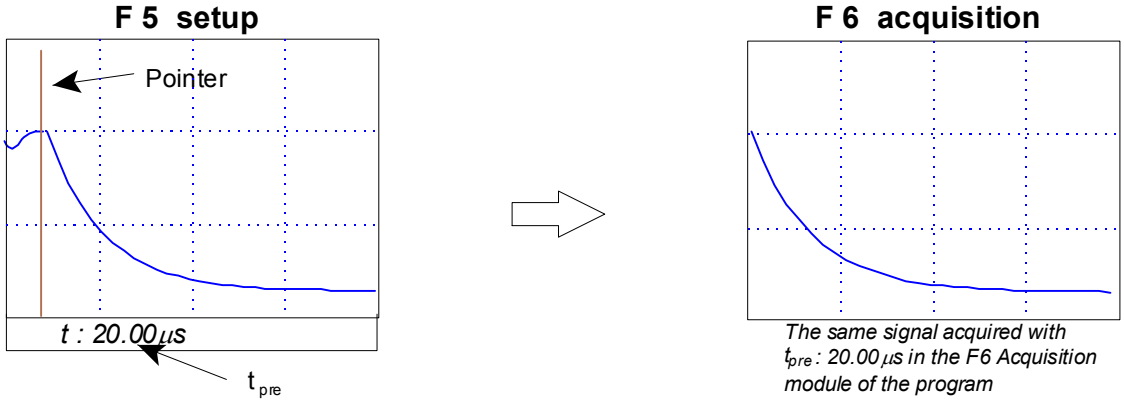
Note:

1) t_p - the pulse width. For most samples a 90° pulse is ~ 1.6µs, (180° pulse ~ 3.4 µs)

2) **t** $_{pre}$ - pre-delay time ~ (20 ± 2) :s.

A short delay, **t** $_{pre}$, is introduced between the end of the pulse and the beginning of the acquisition of data to eliminate ringing in the receiver coil.

This is commonly called pulse break-through. In the Setup mode (F5) data is acquired after the applied pulse. The following figure illustrates the procedure used to determine the starting point for acquiring data. The vertical pointer is useful for setting a value of **t** $_{pre}$. *The <Esc> button activates the pointer.*



The same signal acquired with t_{pre}: 20.00 µs in the F6 Acquisition module of the program

3) Pre-delay time, **t** $_{pre}$, to be set and appears on the sequence pattern after the pulse (P1 or P2) whichever was selected with the <Trig> command to triggering the recording device (i.e. a computer). If the P2 is selected then the **t** $_{pre}$ display setting is shown on a sequence pattern below the letter "R" and above the repetition time.
4) **R** – repetition time delay (1 - 500 s) sets the time to start a whole cycle again. This is the period of time for repeating the cycle which is observed on the display with the setup mode (F5) and time between the runs in the acquisition window.

FID Signals <F1 Field>

The user is encouraged to practice with all the settings. However, there are suggested initial parameters in the F2 and F3 windows for a sample of glycerine in order to focus our attention on the F1 adjustments first.
F2: Step 4.0µs, Sampling 1023 or 511, Channel 0º
F3: Gain 35-40dB, Detection P, Phase 0º, Acq. 1, Time C 5µs, The "Level" sets the vertical position of the signal. Place the signal close to the middle of the display with this setting.

Having turned the system and program on and inserted sample into the probe head, we should see the NMR signal from the sample on the screen. The signal should look something like one of the following

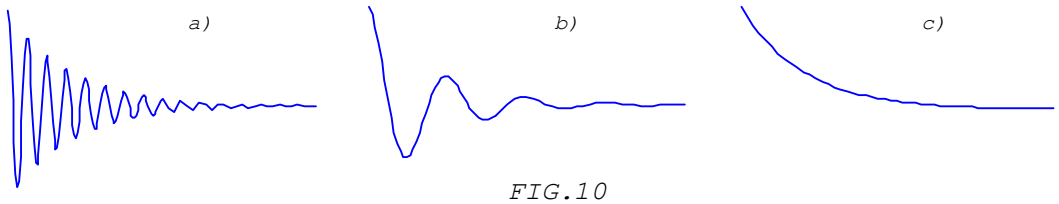
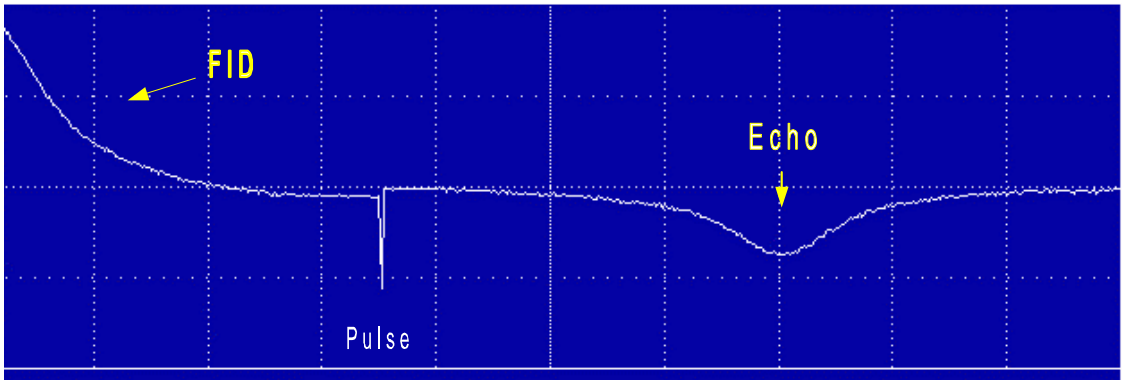
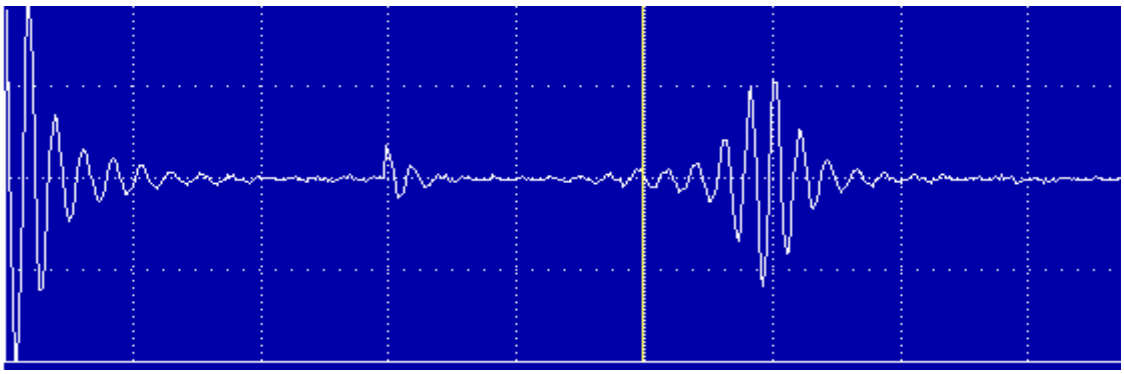


FIG.10

The following images show an example of NMR signals acquired for the same sample





The signals shown in Fig. 10 (and on the above images) appear different although they are produced by the same sample. The signal depends on the nature of the sample and on the characteristics of the instrument. We can assume that the characteristics of the instrument (in particular, the homogeneity of the steady magnetic field) have not been changed while scanning the same sample. Therefore we should be able to determine the same specific characteristic of the sample in all those signals in Fig.10 in spite of the apparent differences in detecting them.

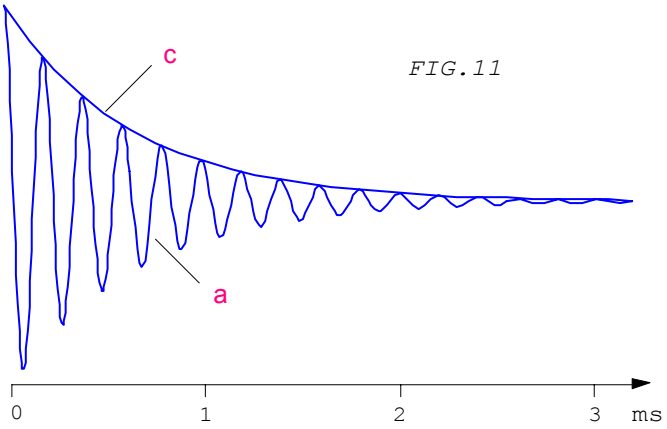


FIG.11

The signal decay parameters characterise the sample. Fig.11 shows a comparison of two signals, a and c, in Fig.10. The amplitudes of the signals decay simultaneously towards zero with a time that depends on the sample under investigation. (For the glycerine sample this time is ~ 3ms).

In each of the a) and b) cases the time response is a simple, damped cosine wave whose frequency is the difference between the proton precessional frequency (the Larmor frequency of M_0) and the spectrometer reference frequency (the frequency of B_1). Fig. 12 illustrates this relationship.

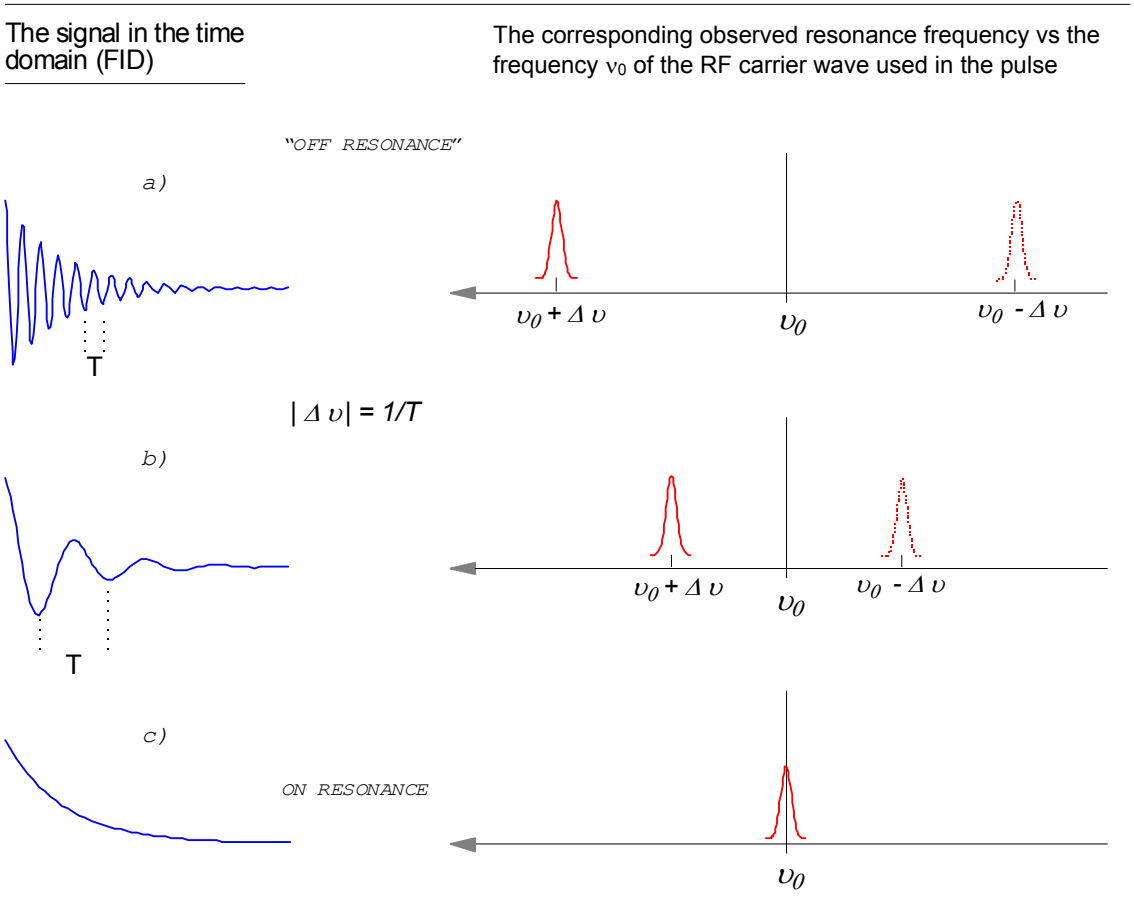


FIG.12

If the RF matches the Larmor frequency exactly, then it is said to be in ***resonance***. $\mathbf{M_0}$, as well as $\mathbf{B}_1$, would appear stationary in the rotating frame of reference and $\mathbf{B}_1$ would be the only field affecting $\mathbf{M_0}$ in this frame. This situation has been described in the "*Basic Principles*" and is illustrated in Fig.7. Note that the xy-component magnetization of $\mathbf{M_0}$ never leaves the xy-plane although it eventually decays to zero. A coil (antenna) placed along the y-axis picks up the x-y component of the magnetization of the precessing vector $\mathbf{M_0}$ and the spectrometer displays the decay envelope of the FID similar to the signal (c) in Figs.10 - 12.

When the frequency of the RF pulse is moved ***off resonance*** the effective and time-independent field, $\mathbf{B}_{eff}$, in the rotating frame is no longer $\mathbf{B}_1$. $\mathbf{B}_{eff}$ is composed of the offset field $\Delta\mathbf{B}$ (along the z-axis)**,** and the RF field $\mathbf{B}_1$. Note that $\mathbf{B}_{eff}$ is not in the xy-plane and it is always larger than $\mathbf{B}_1$ and will cause more rapid precession of the magnetization $\mathbf{M}_0$ around $\mathbf{B}_{eff}$ off resonance, ($\omega = \gamma B$). Compare a ringing pattern of the (a) and (b) signals in Fig.12 and their corresponding offsets, $\Delta\nu$. The reader may also wish to skip a detailed consideration of the effective field and move forward and think about the signal oscillation as a frequency beat between the FID and the RF pulse.

The concept of the rotating frame is exceedingly convenient not only because it removes the need to think about time-dependent fields, but also because NMR spectrometers detect offset frequencies ($\Delta\nu$) rather than the actual frequency (ν). (Our discussion will be based exclusively in the rotating frame). The frequency (offset), $\Delta\nu$, of the signal can be easily determined by measuring the period, T, of the sine wave, $\Delta\nu = 1/T$. Note that the signal will oscillate with the same frequency for both positive and negative offsets with respect to the reference frequency, ν_0.

In summary, the signal can be observed when either the spectrometer is tuned exactly to "resonance" or it is "off resonance". It can be set either by changing the reference frequency of the pulse ($\mathbf{B}_1$) or by changing the Larmor frequency of $\mathbf{M}_0$. Recall that the Larmor angular velocity equation, $\omega_0 = \gamma B_0$, is the direct relationship between the applied magnetic field $\mathbf{B}_0$ and Larmor frequency.

ΔB_0 **-** adjusts $\mathbf{B}_0$ to the desired magnitude and resonance condition.

Note: use the shortest step (0.4μs) for setting and checking the resonance condition.

DETERMINING PULSE TIMES <Method>

The length of time that an rf pulse must be applied to tip the magnetization by a specified angle (e.g. a 90° pulse) is strongly dependent on the amplifier output power level and on the construction and tuning of the rf coil of the probe. However, the user's only concern is to set the pulse duration, t_p. Recall that the angle through which the magnetization $\mathbf{M}_0$ moves during the pulse is given by $\theta = \gamma B_1 t_p$.. A 90° pulse creates the largest amplitude FID (see Fig.6). In the PS-15, a pulse duration $t_p \approx$ 1.6μs produces a 90° pulse, $90^o = \gamma B_1 \times 1.6\mu s$. The procedure is relatively simple and depends on selecting the time that creates a maximum amplitude FID. The vertical pointer can be useful with this procedure (See Fig.9). Its y component shows the amplitude of the signal at the position of the marker. Actually, the 90° pulse is not very sensitive to the duration of the pulse, t. Therefore, in practice one usually adjusts the 180° pulse for a minimum (ideally zero) and sets the 90° pulse width for half of the 180° pulse width.

The procedure for determining the duration of a 90° pulse can be achieved with only one pulse. One procedure for obtaining 90° and 180° pulses is to use a two-pulse sequence in order to observe the results of both pulses simultaneously. For example, tuning the first pulse towards a 90° pulse and the second pulse towards a 180° pulse.

B_0 and B_1 inhomogeneity over the sample makes it impossible in most cases to have accurately set pulses for the entire sample. In practice, all 180° pulses are imperfect and therefore also produce small FID signals.

DETERMINING PULSE DIRECTIONS <Method>

We have seen that the pulse duration determines the motion of magnetization M_0. This motion is also dependent on the direction of B_1. A combination of both various pulse durations and directions at which they can be applied creates an almost unlimited number of complex pulse experiments that can be executed with modern NMR spectrometers. The PS-15 has this capability.

If we apply an rf field B_1 along the x-axis, M_0 will precess about B_1 in the same way that a gyroscopic top precesses in the earth's gravitational field (remember that the nuclei possess *both* a magnetic moment and angular momentum). We can always keep track of the direction in which M_0 moves by remembering the right-hand rule: if the fingers of the right hand point from the direction of M_0 to the direction along which B_1 is directed, then the thumb will point in the direction in which M_0 will move. Thus if B_1 is directed along the +x-axis, M_0 will rotate clockwise about the +x-axis (see Fig.13a). If B_1

is directed along the –x-axis, then, of course, M_0 will rotate in the opposite direction. This is a very important concept and has far-reaching consequences, for it affects the NMR signals that we obtain from our spectrometer. And it provides the basis for the four basic spin-gymnastic exercises, which are shown schematically in Fig. 13a-d.

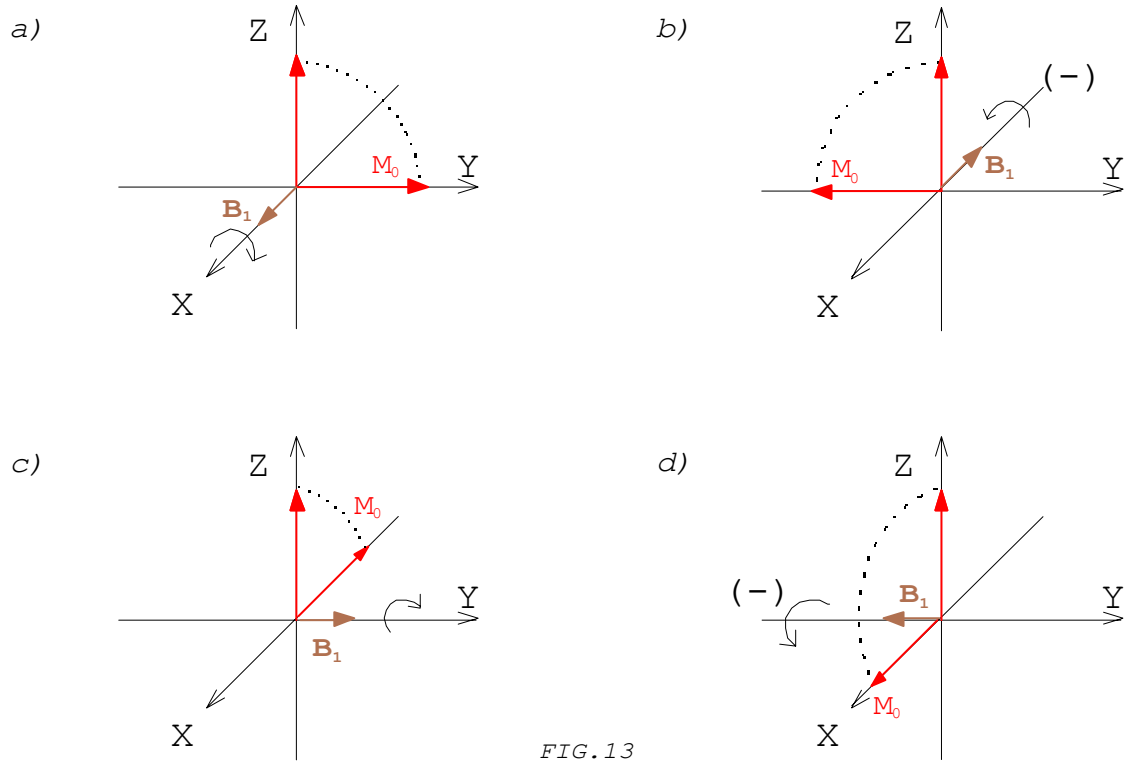
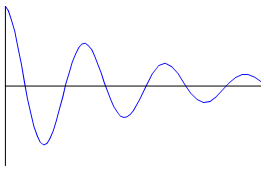
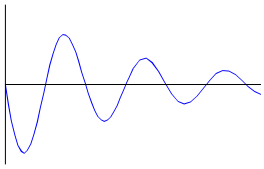
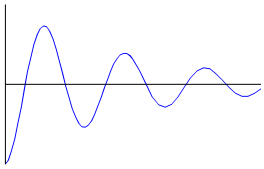
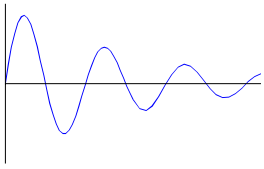


FIG.13

We shall hereafter use the notation for pulses given in Table 1, which is commonly adopted in the NMR literature. The letter +/– x or +/– y denotes the axis along which the pulses of RF energy are applied, and is preceded by the pulse angle in units of radians or degrees. Thus 90°(–x) denotes a 90° pulse along the negative x-axis in the rotating frame (Fig.13b). By a careful choice of the $\mathbf{B}_1$ axis and the duration of the $\mathbf{B}_1$ pulse we can put $\mathbf{M}_0$ at any arbitrary position in our

rotating frame of reference. The position of M_0 in the xy-plane determines the nature of the signal which we observe in the NMR spectrometer. If M_0 lies along the +y-axis, we obtain a positive FID signal as shown in Table 1a. If M_0 lies along the –y-axis, we obtain a negative FID signal, and so on.

Table 1

B_1 Axis	Position of M_0	Form of FID
+X	**+y** axis (Fig.13 a)	
+Y	**–x** axis (Fig.13 c)	
–X	**–y** axis (Fig.13 b)	
–Y	**+x** axis (Fig.13 d)	

The pulse directions in the pulse sequence patterns are denoted as X,Y,–X,–Y.

Flux Stabilizer and FFL (Field-Frequency locking) <F1 Field>

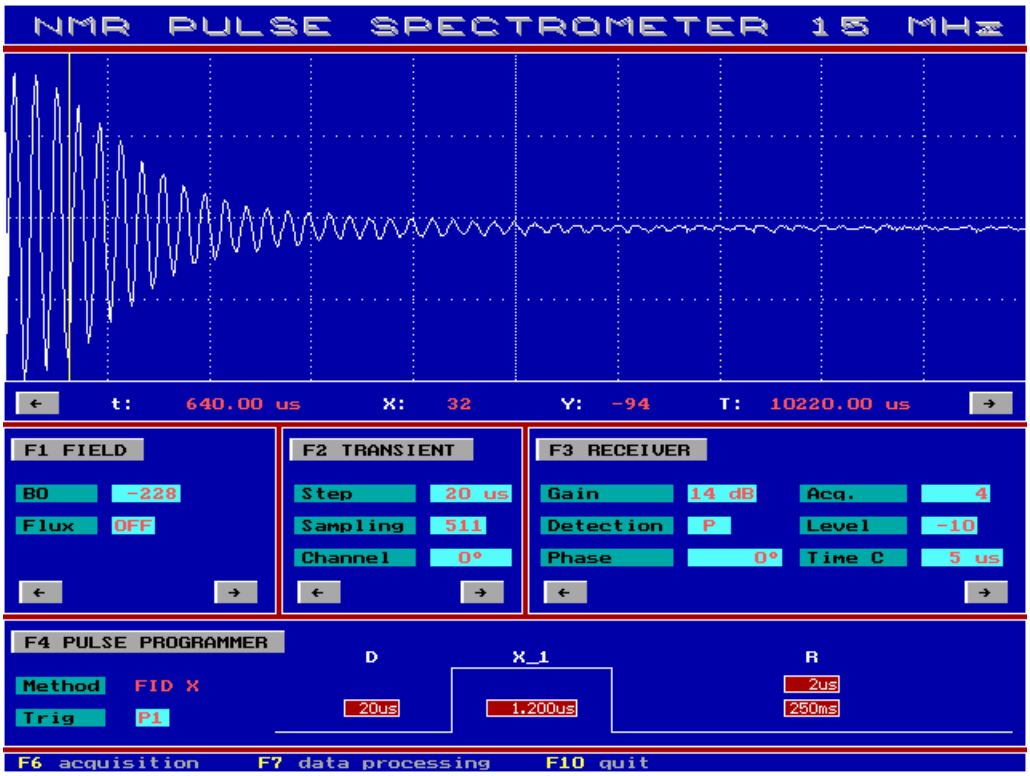
The **F1 Field** module of the program sets the magnetic field $\mathbf{B}_0$ of the electromagnet to the resonance condition via regulation of electric current in the magnet coils. The magnetic field strength also depends on the permeability (which is temperature dependent) of the iron in the magnet. Therefore, high field stability is achieved in the PS-15 spectrometer by employing a field-frequency locking (FFL or NMR lock) system

and a flux stabilizer (often called a “superstabilizer”) system. The flux stabilizer is based on the fact that any change in the magnetic field induces a voltage in separate coils wound around the magnet pole pieces causing a feedback circuit to send a current to the correction coils that compensates for any change in the field since the induced voltage is proportional to dB/dt. This circuit acts like a low pass filter to damp out rapid fluctuations in the field. The flux stabilizer in conjunction with an NMR lock provides both short- and long-term field stability.

The software package includes two programs; “spi.exe” and “spifl.exe”, located in two separate directories the “**user”** and “**service”,** respectively. The spi.exe runs the program with both the flux stabilizer and NMR lock stabilization systems. The magnetic field value, stored in the configuration file, is automatically locked. Using ΔB_0 in F1 window (1024 steps or +/- 512), fine adjustment, in the range of +/- 2.4 mT is available after NMR locking is set. This program is used routinely for NMR experiments at “on-resonance” and “off-resonance conditions.

However, if the spectrometer is moved to a new location or for other reasons the magnetic field requires a larger adjustment than the range available after NMR locking then the magnetic field should be adjusted prior to running the PS-15 program. The spifl.exe is used to adjust the magnetic field to the desire value. The spifl.exe does not activate the NMR lock stabilization and can be used with or without flux stabilizer to adjust the magnetic field in 2048 steps (+/- 1024) that cover the range of +/- 2.5 mT with respect to the existing steady magnetic field B_0, in the magnet. If the magnetic field requires larger change than +/- 2.5 mT, *the potentiometer at the rear of the controller* should be used. It provides an additional change of the field of +/- 5.0 mT.

There is one-to-one relationship between the magnetic field strength and the frequency to satisfy the resonance condition. This means that for a given RF frequency f_R there is only one magnetic field B_R to achieve resonance. The magnet produces a constant magnetic field B_0 . There is a certain range of magnetic field ΔB available with the keyboard in the Setup mode which can alter the magnetic field B_0



. The Setup screen of SPIFL program.

If the matching resonance magnetic field B_R is within the range given by that "window "
(B_0 ± 2.5 mT in 2048 steps) then it is possible with the keyboard to find the resonance by pressing left (field down) or right (field up) arrow key. However, if B_R happens to be beyond that range ("window") then the on-resonance condition cannot be found unless B_0 (the value of the magnet) is changed so that the available range includes the B_R value. To fit the $\pm$2.5 mT range, B_0 can be changed manually with the **flux off** using a potentiometer inside the unit (it changes the base current I_0). In practice, however, this adjustment may be required occasionally, but only by the experienced user.

The pulse probehead also incorporates a CW part with a sealed sample (containing Fluorine, ^{19}F). CW resonance pictures always occurs in the Setup mode when the unit is turned on. It usually takes 2-3 minutes to have FFL locked. (When the stabilizer is ON the green LED on the front panel is active).

The magnetic field magnitude B_0 , seen in the SPIFL program setup window should be set at approximately at -500 units before executing the SPI program because the FFL requires some "head room" to start the sweep and then a certain margin to correct the field.

The FFL system requires the transition of an "error signal" from plus to minus polarity. The DC offset, due to the thermal drift of the electronic circuit, often shifts this signal below or above the zero level. The system corrects it automatically when program starts and compensates it when unit is working. Any uncompensated positive or negative DC level in the FFL is shown by the Stabilizer LED (red) located on the front panel.

If the level of the line is far above or below or above zero level the FFL device cannot generate a correct error signal and finally and cannot lock the magnetic field with respect to that line. FFL is an excellent system for preventing slow drifts but a rapid transient in the field may cause the system to lose track of the resonance and become unlocked. Therefore, NMR locks are usually used in conjunction with a flux stabilizer.

NMR PULSE EXPERIMENTS

This section will guide the new user on how to make pulsed NMR measurements. This tutorial will show the user how to measure relaxation times using different pulse sequences and methods. The relaxation times, T_1 and T_2 , are of paramount importance in pulsed NMR spectroscopy. However, the main goal of "Quick Start" is to familiarize the user with the operation and procedures of the instrument using the hands-on approach. We start with a discussion of how T_1 and T_2 can be measured experimentally. Only general concepts associated with the measurements will be given.

T_1 Relaxation

When $\mathbf{M}_0$ has been deflected from along the $\mathbf{B}_0$ direction by resonance it does not remain in its new position indefinitely, neither does it return to the $\mathbf{B}_0$ direction immediately after the response is over. $\mathbf{M}_0$ returns to its equilibrium position along $\mathbf{B}_0$ in a time that can be quite long compared with the length of the pulse. This is called nuclear relaxation and can be though of in terms of two relaxation processes known as longitudinal relaxation and transverse relaxation. Longitudinal relaxation is also known as spin-lattice relaxation or T_1 relaxation and transverse relaxation is also known as spin-spin relaxation or T_2 relaxation.

FIG.14

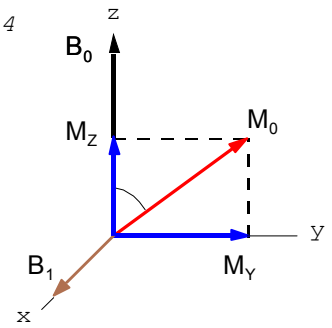


Fig.14 shows $\mathbf{M}_0$ when it is deflected by an angle θ from the z-axis. $\mathbf{M}_0$ can be resolved into two components, $\mathbf{M}_Y$ along the y direction and $\mathbf{M}_Z$ along z direction. It is the magnitude of $\mathbf{M}_Y$ which affects the size of the signal after a pulse. $\mathbf{M}_Y$ is a maximum and equal to $\mathbf{M}_0$ immediately after a 90° pulse (See Fig.6). At this time $\mathbf{M}_Z$ is zero. As nuclear relaxation takes place after a 90^0 pulse and $\mathbf{M}_0$ returns to the $\mathbf{B}_0$ direction, $\mathbf{M}_Y$ gets smaller and smaller and finally disappears, while $\mathbf{M}_Z$ gets bigger and bigger until its magnitude is equal to $\mathbf{M}_0$ when $\mathbf{M}_0$ has finally returned to the $\mathbf{B}_0$ direction.

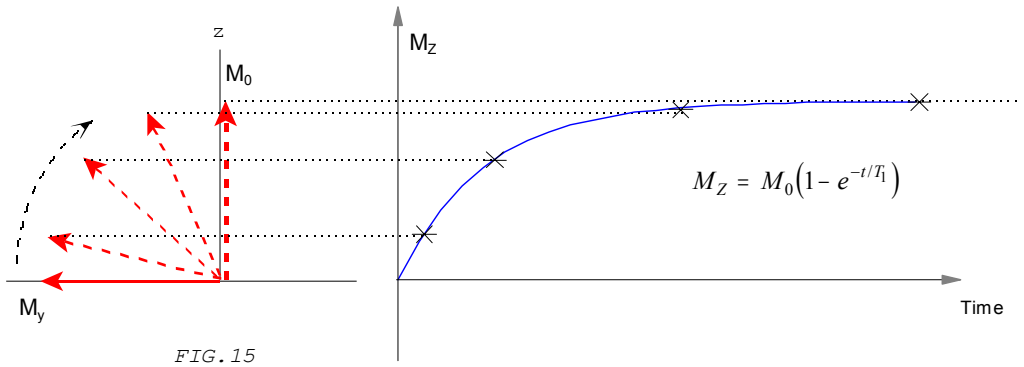


FIG.15

The magnitude of $\mathbf{M}_z$ as function of time after a 90° pulse is shown in Fig.15. In the formula given in Fig.15 T_1 is the time constant of the increase of M_z . T_1 is the time for the longitudinal magnetization $\mathbf{M}_z$ to regrow from 0 to $(1 - e^{-1})$, in a time $t = T_1$, or about 63% of its initial value $\mathbf{M_0}$. The question may arise why we cannot measure T_1 from a single pulse signal. The answer comes from the effects after applying one pulse.

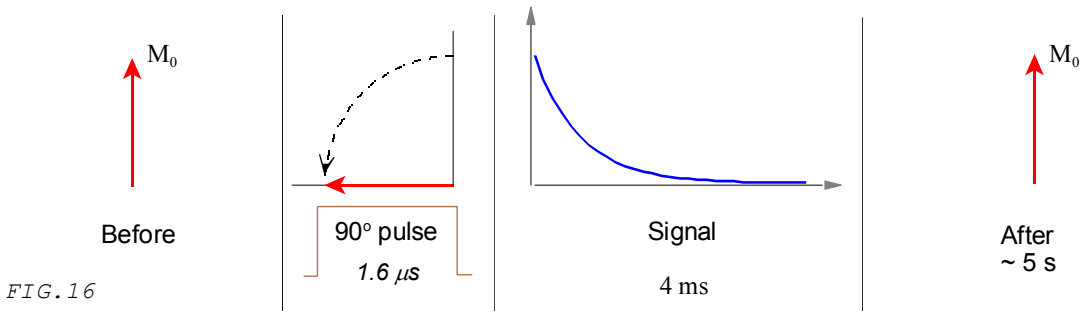


FIG.16

The signal decays much faster than T_1 (due to relaxation T_2 and magnetic field inhomogeneities). Fortunately, one feature of the signal can be used to investigate T_1, namely the amplitude of the FID signal.
Let's return to the previous picture but with some numbers for an easier illustration.

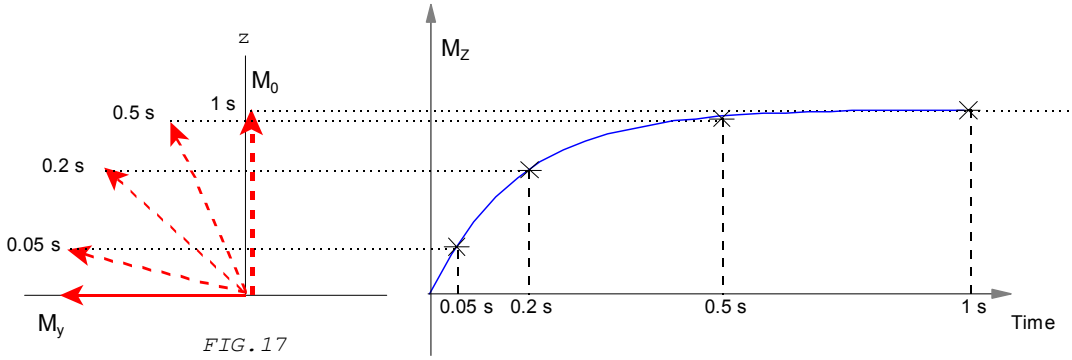


FIG.17

Fig.17 shows the recovery of M after applying one 90° pulse. Note that we know how the process occurs but have said nothing about how it is measured. First, look at the chosen snapshots from this process (Fig.18).

FIG.18

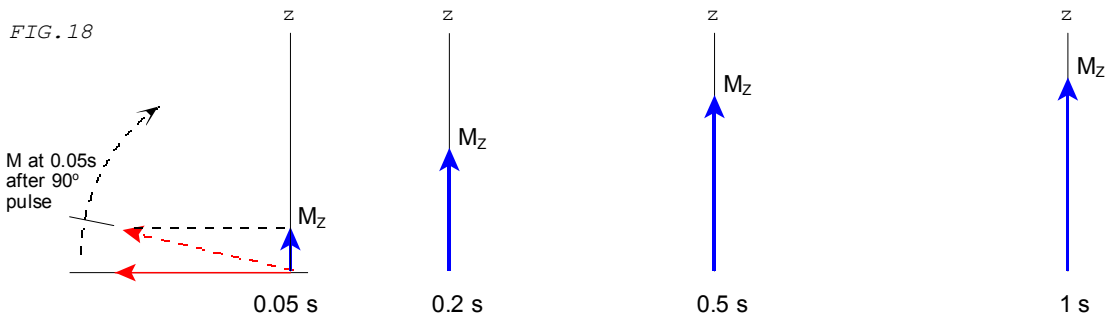
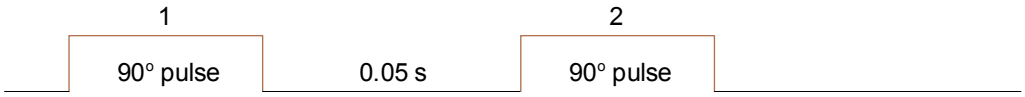


Fig.18 shows the recovery of M at the different times (0.05, 0.2, 0.5 and 1s) elapsed after a 90° pulse. If a 90° pulse is applied again after a few seconds, say 5s, that is after a full recovery of **M** to its initial value ($\mathbf{M}_Z = \mathbf{M}_0$) the same signal (FID) will be produced. The result of a single pulse tells nothing about the relaxation time, T_1. However, what happens if we do not allow M to recover and apply the next pulse earlier than the time required for a full recovery of **M**, say at 0.05s after the first pulse?



Immediately after the first pulse, $\mathbf{M}_0$ starts regaining its initial alignment. If the second pulse is applied 0.05s after the first pulse then

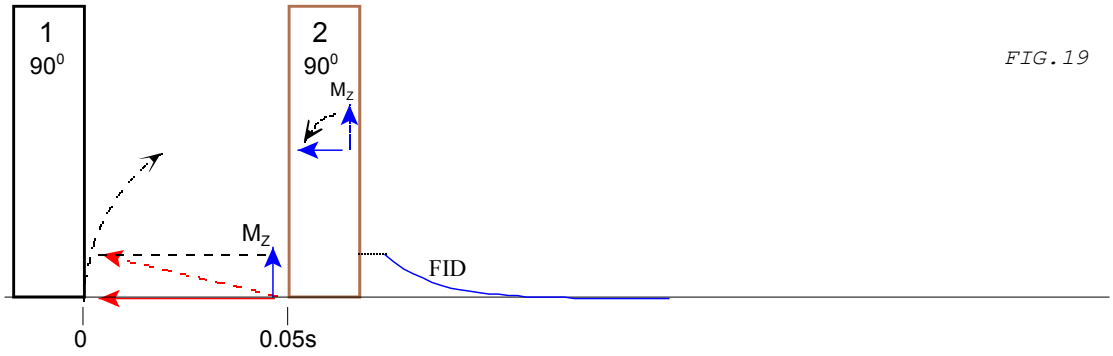


FIG.19

the second pulse rotates $\mathbf{M}_z$ and creates the FID signal in the same way as the first pulse did with $\mathbf{M}_0$. The difference between the FID amplitude of different pulses is in the magnitude (i.e. number) of the **M** vectors aligned along the z axis in the sample at the time when the pulses were applied. The initial amplitude of the FID is proportional to the actual longitudinal magnetization at the moment of pulse application. Hence, if the longitudinal magnetization were given more time to recover then it would produce greater amplitude of the FID signal. Fig.20 illustrates this effect.

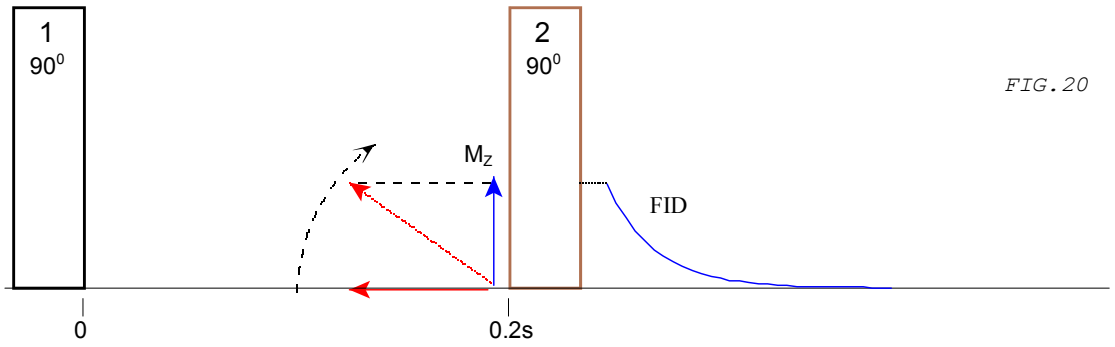
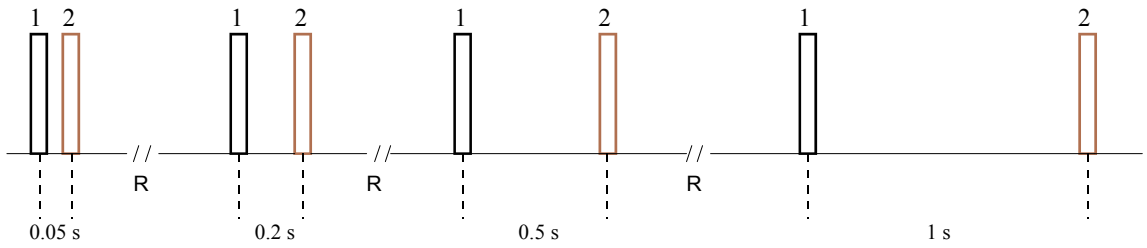


FIG.20

Consider the following pulse sequence comprising a series of pulse pairs each with different separations between the pulses in the pair (also called TR- repetition time).



The sequence can be written as 90° – 0.05s – 90° – **R** – 90° – 0.2s – 90° – **R** – 90° – 0.5s – 90° – **R** - ...
R is a recycle delay that is required for a full recovery of the magnetization before the next pair of pulses is applied.

In conclusion, this pulse sequence, which is called the *Saturation Recovery method* (SR or 90°– 90° method), allows the relaxation time T_1 to be determined by measuring the amplitude of the FID signals produced by a second pulse in each pair. The recycle delay, that is an interval between each pair in sequence, should be equal to at least five times T_1.

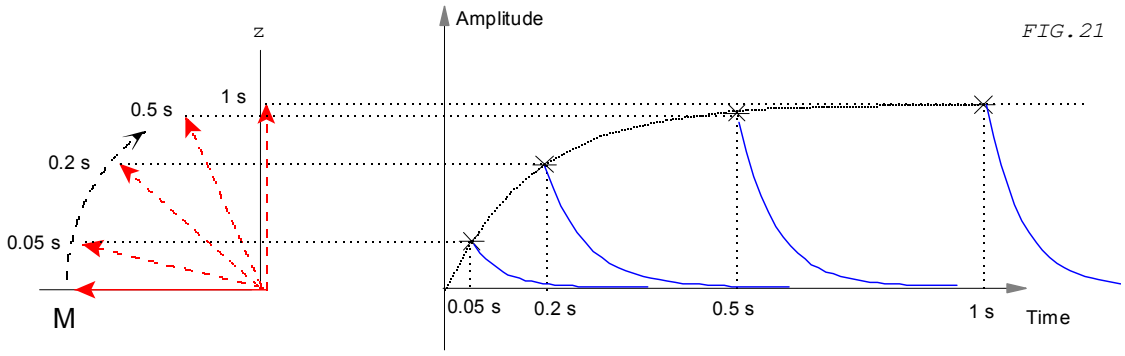


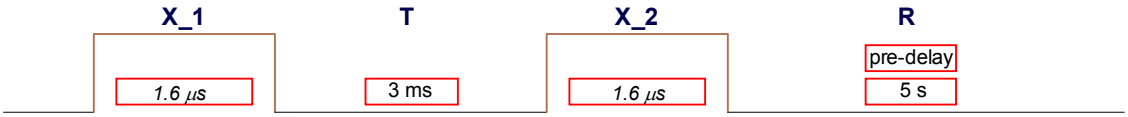
FIG.21

One can obtain data points (x) to plot the relaxation process by measuring the amplitude of the FID signals produced by the second pulse in each pair ("detection pulse") vs time between the pulses in the pair (Fig.21).

Practical Consideration - Measurement of T_1 with the PS-15

Select the "2**P_X_VD**" from the method list.

The following pattern appears in the F 4 Pulse Programmer.



The **P2** selection from the <**Trig**> setting tells the system to start recording the data after the second pulse. The pre-delay display (~22µs) appears after the pulse from which the system will start acquiring data. (*The particular settings may differ from those shown in the figure, the setting of the last run is the default setting*).

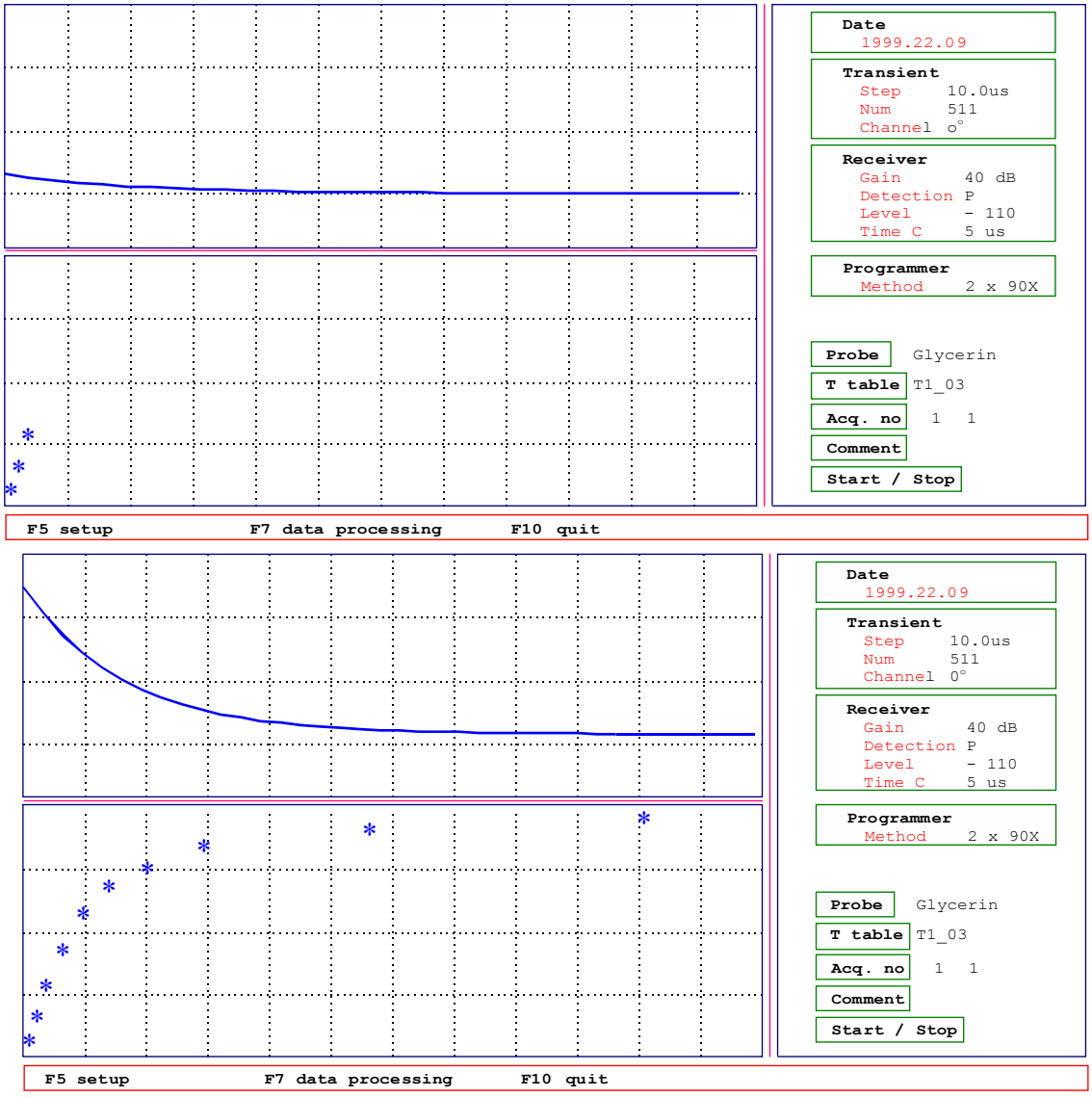
The user can set any delay time (T) between the pulses and can review the resultant signal in the Setup mode (F5). The system repeats the measurements with the recycle delay R. (In the figure R = 5 s).

In the **Acquisition** mode (F6) the system will repeat a series of pulse measurements for different delay times between the pulses. A set of delay times is called a **VD table**. All VD tables are located in VD subdirectory. The prepared list consists of a few tables that are suitable for a wide variety of samples with different relaxation time.

The VD table in our example (Fig.21) would be as follows.
50,
200
500
1000

The spectrometer program assumes that these numbers are in ms (1s = 1000 ms. In practice, the VD table consists of approximately 15 to 20 data points. The table can be edited to meet current experiment requirements regarding number of points and time delays.

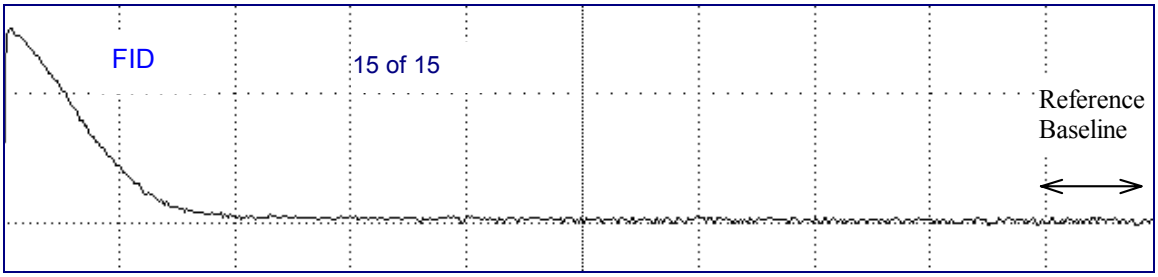
After the Start command the real-time signal (created by the second pulse in each pair, when P2 selected) appears in the upper display and the corresponding data points are plotted in the lower display. The settings used for the glycerin sample are shown in the figures (Transient- 10µs, 511, Channel- 0° , Method –2P_X_VD, Detection-P, Time C- 5µs, Trig-P2, Table-T1_03).

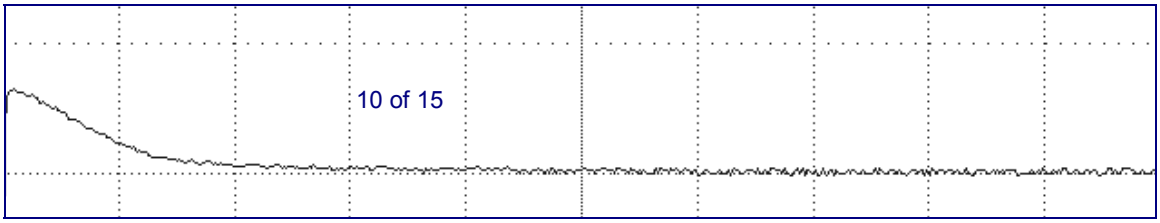


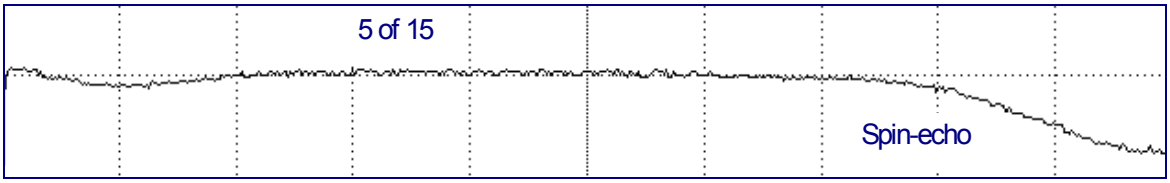
Viewing and saving acquired data with F7 - data processing

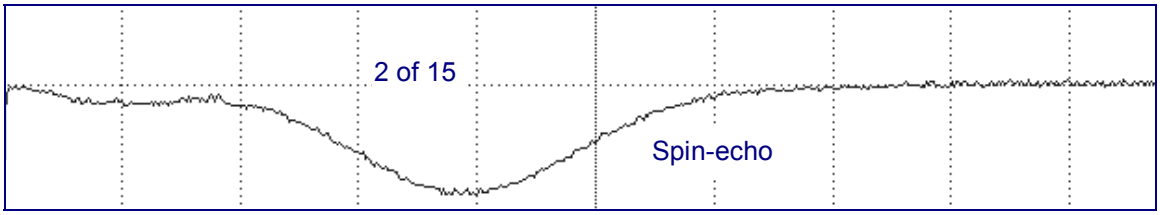
The acquired spectra are saved in the DATA folder. The user can open any file saved in the DATA directory with the **data processing key (F7)**. To download the file press the <F1 File>, double-click Enter, highlight the file on the list with the arrow keys and click Enter to view the file. The file in our example consists of the 15 recorded signals. The first one is shown. To view the other signals, press the Esc key (to

activate the display) and use the Page Up, Page Down, Home and End buttons. Some of the signals are shown below.

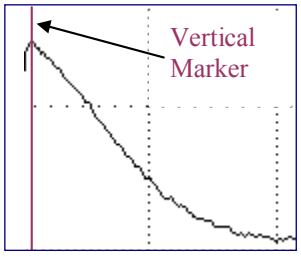








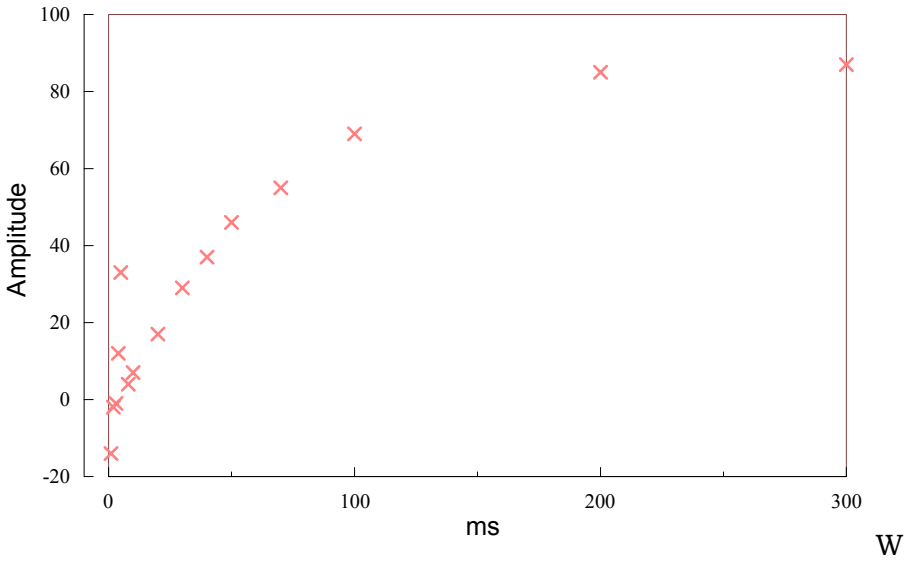
We are interested in determining the relative max amplitude of the acquired FID signals at the same time after the pulse.



Click the Esc key to activate the vertical marker, which can be moved with the arrow keys (and Ctrl to move faster). The y-component is the amplitude of the signals being reviewed. The data points (marked by the vertical pointer) can be acquired and saved. Place the vertical marker on the peak of any signal. Click Enter. It marks the corresponding data points in all signals. Go to the "Save" command (the F1 and arrow down key) to save these data in ASCII format in the DATAOUT folder.

The created file consists of two columns, the time separation between the pulses in a pair (set by the selected T table) and corresponding magnitude of the FID signal. An example is shown below.

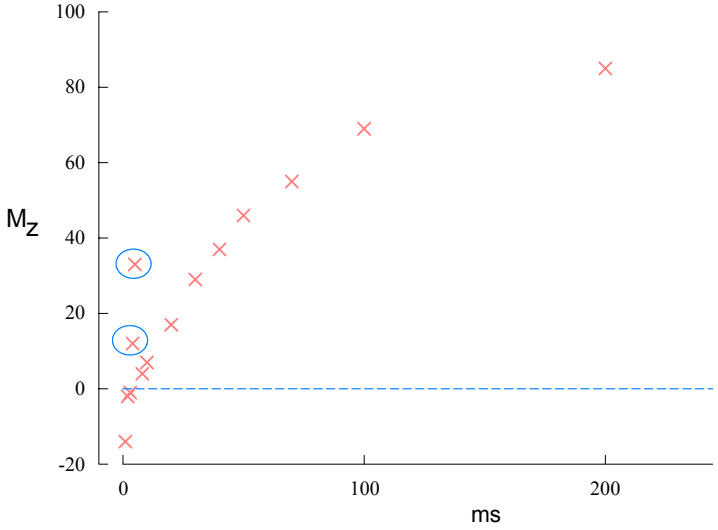
No	ms	Amplitude
1	1	-14
2	2	-2
3	3	-1
4	4	12
5	15	33
6	8	4
7	10	7
8	20	17
9	30	29
10	40	37
11	50	46
12	70	55
13	100	69
14	200	85
15	300	87



W

e have deliberately not presented a “perfect” data set. First, no measurement is ideal. Secondly, we would like the user to note a few aspects related to these measurements and its analysis.

Our goal was to determine a T_1 value for the glycerin sample. At this stage, we are trying to fit the experimental data to the theoretical function $M_Z = M_0[1 - \exp(-t/T_1)]$, see Fig.15.



One can see two groups of data points that do not fit the function describing the process.

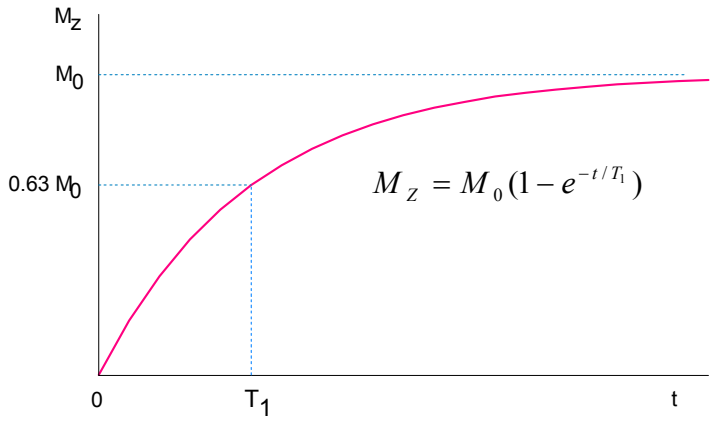
1) The three points with a negative value, for t > 0; M_Z is always > 0.

2) The two circled points are not described by the function.

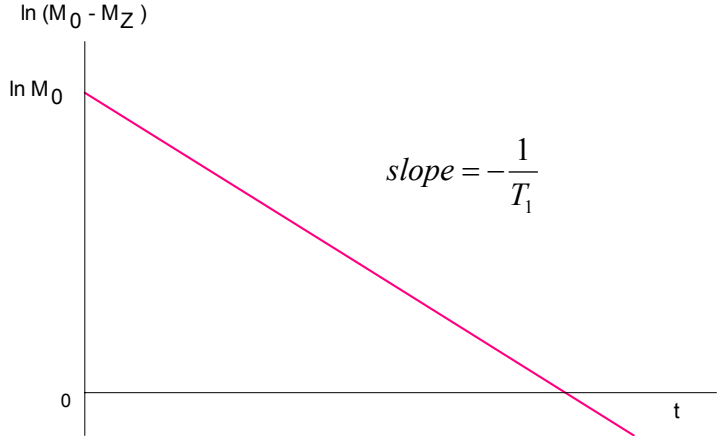
Apparently, these data points should not be used for the fitting process. However, rejecting any experimental data should be justified. For those analyses, it is useful to know how the program calculates the amplitude. The reference level of the baseline is determined by averaging the last 10% of the acquired data points, see Fig. “15 of 15”.

It is easy to explain the results related to the circled points by reviewing the corresponding spectra, the fourth and fifth runs. The later is shown in Fig. “5 of 15”. In this case the signal called a “spin echo” was detected and disturbed at the reference baseline. The rationale of disregarding these two data points is obvious. *We will discuss the spin echo phenomenon later in the T_2 section of this manual. If you operate a pulse NMR instrument and try various pulse combinations, you will find that it is easier to form echoes than not. Almost any two pulses will form an echo. The spin echo signals play an important role and are employed in many experiments. However these echoes are not always a blessing, particularly, for many of the T_1 pulse sequences that we have already encountered.*

The solution regarding the points with negative values is not so obvious. There are two options. The first would be to examine the instrumentation settings; in particular, the accuracy of a 90º pulse employed in the experiment and the repeatability of the measurements. Applying other methods may be also a good idea, for example, the *Inversion Recovery* or S*aturating Comb*. The next approach would be to manipulate the data. For example, one can perform calculations and disregard these points or transform the amplitude column to make all data points positive. A closer examination would indicate that disregarding these data points leads to different T_1-values. A fitting routine is a part of the experiment data analysis and should not be treated in isolation from the experimental conditions. The latter may determine whether a single parameter or multi-parameter fit method should be used. Finally, a T_1-value for the sample can be obtained from fitting the function.



T_1 is the time for the longitudinal magnetization, M_z , to recover from 0 to $(1 - e^{-1})$ or about 63% of its final value M_0 .
In the literature, the T_1 relaxation curve is usually plotted in a semi-logarithmic diagram, i.e., with logarithmic values along the ordinate (y-axis) and time along the abscissa (x-axis).



$$M_Z = M_0(1-e^{-t/T_1})$$

$$M_0 - M_Z = M_0 e^{-t/T_1}$$

$$\ln(M_0 - M_Z) = \ln(M_0 e^{-t/T_1})$$

$$\ln(M_0 - M_Z) = \ln M_0 + \ln e^{-t/T_1}$$

$$\ln(M_0 - M_Z) = \ln M_0 + (-t/T_1)$$

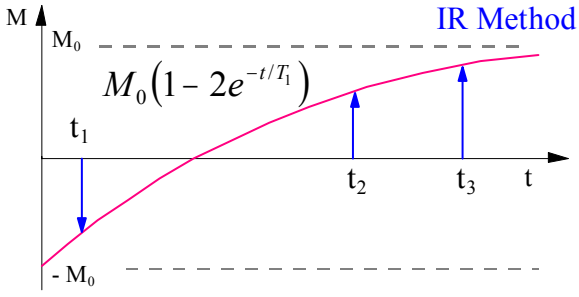
$$\ln(M_0 - M_Z) = -\frac{1}{T_1}\, t \; + \; \ln M_0$$

$1/T_1$ is called the T_1 relaxation rate. A short T_1 means a high relaxation rate and thus a steep negative slope; this means that ln ($M_0 - M_Z$) decreases rapidly and that M_Z rapidly approaches M_0 . The relaxation rate is a useful concept, particularly when considering the effect of paramagnetic substances on the relaxation process.

OVERVIEW OF MEASUREMENTS OF T_1

Measuring a T_1-value for an unknown sample requires that you make an initial estimate about the order of magnitude of the result. You must use this estimate to select delay times for this measurement. Only a small number of measurements will be required to obtain a reasonable value if the initial estimate is close to the actual value. Otherwise, you will need to know how to proceed with a new estimate. This procedure is independent of the NMR spectrometer that you use. The design of the PS-15 and its real-time interface makes this task very easy.

There are two major methods employed for T_1 measurements; the *Inversion Recovery Method* (**IR** or 180°-90° Method) and the *Saturation-Recovery Method* (**SR** or 90°-90° Method).



To determine T_1 via the 180° - t – 90° sequence (IR method), **M** is inverted by a 180° pulse at t = 0. After a delay of t seconds a 90° pulse sampling pulse is used to monitor the recovery of **M**. (In the PS-15 select the "M2_90X" in the method list to perform the IR sequence).

Although there are some variations of these methods the concept remains the same. For example, there is a sequence called the "saturating comb". This method is a generalization of and functionally identical to the 90°-90° sequence in that a number of nearly 90° pulses forming a "comb" sets the magnetization to zero and the recovery is sampled at the later times. Its virtue is that missetting the tip angle so that it is not exactly 90° does not affect the initial condition of $M_z = 0$ provided that the comb is of sufficient length. The saturating comb method can be performed in the PS-15 spectrometer by selecting the "SAT-25" pattern in the method list. The comb length is 25 pulses. The pulse spacing within the comb should be set to $T_2^* < t_1 < T_1$. Note

that the saturating comb is used instead of the first 90° pulse in the described 90°-90°sequence.

For protons in most substances T_1 has values of approx. 0.1 – 5 seconds. For example, water ~ 3.6s, acetic acid ~2.4s, sulfuric acid ~0.7s, glycerin ~ (0.03 – 0.08) s. The values are approximate because T_1 is very sensitive to the state of the sample and a comparison of the independent results should be done with well-defined conditions. It also explains the employment of the relaxation time measurements in the studies of the molecular structure and dynamics.

The following are some examples of the factors affecting the T_1value. Small traces of paramagnetic species in the sample can dramatically reduce the measured relaxation time. The T_1 value of 4s for distilled water can change to 10^{-2} - 10^{-3} s as a result of paramagnetic impurities.
When water is in a partially bonded or restricted state (as when its transiently bonded to proteins and other macromolecules) the T_1-value is much shorter than that of free water, typically about 0.4 to 0.8 s. T_1 depends also on the liquid viscosity; for example, the high viscosity of sulfuric acid and glycerin decreases T_1.

WHAT CAUSES T_1 RELAXATION?

T1, *the longitudinal relaxation time*, is also known as the *thermal* or *spin-lattice relaxation time*. As discussed, regrowth of $\mathbf{M}_0$ occurs via the precessing motion to its initial direction along $\mathbf{B}_0$, See Fig.4. This process requires a net transfer of energy from the nuclear spin system (which creates $\mathbf{M}_0$) to its environment ("the lattice"). Likewise after displacing a compass needle from its equilibrium alignment, oscillation will occur and eventually align the needle with $\mathbf{B}_0$ since friction is always present at the pivot point, this loss of energy through friction is analogous to T_1 relaxation in NMR.
Since NMR relaxation involves a release of energy from the spin system, the Einstein quantum description of the emission of energy from atomic systems applies equally well to NMR. According to Einstein's analysis, emission of energy from an atomic system may be either spontaneous or induced. Einstein showed that the probability of spontaneous emission is strongly frequency dependent (proportional to ω^3). In the visible region of frequency of spectrum, $\omega \approx 10^{12}$ Hz, spontaneous emission is a dominant process. In RF range where NMR energies are found, $\omega \approx 10^7$ Hz, spontaneous emission is extremely improbable. Virtually all energy emission in NMR are stimulated through a direct interaction of a nucleus with its external environment. This interaction may occur through the electrical or magnetic fields generated by other nuclei, electrons, or molecules.

Because T_1 relaxation requires an energy exchange, and because all NMR energy exchanges must be stimulated, *T_1 relaxation can occur only when a proton encounters another magnetic field fluctuation near the Larmor frequency*. The rapid rotational and translation motion of molecules generate fluctuating magnetic fields at or near the Larmor frequency that stimulate recovery of the population of excited protons to equilibrium. As these fluctuations become stronger, T_1 becomes shorter (higher relaxation rate). The source of this fluctuating field is typically another proton or electron, and the interaction is called a dipole-dipole interaction. The two spins may be in the same molecule (intramolecular dipole-dipole interaction) or on different molecules (intermolecular dipole-dipole interaction).
The ability of tiny nuclear magnetic dipoles to influence relaxation seems unlikely at first glance, but is actually very important due to the close proximity that can be achieved between neighboring dipoles. The magnitude of dipolar

interactions has a $1/r^6$ dependence and proton separations in water are of the order of a few Angstroms, which can cause magnetic field perturbations as large as ±1 mT.

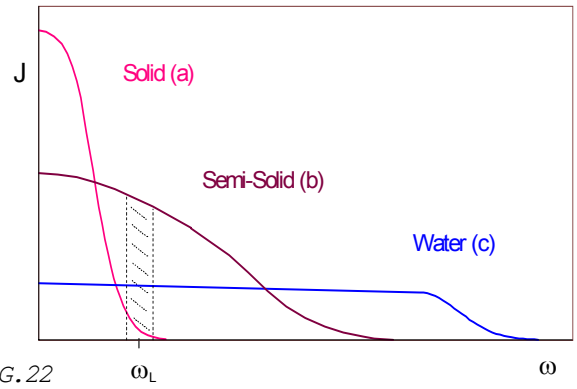


FIG.22

Fig.22 shows the strength of the magnetic fluctuation existing at different frequencies; ω_L is the Larmor frequency. All fluctuations are due to the thermal motions of the magnetic moments. Curve "Solid" corresponds to solids that only have very slow molecular motions and to macromolecules (in fluid) that move very slowly due to their large size. Curve "Semi-solids" corresponds to viscous fluids or to moderately sized molecules, such as lipids, in fluid. Curve "water" corresponds to a non-viscous liquid (e.g. water) and the fastest moving small molecules. The viscous liquid ("Semi-Solid") contains the highest fraction of rotational frequencies and consequently would have the shortest relaxation time. Thus at the Larmor frequency the viscous liquid will relax faster than either the solid or the liquid.

For a proton or electron to produce a fluctuating magnetic field, the molecule in which it resides must be moving or tumbling. To be efficient at T_1 relaxation, the molecule must be rotating near the Larmor frequency. The relationship between T_1 and molecular tumbling rate is represented in Fig.23. Water, with its small molecular size, tumbles much too rapidly to be effective at T_1 relaxation. T_1 values are longer for free water (~4s) than for "bound" or "structured" water (~ 0.4 - 0.8s). At the other extreme, ice has extremely long T_1 values because in the crystalline state its molecular motions are much slower than the Larmor frequency. There are other factors influencing T_1 relaxation, for instance, the presence of paramagnetic centers. Paramagnetic centers do not directly contribute to the NMR signal since their Larmor frequency is much higher than the Larmor frequency of protons. However, their strong local magnetic fields affect nearby protons, substantially increasing their relaxation rates. In general, any mechanism that causes a fluctuating magnetic field at a nucleus is a possible source of relaxation. The graphs in Figs. 22 and 23 correspond to each other. Molecules rotate at all frequencies from zero (static or fixed motion) to a maximum frequency determined by the shortest rotation time, the correlation time, τ_c, which characterizes the material. The NMR relaxation rate in a particular material at the Larmor frequency, ω_L, depends on the fraction of the spectral density, J, at that frequency, relative to those at all other frequencies.

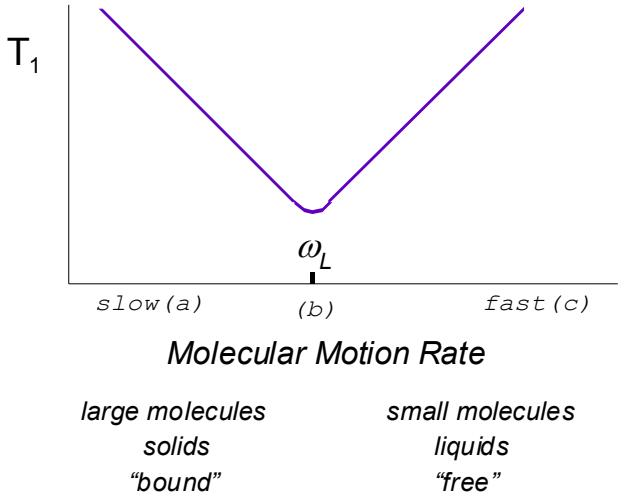


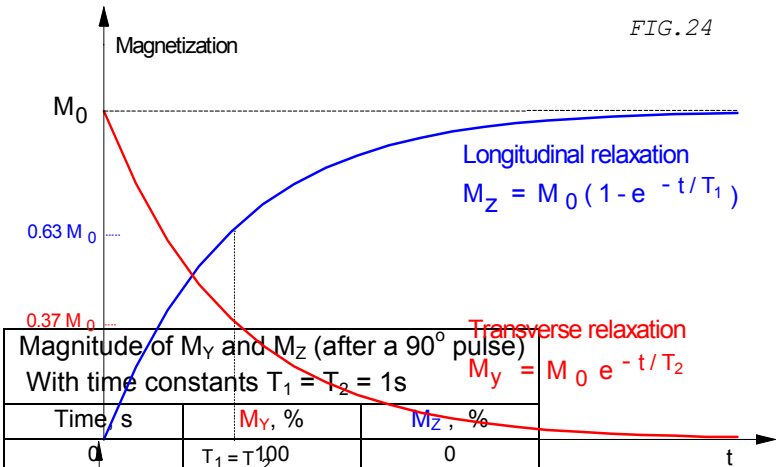
FIG.23

T_2 Relaxation

Fig.14 shows $\mathbf{M}_0$ when it is deflected by an angle θ from the z-axis. $\mathbf{M}_0$ can be resolved into two components, $\mathbf{M}_Y$ along the y direction and $\mathbf{M}_Z$ along z direction. It is the magnitude of $\mathbf{M}_Y$ which affects the size of the signal after a pulse. $\mathbf{M}_Y$ is a maximum and equal to $\mathbf{M}_0$ immediately after a 90° pulse (See Fig.6). At this time $\mathbf{M}_Z$ is zero. As nuclear relaxation takes place after a 90° pulse and $\mathbf{M}_0$ returns to the $\mathbf{B}_0$ direction, $\mathbf{M}_Y$ gets smaller and smaller and finally disappears, while $\mathbf{M}_Z$ gets bigger and bigger until its magnitude is equal to $\mathbf{M}_0$ when $\mathbf{M}_0$ has finally returned to the $\mathbf{B}_0$ direction.

The magnitudes of M_Y and M_Z as a function of time after a 90° pulse are shown in Fig. 24. M_Z represents the longitudinal (spin-lattice, T_1) relaxation and M_Y represents the transverse (spin-spin, T_2) relaxation. Longitudinal relaxation has to do with the component along the $\mathbf{B}_0$ direction. Transverse relaxation has to do with the component at right angles to the $\mathbf{B}_0$ direction.

FIG.24



Magnitude of M_Y and M_Z (after a 90° pulse)
With time constants $T_1 = T_2 = 1$s

Time, s	M_Y, %	M_Z, %
0	100	0
1	37	63
5	0.7	99.3

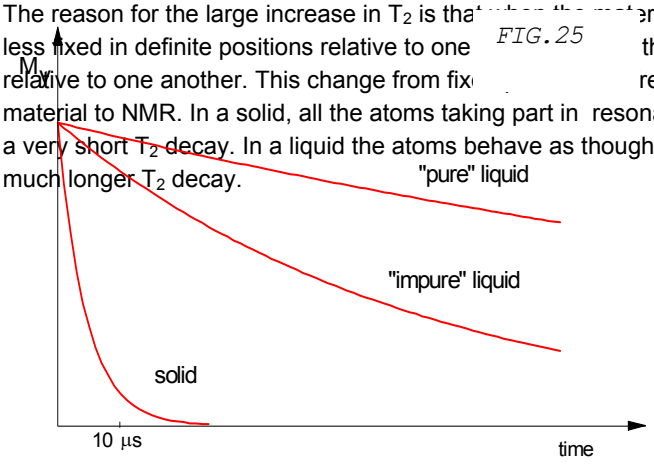
These curves show what happens to the magnetization of the sample after a pulse. The signal giving the FID depends upon the component M_Y but also depends upon other factors which will be dealt with later in this section.
In the formulas given in Fig.24, T_1 and T_2 are the time constants of the increase of M_Z and the decay of M_Y respectively. Table 2 shows how M_Y and M_Z would change if both relaxation processes had the same time constant of one second.

Table 2

The table shows that M_Y after a 90° pulse falls to less than 1% of its initial value after five time constants. The component of the magnetization along the z-axis reaches 99% of its final equilibrium value after five time constants. At first sight these two seem to be rather like the two sides of the same coin. Or, put another way, the sum of the magnitudes of M_Z and M_Y is always equal to M_0 . This is often the case with liquids where $T_1 \approx T_2$ but, with solids, things are not so simple. When a material is in the solid state its behavior is very different from that of the same material when it is in the liquid state. A consequence of this different behavior in the solid state is that the T_2 for hydrogen in a solid can be very much shorter than the T_1. For instance, T_2 for hydrogen in solid fat is about 10 μs whereas T_1 for the same fat is about 1 s. If the fat is melted, both T_1 and T_2 for the liquid fat will be about 100 ms. T_1 has fallen by a factor of about 10 as a result of melting but T_2 has increased by a factor of 10^4 . It is this big change in T_2 between the solid and liquid state that enables NMR to distinguish between the two phases. In general $T_1 \geq T_2$, for a solid $T_1 > T_2$.

The reason for the large increase in T_2 is that when the material is a solid, the atoms and molecules are more or less fixed in definite positions relative to one another. This change from fixed to free movement is reflected in the response of the material to NMR. In a solid, all the atoms taking part in resonance have an influence upon one another. This gives a very short T_2 decay. In a liquid the atoms behave as though they were independent of one another. This gives a much longer T_2 decay.

FIG.25



Measuring T_2 is not as straightforward as measuring T_1. T_2 for solids is too short to be measured easily and T_2 for liquids is often too long to be measured directly from the FID. Nuclei of pure and low viscosity liquids have relatively long relaxation times, whereas signals from nuclei solids have short T_2 values and therefore decay rapidly (Fig.25).

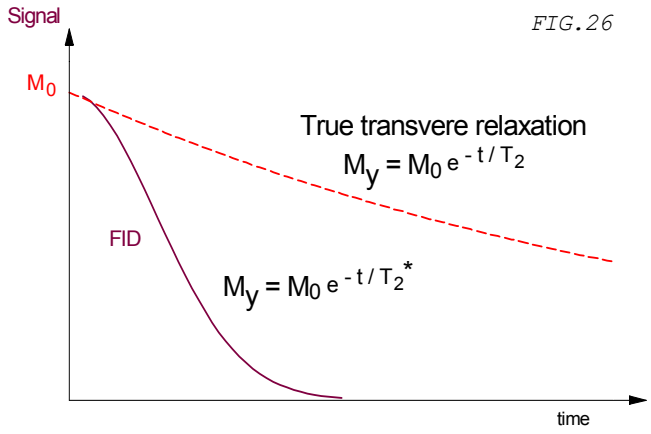


FIG.26

Fig.26 shows the FID obtained from a liquid. The dotted line shows the signal that would be obtained if every part of the liquid sample was in exactly the same steady field. That is, if the magnet had a perfectly uniform field over the whole sample volume. The solid line shows the sort of signal measured in practice. The difference between the two lines is due to the fact that magnets do not produce a perfectly homogeneous field over the whole sample. M_Y and the FID signal decay approximately exponentially. T_2^* is the time constant of the exponential function that describes this decay. The de-phasing of the magnetic moments is the reason for the quick loss of the transverse magnetization (M_{XY}) and the resultant decay of the signal. To understand this process we should start from the origin of the macroscopic magnetization gained by the sample if placed in the magnetic field (Fig.27).

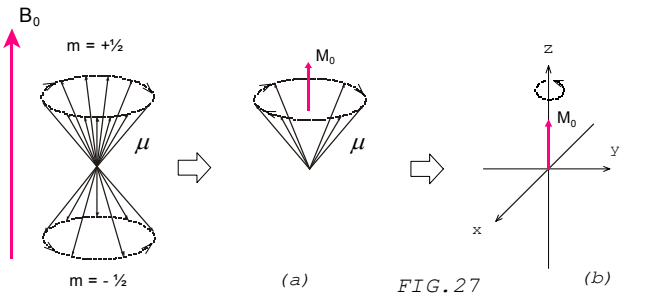


FIG.27

A spin-½ nucleus in a strong magnetic field ($\mathbf{B}_0$) exists in one of two orientations with equal and opposite projections onto the field direction (the z-axis). Depending on its magnetic quantum number (m = ±½) each spin contributes to the total magnetization of the sample. There is a slight excess of nuclei in the m = +½ state (Fig.27 a), the sample therefore has a net magnetization along the z direction. In the perpendicular directions x and y, however, the phases of the individual nuclear magnetic moments (μ) are random, because there is no transverse magnetic field to align them, and their vector sum vanishes. Thus, the total magnetization of the very large number of spins in an NMR sample has magnitude M_0 and is aligned along the positive z-axis (Fig.27 b). (Though made up from the individual quantum mechanical nuclear magnetic moments, the total magnetization of the sample conveniently obeys classical mechanics. We can, therefore, forget about quantum mechanics, at least when discussing basic NMR experiments).

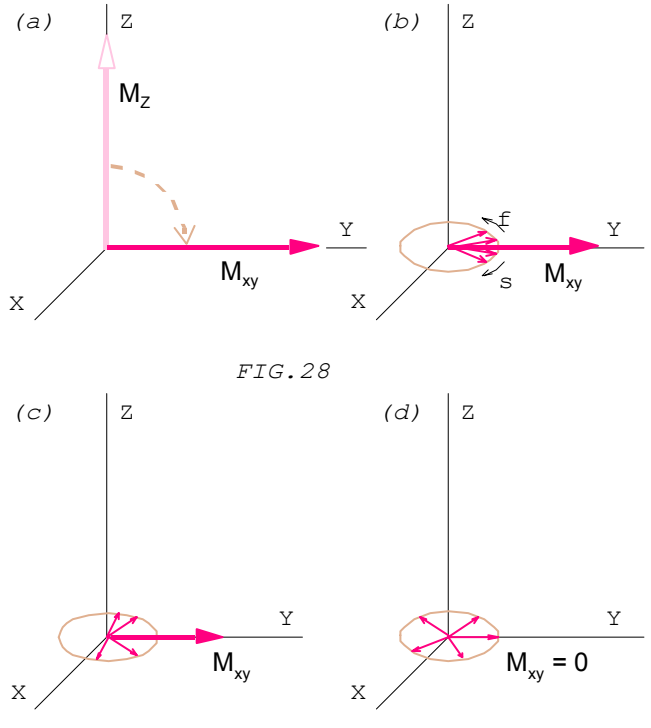


FIG.28

Fig.28 illustrates the dephasing of the proton magnetic moments causing T_2^* decay (see Fig.26) of the transverse magnetization, depicted

in the rotating frame. (a) The application of a 90^0 pulse rotates the magnetization from the z-axis to the x-y plane, without changing its magnitude. (b) and (c) As times passes, the individual proton magnetic moments begin to fan out, as some nuclei precess faster and some slower than the frame and M_{xy} decreases. This figure shows the magnetic moments of five representative protons experiencing slightly different magnetic fields. (d) Complete dephasing results in $M_{xy} = 0$. In (b), (c) and (d) T_1 relaxation also causes a progressive increase in M_z from zero, though this is not shown in the figure.

The key to understanding the dephasing of the protons is the Larmor equation, $\nu = (\gamma/2\pi)B$.

Proton dephasing means that the magnetic moment vectors of different protons are precessing at slightly different frequencies. This is caused by slight differences in the local magnetic field experienced by the individual protons. Such variations in the strength of the local magnetic fields are caused by: (1) variations produced by the magnet, and (2) variations produced by chemical/physical processes in the sample being investigated.

The first cause is due to constant inhomogeneities in the static magnetic field of the magnet. Although NMR is dependent upon a very homogeneous static magnetic field, no magnet is perfect.

Variations in the second case are due to the inherent properties of the sample and are independent of the inhomogeneities produced by the magnet. Even if the static magnetic field of the magnet was perfectly homogeneous, the protons would still dephase due to random variations in the local magnetic field strength created by the physical and chemical environment of the proton. These magnetic field variations are part of the same magnetic fluctuations that was discussed in explaining T_1 relaxation (see Figs. 22 and 23). Proton dephasing, however, is produced by the low frequency part of these fluctuations, in other words by magnetic field fluctuations that are relatively slow (see Chap. *"What Causes T_2 Relaxation?"*) This sample-dependent loss of transverse magnetization is called **T_2** (or **transverse**) **relaxation**. T_2 is the time constant of the exponential function that describes this component of the decay of the transverse magnetization (see Fig.26).

Spin-Echo. Separation of T_2 from T_2*

In describing sample properties we are concerned with T_2 , *not* T_2*. Since T_2* is affected by the degree of inhomogeneity of the magnet, its value for a particular sample will vary when measured with different NMR spectrometers. We will describe a technique that can eliminate the effect of magnet inhomogeneities. This will allow us to measure T_2 directly rather than T_2* even in the presence of magnet inhomogeneities. We can accomplish this with a very ingenious technique that uses a spin-echo pulse sequence. (This way of measuring T_2 was first proposed by Hahn, who named the experiment a "spin-echo" experiment).

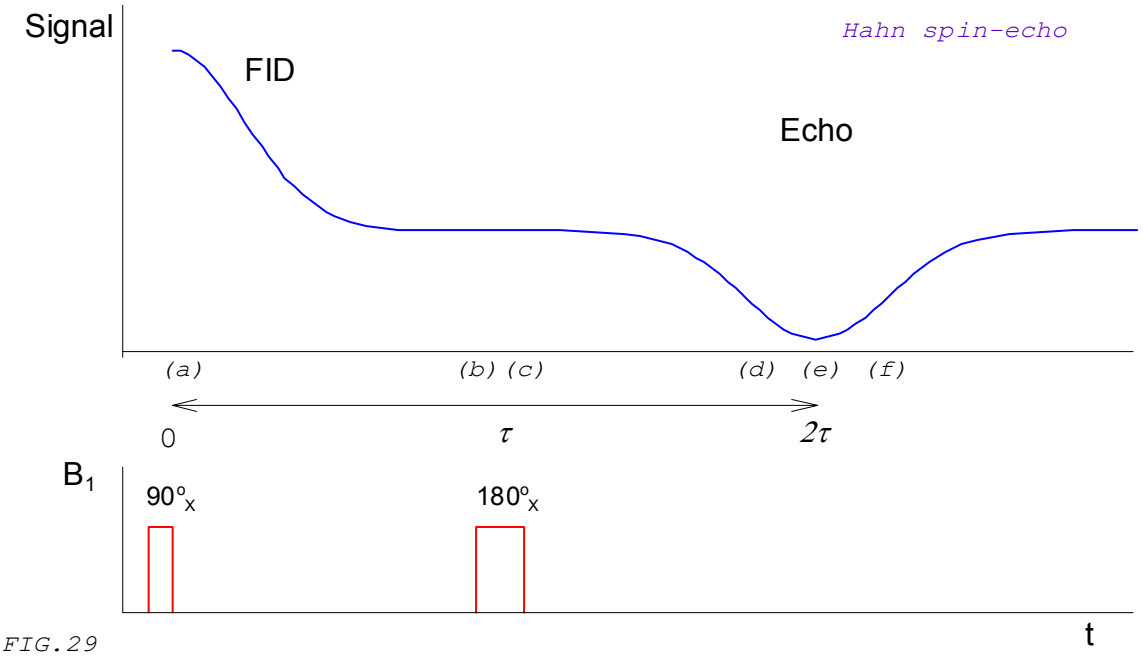


FIG.29

The spin-echo pulse sequence consists of a single 90° pulse followed by one or more 180° pulses. Fig.29 shows the pulse sequence and obtained signals (the Hahn spin-echo experiment).

The application of a 90° pulse produces the FID that quickly disappears as the protons dephase. The application of a 180° pulse at time τ after the 90° pulse produces an echo at time 2τ after the 90° pulse. This time interval of 2τ is called the echo time, TE. The echo is shaped like two FID signals put back-to-back. The height of the echo is determined by the real T_2 of the sample. The 180^0 pulse is halfway between the 90° pulse and the peak of the echo.

To understand the observed process it is necessary to perform some “spin-gymnastic” which is a common practice and is part of the pulsed NMR experiments, Fig.30. This figure shows the same sequence of events as Fig.29. The corresponding phases of the process are indicated with the letters a – f.

(a) A 90° pulse creates a transverse magnetization that begins to dephase.
(b) The nuclear magnetic moments begin to fan out, as some nuclei precess faster and some slower than the frame.
(c) At a time τ after the 90° (x) pulse, a 180^0(x) pulse is applied, also along the *positive x-axis*. The effect of this pulse is to rotate each nucleus moment by 180^0 about the x-axis.
(d) Those moments that are moving faster than the frame naturally continue to move faster but they are behind the slower precessing moments and initiate the ***refocusing***.
(e) The magnetization is refocused along the *negative* y-axis and the *echo* signal reaches its maximum.
(f) The continuing movement of the nuclear magnetic moments causes them to again lose phase coherence.

The rephasing of the magnetic moments causes a FID signal to build up to a maximum at 2τ, but the signal is *negative* relative to the initial FID, since rephasing of the nuclear magnetic moments occurs along the *negative* y-axis.

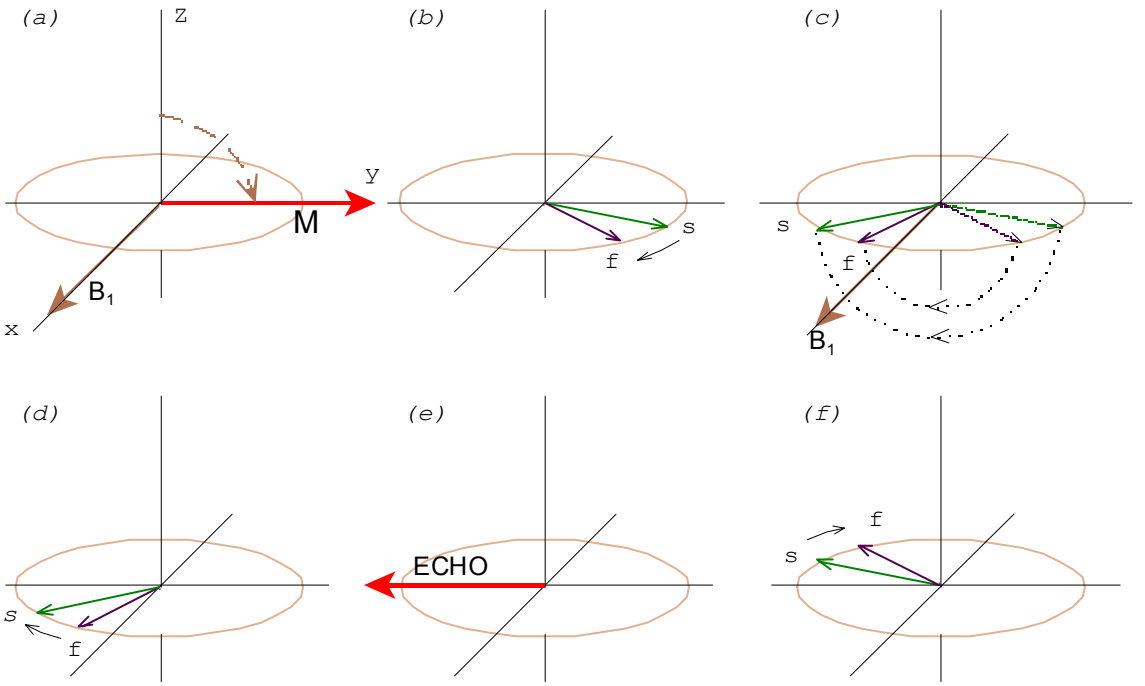


FIG.30 The Hahn spin-echo experiment

If the steady magnetic field were perfectly homogeneous, the echo amplitude might be just as large as the initial FID following the 90° pulse. However, the magnetic moment vectors loose phase coherence not only because of field inhomogeneities, but also because of the inherent processes responsible for transverse relaxation. These "natural" processes, however, are irreversible and cannot be refocused.

It should be noted that the amplitude of the echo is not affected by magnetic field inhomogeneity (provided that diffusion is relatively slow). Consequently, the echo amplitude will decay in time exponentially with a time constant, T_2, which may in principle be determined from a plot of peak echo amplitude versus the time, τ, between the 90° and 180° pulses. In a spin-echo experiment the time τ between the initial 90° pulse and the refocusing 180° pulse is varied from $0.1T_2$ to $2T_2$. As in the measurement of T_1 by the IR or SR methods, it is necessary to carry out a separate pulse sequence for each value of τ and to wait an adequate time between pulse sequences (at least five times T_1) for restoration of equilibrium.

The spin-echo technique is limited in its range of applicability because of the effect of molecular diffusion. The precise refocusing of all magnetic moments is dependent upon each nucleus remaining in a constant magnetic field during the time of the measurements, 2τ. If diffusion causes nuclei to move from one part of field to another, the echo amplitude is reduced. The effect of diffusion in the spin-echo experiment is dependent upon the spatial field gradient (G), the diffusion coefficient (D), and the time during which diffusion can occur. It has been shown that the amplitude of the echo for a pulse separation τ is

$$E_{2\tau} = E_0 \exp\left[-(2\tau/T_2) - (2/3)\gamma^2 G^2 D \tau^3\right], \qquad (1)$$

where E(2τ) and E_0 are the echo amplitudes at time = 2τ and at time zero, respectively. Eq.1shows that the echo amplitude does not decay in a simple exponential way. Because of the τ^3 dependence the effect of diffusion is particularly pronounced for large values of τ and thus affects the measurement of long T_2 relaxation times.

Carr and Purcell showed that a simple modification of Hahn's spin-echo method reduces drastically the effect of diffusion on the determination of T_2. This method can be described as a 90°x –τ – 180°x – 2τ – 180°x – 2τ – 180°x – 2τ …, sequence, or more commonly a *Carr-Purcell* sequence. As in the spin-echo method described previously, all pulses are applied along the *positive* x-axis. The result (Fig.31) is similar to that shown in Fig.29, except that following the dephasing of the magnetic moments shown in Fig.30 (f), another 180°x pulse at time 3τ after the initial 90°x pulse causes a rephasing along the positive y-axis at time 4τ. Subsequent 180°x pulses at 5τ, 7τ, etc., cause echoes at 6τ, 8τ, etc., alternately positive and negative.

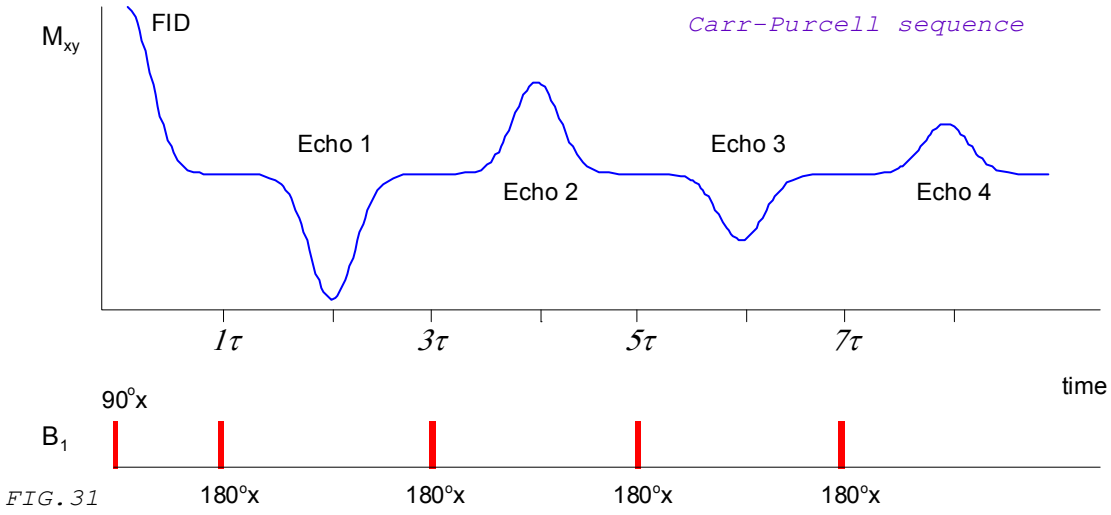


FIG.31

In the *Carr-Purcell-Meiboom-Gill* (CPMG) experiment (Fig.32), which is a phase shifted Carr-Purcell sequence, the 180° refocusing pulses are applied along the positive **y-axis**. All the subsequent refocusing is then along the +y-axis, and all of the echoes are positive. The echoes in this case dephase more slowly because the y-axis refocusing results in substantial cancellation of experimental errors (e.g. pulse width errors or B_1 inhomogeneities problems). Since the τ interval can be made quite short, the effect of spin diffusion can be eliminated.

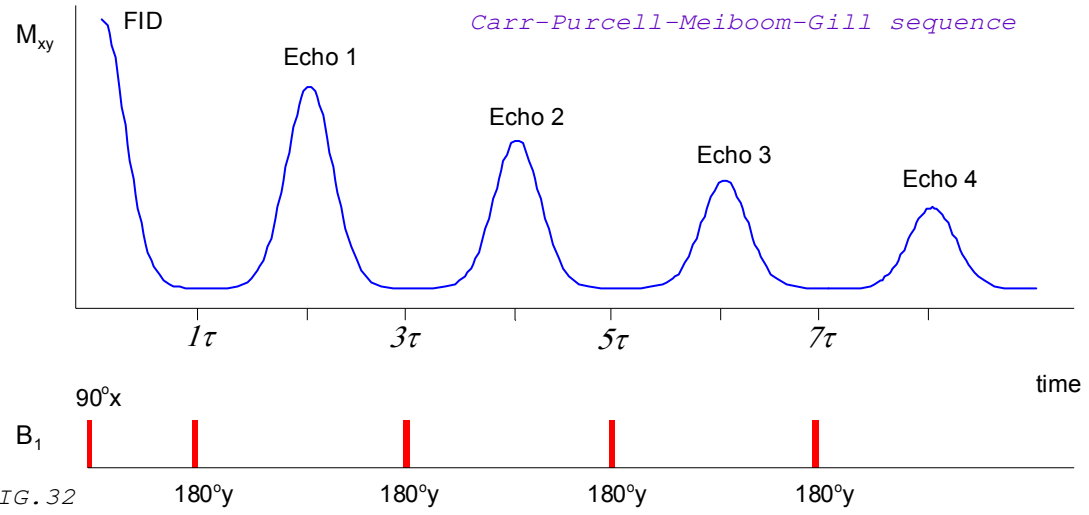


FIG. 32

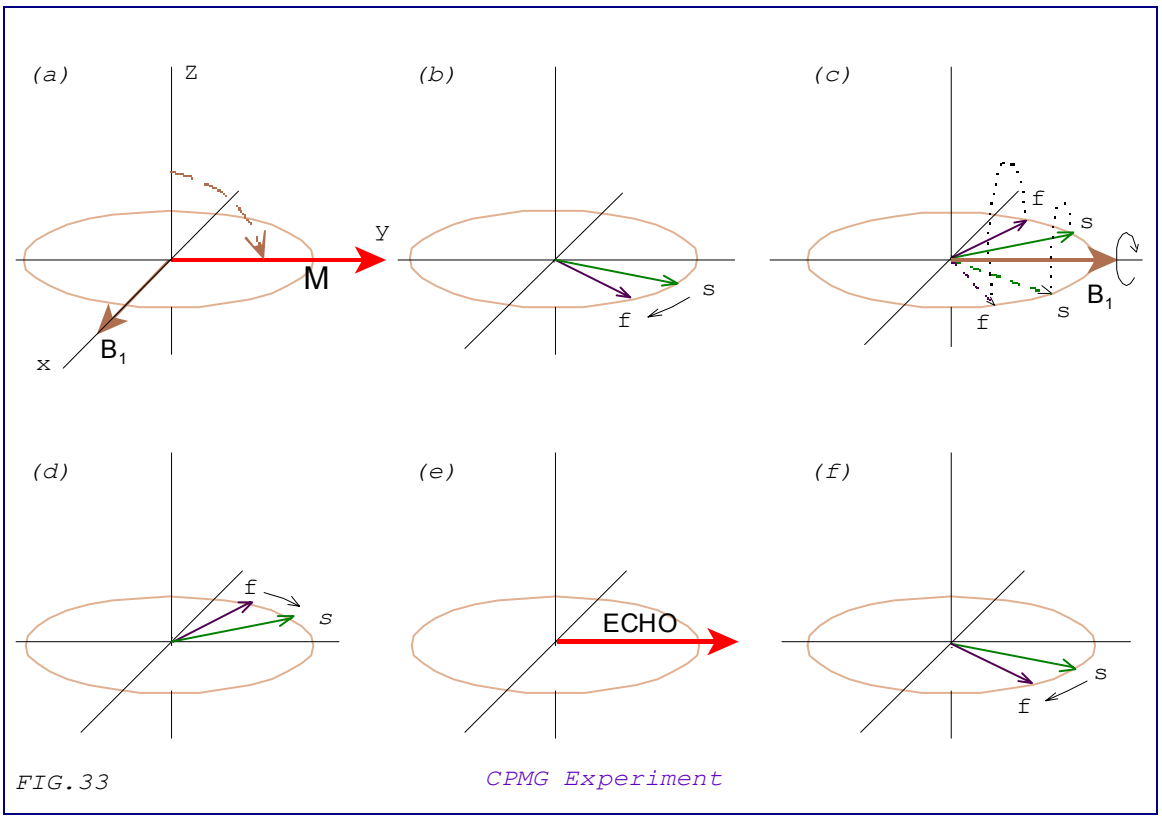


FIG. 33 CPMG Experiment

There are two distinct advantages of the Carr-Purcell method. First, there is a considerable saving in time. A train of n echoes may be obtained in a single sequence, whereas in the unmodified spin-echo method n sequences are required, with a waiting time between sequences that is long relative to T_1 (usually at least five times T_1 is the time between sequences). Second, the effect of diffusion may be virtually eliminated by making τ short, since it is only during a period 2τ that diffusion is effective in reducing the amplitude of an echo. For the Carr-Purcell sequence, equation 1 becomes

$$E_t = E_o \exp\left[-(\tau/T_2) - (1/3)\gamma^2 G^2 D \tau^2 t\right] \qquad (2)$$

This equation tells us that for a series of refocusing pulses spaced 2τ seconds apart, the amplitude of the echo observed at time t is given by E_t . By choosing τ short enough the diffusion term in equation 2 can, in principle, be made as small as desired.

The errors in setting the 180° pulses ("Determining pulse times", p.13) in a Carr-Purcell experiment accumulate and may become troublesome. The CPMG sequence overcomes this problem. For instance, if the pulse is slightly less that 180° , the second and all even-numbered echoes have the correct amplitude, while the odd-numbered echoes are reduced slightly, but not cumulatively. (The reader may try to draw a diagram with some "spin-gymnastic" to show this effect). Since it is virtually impossible to generate accurate 180° pulses over the entire sample, the CPMG is often the method of choice because it eliminates the cumulative errors arising from imperfect pulse width.

The reader may become concerned that we have ignored the T_1 relaxation in our T_2 discussion. Obviously, the 180° pulse does flip over both the transverse and the longitudinal components of magnetization. However, in spin-echo experiments the 180° pulse is usually applied within a few milliseconds, after the longitudinal magnetization has been entirely tipped into the transverse plane by the 90° pulse.

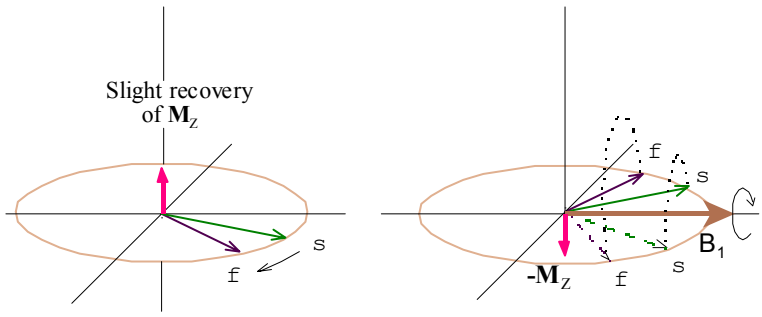
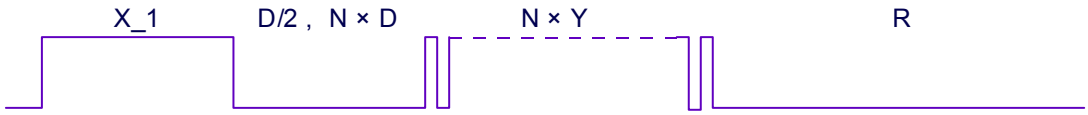


FIG.34

When the 180° pulse is applied, only a tiny amount of longitudinal magnetization has recovered that is available for inversion (Fig.34). As a first approximation, therefore, the 180° pulse appears to be acting only on the transverse components of magnetization. This fact is frequently omitted from most spin-echo diagrams for simplicity.

Practical Consideration - Measurement of T_2 with the PS-15

Select the "**CPMG_25**" from the method list to set the CPMG sequence.
The following pattern appears in the F 4 Pulse Programmer window.



This pattern represents a CPMG sequence and consists of a 90° pulse applied along the positive x-axis (X_1) and a train of 25 180° pulses applied along the positive y-axis, (N x Y). The time intervals between 180° pulses (D) are all the same and equal to twice the interval between the 90° and the first 180° pulse (D/2). The only thing the user must do is to set the D/2 interval and the system will run the sequence. In the PS-15 a pulse duration $t_p \approx 3.4\mu s$ produces a 180° pulse (see "*Determining pulse times*"). Fig.35 illustrates the sequence and setting.

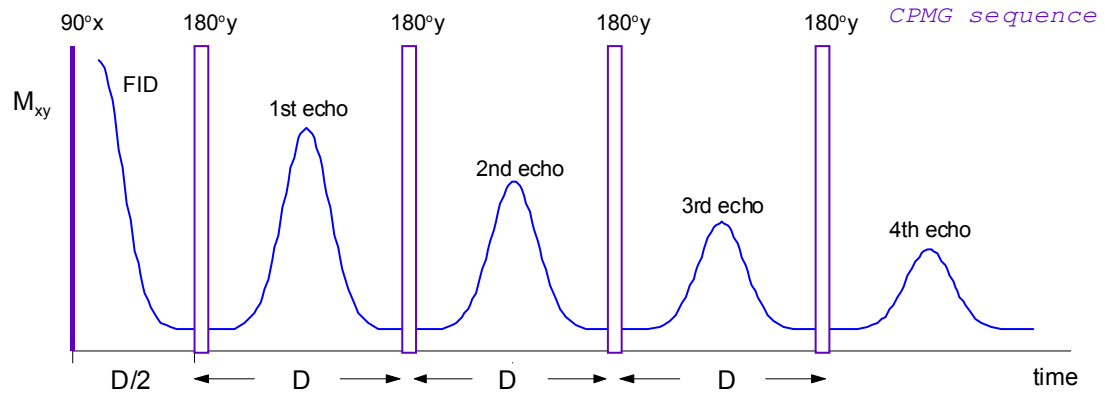
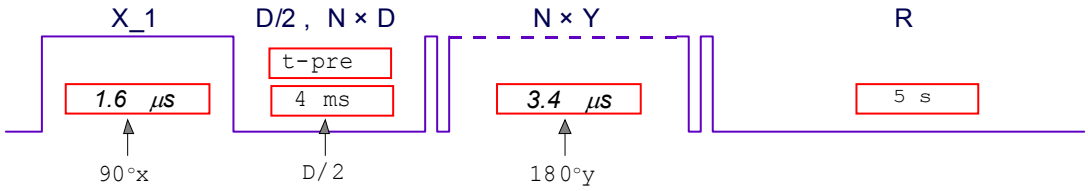


FIG.35

The settings shown in the sequence pattern are similar to the settings for the glycerine sample.



The system repeats the measurements with the recycle delay R (In the figure R = 5 s). It is also the separation period between the runs while a multi-scanning spectral superposition capability of the system is used. That is, when the <Acq.no> is set larger than 1 in the F6 acquisition mode. A multi-scanning option is particularly useful for the samples with a weak signal. The recycle delay should be set at least five times T_1.

The P1 selection from the <Trig> setting tells the system to start recording data following the first pulse. The pre-delay display (~22µs) appears after the pulse from which the system will start acquiring data.

The period of acquiring data can be determined with the <F2 Transient> and should include the entire spin-echo train. Fig.36 shows the spectrum from the glycerine sample obtained with the "on-resonance" condition and the following settings.
TRANSIENT Step – 40.0µs, Sampling – 8191, Channel – 0°
RECEIVER Gain – 34dB, Detection – P, Phase – 0° , Time C - 1µs, Acq. - 1

Once the settings have been selected in the Setup (F5) the acquisition mode (F6) allows recording the spectrum for the sample under investigation to be recorded.

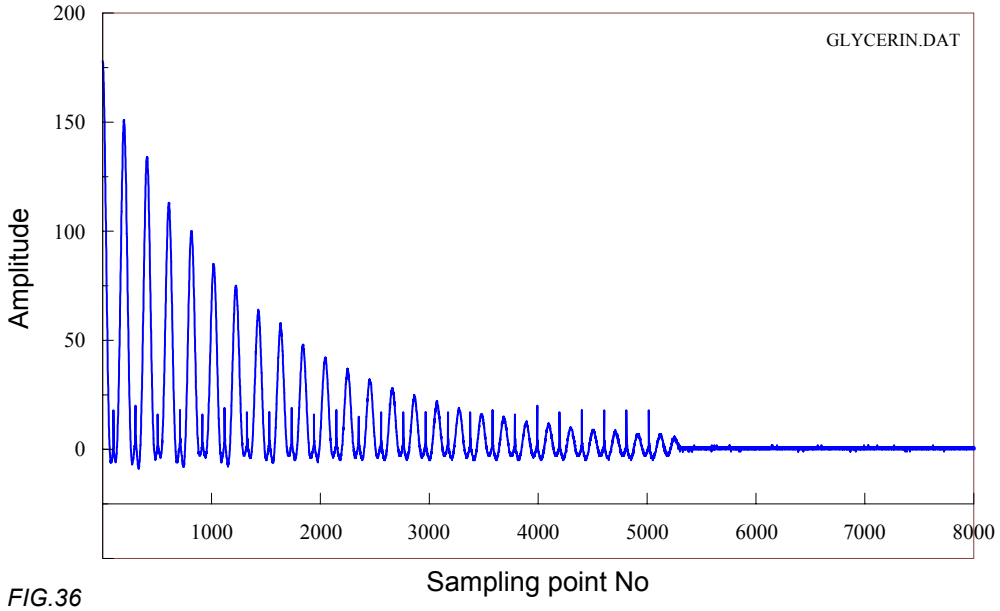


FIG.36

Viewing and saving acquired data with F7 data processing.

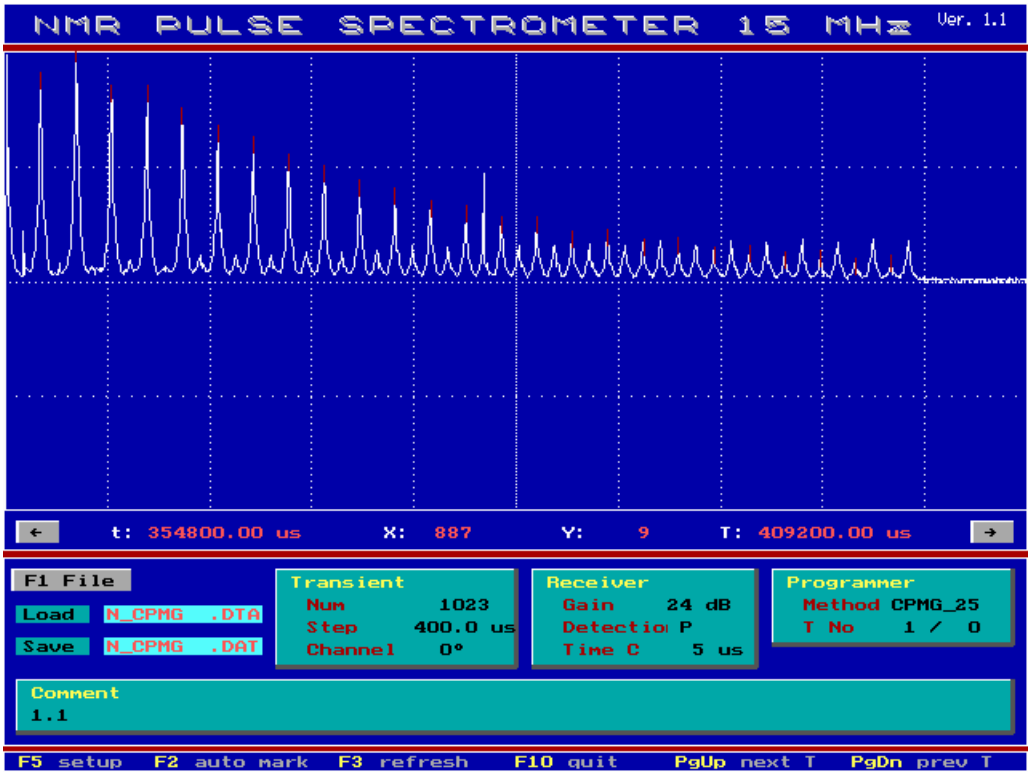
Once the spectrum has been acquired with the F6 acquisition mode it can be saved in the DATA folder. The user can open any file saved in DATA directory with the **data processing (F7)** and perform further signal processing. To download the file press the <F1 File>, double-click Enter, highlight the file on the list with the arrow key and click Enter to view the file.

To save the viewing signals (FIDS or Spin-Echoes) press F1, with the arrow key highlight the save button, click Enter and name your file, click Enter. The file is saved in ASCII format in the DATAOUT folder. Fig.36 was created from such data.

To obtain and save data points for determining T_2 relaxation time

- Click the Esc key (to activate the display commands),
- Place the vertical pointer at the peak of the last echo with the arrow key (pressing Ctrl moves the pointer faster). The y component of the pointer position allows finding the peak easier.
- Click Enter, the program marks the corresponding points on each echo. A small red mark appears on every echo.
- Press F2 <auto mark>, a small yellow window appears on the screen
- Type the number of the echo on which the pointer was placed, a “25” in this case.
- Click Enter to confirm
- Click F1 and with the arrow key highlight the Save display

- Click Enter and type the name of the file (up to 8 letters) with the extension .dat
- Click Enter to save the file.



The created file consists of two columns; time vs corresponding magnitude of the echo signal. An example is shown in Fig.37 (data points obtained from the spectrum in Fig.36).

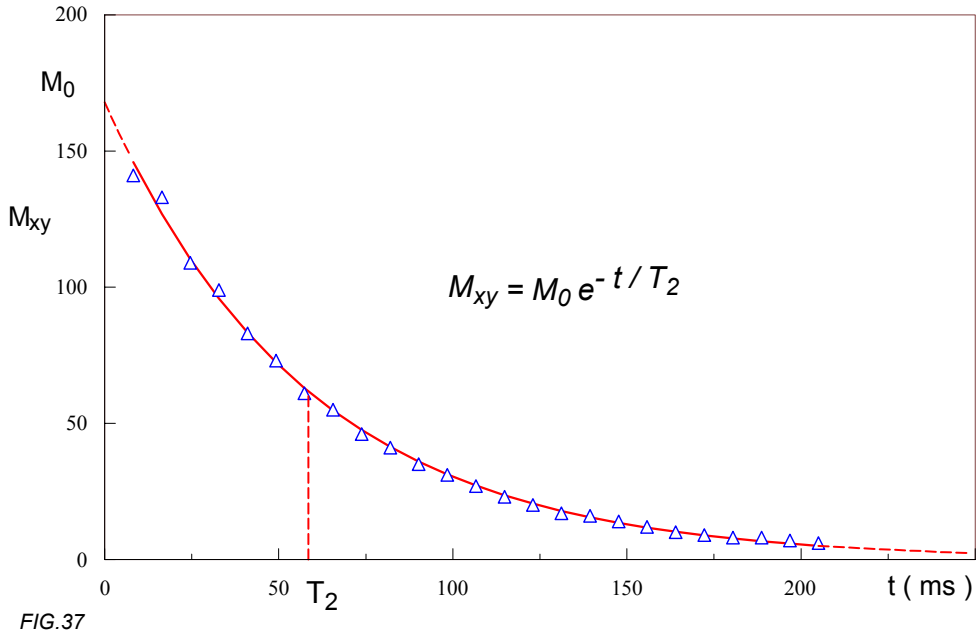


FIG.37

T_2, the transverse relaxation time is the time for the tra

nsverse magnetization, M_{xy}, to decay to e^{-1} (or about 37%) of its initial value. In the literature, the T_2 relaxation curve is usually plotted in a semi-logarithmic diagram, i.e., with logarithmic values along the ordinate (y-axis) and time along the abscissa (x-axis).

Equation in Fig.37 can be transform to $\ln M_{XY} = -\frac{1}{T_2}t + \ln M_0$

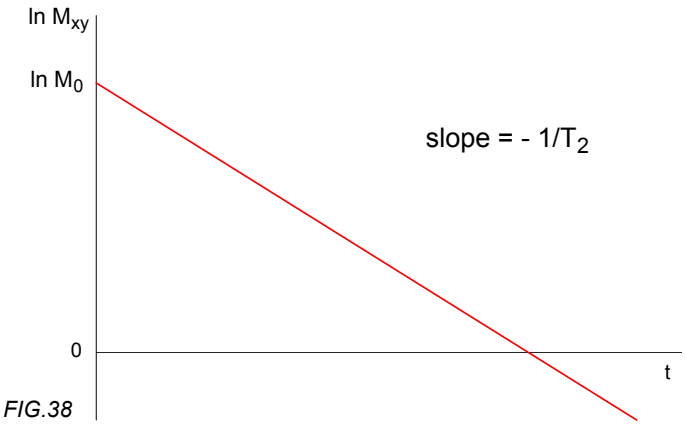


FIG.38

$1/T_2$ is called the T_2 relaxation rate. A short T_2 thus means a high relaxation rate, a fast decay of M_{xy} and steep negative slope for

the line in Fig.38. The CPMG method allows one to determine T_2 .
By measuring the decay time of the FID after a 90º pulse we are actually determining T_2* rather than T_2 since no magnet is perfect. T_2* is always shorter than T_2 . In fact, it can be much shorter if T_2 is long and the magnet is not of high homogeneity.

Similar to the T_2 relaxation, the actual decay of the FID after a 90º pulse can be approximated by the equation:

$$M_{XY} = M_0 \exp(-t/T_2^*)$$

In this case, M_{xy} is the transverse magnetization at a time t after the 90º pulse. Since the FID signal is proportional to , M_{xy} , this equation also gives the approximate shape of the FID. The decay rate is $1/T_2$*. The part of this decay due solely to field inhomogeneities produced by the magnet has a rate $1/T_{inh}$. Thus

$$1/T_2^* = 1/T_2 + 1/T_{inh}$$

Note that there is no such simple, additive relationship between the relaxation times themselves, i.e., $T_2^* \neq T_2 + T_{inh}$.

WHAT CAUSES T_2 RELAXATION?
Comparison of T_2 and T_1 relaxation.

In contrast to T_1 relaxation, where energy transfer from the spin system must occur, T_2 relaxation may take place either with or without energy dissipation. Anything causing T_1 relaxation also causes T_2 relaxation, but T_2 relaxation may occur without T_1 relaxation. Therefore the numerical value of T_2 is always less than or equal to T_1 ; it is never greater.
T_2 relaxation results from any intrinsic process that causes the spins to lose their phase coherence in the transverse plane. Most frequently it results from static or slowly fluctuating local magnetic field variations within the sample itself. If the spin transiently experiences a change in its local static field by a slow interaction with another spin or through an alteration in its chemical environment, that spin temporarily resonates at a slightly different frequency and thereby gains or loses phase compared with the other spins.

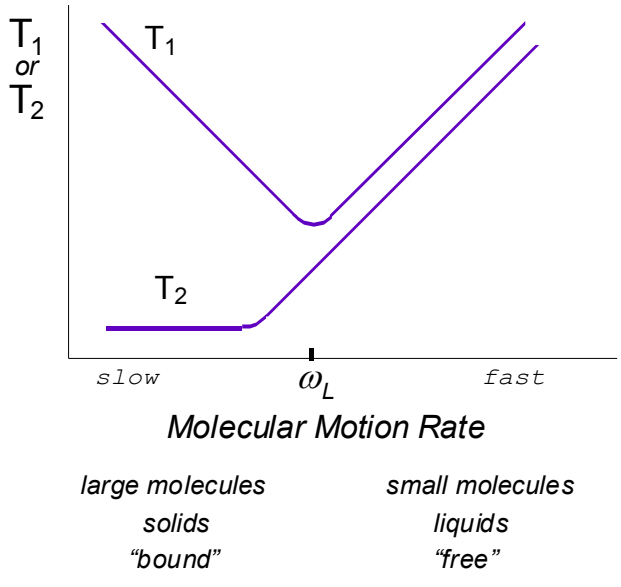


FIG.39

The terms *static* or *slowly fluctuating* include all fields produced by spins

tumbling at rates significantly lower than the Larmor frequency. In practical terms, all molecules tumbling at less than several hundred kHz can be considered static in the NMR frame of reference, where spin precessed at several MHz or more. All molecular motions in this "slow" range are therefore associated with relatively short T_2 values (Fig. 39). In rigid molecules and solids where molecular motions are extremely slow, T_2 relaxation is extremely efficient, and T_2 values may be as short as 5 – 10 μs. These short values
cause the signal to decay so rapidly that the signal from such samples are essentially invisible in an NMR experiment. Several ingenious techniques have been developed to overcome this problem.

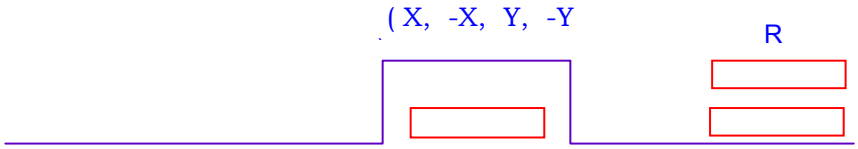
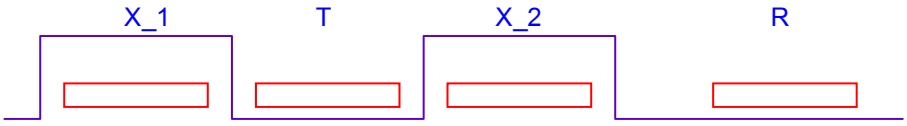
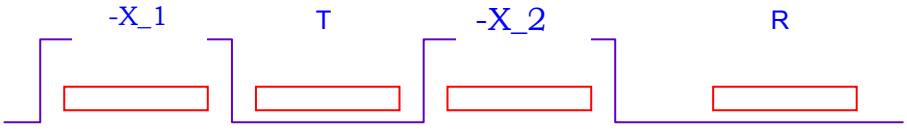
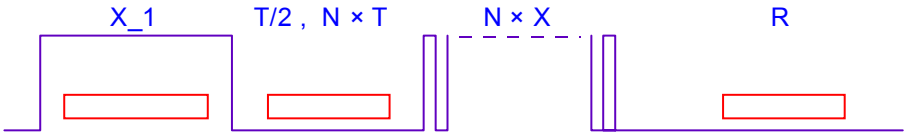
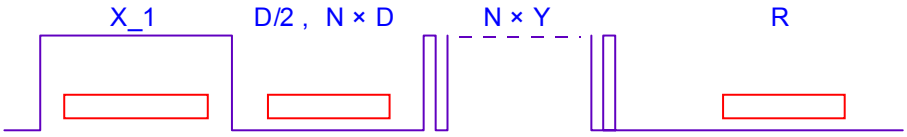
Conversely, when molecular motion is rapid, any local field inhomogeneities experienced by a proton average to zero over a short time. Consequently, these protons experience no consistent or effective static distortions in their local magnetic fields. In rapidly moving molecules, therefore, T_2 relaxation processes are inefficient and T_2 values are correspondingly long.

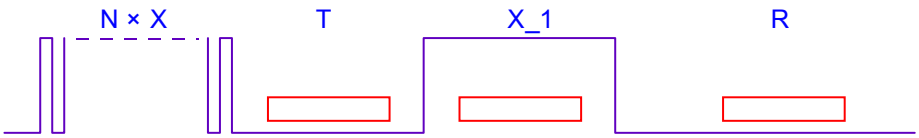
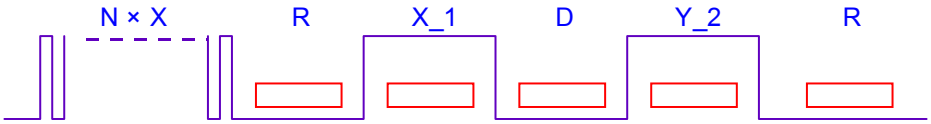
Molecular motions and interactions near the Larmor frequency may contribute to combined T_1 and T_2 relaxation or to T_2 relaxation alone. When a proton's interaction results in the net absorption or emission of energy and a change in state, the proton loses track of its phase relationship with the other spins. Such interaction causes both T_1 and T_2 relaxation. In this mechanism, T_2 relaxation occurs as a consequence of T_1 relaxation, and is therefore sometimes called the "T_1 contribution to T_2."

Two spins may also interact by "exchanging states" with each other in an energy-conserving, spin-spin interaction. This type of interaction at the Larmor frequency contributes to T_2 but not to T_1 relaxation and is sometimes referred to as the "secular contribution to T_2."

Appendix

Method

Method	Sequence Pattern
One-Pulse	
1P_X 1P_-X 1P_Y 1P_-Y	
Two-Pulse	
2P_X_D	
2P_X_VD	
Multi-Pulse Sequence	
CP_25	
CPMG_25	

SAT_25X	
SOLID_E	

Note:

1) The 90° and 180° pulses are pulses most frequently used.
2) The P1 and P2 in the <Trig> command line determines the “detection pulse” after which data points will be acquired. The pre-delay setting display appears accordingly to the <Trig> selection (See Fig.9 for determining the pre-delay time).
3) The “Variable Delay” table can include up to 64 entries ranging from 10µs to several hours but must not include spaces and empty lines.