A Basic Guide to NMR

by

James N. Shoolery

DOI: 10.3247/sl2nmr08.012

Title: A Basic Guide to NMR
Author: James N. Shoolery

Publishers:
First Edition: *Varian Associates*, Palo Alto, CA, USA, 1972.
Second Edition: *Varian AG*, Zug, Switzerland, 1978.
Third, online Edition: *Extra Byte*, Castano Primo, Italy
in Stan’s Library, Vol.II, 2008
Format: scanned PDF
URL: http://www.ebyte.it/library/nmr/ShooleryBasicGuide.html
DOI: 10.3247/sl2nmr08.012
Permanent link via DOI: http://dx.doi.org/10.3247/sl2nmr08.012.

When citing this document, please use the following form:

James Shoolery, *A Basic Guide to NMR*,
3rd Edition, Stan’s Library, Vol.II, 2008, DOI 10.3247/sl2nmr08.012

PREFACE

This booklet was for a long time used by Varian as an educational and promotional handout. In the 70's it amounted to an introductory NMR course for chemistry students and would-be instrument operators. It is widely quoted in NMR literature but it became practically unavailable. Which is a pity because it has a historic value and – despite all those years – many educational merits.

As an Editor, I am grateful to Jim Shoolery and his wife Judith for their enthusiastic endorsement of this freely available re-edition. I also wish to thank warmly the Publisher of the 2nd Edition, Varian Inc, for their courteous permission to proceed with this initiative.

Today, there are two categories of readers who can benefit from this text. The first category are still chemistry students who approach NMR for the first time and like to do so following a historic perspective. The exposition of the intricacies of 1D NMR spectra and of the correlations between NMR parameters and molecular structure are still valid and useful. A present-day student should only skip those Sections which regard instrumental aspects of NMR since they are based on the now obsolete continuous-wave (CW) techniques. In addition, the student should learn to ignore the peak ringing present in practically all the shown spectra. Such "wiggles" are a principal – and ubiquitous - artifact of the CW technique.

The second category of readers comprises those interested in the history of NMR instrumentation who are likely to concentrate nostalgically on exactly those sections which should be skipped by a modern NMR novice. I, for example, have found it interesting to notice in Fig.22 that the early 220 MHz spectra were acquired in CW mode. To think about it, it is logical since Fourier Transform (FT) techniques emerged only at the very end of the 60's, while the Varian 200 and 220 MHz superconducting systems were born in 1964 an 1965, respectively. But I have always tended to associate supercons with FT, and it never crossed my mind that there were times when commercial supercon instruments were still run only in CW mode.

I do not know Jim Shoolery personally – a fact which I sincerely regret. I started doing NMR in 1964 at a polymer research institute in Prague where we had an early *Jeol* 60 MHz instrument. At that time Jim was already a household name for every chemist around the world using a *Varian Associates* NMR instrument but I, as a *Jeol* User and a physicist, was not in his orbit. Nevertheless, I got glimpses of him at conferences, and since my daily work involved service for chemists, I could have hardly done without his "Shoolery Rules". The latter, of course, were nothing less than the first successful advances in the important field which is today known as "prediction" of NMR parameters (chemical shifts and coupling constants) for organic compounds.

But the importance of Jim as a key figure in the early years of NMR goes far beyond his "rules". I had to grow up and talk about him with personalities like Herbert Gutowsky to fully appreciate his historic role. In a sense, he was a prototype of the figure which instrument manufacturers call "application lab chemist" - and he certainly set the standards of that profession very high! The importance of Company application-lab scientists for the development of whole scientific areas and the respective instrumentation does not always receive the credit it deserves. In their daily work, they carry out the highest-quality, innovative measurements on other people's samples and, alas, sometimes receive less than a "thanks" for it, even when the work gets published. Over the years, they accumulate an enormous experience and become essential to plotting the directions in which the instrumentation should evolve.

Jim's position at *Varian Associates*, however, was not exactly that of a plain employee. He joined the Company in 1952 and thus belonged to the core of people who have started it. If I have it right, he was an Associate (though not a Partner) and the only chemist on the team. His foresight and enthusiastic embracement of NMR as a new technique of chemical analysis and his extremely active proselytism (together with *John D.Roberts* at Caltech and a few others) were crucial for the spectacularly rapid acceptance of NMR by the worldwide chemical community. When he retired from *Varian* in September 1990, Jim had on his credit a long list of specific NMR discoveries and hardware advances (one being the introduction of microcoils in 1979), and close to 200 papers. But I am sure that his main and most ever-lasting contribution to the history of NMR is *the bridge he has cast between the physical aspects of NMR and its chemical applications*.

Stanislav Sykora,
November 30, 2008, Castano Primo, Italy,

Further reading about early activities at Varian Associates and about Jim Shoolery's role:

Lenoir T., Lécuyer C.,
Instrument Makers and Discipline Builders: The Case of Nuclear Magnetic Resonance,
Perspectives on Science **3**, 245-276 (1995).
By the courtesy of the Authors, you may download a draft of this article for personal uses at
http://www.ebyte.it/library/downloads/1995_NMR_Lenoir_InstrumentMakers.pdf. See pp.313-321.

Von Hippel E.,
The Sources of Innovation,
Oxford University Press, Paperback Edition 1994.
Available online at http://web.mit.edu/evhippel/www/books.htm. See pp.143-152.

Shoolery J.N.,
NMR in the Beginning,
Analytical Chemistry **65** (17), 731A-741A (1993). DOI 10.1021/ac00065a002.

Reinhardt C.,
A Lead User of Instruments in Science: John D. Roberts and the Adaptation of Nuclear Magnetic Resonance to Organic Chemistry, 1955–1975,
Isis 97, 205-236 (2006). DOI: 10.1086/504732.

A Basic Guide to NMR

James Shoolery, Varian Associates
Palo Alto, California

Reprinted in Switzerland 1978

TABLE OF CONTENTS

INTRODUCTION TO NMR SPECTROSCOPY. I.

1. What is NMR Spectroscopy?

Nuclear Magnetic Resonance Spectroscopy is the name of a technique for quantitatively detecting the presence of certain chemical elements by measuring the amount of energy absorbed from a radio-frequency coil surrounding the sample. Elements with one or more isotopes whose nuclei possess a magnetic moment, i.e., act like a small magnet, can be detected in this way when the experiment is conducted in a strong magnetic field.

If either the magnetic field strength or the radio-frequency is kept constant and the other is swept over a range of values, or spectrum, small shifts in the absorption peaks are observed which can be interpreted in terms of the various chemical environments of the particular element being studied. Splitting up of some of the peaks into multiplets occurs due to interactions between the various magnetic nuclei within the molecules, and this gives further information regarding the structural relationships of the atoms to one another.

2. Which Elements can be Studied?

While over 60 chemical elements have been detected by the NMR technique, some of which have several isotopes with favorable nuclear properties, only a limited number possess sufficiently strongly magnetic nuclei to allow routine practical application to chemical problems. If we confine our interest to these we find that of the common elements listed in Figure 1, only ^{1}H, ^{19}F, and ^{31}P have both high enough natural abundance

ISOTOPE	NMR FREQUENCY at MHz		NATURAL ABUNDANCE %	RELATIVE SENSITIVITY for EQUAL NR. OF NUCLEI	SPIN IN MULTIPLES OF $h/2\pi$
	14.092 kG	23.487 kG			
^{1}H	60.00	100.00	99.985	1.00	$1/2$
^{2}H	9.210	15.351	0.015	9.65×10^{-3}	1
^{11}B	19.250	32.084	80.42	0.165	$3/2$
^{13}C	15.087	25.144	1.108	1.59×10^{-2}	$1/2$
^{14}N	4.334	7.2238	99.63	1.01×10^{-3}	1
^{17}O	8.134	13.56	0.037	2.91×10^{-2}	$5/2$
^{19}F	56.446	94.077	100.0	0.833	$1/2$
^{31}P	24.288	40.481	100.0	0.066	$1/2$

FIG. 1

and sensitivity to justify widespread analytical applications, and consequently Varian's T-60 is designed to study these three elements. Since ^{19}F and ^{31}P are much less frequently encountered than ^{1}H, which is present in all organic substances, the Varian EM-300 and EM-360 NMR spectrometers are designed for proton NMR work exclusively.

3. How is the Sample Prepared?

With few exceptions, solid samples absorb energy over too wide a range of frequencies to provide sharply resolved spectra for solving analytical chemical problems. Samples must therefore be dissolved in a solvent, preferably one with no background absorption due to nuclei of the same type under study, since the solvent is normally present in great excess.

Proton NMR studies are usually conducted in deutero-chloroform, $CDCl_3$, if possible, in order to standardize the interaction between the sample and solvent, which makes comparison of data from model compounds and unknown samples easier and more reliable. The residual $CHCl_3$ in the solvent gives a small peak which is generally easily distinguishable from those arising from the sample. When solubility in $CDCl_3$ is too low, other deuterated solvents are available. Some samples can be run only in water solution in which case it is necessary to accept the intense background absorption by the solvent.

A much wider variety of solvents can be used for studies of ^{19}F or ^{31}P, since the effects of solvent molecules are usually much smaller than the effects of the molecular environment.

4. Why are Nuclei of Some Elements Magnetic?

Fundamental particles such as protons, neutrons, and electrons behave as if they are spinning on an axis, and consequently exhibit the property of angular momentum. If a nucleus contains an even number of protons and an even number of neutrons, the spins pair up, i.e., for each nucleon spinning in one direction there is one spinning the other way, and the total nucleus exhibits no spin. But if there is an odd number of nucleons in the nucleus, as in ^{1}H, ^{19}F, ^{31}P, there will be a net spin angular momentum which will be some multiple of 1/2 ($^{h}/2\pi$), where h is Planck's constant. The spin angular momentum is given the symbol, $\overline{I}$.

Associated with a spinning charged particle will be a magnetic field, since the charge moving in a closed circular path acts like a current flowing in a loop of wire. The nucleus then behaves as if it possesses a magnetic dipole moment which is given the symbol, $\overline{\mu}$.

5. What Happens When You Put the Sample in The Magnet?

In the absence of a magnetic field the nuclei are oriented randomnly, i.e., the north-seeking poles of the tiny nuclear magnetic dipoles can point in any direction with equal probability. Application of a strong magnetic field to the sample containing an ensemble of many such nuclei leads to the magnetic polarization of the sample. For nuclei with $I = 1/2$ there are just two possible energy states; the magnetic moment can align parallel to the field analagous to a compass needle in the earth's field, or anti-parallel to the field. The parallel orientation is the more stable by a very small amount of energy, $2\mu H$. The entire system is in a state of dynamic equilibrium due to the jostling of the molecules by one another associated with their thermal motions. A nucleus cannot remain in one energy state very long without being disturbed enough so that it either absorbs energy and goes from the parallel to the anti-parallel state, or gives up energy with the reverse transition. When equilibrium between the populations of the two states is reached, it is found that the parallel state is slightly more populated. The magnitude of this population difference is small, and inversely proportional to the absolute temperature, and directly proportional to the magnetic moment and the field strength. For ^1H nuclei in a field of 14,092 G at 300°K (room temperature) there are 1,000,010 nuclei parallel to the field for every 1,000,000 anti-parallel. The 10 nuclei out of every 2,000,000 which represent the _excess_ population in the parallel state account for the development of a weak magnetic polarization of the entire sample.

6. How Long Does it Take the Sample to Polarize?

Figure 2 shows that the polarization of the sample grows towards its equilibrium value exponentially with a time constant, T_1, called the thermal relaxation time. This time constant depends essentially on two things:

a) The _amount_ of molecular motion in the sample which is effective in jostling the nuclei from one state to the other. This is affected by _temperature_ and _viscosity_.

b) The strength of the interaction between the nuclei and the rest of the sample. Since nuclei are embedded in the atoms they are protected from direct collisions, and can only be jostled by the interaction between their own magnetic field and the fluctuating magnetic fields of their moving neighbors. Thus, T_1 also depends upon the magnitude of μ and the magnetic moments of other atoms and molecules within the sample. It is further affected by the degree to which the atom being studied is _exposed_ or _shielded_ from close approach by the structure of the molecule containing it.

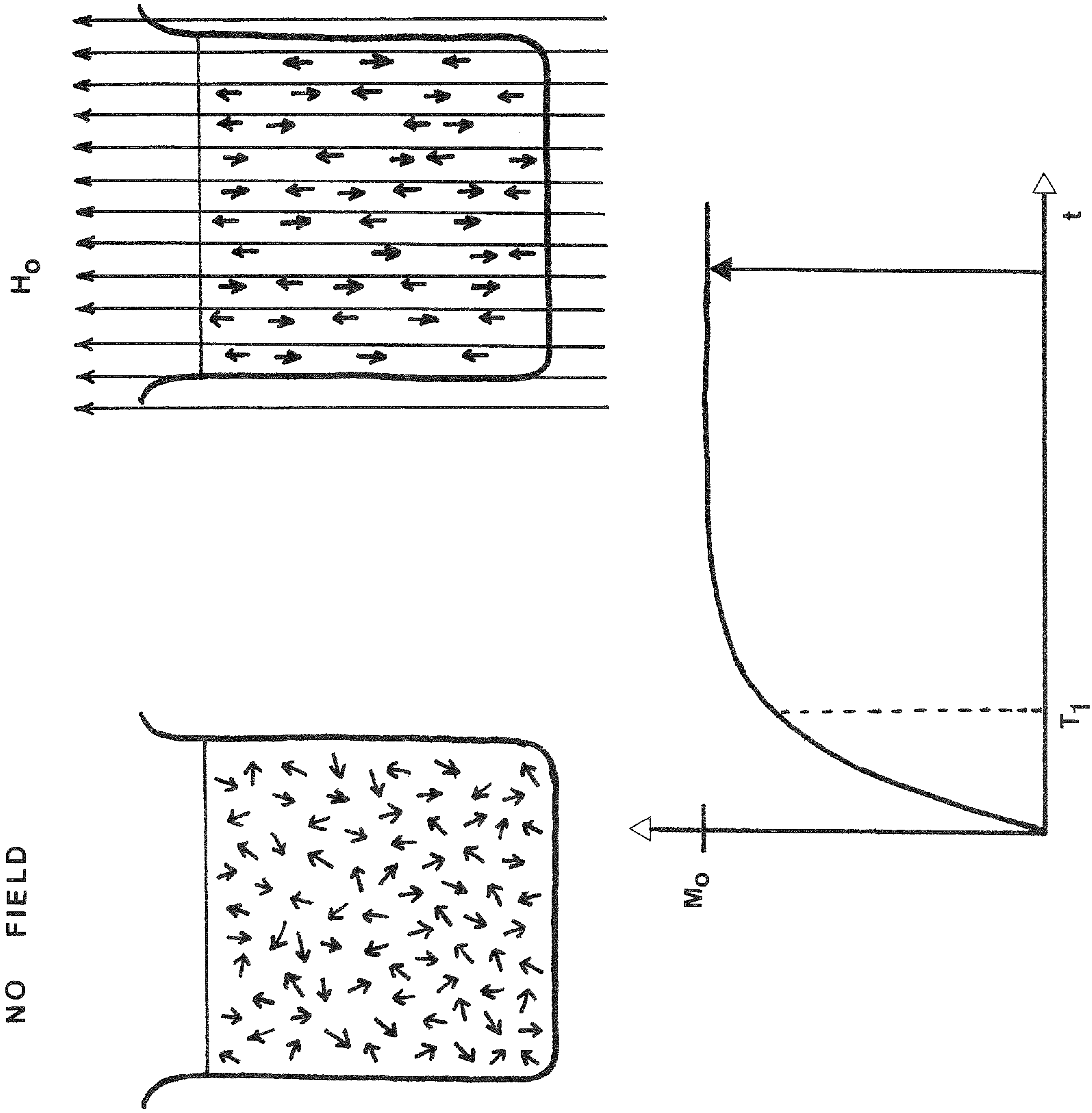


FIG. 2

7. How is the Absorption of Radio-Frequency Energy by the Sample Made to Occur?

The energies of the two states corresponding to parallel and anti-parallel orientation of the nuclei differ by $2\mu H$. Figure 3 shows the energy levels of the two spin states for $I = 1/2$. If electromagnetic radiation with the correct energy is provided, the excess population of nuclei aligned with the field will absorb energy and become opposed to the field. The energy can be supplied by a coil of wire surrounding the sample and carrying an oscillating current of the correct frequency. The oscillating magnetic field associated with this current exerts forces on the nuclei which result in the absorption of energy. The amount of energy absorbed is measured with a detector (radio-receiver) which is sensitive to changes in the voltage across the coil.

Let us calculate the frequency at which the nuclei will absorb energy. If the energy difference is $2\mu H$, and the energy of electromagnetic radiation is given by

$$E = h\nu \qquad \text{Einstein relation}$$

then

$$\nu = \frac{2\mu H}{h}$$

The frequency therefore depends both on the strength of the nuclear magnetic moment and on the strength of the applied field.

In the case of hydrogen nuclei, when $H = 14{,}092$ G., $\nu = 60.000$ MHz. Most analytical NMR instruments operate at this field and frequency.

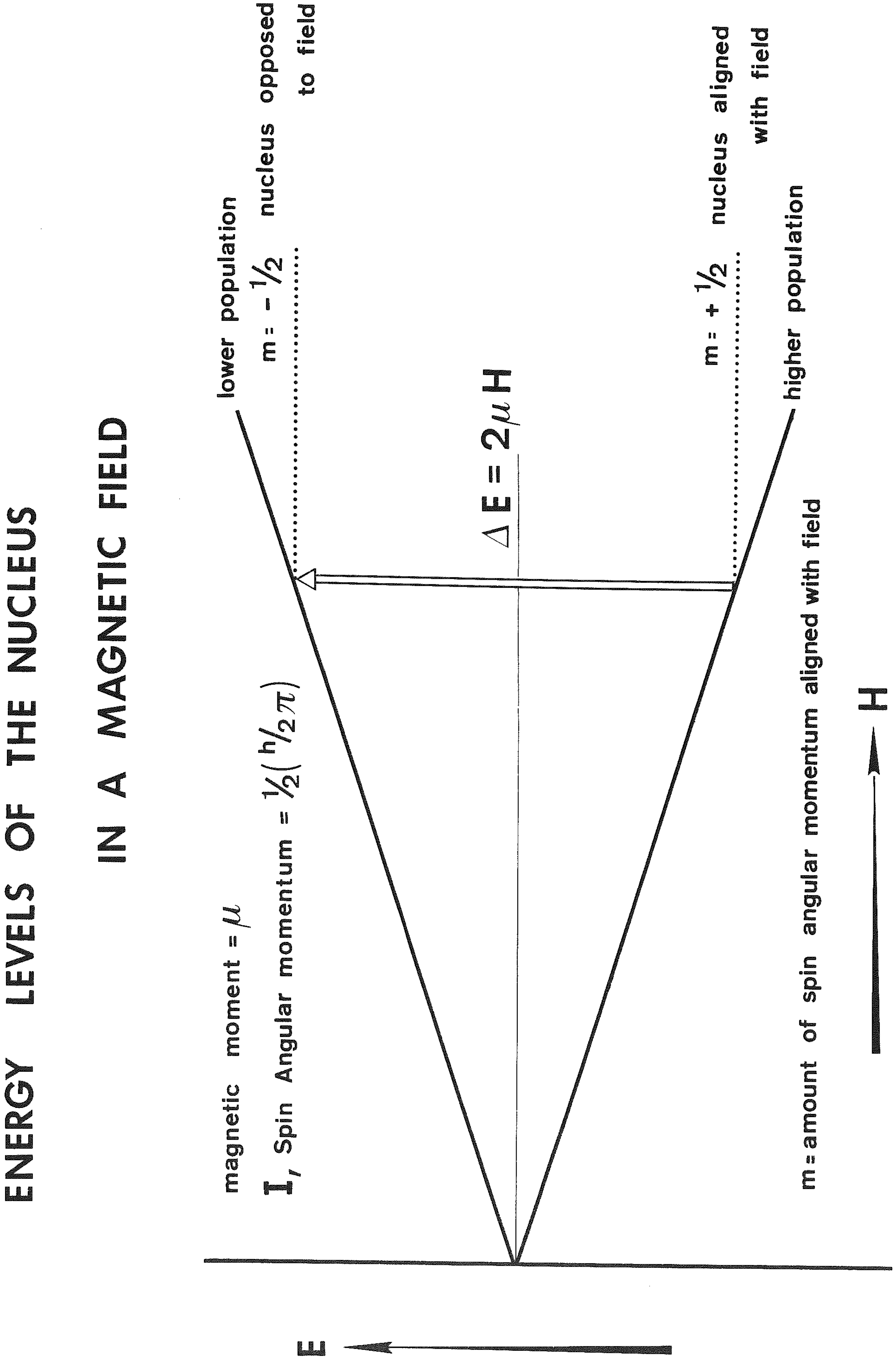

ENERGY LEVELS OF THE NUCLEUS
IN A MAGNETIC FIELD
magnetic moment = μ
I, Spin Angular momentum = $\frac{1}{2}(h/2\pi)$
m = amount of spin angular momentum aligned with field
lower population
m = $-\frac{1}{2}$ nucleus opposed to field
m = $+\frac{1}{2}$ nucleus aligned with field
higher population
$\Delta E = 2\mu H$
H
E

Fig. 3

8. What Factors Affect the Signal-to-noise Ratio, S/N, in the NMR Experiment, and thus Determine the Sensitivity?

To a first approximation, S/N is proportional to the following expression:

$$n \cdot \left(\frac{\nu}{T}\right)^{3/2} \cdot \left(\frac{Q}{B}\right)^{1/2} d_c$$

where

n = number of nuclei/cc.

ν = operating frequency

T = absolute temperature

Q = coil figure of merit

d_c = coil diameter (assuming sample fills coil)

B = bandwidth of detector

ν is determined by the field strength of the instrument and the nucleus being studied. Q and d_c are also determined by the choice of instrument.

This leaves the operator with some control of three factors, namely, the temperature and concentration of the sample, and the filter bandwidth he chooses for the detector.

9. What Basic Instrumentation is Needed to Detect NMR Absorption?

The elements of an NMR spectrometer are very simple. They include:

a) A magnet, providing a strong stable homogeneous field over the sample volume.

b) A stable radio-frequency transmitter.

c) A sensitive radio-receiver and detector.

d) A sensor, consisting of a coil of wire surrounding the sample.

e) A cell containing the sample

f) A method of sweeping through the spectrum.

g) A method of displaying the signals.

Figure 4 illustrates the simplest array of such elements comprising a basic NMR spectrometer.

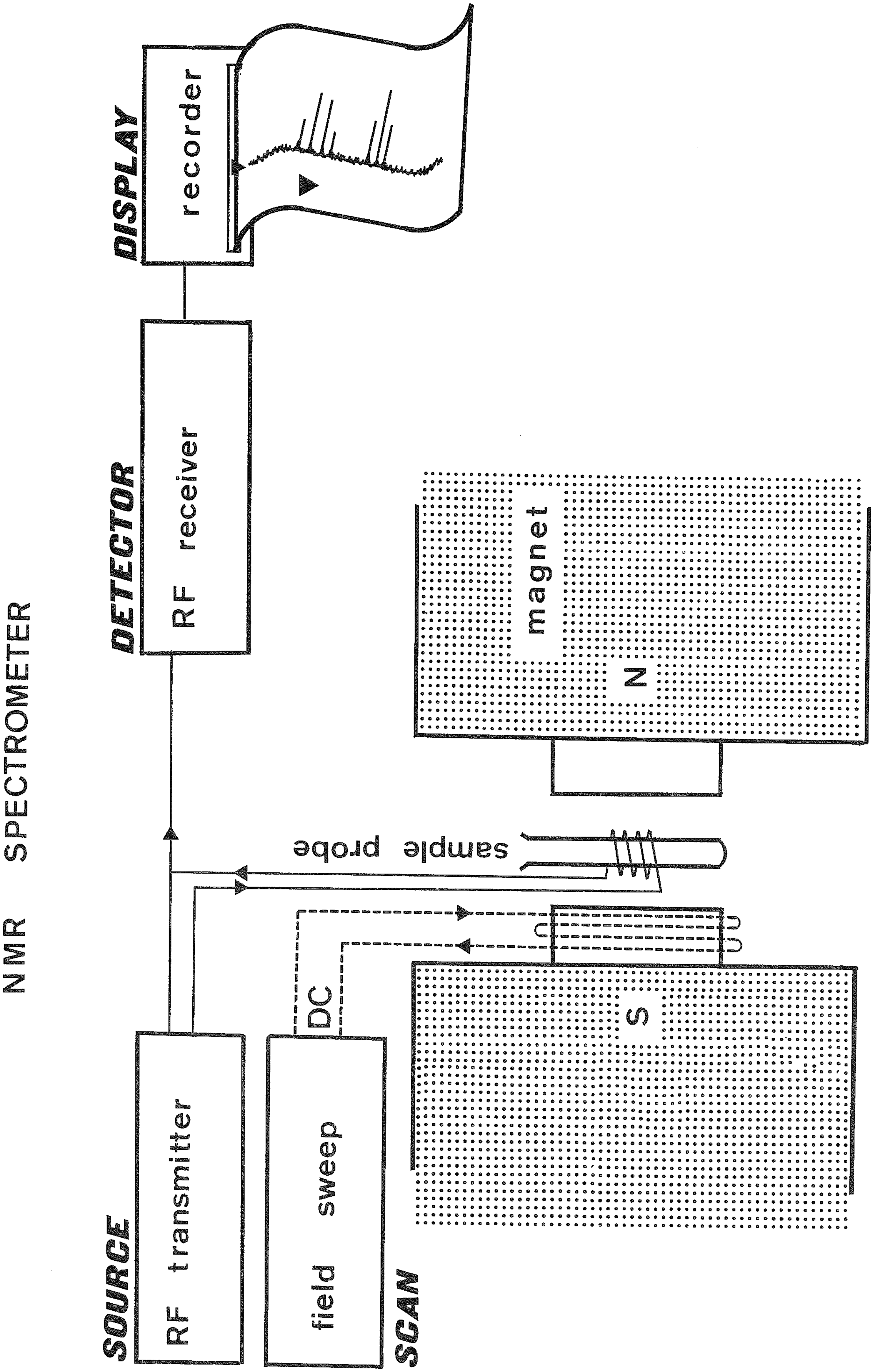

NMR SPECTROMETER
SOURCE
RF transmitter
SCAN
field sweep
DC
DETECTOR
RF receiver
DISPLAY
recorder
sample probe
magnet
N
S

Fig. 4

10. Why are there Small Shifts in the Absorption Peaks from Nuclei in Different Chemical Environments Which Gives Rise to an NMR Spectrum?

The magnetic field at the nucleus is not exactly equal to the field applied to the sample by the magnet. If it were, we would detect only one absorption peak from nuclei of a given species, regardless of their chemical environment. Figure 5 shows, however, that the nucleus exists within a shell of orbiting electrons which are involved in forming bonds to other atoms in the molecule, and that the orbital motions of these negatively charged electrons alter the magnetic field slightly and shield the nucleus to a small extent. In a complex molecule or mixture of molecules we may therefore expect to find a number of peaks if we scan the field or frequency over this range.

The nuclear shielding constant σ is a dimensionless quantity, which is experimentally not accessible. It is defined by

$$H_n = H_o (1 - \sigma)$$

where H_n represents the field experienced by nucleus n. It is therefore advantageous to measure the shielding difference relative to a reference nucleus. This relative shielding is denoted chemical shift.

11. How Are the Chemical Shifts Measured?

The chemical shift in milligauss or in Hz will be twice as large at 60 MHz, i.e. studied with an EM-360, as at 30 MHz with an EM-300. In order to avoid having as many values for the chemical shift for a particular compound as there are NMR instruments operating at different field strengths, the chemical shift δ is also defined as a field-independent quantity like the nuclear shielding constant:

$$\delta_i = \frac{H_r - H_i}{H_o}$$

with H_r and H_i representing the fields experienced by the reference and sample nucleus, respectively. δ is found to vary over a range of about 0.001 percent. Because of these very small variations we normally multiply them by 10^6 in order to report them and they then represent the chemical shifts in <u>parts per million</u> (p.p.m.) of the applied field.

MAGNETIC FIELD AT THE NUCLEUS

$$H_n = H_o (1 - \sigma_n)$$

σ_n = shielding constant of nucleus n

DEFINITION OF CHEMICAL SHIFT

$$\delta_i = (H_r - H_i)/H_o$$

δ_i = chemical shift of nucleus i relative to nucleus r

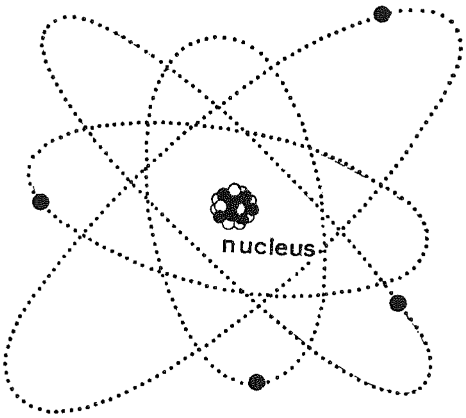


FIG. 5

In order for shift values to have the same meaning in different laboratories we have to agree on the starting or reference point for the measurement. After years of controversy, chemists have nearly universally adopted the position of the resonance from tetramethylsilane (TMS) as the value 0.0 on the chemical shift scale and designated shifts to lower applied magnetic field (←) as positive and those to higher applied magnetic field (→) as negative. This is because the great majority of chemical substances are found to the left of TMS. Figure 6 shows the chemical shifts of a number of compounds whose symmetry makes all the protons in the molecule indistinguishable from one another, thus giving only a single line per compound. Figure 7 is the spectrum of a real mixture of this type.

12. Why Was TMS Chosen as a Reference?

TMS has been selected for the following reasons.

a) It is cheap and readily available

b) Chemically very unreactive

c) It is volatile (b.p. 20°C) thus easily removed from a sample

d) Contains 12 equivalent protons per molecule, giving an intense single absorption line, even at low concentrations

e) Very few other resonances fall close to it

13. Do All Spectra, then, Consist of a Series of Single Lines, One for Each Chemically Non-equivalent Structural Site or Functional Group?

If the shielding which gives rise to the chemical shift were the only effect which could change the field strength at the nucleus, this would be true. However, there are interactions between the magnetic nuclei themselves which must also be taken into account since they give rise to non-vanishing contributions to the field at the nucleus.

Fig. 6

SOME CHEMICAL SHIFTS IN DILUTE CCl_4 SOLUTION

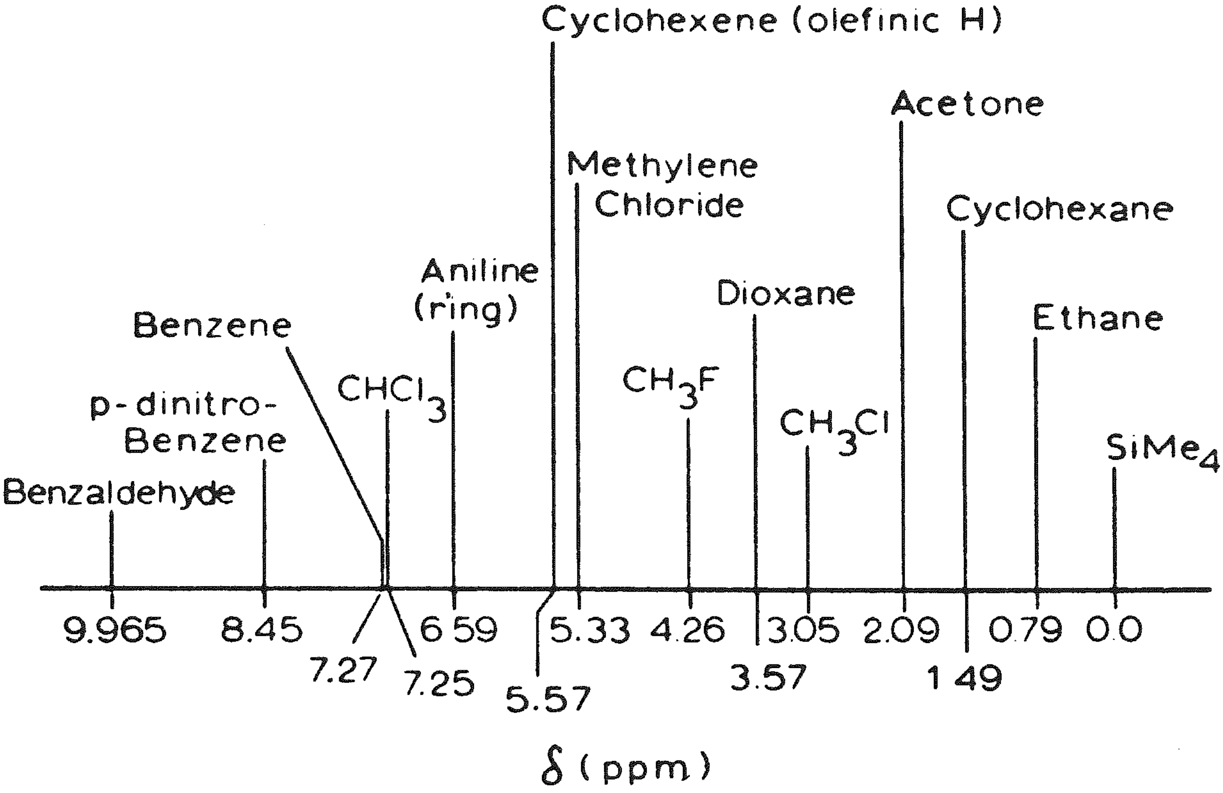
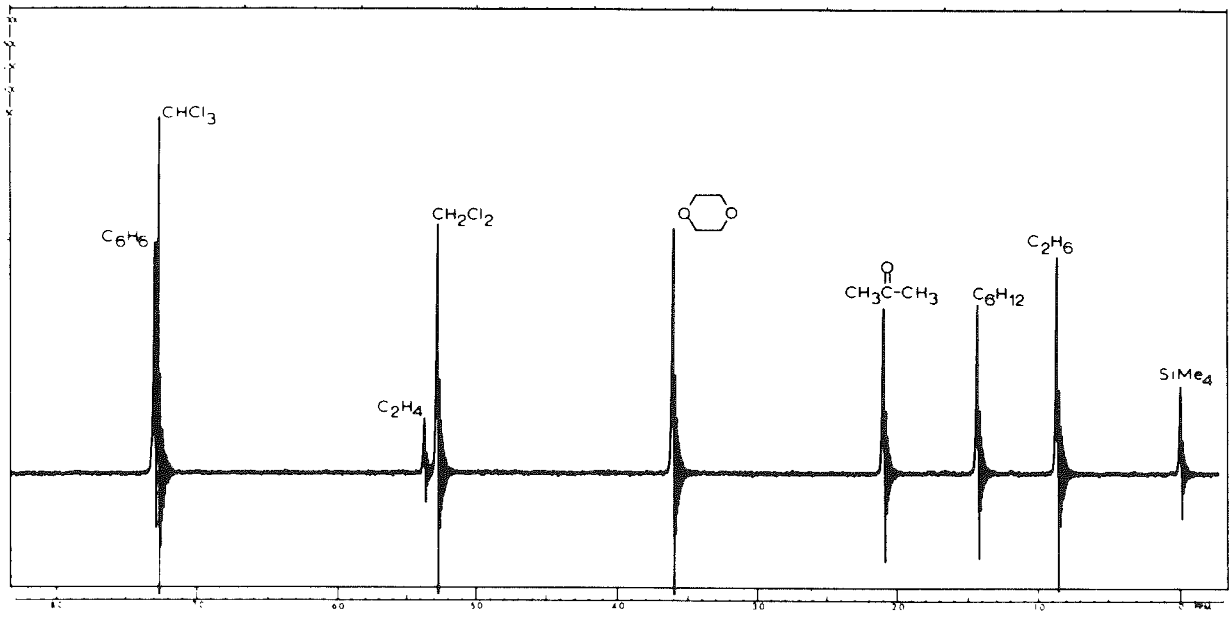


Fig. 7



14. What is this Effect Called?

All nuclei which are magnetic affect each other at any distance through the interaction between their dipolar magnetic fields. In a liquid sample with rapid molecular tumbling, all such interactions average to zero, so that the nuclei behave as if there were no net forces between them. However, nuclei which are chemically bonded together, or bonded to other atoms which, in turn are bonded together, exhibit an interaction which is not dipolar, but which is very preferentially propagated through the chemical bonds due to the interaction between the nuclei and the strongly magnetic electrons in the bonds. This interaction does not average to zero. It is called spin - spin coupling and its effect is to split the absorption peaks into multiplets with predictable numbers of lines, spacing, and relative intensities.

15. Why Do Multiplets Arise?

Figure 8 shows the simplest case of two nuclei whose spins are coupled together by spin - spin coupling. If we recall that there are two nearly identically populated energy states for nuclei in a magnetic field, then in the example chosen, half of the molecules will have nucleus A with spin parallel to H_o, and half with spin anti-parallel to H_o. Similarly, half of the molecules will have nucleus B with spin parallel to H_o, and half will be anti-parallel. These two states interact with the electrons differently with the result that in half of the molecules the field at nucleus A is increased slightly and in the other half it is decreased slightly, due to the two possible orientations of nucleus B. We therefore detect two resonances and we say the peak has been split into a doublet. Similarly, nucleus B experiences a splitting of identical magnitude by the two spin

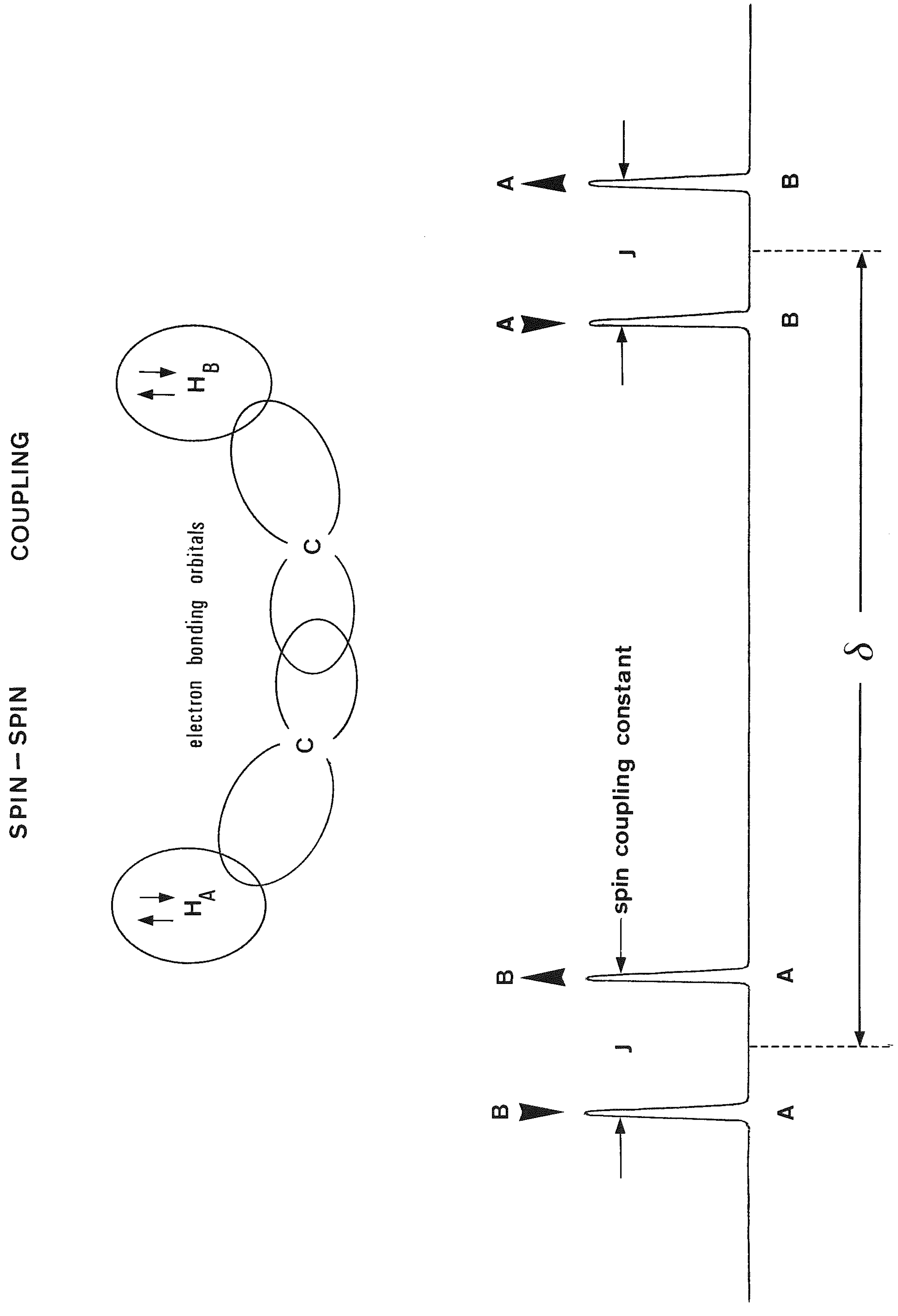

SPIN – SPIN COUPLING
electron bonding orbitals
H_A
H_B
C
C
spin coupling constant
J
J
δ
A
B

FIG. 8

states of nucleus A. Therefore, we find our spectrum is a pair of doublets with spacing J, called the spin-coupling constant. Since the coupling is due to an interaction entirely within the molecule, it is independent of the applied field H_o. It is usually measured and reported in Hz (cycles per second).

16. What Happens if the Nucleus Has More Than One Neighbor?

If a second magnetic nucleus is attached to a neighboring atom, the two spins can align three ways with statistical probabilities in the ratio 1:2:1 as shown in Figure 9. Half of the molecules have the spins opposed which results in no shift of the line, and one line of the triplet coincides with the chemical shift of nucleus A.

As additional neighbors are added, the number of lines increase as shown in Figure 10. If the couplings are equal in magnitude, overlap of the lines leads to the result that η neighbors give rise to a multiplet of $\eta + 1$ lines.

The relative intensities of the lines are given by the binomial coefficients which are as follows for multiplets with increasing numbers of lines.

1
1 1
1 2 1
1 3 3 1
1 4 6 4 1
1 5 10 10 5 1
1 6 15 20 15 6 1

An example of a real molecule with two spins coupled to three spins is diethyl ether. The two ethyl groups are symmetrical and each has the structure $-CH_2-CH_3$ Figure 11 shows the spectrum of this molecule. The CH_2 is split into a quartet as predicted and the CH_3 is split into a triplet.

THREE SPINS, AX_2

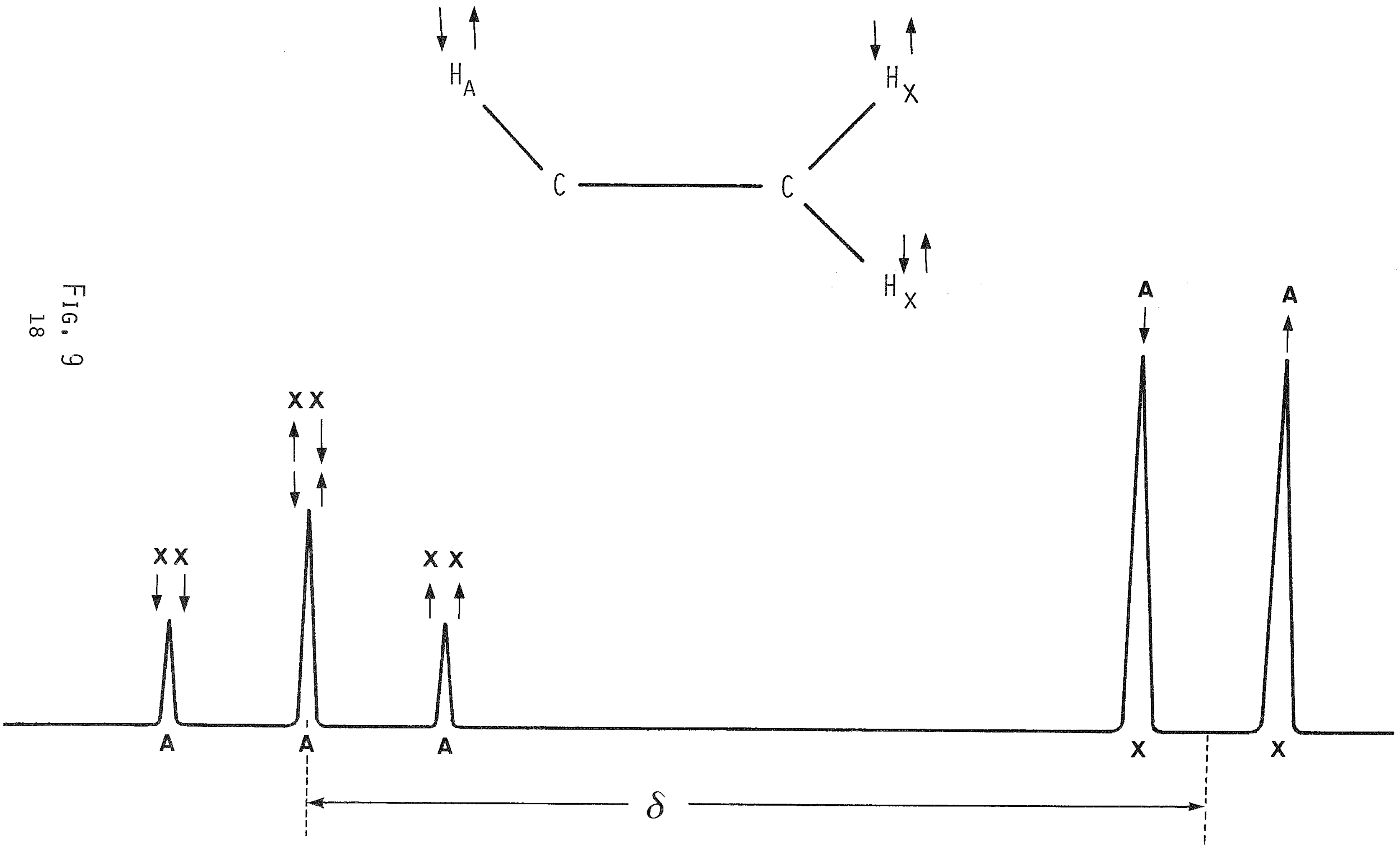


FIG. 9

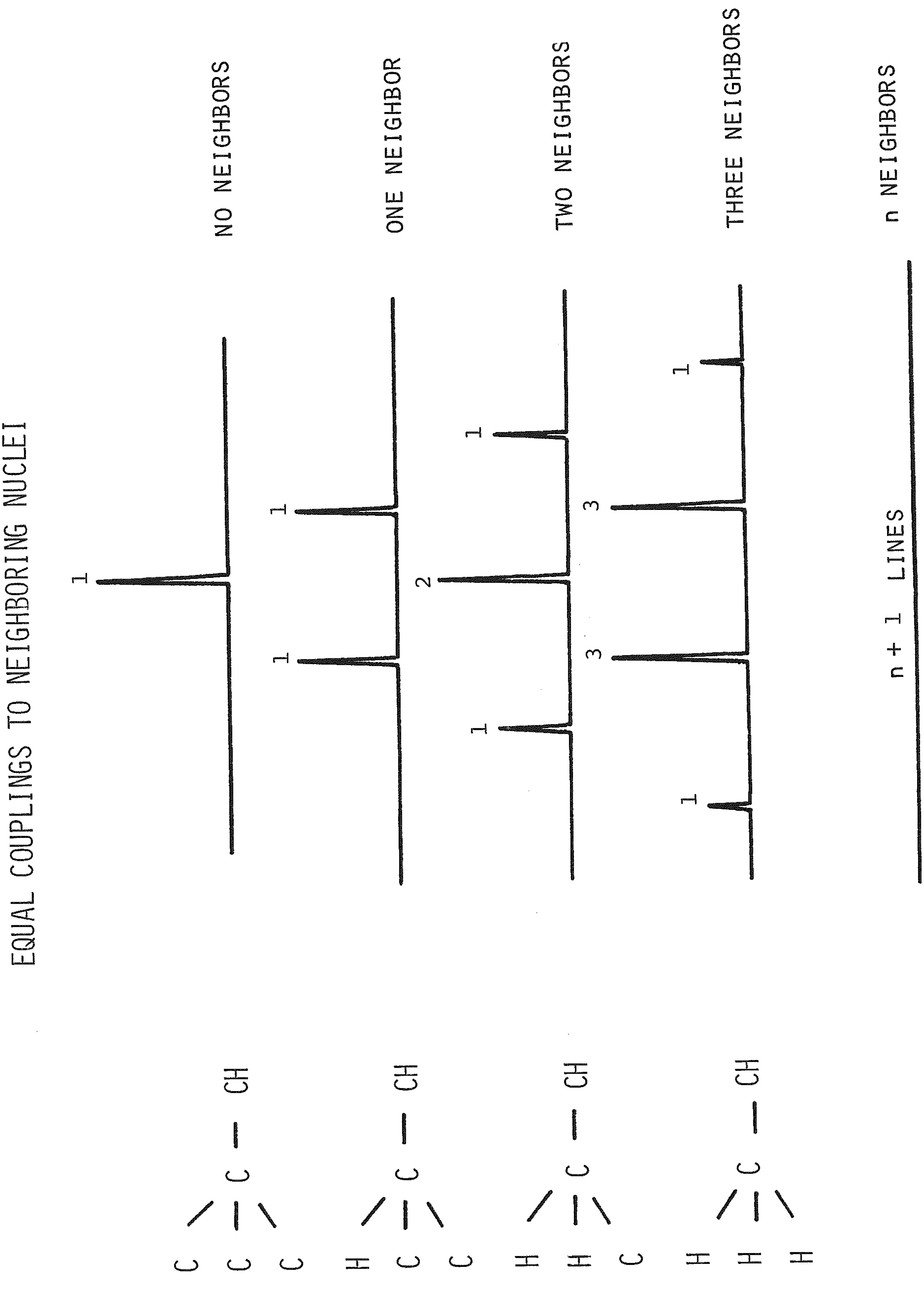
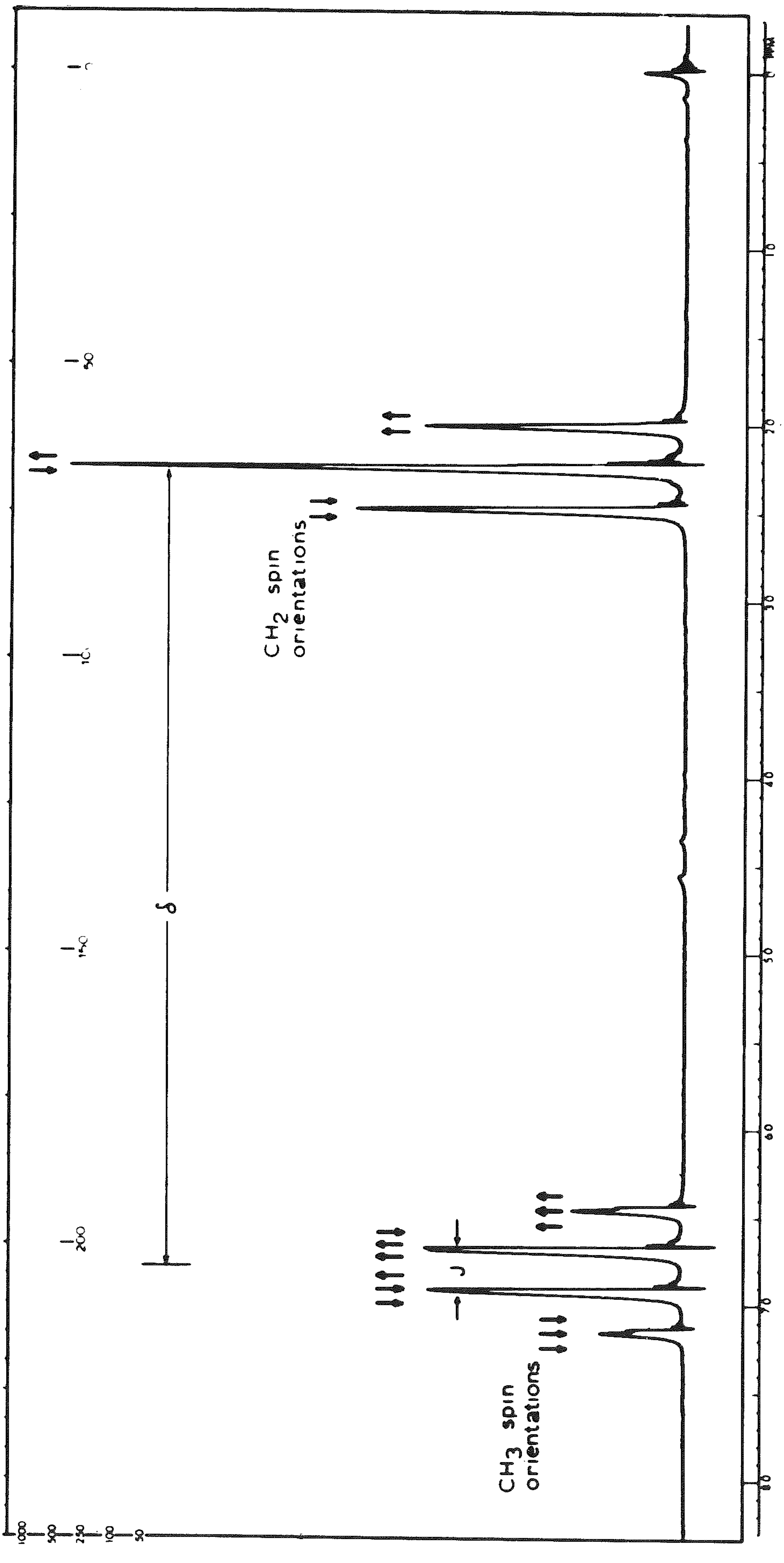

EQUAL COUPLINGS TO NEIGHBORING NUCLEI
NO NEIGHBORS
ONE NEIGHBOR
TWO NEIGHBORS
THREE NEIGHBORS
n NEIGHBORS
1
1
1
1
2
1
1
3
3
1
n + 1 LINES
C C C C CH
H C C C CH
H H C C CH
H H H C CH

FIG. 10

FIG. 11


CH$_2$ spin orientations
CH$_3$ spin orientations
δ
J
0
50
100
150
200
0
10
20
30
40
50
60
70
80
1000
500
250
100
50

INTRODUCTION TO NMR SPECTROSCOPY. II.

A. Basis of the Chemical Shift

1. What kind of electronic motions within a molecule contribute to the chemical shifts of nuclei within that molecule?

Three types of electronic motions contribute to intra-molecular shielding. These are:

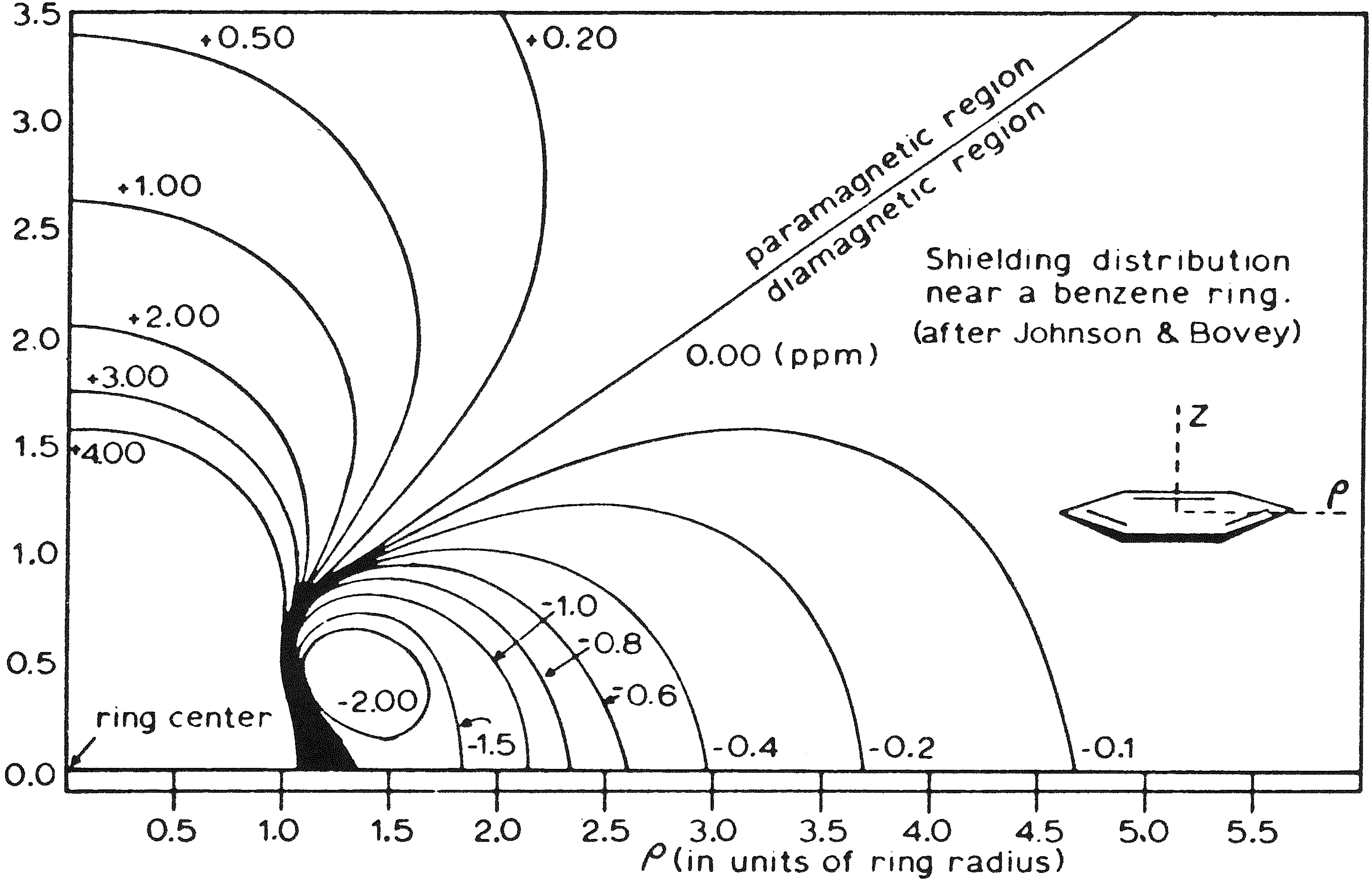
a. Spherically symmetric circulation of electrons around the nucleus. This leads to diamagnetic shielding which reduces field at nucleus. More field must be applied to satisfy resonance condition, so signals move to higher applied field or to the right.

Giving up an electron reduces this shielding; gaining an electron increases it.

b. Non-symmetric circulation of electrons as in case of sharing electrons leads to orbital magnetic moments whose average contribution to the field at the nucleus leads to a paramagnetic shielding.

c. Circulation of delocalized electrons. The π-electrons in the benzene ring represent the best example of long range shielding arising from currents circulating within a molecule. Due to the dependence of the current upon the orientation of the molecule to the field, these effects do not average out with random tumbling but make the aromatic ring appear to be surrounded by a magnetic field. The field opposes the applied field within the ring and reinforces it in the plane of the ring outside of the ring. Figure 12

FIG. 12



Shielding distribution near a benzene ring. (after Johnson & Bovey) shows the distribution of the field around the benzene ring, based on a theoretical calculation.

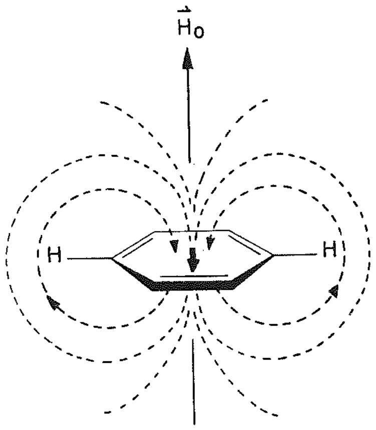


Figure 13 illustrates the effects of all three types of electronic motions.

2. Are chemical shift values characteristic of particular chemical functional groups?

Studies of large numbers of chemical compounds have shown that the chemical shifts of each type of functional group do indeed fall into ranges of values which can be quite narrow or quite large, depending on how strongly the functional group in question interacts with the rest of the molecule in which it is located or with neighboring molecules in the solution under study. Figure 14 shows some characteristic proton chemical shift ranges.

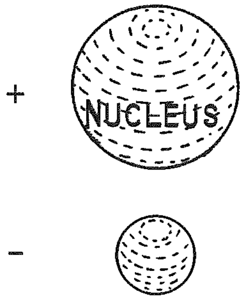
B. Spin-spin coupling.

3. Do the spin-spin multiplet patterns always have n + 1 lines for a nucleus with n neighbors?

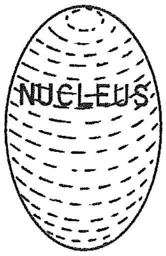
This is true only when the coupling constant to all of the neighbors is the same, which it frequently is. However, the spin-spin coupling between two protons is transmitted differently through different chemical bonds, and is strongly dependent on the geometry of the molecule. Therefore, it is not uncommon to find multiplets arising from a nucleus which is coupled unequally to n neighbors. Figure 15 shows the case of unequal coupling.

INTRAMOLECULAR SHIELDING

IONIC BOND



ELECTRON
DENSITY
REDUCED



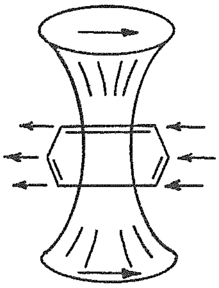
SPHERICAL
SYMMETRY
DISTORTED

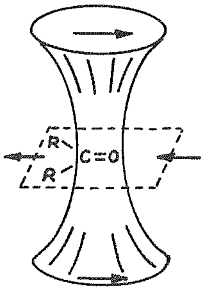
DIAMAGNETIC TERM IN SHIELDING REDUCES FIELD AT NUCLEUS, SHIFTS SIGNALS TO HIGHER APPLIED FIELDS

PARAMAGNETIC TERM IN SHIELDING INCREASES FIELD AT NUCLEUS, SHIFTS SIGNALS TO LOWER APPLIED FIELDS

MAGNETIC ANISOTROPY

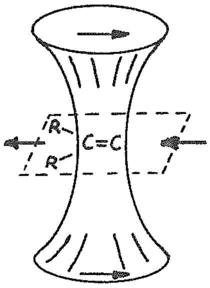
aromatic ring

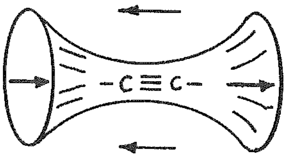




ketones

olefins





acetylenes

FIG. 14

CHARACTERISTIC PROTON CHEMICAL SHIFT RANGES

δ (p.p.m from TMS)	
0.0 - 0.5	3-MEMBERED RINGS , $-\overset{|}{\underset{|}{\mathrm{Si}}}-\overset{|}{\underset{|}{\mathrm{C}}}-\underline{\mathrm{H}}$
0.5 - 2.0	$C-CH_3$, $C-CH_2$, $C-CH$.
2.0 - 3.5	$R-CH_3$, $R-CH_2$, $R-CH$; $R = N, C{=}O, Br$.
3.5 - 4.5	$X-CH_3$, $X-CH_2$, $X-CH$; $X = F, Cl, O$.
4.5 - 6.0	CH =
5.5 - 6.5	CH = (conjugated)
6.5 - 8.5	ϕH
9.0 - 10.0	$-\mathrm{C}\genfrac{}{}{0pt}{}{\nearrow \mathrm{O}}{\searrow \mathrm{H}}$
10.0 - 10.5	- COOH
1.5 - 15	- OH (depending on H-bonding)

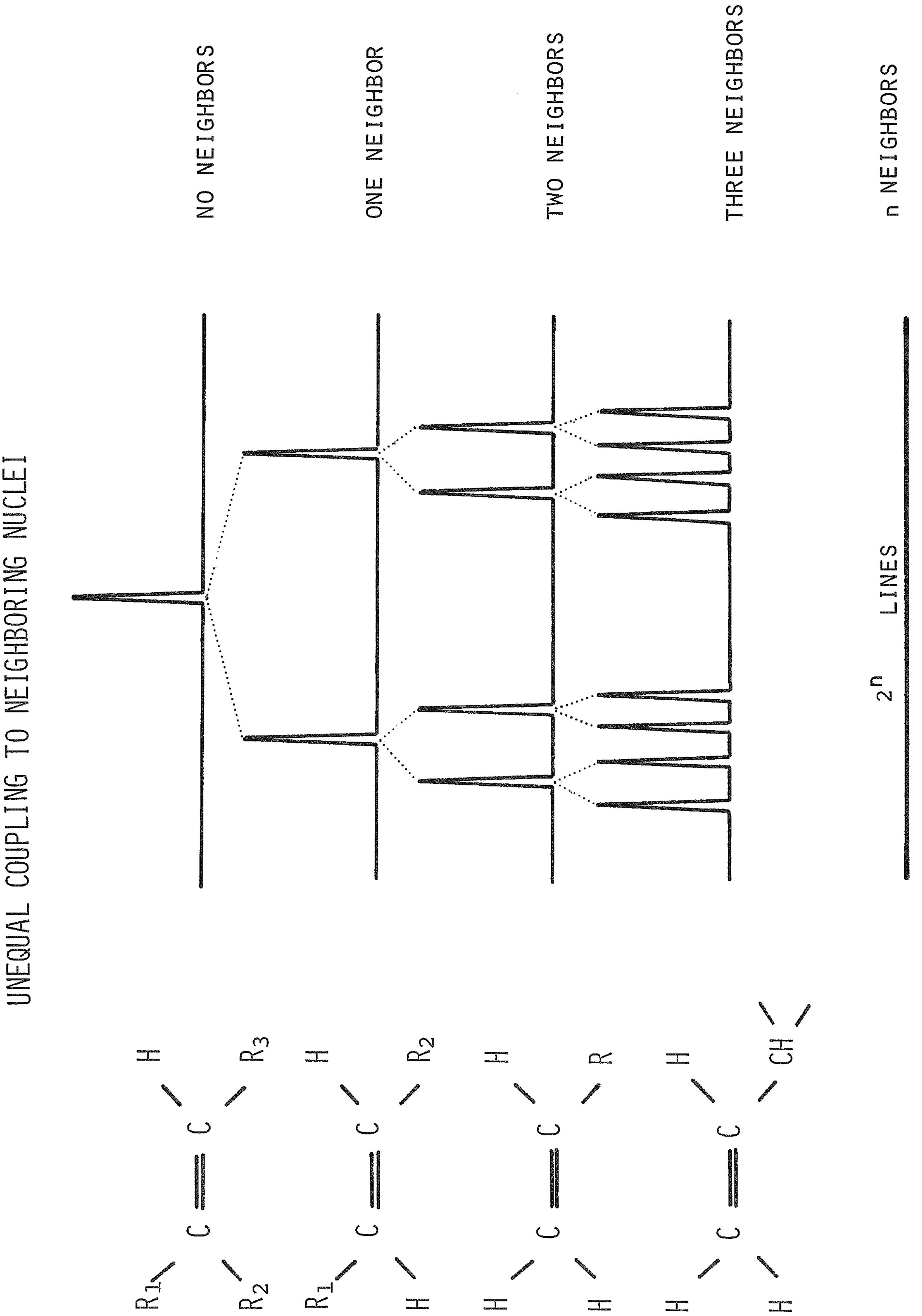

UNEQUAL COUPLING TO NEIGHBORING NUCLEI
NO NEIGHBORS
ONE NEIGHBOR
TWO NEIGHBORS
THREE NEIGHBORS
n NEIGHBORS
2^n LINES
R_1
R_2
R_3
H
C
R
CH

FIG. 15

4. What factors influence the magnitude of the coupling constant, J.

The coupling constants are attenuated in passing through chemical bonds so that in general

$$J_{H-C-H} > J_{H-C-C-H} > J_{H-C-C-C-H}$$

Double or triple bonds attenuate the coupling less than single bonds.

In addition to the number of intervening bonds, a number of other factors influence the coupling constants. These are listed in Figure 16.

5. Do the coupling constants fall within any characteristic ranges for particular arrangements of neighboring nuclei?

The Table shown in Figure 17 lists a number of characteristic coupling constants. These values are very helpful in deciding between possible alternative structures of molecules being studied.

The theoretical curve for the dependence of coupling constant as a function of H-C-H angle matches experiment perfectly in magnitude but gives the wrong sign. The theory is faulty, but the data are useful.

A particularly valuable correlation is shown in Figure 18 in which the angular dependence on the coupling constants of vicinal protons, i.e., $J_{H-C-C-H}$, is given. The conformations, or geometrical shapes of complex molecules can sometimes be determined by measurement of J values which would be sensitive to changes in H-C-C-H angles. Some caution is required since, as we saw in Figure 16, other factors also affect J.

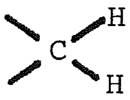
6. Are couplings found between nuclei separated by more than three chemical bonds?

Such couplings are called long range couplings and seldom exceed 2-3 Hz. Figure 19 shows some typical long range coupling constants.

FIG. 16

FACTORS AFFECTING COUPLING CONSTANTS

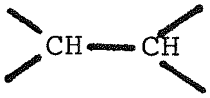
Geminal Protons



J_{gem} : +5 to -30 cps

a) Electronegativity of substituente
b) Angle dependence
c) π - Electron contribution

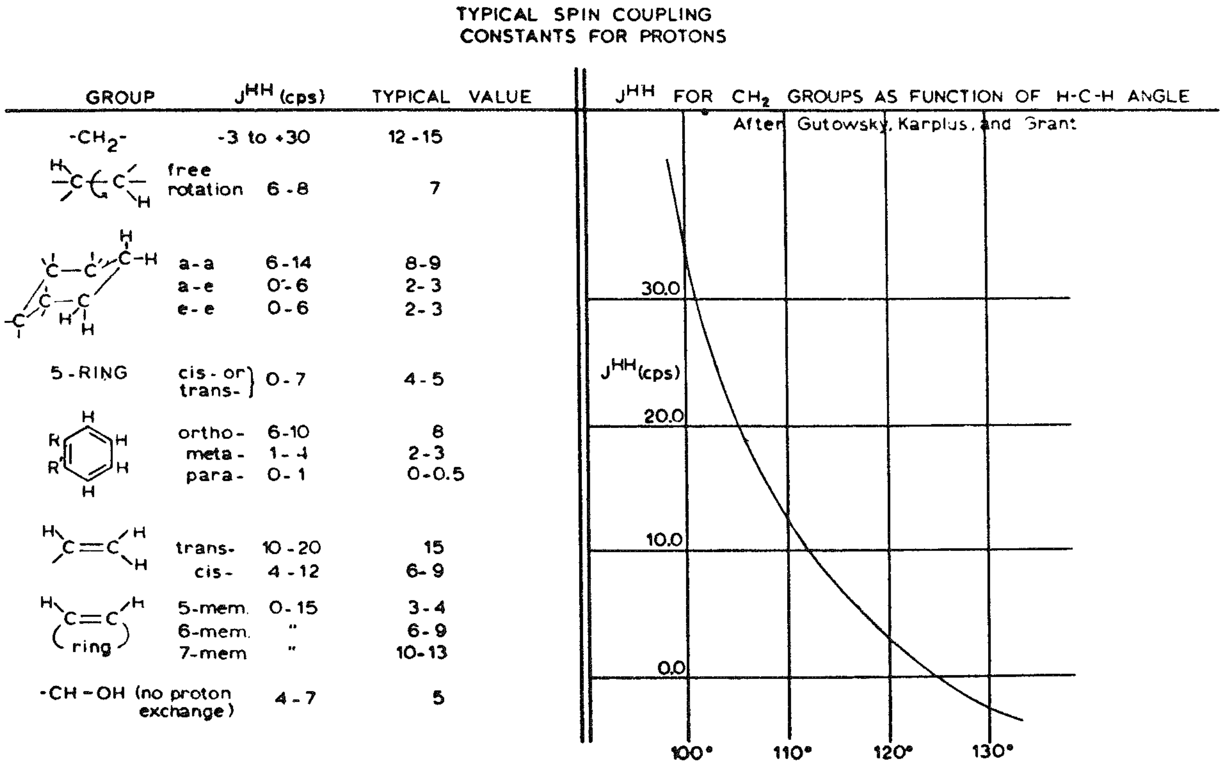
Vicinal Protons



J_{vic} : 6 - 8 cps (free rotation)
: 0 - 14 cps (in fixed positions)

a) Dihedral angle
b) Electronegativity of substituente
c) C-C-H Bond angles
d) Bond length

FIG. 17


TYPICAL SPIN COUPLING CONSTANTS FOR PROTONS

GROUP		J^{HH} (cps)	TYPICAL VALUE
$-CH_2-$		-3 to +30	12 - 15
	free rotation	6 - 8	7
	a - a	6 - 14	8 - 9
	a - e	0 - 6	2 - 3
	e - e	0 - 6	2 - 3
5 - RING	cis - or trans -	0 - 7	4 - 5
	ortho -	6 - 10	8
	meta -	1 - 4	2 - 3
	para -	0 - 1	0 - 0.5
	trans -	10 - 20	15
	cis -	4 - 12	6 - 9
	5-mem.	0 - 15	3 - 4
	6-mem.	"	6 - 9
	7-mem	"	10 - 13
-CH - OH (no proton exchange)		4 - 7	5

FIG. 18

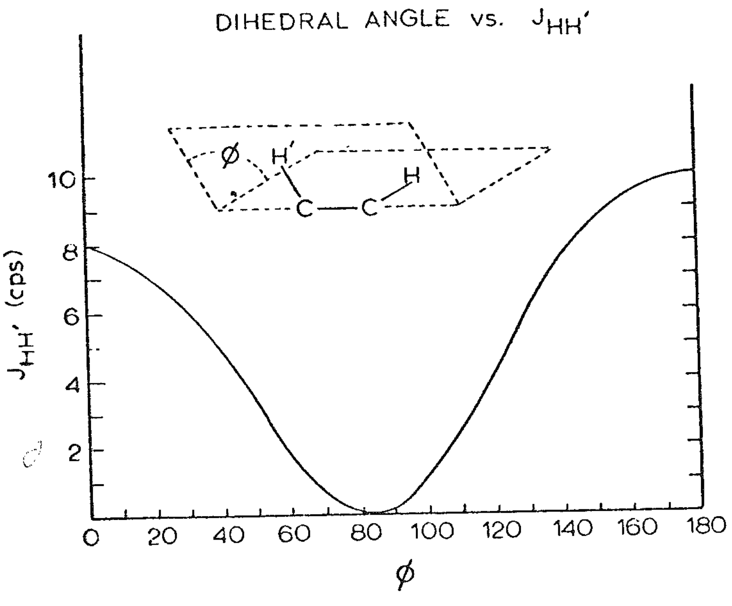
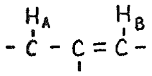
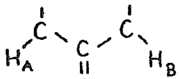
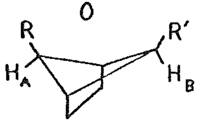
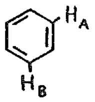
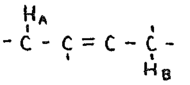


FIG. 19

LONG RANGE H-H COUPLING CONSTANTS

		J_{AB} (Hz)
4-BONDS	 $-\overset{H_A}{C}-C=\overset{H_B}{C}-$	1-2
		0-1
		5-7
		2-3
5-BONDS	 $-\overset{H_A}{C}-C=C-\underset{H_B}{C}-$	0-2
>5-BONDS	$C\underline{H}_3-C\equiv C-C\equiv C-C\equiv C-C\underline{H}_2\text{-}OH$	0.4

7. Why do the intensities of the lines in some spin-spin multiplets depart from the values predicted by the simple theory?

This effect is found when the coupling being observed is between nuclei whose chemical shift difference is not large compared to the coupling between them. Such nuclei no longer behave as isolated, separated sets of nuclei, but influence each other in a way which results in the growth of some spectral lines at the expense of others. The total intensity of the multiplet is unchanged, since it is proportional only to the number of nuclei being observed.

In addition to changes in the relative intensities, extra lines may appear in the multiplet. However, as the chemical shift difference approaches zero, the intensities of all lines but one approach zero, and when the chemical shift is zero between two sets of nuclei, only a single sharp line can be observed.

This perturbation of intensities and extra complexity in the number of lines can make coupling patterns virtually unrecognizable and is the main reason why proton NMR spectroscopy benefits so much from high magnetic field strength. Figures 20 and 21 show how much improved the spectra are as the magnetic field and frequency are increased.

C. Quantitative Studies by NMR

8. What is the significance of the total area under the NMR absorption peaks?

It can be easily shown that once we have set the r.f. level (H_1) and the sweep rate of the spectrometer, the areas under the absorption peaks which arise from a given set of nuclei are proportional only to the concentration of those nuclei in the solution. This has great significance for applications in structural studies and quantitative analysis. It means that the relative intensities of the NMR absorption peaks in the

FIG. 20

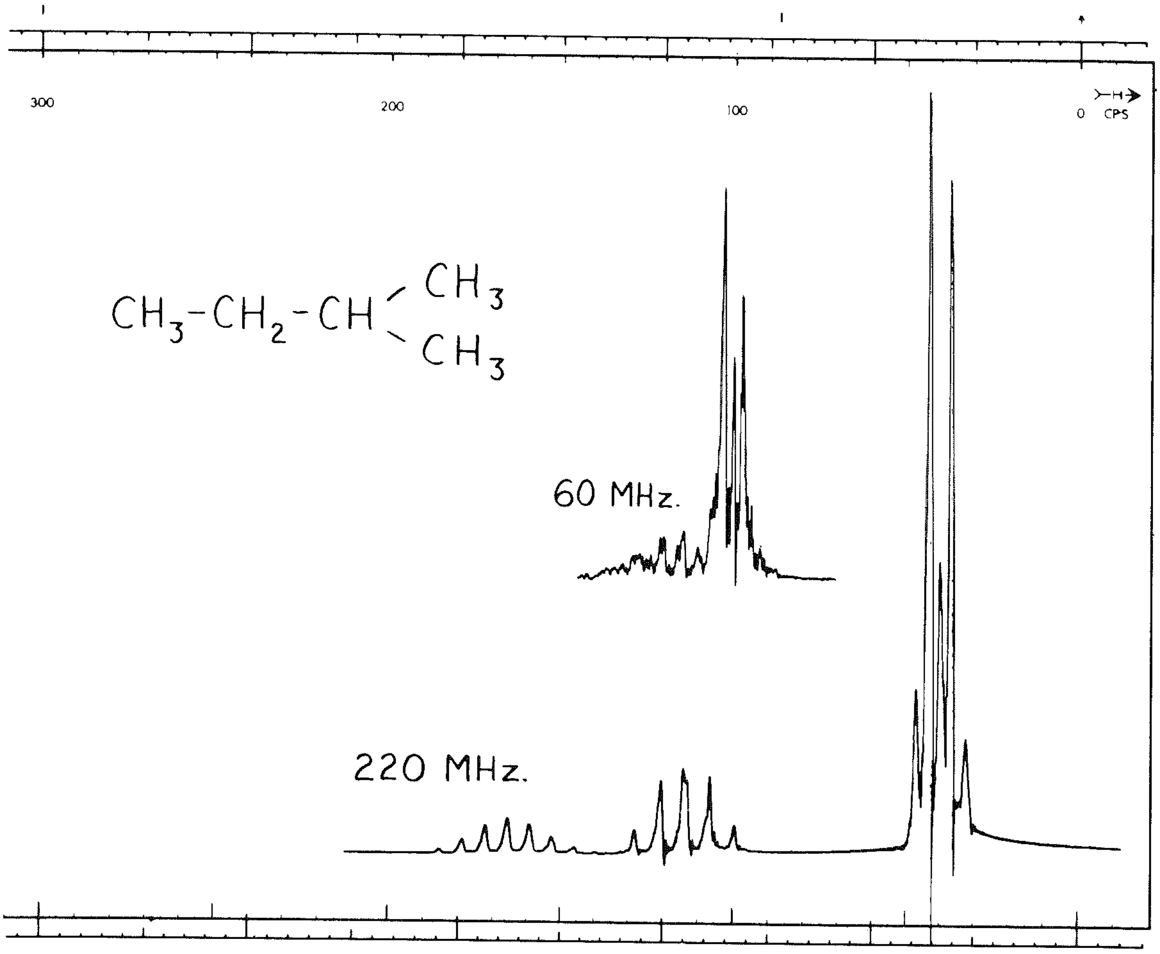
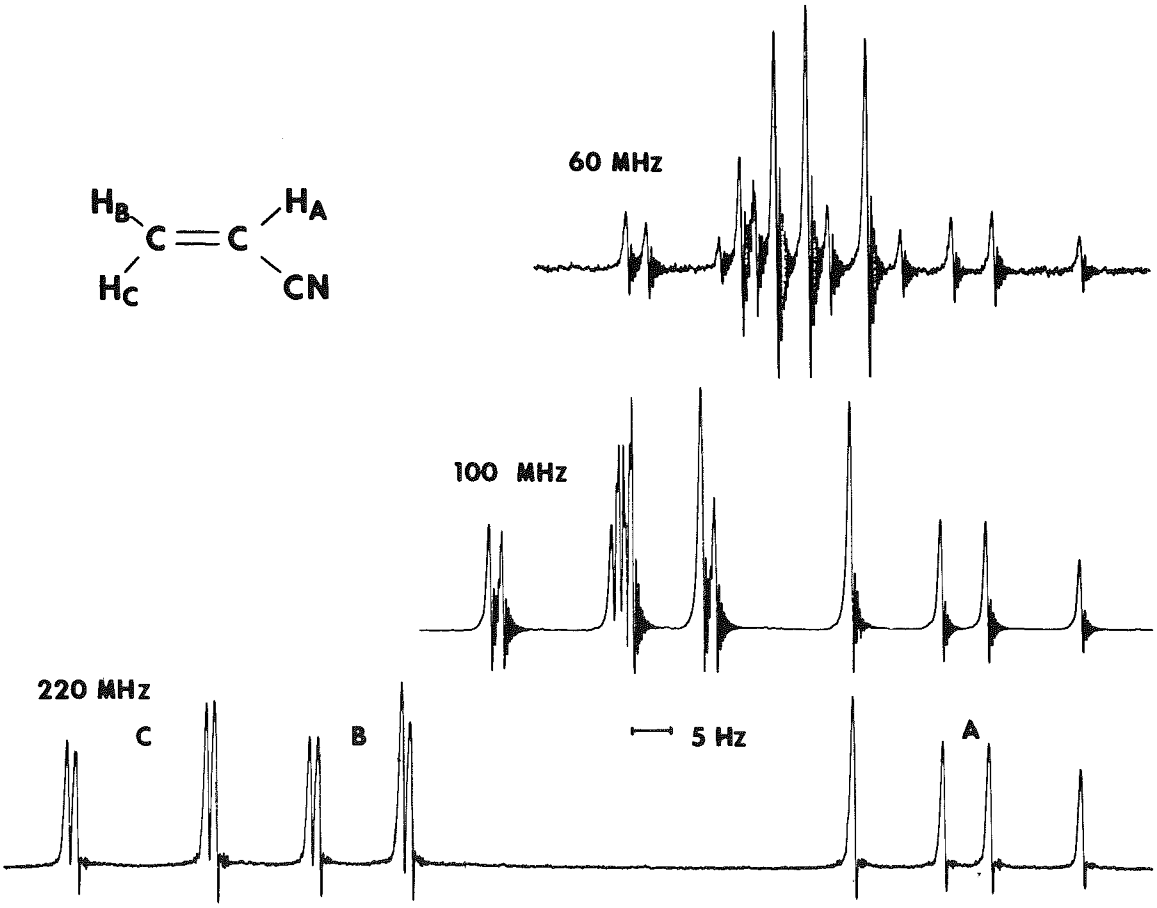

300
200
100
0 CPS
CH3-CH2-CH(CH3)CH3
60 MHz.
220 MHz.

FIG. 21


HB
HA
C=C
HC
CN
60 MHz
100 MHz
220 MHz
C
B
5 Hz
A

spectrum reflect the relative numbers of nuclei in a pure substance, or the relative numbers of molecules of each type in a mixture. Unlike optical spectra in which each peak is proportional not only to the concentration but also to an absorption coefficient which must be measured by having a known concentration of the substance being studied, NMR spectra can be interpreted quantitatively even when they have been obtained from unseparated, crude mixtures.

Figure 22 shows the spectrum of a complex alkaloid whose molecular formula is $C_{20}H_{22}N_2O_2$. Many of the peaks due to structurally non-equivalent protons with overlapping spin-spin coupling patterns are uninterpretable. The integral of the spectrum is also shown as an ascending step function, the size of each step being proportional to the relative number of nuclei contributing to the corresponding absorption peaks.

A pure substance must have integral numbers of protons in different structural sites. Therefore, the ratios of the areas must be the ratios of integers. The smallest step cannot correspond to less than one proton; therefore, if the larger steps are expressed in multiples of the smallest step and all are added together we will have the total number of protons in the molecular formula. The total of all the steps in this case adds up to 22 which is in accord with the formula.

9. How is the analysis of a mixture carried out?

Figure 23 shows the spectrum of a commercial mixture of aspirin, caffeine, and phenacetin known as APC. The quantitative analysis of the mixture is easily accomplished by integrating the region of the spectrum containing the N-methyl groups in caffeine, the N-acetyl group group in phenacetin, and the acetate methyl of aspirin. The molar ratios can be calculated from the relative concentrations of the three molecular species based on the relative numbers of methyl groups which serve to identify them.

FIG. 22

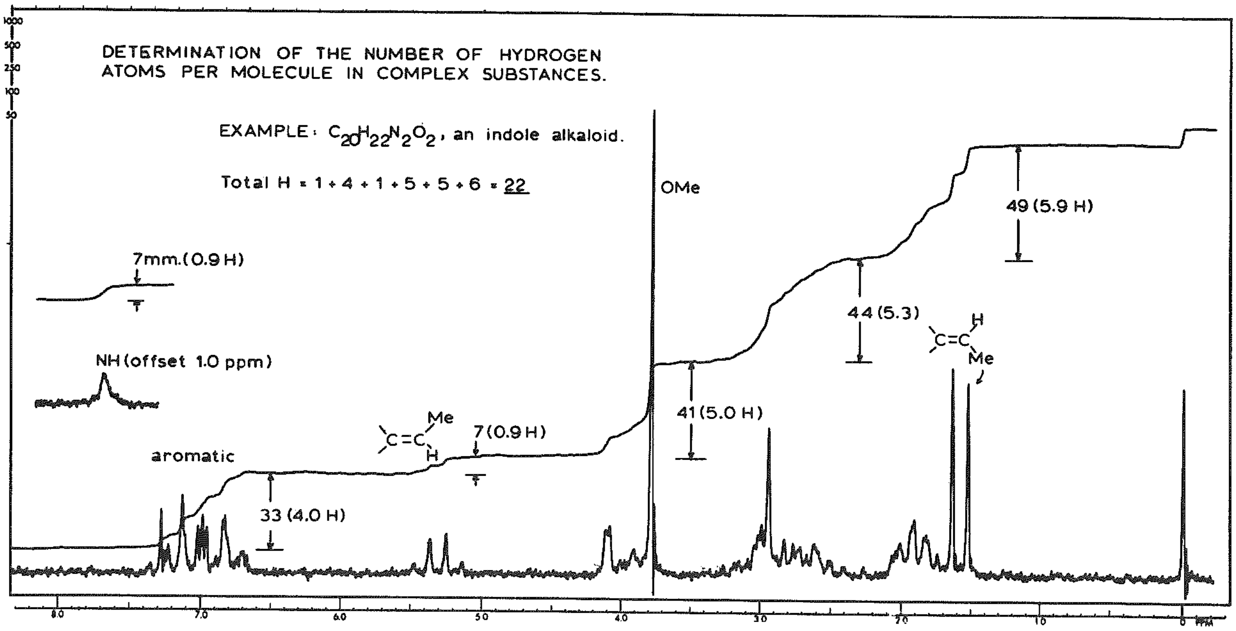
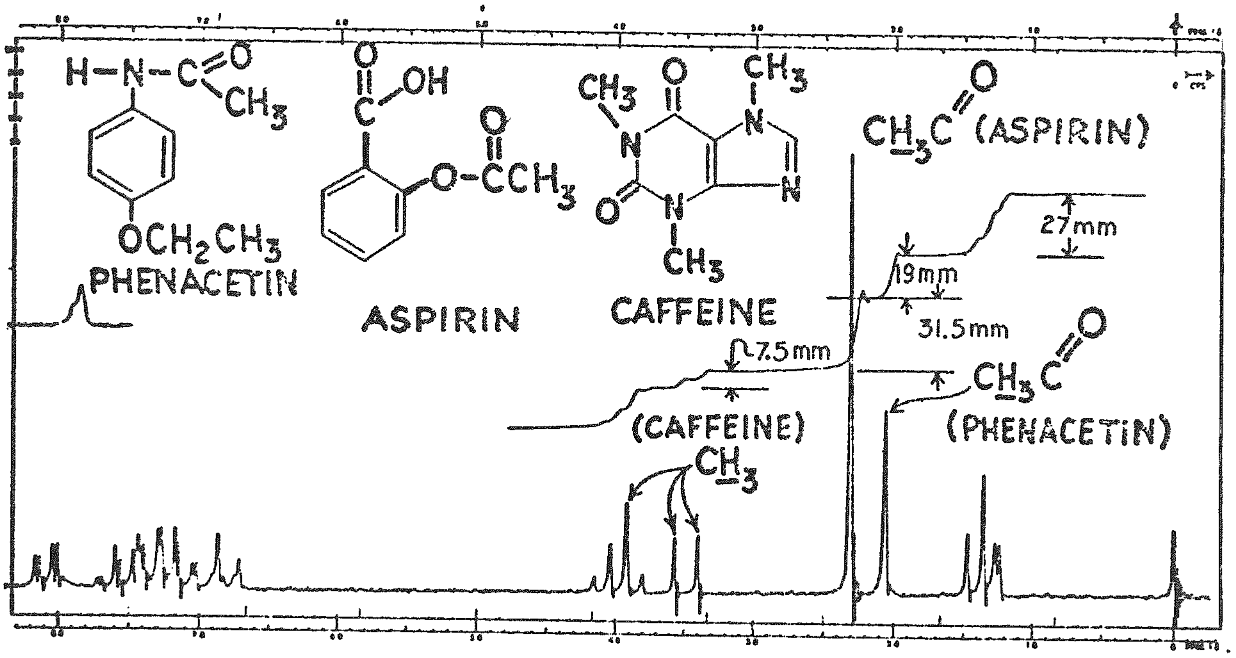


FIG. 23



SAMPLE: COMMERCIAL APC PREPARATION

100 Mg SAMPLE IN .4cc $CDCl_3$

TEMP: 55°C ANALYSIS: Mg / 100 Mg SAMPLE

ASPIRIN	56.5 Mg
PHENACETIN	34.0 "
CAFFEINE	7.0 "

D. Spin Decoupling

10. What can be done to simplify assignments of NMR spectra and to unravel complex overlapping patterns of spin-spin multiplets?

The most effective approach is to run the spectrum at a sufficiently high field strength so that all the groups are separately visible and display first-order type relative intensities within the multiplets. However, a sufficiently high field instrument may not exist in many cases, or at least may not be available, and in cases where several nuclei are coupled together with nearly identical coupling constants it may still not be possible to decide which groups are coupled, and consequently, which groups in the molecule are neighbors.

A very powerful method of determining the presence of coupling between two sets of nuclei, and for eliminating the effects of such coupling in favorable cases is double irradiation, i.e., irradiation of the sample at either two simultaneous different radio-frequencies or one radio-frequency modulated by audio-modulation to give a carrier and sidebands in the presence of gyromagnetic nuclei. When the second r.f. frequency is applied at a lower power level it might be called spin-perturbation, or more commonly, spin-tickling. When applied at a higher level of power, it is called spin-decoupling.

The effect of spin-decoupling is to collapse a multiplet of lines from a group of nuclei, A, which are coupled to a group of nuclei, B, to a singlet (under favorable conditions) when a second r.f. frequency strongly irradiates group B at its resonance frequency while we are applying the normal amount of H_1 to the group A and recording the absorption from it.

The energy level diagram is frequently used to explain spin-decoupling on the basis that since absorption and stimulated emission are equally probably, a strong r.f. field at resonance will cause the nuclei to flip rapidly from parallel to anti-parallel orientations. When the flipping rate becomes rapid compared to the separation of the lines in the spin-

spin multiplet, i.e., 50 times per second for a multiplet whose spacing is 5 cycles/sec (Hz), the nuclei in group B appear to those in group A to be neither parallel nor anti-parallel, but to possess no effective spin or magnetic moment.

A more precise explanation involves the concept that the nuclei only couple their spins together because some component of their magnetic moments point in the same, or opposite directions. In the absence of an r.f. field of the correct resonance frequency for group B, this is true. But when the r.f. field is applied, the nuclei are perturbed from their equilibrium direction and can be made to point their magnetic moment at right angles to the direction of the magnetic moment due to group A. The coupling then goes to zero.

In actual practice, the coupling cannot be completely collapsed for two groups of protons, but if the relative chemical shift of the groups, δ_{AB}, is $>10\ J_{AB}$, the collapse can be made nearly complete. If $\delta_{AB}<10\ J_{AB}$, partial collapse may be effected and coupling between two groups can be verified.

The application of a strong r.f. field at group B also causes a shift of the resonance of group A, and all other resonances in the spectrum, called the Bloch-Siegert shifts.

The Bloch-Siegert shift is given by

$$\text{Shift} = \sqrt{(\gamma H_2/2\pi)^2+(\Delta f)^2}-\Delta f$$

where H_2 is the r.f. field at group B, and Δf is the frequency separation of group B from the point at which the shift is observed.

Note that for large Δf, the Bloch-Siegert shift is negligible, but can become quite large for small Δf, where it approaches a linear dependence on H_2. This leads to an effect called Bloch-Siegert broadening, due to inhomogeneity of the strong r.f. field, H_2. If H_2 is not uniform over the sample, the shift also varies over the sample volume and this leads to broadening. The *crossed-coil* spectrometer has an advantage over the single coil for spin decoupling since the design of an optimum receiver coil precludes the generation of a homogeneous r.f. field with the same coil.

Figure 24 shows the application of spin-decoupling to a chemical problem. A molecule with structure I or II gives the proton spectrum shown at the top, which displays a triplet at the far left due to a proton of the type $CH_2 - C\underline{H} = \underset{1}{C} - C = O$. This could arise from either structure, but in I the CH_2 is adjacent to R, and in II it is adjacent to an oxygen atom. The complexity of the spectrum prevents assigning the CH_2 group with certainty to any region of the spectrum. When, however, the spin decoupler is set around 2.4 ppm from TMS the triplet collapses to a singlet as shown in the lower trace. This conclusively proves the location of the CH_2 and shows it would *not* be next to oxygen, confirming structure I.

E. *Dynamic Effects in NMR Spectra*.

11. What effects are noted in high resolution NMR spectra due to changes in molecular motion?

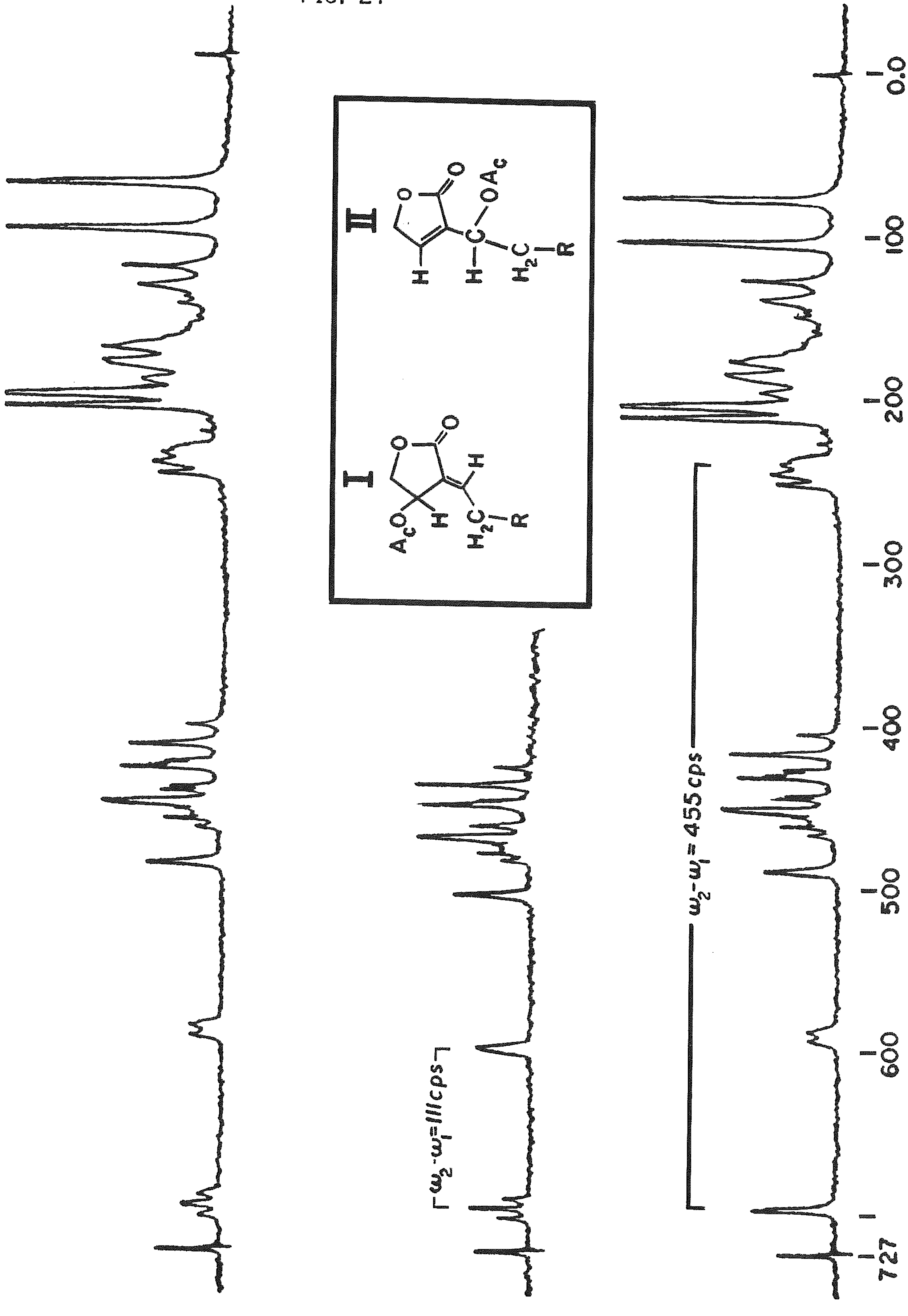
All solutions consist of a mixture of molecules in violent motion, translational, vibrational, and tumbling. The vibrational motions are of such high frequency that the molecules behave as if the nuclei were rigidly located at their *average* positions. Isotopic substitution *can* change this average and produce *isotope* shifts in the chemical shifts. But generally vibrational motions can be neglected.

Translational and rotational motions have a profound effect upon the spectra. If there were *no* molecular motion, NMR spectroscopy would be impossible for the following reasons.

a. The populations in the upper and lower state could be rapidly equalized, with no way for the excess population in the lower state to be reestablished. In effect, the

FIG. 24

PROTON - PROTON SPIN DECOUPLING



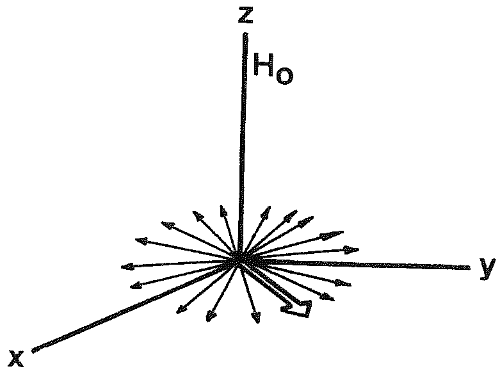
nuclear spin system would be heated up by the r.f. energy, with no thermal contact with its surroundings to dissipate the heat, so it would stop absorbing energy.

b. Each nucleus would experience the static dipolar magnetic field of all the other nuclei in the sample. This would lead to very broad lines.

The component of translational and rotational motions at the precessional frequency, $\nu = \frac{2\mu}{h}H$, is effective in establishing thermal equilibrium, or in reestablishing it when it is disturbed by the application of the r.f. field, H_1. These molecular motions, therefore, determine T_1. The temperature and viscosity affect the translational motions, while the molecular size and shape and the viscosity determine the rotational ones. Small molecules reorient rotationally in times of the order 10^{-11} sec., 1000 times shorter than the nuclear precession cycle. Energy transfer is therefore quite inefficient and T_1 is long, several seconds or more.

Polymers and proteins reorient in times comparable to the nuclear precession cycle. These motions are effective relaxation mechanisms and lead to short T_1's, which correspond to <u>broad</u> lines. Studies over a range of temperature can give information about the shapes of polymer molecules, or changes in the shape of the molecules.

We have seen that the NMR signal arises from the precessing macroscopic nuclear moment, M_o. The voltage induced in the coil arises from the rotating component of M_o in the X-Y plane, under the influence of the driving r.f. field, H_1. If all of the nuclei in the sample are located in the same field strength, the individual nuclear moments continue to precess <u>in-phase</u>, and the rotating component disappears at the rate determined by T_1 which tends to re-align M_o along the field axis (Z-axis). If, however, the nuclei are distributed over a range of field strengths, the individual moments precess at different rates and gradually get out-of-phase with one another.



The net rotating moment becomes zero, even though repolarization has not yet taken place via the T_1 mechanism. The time for this process to occur is called T_2, the transverse, or spin-spin relaxation time. It can be shorter than T_1, but never longer.

T_2 is determined not only by static inhomogeneities in the field but by momentary inhomogeneities due to the fields at the nuclei due to their magnetic neighbors cruising past.

12. How do changes in temperature or concentration of the sample affect the spectra?

Hydrogen atoms attached to oxygen or nitrogen atoms in a molecule are loosely held and can become involved in both association and exchange. In the first case the hydrogen atom remains bonded to the atom it belongs to, but is attracted to the negative end of a dipole in another molecule (or part of the same molecule) and in the process its chemical shift is changed. If the association persists for a long time compared to the time difference in the precession cycle for the associated and unassociated state, we find a shift in the absorption peak but little or no broadening. On the other hand, if the association forms and breaks in a time scale comparable to the frequency difference, the proton precession frequency is randomly shifted from one value to another and the nuclei can get badly out-of-phase, leading to a broadening of the absorption peak due to the shortening of T_2.

In the second case of exchange, the proton actually comes loose from its bond and migrates to another site, being replaced by one from somewhere else. Protons thus randomly jump between two or more sites with different chemical shift values. As the process increases in speed with increasing temperature, the lines broaden and gradually merge into a broad line at the center of gravity of the resonances between which exchange is occurring, finally sharpening up again as the exchange becomes rapid. Figure 25 illustrates this process. The lower spectrum is an expanded view of the region of the spectrum of dimethyl formamide which contains the methyl resonances. Due to partial double bond character of the C-N bond, rotation about that bond is hindered by a barrier of nearly 20 kilocalories/mole. At low temperatures this makes the exchange time of the methyls long compared to the difference in chemical shift arising from their different spatial relationship to the carbonyl group. As the temperature is raised the lines broaden and merge.

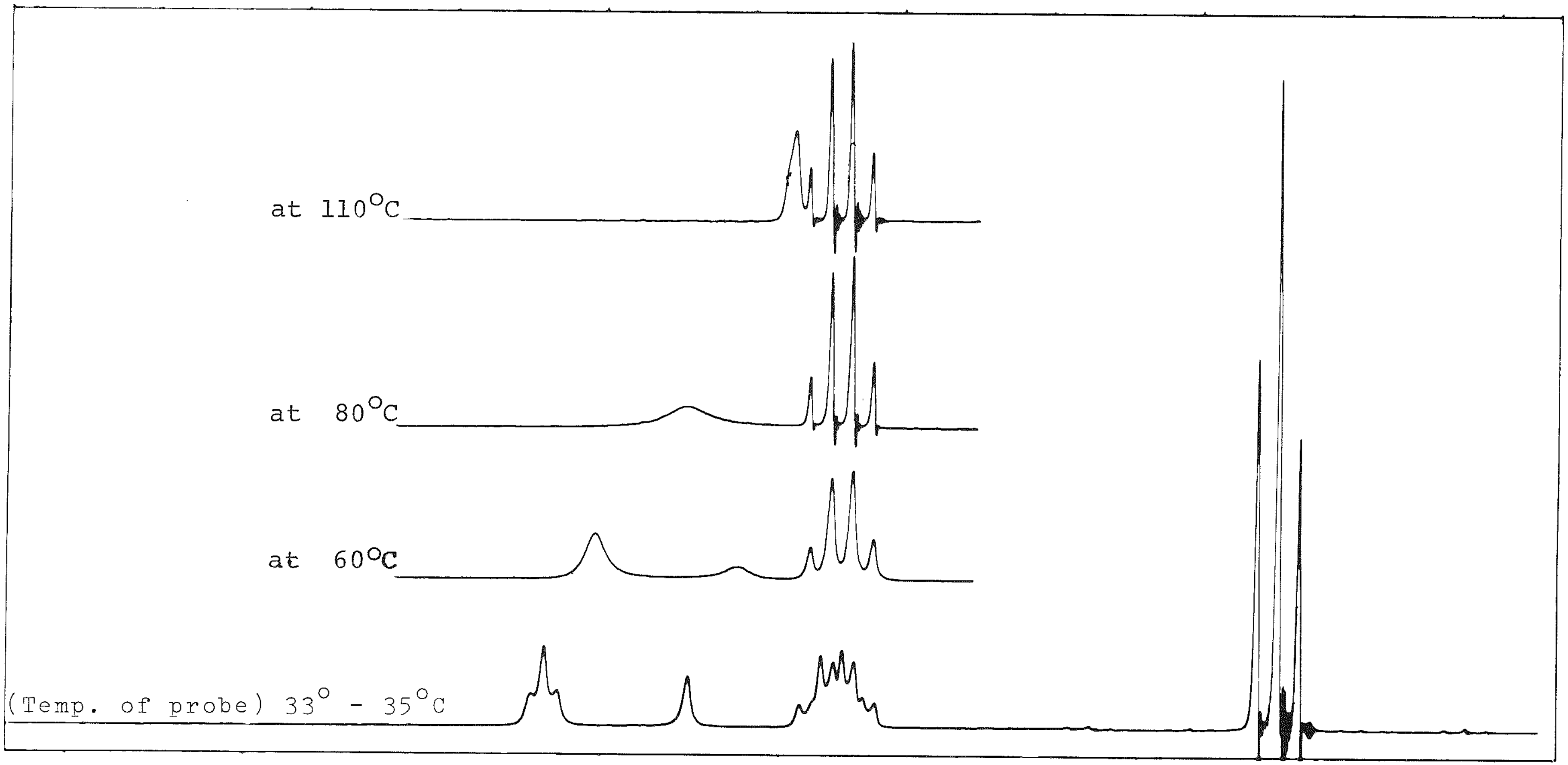
The upper spectrum of Figure 25 illustrates intermolecular exchange and two effects that it produces. The full scan shows the spectrum of 95% alcohol with 5% H_2O at room temperature. The methylene peak of the ethanol appears as an eight-line pattern due to a coupling of about 7 Hz to the CH_3 protons and a doubling of the resulting quartet with a $J_{CH_2\text{-}OH}$ of approximately 5 Hz.

At a temperature of 60°C, the rate at which OH protons exchange between H_2O and C_2H_5OH has increased to the point where:

a. The 5 Hz coupling has been averaged out, since a departing hydroxyl proton may have either parallel or anti-parallel spin and be replaced by one with opposite orientation in a random way.

b. The OH resonance in both H_2O and ethanol is broadening.

FIG. 25



At still higher temperatures the OH resonances merge together and continue to get sharper as more complete averaging takes place.

It will be noted that the center of gravity of the hydroxyl resonances moves to the right with increasing temperature. This reflects a gradual breakdown in the hydrogen-bonding type association in the solution.

F. Summary

We have seen that the information from high resolution NMR spectra resides in the following parameters which we can measure:

a. Chemical shifts. Indicate type of functional groups present.

b. Spin-spin coupling constants. Indicate which functional groups are neighbors, number of neighboring protons, and angular configurations of bonds.

c. Integrated intensities. Measure relative concentrations of protons in pure samples or mixtures.

d. Line widths and shifts as function of parameters like temperature or concentration. Indicate dynamic processes occurring in sample and allow measurement of equilibrium constants, barriers to rotation, exchange rates, etc.

BASIC NMR INSTRUMENTATION. I.

1. Is NMR Spectroscopy completely different from other well known forms of spectroscopy?

Many people have thought of NMR Spectroscopy as a mysterious technique only suited to the dark basements of University Physics Departments. However true this might have been during its infancy, NMR has now become a well behaved member of the family of spectroscopic instruments, which include IR, Raman, and UV Spectrophotometers. All of these instruments have certain elements in common. These include the following:

a) A source of electromagnetic radiation of suitable wavelength.
b) A means of scanning the wavelength over a selected range.
c) A cell, transparent to the radiation, which contains the sample which absorbs certain wavelengths.
d) A detector which measures the radiation passing through the cell, and thus the amount absorbed.
e) An amplifier to drive the display system.
f) A display which is usually a graphic recorder which plots absorption of energy as a function of wavelength.

Figure 26 shows the elements of a typical optical spectrophotometer. The frequency of the radiation is of the order of 10^{14} Hz. It is usually generated in an incoherent manner by a lamp or filament of some kind. Thermal detectors are usually used.

By way of comparison, Figure 27 shows the elements of a typical NMR spectrometer. The main differences are the following:

a) The frequency is of the order of 10^8 Hz. Therefore the wavelength is approximately a million times longer than optical wavelengths. It is generated in a coherent manner by electronic oscillators.

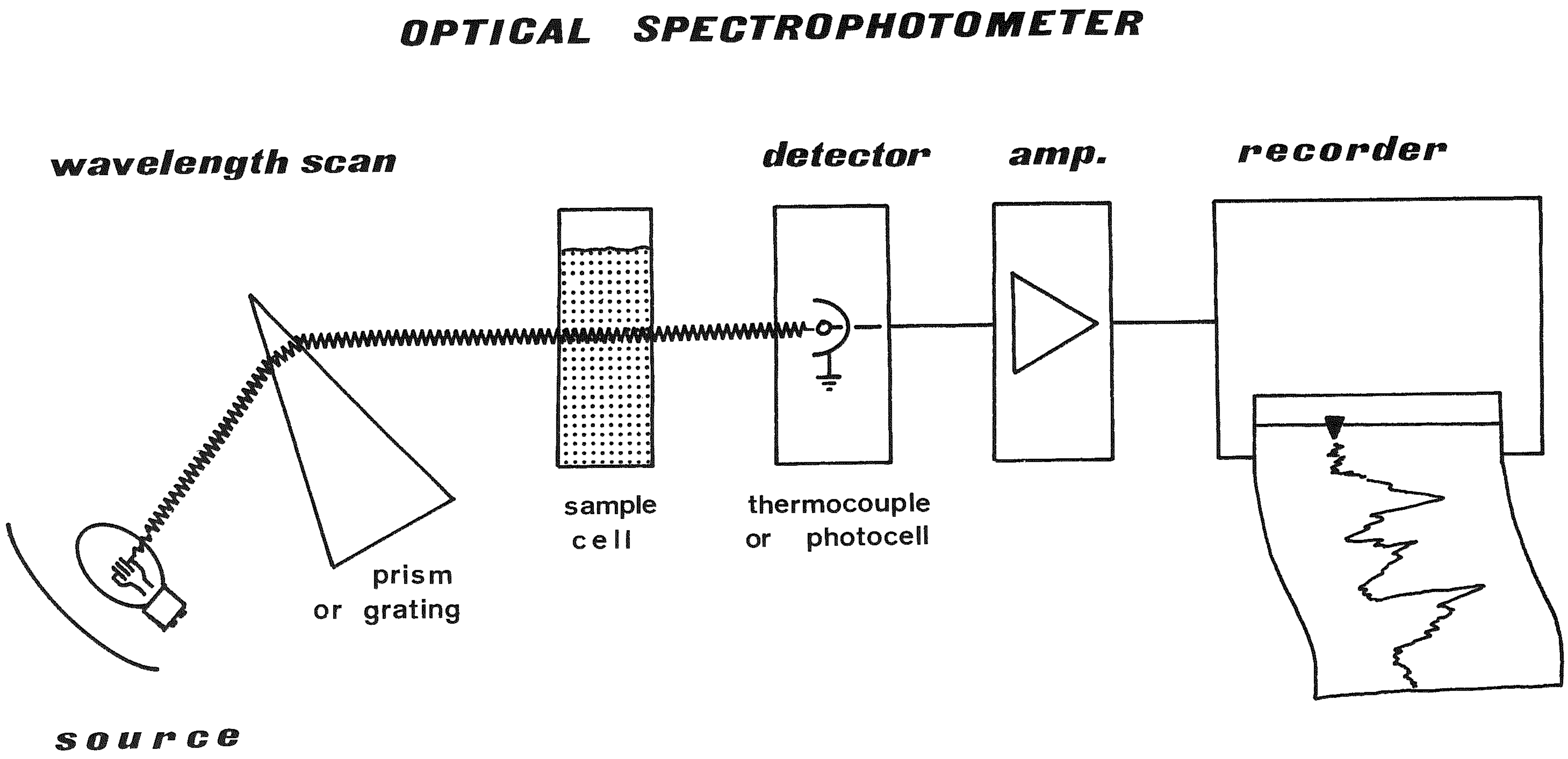

OPTICAL SPECTROPHOTOMETER
wavelength scan
detector
amp.
recorder
sample cell
thermocouple or photocell
prism or grating
source

FIG. 26

NMR SPECTROMETER

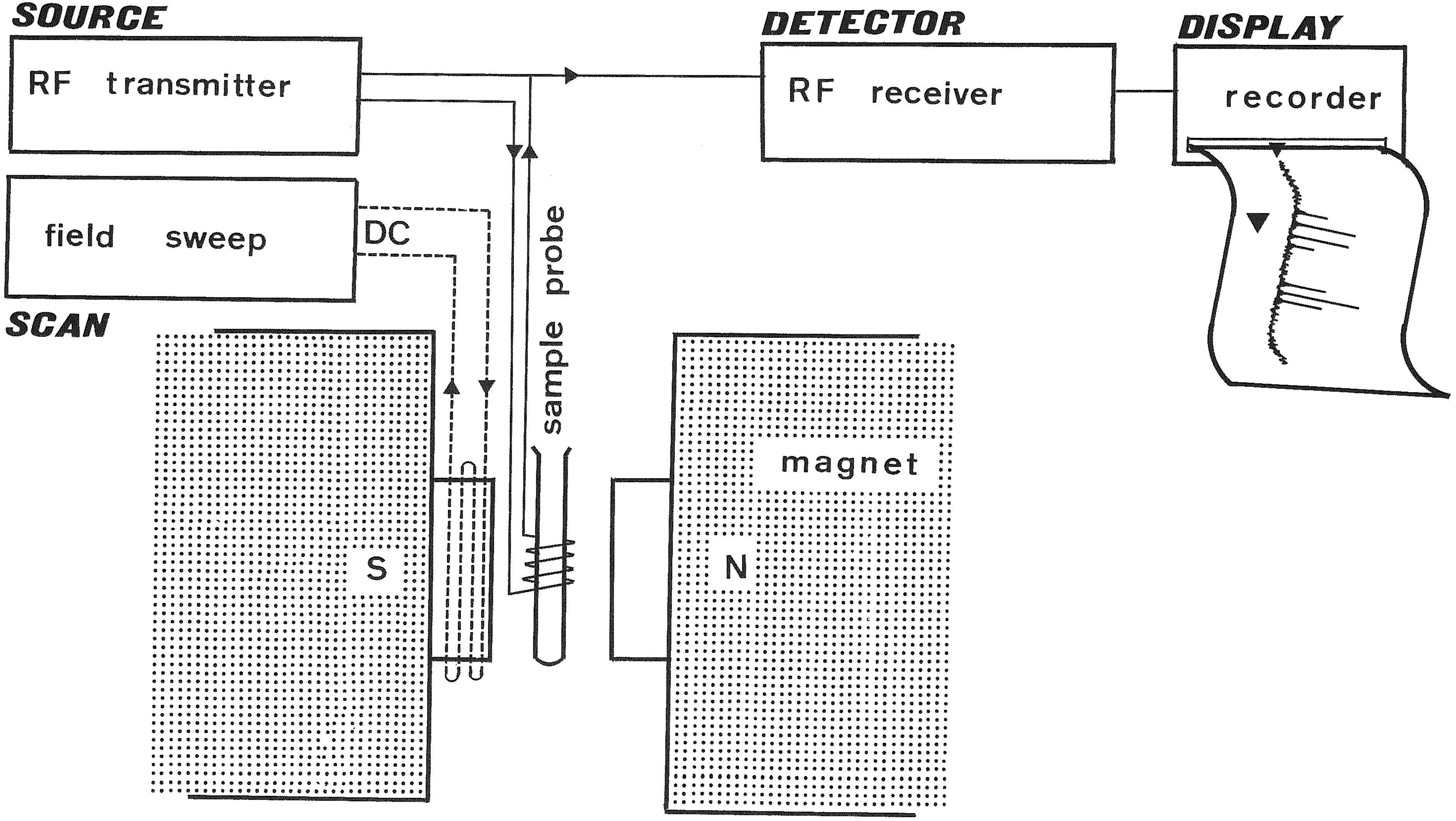


FIG. 27

b) The detector is coherent, tuned to the same frequency as the source.

c) The scan of wavelength can be accomplished either by varying a frequency, again coherently, or by varying the strength of the magnetic field which we have seen is proportional to frequency by the relation

$$\nu(\text{fre.}) = \frac{2\,\mu}{h} H$$

2. What is the most striking difference in NMR from other spectroscopic techniques?

We have seen that the energy levels in NMR depend for their existence upon the presence of a strong magnetic field. In optical spectroscopy the levels studied are intrinsic properties of the molecules resulting from their electronic, vibrational, and rotational motions. Thus, NMR instruments require an additional element which can be large, heavy, complex, and expensive, namely, the magnet. A great deal of applied research has been devoted to developing magnets of sufficient strength which are smaller, lighter, simpler, and cheaper.

3. What are the objectives in the design of an NMR Spectrometer system?

Generally, the major objective in designing an instrument is to achieve the highest performance possible consistent with the marketing objectives of cost, size, complexity, and style. Secondary objectives involve ease of operation, reliability, and ease of maintenance and servicing. Finally, we shall see that since a great variety of NMR experiments are possible, versatility has high value and as far as possible instruments should be designed to be compatible with the later addition of known techniques as accessories, and with as modular a concept as possible to accommodate new developments.

Because in many ways NMR is a marginal technique, i.e., the levels of performance at which useful information is obtained are not too far from the best that can be done, trade-offs of performance against other objectives cannot be carried too far. However, except for the state-of-the-art research instruments, the designer is usually forced into a performance vs. marketing objective trade-off.

4. How is NMR Spectrometer performance characterized?

The parameters which are of paramount importance are:

a) Resolution, measured as line width at half maximum.
b) Sensitivity, measured as signal-to-noise ratio, S/N, for a standard sample.
c) Short term stability, or drift during time required to scan a spectral line.
d) Long term stability, measured as drift in the ratio of field strength to frequency over some specified period of time.
e) Resolution stability, measured as the time required for resolution to degrade by a specified amount.

5. What instrumentation is used to detect the absorption of radio-frequency energy by the sample?

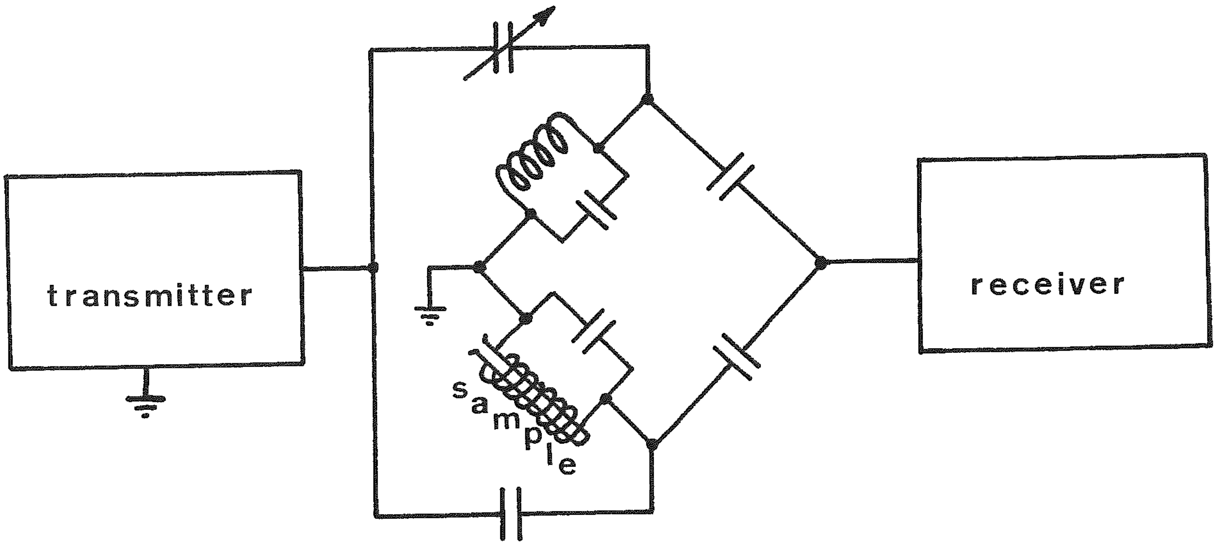
Electromagnetic radiation in the radio-frequency range can be generated coherently by an electronic oscillator and applied uniformly over the volume of the sample by means of a coil of wire wound around the sample called the <u>transmitter coil</u>. This coil must have its axis at right angles to the applied field H_o. Unlike other forms of spectroscopy, NMR absorption takes place coherently, or in a cooperative manner, since all the nuclei are excited in phase. We shall be concerned only with the excess population in the lower energy state, i.e., 10 nuclei out of every 2,000,000, which we shall regard as giving rise to a small macroscopic spin angular momentum, as if the sample could be represented by a small spinning magnet.

Two basic approaches to detecting the absorption of r.f. energy by the sample have been developed, called the crossed-coil and the single-coil techniques. Further variations of the single-coil approach include unbalanced direct observation, and balanced-bridge circuits.

a) Crossed-coil. A second coil of wire, mutually perpendicular to H_o and the transmitter coil, called the receiver-coil, is connected to a sensitive receiver tuned to the transmitter frequency. Direct coupling between the coils is minimized by the orthogonality of the coils, and can be made to pass through zero by balancing paddles located near the coils. With a sample in the coil, signal voltages appear across the receiver coil when the transmitter coil is excited with r.f. at the frequency

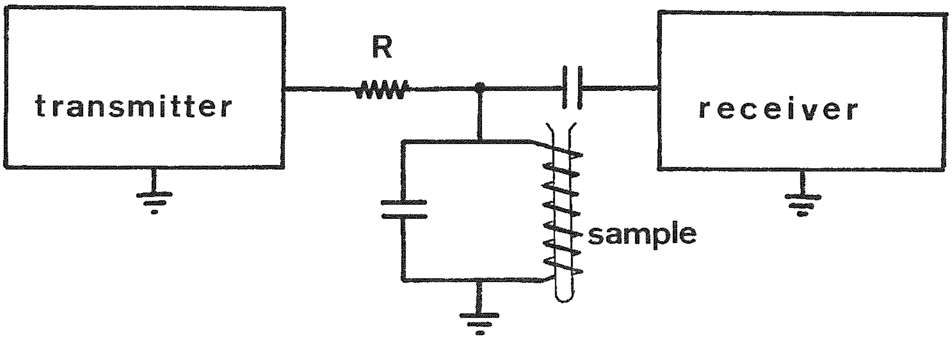
$$\nu = \frac{2\mu H}{h} .$$

b) Balanced-bridge. Another type of balanced circuit is shown below.



The presence of a sample in one arm unbalances the bridge when $\nu = \frac{2\mu H}{h}$. This circuit and the crossed coil system are useful when very weak NMR signals ($<10^{-9}$ volts) are detected in the presence of high transmitter power. It is then possible to amplify the r.f. signals a large amount before detecting them without overloading the detector. However, adjustment of balance controls is necessary when changingsamples which requires an extra operation. The bridge circuit is electrically balanced while the crossed coil is geometrically balanced to first order and trimmed up electrically, which makes it somewhat more stable at high power levels. Bridge circuits are used in some commercial high resolution NMR instruments.

c) Unbalanced direct-observation. A simpler circuit can be employed if low transmitter power levels are used, which is the case in high resolution NMR spectroscopy. One type is shown below.

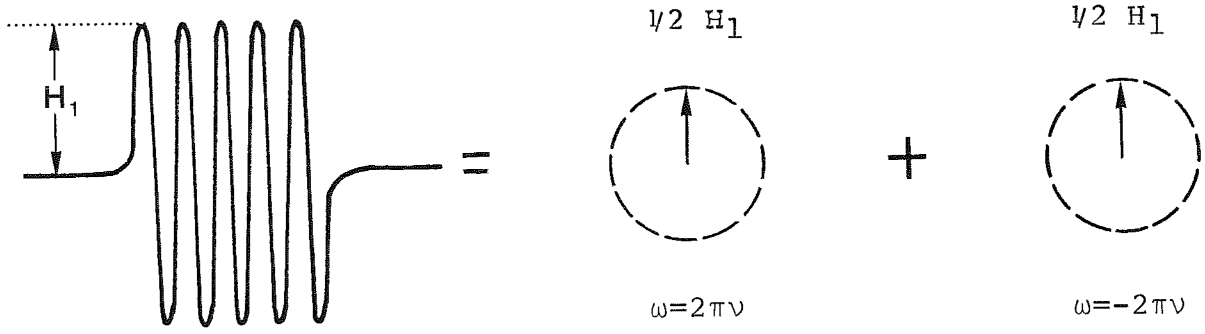


When $\nu = \frac{2\mu H}{h}$, the sample absorbs energy and the voltage across the coil drops. No balance controls are necessary, and the mechanical construction is simpler. Variations of this circuit are used in the Varian EM-300, EM-360, and T-60.

6. What happens to the nuclei when the transmitter is turned on and the field scanned through the absorption peak?

At equilibrium the macroscopic magnetic moment of the sample points parallel to H_o, ie., the nuclei polarize and line up with the field. If an oscillating magnetic field of strength H_1 is applied along a perpendicular axis by a transmitter coil, the instantaneous value of the field experienced by the nuclei is no longer H_o, but the vector sum of H_o and H_1. Since H_o is 14096 gauss, and H_1 may be a fraction of a milligauss, the perturbation is a very small one. Nevertheless, the nuclear magnetic moment, M_o, is out of equilibrium and experiences a small torque tending to align it in the new direction, which tilts it out of the YZ plane slightly. But, since there is angular momentum associated with the nuclei, the motion is gyroscopic, i.e., at right angles to the force. This leads to a <u>precessional</u> motion whose angular velocity $\omega = 2\pi\nu$ is determined by $\nu = \frac{2\mu}{h}H_o$, which is the same frequency as that which corresponds to the separation of the parallel and anti-parallel energy levels.

Since H_1 oscillates at a radio-frequency, its amplitude reverses twice during each cycle. Mathematically, H_1 can be regarded as two superimposed counter-rotating magnetic fields at the frequency ν, each with 1/2 the amplitude of H_1.



One component rotates in the same direction as the precession of the nuclear magnetic moment. The other rotates in the opposite direction and can be disregarded.

When the frequency of H_1 is exactly equal to the precession frequency of the nuclear magnetic moment, the nuclear moment is in resonance with the torque exerted by the field H_1. That is, as the nuclear moment precesses, the rotating component of H_1 rotates in step with it and there is a constant force tilting the nuclei away from the Z-axis. The nuclear moment spirals around H_o until is is precessing with a component in the X-Y plane. M_o comes to a dynamic equilibrium due to the thermal relaxation time, T_1, which acts as a restoring force to align M_o with H_o. At the point, the strength of the signal is determined by the component of M_o which rotates in the X-Y plane and whose flux custs the receiver coil on the Y-axis, generating an r.f. voltage. The magnitude of this voltage as a function of field strength is given by the expression

see FIG 28

$$u + iv$$

where u is the component of the rotating moment on the XY plane which is in phase with the driving r.f. voltage, and v is the component which is 90° out-of-phase with the driving r.f. voltage.

By adjusting the phase of the voltage coupled directly from transmitter to receiver coil in a crossed coil system, we can detect either u, v, or a mixture of the two. The component u is called the dispersion mode and is an asymmetric signal with the character shown in Figure 29. At large values of H_o-H the signal falls off as the inverse of the distance from the center of the resonance. The component v is called the absorption mode and has the character that at large values of H_o-H it falls off as the inverse square of the distance from the center of the resonance, and is symmetric about it.

EFFECT OF RADIO FREQUENCY FIELD H_1

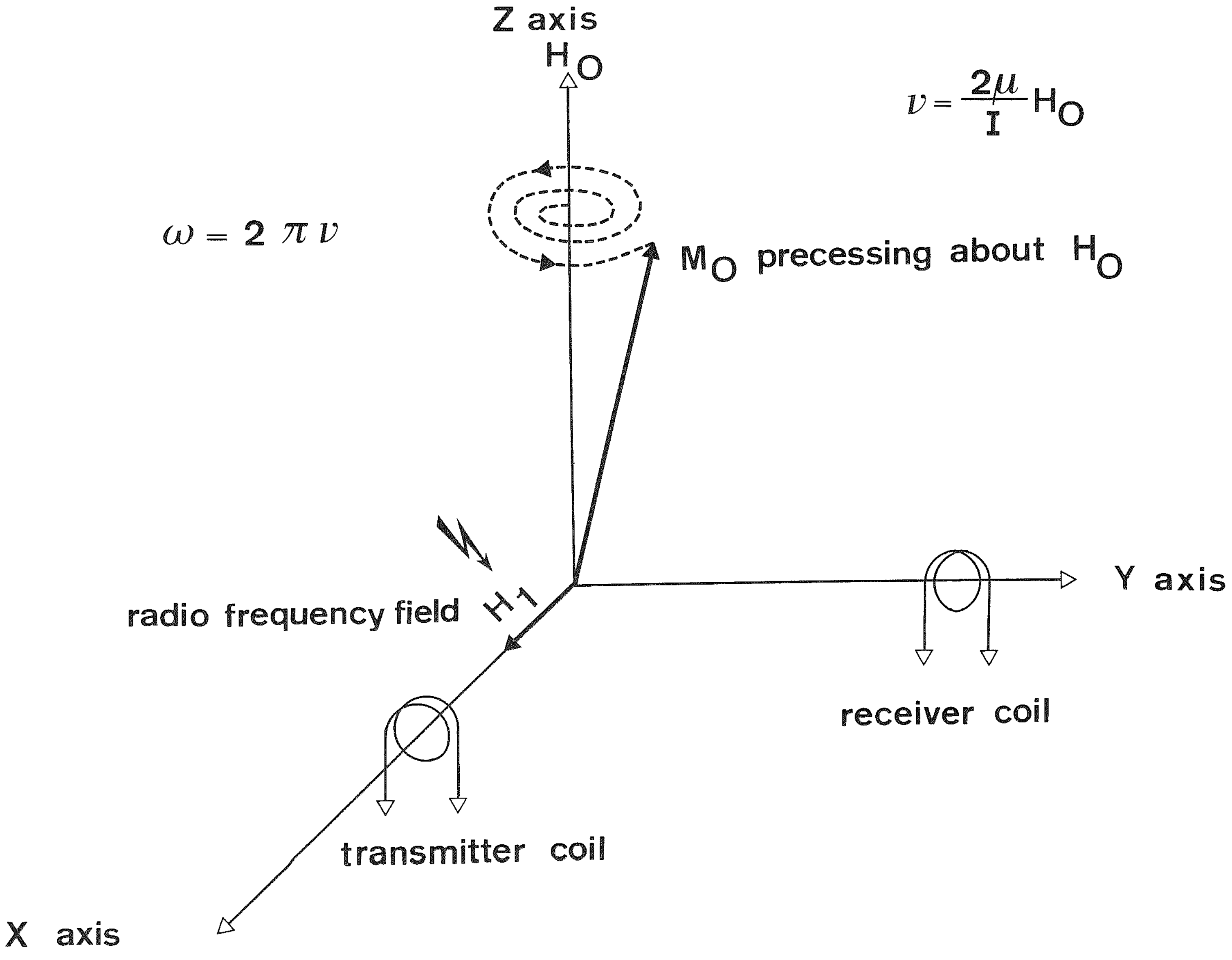
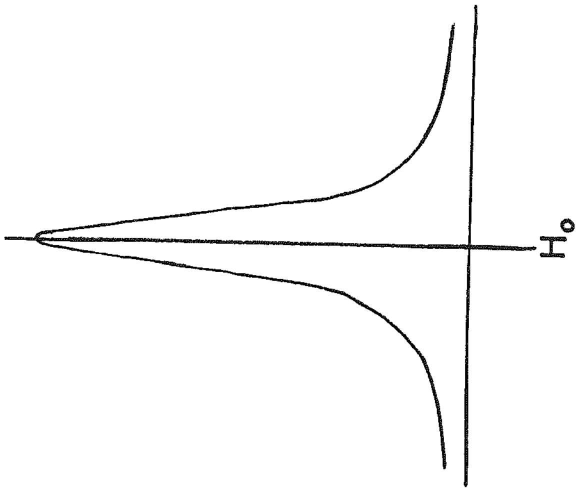


FIG. 28

FIG. 29

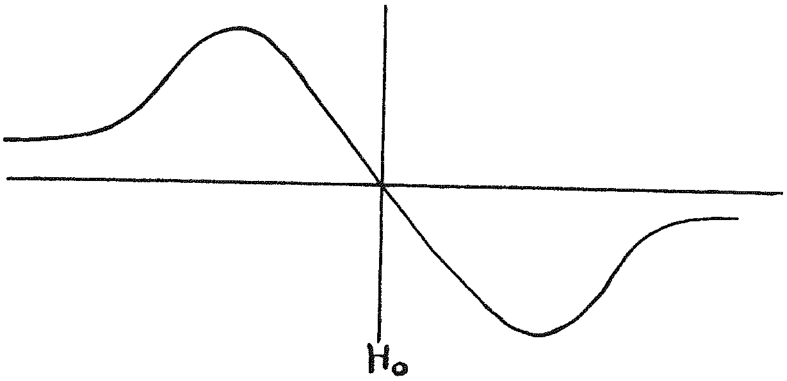
ABSORPTION MODE

$$V = \frac{M_0 H_1 \gamma T_2}{1 + (\gamma H_1)^2 T_1 T_2 + [\gamma T_2 (H_0 - H)]^2}$$



DISPERSION MODE

$$u = \frac{M_0 H_1 T_2^2 \gamma (H_0 - H)}{1 + (\gamma H_1)^2 T_1 T_2 + [\gamma T_2 (H_0 - H)]^2}$$



A single coil spectrometer without additional refinements can detect only the absorption mode signal, since the phase of the driving r.f. voltage dominates the detection of the signals from the precessing nuclear magnetic moment.

7. How is the instrument normally adjusted for high resolution NMR operation.

The absorption mode signals are much sharper than the dispersion mode, and interfere much less with one another in a complex spectrum. We shall also see that the integrated area under the absorption signals is significant, while for the dispersion signals it is zero. For these reasons, the phase control of the spectrometer is nearly always adjusted to produce an absorption spectrum with a minimum admixture of dispersion.

8. Why does a single-coil instrument have a phase control?

Scanning NMR spectrometers of the type used for analytical work cannot be scanned very rapidly through the spectrum without distortion of the NMR signals and loss of sensitivity, since the bandwidth of the detector must pass the frequency components of the signal. Fast sweeps require a larger bandwidth and permit more noise to appear in the spectrum. The slow sweeps necessary for clearly resolved spectra result in low frequency components in the envelope of the absorption lines after passing through the r.f. detector. These cannot be distinguished from changes in r.f. leveldue to instabilities in transmitter level or coil tuning and balance. With very weak signals, these problems become insurmountable. For this reason, NMR spectrometers are provided with auxiliary coils on the magnet or in the air gap to superimpose a small audio-frequency modulation on the field H_0. Since the magnetic field and frequency are related by $\nu = \frac{2\mu}{h} H$, when nuclei are present they are affected by the field modulation just as if the transmitter were frequency modulated.

This has the effect of having a carrier at the r.f. frequency (for example, 60.000 MHz) and side-bands located at $\pm \nu_{mod}$ on either side. A low index of modulation is chosen in the range 5-10 percent so that only one set of sidebands is normally present. The spectrometer therefore acts as if the r.f. frequency of the transmitter is either ν, $\nu - \nu_{mod}$, or $\nu + \nu_{mod}$. A typical value for ν_{mod} is 5-10 kilohertz which is considerably more than the total extent of the high resolution NMR spectrum in the case of proton NMR.

The absorption of r.f. energy by the precessing nuclear magnetic moment of the sample now takes place at a slightly different applied field, $H_o \pm \frac{h\nu_{mod}}{2\mu}$, and has an audio-frequency sinusoidal variation superimposed upon the r.f. voltage induced in the receiver coil. This has the advantage that the amount of audio-frequency modulated r.f. voltage present in the coil is proportional only to the number of magnetic nuclei present and not to any variations in the r.f. source or coupling, assuming a sufficiently mechanically rigid coil assembly to eliminate mechanical coupling of r.f. and audio modulation. A further advantage is that part of the signal amplification takes place at radio frequency and part at audio frequency, which avoids overloading and extends the dynamic range of the amplifier.

The u-mode and v-mode signals are affected differently by the modulation, so that when modulation is applied to a single-coil system which would otherwise detect only the v-mode, the character of the detected signal depends upon the adjustment of the audio-phase in the phase sensitive detector. As in a crossed-coil or bridge system, the dispersion mode is usually minimized by the phasing adjustment. (See Figure 30.)

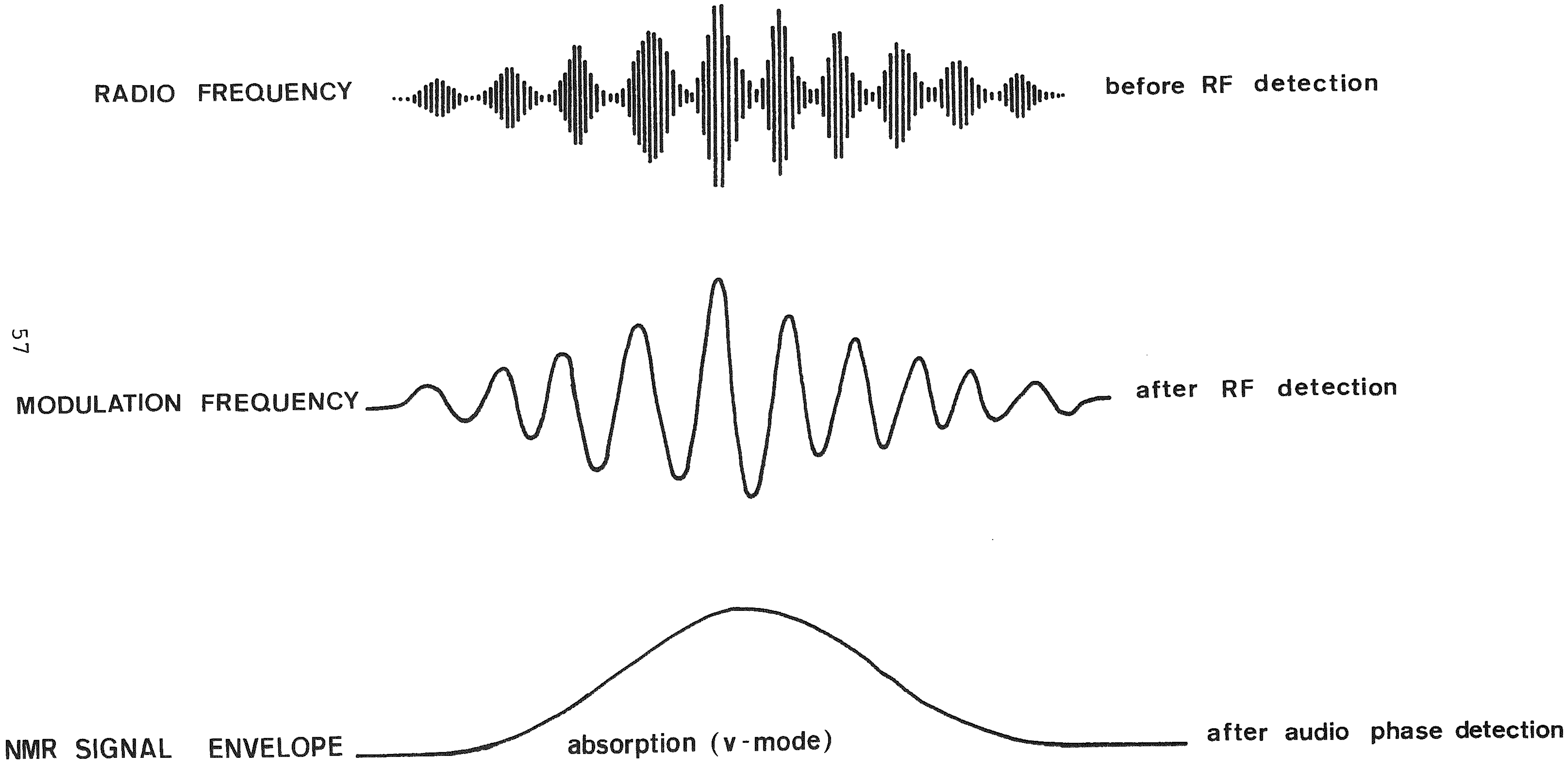
9. How does the NMR signal depend upon the transmitter power level control which determines the amplitude of H_1?

We have seen that

$$v = \frac{M_o H_1 \gamma T_2}{1 + (\gamma H_1)^2 T_1 T_2 + [\gamma T_2 (H_o - H)]^2}$$

FIG. 30

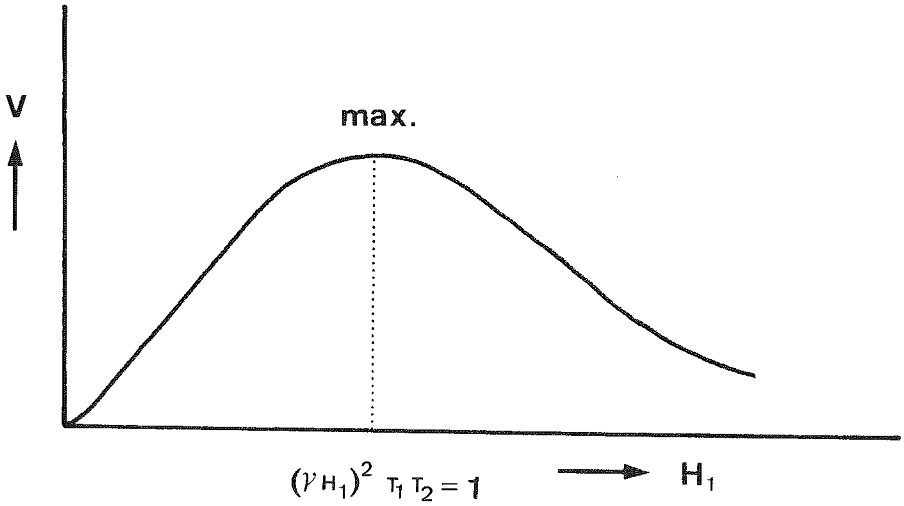
DETECTION OF NMR SIGNALS



T_1 and T_2 are the thermal relaxation and the transverse (sometimes called spin-spin) relaxation times and are properties of the sample The constant γ is called the magnetogyric ratio and is $\frac{4\pi\mu}{h}$ for a nucleus whose spin = 1/2. M_o is a constant determined by the concentration of the sample -- the more nuclei/cc the greater the net macroscopic magnetic moment. At the center of the absorption peak, the expression reduces to

$$V = \frac{(\text{const.})\ H_1}{1 + (\gamma H_1)^2 T_1 T_2} = \frac{C_1 H_1}{1 + C_2 H_1^2}$$

The dependence of signal strength on H_1 is shown in the following graph:



The signal increases linearly with H_1 at low power levels, then turns over and decreases as $1/H_1$ at high power levels. The maximum is rather broad and one can choose to work either at the maximum or a little below for weak samples. Strong samples should be studied on the linear part of the curve, since the width of the absorption peak at half maximum is

$$\Delta H = \frac{2}{\gamma T_2} \sqrt{1 + (\gamma H_1)^2 T_1 T_2}$$

and increases by $\sqrt{2}$ in going from low H_1 to $(\gamma H_1)^2 T_1 T_2 = 1$. Beyond this point ΔH continues to increase, eventually becoming linearly proportional to H_1.

The phenomenon of going through a maximum is called saturation. Figure 31 shows how it is related to the energy level diagram. The probabilities of a nucleus absorbing energy or being stimulated to emit energy are equal. Therefore, the energy absorbed is proportional to the excess population in the lower state. As the level of H_1 increases, the populations are equalized since nuclei in the upper state cannot be returned to the lower state by thermal relaxation processes as rapidly as it is depopulated.

10. Is it better to sweep the magnetic field strength or the frequency?

It is somewhat simpler to generate a linearly increasing current than a linearly increasing or decreasing frequency. Furthermore, frequency sweep results in a constantly changing phase shift over the spectrum which causes some difficulties. Therefore, for most work it appears to be preferable to sweep the magnetic field.

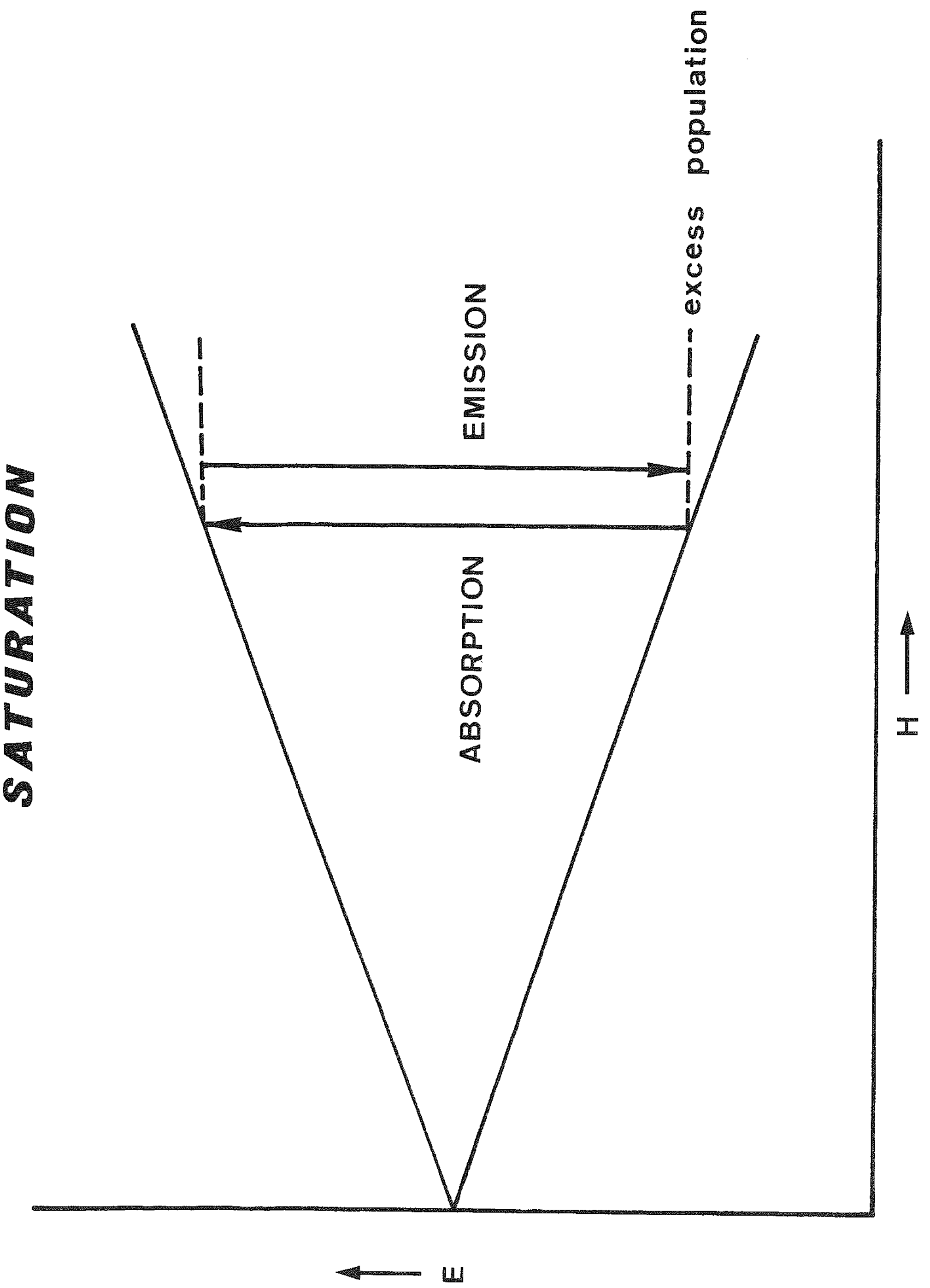
11. What effect does the sweep rate have on the appearance of the absorption lines.

The shape of the absorption mode signal given by

$$v = \frac{M_o H_1 \gamma T_2}{1 + (\gamma H_1)^2 T_1 T_2 + [\gamma T_2 (H_o - H)]^2}$$

is valid only for very slow sweeps, called slow-passage conditions where the nuclei remain in equilibrium. It is usually impractical

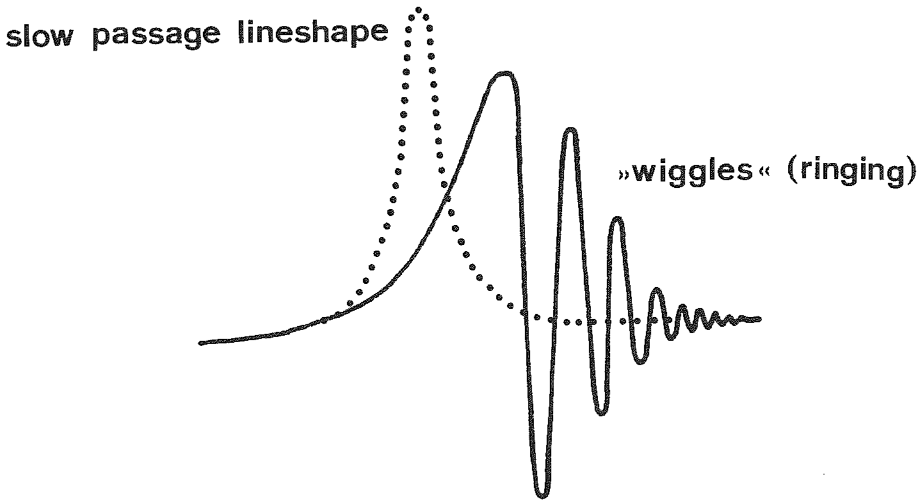
SATURATION



absorption mode signal reaches maximum strength when $(\gamma H_1)^2 T_1 T_2 = 1$

FIG. 31

to sweep so slowly; therefore, most spectra are run under conditions which do not permit the nuclei to stay in equilibrium with the static field H_o and the driving r.f. field, H_1. In particular, after passing through the resonance the nuclei continue to precess at the frequency $\nu = \frac{2\mu H}{h}$ where H is now larger than H_o. The precession dies out with the time constant characteristic of the natural relaxation processes in the sample which re-establish thermal equilibrium, or which cause the nuclei to lose phase-coherence with one another, i.e., T_1 and T_2. During this time a decaying r.f. voltage is induced in the receiver coil which beats with the frequency $\nu_o = \frac{2\mu H_o}{h}$ which is the nominal 60.000 MHz operating frequency of a spectrometer of the T-60 or EM-360 type. This beat frequency has been called "the wiggles", or "ringing", and has the following appearance



The line width is increased, the line is shifted in the direction of the sweep, and is followed by an oscillatory decay.

These effects are unavoidable with very sharp lines but can be minimized by very slow scan rates. Slow scan rates place greater demands for stability on the spectrometer.

12. What problems are caused by "ringing".

a) In closely spaced multiplets the ringing from one line may distort the next line or even eliminate it.

b) The shift in the peak can lead to inaccuracies of chemical shift measurement. However, if the line width of the line being measured is similar to that of the TMS reference, the errors cancel.

13. What considerations are involved in the design of magnets for high resolution NMR.

Both permanent (alnico) and electromagnets are used for high resolution NMR. Both types can be made to meet the following requirements:

a) High enough field strength (14,100 gauss) to give good sensitivity and enough separation of typical chemical functional groups.

b) Sufficient resolution to separate lines in closely spaced spin-spin multiplets.

c) Short term and long term field strength stability.

d) Stability of magnet homogeneity - ability to maintain good resolution.

e) Reasonable size, weight, and cost.

f) Reasonable reliability.

14. What are the advantages of each type of magnet?

A. Electromagnets

a) Smaller size for same field strength, especially at high fields.

b) Field can be varied. Actually not very practical in high resolution work.

c) Much less sensitive to temperature changes, thus more easily adapted to variable temperature accessories Needs no elaborate thermostat system.

B. Permanent Magnets

a) Requires no power supply. Consumes no electric power or cooling water. Needs no dynamic stabilization.

b) Quiet. Generates no heat, noise, water leaks, etc.

c) Less adjustment required. Since permanent magnet must be protected against change of temperature, it normally comes to an equilibrium which remains stable over long periods of time.

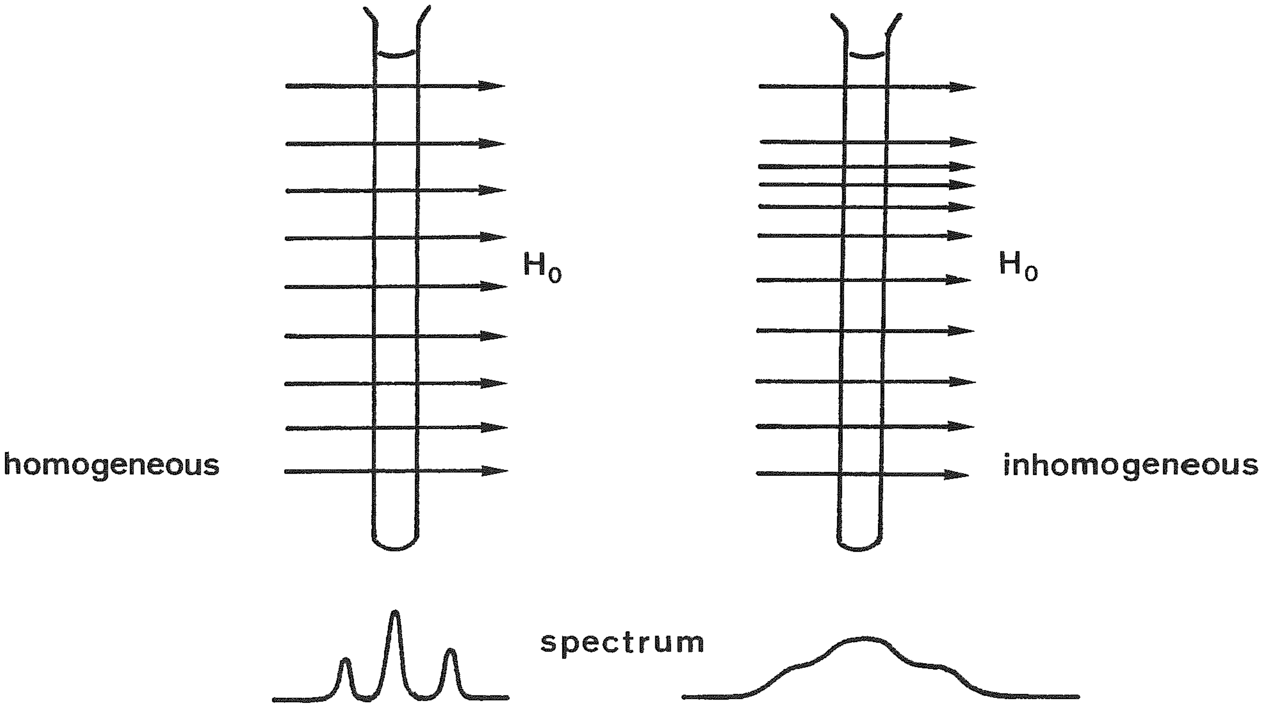
d) Only momentarily affected by power failure.

15. Which is better for analytical NMR work?

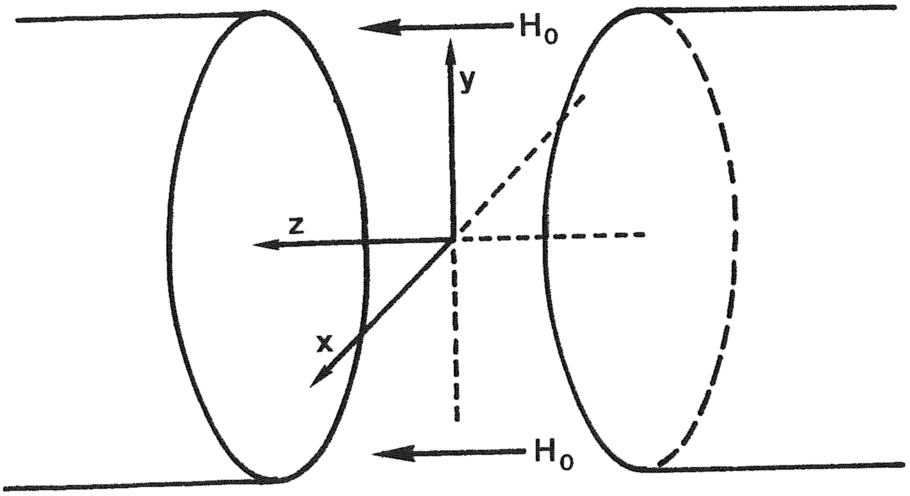
Although both types are commercially available, the majority are of the permanent magnet type. The advantages of the permanent magnet are much more relevant to the applications and the operator needs than those of the electromagnet.

16. What considerations are involved in obtaining and maintaining a homogeneous field over the sample volume.

If the nuclei in different parts of the sample tube experience slightly different field strengths, the signal from those nuclei will be smeared out into a broadened line, overlapping and merging with other lines due to chemical shifts and spin-spin coupling, with the result that the desired information is obscured or lost.



The problem of specifying magnet homogeneity is simplified by defining a set of coordinate axes whose center is midway between the pole faces at the center of the cylindrically symmetric air gap.



The axis parallel to the field is designated the Z-axis, while the vertical axis is called the Y-axis and the horizontal axis the X-axis.

The transmitter coil in crossed-coil systems will apply a field along the X-axis and the receiver coil will be wrapped around the Y-axis.

The following factors affect the homogeneity.

a) Face-to-gap ratio.

b) Flatness of pole cap faces.

c) Homogeneity of magnetic materials in the pole caps.

d) Design of the pole cap shape.

e) Magnetic susceptibility of materials placed within the gap, including the sample cell and the sample itself.

f) Design and adjustment of current carrying correcting coils used to offset inhomogeneities remaining after factors a-e are optimized.

g) Rotation of the sample.

17. How do these factors affect homogeneity?

a) Face-to-gap ratio. Narrow gaps are advantageous for maximizing field and give better first order homogeneity by minimizing flux leakage. They put greater demands on flatness and magnetic homogeneity of pole caps since the surfaces are closer to the sample. The accessibility and sample size become more limited, so a trade-off is necessary.

b) Pole cap surfaces. Grinding and lapping to optical flatness gives best performance and least test time, but must be considered against cost of simple machining.

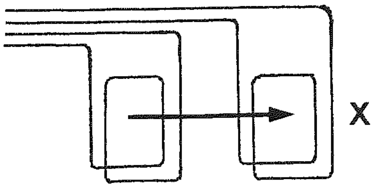
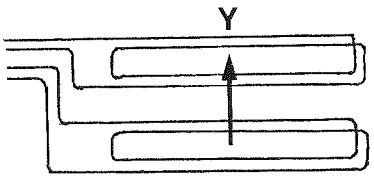
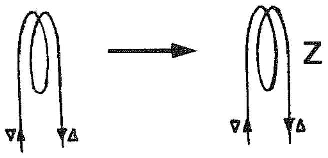
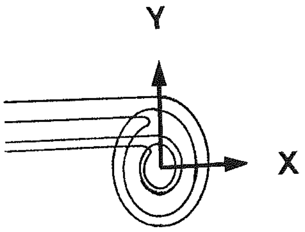
c) Pole cap materials. Special steels give best results but must be considered in terms of cost.

d) Special mathematically designed shapes give better results at high field strengths where saturation of iron affects flux paths. Cost of machining rises sharply.

e) Special high purity metals, plastics, cements, and wire are needed in probe construction. Frequent testing of magnetic contamination required. Assembly must be performed in clean rooms by skilled workers. There is a high reject rate and high inspection costs.

Even a well designed spectrometer can be defeated by use of uneven sample tubes or by magnetic contaminants in the sample. Most common are small nickel chips from scraping fritted glass filters with nickel spatulas in the process of synthesizing and purifying the chemical samples studied in NMR.

f) A set of current shims is normally used to correct the small inhomogeneities which remain. These inhomogeneities can be regarded as consisting of the following types:

Type	Description	Correcting Coils
X-gradient	Field higher in front or back	
Y-gradient	Field higher at top or bottom	
Z-gradient	Field higher on one side	
Y^2-curvature	Field higher in center or edges	
XY, XZ, Y^3, Y^4	More sharply varying fields or skewed fields	Special Shapes

Coils can be located either on magnet or probe. Stability of current supply and condition of potentiometers controlling shim currents is very important. Short circuits or loose connections in these circuits can cause trouble which is difficult to find.

The currents in the shim coils are normally set at the factory in permanent magnets. This is not possible in electromagnets which present a new shimming problem each time they are energized. After the permanent magnet settles in after installation the shim currents are readjusted. After this procedure, only the Y-gradient and Y^2-curvature should need frequent adjustment.

Changing from one probe to another may require extensive reshimming. This would occur when:

a) Changing from room temperature probe to variable temperature probe.

b) Changing T-60 to ^{31}P or ^{11}B from ^{1}H to ^{19}F.

Sudden large changes in shim current requirements may indicate:

a) Loss of temperature control (accompanied by drift)

b) Dirt in probe

c) Failure in shim coil circuitry

g) Sample Spinning. Advantage is taken of the fact that the nuclear relaxation times are long -- of the order of seconds -- so that the observations reflect the average nuclear environment over this time scale. Therefore, rapid rotation of the tube containing the sample averages out the field gradients along the two axes perpendicular to the axis of rotation. Nuclei in high and low fields exchange places rapidly and the average field strength experienced by both becomes the same.

Sample spinning is accomplished by a small air driven turbine driving a plastic rotor pressed onto the sample tube. Precision tubes are used to give smooth operation. Rates of rotation normally must be greater than 30 r.p.s. and less than 50 r.p.s. The rate is measured by a photosensitive tachometer in the T-60, and revolutions per second read directly on a panel meter. On other spectrometers, the sample rotation rate is usually determined from indications in the NMR spectrum.

One detrimental effect of spinning the sample is that rotation in a magnetic field gradient results in a weak modulation of the field strength at the rotation frequency and leads to the appearance of <u>spinning side bands</u> on either side of each spectral line a distance ν_{spin} away from the line. The amplitude of these side bands depends upon the magnitude of the field gradient and the sample rotation rate. The higher the rotation rate, the smaller the index of modulation and the smaller the side bands. Above 50 r.p.s., however, a vortex tends to form which causes a column of air to penetrate the active volume of the sample, and the resulting discontinuity in the magnetic susceptibility distorts the field and deteriorates the resolution.

The side bands surrounding a strong line do directly indicate the spinning rate and can be used to adjust the spin rate. If larger than 0.5-1.0 percent of the parent peak, they can complicate interpretation of spectra and introduce serious errors in quantitative measurements. Some analyses require that the spinning be stopped completely, but usually careful adjustment of X and Y gradients and spinning sufficiently rapidly suffice to give satisfactory spectra.

18. How are high resolution NMR Spectra displayed and recorded.

There are two common approaches to display of spectra. These are:

a) Oscilloscope
b) Graphic recorders
    i) strip chart
    ii) X-Y type

The use of an oscilloscope is necessary for making resolution adjustments in some commercial spectrometers. It can also be convenient for quickly surveying the spectral region for peaks, for observing changes in the spectrum over short time periods, and for checking circuit performance and wave forms.

Varian Associates has taken the position with the EM-360 and T-60 that an oscilloscope is not necessary and is therefore an optional accessory. This is made possible by a technique to be described later of optimizing resolution by maximizing recorder pen deflection.

Since modern spectrometers all utilize precalibrated charts, it is necessary to register the TMS reference on the zero point of the chart. With strip chart recorders, this is done by moving the chart zero point under the pen and then engaging the chart drive when the fine field strength adjustment gives a pen deflection from the TMS resonance. Flat bed X-Y recorders such as those in the EM-360 and T-60 accept a pre-printed chart with 0.0 ppm on the right. The pen is moved to this location and the zeroing control adjusted until TMS deflects the pen.

The T-60 utilizes an 8-1/2 x 11" notebook-sized chart popular with bench chemists. Spectroscopists seem happy with the 11 x 17" chart size of the EM-360.

Most commercial instruments have the field scan geared to the chart drive or X-axis pen drive. The Y-axis displays the NMR absorption.

19. Summary

After installation and initial adjustment of an NMR Spectrometer, the operator will normally be concerned with the following operating procedures and controls. Parentheses indicate the operation is not necessary with every sample.

1. Check spinner speed.
(2.) Adjust spinner drive if necessary.
3. Move recorder arm to zero position.
(4.) Adjust SWEEP ZERO control for TMS pen deflection. Trim homogeneity for maximum pen deflection with Y-gradient control.
(5.) Select SWEEP SPEED.
(6.) Select SWEEP RANGE.
(7.) Select SWEEP OFFSET (end point of spectral scan).
(8.) Select r.f. power setting.
(9.) Find a strong sharp line, possibly TMS, and adjust r.f. PHASE for minimum dispersion.
10. Find largest peak. Scan through it at selected sweep rate and adjust SPECTRUM AMPLITUDE (signal amplification) to fill chart.
(11.) Set FILTER BANDWIDTH for trade-off between minimum noise level and distortion of the spectral peaks.
12. Move recorder to left side of chart. Lower pen.
13. Start spectral scan.

BASIC NMR INSTRUMENTATION. II

A. Integration

1. What considerations are important in measuring the integrals of the absorption peaks?

The integral (area) of the absorption peak under slow passage conditions is:

$$\int v\,dH = \frac{(\text{const.})\ M_0 H_1}{[1 + (\gamma H_1)^2 T_1 T_2]^{1/2}}$$

M_o, the nuclear moment is inversely proportional to temperature, and directly porportional to the concentration of nuclei, N.

It is necessary to integrate against time to take advantage of electronic integration techniques, although research NMR spectrometers with accessory digital computers can be programmed to do a digital sum giving $\int v\,dH$ directly.

$$\int v\,dt = \frac{(\text{const.})\ H_1 N}{T(dH/dt)[1 + (\gamma H_1)^2 T_1 T_2]^{1/2}}$$

where $\frac{dH}{dt}$ is the sweep rate of the spectrometer.

We can see that the integrals are dependent upon the relaxation times T_1 and T_2 and since these are often different for different nuclei in the same molecule, and vary over a wide range, we must find a way to avoid this dependence.

To a good approximation it can be shown that intermediate passage conditions can be set up rather easily to meet the following conditions:

$$\frac{4\pi\mu}{h} H_1^2 \ll \frac{dH}{dt}$$

That is, for a given sweep rate the r.f. power level has to be limited as shown above. If we leave the power level optimum for recording the V-mode absorption peaks, we must greatly increase the sweep speed; if we leave the sweep speed the same, we must reduce H_1.

A practical consideration involving the stability of the baseline results in a rapid sweep at higher power being preferable to a slow sweep at low power. This places fewer demands on the baseline stability (Integrator Balance) and also allows several integrals to be measured in the same time period and these can be analyzed statistically to give some confidence limits.

Under these conditions,

$$\int v dt = \frac{(\text{const.})\ N\ H_1}{T\ (dH/dt)} \left[1 - 1/2\ \gamma\ H_1^2 / \frac{dH}{dt} + \ldots\ldots \right]$$

The error thus can be kept to $1/2\ \gamma H_1^2/dH/dt$ even though relaxation time differences are neglected.

An example of the accuracy attainable in spite of neglect of relaxation time differences is shown in Figure 32. The agreement between the total hydrogen content of several substances and the NMR measurement of this quantity is very satisfactory.

Instrumentation for integration is very simple. A Miller-integrator type circuit is used as follows:

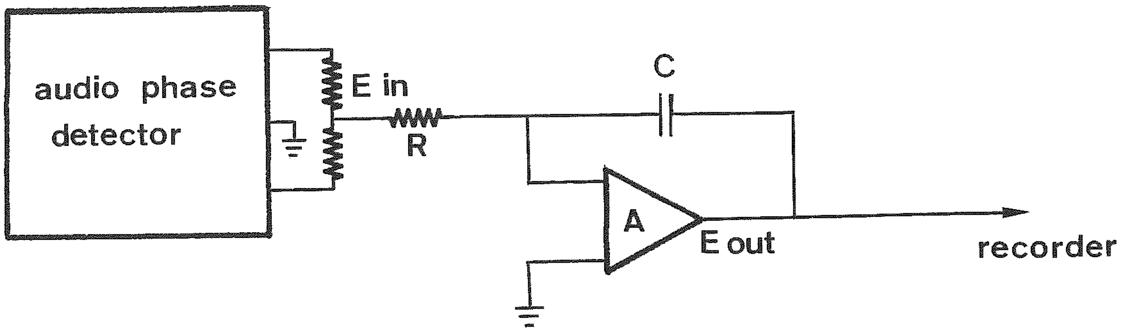


FIG. 32

TOTAL HYDROGEN ANALYSES BY NMR FOR COMPLEX ORGANIC MOLECULES

SAMPLE	WT.(mg)	% H by wt.	% H (NMR)[a]
$C_{10}H_8$ (A)	25.1	6.29	6.31 ± 0.03[b]
$C_{10}H_8$ (B)	25.3	6.29	6.28 ± 0.01
$C_{10}H_8$ (C)	25.3	6.29	6.28 ± 0.02
$C_{27}H_{44}O_3$	25.1	10.65	10.58 ± 0.05
$C_{15}H_{18}O_6$	25.2	6.26	6.27 ± 0.04
$C_{15}H_{20}O_6$	25.1	6.80	6.81 ± 0.03

a) Based on average integral of napthalene solutions

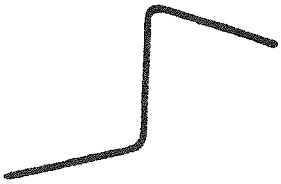
b) Standard deviation

A stable, high gain operational amplifier across a capacitor, C, effectively increases the value of C by the gain of the amplifier, making RC very long ($> 10^5$ seconds). The integral of the input voltage thus appears at the output, and the response is linear.

The value of the integral can be read from the record on the chart or measured with an auxiliary digital voltmeter. The latter has the advantage of 3-decimal place accuracy.

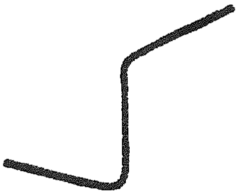
The integrator balance control must be set when the spectrometer is not detecting any signals. Usually, the recorder arm is moved to a region where there are no NMR signals.

The most important adjustment for integration is the phase control. This is set for minimum dispersion signal. The presence of a dispersion signal is denoted by asymmetry in the integral as shown below:

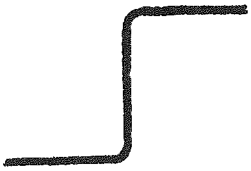


v-u mode

or



v + u mode



v-mode

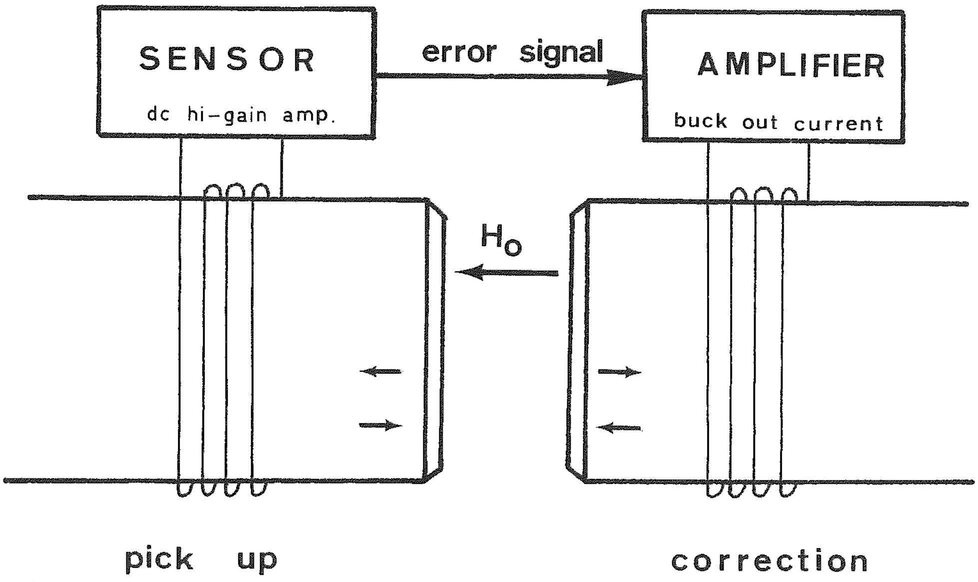
B. Magnetic field and Frequency Stabilization

2. What are the requirements on stability?

Since the NMR signals can be only a few tenths of a Hz in width at a frequency of 60.000 MHz, the true shape will be measured only if the stability is of the order of 1 part in 10^9 for the ratio of the field and frequency. Rather than control each separately to the requisite degree, it is nearly always preferable to measure the ratio, using an NMR signal, and by appropriate feedback to maintain a fixed value of that ratio.

3. Is preliminary stabilization needed?

In the case of electromagnets it frequently is desirable to stabilize against high frequency fluctuations of the field by a device called a flux stabilizer.



A number of problems connected with flux stabilizers exist. These include:

a. Cost
b. Complexity
c. Drift
d. Adjustment
e. Maintenance

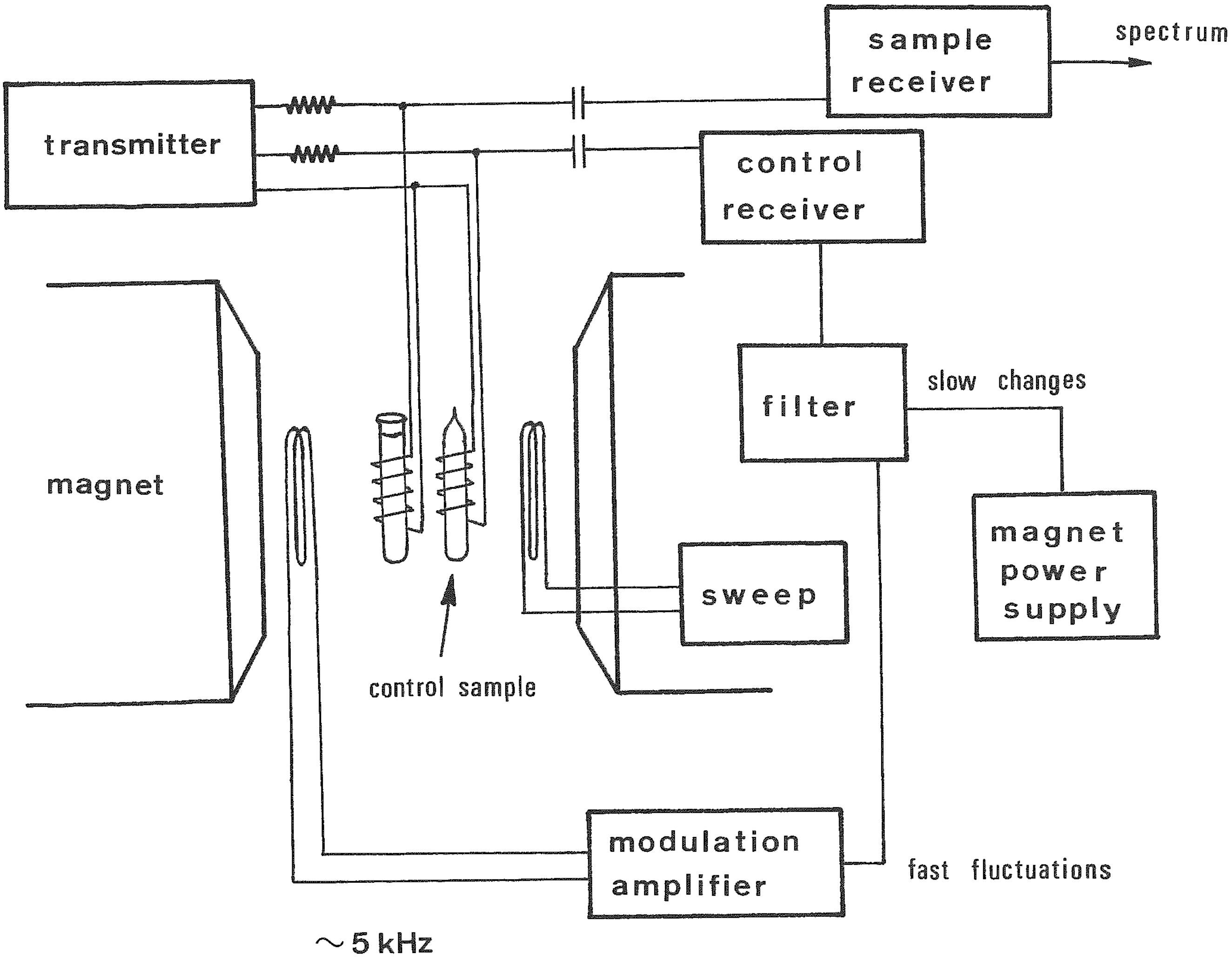
Permanent magnets have no need <u>either</u> for current regulators or flux stabilization. NMR field frequency control can be applied directly.

4. Is a flux stabilizer the only way to take out short term field fluctuations?

Because of the disadvantages listed above, the Varian A-60 was based on a new concept -- the Nuclear Sideband Oscillator frequently abbreviated NSBO. In this device a sample of nuclei are included in the feedback loop of an oscillator which can oscillate over a short range around 5-10 KHz. In the presence of a magnetic field, oscillation takes place only at the correct frequency for the nuclei such that the following condition is met:

$$60.000 \text{ MHz} + \nu_{\text{mod}} = \frac{2\mu}{h} H.$$

The circuit for accomplishing this is shown below:



If the magnet field strength changes, the modulation on both samples changes to keep the system on resonance. However, a differential sweep coil which changes the field locally only at the control sample allows a sweep through the spectrum to be accomplished.

5. Is the NSBO used in permanent magnets?
Complexity in the probe, due to the many coils in the vicinity of the sample is a disadvantage of the NSBO. It is also difficult to adapt to a second frequency for spin decoupling. Since permanent magnets have only slow drift to contend with, a simpler feedback loop for locking the field can be used.

The NSBO has survived, in simplified form, for making homogeneity adjustments. For this purpose, the sample coil and sample receiver can be switched into a feedback loop of the NSBO type. The recorder pen monitors the level of oscillation which depends on the narrowness of the NMR line. A special sample for tuning up resolution is used. This is often a water sample with T_1 purposely shortened by paramagnetic ions to avoid relaxation problems. This system allows very rapid and precise adjustment of current shims to values close to optimum.

6. What type of lock system is used on the T-60/EM-360/EM-390 for critical work requiring high stability?
Slow drift is encountered in a permanent magnet due to temperature changes. These are difficult to eliminate completely by thermal regulators and become uncontrollable when variable temperature work is being done and heat is leaking into the magnet. A dynamic lock system is then needed. Another application of the lock system is for repetitive scanning of a spectrum for time averaging where exact registration of many successive scans is required.

For simplicity and high performance, the internal lock system shown in Figure 33 is found to be satisfactory. In this system two

INTERNAL LOCK SYSTEM (T-60, EM-360, EM-390)

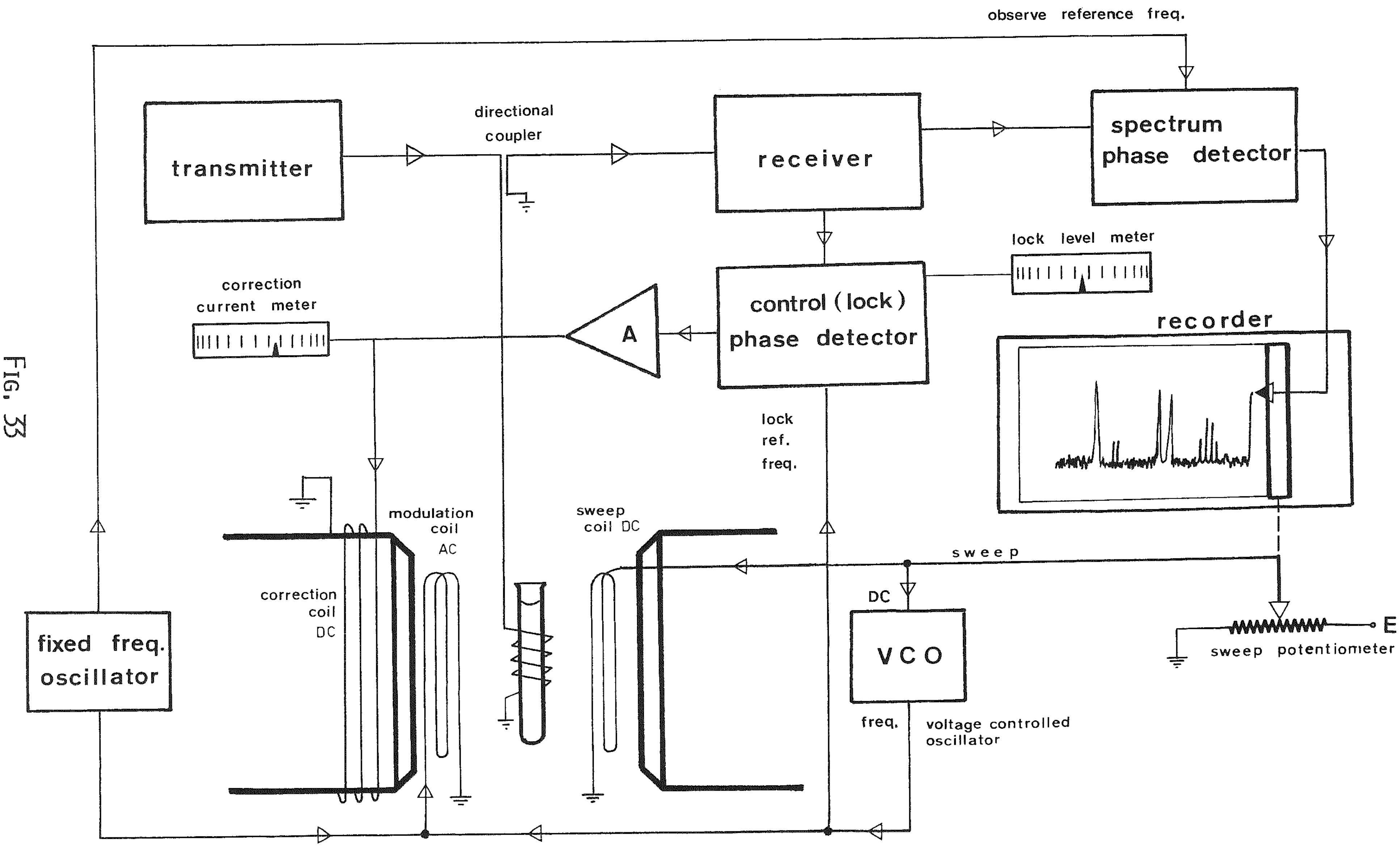
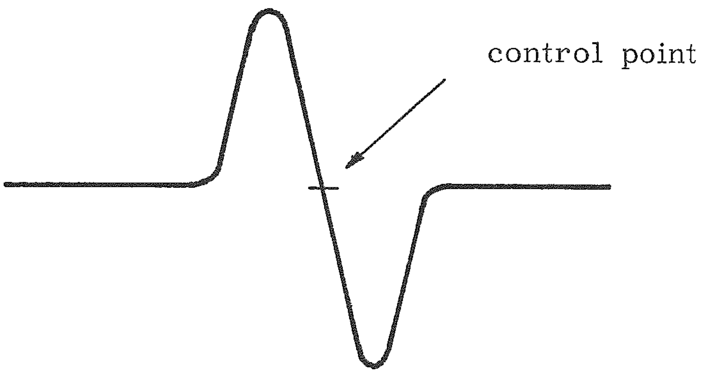


FIG. 33

sidebands and two phase detectors are used -- one at a fixed frequency and one generated by a voltage controlled oscillator (VCO). In operation the spectrum is detected by the fixed frequency phase detector with phase set to detect the absorption mode. The arm of the recorder is tied to a potentiometer which generates a field sweep through a sweep coil. The VCO changes the frequency of modulation in the control circuit so that as the field changes, $\nu_o + \nu_{mod}$ changes along with it, with the result that the spectrometer receiver also has an output from a line in the spectrum which, in effect, is not swept. This is the control line, often TMS, but can be any strong line. The phase detector operating at the frequency of the VCO is set to detect the dispersion signal, which has the necessary discriminator shape shown below:



The output of the control phase detector drives correction coils in the correct phase to cancel out any change of field strength.

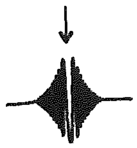
C. Spin Decoupling

7. What instrumentation is needed for double resonance experiments such as spin tickling and spin decoupling?

Two types of experiments are commonly performed. The first is called field sweep decoupling and the second is called frequency sweep decoupling. They differ in the following way. Field sweep decoupling employs two fixed side band frequencies, one operated at a much higher index of modulation so that H_2 is stronger than the observing r.f., H_1. The field is swept through the spectrum. If the frequency separation, $\nu_2 - \nu_1$, is equal to the chemical shift, decoupling occurs at ν_1. Only sets of nuclei separated by $\nu_2 - \nu_1$ will be decoupled.

Frequency sweep decoupling allows a set of nuclei to be decoupled from all others in the spectrum to which they are coupled. The frequency ν_2 can be fixed and the field fixed. Then ν_1 is swept through the spectrum and all nuclei coupled to the group at ν_2 will be decoupled. However, frequency sweep of ν_1 leads to phase shifts across the spectrum, so a pseudo-frequency sweep system is actually employed in the T-60. The frequency ν_2 is varied along with the field sweep by a VCO as in the lock system, so that the selected group is in effect not swept, but irradiated by H_2 continuously. Since the field is swept and ν_1 is fixed, the spectrum is scanned in the usual way. All nuclei coupled to the group at ν_2 will appear decoupled in the resulting spectrum.

As the field is swept, ν_2 varies with it and passes through ν_1. At this point a zero beat occurs on the chart which looks like the illustration below:



This shows where in the spectrum the double irradiation is taking place.

Controls are provided for the index of modulation for ν_2, since the decoupling experiment requires adjustment of both ν_2 and H_2 to optimum values.

D. Variable Temperature

8. How is the sample temperature varied in NMR.
Since permanent magnets are temperature sensitive, varying the sample temperature creates a problem due to leakage of heat into the magnet. This can be reduced by providing either a vacuum jacket for the sample or some other form of insulation. Heated or cooled air or dry nitrogen is usually used as a heat transfer fluid. A temperature sensor in the gas stream controls the temperature to about 0.5°C over a range from about -50°C to +100°C.

Since the unlocked magnet will drift, the internal lock system is of great value in variable temperature work.

E. Summary

In addition to the basic high resolution NMR instrument which involves only the following:

- a. Homogeneous stable magnet with current shims
- b. Transmitter
- c. Probe (r.f. coil and sweep coils)
- d. Receiver
- e. Recorder with sweep potentiometer

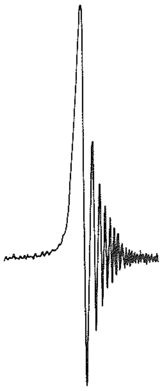
it is possible to add further refinements for various purposes. These include:

- a. Integrator. For quantitative analysis.
- b. Internal Lock. For increased stability and variable temperature work.
- c. NSBO. For simplified homogeneity adjustment.
- d. Double resonance facilities. For spin decoupling.
- e. Variable temperature probe and controller.

The total array of instrumentation permits a wide variety of applications of NMR spectroscopy which will be discussed in the next section.

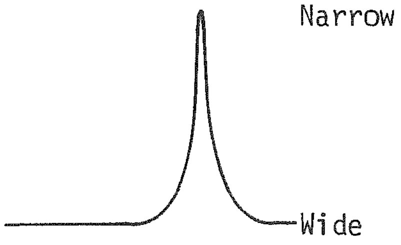
NMR TERMS

1. Ringing

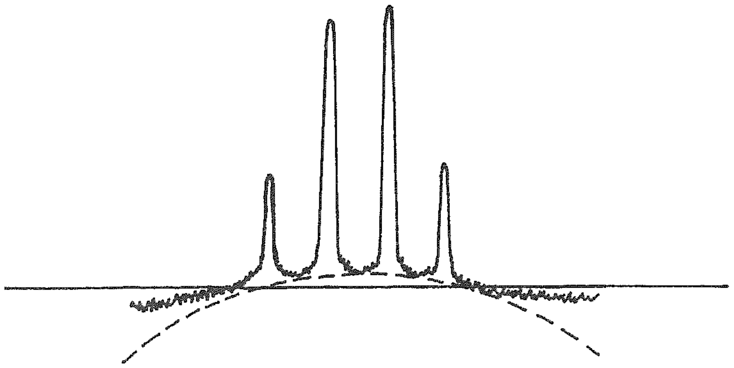


The decay pattern considered a characteristic of good resolution should be exponential and symmetrical.

2. Hump (lineshapes)

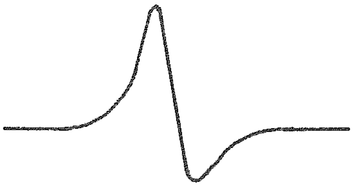


Hump in a single line

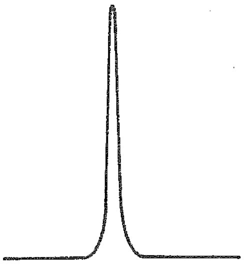


Base line due to magnetic inhomogenieties.
Base line should be as flat as possible.

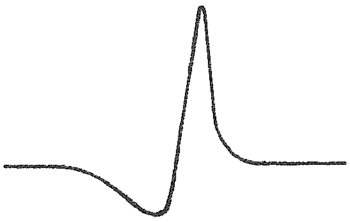
3. Phasing



Partial dispersion

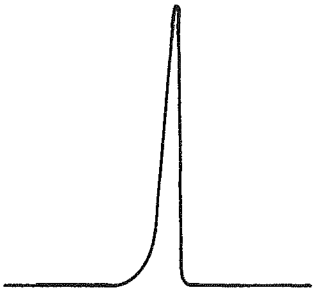


Good
Pure absorption



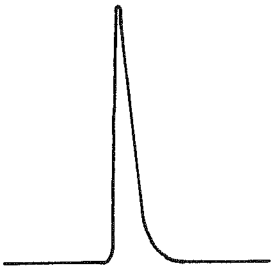
Partial dispersion

4. Front Porch
(Leading Peak)



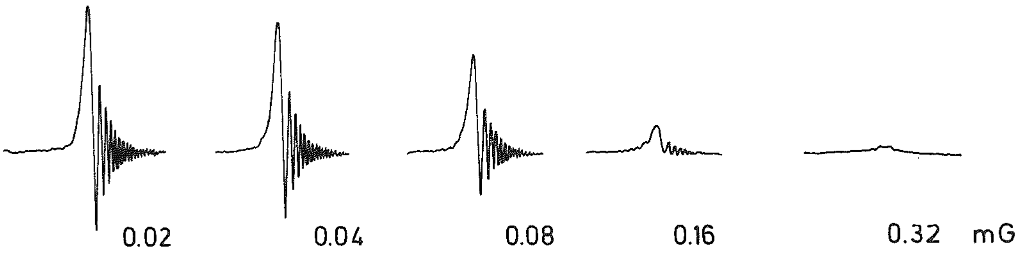
Over Cycled - Dished Field

5. Back Porch
(Trailing Peak)



Under Cycled - Domed Field

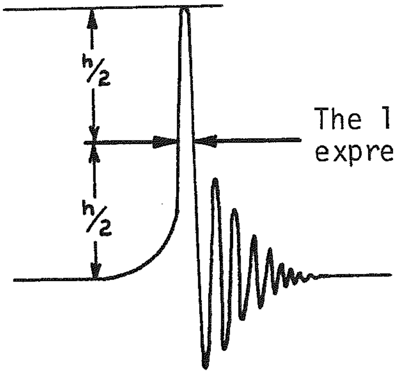
6. Saturation



RF Power Increasing →

When the peak amplitude starts to drop and broaden with increasing RF power, it has reached the point of saturation.

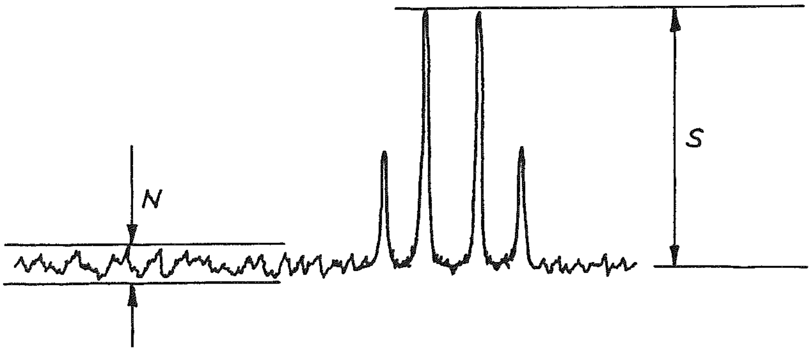
7. Resolution



The line width at half height, normally expressed in Hz, could be expressed in ppm.

For this test, the natural line width of the line must be less than the resolving power of the spectrometer.

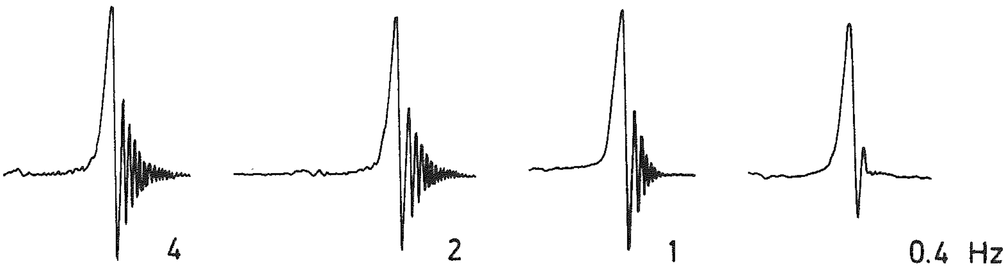
8. Sensitivity



$s/n = \frac{s}{n} \times 2.5$

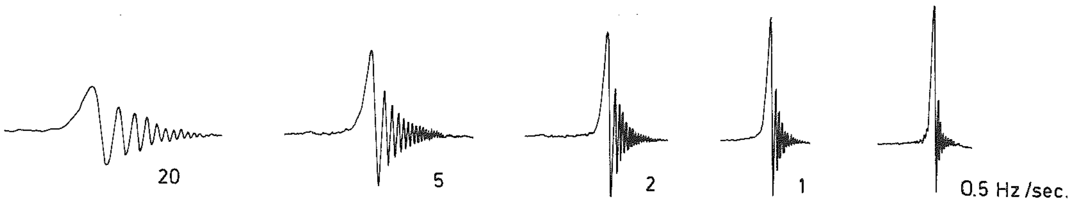
The spectrum of the methylene protons, a quartet, is run at standard s/n parameters for that system. S/n is the tallest peak of the quartet multiplied by 2.5 and divided by the peak-to-peak noise. This is, in reality, signal-to-average noise.

9. Filtering



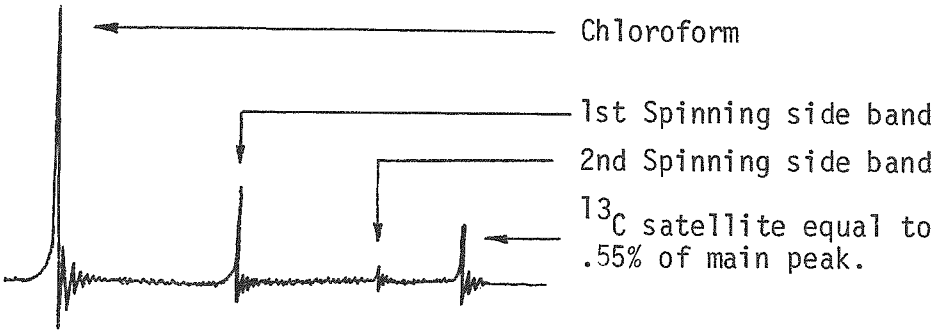
Increasing filter time constant →
← Increasing filter band width.

10. Sweep Broadening



If sweep is too rapid, a narrow line will appear broad. The longer the relaxation time, the more pronounced is the effect.

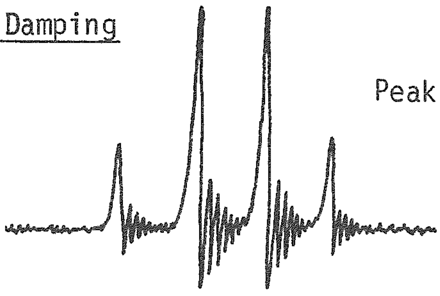
11. Spinning Side Bands and ^{13}C Satellites



Spectrum Amplitude X 30

Spinning side bands are displaced from the main peak by the spin rate. The 13C satellite is due to a coupling constant of a ^{1}H - ^{13}C.

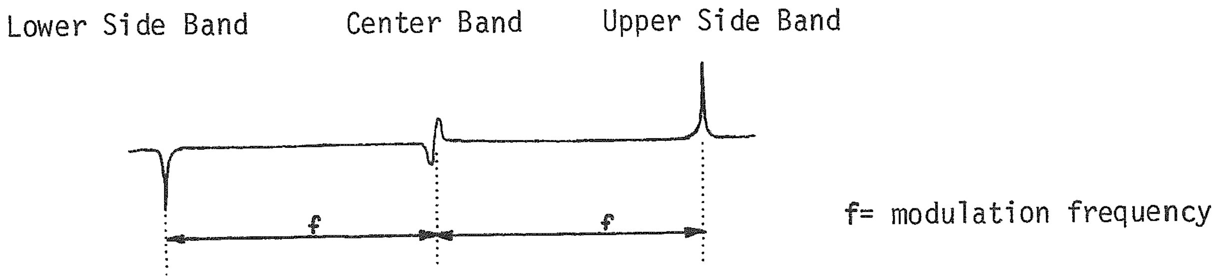
12. Radiation Damping



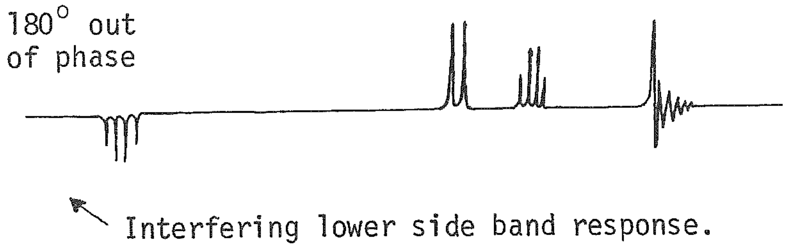
Peak Ratios: 1:2.5:2.5:1 theoretically is 1:3:3:1. Line width of inner peaks is greater than outer peaks.

Strong signals in presence of weak signals suffer loss of amplitude and are broadened.

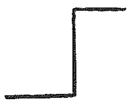
13. Modulation Side Bands



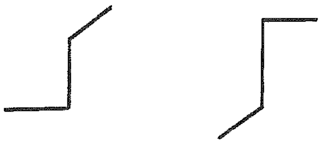
RF observing energy is available at both side band positions. If peaks are chemically shifted by ~ 2f, you will get a spectrum similar to --



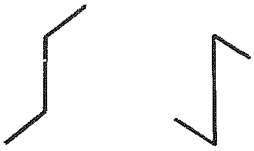
14. Integral Problems



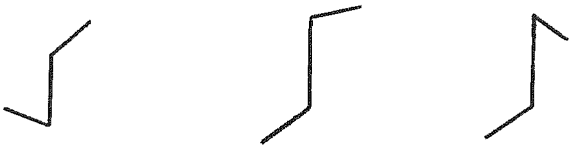
Correct



Wrong Phase



Wrong Drift



Wrong Phase and Drift