A Basic Guide to

N M R

by James Shoolery

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Amezquita

James N. Shoolery

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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 ^1H, ^{19}F, and ^{31}P have both high enough natural abundance

Isotope	NMR Frequency MHz. at 14,092 G.	Natural % Abundance	Relative Sensitivity For Equal Numbers of Nuclei.	Spin in Multiples of $h/2\pi$
^1H	60.000	99.985	1.00	$\frac{1}{2}$
^2H	9.210	0.015	9.65×10^{-3}	1
^{11}B	19.250	80.42	0.165	$\frac{3}{2}$
^{13}C	15.087	1.108	1.59×10^{-2}	$\frac{1}{2}$
^{14}N	4.334	99.63	1.01×10^{-3}	1
^{17}O	8.134	0.037	2.91×10^{-2}	$\frac{5}{2}$
^{19}F	56.446	100.	0.883	$\frac{1}{2}$
^{31}P	24.288	100	0.066	$\frac{1}{2}$

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 ^1H, 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

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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 ^{1}H 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.
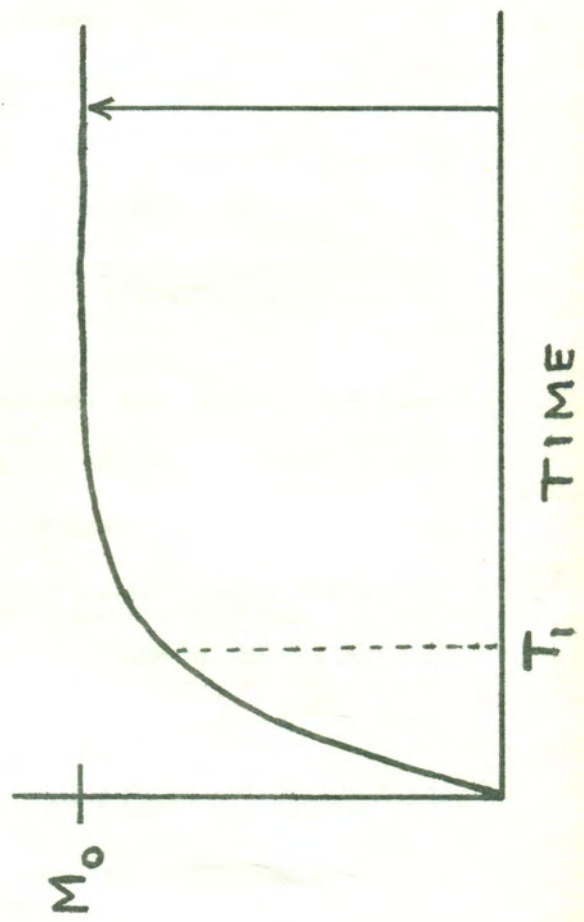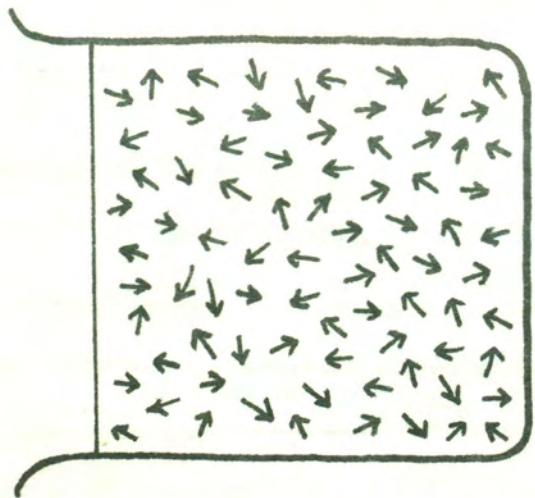
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FIG. 2

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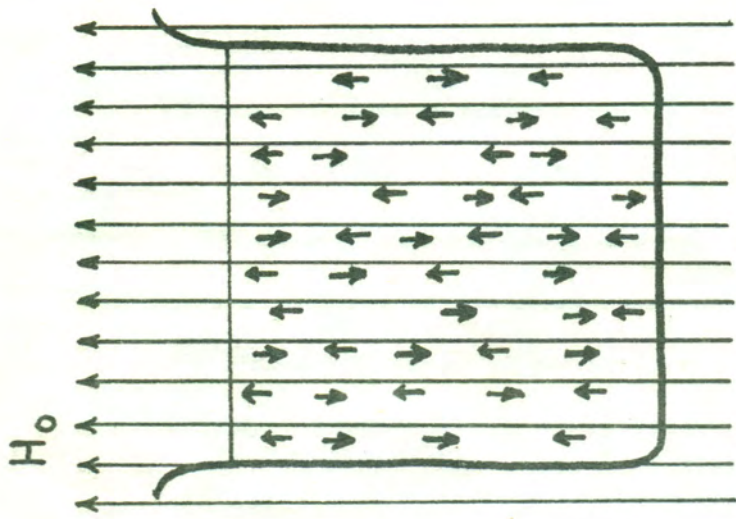
7. How is the Absorption of Radio-Frequency Energy by the Sample Made to Occur?

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.

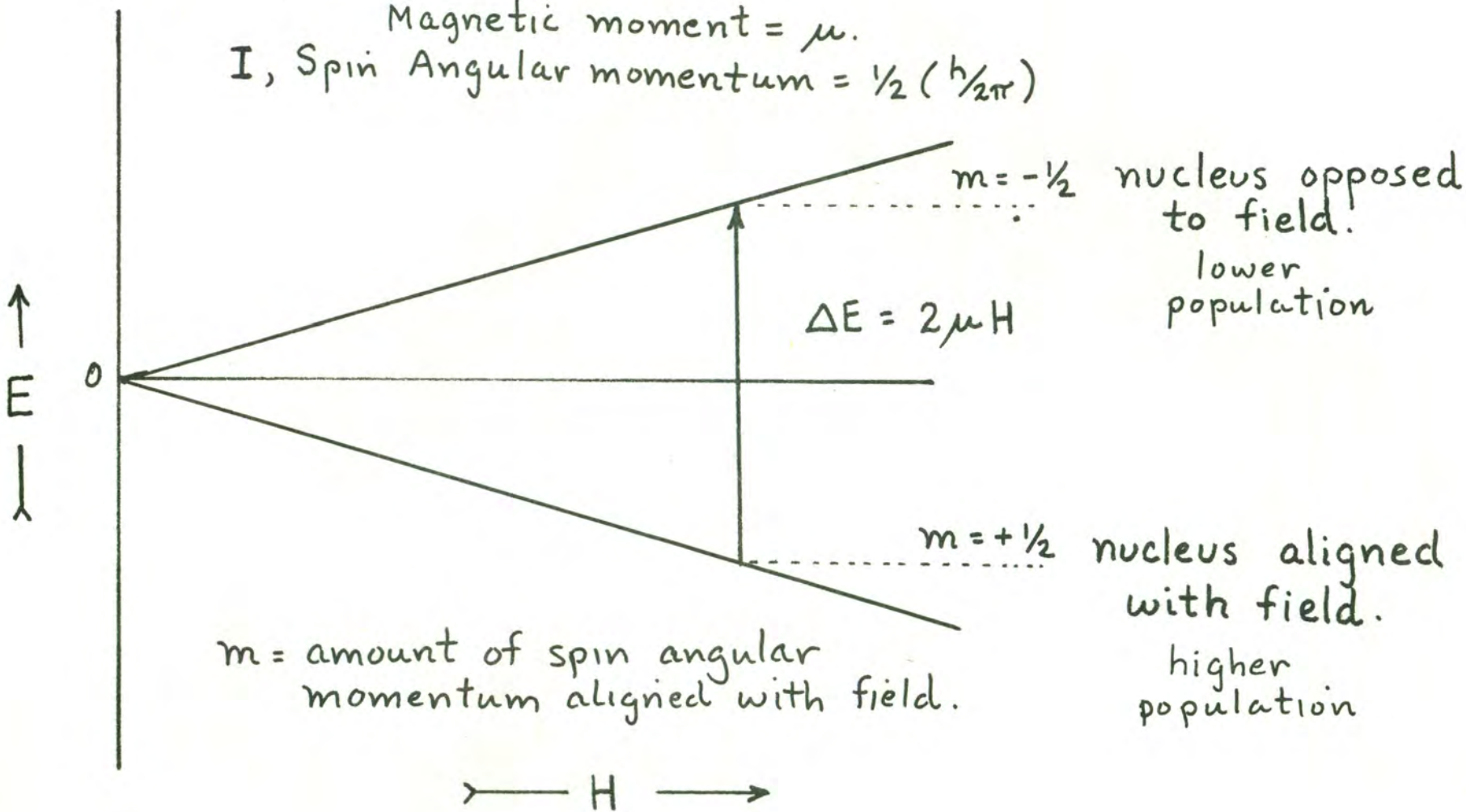I, Spin Angular momentum = $\frac{1}{2}\left(\frac{h}{2\pi}\right)$

E

0

$m = -\frac{1}{2}$ nucleus opposed to field!

lower population

$\Delta E = 2\mu H$

$m = +\frac{1}{2}$ nucleus aligned with field.

higher population

m = amount of spin angular momentum aligned with field.

$\longrightarrow H \longrightarrow$

Fig. 3 8

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.
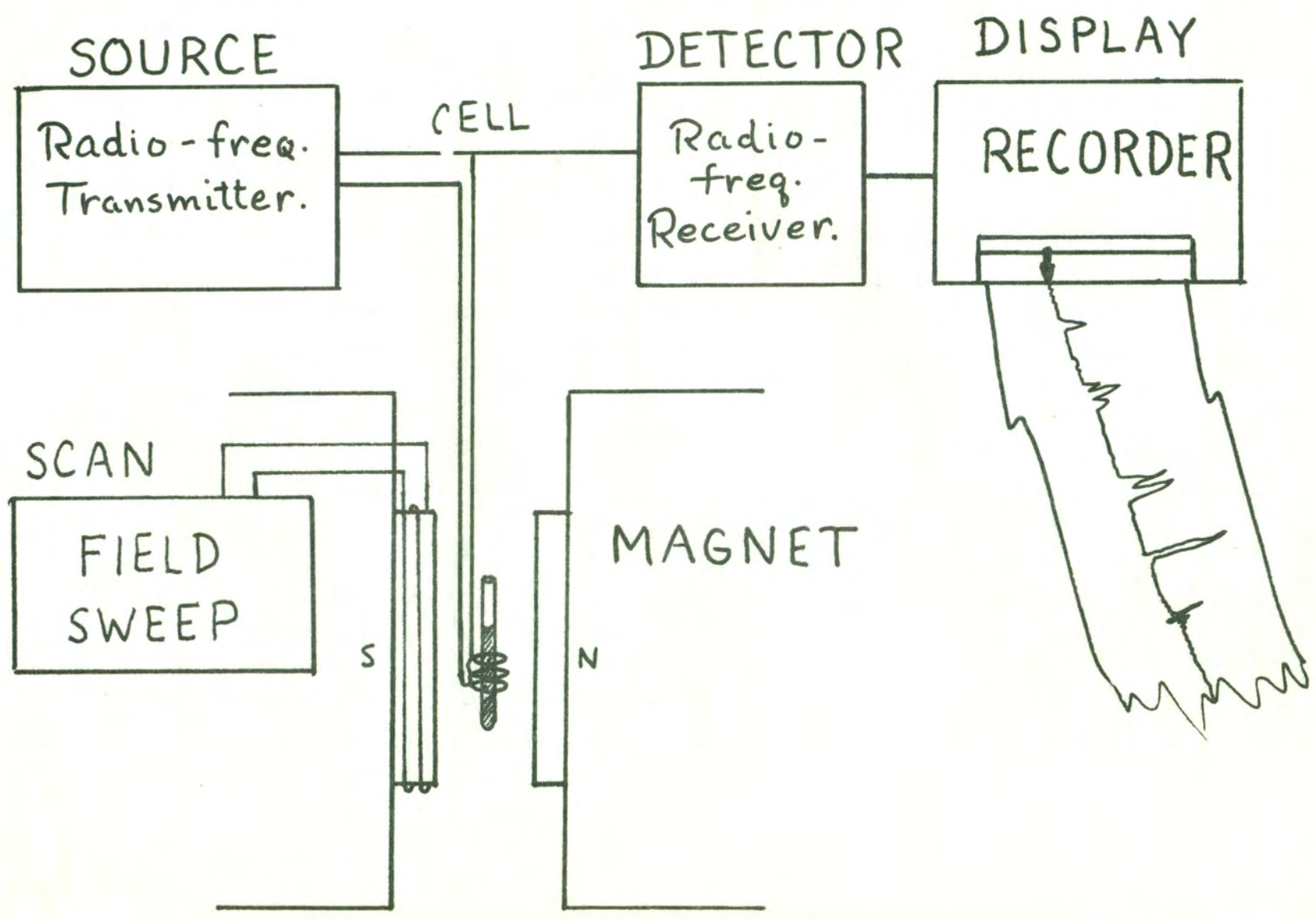
NMR SPECTROMETER

Fig. 4
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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 <u>charged</u> electrons alter the magnetic field slightly and <u>shield</u> the nucleus to a small extent. The chemical shift, δ, is proportional to the applied field, H_o, and for protons it varies over a range which is about 0.001 percent of the applied magnetic field. In a complex molecule or mixture of molecules we may expect to find a number of peaks if we scan the field or frequency over this range.

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 of a particular chemical compound as there are NMR instruments operating at different field strengths and frequencies, we usually report values of σ rather than δ, i.e., we divide the measured shift values by the field strength or frequency, depending on whether we have measured them in magnetic field or frequency units. Since the values of σ are very small, we normally multiply them by 10^6 in order to report them, and they then represent the chemical shift in <u>parts</u> <u>per</u> <u>million</u> (p.p.m.) of the applied field.

MAGNETIC FIELD AT THE NUCLEUS.

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

↑ shielding constant

· nucleus

$$H_n = H_o - H_o \sigma$$

$$H_n = H_o - \delta \text{ (chemical shift)}$$

δ is proportional to H_o

Fig. 5
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In order for shift values to have the same meaning in different laboratories

estar de acuerdo

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.

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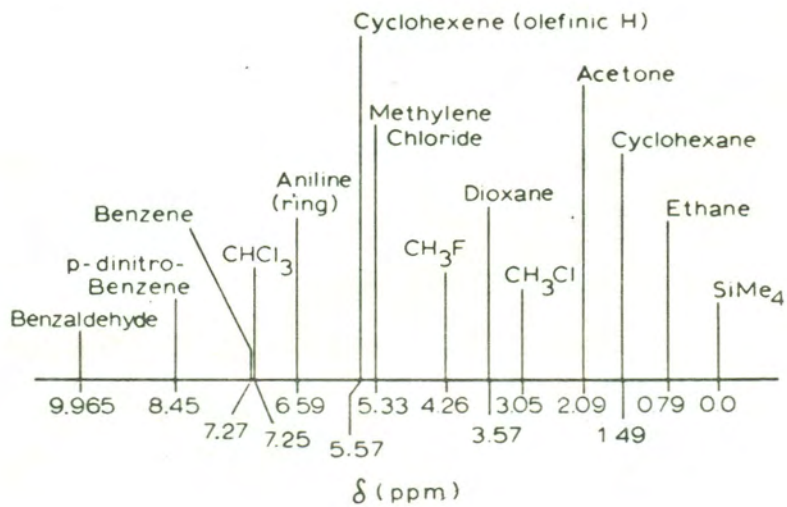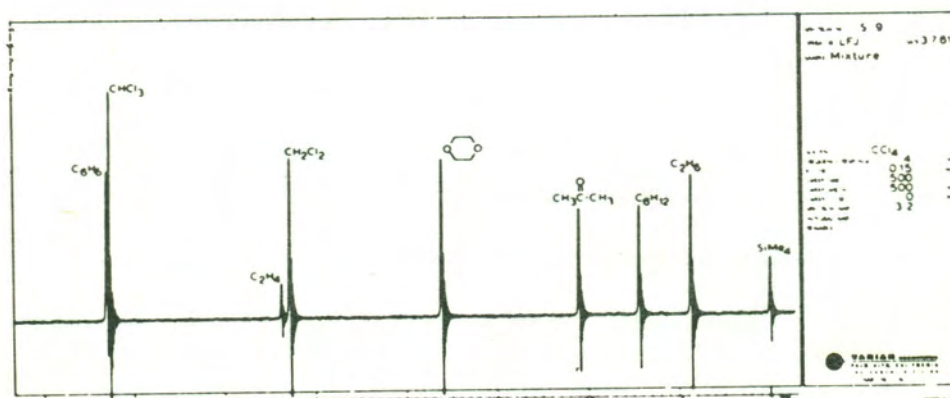*to.*

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FIG. 6

SOME CHEMICAL SHIFTS
IN DILUTE CCl_4 SOLUTION

Chart showing chemical shifts of various compounds plotted against δ (ppm): Benzaldehyde, p-dinitro-Benzene, Benzene, $CHCl_3$ (7.27), Aniline (ring), Methylene Chloride, Cyclohexene (olefinic H), CH_3F, Dioxane, CH_3Cl, Acetone, Cyclohexane, Ethane, $SiMe_4$. Scale markings: 9.965, 8.45, 7.27, 7.25, 6.59, 5.57, 5.33, 4.26, 3.57, 3.05, 2.09, 1.49, 0.79, 0.0.

δ (ppm)

FIG. 7

NMR spectrum of a mixture in CCl_4 showing peaks labeled: C_6H_6, $CHCl_3$, C_2H_4, CH_2Cl_2, dioxane, $CH_3C\cdot CH_3$, C_6H_{12}, C_2H_6, $SiMe_4$.

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_0, and half with spin anti-parallel to H_0. Similarly, half of the molecules will have nucleus B with spin parallel to H_0, 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
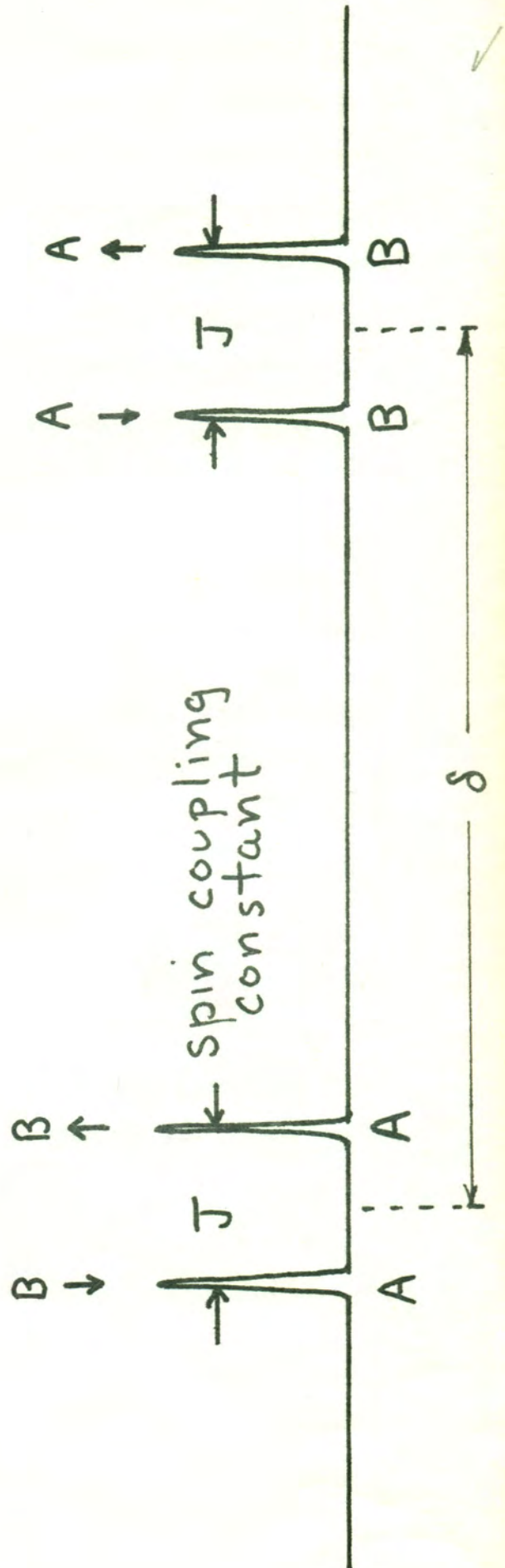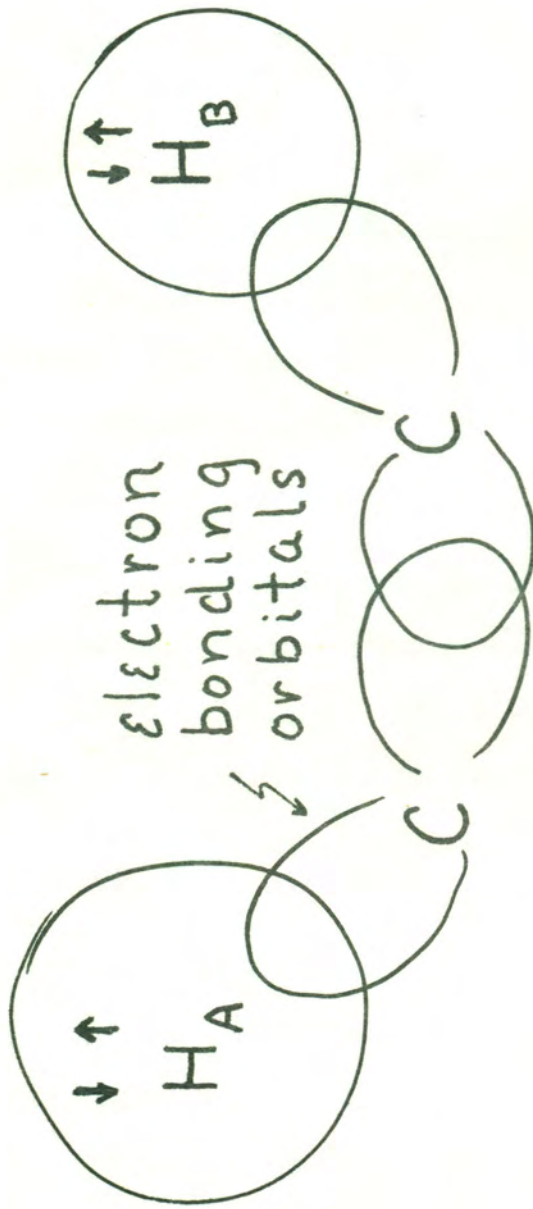
SPIN - SPIN COUPLING.

FIG. 8

states of nucleus A. Therefore, we find our spectrum is a <u>pair</u> of <u>doublets</u> with spacing J, called the spin-coupling constant. Since the coupling is due to an interaction entirely within the molecule, it is <u>independent</u> 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.

$$
\begin{array}{c}
1 \\
1\ 1 \\
1\ 2\ 1 \\
1\ 3\ 3\ 1 \\
1\ 4\ 6\ 4\ 1 \\
1\ 5\ 10\ 10\ 5\ 1 \\
1\ 6\ 15\ 20\ 15\ 6\ 1
\end{array}
$$

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.
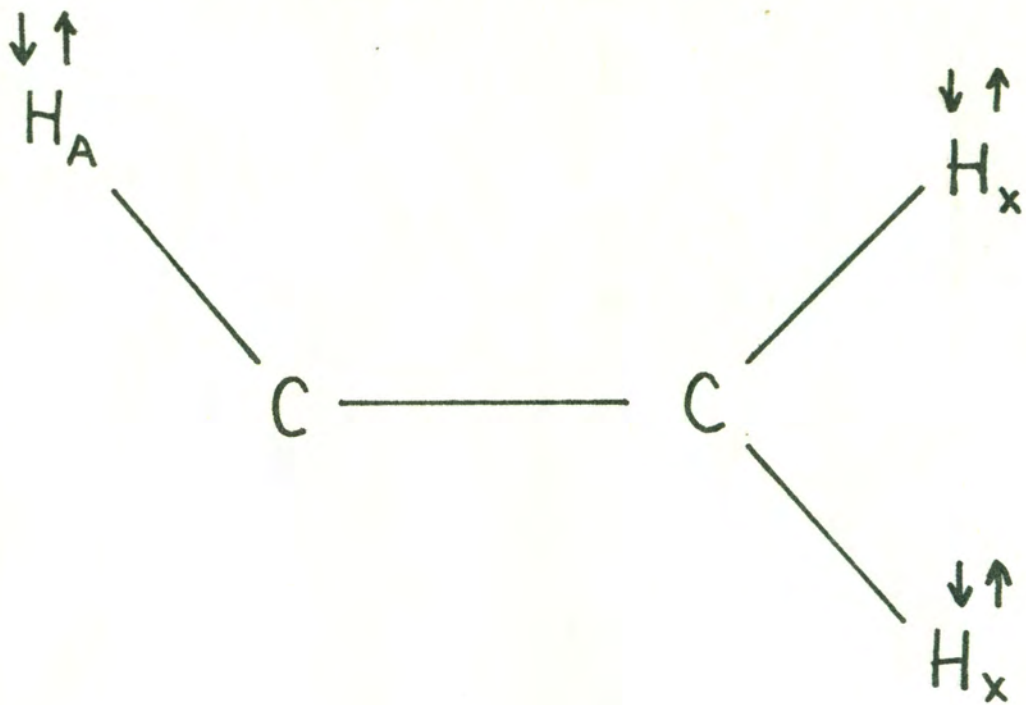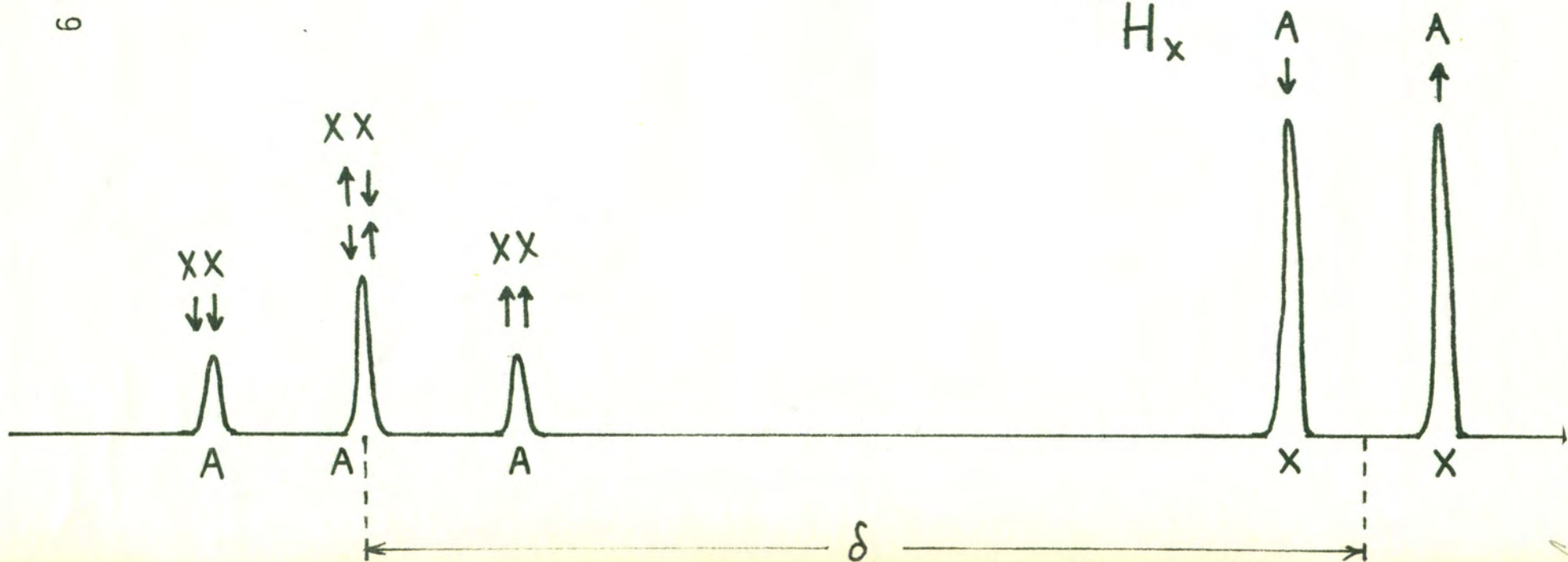
THREE SPINS, AX$_2$

Fig. 9
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FIG. 10

EQUAL COUPLING TO NEIGHBORING NUCLEI.

no neighbors

one neighbor

two neighbors

three neighbors

n+1 lines — n neighbors

FIG. 10
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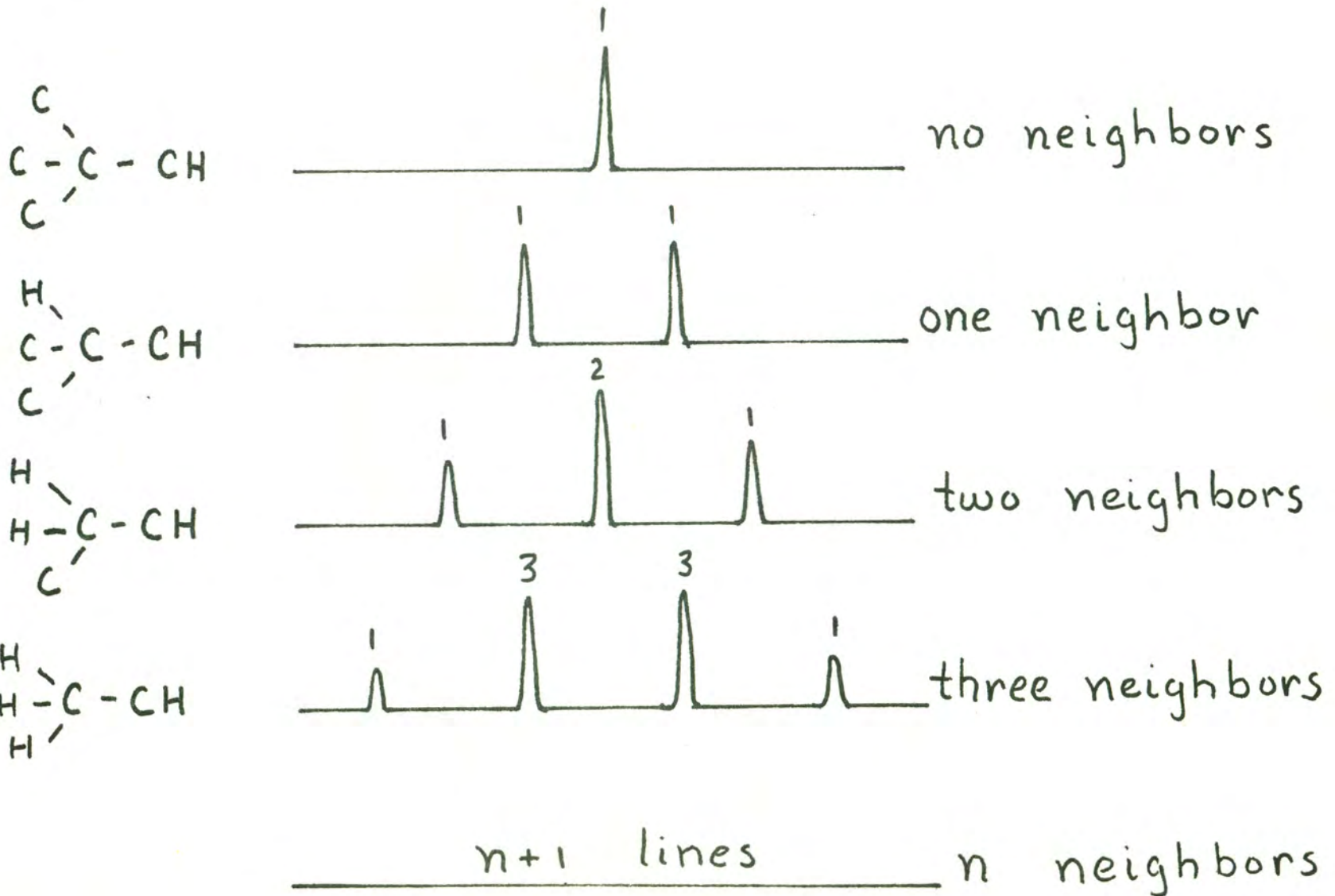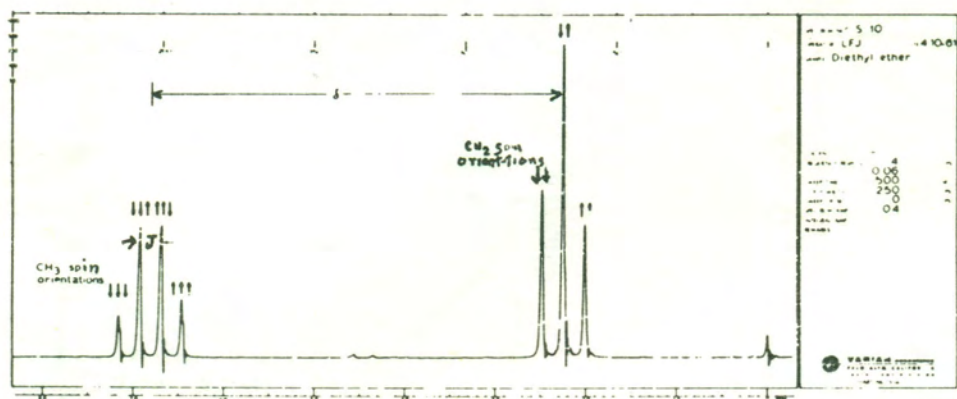
FIG. 11

A. <u>Basis of the Chemical Shift</u>

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 <u>intra-molecular</u>
<u>shielding</u>. These are:

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

   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 <u>paramagnetic</u> 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)
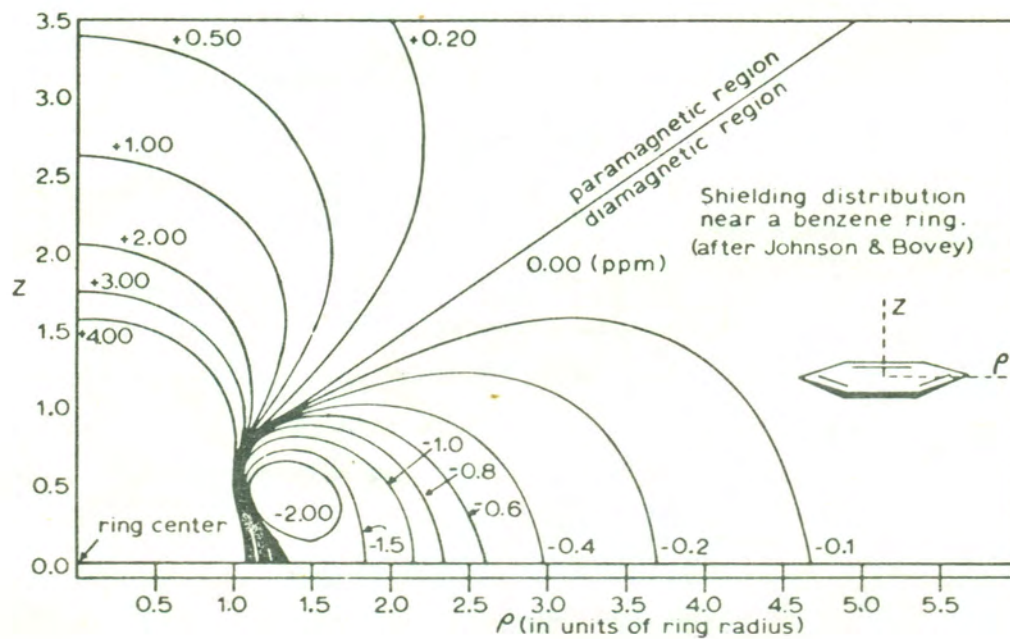
shows the distribution of the field around the benzene ring, based on a theoretical calculation.
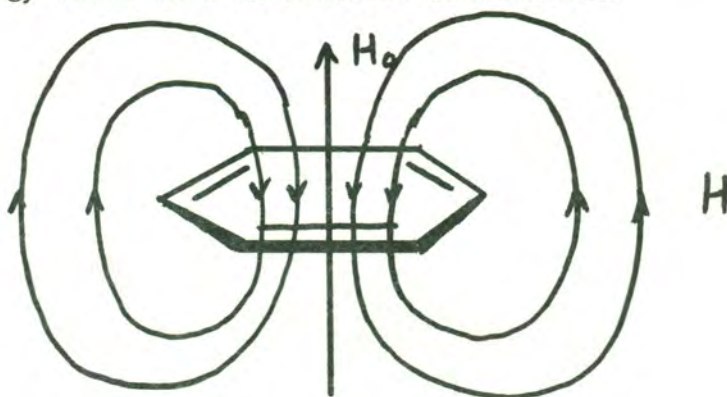

Figure 13 illustrates the effects of all three types of electronic motions.

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.
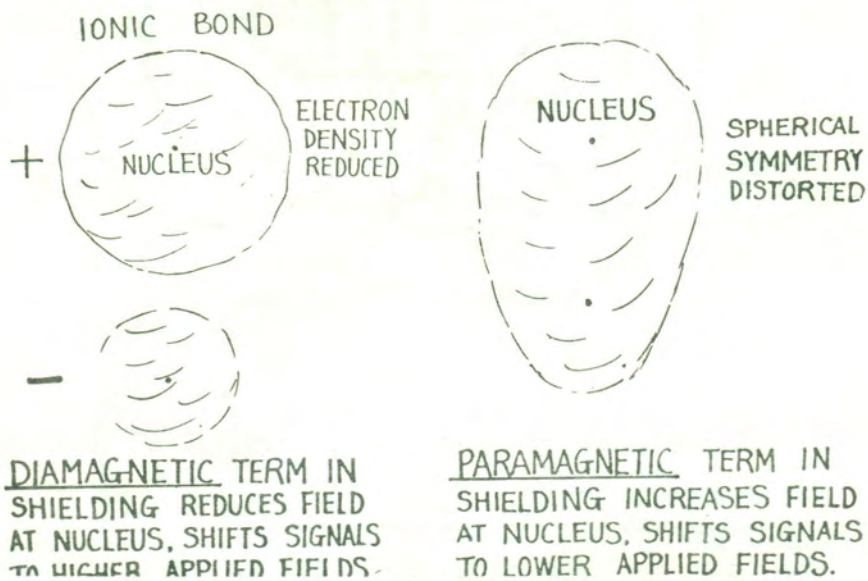
B. Spin-spin coupling.

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.
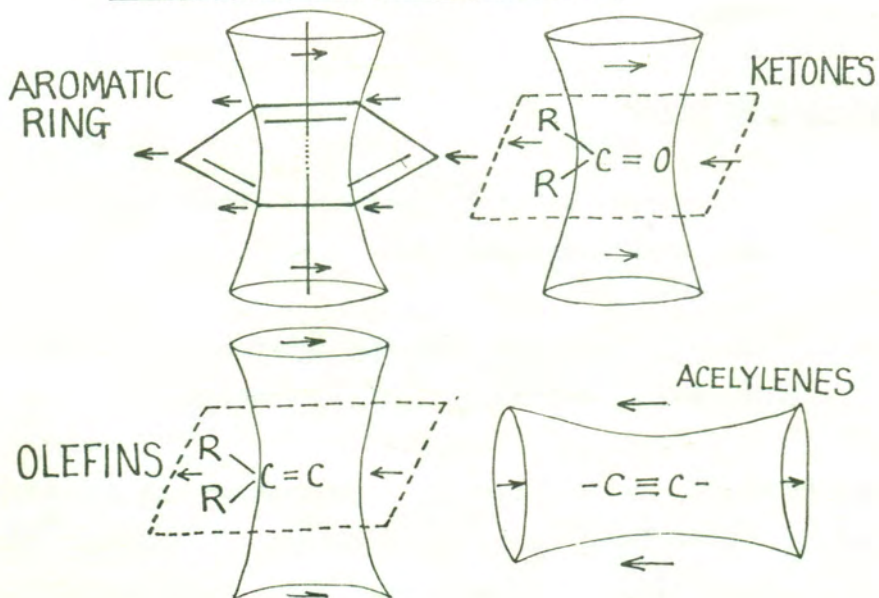
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Fig. 13

INTRAMOLECULAR SHIELDING

IONIC BOND

ELECTRON
DENSITY
REDUCED

NUCLEUS

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

R
R
$C = O$

OLEFINS
R
R
$C = C$

ACELYLENES

$-C \equiv C-$

FIG. 14

CHARACTERISTIC PROTON CHEMICAL SHIFT RANGES

δ (p.p.m from TMS)

0.0 – 0.5	3- MEMBERED RINGS , $-\overset{\shortmid}{S}i - \overset{\shortmid}{C} \cdot \underline{H}$
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	$-C \overset{O}{\underset{H}{\lessgtr}}$
10.0 – 10.5	$- COOH$
1.5 – 15	$-OH$ (depending on H-bonding)

UNEQUAL COUPLING TO NEIGHBORING NUCLEI.
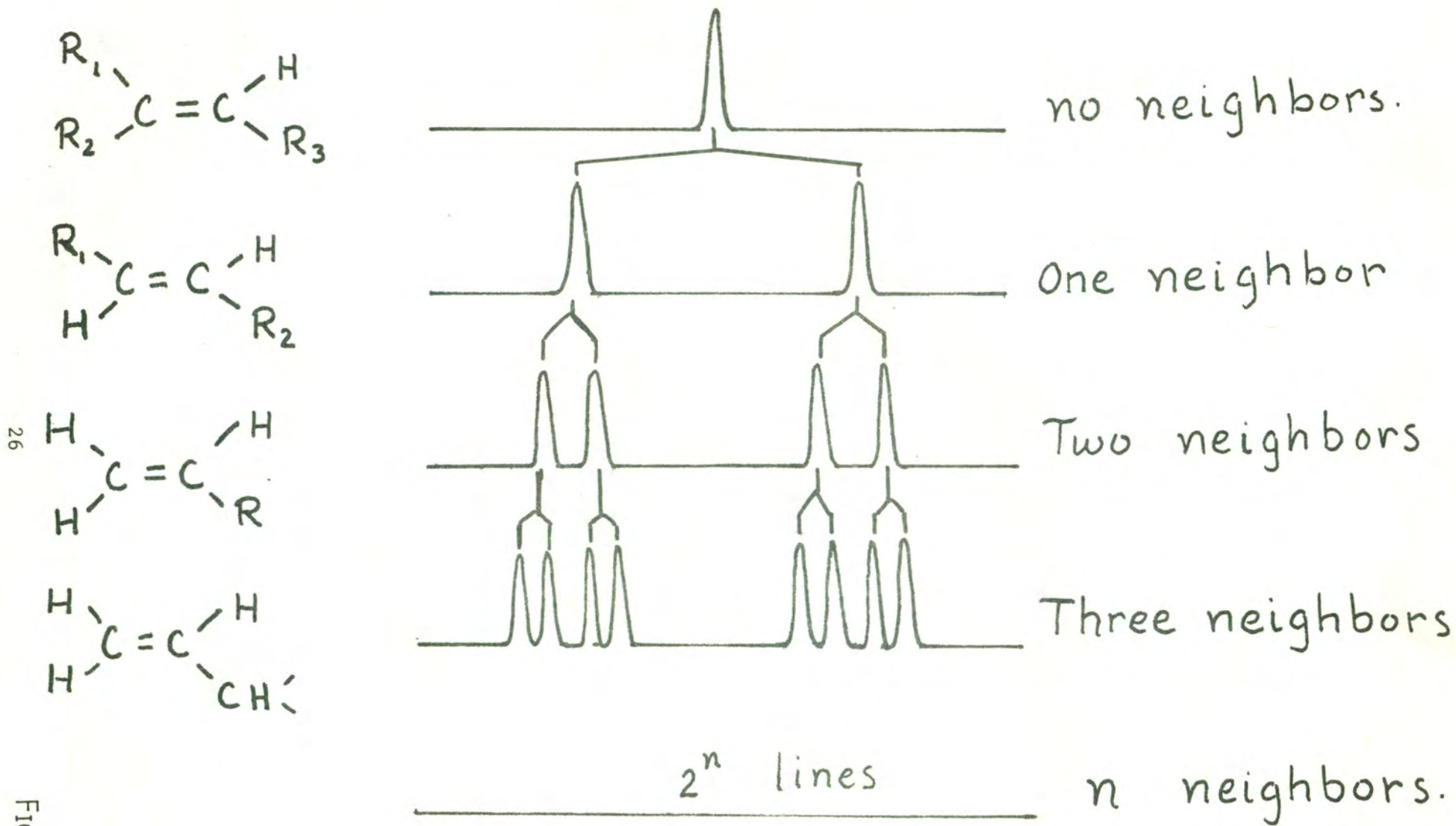
FIG. 15

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 substituents
b) Angle dependence
c) π - Electron contribution

Vicinal Protons

CH—CH J_{vic} : 6 - 8 cps (free rotation)

: 0 - 14 cps (in fixed positions)

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

FIG. 17

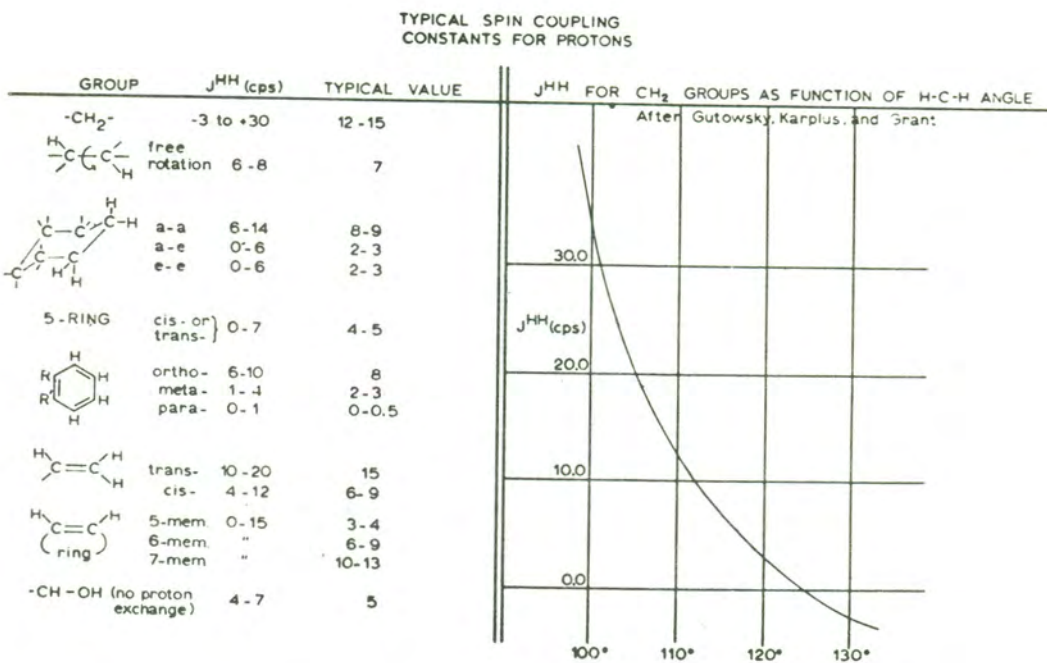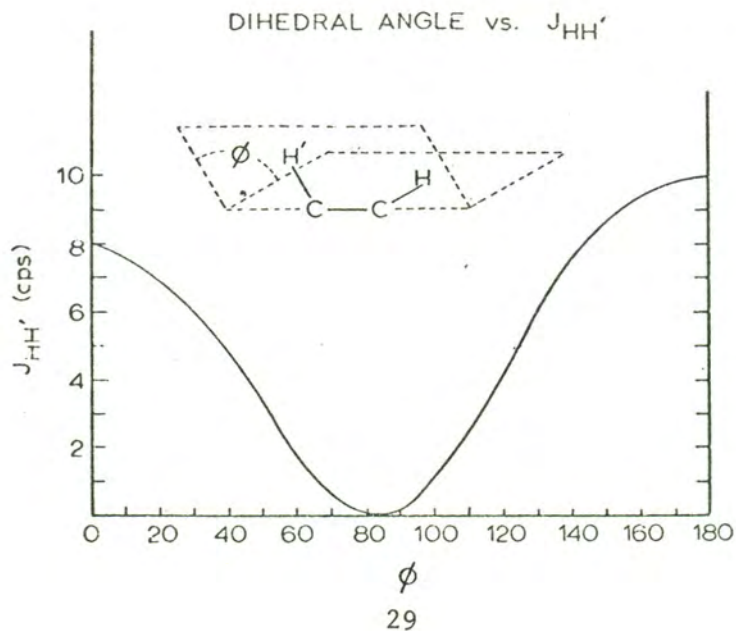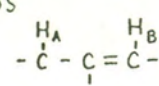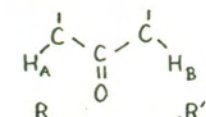## TYPICAL SPIN COUPLING CONSTANTS FOR PROTONS

GROUP	J^{HH} (cps)	TYPICAL VALUE
-CH₂-	-3 to +30	12 - 15
H—C—C—H (free rotation)	6 - 8	7
(chair ring)	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
(benzene ring)	ortho-: 6-10	8
	meta-: 1-4	2-3
	para-: 0-1	0-0.5
H—C=C—H	trans-: 10-20	15
	cis-: 4-12	6-9
H—C=C—H (ring)	5-mem.: 0-15	3-4
	6-mem.: "	6-9
	7-mem.: "	10-13
-CH-OH (no proton exchange)	4-7	5

J^{HH} FOR CH₂ GROUPS AS FUNCTION OF H-C-H ANGLE
After Gutowsky, Karplus, and Grant

FIG. 18

DIHEDRAL ANGLE vs. $J_{HH'}$

29

FIG. 19

LONG RANGE H-H COUPLING CONSTANTS

	J_{AB} (Hz)
4-BONDS	

4-BONDS $-\overset{H_A}{\underset{|}{C}} - \underset{|}{C} = \overset{H_B}{C} -$	1 - 2
$H_A\overset{|}{C} - \underset{\overset{||}{O}}{C} - \overset{|}{C}H_B$	0 - 1
$\overset{R}{H_A} \quad \overset{R'}{H_B}$	5 - 7
benzene ring H_A / H_B	2 - 3
5-BONDS $\quad -\overset{H_A}{\underset{|}{C}} - \underset{|}{C} = C - \overset{|}{\underset{H_B}{C}} -$	0 - 2
>5-BONDS $\quad C\underline{H}_3 - C \equiv C - C \equiv C - C \equiv C - C\underline{H}_2 - OH$	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
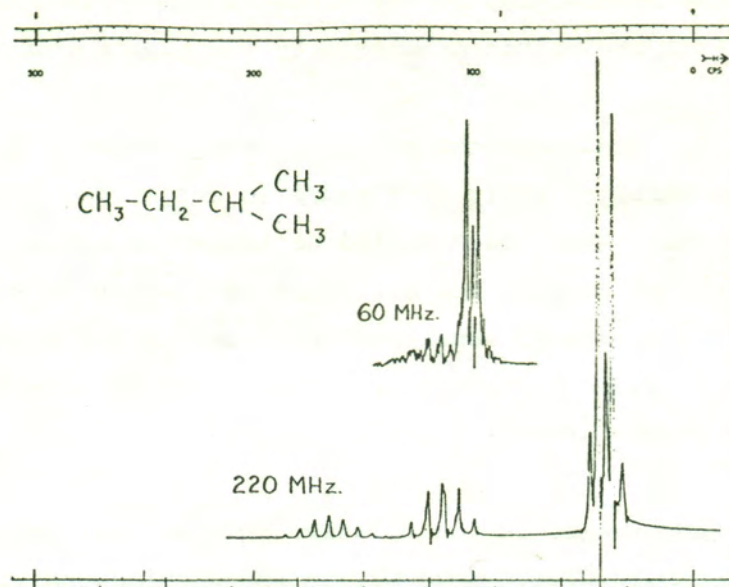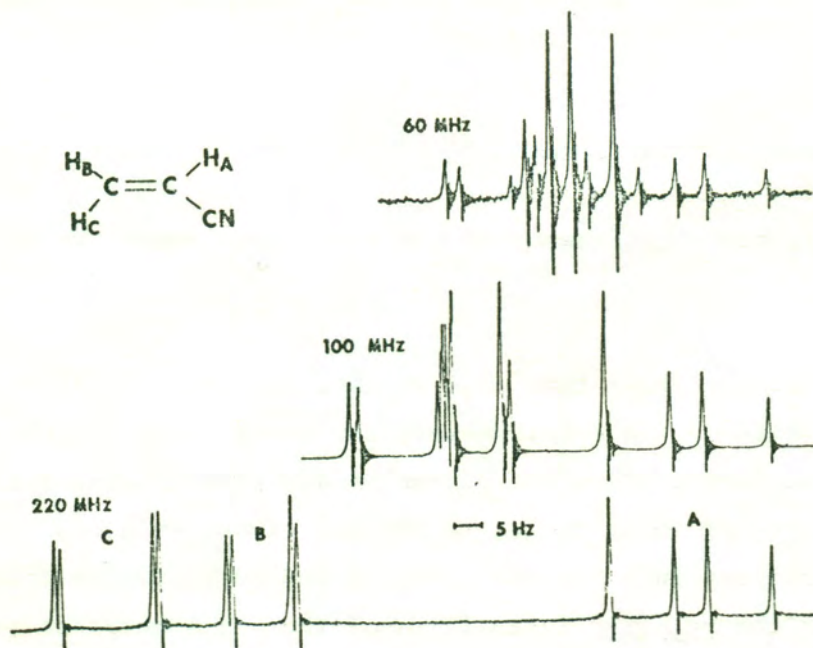
Fig. 20

CH₃–CH₂–CH⟨CH₃/CH₃

60 MHz.

220 MHz.

Fig. 21

60 MHz

100 MHz

220 MHz

C   B   ⊢ 5 Hz   A

spectrum reflect the relative <u>numbers</u> of nuclei in a <u>pure</u> <u>substance</u>, or the relative <u>numbers</u> of <u>molecules</u> of each type in a <u>mixture</u>. 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 <u>known</u> concentration of the substance being studied, NMR spectra can be interpreted quantitatively even when they have been obtained from <u>unseparated, crude</u> 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 <u>integral</u> 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

DETERMINATION OF THE NUMBER OF HYDROGEN ATOMS PER MOLECULE IN COMPLEX SUBSTANCES.

EXAMPLE: $C_{20}H_{22}N_2O_2$, an indole alkaloid.

Total H = 1+4+1+5+5+6 = 22
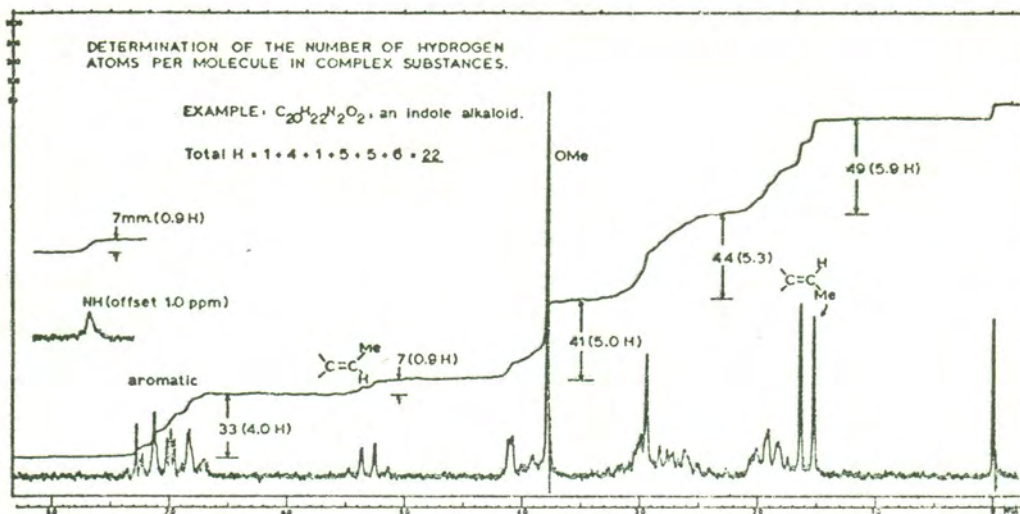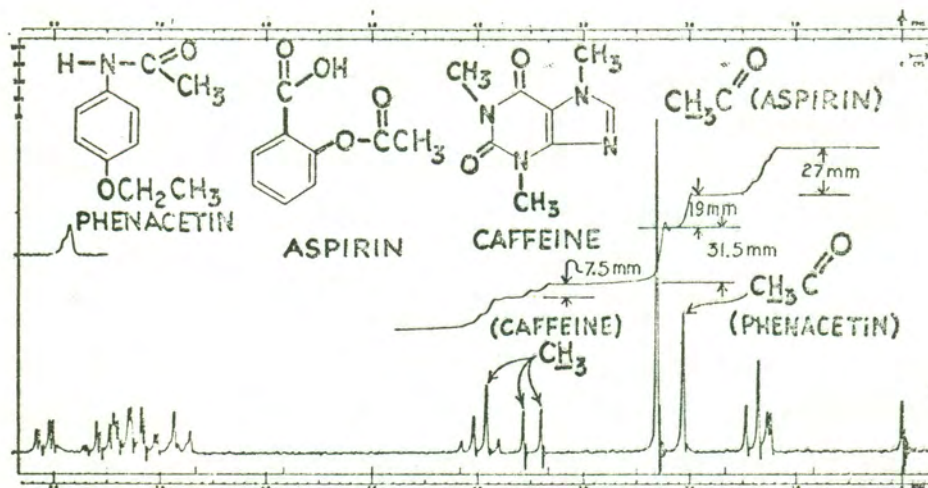
FIG. 23

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 δ_{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 Block-Siegert shifts.

The Block-Siegert shift is given by

$$\text{Shift} = \sqrt{(\text{const. } H_2{}^2) + (\Delta f)^2} - \Delta f$$

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 Block-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 Block-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 <u>crossed-coil</u> 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 $CH_2 - C\underline{H} = C - C = 0$. 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 <u>not</u> be next to oxygen, confirming structure I.

E. <u>Dynamic Effects in NMR Spectra.</u>

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 <u>average</u> positions. Isotopic substitution <u>can</u> change this average and produce <u>isotope</u> 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 <u>no</u> 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
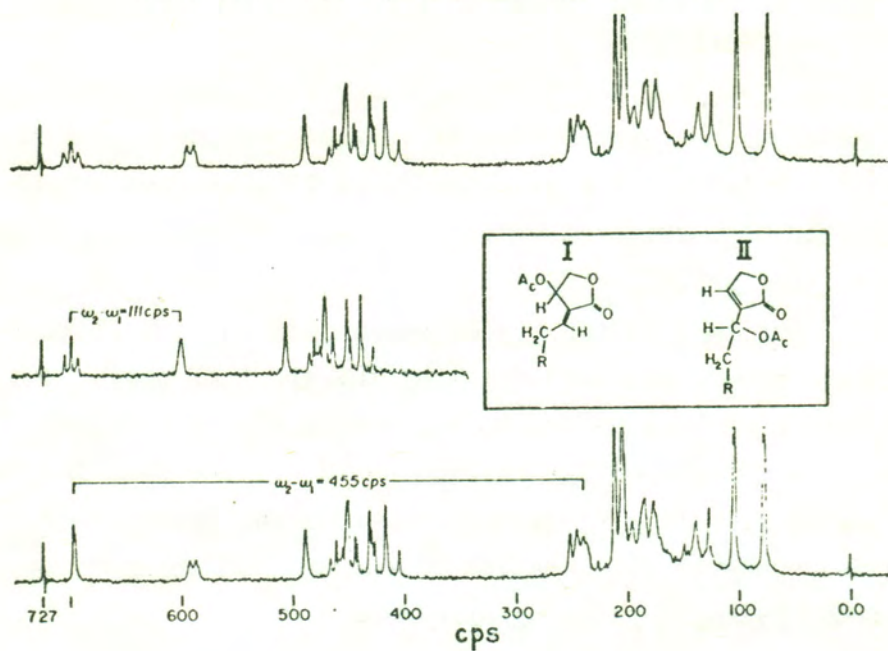
nuclear spin system would be heated up by the r.f. energy, with no thermal contact with its surroundings to dissipate the heat, so it would stop absorbing energy.

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 broad 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 in-phase, 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.

39
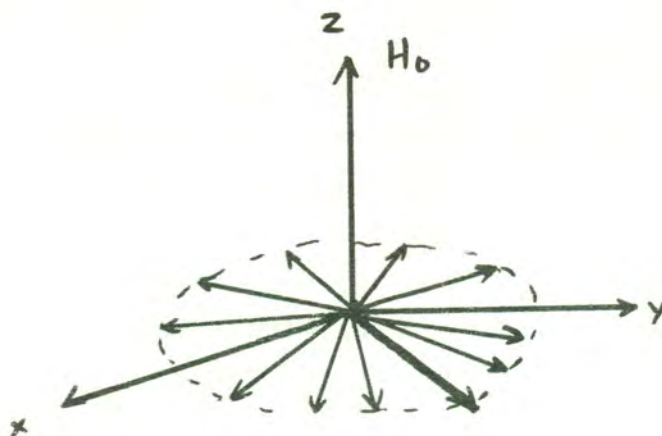
The net rotating moment becomes zero, even though repolarization has not yet taken place via the T_1 mechanism. The time for this process to occur is called T_2, the transverse, or spin-spin relaxation time. It can be shorter than T_1, but never longer.

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

40

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-OH} of approximately 5 Hz.

At a temperature of $60^{\circ}C$, 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 110°C

at 80°C

at 60°C

NMR OF THE PROTON AT 20°C

CH$_3$CH$_2$OH
AND H$_2$O

42
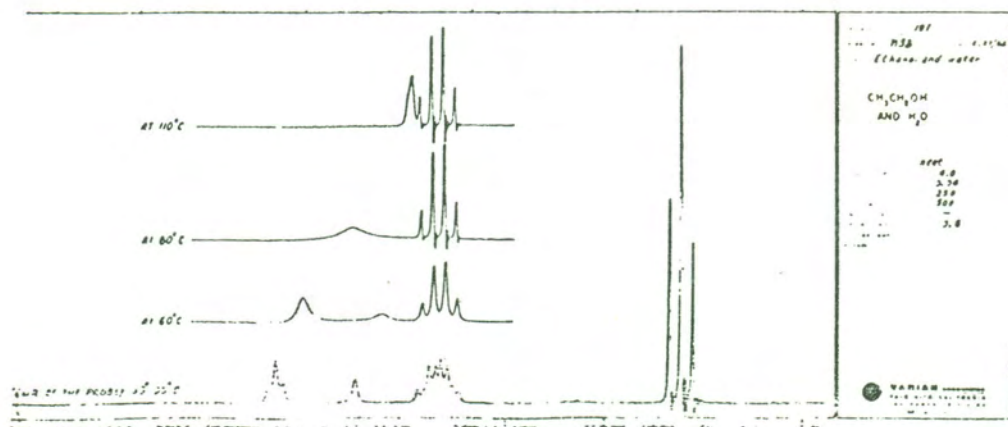
At still higher temperatures the OH resonances merge together and continue to get sharper as more complete averaging takes place.

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
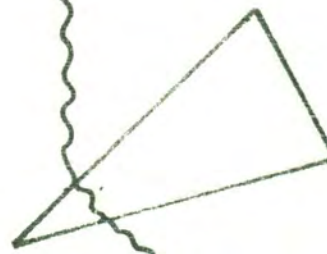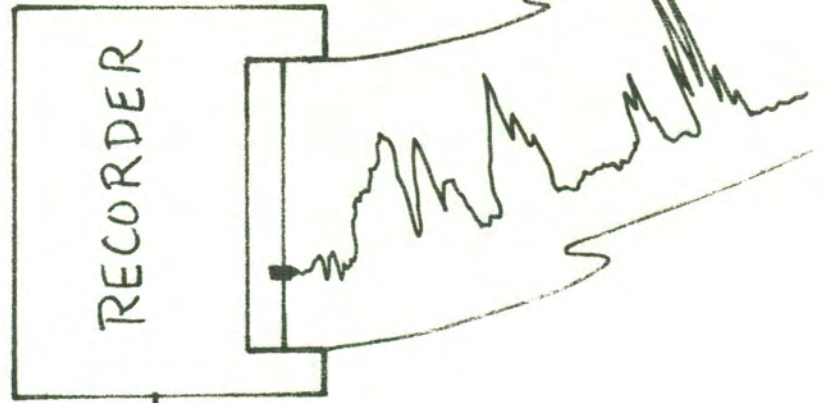spectrometer. The main differences are the following:

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

DISPLAY

RECORDER

DETECTOR AMP.

thermocouple or photocell

Sample CELL

prism or grating

WAVELENGTH SCAN

SOURCE

FIG. 26   45

NMR SPECTROMETER
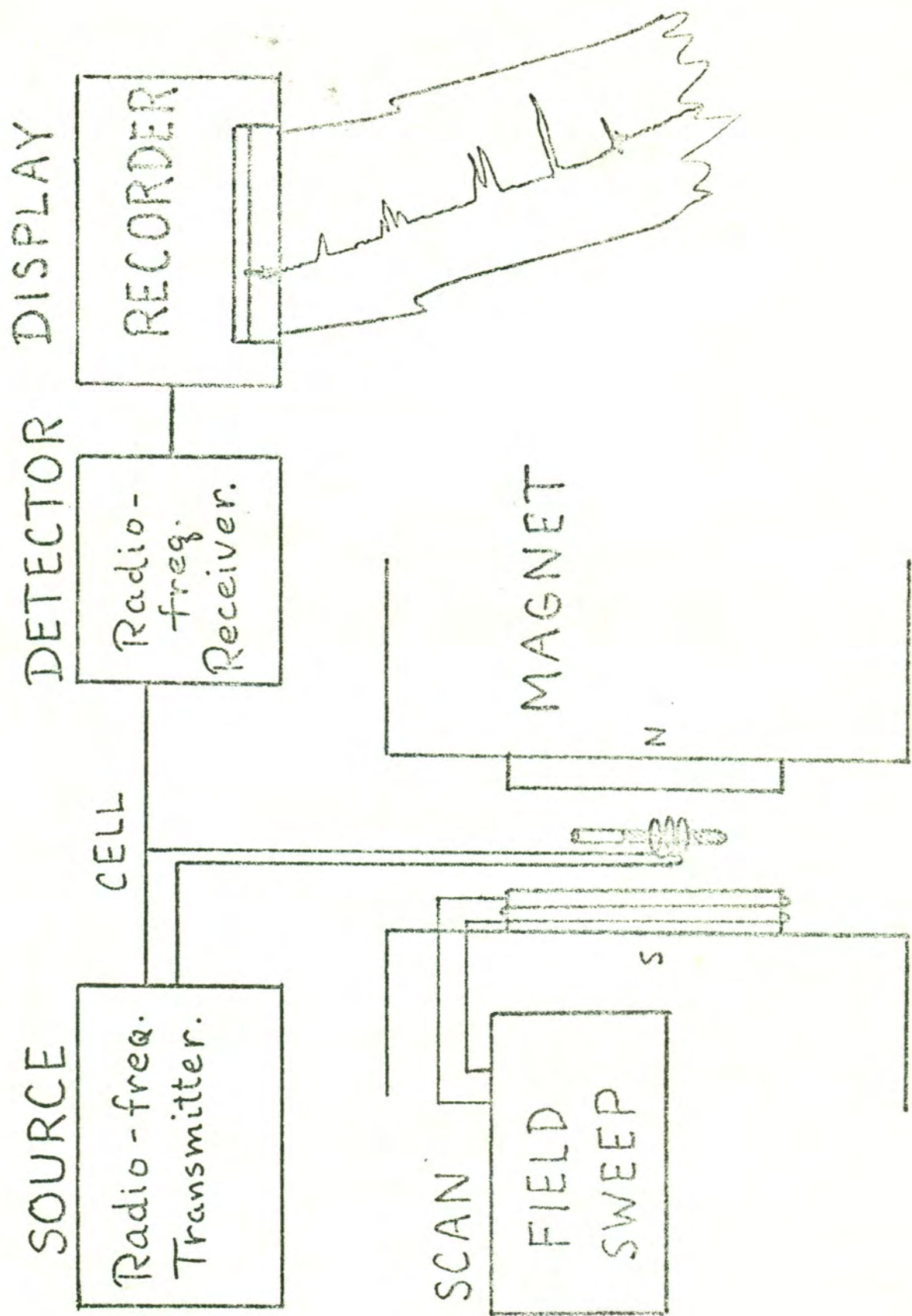

Fig. 27

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 transmitter coil. 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.
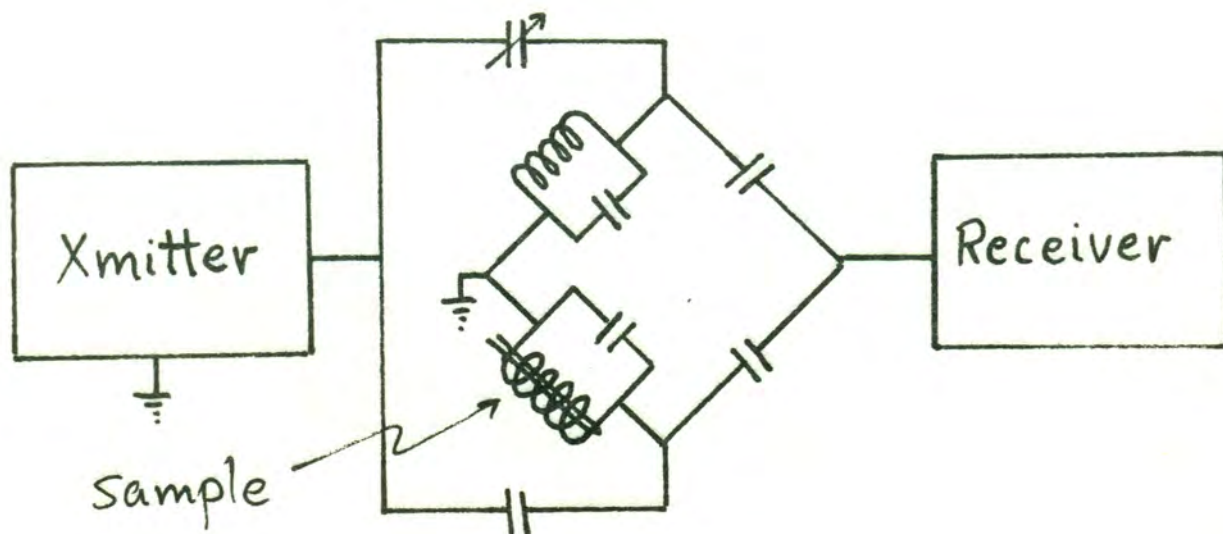
The presence of a sample in one arm unbalances the bridge when $\nu = \frac{2\mu H}{h}$. This circuit and the crossed coil system are useful when very weak NMR sigaals ($<10^{-9}$ volts) are detected in the presence of high transmitter power. It is then possible to amplify the r.f. signals a large amount 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) <u>Unbalanced</u> <u>direct-observation</u>. 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.
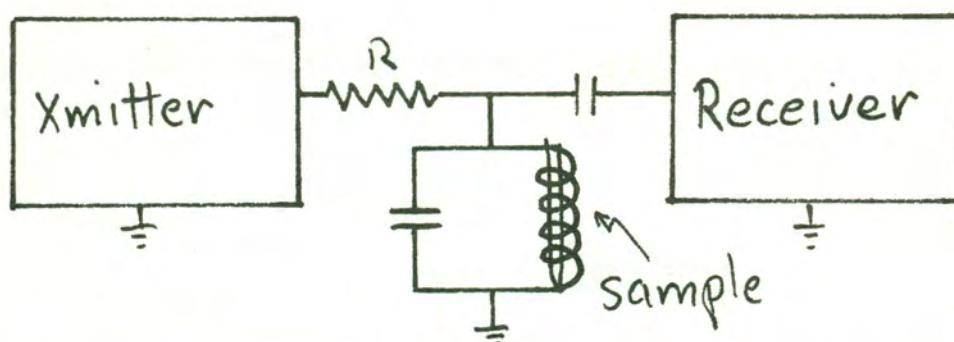

When $\nu = \frac{2\mu H}{h}$, the sample absorbs energy and the voltage across the coil drops. No balance controls are necessary, and the mechanical construction is simpler. Variations of this circuit are used in the Varian EM-300, EM-360, and T-60.

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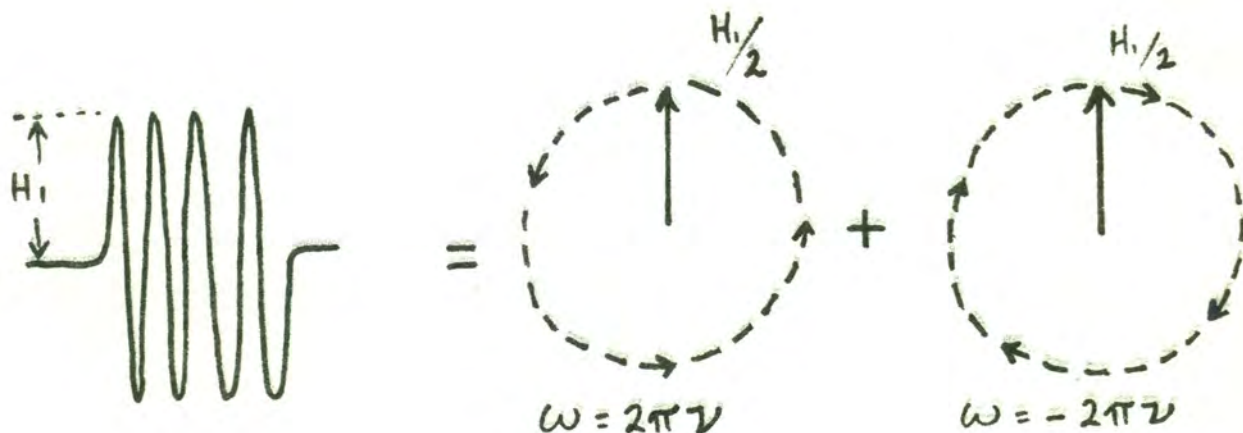
One component rotates in the same direction as the precession of the nuclear magnetic moment. The other rotates in the opposite direction and can be disregarded.

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_0 until is is precessing with a component in the X-Y plane. M_0 comes to a dynamic equilibrium due to the thermal relaxation time, T_1, which acts as a restoring force to align M_0 with H_0. At the point, the strength of the signal is determined by the component of M_0 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_0-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_0-H it falls off as the inverse square of the distance from the center of the resonance, and is symmetric about it.

FIG. 28

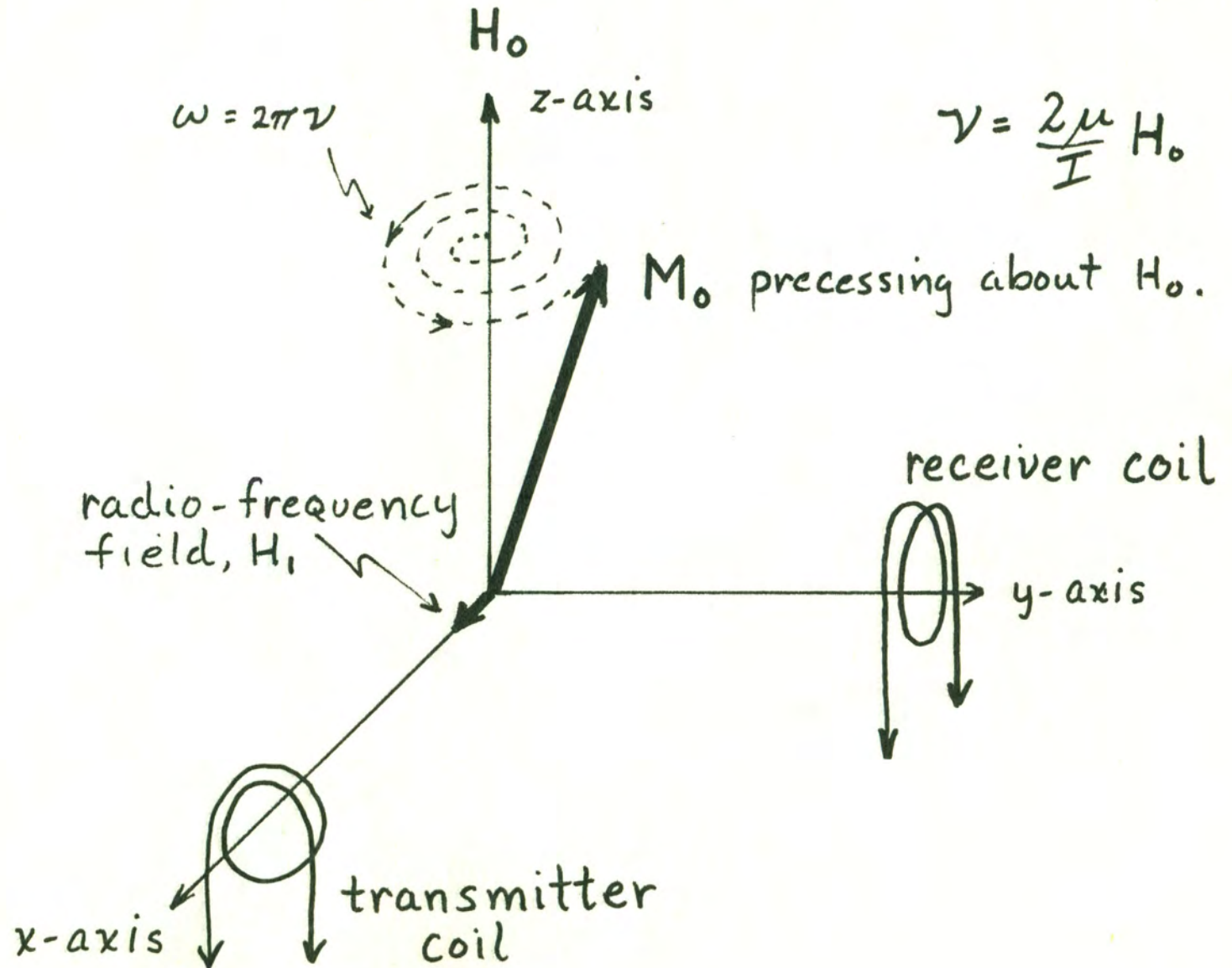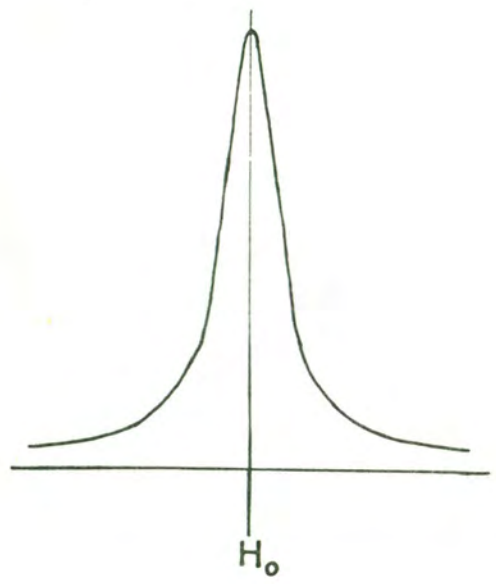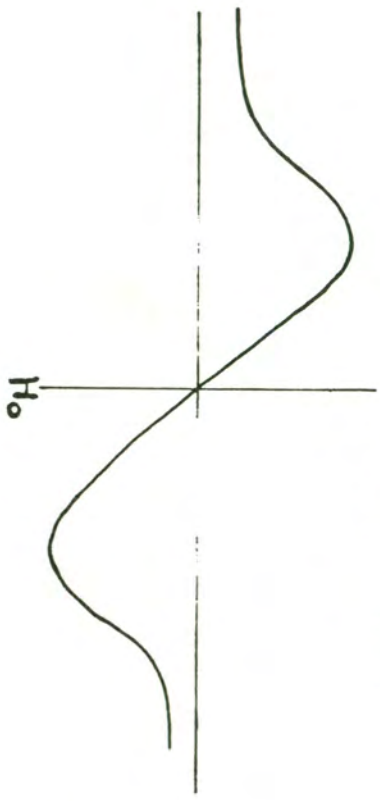# EFFECT OF RADIO - FREQUENCY FIELD , H_1.

H_0

z-axis

$\omega = 2\pi\nu$

$\nu = \dfrac{2\mu}{I} H_0$

M_0 precessing about H_0.

receiver coil

radio - frequency
field, H_1

y-axis

x-axis

transmitter
coil

FIG. 28

ABSORPTION MODE

$$V = \frac{M_0 H_1 rT_2}{1 + (rH_1)^2 T_1 T_2 + \left[rT_2(H_0 - H)\right]^2}$$

DISPERSION MODE

$$u = \frac{M_0 H_1 T_2^2 r (H_0 - H)}{1 + (rH_1)^2 T_1 T_2 + \left[rT_2(H_0 - H)\right]^2}$$

FIG. 29

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_o. 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.
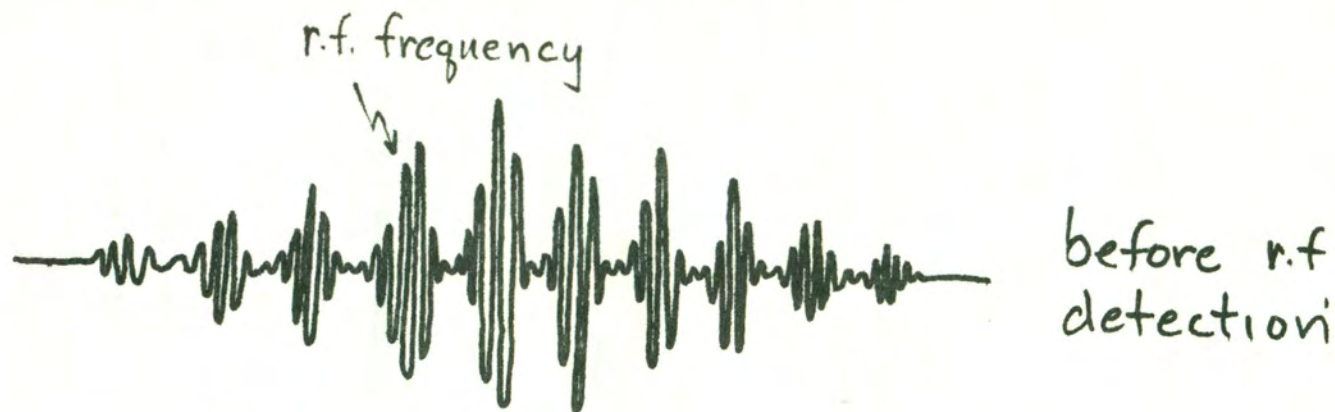
The u-mode and v-mode signals are affected differently by the modulation, so that when modulation is applied to a single-coil system which would otherwise detect only the v-mode, the character of the detected signal depends upon the adjustment of the audio-phase in the phase sensitive detector. As in 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
$$v = \frac{M_o H_1 \gamma T_2}{1 + (\gamma H_1)^2 T_1 T_2 + [\gamma T_2 (H_o - H)]^2}$$

DETECTION OF NMR SIGNALS.

r.f. frequency

before r.f detection

modulation frequency

after r.f. detection

absorption line envelope (v-mode)

after audio-freq. phase-sensitive detection.

Fig. 30

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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 <u>magnetogyric ratio</u> 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:
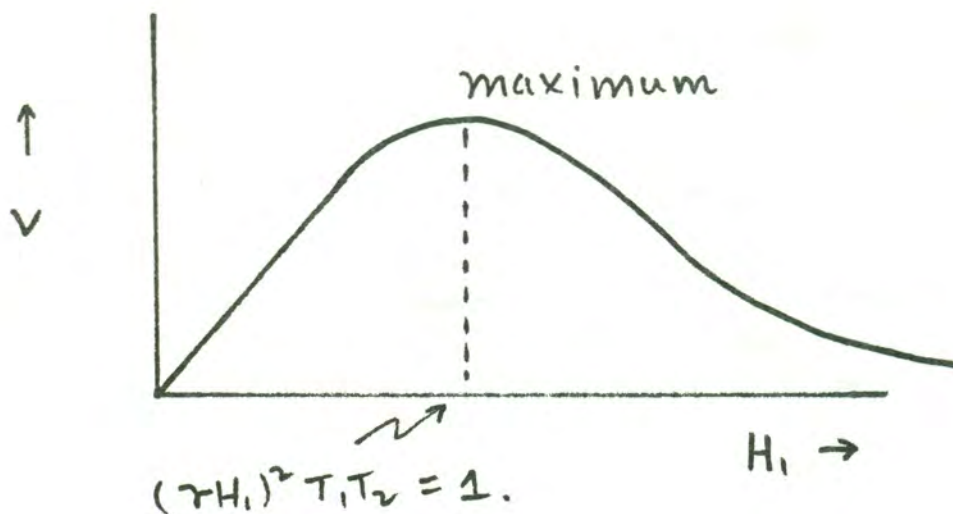
The signal increases linearly with H_1 at low power levels, then turns over and decreases as $1/H_1$ at high power levels. The maximum is rather broad and one can choose to work either at the maximum or a little below for weak samples. Strong samples should be studied on the linear part of the curve, 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 <u>saturation</u>. 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.
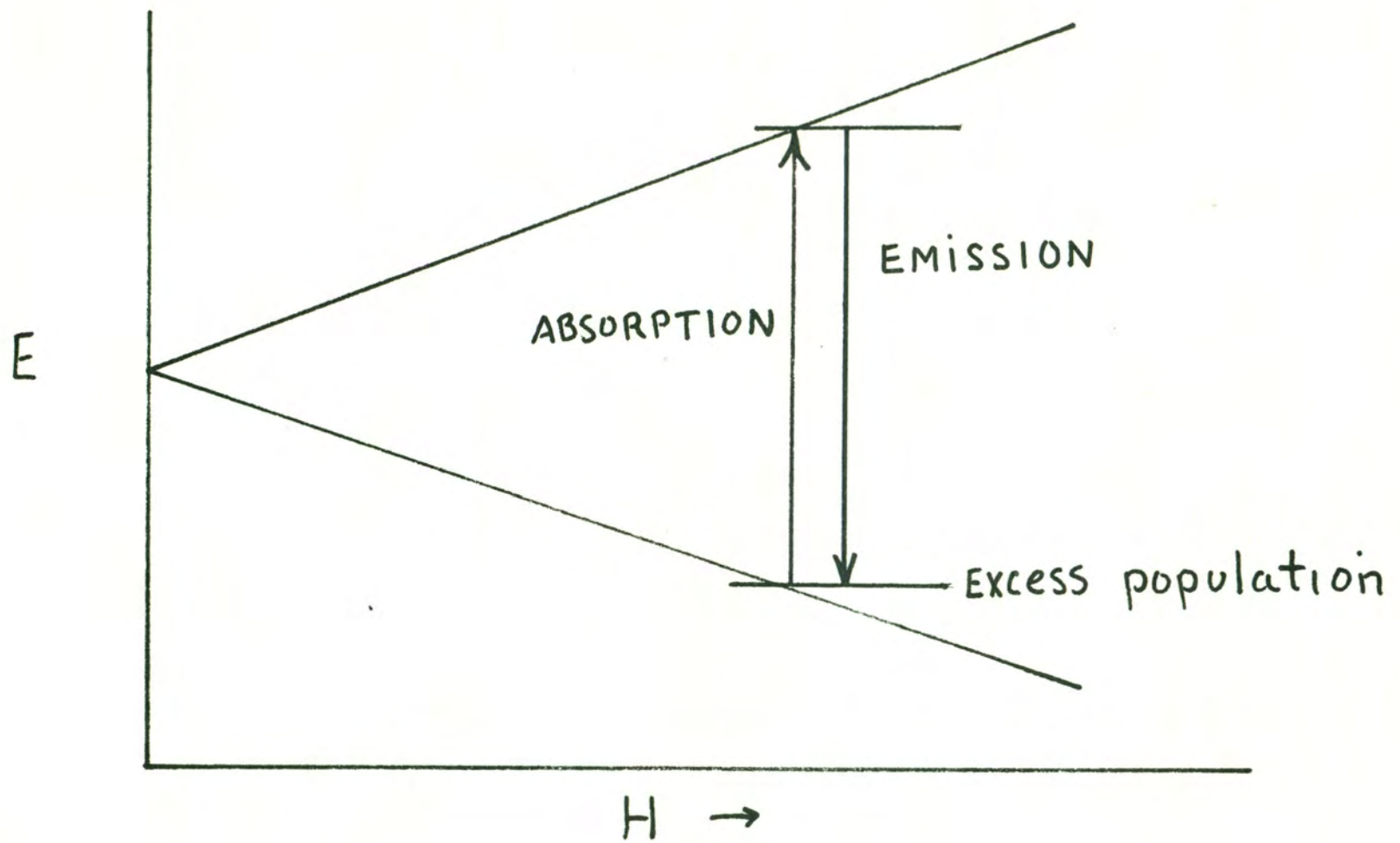
11. What effect does the sweep rate have on the appearance of the absorption lines.

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 <u>slow-passage</u> conditions where the nuclei remain in equilibrium. It is usually impractical

SATURATION

Fig. 31

ABSORPTION MODE SIGNAL REACHES MAXIMUM
STRENGTH WHEN $\quad (\gamma H_1)^2 T_1 T_2 = 1$

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

slow passage
lineshape 2 →

"wiggles" (ringing)
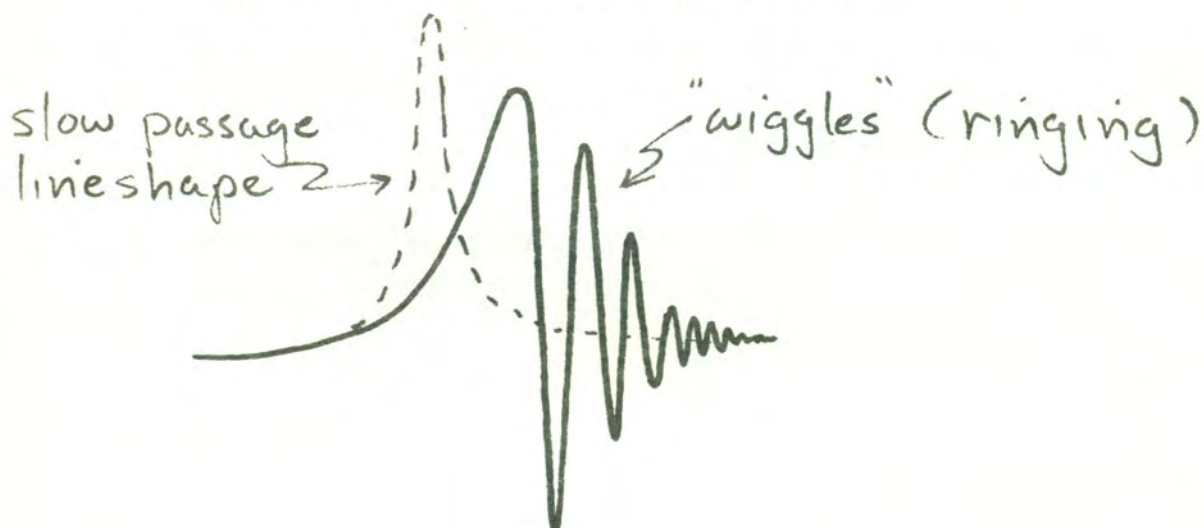
The line width is increased, the line is shifted in the direction of the sweep, and is followed by an oscillatory decay.

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.

HOMOGENEOUS   INHOMOGENEOUS

H_0 H_0

SPECTRUM
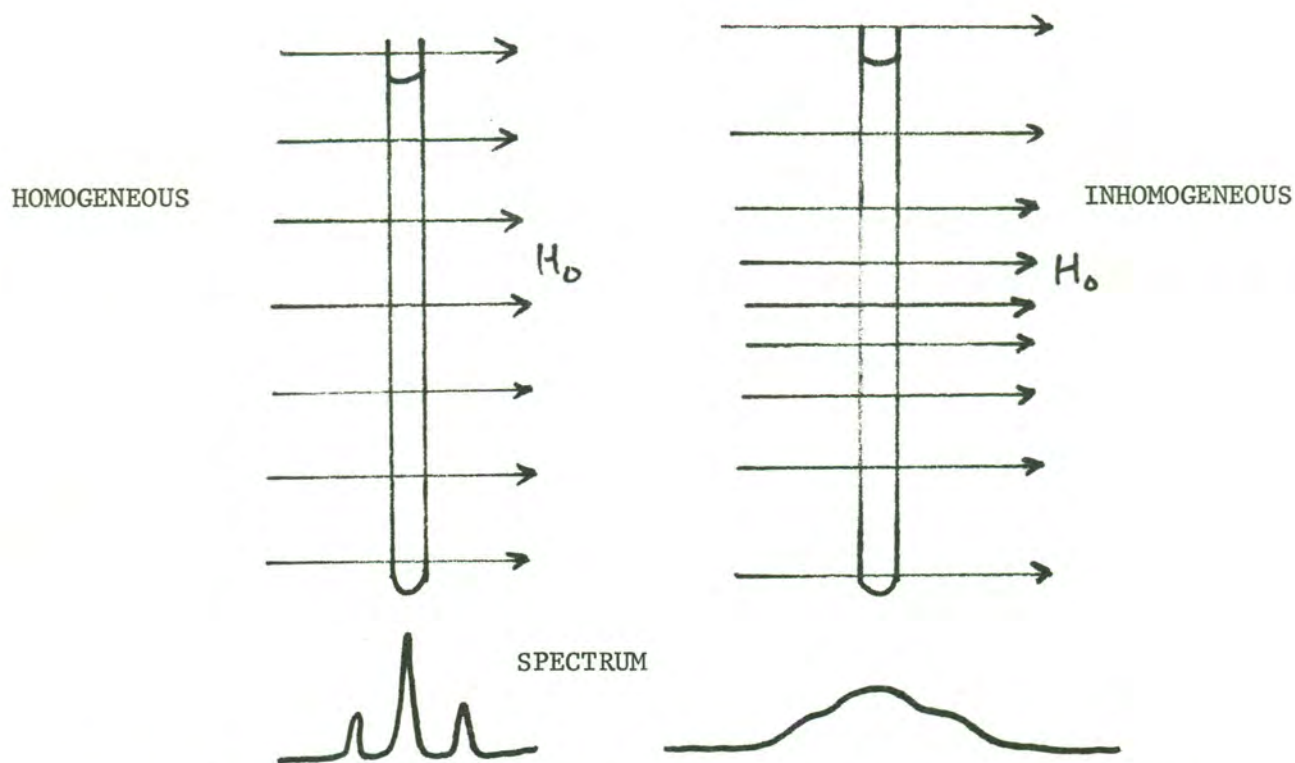
The problem of specifying magnet homogeneity is simplified by defining
a set of coordinate axes whose center is midway between the pole faces
at the center of the cylindrically symmetric air gap.
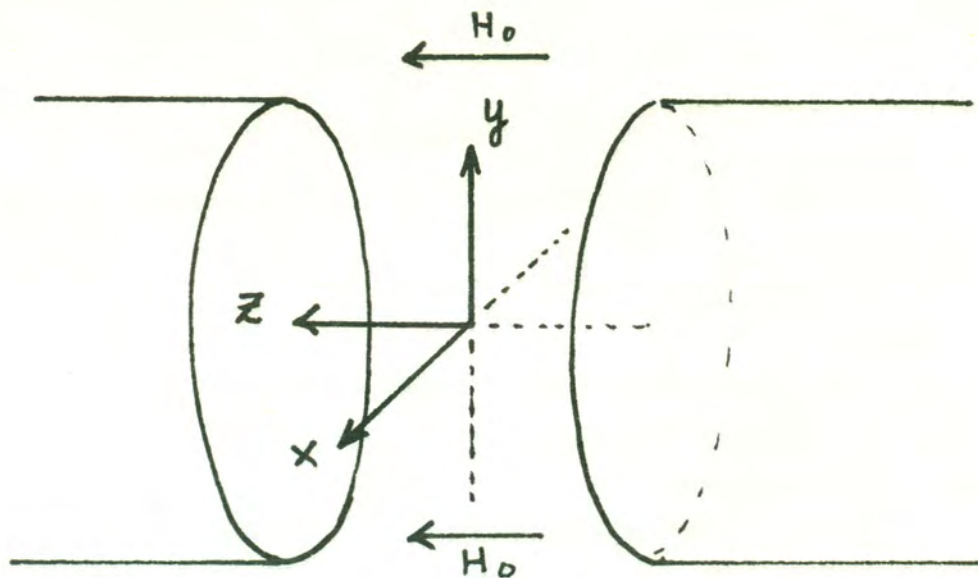
The axis parallel to the field is designated the Z-axis, while
the vertical axis is called the Y-axis and the horizontal axis
the X-axis.

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
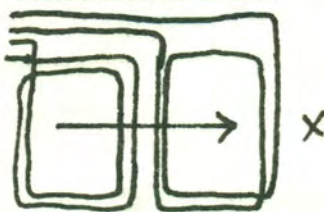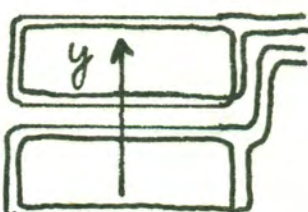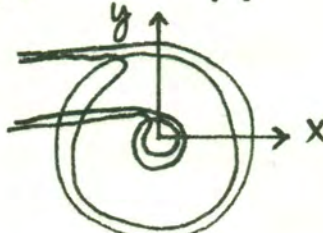   are optimized.

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 ^1H 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 spec-
trometers, 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</u> <u>side</u> <u>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 quan-
titative 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. The EM-300, however, for simplicity uses a y-t recorder. The chart is driven at a fixed rate and the field is scanned at various selected rates. Any product of sweep time (min.) and chart rate (inches/min.) which gives a spectrum 8" long can be used with a transparent overlay calibrated in ppm from TMS.

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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 <u>slow passage</u>
conditions is:

$$\int vdH = \frac{(const.)\ M_oH_1}{[1 + (\gamma H_1)^2 T_1 T_2]^{1/2}}$$

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 spec-
trometers with accessory digital computers can be programmed to
do a digital sum giving $\int vdH$ directly.

$$\int vdt = \frac{(const.)\ H_1N}{T(dH/dt)[1 + (\gamma H_1)^2 T_1 T_2]^{1/2}}$$

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 <u>intermediate</u> passage
conditions can be set up rather easily to meet the following conditions:

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

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,

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

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.

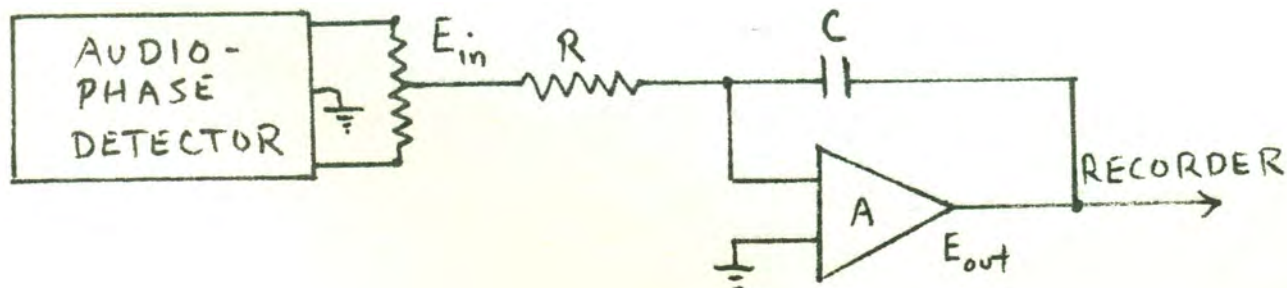Instrumentation for integration is very simple. A Miller-integrator type circuit is used as follows:

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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 <u>phase</u> 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
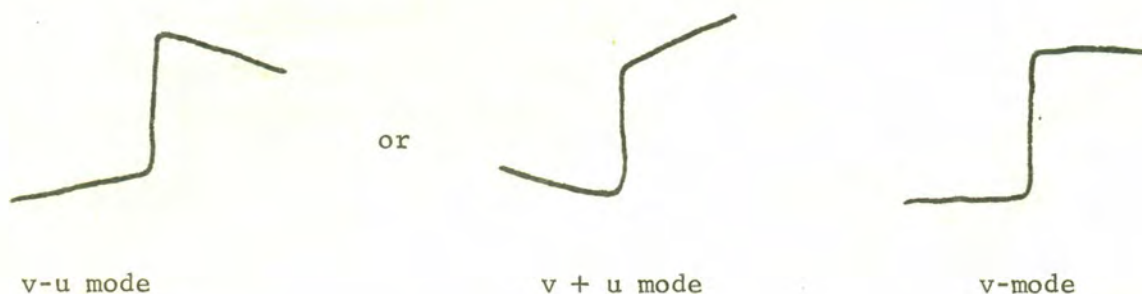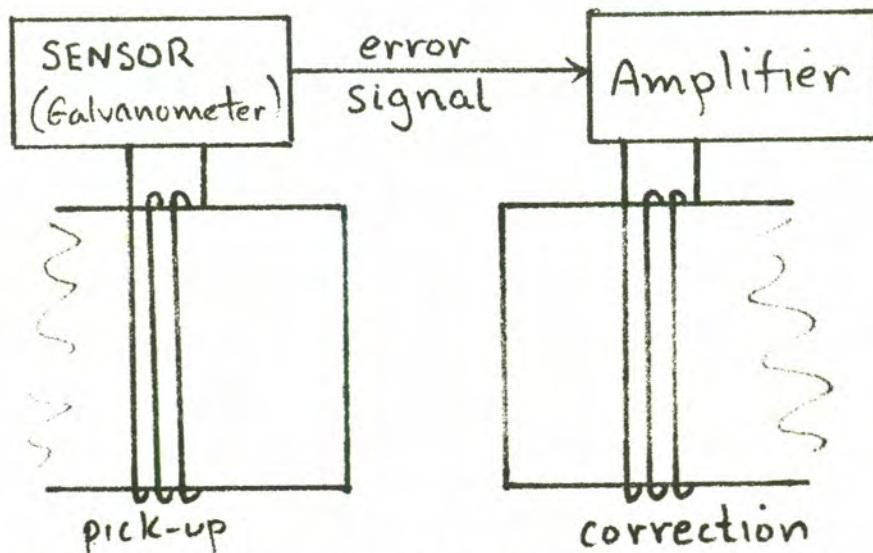
B. Magnetic field and Frequency Stabilization

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 feed-
back 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 either 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:

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

The circuit for accomplishing this is shown below:
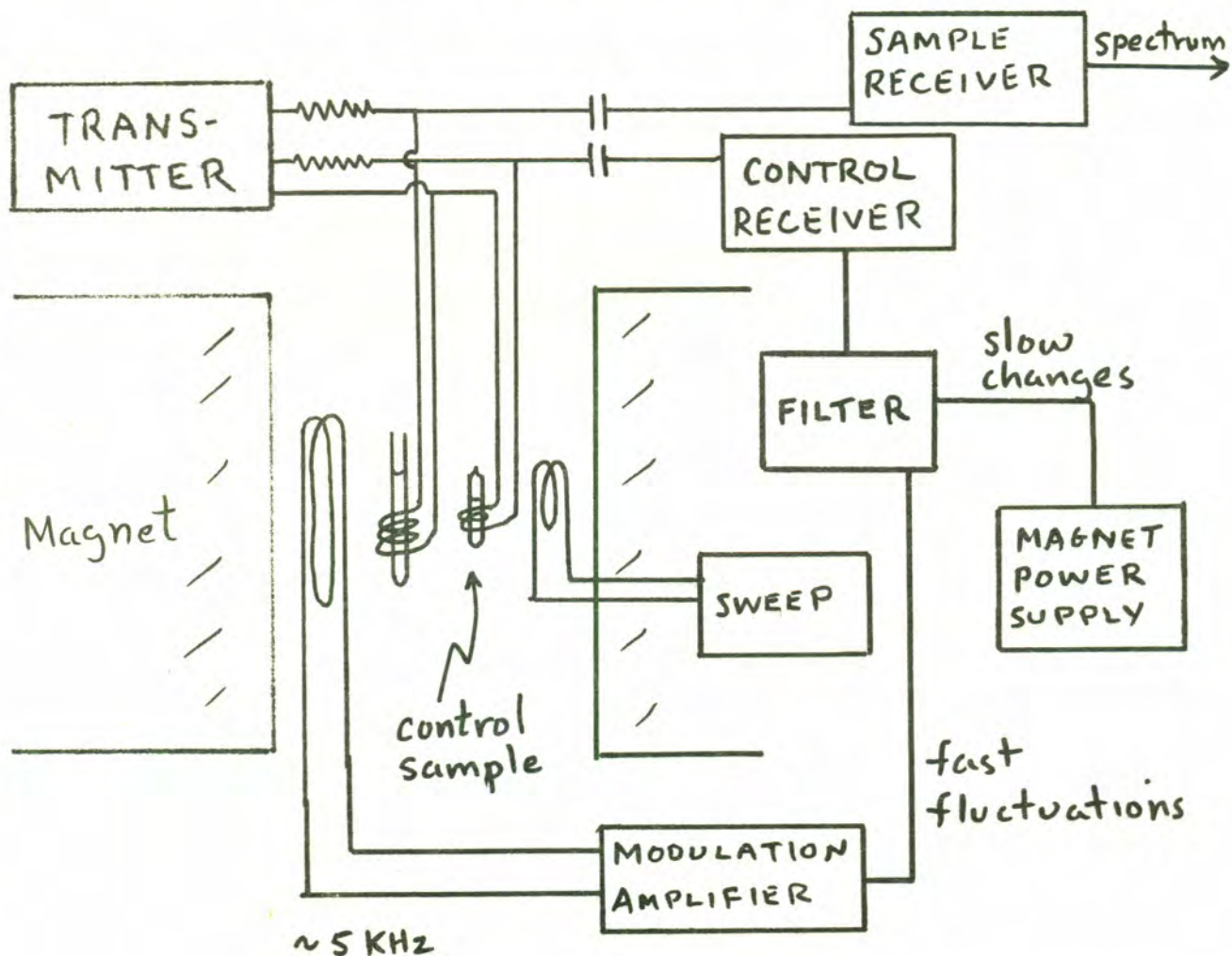
If the magnet field strength changes, the modulation on both samples changes to keep the system on resonance. However, a differential sweep coil which changes the field locally only at the control sample allows a sweep through the spectrum to be accomplished.

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 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 SIMILAR
TO TYPE USED IN T-60

Fig. 33
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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 <u>changes</u>
the frequency of modulation in the control circuit so that as
the field changes, 60.000 MHz + ν_{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 <u>not</u> <u>swept</u>. 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 <u>dispersion</u> signal, which has the necessary discriminator
shape shown below:
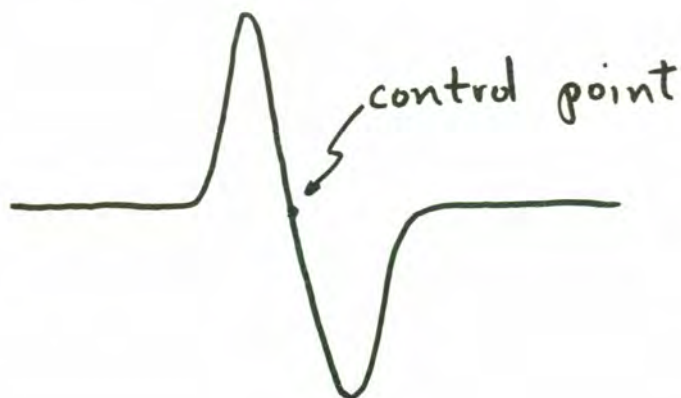
The output of the control phase detector drives correction coils
in the correct phase to cancel out any change of field strength.

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 deccupling 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 con-
tinuously. 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 $0.5^{\circ}C$ over a range from about $-50^{\circ}C$ to
$+100^{\circ}C$.

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
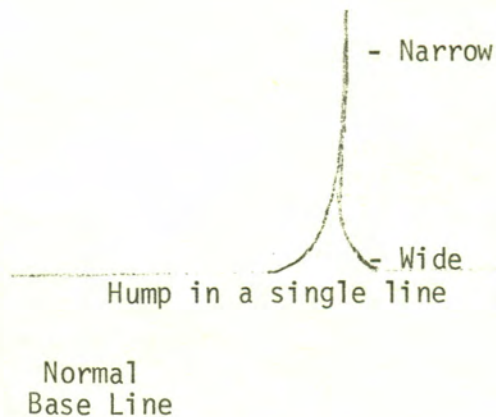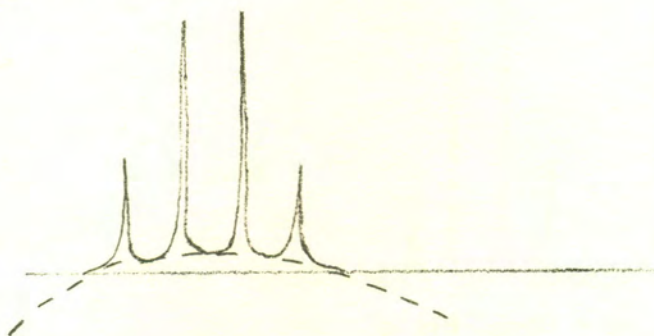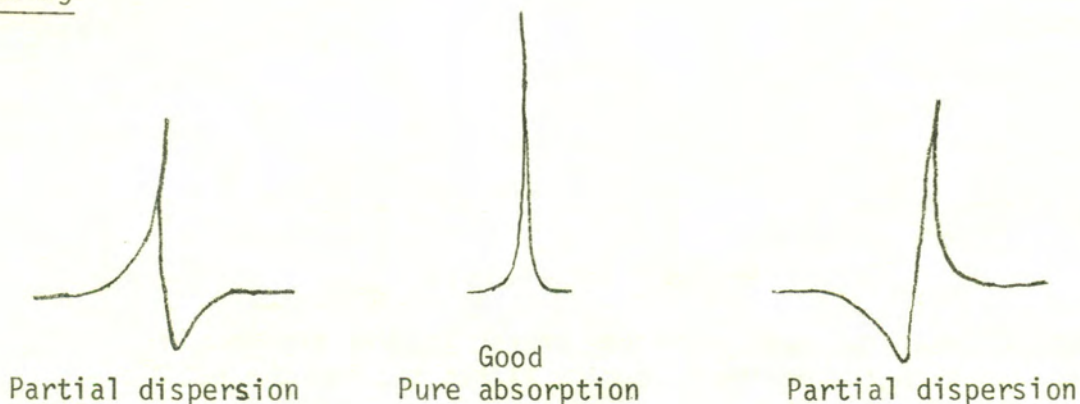of NMR spectroscopy which will be discussed in the next section.

1. <u>Ringing</u>
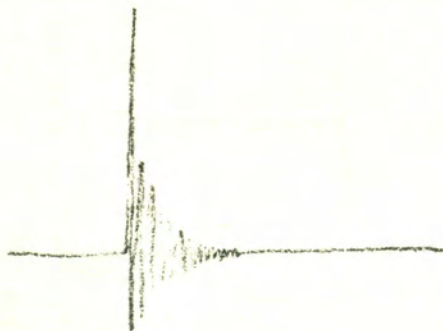
The decay pattern considered a characteristic
of good resolution should be exponential and
symmetrical.

2. <u>Hump</u> (lineshapes)

- Narrow

- Wide
Hump in a single line

Normal
Base Line

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

3. <u>Phasing</u>

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
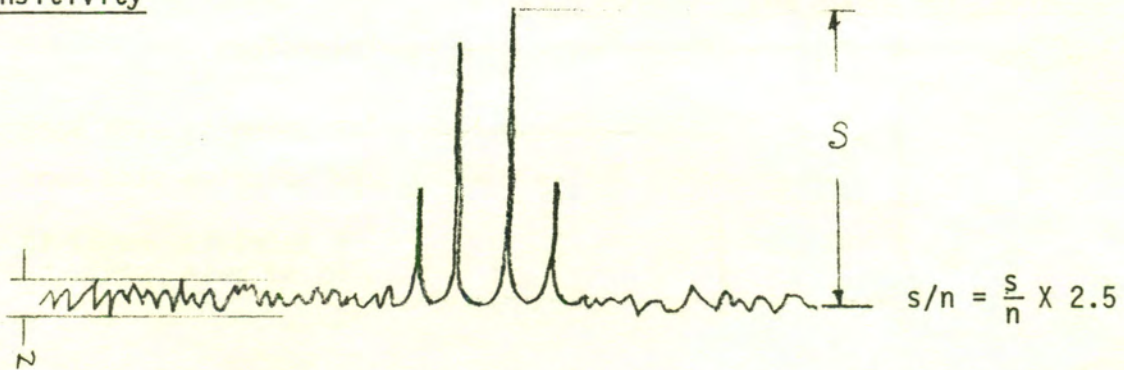of saturation.

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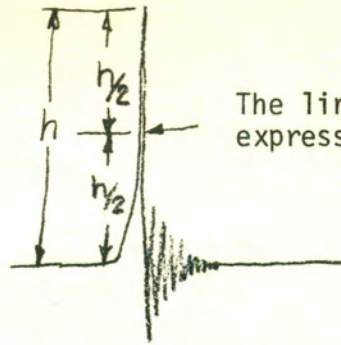
The line width at half height, normally expressed in Hz, could be expressed in ppm.

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.
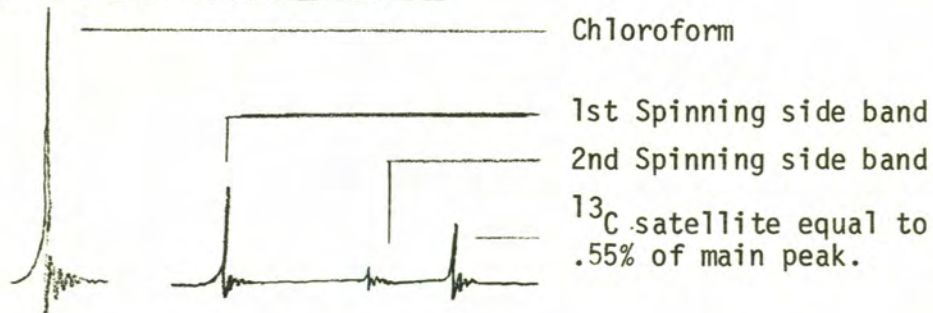
9. Filtering
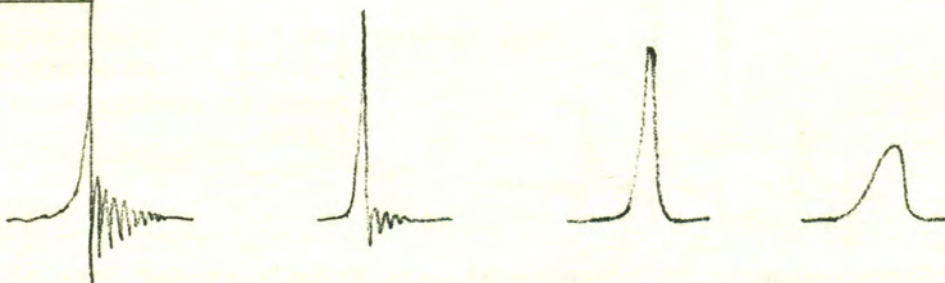
Increasing filter time constant →
← Increasing filter band width.

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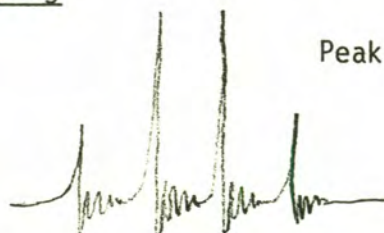
11. Spinning Side Bands and ^{13}C Satellites

———————————————————————— Chloroform

———————————— 1st Spinning side band

———————————— 2nd Spinning side band

^{13}C satellite equal to .55% of main peak.

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 Rations 1:2.5:2.5:1 theoretically is 1:3:3:1. Line width of inner peaks is greater than outer peaks.
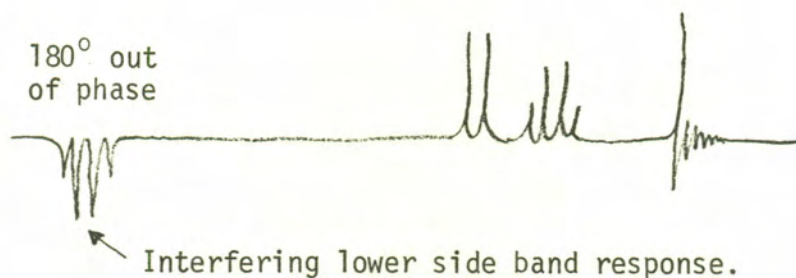
Strong signals in presense of weak signals suffer loss of amplitude and are broadened.

13. Modulation Side Bands

Lower Side Band   Center Band   Upper Side Band

f = modulation frequency

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

180° out
of phase

↗ Interfering lower side band response.

14. Integral Problems

Correct   Wrong Phase   Wrong Drift

Wrong Phase and Drift