CHAPTER 7

Nuclear Magnetic Resonance Spectroscopy

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7.1 INTRODUCTION

The intensity of research activity centered on nuclear magnetic resonance as a tool or delicate probe used to determine the fine structure of molecules is incredible when one considers the fact that the first experiment was performed in 1946. Nuclear magnetic resonance was not considered to have broad application until several years later when high resolution studies on ethanol revealed the absorption peaks of the ethanol hydrogens were not alike but were dependent on the environment and the nature of the atom to which they were bonded. The outcome of these initial experiments was the development of stable high resolution instruments capable of probing the fine structure about hydrogen, fluorine, phosphorus, boron, and other isotopes with a non-zero spin number.

Nuclear magnetic resonance like infrared and ultraviolet is the process whereby energy from an external source is absorbed and causes a change or resonance to an "excited" or high energy state. The energy required for nuclear magnetic resonance is in the low energy or long-wavelength radiofrequency end of the electromagnetic spectrum. The equivalent of the monochromator in other forms of spectroscopy is an electrically varied radiofrequency or a variable magnetic field. The detector, unlike the usual

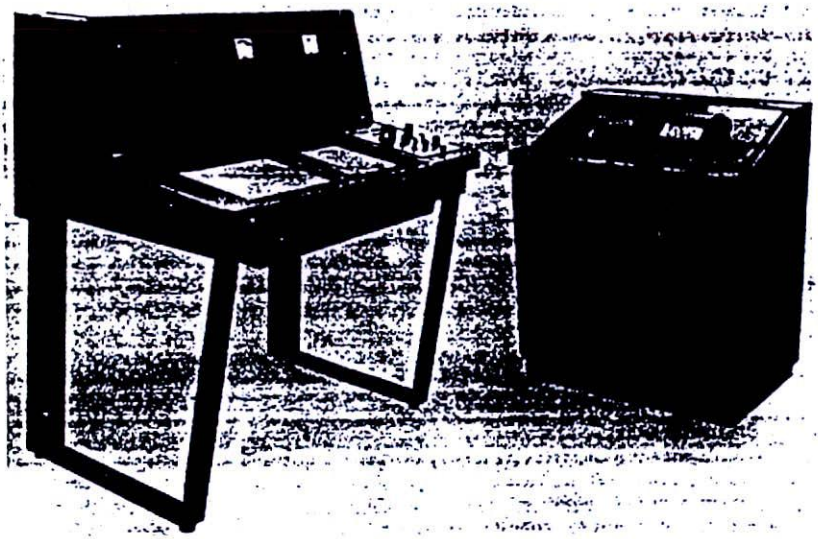


FIGURE 7.1: Varian A-60 A nuclear magnetic resonance spectrometer. Courtesy of Varian Associates, Palo Alto, California.

photomultiplier of ultraviolet or infrared, is a radio receiver. Thus, nuclear magnetic resonance requires a source of energy that resonates or is in tune with the nuclear magnetic and a detector. Figure 7.1 illustrates a nuclear magnetic resonance spectrometer; a schematic diagram of the same instrument is presented in Fig. 7.2.

The prerequisite for application of nuclear magnetic resonance is that the atom under examination has a nuclear spin number I greater than 0. When this condition is satisfied ($I > 0$), as, for example, in ^{1}H, ^{13}C, ^{15}N, ^{19}F, and ^{31}P, then magnetic resonance can be induced. The peculiar property associated with a spinning nucleus with $I = 1/2$ is that, although the nucleus is a symmetrically charged sphere, a spinning charged body creates a magnetic field. In effect, the spinning nucleus acts as a tiny bar magnet. If this spinning nucleus is placed in a strong external magnetic field, it will align with or

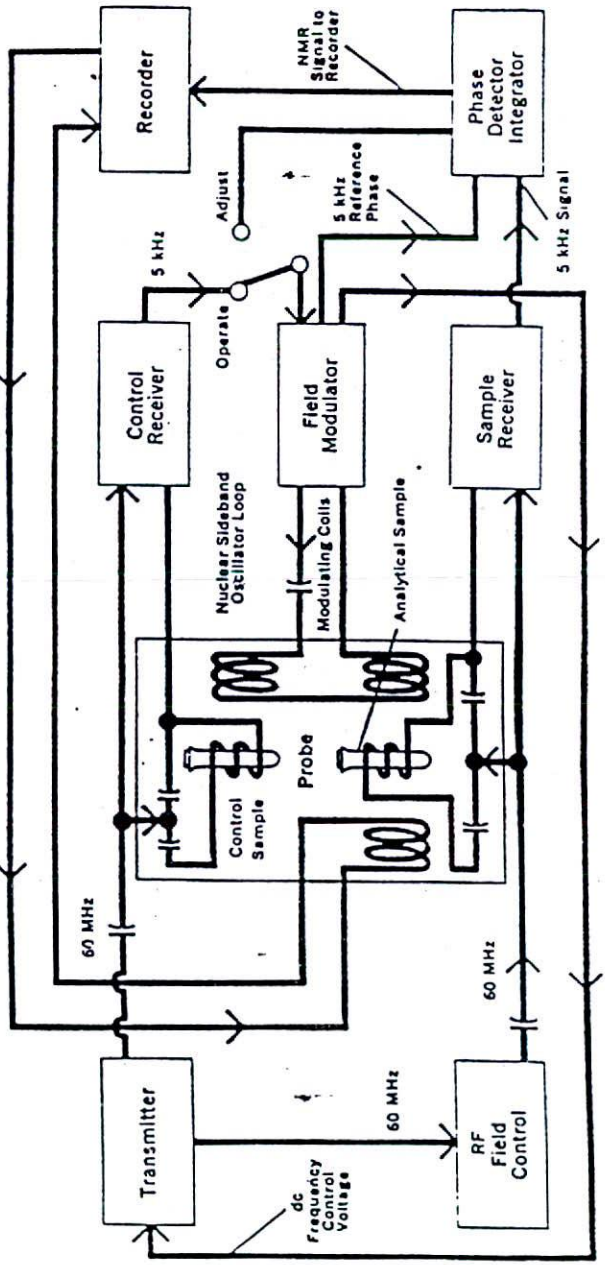


FIGURE 7.2: Schematic block diagram of an A-60 A spectrometer. Courtesy of Varian Associates, Palo Alto, California.

against the external field much the same as the needle on a compass aligns with the earth's magnetic field. It should be noted that alignment with the field represents the stable or low energy state (−1/2) and alignment against the field (+1/2) is the high energy or excited state. An upward transition from the lower state can be accomplished if a discrete energy "packet" can be supplied to the low energy spinning nucleus.

Proceeding into the theory of nuclear magnetic resonance, what property of this spinning nucleus can be utilized and externally supplied with this

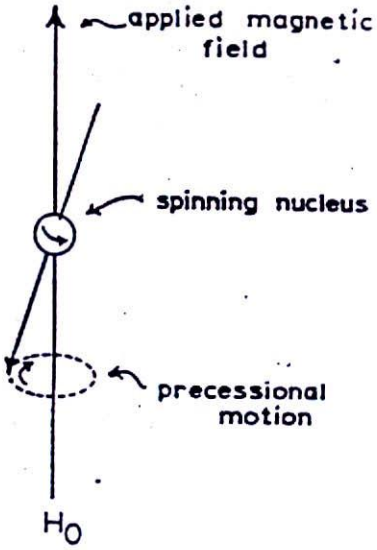


FIGURE 7.3: Precessional motion of a spinning nucleus aligned with an applied magnetic field (H_0).

discrete energy to cause resonance? In other forms of spectroscopy "resonance" with electronic energy levels or natural bond vibrations gives rise to absorption of energy and promotion to an excited state. In nuclear magnetic resonance a property of a spinning nucleus, in contrast to a static bar magnet, is that the magnetic vector is not perfectly aligned or parallel with the externally applied field vector, but rotates around the applied vector much as a freely suspended gyroscope rotates about the earth's gravitational field vector (Fig. 7.3). Further this "precession" about the applied magnetic vector occurs with a frequency that is proportional to the applied magnetic field, according to the Larmor precession theorem.

$$\omega = \gamma H_0 \tag{7.1}$$

The constant of proportionality γ, known as the gyromagnetic constant, is dependent on the nucleus; H_0 is the applied field strength in gauss, and ω, the angular momentum, is 2π times the frequency (ν) of precession. Rearranging to give

$$\gamma = \frac{\omega}{H_0} = \frac{2\pi\nu}{H_0} \tag{7.2}$$

$$\gamma/2\pi = \frac{\nu}{H_0} \tag{7.3}$$

we find that for a given nucleus ($I = 1/2$) the precessional frequency ν increases as the externally applied field increases. For the hydrogen nucleus the

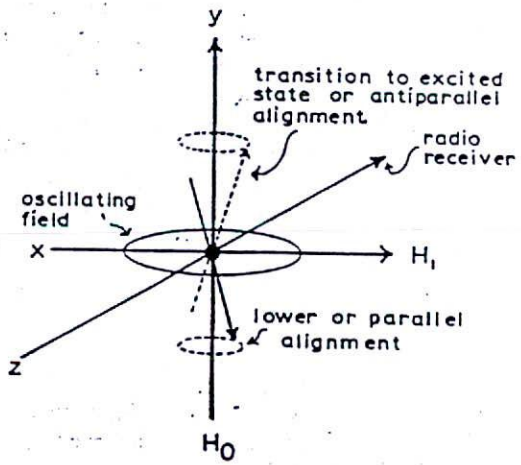


FIGURE 7.4: Alignment of a spinning charged nucleus in the applied field H_0; resonance of the oscillating field H_1 with the precessing nuclear vector induces a transition to the high energy state.

following values for frequency: 20, 40, 60, and 100 MHz correspond to applied fields of 4700, 9400, 10,400, and 17,300 G respectively. Examination of an idealized model in Fig. 7.4 reveals that a hydrogen nucleus aligned with an external field (H_0) of 10,400 G precesses with a certain frequency. If another electrically generated oscillating field (H_1) is now applied perpendicular to H_0, then at the frequency of oscillation in H_1 that corresponds exactly to the frequency of precession of the nuclear vector, the latter will absorb energy and "flip" to the high energy antiparallel or excited state. In the process of flipping, the precessing nuclear magnet induces a current in a radio receiver oriented perpendicular to the other two fields and tuned to the nuclear precessional frequency (60 Mc).

The idealized model does not account for the disturbing effect of thermal motion which effectively reduces the number of protons that align in the applied field. This limitation can be resolved by using stronger applied fields such as the 17,300-G magnet in the 100-mC instrument, which gives a greater proportion of aligned protons. While aligned in this strong field, all of the nuclear magnets theoretically would align parallel or in the low energy state $(-1/2)$. This would be the situation at 0°K, but at room temperature thermal motions allow only a slight excess aligned in the low energy state. Irradiation of this collection of nuclear magnets with the proper radiofrequency gives equal probability of an upward transition and a downward transition. Fortunately there is a slight excess in the lower state and a signal can be detected from the finite excess of upward transitions.

After irradiation the two states have been equally populated, and unless the lower state can be repopulated in excess, there is no net absorption of energy and the signal fades. In infrared and other spectroscopic methods of analysis the return to ground state is rapidly achieved by emission of the excess energy and by thermal motions. The process of return in nuclear magnetic resonance to an excess population in the lower state requires some mechanism for dissipation of the excess energy. Emission of energy only takes place with an exchange of nuclear states and no net increase in the lower state.

Transition between states that occur by mechanisms other than emission are responsible for the return to an excess in the ground state, an essential feature for nuclear magnetic resonance. These relaxation processes can be divided into spin-spin relaxation and spin-lattice relaxation. In its simplest form the former process, often referred to as "transverse relaxation" (T_2), is a transfer of energy by a mutual exchange of spin states contributing little to the return to equilibrium. Spin-lattice or longitudinal relaxation (T_1) is the principal mode of establishing the initial equilibrium state. Violent thermal motions of nuclei produce random oscillatory magnetic fields some of which have frequency components equal to the precessing, excited nuclear magnet. By a transfer of the excess energy from the precessing excited nucleus to the reasonating random thermal fields dissipation as thermal energy is accomplished and the ground state is repopulated.

The natural line width observed for absorption of energy is proportional to the reciprocal of the relaxation time. If the latter is short, for example, in solids, or viscous liquids, or in the presence of paramagnetic molecules (O_2), the resonance signal is broad. Accordingly, moderately long relaxation times give rise to sharp peaks.

Examination of this simplified view of the theory of nuclear magnetic resonance gives some indication of the instrumental design. The strongest, homogeneous magnet obtainable is desirable to achieve the greatest field alignment and highest resolution in the spinning nuclei. The gyromagnetic ratio of the particular spinning nucleus being examined dictates the oscillating radiofrequency that will resonate with the precessing nuclear magnetic vector.

The detector, situated at a right angle to the oscillating input, is tuned to receive radio frequency generated by the moving nuclear magnet as it undergoes an upward transition. In the center is placed the sample, which responds in part to each of the components just discussed. Alignment of the nuclear spin state with the applied field (H_0) on the y axis is followed by irradiation of the sample by a component of oscillating current on the x axis. When the second field (H_1) oscillates at the same frequency as the precessing nuclear magnet, energy is absorbed in the lower spin to flip to the higher energy spin

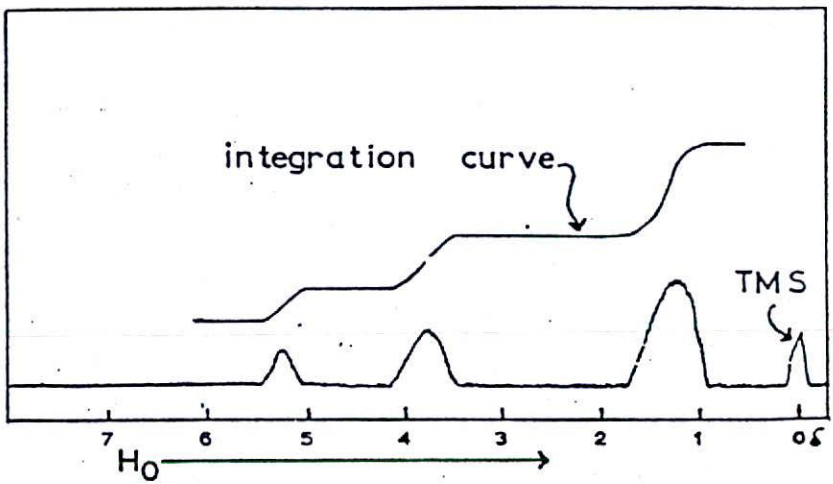


FIGURE 7.5: Low resolution spectrum of ethanol.

state. During the transition a signal is generated by the flipping nuclear magnet that is detected by the receiver installed on the z axis. The entire process of alignment, irradiation, detection, and recording the absorption complete the sequence. The theory of nuclear magnetic resonance predicts that a proton or any nucleus with nonzero spin should have an absorption peak at a frequency of radiation that varies with field strength according to the gyromagnetic ratio. If this is true, then all protons should resonate at a certain frequency in a given magnetic field. Several years after the initial demonstration of nuclear magnetic resonance an experiment utilizing a stronger magnet revealed that under higher resolution not one line for all protons but three resonance lines were observed for ethanol Fig. 7.5. The three lines, with relative areas under the curve of 1-2-3, were separated by only a few milligauss in a strong magnetic field. Almost immediately the significance of this finding was realized—the resonance absorption lines of protons varied over a narrow range because local magnetic fields in the environment

of the proton affected the resonance frequency. The three observed peaks for ethanol represented the protons in different environments—on oxygen, C_1, and C_2; the distances between lines were termed the "chemical shift."

High resolution nuclear magnetic resonance thus required not only a homogeneous applied field and a stable applied oscillating radio frequency for irradiation but also a means of varying and measuring either the field or frequency over a very small range with a high degree of accuracy. Commercial instruments presently available utilize both methods—that is, varying radio-frequency in a stable field or varying field at a stable frequency.

7.2 CHEMICAL SHIFT

Application of nuclear magnetic resonance for qualitative analysis is dependent on the chemical shift between protons and the nature of the forces causing the chemical shift. Examining the protons of the classical example, ethanol, there are six protons in three different environments or rather bonded to three different atoms. The chemical shift is the relative position of the resonance signals for the three types of protons. Since absolute measurement of a milligauss in a field of several thousand gauss is not practical, the relative position of an absorption signal is usually assigned in reference to a standard mixed with the sample. The requirements for this standard are such that it should be stable, easily purified, readily available, soluble, and easily removed from the sample. Of the many possible standards considered it appears that tetramethylsilane, $(CH_3)_4Si$ (TMS), is the standard of choice. In addition to meeting the requirements just stated it gives rise to a very sharp resonance signal at a higher field than most other protons. Thus a proton is designated as having a chemical shift at a lower field by some arbitrary value from the TMS absorption peak.

Assuming operation of an instrument with a variable magnetic field and a stable frequency, examination of a solution of ethanol and tetramethylsilane in an inert, nonprotonated solvent (see Fig. 7.5) gives three major peaks in addition to the TMS reference peak. The oxygen-bound proton appears at the lowest magnetic field, followed in order by the protons on C_1 those on C_2, and the methyl protons of TMS, the latter appearing at the highest field. According to theory, the protons absorb radio frequency of a certain value that is dependent on the applied magnetic field.

The protons of ethanol, although subjected to the same applied field, experience the effect of very minute local fields that varies with the immediate chemical environment. For example, the field about the CH_3 protons of ethanol is the summation of applied field and the local field created by the bonding electrons about the proton nucleus. In addition to aligning the nucleus the spinning electrons also align with the applied field, but usually in the opposite direction. The electron motion creates a tiny induced field in the

applied field H_0 and changes the field experienced by the proton H_{eff} according to the following:

$$H_{eff} = H_0 - \sigma H_0 \tag{7.4}$$

where σH_0 is the induced field generated in the applied field H_0. This "shielding" alters the effective field in the immediate vicinity of the proton nucleus to some value either higher or lower than the applied field. The protons attached to C_2 experience "shielding" relative to the protons on C_1 and oxygen.

Regarding the values used in assigning relative chemical shifts, certain conventions have been adopted to adjust for the various commercially available instruments. Table 7.1 lists several nuclei with their resonating frequency

TABLE 7.1: Resonating Frequency at Various Field Strengths

NMR frequency			Frequency at various fields		
Nucleus	Spin no.	Megacycles/ kilogauss field	9400 G	14,092 G	23,490 G
^1H	1/2	4.26	40.00	60.00	100.00
^2H	1	0.65	6.15	9.21	15.35
^{11}B	3/2	1.37	12.93	19.25	32.08
^{13}C	1/2	1.07	10.06	15.08	25.14
^{14}N	1	0.308	2.89	4.33	7.22
^{17}O	5/2	0.577	5.42	8.13	13.55
^{19}F	1/2	4.00	37.65	56.44	94.07
^{31}P	1/2	1.72	16.19	24.29	40.48

at various fields. Although most routine examinations utilize a 60-Mc instrument with a field of 14,092 G and employ TMS as the reference, a method of comparison to results obtained using instruments at other field strengths is used in assigning chemical shifts.

Three parameters have been used to relate the position of a resonance signal relative to TMS. The recording of a nuclear magnetic resonance spectrum employs an xy recorder. Using the common 60-Mc instrument with a field that can be varied several hundred milligauss on either side of 14,092 G the x axis represents increasing field strength in moving from left to right on the chart. Since the field is varied and the radio frequency is stable, it would be logical to report peak positions (chemical shift) relative to the standard in milligauss. This method of assignment has not been adopted, but rather the resonance peak is recorded in units of cycles per second (hertz) shift from the reference; almost all protons can be observed at 60 Mc to appear over a range of about 700 Hz. If an absorption peak was observed at a $\Delta\nu$ of -100 Hz (the negative sign indicates a lower field) relative to tetramethylsilane at 60 Mc, this would be the same as a $\Delta\nu$ of -67 Hz recorded at 40 Mc or -167 Hz recorded at 100 Mc. Therefore the position of the chemical shift recorded in cycles per second is field dependent. A

better method of reporting or assigning resonance signals that is field independent is based on the following:

$$\delta = \frac{H_r - H_s}{H_r} = \frac{\Delta\nu \times 10^6}{\text{Oscillator frequency (Hz)}} \tag{7.5}$$

The chemical shift δ is the difference in field positions of the reference (H_r) and the sample (H_s) divided by the field strength of the reference in gauss. Since the positions are recorded in cycles per second, the ratio of the shift from TMS in cycles per second ($\Delta\nu \times 10^6$) to the oscillator frequency of the instrument also gives δ, the chemical shift, expressed in parts per million (ppm). Applying this to the example of a −100-Hz resonance signal at 60 Mc

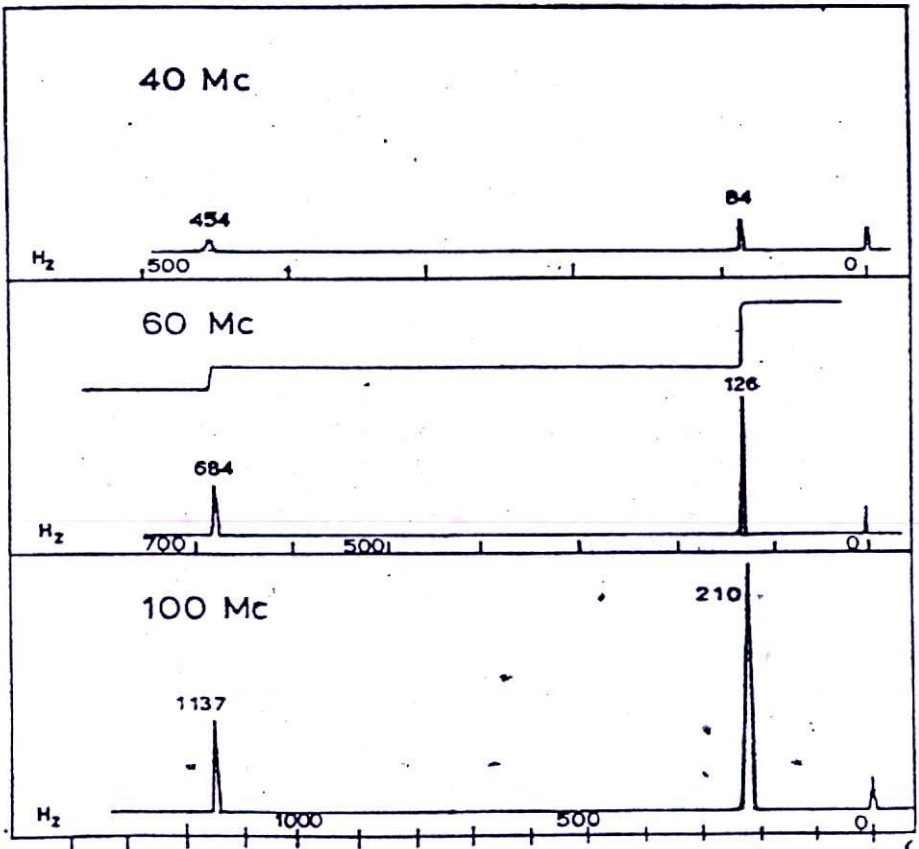


FIGURE 7.6: 40, 60, and 100 Mc nuclear magnetic resonance spectra of acetic acid in $CDCl_3$ with TMS standard.

the δ is 1.66 ppm. Examination will show that δ, independent of field strength and oscillator frequency, is 1.67 ppm in the example just given for all instruments using TMS as a standard. A further modification has been introduced that assumes TMS as the standard:

$$\tau = 10.00 - \delta \tag{7.6}$$

This simply states that the field position τ is relative to the TMS peak, which is at the highest field, +10.00. Most organically bound protons fall at a lower field position ranging from −1.0 to 9.5 τ. In the example shown in Fig. 7.6 acetic acid has absorption signals at −84 and −454 Hz in the top spectra (40 Mc), at −126 and −684 Hz when examined at 60 Mc, and at −210 and −1137 Hz run at 100 Mc. The chemical shift for these peaks is $\delta = 2.10$ and 11.37 ppm or $\tau = 7.80$ and −1.37. The reference TMS peak appears to 0 Hz on the right-hand or high field side of all three spectra.

Another property of nuclear magnetic resonance that has great utility is that the strength of the signal or rather the total area under the resonance peak is proportional to the number of protons resonating at that frequency. All commercial instruments are equipped with an integrator or a device to measure the area under a curve. The horizontal line in Fig. 7.5 and across the middle spectra in Fig. 7.6 is a recording of the area under the respective resonance peaks. The area encompassed by the low field peak (11.37 ppm) is one-third that of the 2.10-ppm signal.

A. EXPERIMENTAL METHOD

Instrument design and the properties of nuclear magnetic resonance, like ultraviolet and infrared, place certain restrictions on sample preparation and examination. Short relaxation times and the resulting broad, poorly resolved resonance signals observed in solids and viscous liquids recommends the use of dilute solutions. Solvents commonly used are carbon tetrachloride, carbon disulfide, deuterochloroform ($CDCl_3$), deuterium oxide, deuteroacetone (CD_3COCD_3), d_6-dimethylsulfoxide ($CD_3—SO—CD_3$), and other deuterated liquids. Almost any hydrogen-containing solvent can be used if the resonance signals for the solvent protons do not interfere or overlap with the sample signals; the signals due to the high concentration of solvent will be very strong.

The solutions are normally prepared in the range of 15–20% w/v in a minimum volume of 0.5 ml. The reference, tetramethylsilane, is usually prepared as a 1% solution in the solvent to be used. In polar solvents the sodium salt of a trimethylsilylalkane sulfonate is used. The sample is put in a high precision tube designed for the instrument and inserted in the sample holder (probe). After the instrument has been stabilized, scanning the sample and integration usually can be accomplished in 15 min.

B. INTERPRETATION

The first cursory glance at a nuclear magnetic resonance spectrum places it somewhere between an infrared and an ultraviolet spectra in appearance—certainly more complicated than an ultraviolet spectrum, but not quite as formidable as infrared. The first attempts to assign peaks in any of the three forms of spectroscopy can be an awkward experience. Although all three analytical methods are essential in structure determination, it is generally

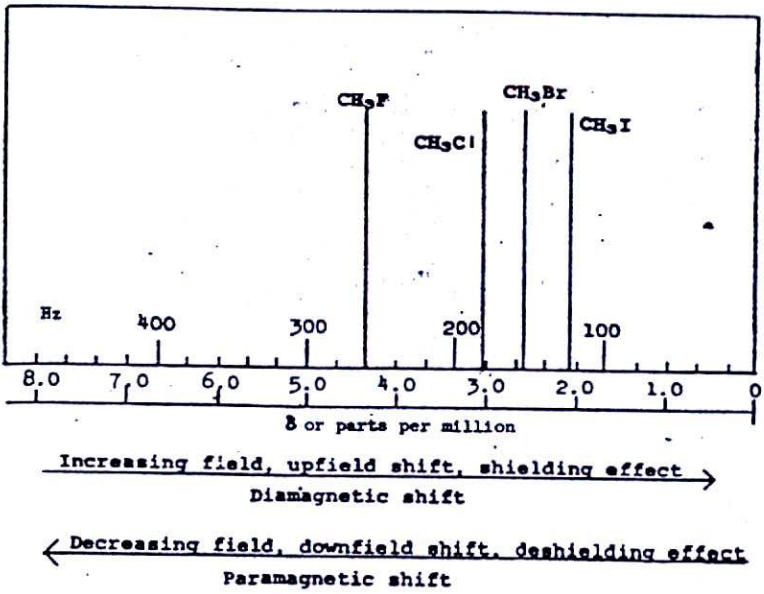


FIGURE 7.7: Scan from low to high fields.

agreed that nuclear magnetic resonance yields the most information about the structure of an organic molecule.

Before proceeding to the theory behind the chemical shift and interpretation of spectra the student should be aware of the terms used in nuclear magnetic resonance. As noted in the introduction, the spectrum in Fig. 7.7 recorded from left to right represents a "scan" or "sweep" from lower to high fields usually terminating at the TMS peak on the extreme right-hand side (high field or upfield), which is arbitrarily set at 0 Hz or 0 ppm (δ). Almost all proton peaks will appear at lower field (downfield or to the left of the TMS peak. The range in cycles per second that usually is examined is either -1000 or -500 Hz for a 60-Mc instrument and should include all but the most unusual proton resonance signals. Various mechanisms give rise to "shielding" the nucleus from the applied field. A diamagnetic shift or shielding

effect is a chemical shift to a signal at high field (to the right). A downfield shift to absorption at lower applied fields is indicated by a paramagnetic shift or deshielding. Interpretation of the spectra and assignment of peaks will be greatly simplified if a qualitative understanding of the shielding mechanisms is acquired. With the aid of empirical rules and the published data on nuclear magnetic resonance often complete assignment of the structural environment of protons can be made.

Shielding or deshielding of the proton nucleus is caused by local magnetic fields generated in the applied field by the electrons in the immediate vicinity of the resonating nucleus. Tetramethylsilane gives a single sharp peak at high field because of strong shielding. Silicon, having relatively low electronegativity, permits the generation of strong local fields caused by the circulating silicon electrons. These fields are in opposition to the applied field and in effect partially neutralize the latter. This requires the application of a higher field to overcome the neutralization and induce resonance in the methyl protons, hence the term "shielding." Analogy can be seen in the methyl halides, where progressing to less electronegativity F → Cl → Br → I gives an increase in the shielding; the methyl proton peaks in the respective halides appear as seen in Fig. 7.7 at 4.36, 3.05, 2.68, and 2.16 ppm. An important feature of the opposing field caused by circulating electrons is that the strength of this induced field is proportional to the applied field, therefore the chemical shift in cycles per second for a given proton increases as the applied field is increased: A shift of −100 Hz at 60 Mc moves to −167 Hz at 100 Mc.

Many additional examples of a deshielding effect (downfield shift) with increasing electronegativity can be shown, for example, in a comparison of methane, methyl alcohol, and methylamine. The C—H of methane absorbs at high field (0.3 ppm) compared to the C—H of methylamine (2.5 ppm) and methanol (3.6 ppm).

Further consideration of the effect of the applied field on electrons reveals another mechanism contributing to the chemical shift. In the presence of an applied field, electrons circulate in a perpendicular path and generate a magnetic field opposite to the applied field in the center of the electron "coil" (Fig. 7.8). A proton or any nucleus within the coil is shielded in proportion to the strength of the electron field. In contrast a nucleus outside or on the periphery of the circulating electrons experiences a reverse field. In Fig. 7.8 a nucleus X within the "coil" is shielded from the applied field; a nucleus Y outside of the coil is deshielded. This deshielding is the summation of both the applied and induced fields, and less applied field is required to resonate the nucleus. Deshielding by this mechanism is termed a "paramagnetic effect."

Paramagnetic effects are particularly important in relatively rigid molecules that are oriented in the applied field. As an illustration consider the circulating [illegible] of Fig. 7.9 [illegible] the electrons of toluene; both the ring and

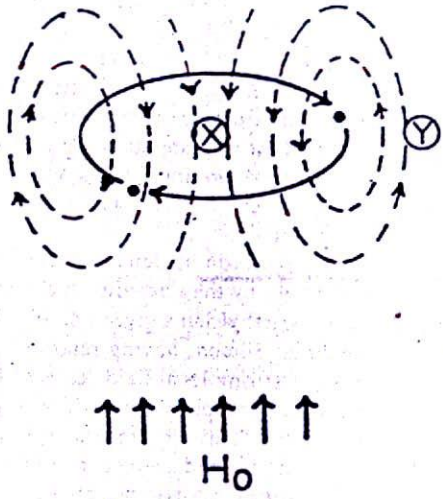


FIGURE 7.8: Effect of an applied field. Dashed lines represent fields generated by circulating electrons. Nucleus X is shielded and at a higher field. Nucleus Y is deshielded and resonates at a low applied field.

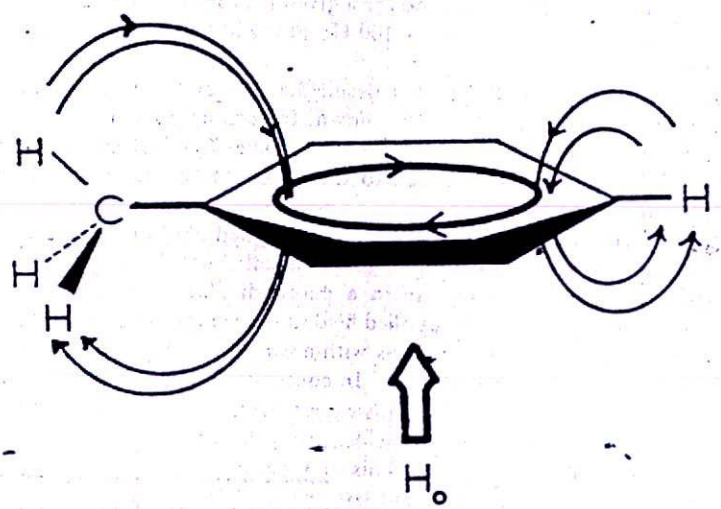


FIGURE 7.9: Paramagnetic effects in relatively rigid molecules. Deshielding in aromatic systems. The methyl group of toluene resonates at 2.32 δ and the ring protons are at 7.17 δ.

methyl protons experience the additive effect of the applied and the paramagnetic field. Subsequently, less applied field is required to resonate both types of protons; the aromatic signals are at 7.2 ppm and the methyl at 2.3 ppm. When this mechanism, specific proton orientation with respect to an induced field, is operative it is termed an "anisotropic effect." The electron current in an oriented molecule such as an aldehyde, being diamagnetic within the coil, has a strong paramagnetic component in the region of the aldehydic proton, sufficient to allow resonance of this proton in a very low applied field, about 9 to 10 ppm. Anisotropic effects can be observed in many types of organic molecules such as acetylene, olefins, ketones, esters, acids, and nitriles, and the magnitude of the effect is dependent on the specific orientation of the proton in the field.

In summary, many factors in addition to electronegativity contribute to the chemical shift; paramount are the diamagnetic (shielding), paramagnetic (deshielding), and anisotropic effects.

Complete assignment of resonance signals to particular protons in a given molecule is relatively easy if some prior knowledge of the structure is available. Examination of an "unknown" will reveal the nature of the groups to which the protons are bonded and integration will give the relative numbers of each type of proton. Particularly in relatively simple molecules, complete structural determination often can be made on the basis of the nuclear magnetic resonance spectrum.

Many reference tables are available for assistance in correlation of proton peaks and the assignment of structure. The student is referred to the bibliography for excellent sources of reference tables and charts.[1-7] Experience gained in assignment peaks can be invaluable in interpretation, and for this reason the student is referred to the *Varian Associates Catalog of Spectra*, volumes I and II, for a diverse range of examples.[7]

Much of the value of nuclear magnetic resonance as a tool for structure determination is because of the relatively narrow region for absorption by a particular proton. It will be apparent throughout spectral interpretation that the shielding or deshielding mechanisms are only operative over a very short distance and the effect is rarely extended beyond two saturated carbon atoms. A further consideration that will be obvious is that protons on flexible or freely rotating groups or molecules will experience an averaging of the local field effects, whereas rigid molecules or conformationally restricted protons, particularly in cyclic systems, will have unique field effects not shared by the flexibly system.

The conventions adopted in Figs. 7.10 and 7.11 and discussion have been to refer to peak positions in δ (ppm) relative to TMS at 0: Conversion to τ values is a simple matter since $\tau = 10 - \delta$. Several examples will be noted, but it is generally safe to assume that progression from a methyl ($CH_3-C\leq$) to a methylene ($\geq C-CH_2-C\leq$) and finally a methine $CH-C_3\leq$, all other parameters remaining constant, results in a slight downfield shift. Further,

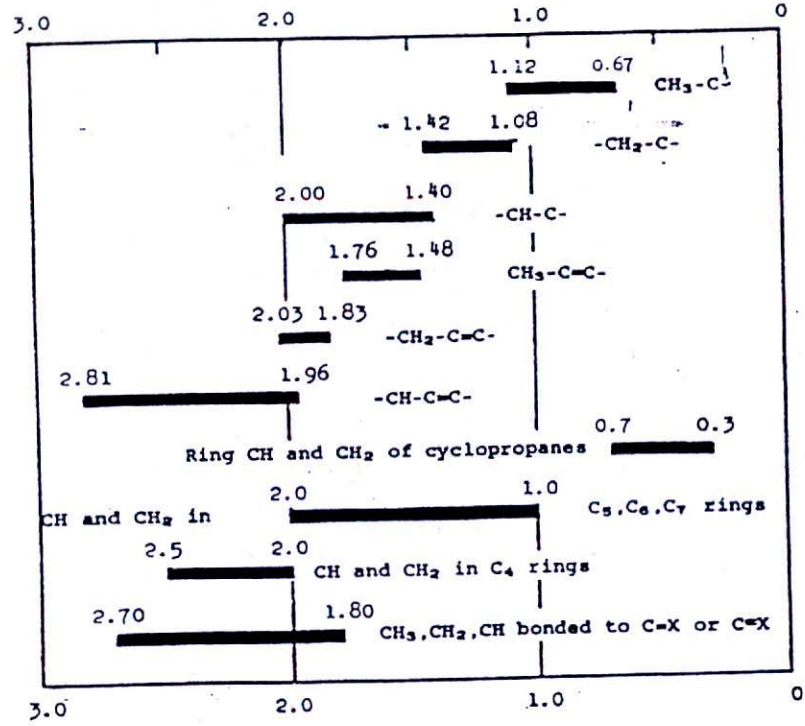
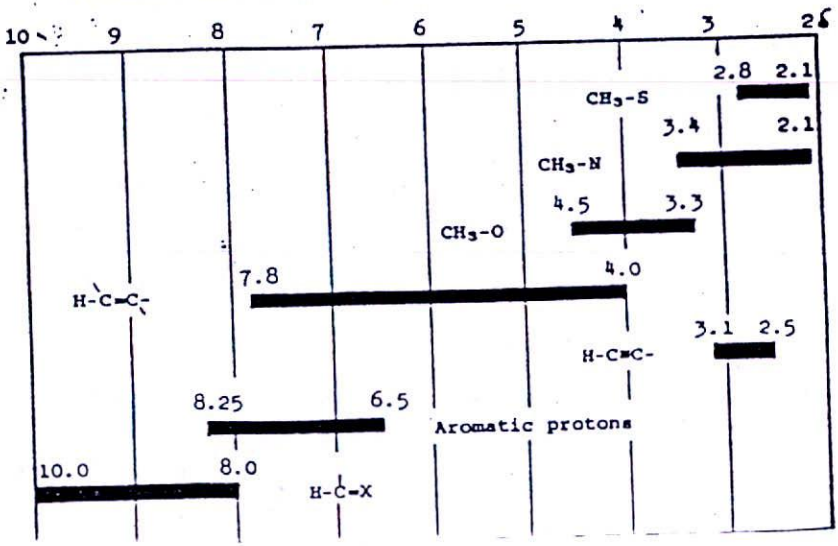


FIGURE 7.10: Resonance peak assignments for CH_3—C, CH_2—C, and CH—C.

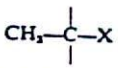


in addition to conformational effects, other factors such as ring currents will alter the positions of methylene and methine protons; therefore, cyclic systems will not be analogous to their paraffinic counterparts.

C. ALIPHATIC PROTONS

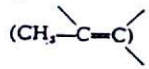
Methane protons resonating at 0.3 ppm undergo a downfield shift to 0.8 ppm when one of the protons is replaced by carbon. Successive replacement gives absorption at 1.3 and 1.4 ppm for the methylene and methine protons, respectively (Fig. 7.10). Precise assignment of the methyl can be made, but in general a range from 0.6 to 1.12 ppm will include all paraffinic methyls at least one saturated carbon atom removed from a group other than a saturated carbon. The inclusive range for methylenes is 1.08–1.42 ppm and for methines is 1.40–2.0 ppm.

When these groups are situated one carbon removed from an electronegative group or atom X,

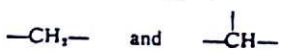


where X represents halogenic, olefinic group, oxygen, nitrogen, or a similar group other than a saturated carbon, a slight paramagnetic shift is experienced and in general the respective field positions for the CH_3, CH_2, and CH will be shifted downfield about 0.1 to 0.2 ppm from their paraffinic counterpart.

A methyl attached alpha to an olefin



appears in the region from 1.48 to 1.76 ppm; corresponding shifts for



are in Fig. 7.10. Direct substitution on an aromatic ring results in deshielding to between 1.95–3.15 ppm. The toluene methyl appears at 2.44 ppm, the CH_2 of ethylbenzene at 2.62 ppm, and the methine of isopropylbenzene at 2.87 ppm. Methylenes or methine protons in cyclic systems experience deshielding due to the ring currents, and the shift is dependent on ring size. Large shielding effects are noted in cyclopropanes with signals appearing between 0.3 and 0.7 ppm. Protons attached to four-membered cyclic system resonate near 2.5 ppm with C_5 and larger rings at an intermediate position, between 1.0 and 2.0 ppm. Double bonds in the ring enhance the deshielding of methylenes to a range between 2.0 and 2.7 ppm.

Substitution of a methyl alpha to a carbonyl or nitrile (ketone, aldehyde, ester, amide, nitrile, oxime, etc.) creates a relatively strong field in the proton region that gives a large downfield shift to between 1.80 and 2.70 ppm. For example, the methyl protons of acetic acid, acetone, and acetaldehyde resonate at 2.10, 2.17, and 2.20 ppm, respectively; the usual region for methyls

alpha to a carbonyl is between 2.0 and 2.6 ppm, for methylenes 2.1–2.4 ppm, and methines 2.4–2.6 ppm.

Protons on carbon bonded to halides, nitrogen, oxygen, sulfur, and other atoms experience strong deshielding (see Fig. 7.11). The role of electronegativity in deshielding mechanisms cannot be discounted as evidenced by the correlation between the electronegativity of the halide in the halomethanes and the chemical shift (Fig. 7.7). Methyl sulfides or mercaptans resonate between 2.1 to 2.8 ppm. Variations in the substitution on nitrogen may

TABLE 7.2: Shoolery's Effective Shielding Constants

Group	σ_{eff}, ppm[a]
Cl	2.53
Br	2.33
OR	2.36
I	1.82
CR=O	1.70
C≡N	1.70
SR	1.64
$NR'R^2$	1.57
C≡CH	1.44
$CR'=CH'R^2$	1.32
CH_3	0.47

[a] $\delta = 0.233 + \Sigma\sigma_{eff}$.

affect the signal; for example, a simple aminomethylene near 2.5 ppm is deshielded to between 3.2 and 3.1 ppm in *N*-methylacetamide and sulfonamides and in quaternary salts moves to 3.4 ppm. Protons on carbons alpha to oxygen normally absorb between 3.3 and 4.5 ppm. The magnitude of the paramagnetic shift is determined in part by the nature of the oxygen; a methylene in a dialkyl ether at 3.4 ppm shifts to 3.6 ppm for the corresponding alcohol and further in a phenolic ether (3.95 ppm), aliphatic ester (4.1 ppm), aromatic ester (4.2 ppm), and finally is found at 4.3 ppm in ethyl trifluoroacetate.

The relative predictability of the shift associated with alkyl protons alpha to atoms other than saturated carbons has been used in the formulation of constants that are useful in structural assignment.[1-3] Assuming either a methylene or methine proton attached to two or three other groups or atoms, the following equation can be used to calculate the position of absorption of the protons:

$$\delta = 0.233 + \Sigma\sigma_{eff} \tag{7.7}$$

In this equation the shift δ for an alkylproton is the position of methane proton absorption, 0.233 ppm, plus the sum of the effective shielding constants (σ_{eff}) shown in Table 7.2. The observed and calculated position of the resonance signal for methylenes usually agree within ± 0.05 ppm.

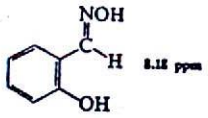
Protons joined to multiple-bonded carbons are found in olefinic, acetylenic,

and aromatic compounds, where the multiple bond is to another carbon and in aldehydes, aldoxines, and formic acid and its derivatives, where multiple bonding to oxygen or nitrogen is involved. Simple correlation with electronegativity or inductive effects are not applicable in these compounds because strong anisotropic effects often are responsible for unusually large chemical shifts. The exact position of the chemical shift for an olefinic proton within the expected range of 4.6 to 6.4 ppm is often used in assigning the orientation of the protons. In a monosubstituted vinyl system the terminal methylene normally is near 4.9 ppm with a slightly higher field signal for the terminal proton *cis* to the adjacent proton. The nonterminal proton absorbs near 5.7 ppm. Cyclic olefinic protons are shifted to lower field compared to their acyclic analogs. Likewise conjugation enhances the deshielding to even lower field values extending the total range for all types of olefinic protons to between 4.0 and 7.8 ppm. The acetylenic proton is subjected to a rather large shielding effect due to diamagnetic anisotropic effects and resonates between 2.5 and 3.1 ppm.

Correlations of the chemical shift with structure in aromatic proton systems have been very useful in assigning aromatic substitution patterns. Absorption in benzenoid systems in the range of 6.5 to 8.0 ppm follows the predictable effects of ring substitution. Diamagnetic shielding due to the electron density on the carbon to which the proton is attached has the greatest effect on the chemical shift; electron-donating substituents increase the electron density at the ortho and para carbons and result in a shift in absorption of these protons to higher fields relative to benzene. Low-field shifts result when the electron density at the C—H is lowered by electron-withdrawing substituents. The diamagnetic anisotropic effect, which falls rapidly as the distance between the proton and the oriented group increases, is influential only in causing an upfield shift of the ortho proton signals. The third factor that modifies the resonance absorption of aromatic protons is the paramagnetic deshielding caused by ring currents and the magnitude of the induced field; electron-donating groups increase the electron density, giving larger fields that are parallel to the applied field in the vicinity of the proton and cause a downfield shift. The overall shift therefore is the combined total of these factors and is strongly influenced by ring substituents. As a rule protons on aromatic rings containing an electron-withdrawing substituent are shifted a maximum of 1.0 ppm downfield from the benzene proton peak at 7.27 ppm; the protons ortho to nitro and carbonyl groups experience the strongest shift. Electron donors cause a shielding effect on the aromatic protons and with the exception of iodobenzene and aminobenzenes the shift of the ortho, meta, and para protons is approximately the same.

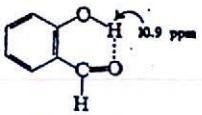
In contrast, the protons on heterocyclic systems undergo much larger shifts, particularly when the protons are alpha to the heteroatom. Correlation of the nuclear magnetic resonance spectra of heterocyclic molecules is complicated by the effects of both the ring substituents and the hetero atom.

Aldehydes, aldehyde derivatives, formic acid derivatives, and aldoximes offer a special case of protons attached to sp_2 carbon since the double bond is to a hetero atom. The strong paramagnetic shielding caused by anisotropic effects shifts the aldehyde proton to the region 9.4 to 10.0 ppm, a unique shift very useful in structural assignment. Low field absorption is characteristic of all protons attached to systems containing multiple bonding to a hetero atom; the single proton peak for methylformate is observed at 8.08 ppm, that for salicylaldoxime is at 8.18 ppm.

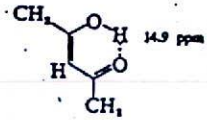


salicylaldoxime

Protons attached to atoms other than carbon are encountered in amines, amides, mercaptans, halogen acids, metal hydrides, alcohols, phenols, carboxylic acids, water, and other molecules such as SiH_4, PH_3, H_2S, NH_3, and H_2. However, the discussion will be limited to the use of nuclear magnetic resonance primarily in structure determinations in organic molecules containing alcohols, phenols, acids, amines, amides, and mercaptans. Two characteristic properties associated with protons bonded to oxygen, nitrogen, and sulfur are (1) their rapid chemical exchange, which is responsible for sharp peaks, and (2) the ability to hydrogen bond. Hydrogen-bonded protons resonate at lower fields than the corresponding unassociated protons. Intramolecular hydrogen-bonded hydroxyl groups are strongly deshielded; for example, the phenolic proton of salicyaldehyde is at 10.9 ppm, and in



salicylaldehyde



acetylacetone

enols such as acetylacetone the enolic proton is at 14.9 ppm. The characteristic properties of intermolecular hydrogen bonds—their concentration and temperature dependence—are readily observed in nuclear magnetic resonance. The resonance signal for protons bonded to oxygen is variable, appearing at higher fields on dilution with inert solvents or as temperature is increased. The predominance of the dimeric form in carboxylic acids and the strength of the hydrogen bonds stabilizes the signal in the region of 10 to 12 ppm, even on dilution with inert solvents.

Amines undergoing rapid chemical exchange in neutral or basic solvents exhibit a sharp absorption peak that changes in appearance to a broad band for the ammonium salt. Although there are exceptions, the normal range for

the N—H of aliphatic and aromatic amines is between 1 and 5 ppm with the aromatic amine protons in the low field end of this range (3.5–5.0 ppm). Amide N—H resonates in the region of 5 to 9 ppm as a broad peak.

Aliphatic mercaptan protons absorb in the relatively high field range of 1.2 to 1.6 ppm; propane-1,3-dithiol has the S—H resonance signal at 1.35 ppm. Benzylic mercaptans are found near 2 ppm, i.e., furfuryl mercaptan at 1.90 ppm, while aromatic mercaptans appear at lower fields; the S—H of *p*-chlorothiophenol is assigned at 3.45 ppm, that of *p*-methylthiophenol at 3.27 ppm.

The property of rapid chemical exchange associated with O—H, S—H, and N—H groups is employed in the interpretation of nuclear magnetic resonance spectroscopy. Since deuterium does not resonate (absorb) in the proton region, after obtaining the spectrum of such a compound, the addition of several drops of deuterium oxide to the sample tube results in exchange of the O—H, N—H, or S—H protons to give the corresponding O—D, N—D, or S—D function. The latter does not give a signal, and by differences in the two spectra the absorption peak for the exchangeable proton is easily assigned.

D. INTERACTIONS BETWEEN NUCLEI

Prediction of the nuclear magnetic resonance of a simple molecule should not be too difficult after the fundamentals of the mechanisms causing the chemical shift are understood. A halide causes a certain amount of deshielding of the methyl protons that is dependent on the relative electronegativity of the halide; shifts to lower fields (deshielding) accompany increases in electronegativity. Thus, the methyl protons of CH_3I appear in the high field end of the alkyl halide region ($\sim$2–4 ppm); it is found at 2.15 ppm. Likewise, in the spectrum of iodoethane, the methylene flanked by a methyl and an iodine should fall at even lower fields and is observed at 3.20 ppm, perhaps somewhat more deshielded than expected. The methyl, adjacent to a CH_2, should be near the region 1–2 ppm; it is at 1.83 ppm. On examination, the spectrum for iodoethane (Fig. 7.12) appears to be complicated by multiple peaks for both the methyl and the methylene, in contrast to the sharp single peak observed for the methyl of CH_3I. Integration of the spectrum for iodoethane results in a 2:3 ratio for the multiplets, the centers of which are 3.20 and 1.83 ppm, respectively. Returning to the theory of nuclear magnetic resonance, each proton must be examined in detail with respect to the field created by a neighboring proton.

Inspection of the two types of protons on iodoethane will be greatly simplified if each proton is designated in turn H_{A_1} and H_{A_2} for the two methylene protons and H_{B_1}, H_{B_2}, and H_{B_3} for the three methyl protons. Each of the five protons oriented in the applied field can be aligned with the field ($\downarrow$), in which case it has a low energy of $-1/2$ spin or antiparallel ($\uparrow$) for a $+1/2$ spin. The three equivalent methyl protons resonate in the applied field by

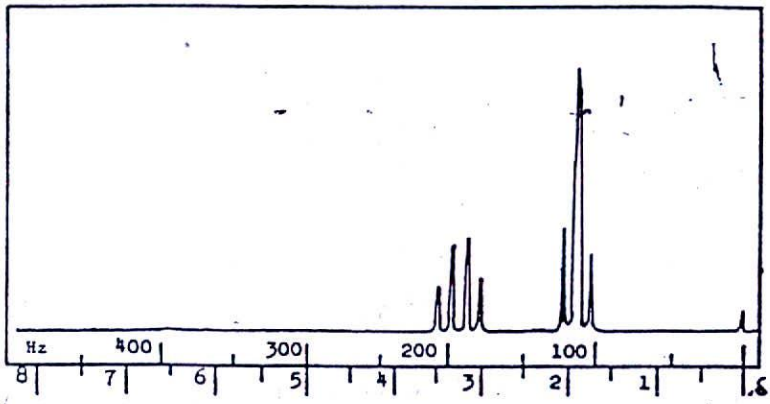


FIGURE 7.12: 60-mC spectrum of iodoethane.

flipping from the $-1/2$ spin to $+1/2$ spin orientation and in iodomethane, absorbed at 2.15 ppm, as a sharp singlet peak. In contrast, the methyl of iodoethane is a triplet ($\sim$ 1.83 ppm) with relative areas 1:2:1 for the three peaks of the triplet. The chemical shift or the field position of the resonance signal is determined by the effective field, which is the summation of the applied field (H_0) and local fields generated by circulating electrons. The additional induced field that is now operating in splitting a peak in this instance into a triplet is that generated by neighboring nonequivalent protons aligned with or against the field. In Table 7.3 all of the possible and equally probable combinations of spin states for H_{A_1} and H_{A_2}, the methylene protons, are given. Therefore the effective field experienced by the methyl protons is that due to the applied field, the induced electron field, and the neighboring nuclear or proton field. Taken in order, state 1 in Table 7.3 has both neighboring protons H_{A_1} and H_{A_2} parallel with and partially neutralizing the applied field; this spin orientation requires a slightly higher applied field to resonate the three methyl protons. States 2 and 3 each total zero in their effective field and therefore do not change the applied field; this methyl peak

TABLE 7.3: Spin States of the Methylene Protons of Iodoethane

	Orientation		Spin state		
	H_{A_1}	H_{A_2}	H_{A_1}	H_{A_2}	Total
1	↓	↓	$-1/2$	$-1/2$	-1
2	↓	↑	$-1/2$	$+1/2$	0
3	↑	↓	$+1/2$	$-1/2$	0
4	↑	↑	$+1/2$	$+1/2$	$+1$

is at intermediate field. In the last orientation, both methylene protons in the high energy state add to the applied field, resulting in slightly less applied field necessary to resonate the methyl protons. Both 1 and 4 are equal but opposite in their effect, and the methyl peaks for these two are equally separated from the central intermediate peak. Integration of the methyl triplet reveals a ratio of 1:2:1: the central peak is twice the area of the two satellite peaks because two states, 2 and 3, contribute to the intermediate peak. The methyl peak has been split into a triplet by a mechanism termed "spin-spin splitting." The magnitude of the splitting or the distance between the lines, in contrast to the chemical shift, is independent of the field strength and subsequently is

TABLE 7.4: Spin States of the Methyl Protons of Iodoethane

	Orientation			Spin state			
	H_{a_1}	H_{a_2}	H_{a_3}	H_{a_1}	H_{a_2}	H_{a_3}	Total
1	↓	↓	↓	−1/2	−1/2	−1/2	−3/2
2	↓	↓	↑	−1/2	−1/2	+1/2	−1/2
3	↓	↑	↓	−1/2	+1/2	−1/2	−1/2
4	↑	↓	↓	+1/2	−1/2	−1/2	−1/2
5	↓	↑	↑	−1/2	+1/2	+1/2	+1/2
6	↑	↓	↑	+1/2	−1/2	+1/2	+1/2
7	↑	↑	↓	+1/2	+1/2	−1/2	+1/2
8	↑	↑	↑	+1/2	+1/2	+1/2	+3/2

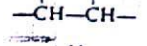
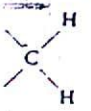
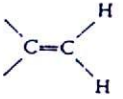
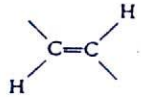
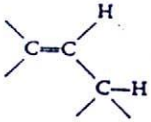
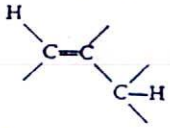
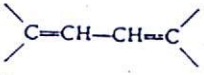
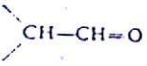
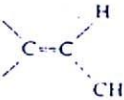
measured in cycles per second (hertz), not parts per million (δ). This measurement, the spin-spin coupling constant (J), for the methyl of iodoethane is 7 Hz.

Analysis of all the possible spin states for the methyl-group protons (Table 7.4) reveals eight possible orientations, theoretically an eight-line multiplet. On inspection of the total spin effect only four field effects are possible: a −3/2 effect; 2, 3, and 4 are the same at −1/2; 5, 6, and 7 are +1/2; and 8 is +3/2. The total relative areas of the multiplet therefore are 1:3:3:1 with a coupling constant $J = 7$ Hz. The mutual splitting of H_{a_1} and H_{a_2} results in the same J for both protons.

Assignment of the chemical shift for the methylene is made in the middle of the quartet, 3.20 δ. The number of lines in the spectrum of the methyl is predictable from the rule $n + 1$, where n is the number of equivalent protons on an adjacent group.

Coupling rarely extends further than between two neighboring proton-bearing groups unless an additional effect such as a double bond is present between the mutually splitting groups. In any event the magnitude of the coupling constant J falls rapidly with distance. The simple rule $n + 1$ for predicting the number of lines in the multiplet applies to those instances where the difference between the chemical shift ($\Delta\nu$) for mutually splitting groups

TABLE 7.5: Common Spin-Spin Coupling Constants

Group	J, Hz
	2–9
	12–15
	5–10 axial-axial; ∠ = 180° 2–4 axial equatorial; ∠ = 60° 2–4 equatorial-equatorial; ∠ = 60°
	0.5–3
	7–12
	13–18
	4–10
	0.5–2.5
	9–13
	2–3
	1–3
	6–8

$(\delta_A - \delta_B)$ is much greater than the coupling constant J of these groups. When $\Delta\nu$ approaches J in magnitude (overlapping), a much more complex multiplet is encountered and the multiplicity of lines, δ, J, and intensity for each line must be calculated.

Table 7.5 gives the expected or predicted spin-spin coupling constants for commonly encountered groups. The usual J for adjacent paraffinic protons is 2–9 Hz; when nonequivalent protons are attached to the same group, usually in rigid or cyclic systems, geminal splitting occurs to give $J = 12 - 15$ Hz.

TABLE 7.6: Spin-Spin Coupling Constants

Aromatic	Spin-spin coupling constants		
	ortho 6–9 meta 1–3 para 0–1		
	X = O	X = NH	X = S
	$\alpha\beta$ 1.6–2.0	2.0–2.6	4.6–5.8
	$\alpha\beta'$ 0.6–1.0	1.5–2.2	1.0–1.8
	$\alpha\alpha'$ 1.3–1.8	1.8–2.3	2.1–3.3
	$\beta\beta'$ 3.2–3.8	2.8–4.0	3.0–4.2
	$\alpha\beta$ 4.9–5.7		
	$\alpha\gamma$ 1.6–2.6		
	$\alpha\beta'$ 0.7–1.1		
	$\alpha\alpha'$ 0.2–0.5		
	$\beta\gamma$ 7.2–8.5		
	$\beta\beta'$ 1.4–1.9		

For substituted cyclohexanes the dihedral angle between the adjacent protons determines the J, and nuclear magnetic resonance is valuable in rigid, cyclic, and olefinic systems in the determination of stereochemistry. It can be readily observed in Table 7.6 that in aromatic systems the magnitude of the splitting constant decreases with increased distance. The magnitude of the J and the possibility of small chemical shift differences often results in very complicated spectra.

Analysis of spin-spin splitting is relatively easy when the chemical shift (δ) is much greater than the coupling constant (J); in these instances the two splitting nuclei are termed an "AX system" since the chemical shift for nuclei A is quite different than nuclei X. Iodoethane is an example of an A_2X_3 system—A_2 represents the methylene protons, X_3 the methyl protons, and the multiplets observed follow the $n + 1$ rule. An AX system would give two doublets, while an A_2X_2 gives two triplets, and finally an A_3X_3 results in two quartets.

When the magnitude of the chemical shift, ν measured in cycles per second (hertz), between the interacting nuclei ($\Delta\nu$) is small and approaches the magnitude of the splitting constant (J), then the splitting becomes complicated.

As an example of an AB system, a closely spaced four line spectrum is predicted. As the ratio $J_{AB}/\Delta\nu_{AB}$ becomes larger, the character of the four lines changes—the inner two lines increase at the expense of the outer lines, and the distances in cycles per second between the inner and outer lines increases (Fig. 7.13). Assignment of the δ and J is accomplished by the following formula:

$$|B_2 - A_2| = |B_1 - A_1| = \sqrt{\Delta\nu_{AB}^2 + J_{AB}^2} \tag{7.8}$$

where the lines for the protons are labeled with increasing field B_2, B_1, A_2, and A_1.

Three interacting nuclei can be assigned: AX_2, AB_2, AMX, and ABX. In the first instance, where the difference in the chemical shift between the coupling nuclei A and X is large relative to the coupling constant ($\Delta\nu_{AX} > J_{AX}$), a simple triplet-doublet spectra is seen (Fig. 7.14). The A_2B example of Fig. 7.14 is complicated by the fact that the multiplet pattern is dependent on the ratio $J_{AB}/\Delta\nu_{AB}$.

The AMX spectrum is a multiplet of three quartets since in all states the $\Delta\nu \gg J$. In Fig. 7.15 the observed pattern is valid for $J_{AM} > J_{AX} > J_{AM}$. Analysis of ABX and ABC spectra gives rise to complicated spectra that must be analyzed with care; many examples of this type are found in unsaturated and aromatic compounds.

Examples of four (A_2X_2, A_2B_2, A_3B) or five (A_2B_3) interacting nuclei can be simple, easily recognizable patterns when J_{AA}, J_{XX}, and J_{BB} equal zero and the $\Delta\nu$ is much greater than J. The ethyl group represents such an example of an A_2B_3 spectra.

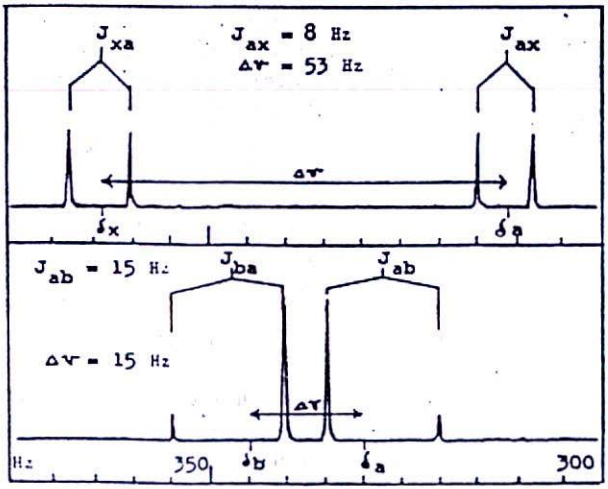


FIGURE 7.13: Coupling between AX and AB protons.

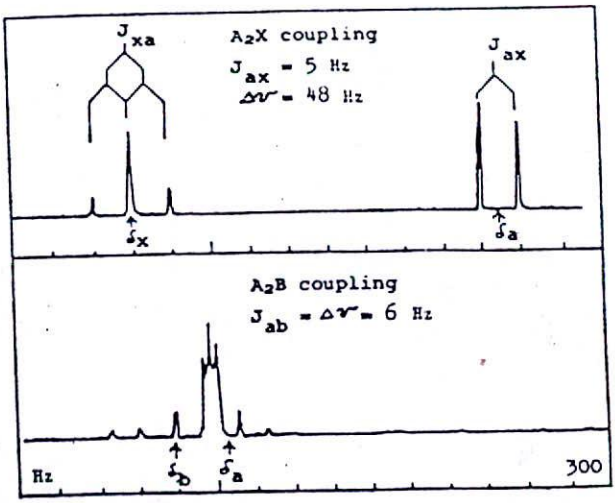


FIGURE 7.14: Coupling between A_2X and A_2B protons.

The analysis and interpretation of complex spectra that are observed in ABX, ABC, and other systems often requires mathematical treatment and a prior knowledge of the structural possibilities for each pattern. Since the magnitude of the coupling constant is independent of the field strength, resolution and assignment of complex multiplet patterns are simplified by examination at higher field strengths where the chemical shift difference ($\Delta\nu_{AB}$) increases while the coupling constant (J_{AB}) remains constant, effectively decreasing the $J/\Delta\nu$ ratio.

The application of spin-decoupling and double-resonance techniques, particularly at higher fields, is routinely employed in analysis of such systems. Excellent discussions of complex splitting patterns and their analysis are available and should be referred to for additional information.[3]

Nuclear magnetic resonance of isotopes other than hydrogen has been of great interest to chemists, particularly the resonance associated with fluorine, phosphorus, boron, and carbon-13. [4]

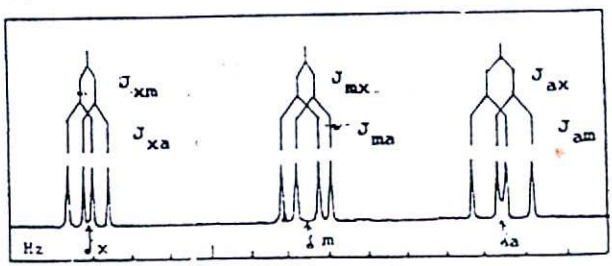


FIGURE 7.15: Coupling between AMX protons; J_{AM} = 6 Hz, J_{AX} = 4 Hz, J_{MX} = 2 Hz

E. APPLICATIONS IN PHARMACEUTICAL ANALYSIS

The analysis of pharmaceuticals from a qualitative view is concerned with the identification of medicinals and the detection of impurities. Quantitative analysis is applied in determination of the concentration of a drug in dosage form, often in the presence of other drugs or compounding agents. Development of methods in drug analysis employs the entire spectrum of analytical techniques.

Application of modern instrumental methods such as infrared and ultraviolet spectroscopy, polarography, and chromatographic procedures in the analysis of drugs is increasing at a remarkable pace. Although the role of nuclear magnetic resonance in research for structural determination is firmly established, the applications to drug analysis only recently have been explored.[8]

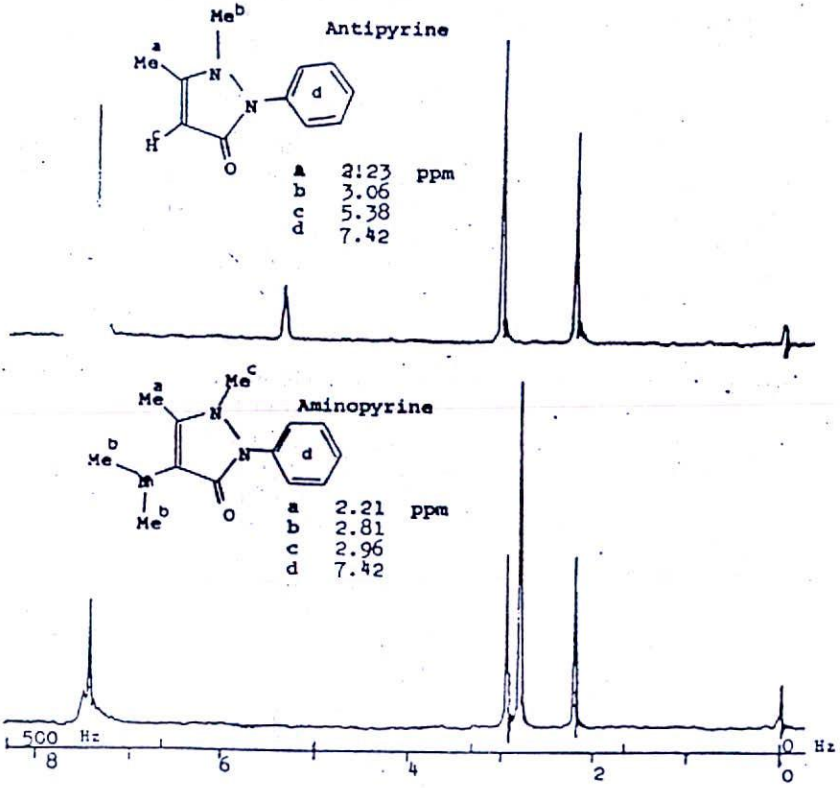


FIGURE 7.16: Nuclear magnetic resonance spectra of antipyrine and aminopyrine. Courtesy of Varian Associates, Palo Alto, California.

The identification of a particular drug using nuclear magnetic resonance often is not possible due to the close chemical relationship among the various analogs in a particular class of drugs; however, as the first approach, the general structural features can be determined. Thus, limiting the unknown to a particular class of drugs, for example, the phenothiazines, comparison to standards using infrared or other "fingerprinting" methods leads to rapid identification. The inherent limitations of nuclear magnetic resonance stem partially from the fact that relatively large quantities of the sample are required—10 to 40 mg. The detection of impurities in the sample is hampered by the insensitivity of the detection method, requiring more than 5% of the impurity in the sample, and furthermore, the impurity must have a strong resonance signal in a region where the sample does not absorb. These requirements are not often encountered in drugs for two reasons: first the potency of drugs is such that a small quantity of impurity may have strong undesirable pharmacological effects; second the impurity, usually a side product in the synthesis, is closely related structurally to the drug and would exhibit proton resonance in the same regions. Efforts to solve the problem of low sensitivity and relatively low signal-to-noise ratio in nuclear magnetic resonance have centered on computer techniques. Since random noise detection will average to zero over many sweeps of a particular sample and a true resonance signal is additive, by repetitive scans of a sample and storage of the resultant signal in a computer the intensity of a weak signal can be increased manyfold, while the signal due to noise is cancelled to zero. An accessory called a "computer of average transients" (CAT) has been designed for use in increasing the signal-to-noise ratio.

As an example of qualitative analysis of drugs by nuclear magnetic resonance, the spectra of antipyrine and aminopyrine (Fig. 7.16) exhibit only one obvious difference. In antipyrine the sole heterocyclic ring proton, which is replaced by a dimethylamino group in aminopyrine, resonates at 5.38 ppm; the protons of the two methyls on the nitrogen in aminopyrine introduce a peak at 2.81 ppm.

As an aid in characterization of phenothiazines the infrared, ultraviolet, and nuclear magnetic resonance spectra of twenty-three analogs have been published.[9] The fingerprint like spectra in Fig. 7.17 represent three classes of molecules—colchicine, eserine, and procaine. The respective protons have been assigned in Table 7.7.

Quantitative analysis of mixtures has employed ultraviolet and infrared spectroscopy where the Beer-Lambert law or calibration plots are applicable. The counterpart used for quantitative analysis in nuclear magnetic resonance is the proton integration curve where the relative areas under a resonance peak can be compared to standards; by this procedure the ratio of the components in the mixture can be estimated. In analogy to the Beer-Lambert law, where the absorbance is related to the number of molecules, the area under the resonance peak as measured by an integrator gives the relative number of

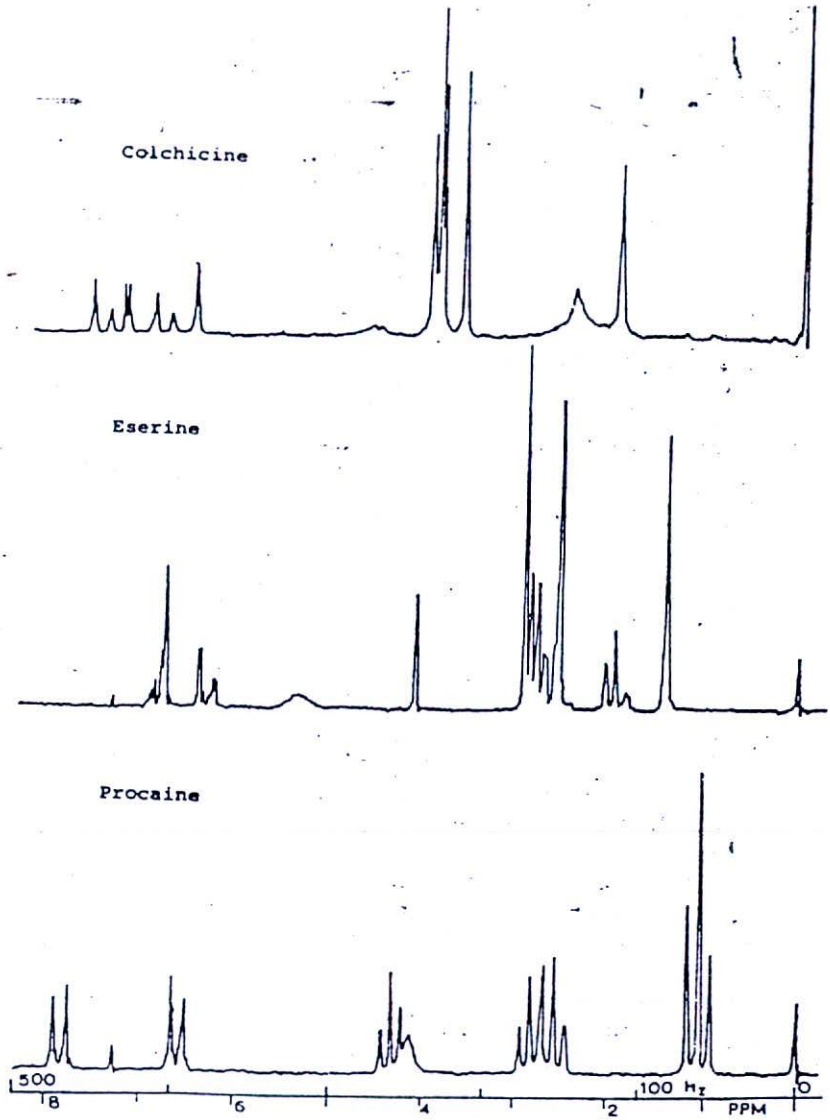
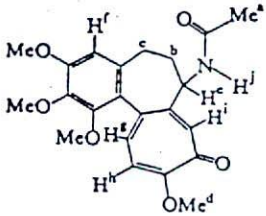
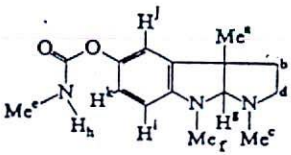
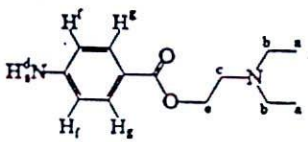


FIGURE 7.17: Nuclear magnetic resonance spectra of colchicine, eserine, and procaine. Courtesy of Varian Associates, Palo Alto, California.

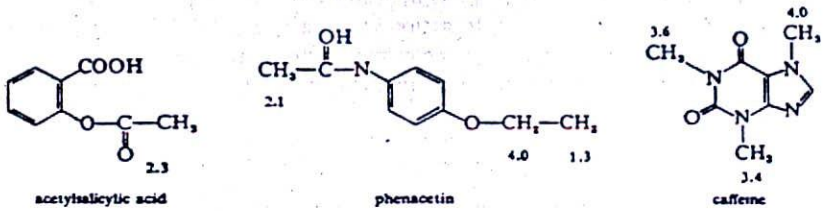
protons in the respective peaks. For example, examination of an equimolar mixture of methanol and acetone gives a spectrum having absorption signals at 2.2 ppm for the six methyl protons of acetone and at 3.5 ppm for the three methyl protons of methanol. Integration shows a ratio of 2:1 for the proton area under the respective peaks, which calculates for a molecular ratio of 1:1. By careful experimentation the error in such an application usually is less than 3%.

TABLE 7.7: Assignment of Proton Values

	Assignments, ppm
	**Colchicine** a 1.96 g 6.92 b 2.43 h 7.37 c 2.43 i 7.63 d 3.67 j 8.38 e 4.62 f 6.55
	**Eserine** a 1.42 g 4.12 b 1.95 h 5.33 c 2.55 i 6.37 d 2.70 j 6.78 e 2.82 k 6.87 f 2.92
	**Procaine** a 1.05 g 7.83 b 2.62 c 2.82 d 4.13 e 4.33 f 6.63

Acetylsalicylic acid-phenacetin-caffeine combinations have been analyzed by nuclear magnetic resonance.[10] In the reported procedure it was found that all three components give unique resonance peaks. Acetylsalicylic acid has a sharp methyl peak at 2.3 ppm, phenacetin has the ethyl peaks centered at 1.3 ppm (CH_3) and 4.0 ppm (CH_2). Caffeine has peaks at 3.35, 3.55, and 4.0 ppm for the three methyl groups. The peaks selected for quantitative analysis were 2.3 ppm for acetylsalicylic acid, 3.4 and 3.6 ppm for caffeine, and 4.0 ppm for phenacetin. The area under the acetylsalicylic acid signal has three protons, representing one molecule of that compound. Since the concentration of caffeine is low relative to the other ingredients, measurement of both the 3.4 and 3.6 ppm signals gives six protons representing one molecule of caffeine. The phenacetin methylene quartet at 4.0 ppm is complicated

by the N_7 methyl of caffeine, which also absorbs at 4.0 ppm. Thus measurement of the area under the 4.0-ppm multiplet gives two protons representing each phenacetin molecule and three protons for each caffeine molecule.



Since the number of caffeine molecules has been measured independently at the 3.4- and 3.6-ppm regions, the 4.0-ppm integration can be corrected for phenacetin by subtracting one-half of the 3.4 and 3.6-ppm integrated areas. Correcting all ratios to give equimolar equivalents, the molecular ratios of

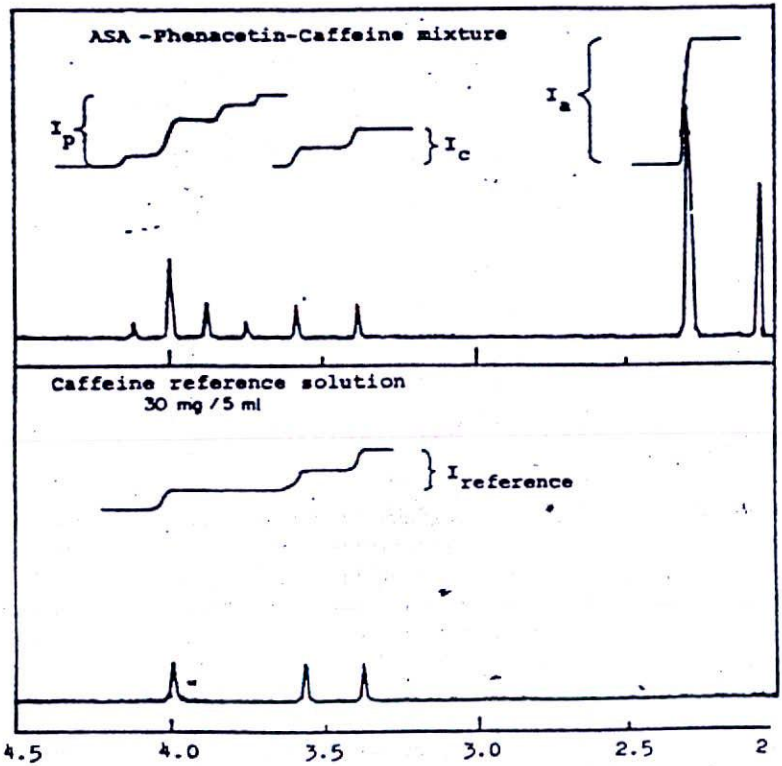


FIGURE 7.18: Spectrum of ASA–Phenacetin–Caffeine mixture and caffeine reference solution.

each ingredient are given by the following equation where I is the ratio of the integrated area for each peak

$$\text{Acetylsalicylic acid-caffeine-phenacetin} = (I_A):(\tfrac{1}{2}I_C):\tfrac{2}{3}(I_P - \tfrac{1}{2}I_C)$$

In the example given in Fig. 7.18 the areas for each region I_A, I_C, and I_P are 101:28:58; accordingly, the molecular ratios are:

$$A:C:P = 101:14:66 = 7.2:1:4.7$$

The relative concentrations c can be calculated from the molecular weight:

$$c_A:c_C:c_P = 7.2 \times 180:1 \times 194:4.7 \times 184$$
$$= 1295:194:865 = 6.67:1:4.46$$

The absolute concentrations can be calculated from comparison to a standard. A sample containing 30 mg of caffeine in 5 ml of solvent was examined under the same conditions as the unknown and was found to give an integral area (I_R) of 24 mm for the 3.4- and 3.6-ppm signals. The concentration of caffeine unknown (C_S) in milligrams per 5 ml is proportional to the integrated areas for the reference and the sample:

$$C_S = \frac{I_S}{I_R} C_R = \tfrac{28}{24}(30 \text{ mg/5 ml}) = 26 \text{ mg/5 ml}$$

The concentration of the acetylsalicylic acid (C_A) and the phenacetin (C_P) in milligrams per 5 ml then can be determined

$$C_A:C_C:C_P = 7.2:1:4.7 = 187:26:122 \text{ mg/5 ml}$$

The advantages are speed and the nondestructive nature of the method; the 1% error found in the integration curve is the chief disadvantage, particularly when there is a wide difference in concentration of the ingredients in the mixture. By this method and application of a slight correction introduced for the resonance signal of carbon-13 Hollis reported deviations of only 1.1% for acetylsalicylic acid, 2.2% for phenacetin, and 3.2% for caffeine.

Although few applications of nuclear magnetic resonance to analysis of pharmaceutical systems have been reported, the possibilities for the future in both qualitative and quantitative analysis of pharmaceuticals are encouraging. Nuclear magnetic resonance certainly will not replace infrared and ultraviolet, but will add another dimension to pharmaceutical analysis.

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