15

Nuclear magnetic resonance spectroscopy

Following on from the pioneering work of physicists Bloch and Purcell around 1945 nuclear magnetic resonance (NMR) very quickly became a flexible and irreplaceable method of spectroscopy for a variety of areas in chemistry. NMR is a powerful and theoretically complex analytical tool that allows the study of compounds in either solution or in the solid state and serves equally in quantitative as in structural analysis, though it is especially in the latter where it is very efficient in gathering structural information concerning molecular compounds. It is therefore of particular practical importance to organic chemistry and biochemistry. Used as a complementary technique to methods of optical spectroscopy and mass spectrometry, it leads to precise information concerning the structural formula, stereochemistry and in some cases the preferred conformation of molecules and even to the identity of the compounds studied. For all of these reasons, NMR has become one of the principal study techniques for inorganic crystals as well as molecular structures. Although NMR has for a long time been considered as not sensitive enough to be adaptable to environmental analysis, this situation has changed, particularly in view of the linking of liquid chromatography with NMR spectroscopy.

15.1 General introduction

Nuclear magnetic resonance has given its name to a very remarkable method of solving the problems of structural determination for organic compounds and for certain types of inorganic material. NMR spectrometers are therefore often located in research laboratories although more trouble-free instruments exist exploiting the same principles for routine applications (see Figures 15.32 and 15.33, at the end of this chapter).

This method for studying matter can be described by choosing solely relevant examples from organic chemistry, the elucidation of molecular structures having

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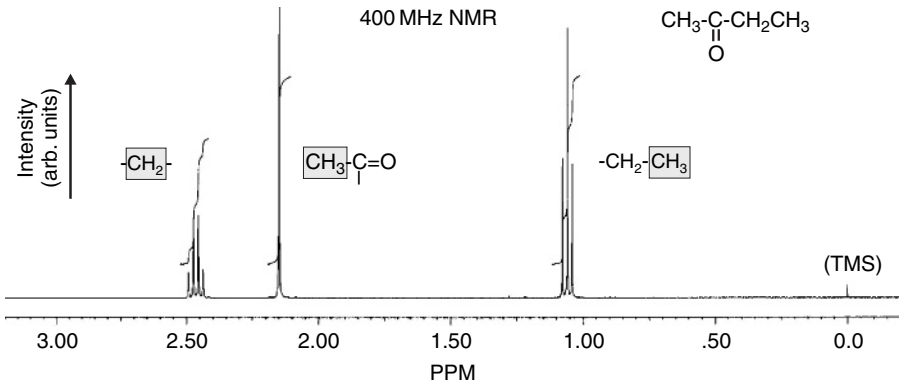


Figure 15.1 *Conventional representation of the* ^1H *NMR spectrum of butanone* $[CH_3(C=O)CH_2CH_3]$. The corresponding integration superimposed over the peaks permits an evaluation of the relative areas of the signals. The meaning of the abscissa will be explained later on.

always served as a driving force for NMR development and to the numerous technical improvements effected since the origin of the technique.

NMR collects information concerning interactions between the nuclei of certain atoms present in the sample when they are submitted to a static magnetic field which has a very high and constant intensity and exposed to a second oscillating magnetic field. The second magnetic field, around 10 000 times weaker than the first is produced by a source of electromagnetic radiation in the radiofrequency domain.

The basic document delivered by these instruments is the *NMR spectrum* (Figure 15.1), a graph representing signals of resonance. It results from the absorption by the sample of definite frequencies sent by this electromagnetic source. The origin of these spectra, which are quite different from optical spectra, can be best understood by consideration of nuclear spins.

■ The spectrum of Figure 15.1 is an example of one-dimensional NMR (the intensities of the signals not being counted as a second dimension). More sophisticated studies, in two, three and four dimensions enable a more precise localization of the position of all of the atoms in the molecule studied. This chapter only describes one-dimensional NMR (1D-NMR), for samples in solution.

15.2 Spin/magnetic field interaction for a nucleus

All atomic nuclei – as well as each particle – are characterized by a number of intrinsic parameters, spin $\vec{I}$ being one of them. This vector quantity introduced by quantum mechanics is used to explain the behaviour of atoms in media where there is a preferential direction. A magnetic field creates just such an orientation

for all atoms within this field. The spin of the nucleus has the same dimensions (J s) as the kinetic moment $\vec{L}$ of classic mechanics. The norm of the spin varies from one nucleus to another because it is defined from the spin quantum number I, a characteristic of each nucleus considered, whose value can be zero or a positive multiple of 1/2.

An isolated nucleus with a non-zero spin number behaves like a small magnet with a magnetic moment $\vec{\mu}$ (JT^{-1}) such that:

$$\vec{\mu} = \gamma \cdot \vec{I} \tag{15.1}$$

This *nuclear magnetic moment* $\vec{\mu}$ is represented by a vector co-linear to $\vec{I}$ (Figure 15.2), and has the same or the opposite direction, according to the sign of γ the *gyromagnetic ratio* (also called the *gyromagnetic constant*).

If an isolated nucleus with a non-zero spin number, which can be compared to a kind of microscopic magnetic needle, is submitted to a magnetic field $\vec{B}_0$ which makes an angle θ with the spin vector, an interactive coupling occurs between $\vec{B}_0$ and $\vec{\mu}$; this modifies the potential energy E of the nucleus.

If $\vec{\mu}_Z$ represents the projection of $\vec{\mu}$ on the z-axis, which, by convention, is oriented as $\vec{B}_0$, then the energy of the interaction (also called the Hamiltonian) is:

$$E = -\vec{\mu} \cdot \vec{B}_0 \quad \text{or} \quad E = -\mu \cos(\theta) B_0 \quad \text{or, finally} \quad E = -\mu_z \cdot B_0 \tag{15.2}$$

According to quantum mechanics, μ_z, for a nucleus, can only have $2I + 1$ different values. This means that in a magnetic field $\vec{B}_0$, the potential energy E can also only take $2I + 1$ values. The same goes for angle θ. The quantification

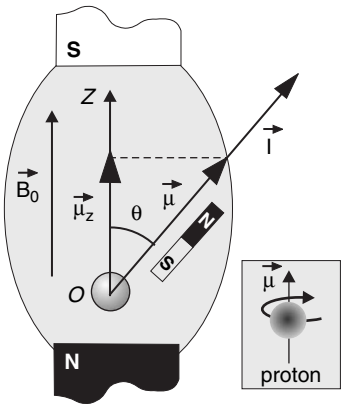


Figure 15.2 *Basic NMR vectors*. When a spinning nucleus is placed in a magnetic field, the nuclear magnet experiences a torque which tends to align it with the external field. In the conventional NMR coordinate system, the external magnetic field is along the z-axis.

of μ_z are given by the magnetic spin number m which can takes the values (in $h/2\pi$ units) expressed by the following series:

$$m = -I, -I+1, \ldots, I-1, I$$

By using relations 15.1 and 15.2, the $2I+1$ allowed energy values are given by the following relations:

$$E = -\gamma \cdot m \cdot B_0 \tag{15.3}$$

For $I = 1/2$, the two possible values of E (in joules, and subscripted as α and β) correspond to $m = +1/2$ and $m = -1/2$. They are given as follows:

$$E_1(\text{or}E_\alpha) = -\gamma \frac{1}{2} \frac{h}{2\pi} B_0 \quad \text{and} \quad E_2(\text{or}E_\beta) = +\gamma \frac{1}{2} \frac{h}{2\pi} B_0$$

This splitting in several energy levels recalls the Zeeman effect (cf. Chapter 14) which concerns the separation of electronic states in a magnetic field. It is sometimes referred to in NMR as the Zeeman nuclear separation.

The quantification of the spin vector projection along the z-axis has for effect that $\vec{I}$ traces the surface of a cone of revolution with an angle between the axis and atom that can be calculated if $\vec{\mu}$ and $\vec{\mu}_Z$ are known. This *precession movement*, around an axis parallel to that of the magnetic field (Figures 15.3 and 15.5a) is characterized by a frequency which increases with the field intensity.

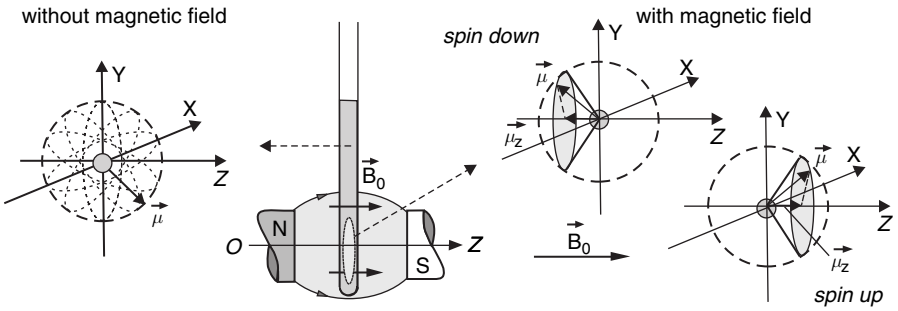


Figure 15.3 *Effect of a magnetic field upon a nucleus of spin number 1/2 for an atom in a compound in solution*. If the nucleus is in the upper part of the sample tube, i.e. not submitted to the external magnetic field, $\vec{\mu}$ has no preferential orientation with time. Alternatively, when the nucleus is in the strong magnetic field of the central section, it lines up, precessing in the direction of the applied field. The projection of $\vec{\mu}$ is in the same or the opposite direction to $\vec{B}_0$. Unfortunately, here and next figures the dynamic concepts of NMR spectroscopy are difficult to understand whith static diagrams.

■ To give a concrete idea of nuclear spin, allusion is often made to the movement of a spinning top rotating around its axis, which makes at a given instant, an angle θ with respect to the direction of the Earth's gravitational field. The spinning top describes a gyroscopic movement about this direction which arises from the combination of its movement of rotation about its own axis and coupling with the gravitational vector. As friction slows down the rotation, the angle θ will increase continually. In contrast to the principle of the spinning top, for a nucleus in a vacuum, the angle is maintained with time, whatever the value of the applied field, because the values of the magnetic moment and of its projection are quantized. The rotational axis of the spinning nucleus cannot be orientated parallel, or anti-parallel (although these terms are used for nuclei) with the direction of the applied field $\vec{B}_0$. Therefore, for a nucleus with a spin number I =1/2, calculation leads to the only angles permitted for $\vec{I}$ with respect to z-axis which are approximately 55° and 125°. This takes into account the value of the spin total angular momentum ($\sqrt{I(I+1)}$), and that of the intrinsic angular momentum (I). The angle of 54.74°, used in certain NMR experiments is called the 'magic angle'.

15.3 Nuclei that can be studied by NMR

In a nuclide represented by ${}^{A}_{Z}\mathrm{X}$, the nucleus will have a non-zero spin number I, if the number of protons Z and the number of neutrons A, are not both even numbers. ${}^{1}_{1}\mathrm{H}$, ${}^{13}_{6}\mathrm{C}$, ${}^{19}_{9}\mathrm{F}$, ${}^{31}_{15}\mathrm{P}$ have, for example, a spin number $I = 1/2$ while $I = 1$ for ${}^{2}_{1}\mathrm{H}$ (deuterium D) or ${}^{14}_{7}\mathrm{N}$. All these nuclei will give an NMR signal. Alternatively, the nuclei ${}^{12}_{6}\mathrm{C}$, ${}^{4}_{2}\mathrm{He}$, ${}^{16}_{8}\mathrm{O}$, ${}^{28}_{14}\mathrm{Si}$, ${}^{32}_{16}\mathrm{S}$ which have a spin number of zero, cannot be studied by NMR. In fact, more than half of the stable nuclei known (at least one isotope per element) leads to an NMR signal although the sensitivity varies enormously depending upon the nucleus.

Hence, the protons (the common name for ${}^{1}\mathrm{H}$), or ${}^{19}\mathrm{F}$, are easier to detect than ${}^{13}\mathrm{C}$, which is much less sensitive and also for the reason that it represents only 1 per cent of elemental carbon.

15.4 Bloch theory for a nucleus of spin number $I = 1/2$

At a macroscopic scale, even the smallest quantity of a compound is composed of a gigantic number of individual molecules. The number of nuclei being so high, the NMR signal reflect their statistical behaviour, as in optical spectroscopy.

Let us consider a collection of identical nuclei whose spin number is $I = 1/2$. In the absence of an external magnetic field, the individual spin vectors will be randomly oriented and vary constantly with time. From an energy point of view, these nuclei form a population that is considered a *degenerated* state (Figure 15.3).

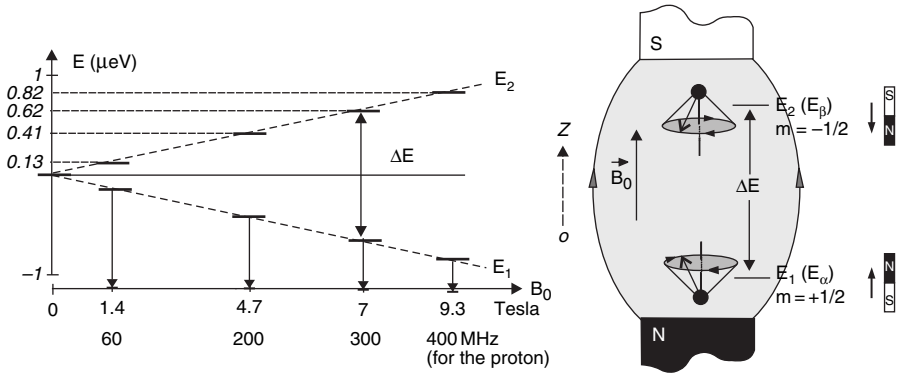


Figure 15.4 *Representation of the energy split for a nucleus with a spin number $I = 1/2$ placed into a magnetic field.* The parallel alignment (E_1) is slightly lower in energy than the antiparallel one (E_2). The four values chosen for the field $\vec{B}_0$ correspond, for the proton, as it will be see later, to commercial instruments of 60, 200, 300 and 400 MHz respectively. $\vec{B}_0$ represents the magnetic field strength, measured in Tesla (T). The common values are above 1 T. For comparison, the Earth's magnetic field is approximately 5×10^{-5} T.

When these nuclei are placed in a strong external magnetic field $\vec{B}_0$ (OZ orientation) an interaction will occurs between each individual nuclear magnetic vector and this field.

There appears therefore two groups of nuclei according to the direction of their spin vector along the z-axis, whose energies correspond to low energy state E_1 and to high energy state E_2, as previously defined (Figure 15.4). These orientations are called 'spin up' (magnet image N-S-N-S) and 'spin down' (N-N-S-S).

The difference in energy ΔE between the two states is:

$$\Delta E = E_2 - E_1 = \gamma \frac{h}{2\pi} B_0 \tag{15.4}$$

ΔE is proportional to the field $\vec{B}_0$ (Figure 15.4). Thus, for the proton (^{1}H), if $B_0 = 1.4$ T, the difference in energy is very small: 3.95×10^{-26} J or 2.47×10^{-7} eV. The ratio $(E_2 - E_1)/B_0$, depends only upon the value for γ, for the nucleus being studied (Table 15.1).

Table 15.1 Values of γ for the most commonly studied nuclei in NMR

Nuclei N	γ(rad · s^{-1} · T^{-1})	Sensitivity
^{1}H	2.6752×10^8	1
^{19}F	2.5181×10^8	0.83
^{31}P	1.084×10^8	6.6×10^{-2}
^{13}C	0.67283×10^8	1.8×10^{-4}

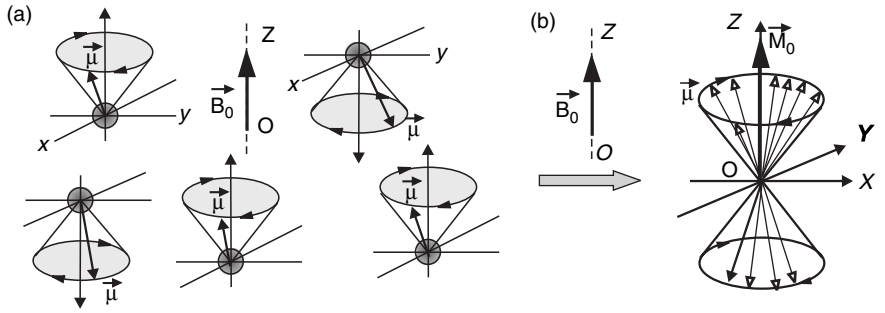


Figure 15.5 *Precession and magnetization.* (a) A snapshot illustrating the precession movement of the spin vector of five independent nuclei in a magnetic field; (b) The macroscopic magnetization vector which results from the individual orientations of a large number of nuclei. If $B_0 = 1.5\,\mathrm{T}$, there are less than 10 spin-up more than spin-down per million ^{1}H nuclei. The values of this very weak net magnetization depends on the excess of population E_1 versus E_2. By convention the external magnetic field and the net magnetization vector at equilibrium are both along the z-axis.

The population of nuclei located in energy state E_2, is slightly less numerous than that in the more stable state E_1. Expression 15.5 calculates the ratio of these two populations (Boltzmann distribution equilibrium).

$$R = \frac{N_2}{N_1} = \exp\left[-\frac{\Delta E}{kT}\right] \tag{15.5}$$

(for ^{1}H, $R = 0.999\,964$ if $T = 300\,\mathrm{K}$ and $B_0 = 5.3\,\mathrm{T}$ where $k = 1.38 \times 10^{-23}\,\mathrm{J\,K^{-1}\,at^{-1}}$).

As a result, it is only the *slight excess* in population E_1 which is responsible for the NMR signal. By increasing the value of the magnetic strength of the magnet, the difference ΔE will be greater and thus sensitivity will increase since the signal is proportional to the populations difference.

On a graphic representation, the weak magnetization of the sample solution is accounted for by using the vector $\vec{M}_0$, which is the vector sum of all of the individual vectors $\vec{\mu}$ (Figure 15.5). At equilibrium, this vector lies along the direction of the applied magnetic field $\vec{B}_0$ and is called the equilibrium magnetization or *net magnetization*. $\vec{M}_0$ is used to describe pulsed NMR, and to explain the appearance of signals when nuclei enter resonance. $\vec{M}_0$ is an entity to which the laws of electromagnetism can be applied.

15.5 Larmor frequency

From an analytical point of view, a nuclide can be identified if ΔE, in a field $\vec{B}_0$, could be measured (assuming $I = 1/2$), by knowledge of the gyromagnetic

constant γ. As in optical spectroscopy, this difference in energy can be determined under conditions where species can pass between one state and the other, by absorption or emission of a photon.

A basic experiment consist of irradiating the nuclei present in a magnetic field with a source of electromagnetic radiation of variable frequency ν with a propagation direction perpendicular to the external field. Absorption will occur if:

$$h\nu = \Delta E = E_2 - E_1 \tag{15.6}$$

The energy of this photon must exactly match the energy difference between the two states. Expression 15.4 leads to the fundamental relation of resonance (15.7):

$$\nu = \frac{\gamma}{2\pi} B_0 \tag{15.7}$$

This important and general expression, irrespective of the value of I, gives the frequency *Larmor frequency*. It links the magnetic field in which the nuclei being studied are located and the frequency of electromagnetic radiation that induces resonance, hence a signal in the spectrum when the lower energy nuclei spin-flip to the higher state (Table 15.1).

The radiofrequency which induces the exchange between the two levels is equal to the Larmor frequency at which the spin vector rotates about the average direction of the z-axis. Larmor, an Irish physicist whose work preceded that of NMR has shown, by an independent reasoning that ω, the angular rotation frequency of the spin vector about the z-axis, has a value of:

$$\omega = \gamma \vec{B}_0$$

This is the Larmor equation. Since $\omega = 2\pi\nu$, this expression is equivalent to Equation 15.7. The two approaches are different: one leads to the frequency of the quanta which separates both energy states and the other to the mechanical precession frequency. These two frequencies have the same value.

The Larmor frequency of a given nucleus increases with $\vec{B}_0$. It is situated in the microwave region of the electromagnetic spectrum and varies, for a field of 1 T, from 42.5774 MHz for the hydrogen ^{1}H nucleus (proton) to 0.7292 MHz for gold $^{197}_{79}$Au (Table 15.2). The instruments are specifically designed for the frequency at which protons resonate, even if they are of able to study other nuclei.

Table 15.2 Values in MHz of some resonance frequencies for $\vec{B}_0 = 1$ T

Nuclei	^{1}H	^{19}F	^{31}P	^{13}C	^{15}N
Frequency ν	42.58	40.06	17.24	9.71	4.32

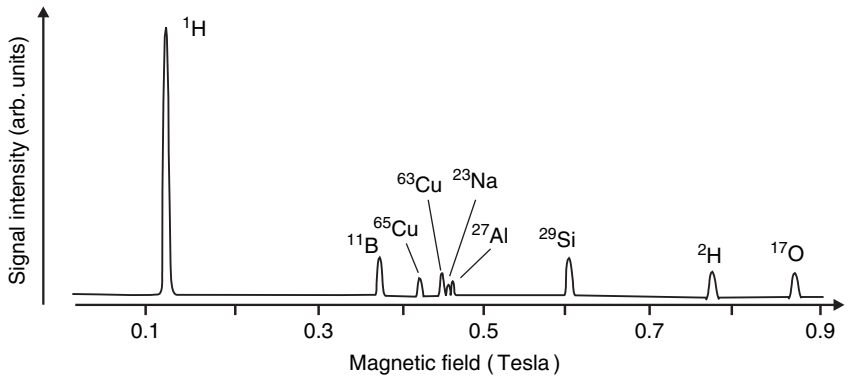


Figure 15.6 *NMR spectrum of a water sample placed in a borosilicate glass container, observed at a frequency of 5 MHz* (the field is expressed in gauss; Varian document). There are no commercial instruments of this type because NMR is not sensitive enough to solve many of the problems encountered in elemental analysis.

The construction of experimental multi-nuclei instruments was initiated from these principles. These instruments can sweep over a wide range of the field while maintaining a fixed radiofrequency. This leads to a qualitative screening of the easiest elements to detect within the sample through their characteristic resonance (Table 15.2 and Figure 15.6).

15.6 Pulsed NMR

$\vec{M}_0$ that is shrouded in the strong magnetic field $\vec{B}_0$ is too weak to be measured. So, to get a signal, resonance of the nuclei is obtained by superimposing upon $\vec{B}_0$, in the probe area, a weak oscillating field $\vec{B}_1$ which originates from a coil that is fed with alternating current radiofrequency.

To understand the interaction of the nucleus with the field $\vec{B}_1$ in the laboratory frame of reference, it is considered as resulting from the composition of two vectors rotating in opposite directions (clockwise and counter clockwise) in the *xy*-plane with the same angular velocity (Figure 15.7). If the frame of reference rotates with the precession frequency, the vector component that also rotates clockwise in the laboratory frame of reference will appear stationary. The second vector component that rotates counter clockwise is moving around the *z*-axis far from the resonance frequency of the spins. It can be ignored. Only the vector turning in the same direction as the precesion movement can interact with the nucleus. As transverse magnetization rotates about the *z*-axis, it will induce a current in a coil of wire located in the *xy*-plane.

For $\vec{B}_1$ to be suitably aligned, the propagation axis of the radiofrequency needs to be perpendicular to the z-axis (Figure 15.8). The transfer of energy from the source

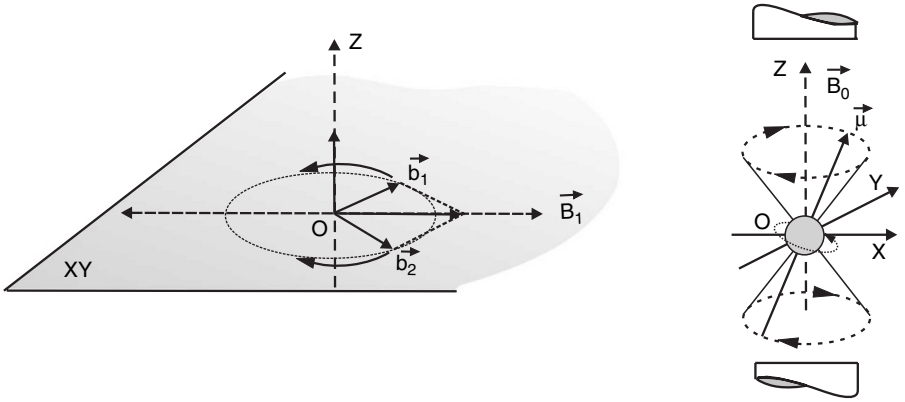


Figure 15.7 *Representation of magnetic field* $\vec{B}_1$. In all points of the plane xy the magnetic component of the wave can be dissociated into two half vectors $\vec{b}_1$ and $\vec{b}_2$ rotating in phase with opposite velocities. Here, only $\vec{b}_2$ can interact with the nuclei of population E_2.

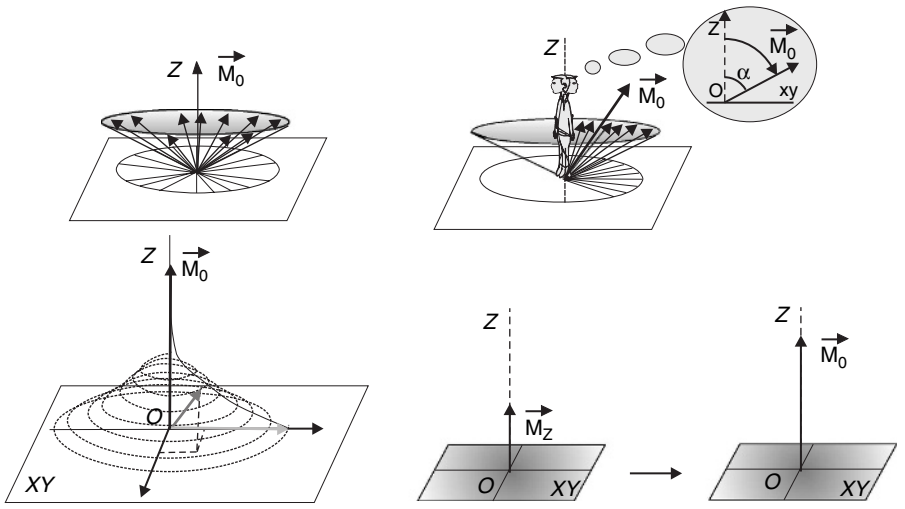


Figure 15.8 *Deviation of* $\vec{M}_0$ *with irradiation and relaxation of the transverse and longitudinal relaxations of the system following resonance*. Schematic representation in which the individual vectors form a spin packet resulting from the non-equilibrium of the populations are shown (laboratory frame of reference). $\vec{B}_1$ has a double action, flipping the nuclei and packing them in phase. An observer situated in a rotating frame at the precession frequency, would see the magnetization vector tilted by an angle α. Relaxation of $\vec{M}_0$ to its original position. If $\vec{M}_Z = 0$, the spin system is saturated. As transverse magnetization rotates about the z-axis, it will induce a current in a coil of wire located around the x-axis. Plotting current as a function of time gives a sine wave.

to the nuclei occurs when the radiofrequency of the source and the frequency of precession are identical. Under these conditions the spin of some nuclei adopts the second alignment allowed (in the case where $I = 1/2$). Nuclei in energy state E_1 pass to energy state E_2: in this way, the populations are modified. Saturation is attained when they become equal.

Before irradiation, the individual vectors $\vec{\mu}$ are out of phase with respect to each other, which is represented by the vector $\vec{M}_0$, aligned in the direction OZ (Figure 15.5). As the resonance condition is reached, all the individual vectors *pack together and rotate in phase* with $\vec{B}_1$. Thus, the direction of $\vec{M}_0$ changes by an angle α (relative to z-axis), which can be controlled from 0 to π, by adjusting the time and the intensity of the applied radiation. Figure 15.8 illustrates this phenomenon with several individual vectors which join together. It is for this reason that $\vec{M}_0$ acquires a $\vec{M}_{XY}$ component in the horizontal plane which is maximum when $\alpha = \pi/2$ and conserves a variable component $\vec{M}_Z$ in the z-axis direction (except when $\alpha = \pi/2$). The frequency of the rotation of the magnetization vector is always equal to that of the precession movement. When the irradiation ceases the system returns progressively to the initial state of equilibrium. A coil detects the component in the y-direction (Figure 15.8).

What happens in the probe of the apparatus? A common interpretation suggests imagining an external observer linked to a rotating frame about the z-axis at the frequency of precession of the concerned nuclei (Figure 15.8). For example, let us assume the case of a single precession frequency. When the radiofrequency has this particular value, the nuclei seem, to the observer, to feel only the effects of the rotating component of the field (i.e. perpendicular to z-axis). The magnetization vector deviates until an angle α. After the pulse the individual magnetic moments lose their phase coherence through interaction with the spins of the neighbouring nuclei, faster than they can re-align themselves according to the Boltzmann distribution function which described the populations before the pulse. Thus, $\vec{M}_0$ loses its xy-plane component, without having returned to its initial value in the OZ direction, which can take a longer period of time.

■ The actual process for obtaining the whole range of frequencies by computation did not exist when the first generation of NMR machines were built. In the earliest NMR instruments, the former approach consisted of obtaining resonance by sligthly modifying $\vec{B}_0$ using a coil wrapped around the pole piece of the magnet (Figure 15.9). Because these instruments used to sweep the magnetic field, they operated at a single frequency (CW-NMR). The spectrum was nevertheless recorded in Hz since the Larmor equation corralates field with frequency. This form of absorption NMR can also be performed with a constant magnetic field and a frequency which is varied. This process for finding the signal is similar to the sequential method of recording optical spectra, or even in daily life, when seeking an FM radio station on an analogue receiver transistor.

In this mode of acquisition, the quality of the spectrum depends on a small fraction of the total recording time. Fourier transform spectrometers make much better use of the recording time.

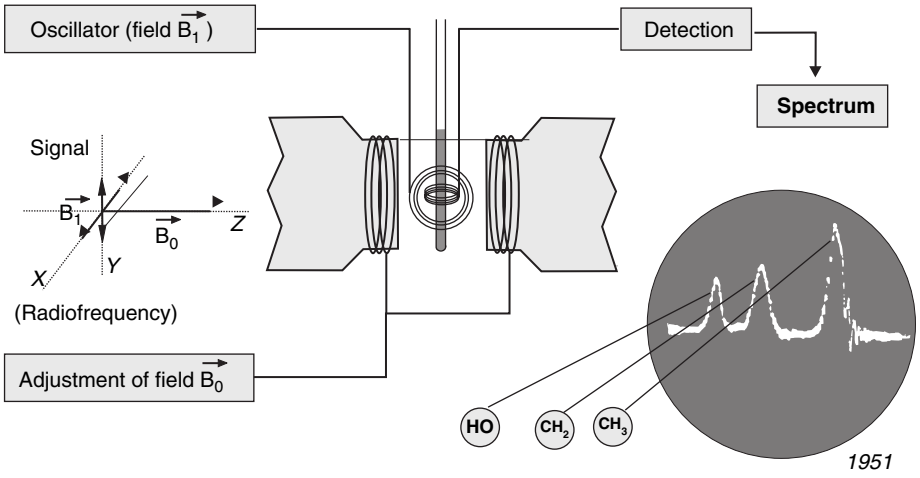


Figure 15.9 *CW-NMR. Arrangement of the different coils around the probe between the poles of the magnet.* Signal is detected along x or y axes. Right, an historic recording (1951) of the proton spectrum of ethanol. The three signals correspond to the six hydrogen atoms of CH_3, CH_2 and OH. This will be better understood after reading Section 15.8.

Technically speaking, the sample is irradiated for a few microseconds with an intense pulse, which is a means of exposing the sample to all frequencies included in the domain to be sampled. This can be compared to a flash of white light (comparing a polychromatic light with respect to a monochromatic source). For example, when working at 300 MHz, in order to irradiate all of the protons whatever their environment, a frequenciy range covering at least 6000 Hz is required. Under these conditions a small fraction of each type of proton (but not all the nuclei), will absorb the resonance frequency.

To find a schematic representation for a compound absorbing tens of frequencies is obviously impossible. In such cases, $\vec{M}_0$ can be dissociated into many vectors, each of which precesses around the field with its own frequency (Figure 15.8, for a simplified situation). During the return to equilibrium, which can take several seconds, the instrument records a complex signal due to the combination of the different precession frequencies present, of which the intensity decreases exponentially with time (Figure 15.10).

This damped interferogram is called *free induction decay* or FID. The signal corresponds at each instant to an overall numerical value which informs on the frequencies of the nuclei that have attained resonance at that particular instant. These values can be transformed by Fourier calculations that converts the signal from the *time domain* into a classically spectrum of the *frequency domain*.

This *pulsed wave* technique, is a simultaneous method in the sense that the apparatus acquires, at each instant, information concerning all of the frequencies present. The major developments of current NMR result from the generalization of this procedure which permits the study of less sensitive nuclei, such as ^{13}C.

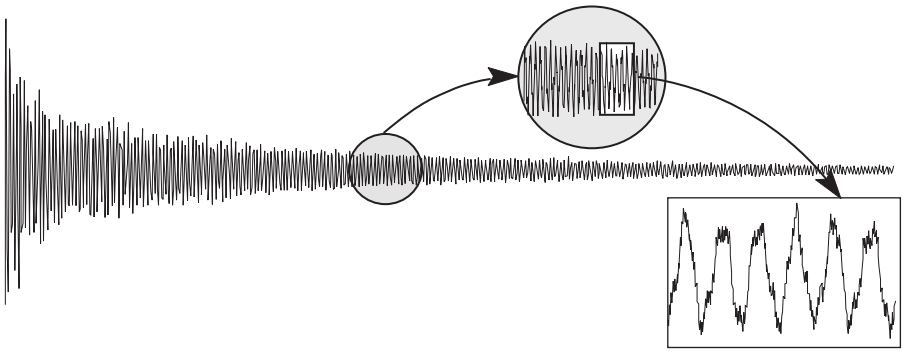


Figure 15.10 *Interferogram of fluroacetone obtained with a pulsed wave NMR instrument.* The signal $I = f(t)$, after conversion to a classic spectrum $I = f(\nu)$ by Fourier transform, is that of the spectrum shown in Figure 15.18. The interferogram, recorded over of a few seconds can be accumulated many times to improve the signal/background noise ratio. The computer does not record a continuous FID, but a FID that is sampled at a constant interval.

15.7 The processes of nuclear relaxation

After the pulse of radiofrequency, $\vec{M}_0$ will regain its equilibrium value after a relaxation time which is depending upon the element and the medium (Figure 15.11). This relaxation period depends on both the reconstitution of the populations to their initial state (time constant T_1), named *longitudinal relaxation* (spin lattice relaxation) and on the loss of phase coherence (time constant T_2), called *transverse relaxation* (spin–spin interactions). Each of the spin packets is experiencing a slightly different magnetic field and rotates at its own Larmor frequency. In solution, T_1 is never longer than a few seconds (for ^{1}H), while it

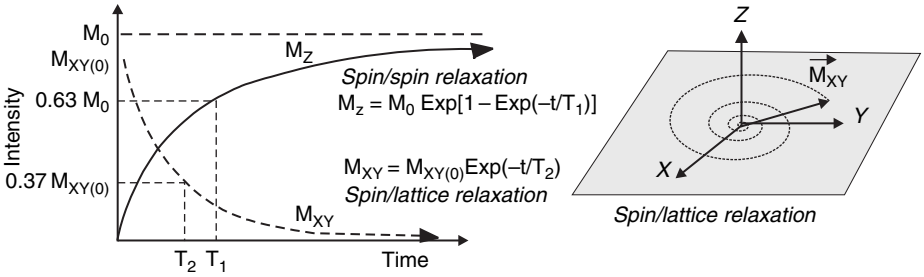


Figure 15.11 *The two processes of nuclear relaxation.* Evolution over time of spin–spin and spin/lattice relaxation. T_1 is defined as the time required to change the z-component of magnetization by a factor of e. The greater the rigidity of the medium, the smaller T_2 will be. T_2 is always less than or equal to T_1. The net magnetization in the xy-plane goes to zero and then the longitudinal magnetization grows in until to get $\vec{M}_0$ along the z-axis.

can be as long as several hours with solids. These two components of $\vec{M}_0$ must therefore be dissociated. Knowledge of the lifetimes of T_1 and T_2 yields very useful information on the structure of the samples analysed. T_1 decreases when the viscosity of the medium increases, which causes signal broadening. Changes in T_2 also has an effect upon the width of signals. A neat liquid compound leads to broader signals than they would be if the compound was in a dilute solution.

15.8 Chemical shift

So far as NMR is concerned, molecules present in dilute solutions form independent entities that do not interact noticeably between themselves. Alternatively, within a given molecule, the electronic and steric environment of each nucleus creates a very weak local magnetic field which shields it more or less from the action of the external field $\vec{B}_0$. Each atom has a particular environment – at least if there are no specific elements of symmetry in the molecule. According to the Larmor equation, these very weak local variations in the intensity of the field will affect the frequency of resonance as compared to what would be seen in a vacuum. This effect is called *shielding* or *deshielding* of the nuclei.

This observation is the basis of the principal exploitation of NMR: rather than observing all of the nuclei present, spread over a wide range of frequencies (several tens of megahertz), the study focuses on a single type of nucleus at a time. In other words the technique zooms over a very narrow range of frequencies (for example 1000 Hz) in order to record the different signals which result from specificities of each compound (Figure 15.1). This screening effect is quantified by the *shielding constant* σ which appears in equation 15.8 linking the effective field B_{eff} which reaches the nucleus with the external field B_0:

$$B_{\text{eff}} = B_0(1 - \sigma) \tag{15.8}$$

All variations in σ affect the resonant frequency of the corresponding nucleus. This phenomenon is called a *chemical shift*. As many chemical shifts are observed as there are molecules containing different shielding constants σ. Consequently large molecules lead to complex spectra as a result of their numerous σ values. For a nucleus i for which $I = 1/2$, the Larmor equation becomes, on the introduction of the effective field B_{eff}:

$$\nu_i = \frac{\gamma}{2\pi} B_0(1 - \sigma_i) \tag{15.9}$$

Concerning the proton ^{1}H, expression 15.9 leads to a difference of 1 Hz for the resonance frequency when the surrounding field varies of 2.3×10^{-8} T, while a variation of 10^{-4} T (1 gauss) induces a shift of 4258 Hz. For this reason the setting

of the magnet must be perfectly controlled in order to maintain the stability of the field B_0 in the NMR instrument. Thus, the temperature has to be controlled to within a 1/100th of a degree. Furthermore, the sample is rotated in a tube with very thin walls to reduce the heterogeneity of the field. Considering these constraints, the construction of mobile NMR instruments is a real achievement (Figure 15.33).

15.9 Measuring the chemical shift

According to the Larmor equation, the slightest variation in the field has repercussions on the resonance frequencies. Thus, it would be inadvisable to try to compare spectra or identify compounds using the absolute signal frequencies obtained with different instruments. For this reason the chemical shift is referenced by a relative scale $\Delta\nu/\nu$, which is independent of the instrument. That is why a compound acting as an internal standard is used and can therefore serve as a fixed reference, as well as the frequency ν_{app} of the instrument. For the nucleus considered, the different chemical shifts can then be obtained by dividing the frequency difference $\Delta\nu$ between each signal of the compound studied and that of the standard, by the characteristic frequency ν_{app} of the instrument (expression 15.10).

The values obtained are expressed in parts per million (ppm). In order to calculate the chemical shift δ_i (ppm) corresponding to a signal of frequency ν_i with respect to an internal standard (ν_{ref}), there is no need to know the absolute frequencies, only $\Delta\nu$ is necessary.

$$\delta_i = \frac{\nu_i - \nu_{ref}}{\nu_{app}} \cdot 10^6 = \frac{\Delta\nu}{\nu_{app}} \cdot 10^6 \qquad (15.10)$$

The internal standard used for both ^{1}H and ^{13}C NMR is tetramethylsilane (TMS). This inert and volatile compound (boiling point = 27 °C), yields a single signal in both ^{1}H NMR (for the 12 equivalent protons) and in ^{13}C NMR (for the four equivalent carbons). TMS signal is positioned at origin of the δ scale (Figure 15.11). Almost all organic compounds have positive values for δ, ranging for ^{1}H from 0 to 15, to 250 for ^{13}C.

■ The TMS signal is located on the right-hand of the spectrum, respecting the convention according to which, in spectroscopy, the energy parameter, positioned along the abscissa, decreases when moving towards the right of the spectrum.

The δ scale has enabled the generation of empirical correlation tables based on the chemical shifts as a function of chemical structure, which can be used irrespective of the NMR instrument (Tables 15.5 and 15.6 at the end of this chapter). Expression 15.10 shows that if the spectra of the same compound,

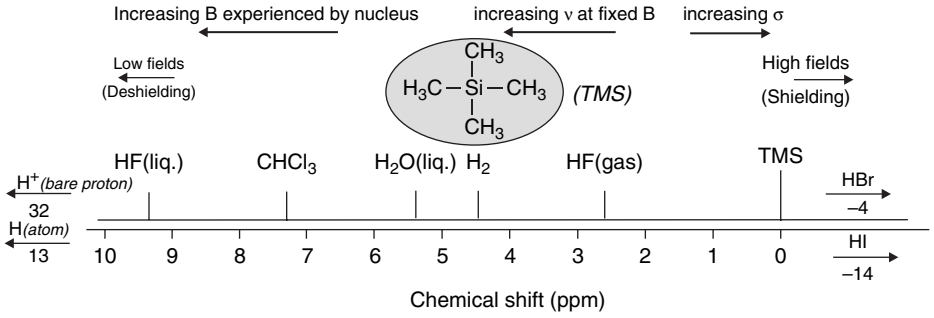


Figure 15.12 *Chemical shifts of certain compounds in proton NMR*. Shielding effects recorded with a fixed frequency instrument.

recorded with two instruments whose fields B_0 make a ratio between them of k are compared, then the frequencies of the homologous signals for the two spectra will be in this same ratio k, the values of δ remaining the same. However, these tables are not always sufficient to make a correct attribution of the signals. Software programs have been developed to help in this regard.

15.10 Shielding and deshielding of the nuclei

The more marked the *screening* effect, the more the nuclei are said to be *shielded*: in order to obtain resonance the value of the field $\vec{B}_0$ must be increased, at least when referring to a continuous wave instrument operating at fixed frequency. Signals situated to the right of the spectrum are said to be resonant at *high field* and by contrast signals observed to the left of the spectrum correspond to deshielded nuclei and are said to be resonant at *low field* (Figure 15.12).

15.11 Factors influencing chemical shifts

Examination of a large number of NMR spectra allows to highlight the factors that are responsible for predictable and cumulative effects on the chemical shifts.

15.11.1 Effects of substitution and hybridization

The simple replacement of a hydrogen atom by a carbon-containing group R produces a deshielding of the remaining protons. This substituent chemical shift reaches 0.6 ppm when comparing RCH_3 to CHR_3. This effect can attain 40 ppm for ^{13}C NMR. The state of hybridization of the carbon atoms greatly influences also the position of the signals.

These *anisotropic* variations are due to the chemical bonds, that is, to the non-homogeneous electronic distribution around bonded atoms, to which can be added the effect of unimportant magnetic fields induced by electron circulation. Ethylenic protons are therefore deshielded as they are positioned in an electron-poor plane. Inversely, acetylenic protons are shielded, because they are located in the axis of the C–C bond and so, plunged into an electron-rich environment. Aromatic protons are displaced towards lower fields because, as well as the anisotropic effect, a local field produced by the movement of the aromatic electrons or the 'ring current,' is superimposed to the main magnetic field (Figure 15.13).

15.11.2 Resonance and inductive effects

The chemical shifts of organic compounds are sensitive to the delocalization of electrons bonding. Particularely, the existence of mesomeric forms provokes large shifts of the signals (Figure 15.14).

Thus, it is comprehensible that the electronic effects which modify the polarity of the bonds have an influence upon the chemical shifts. Table 15.3 illustrates the effect of electronegativity upon the position of the methyl signal in the halomethane series CH_3-X where the chemical shift of the carbon atom signal increases with the electronegativity of the halogen X.

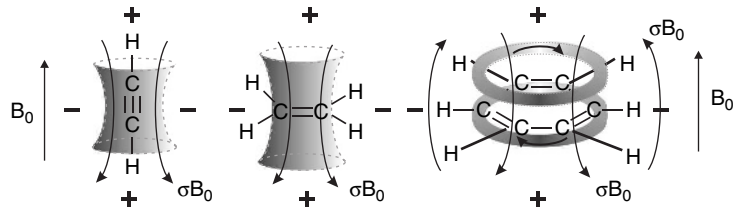


Figure 15.13 *Anisotropic effects and induced local fields.* The presence of π-bonding can be translated as zones of either a (+) shielding or a (−) deshielding effect. The ethylenic protons are at the outside of a kind of double cone of protection while the aromatic protons undergo the effect of the electrons moving in two parallel circular clouds.

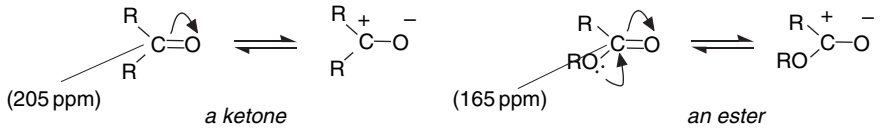


Figure 15.14 *Effects on NMR signals of the resonance effect in mesomeric forms of a carbonyl.* If the carbonyl of a ketone is compared with that of an ester, then it should be noted that for the ketone, the more electropositive of the two, the carbon has less protection than the ester. In ^{13}C NMR the carbonyl signal for a ketone is around 205 ppm while for an ester it is around 165 ppm.

Table 15.3 Influence of the electronegativity χ^* of a halogen on δ (TMS ref.)

	$CH_3F(\chi = 4)$	$CH_3Cl(\chi = 3.2)$	$CH_3Br(\chi = 3)$	$CH_3I(\chi = 2.6)$
δ_H (ppm)	4.5	3	2.7	1.3
δ_C (ppm)	75	30	10	−30

* electronegativity λ in eV according to the pauling scale

15.11.3 Other effects (solvents, hydrogen bonding and matrix)

^{1}H NMR spectra of organic compounds are usually obtained in solvents not containing hydrogen atoms. The solvent, far more abundant than the solute of which the concentration is only of several percent, leads to associations of which the stability depends upon the respective polarities. Consequently, the sample concentration and solvent used must be provided with the correlation tables.

The most widely used solvent is deuterated chloroform ($CDCl_3$) which is sufficiently polar to dissolve the majority of organic compounds. Also used are acetone-*d6*(C_3D_6O), methanol-*d4*(CD_3OD, pyridine-*d5*(C_5D_5N) or heavy water (D_2O).

■ In ^{1}H NMR, the position of the chloroform signal in mixtures chloroform/toluene varies from 7.23 ppm (90 per cent chloroform/10 per cent toluene v/v.) to 5.86 ppm (10 per cent chloroform/90 per cent toluene). This shift towards a higher field, for toluene-rich mixtures rich is explained by the presence of complexes causing the proton of chloroform to be located in the protecting zone of toluene's aromatic nuclei.

When the studied compounds have labile hydrogen atoms, D ↔ H exchanges can occur in certain solvents. These exchanges will cause alterations to the intensity and position of the relevant signals. Likewise, hydrogen bonding is able to modify the electronic environment of some protons making their chemical shift sometimes difficult to predict.

Finally, interactions between neighbouring molecules and also the effect of viscosity will alter the resolution of the spectrum through the spin/lattice relaxation time.

15.12 Hyperfine structure – spin–spin coupling

As a general rule, NMR spectra contain more signals than there are nuclei with different chemical shifts. This phenomenon is due to the external magnetic field which influences all of the atoms of the sample compound, causing an alignment of all nuclear spin and that the orientation taken in the magnetic field by one

nucleus affects the neighbouring nuclei, through valence electrons. If the distance between non-equivalent nuclei is less than or equal to three bond lengths, this effect called spin–spin coupling or J coupling is observable. *Homonuclear coupling* (between nuclei of the same type) or *heteronuclear coupling* (between nuclei of different types, as $^{1}H/^{13}C$)) gives rise to small shifts in the signal. This hyperfine structure of the spectrum brings additional information about the compound which is studied. Homonuclear coupling between protons is quite frequent. The presence of ^{13}C, ^{31}P and ^{19}F leads also to heteronuclear couplings with the protons.

■ This phenomenon must not be confused with the interaction through distance between two nuclei which exchange their magnetization because the structure of the molecule is such that they are in close proximity in space, although a great number of bonds separate them. This is the Nuclear Overhauser effect which manifests itself by modifications in signal intensities.

15.13 Heteronuclear coupling

15.13.1 Hydrogen fluoride: a typical heteronuclear coupling

Hydrogen fluoride (HF) is a simple molecule that allows the observation of a spin–spin heteronuclear coupling between the two atoms that are linked by a covalent bond. When the ^{1}H spectrum of this compound is examined, two signals of equal intensity are observed though the molecule itself contains only one hydrogen atom (Figure 15.15). The reason for this is the following: when a sample of this compound is placed in the probe of the instrument, that is equivalent to insert a very large number of individual hydrogen fluoride molecules into the magnetic field $\vec{B}_0$. Knowing that both H and F have the spin number $I = 1/2$, the molecules are distributed at thermal equilibrium into four populations E_1 to E_4, that correspond to the following spin combinations given on Figure 15.15.

If there was no interaction between the H and F nuclei, the populations E_1 and E_2 on one side and E_3 and E_4 on the other, would have the same energy and consequently in ^{1}H NMR, only a single signal would be seen (Figure 15.16). However the reality is different as an interaction exists between H and F. With

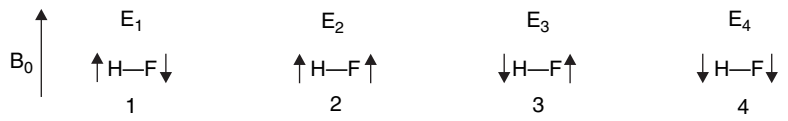


Figure 15.15 *HF sample in ^{1}H NMR – the four populations in the magnetic field $\vec{B}_0$.*

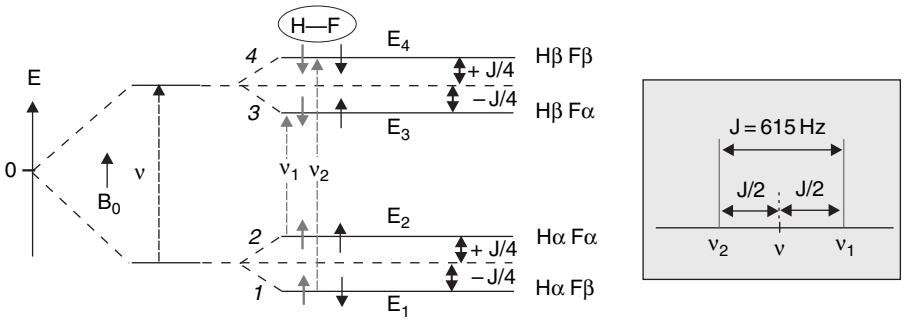


Figure 15.16 *Coupling diagram for the HF molecule in* ^1H *NMR*. Hypothetical comparison of the situation in which there is no coupling with the fluorine atom and the real situation. The values for ν_1 and ν_2 differ in length by J Hz.

respect to the situation in which no coupling arises, a theoretical development leads to the conclusion that the two populations E_1 and E_2 correspond now to two different energy states: it follows that there will be a spliting-up of the peak as supposed previously. The energy required to go from E_2 to E_3 is not the same as from E_1 to E_4. The spin orientation taken by the fluorine atom has an effect on the transition energy observed in ^{1}H NMR. The spectrum of this compound will therefore contain two peaks corresponding to the transitions situated on Figure 15.16.

The *coupling constants*, expressed in hertz, are available from the NMR spectrum. They are designated by the letter J with an index giving the name and the number of bonds which separate these atoms. If we consider the case of HF, the coupling constant can be written:

$$^1J_{\mathrm{FH}} = 615\,\mathrm{Hz}$$

The spin orientation of the fluorine atom, which has a resonance frequency very far from that of the proton, will not be disturbed by the resonance occuring in protons : in a field of 2.35 T ^{1}H resonates at 100 MHz while ^{19}F resonates at 94 MHz. The two peaks of the ^{1}H signal are separated by 615 Hz, irrespective of the intensity of B_0. The ^{19}F NMR spectrum for this molecule would lead to a similar spectrum. Two peaks would be observed separated by 615 Hz, due this time, to the coupling with the proton following one or other of the orientation chosen.

When the number of bonds separating the atoms concerned increases, the spin–spin couplings weaken very quickly, assuming that there are no multiple bonds (double or triple) which could propagate the spin effect through the π-bond electrons.

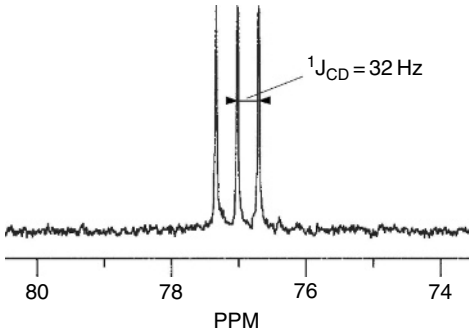


Figure 15.17 *^{13}C Spectrum in $CDCl_3$ showing the coupling $^1J_{CD}$.* The three signals of this spectrum are caused by the heteronuclear coupling between the ^{13}C and the atom of deuterium ^2H (or D), (coupling $^1J_{CD} = 32$ Hz). Since the deuterium has a spin number $I = 1$, there are three possibilities for m: $-1, 0, +1$, which leads to the triplet. The three components are of equal intensity as there is only a single atom of deuterium. This triplet is present on almost all ^{13}C spectra, in superimposition, when this solvent is used.

15.13.2 Heteronuclear coupling in organic chemistry

The presence in a molecule of atoms such as ^{13}C, ^1H or ^{19}F which possess a spin number of 1/2, is at the origin of numerous heteronuclear couplings, exploited for their structural information (Figures 15.17 and 15.18).

15.14 Homonuclear coupling

Signal splitting, as studied in the case of hydrogen fluoride, is often encountered in organic compounds with adjacent hydrogen atoms if their chemical shifts are different.

15.14.1 Weakly coupled systems

Nuclei are said to be weakly coupled when the coupling constants are much smaller than the differences in chemical shifts of the nuclei concerned (after conversion in Hz). The ethyl group of butanone (Figure 15.1) illustrates this situation. These five protons – 3 and 2, weakly coupled give rise to three peaks (a *triplet*) towards 1.1 ppm and to four peaks (a *quadruplet*) towards 2.5 ppm.

The origin of these peaks in the spectum can be ascribed by their chemical shifts (Table 15.5). The triplet is due to the CH_3 and the quadruplet to the neighbouring

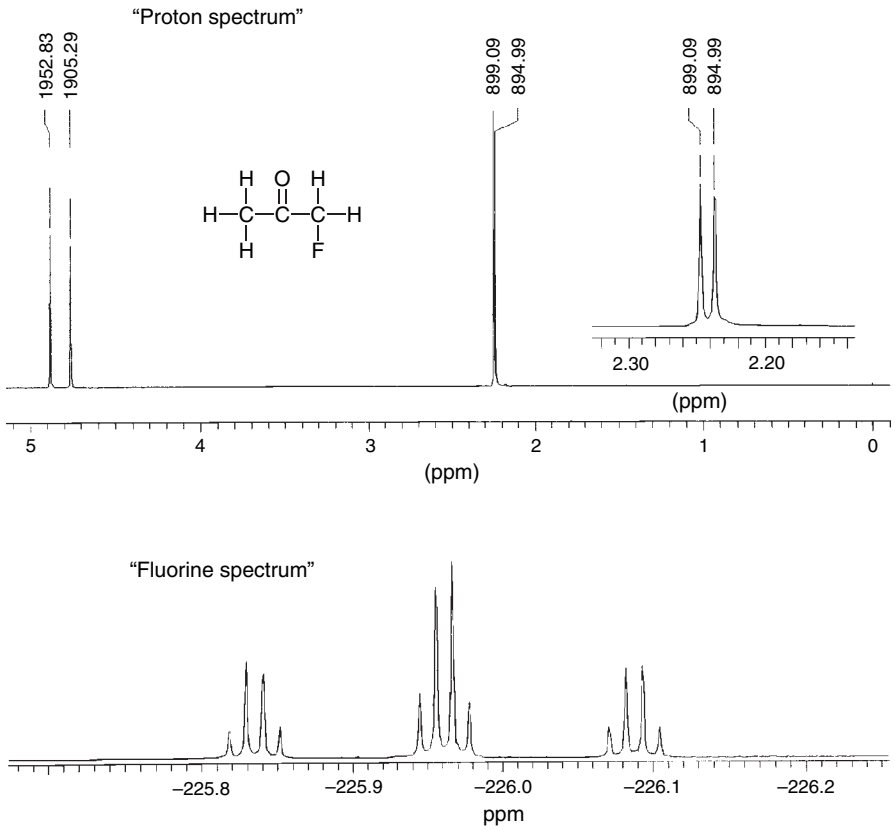


Figure 15.18 *NMR spectra of monofluoroacetone.* An example of heteronuclear coupling. Above, the ^{1}H NMR spectrum. The presence of the fluorine atom leads to a doublet for the methyl group ($^{3}J = 4.1$ Hz) as well as for the CH_2 ($^{2}J = 47.5$ Hz). Below, spectrum of ^{19}F. The single fluorine atom of this molecule leads to a triplet with the CH_2 group and a quadruplet with the methyl (explanation in next section). The resulting signal is therefore a triplet of quadruplets. With the aid of table 15.4 the coupling constants can be calculated and compared for the two spectra (the scale for the chemical shifts is positioned with respect to $FSiCl_3$.

CH_2. The relative intensities of the components, within each of these multiplets can be deduced by the laws of statistics (Figure 15.19 and Table 15.4).

As a general rule for weak couplings, if n nuclei (spin number I), are placed in the same magnetic environment, influencing in an identical manner one or several adjacent nuclei, then the signals observed for the latter will be formed of $2nI + 1$ peaks regularly spaced – i.e. $(n + 1)$ signals in the case where $I = 1/2$. The T_1 and T_2 values of all the spins must be equal. The peak intensities in a multiplet will follow the same pattern found in each row of Pascal's triangle (Table 15.4). However, if a group of protons is submitted to the effect of neighbouring nuclei for which the chemical shifts and the coupling constants are different, the previous

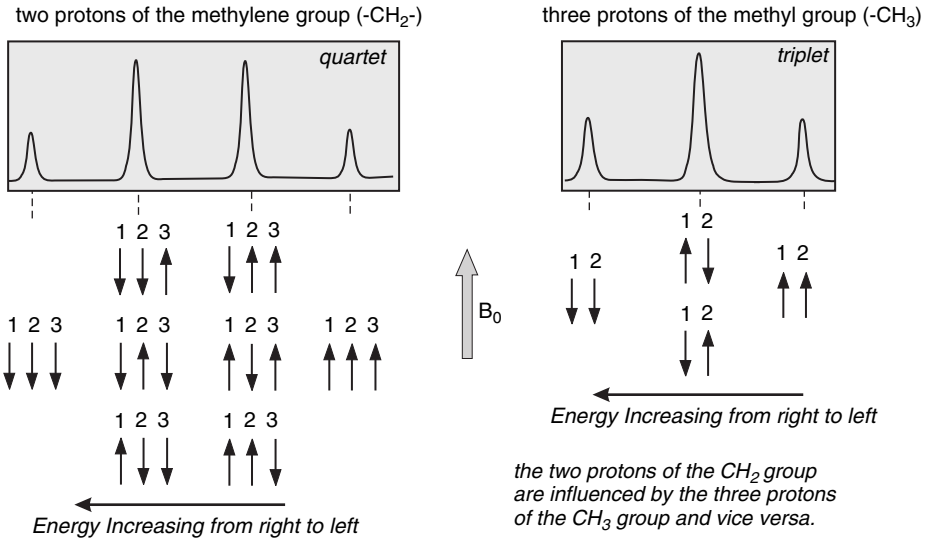


Figure 15.19 *Representation of the different spin states for the five protons of an ethyl group*. In the same row are found the spin states which produce the same effect on neighbouring nuclei. The sample contains a large number of identical molecules, these are distributed across several populations giving each one a statistically weighted signal like the number of states per row of this scheme.

Table 15.4 Pascal's triangle and its application to NMR for $I = 1/2$

Neighbouring hydrogens	Multiplicity	Intensity						
0	singlet	1						
1	doublet	1	1					
2	triplet	1	2	1				
3	quadruplet	1	3	3	1			
4	quintuplet	1	4	6	4	1		
5	sextuplet	1	5	10	10	5	1	
6	septuplet	1	6	15	20	15	6	1

approximation is no longer applicable: the multiplicity of the signals and the intensity of the peaks cannot be deduced so easily.

■ *Nomenclature for coupled systems in* ^{1}H *NMR*. Spectral interpretation for a molecule with many hydrogen atoms is easier when sub-groups of signals correspond to classic situations. These particular cases are classified by means of a nomenclature using the letters of the alphabet, selected with respect to the chemical shift (Figure 15.20). Protons with the same chemical environment or chemical shift are called equivalent. If the chemical shifts are too little different, the protons are

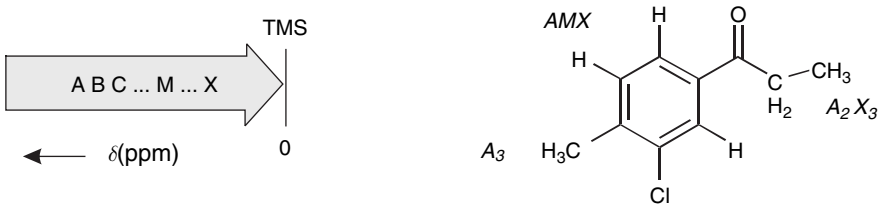


Figure 15.20 *NMR nomenclature of spectra.* The ^{1}H spectrum of 3-chloro-4-methylpropiophenone is the complex result of the superimposition of the signals of several independent yet easily recognized sub-groups.

designated by the first letters of the alphabet (AB, ABC, A_2B_2, A_3), while protons with very different chemical shifts are designated by letters such as A, M, and X (i.e. AX, AMX, AX_3, etc.). Like this, the ethyl group (as ethoxyl), constitutes a system A_2 X_3; the protons of ethanal represent an AX_3 system while a vinyl group will be ABC, AMX or ABX depending upon the example studied.

In ^{13}C NMR, as the coupling constants $^{1}J_{C\text{-}H}$ being of the order of 125 to 200 Hz, there are frequent signal overlappings, which makes the spectrum more difficult to interpret. Technically it is possible to eliminate the entire set of couplings for all of the protons (see Figure 15.21).

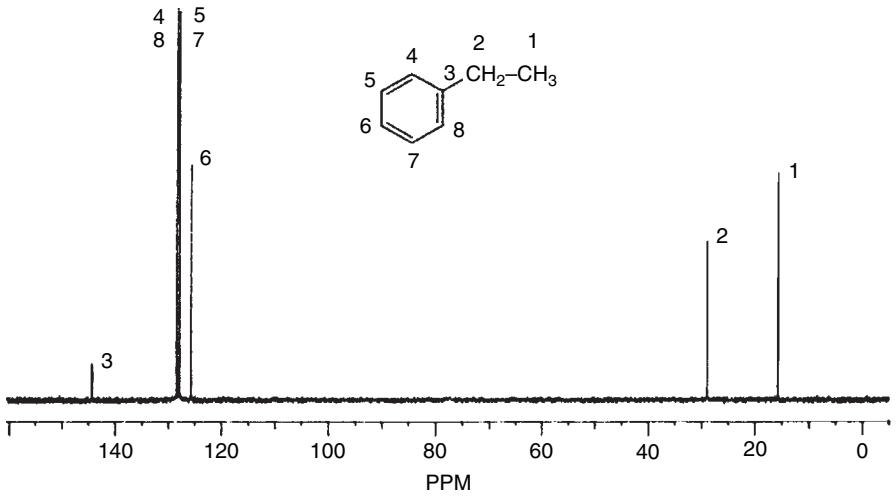


Figure 15.21 *Proton decoupled ^{13}C spectrum of ethylbenzene.* Each carbon atom gives a single signal consisting of a singlet. These simpler 'broad band,' decoupled spectra yield less information.

15.14.2 Strongly coupled systems

When the ratio $\Delta\nu/J$ is small the protons become strongly coupled. The intensities of the multiplets are then very disturbed. New peaks arise from combinations of frequencies hindering the interpretation of spectra. This is one of the reasons for the development of instruments operating at higher frequencies (300 → 750 MHz) with supraconducting magnets to obtain the required magnetic fields. Given that $\Delta\nu$ is proportional to the operating frequency, while J remains constant, the ratio $\Delta\nu/J$ increases and again nuclei appear as weakly coupled (Figure 15.22). However there are other phenomena that complicate the spectra when the frequency exceeds 600 MHz.

AB system. When the difference in resonance frequencies between two protons is comparable with their coupling constant J, then this system gives rise to a total of four peaks. This is the simplest strongly coupled system. The signal for each proton contains two peaks separated by J Hz. However the intensities are no longer equal and the chemical shifts, which can no longer be read from the spectrum, must be calculated from the expressions indicated on Figure 15.23.

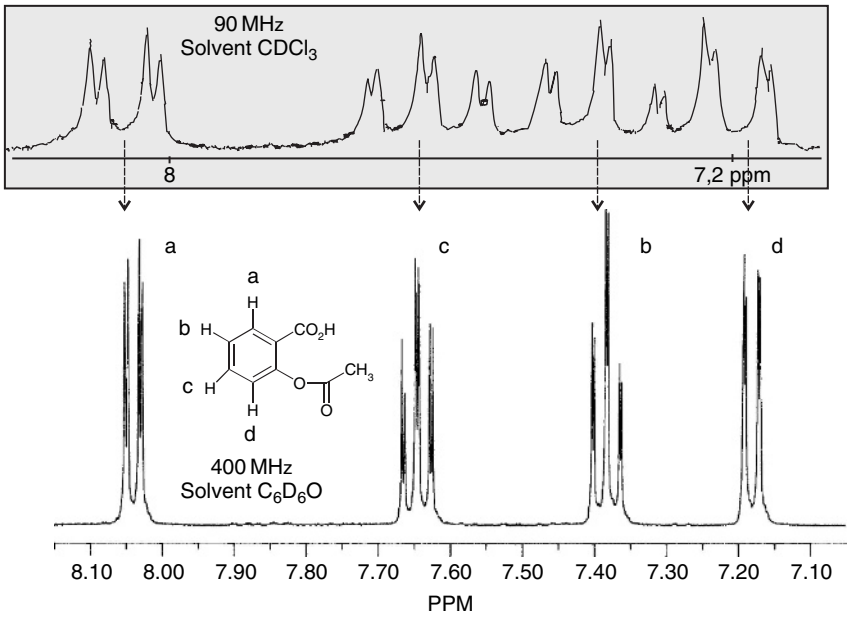


Figure 15.22 *Spectrum of the four aromatic protons of aspirin*. The figure above reproduces, with a similar scale, aspirin spectrum obtained on two different instruments, one running at 90 MHz (solvent $CDCl_3$) and the other at 400 MHz (solvent C_3D_6O). The sensitivity of NMR grows as $\vec{B}_0^{3/2}$.

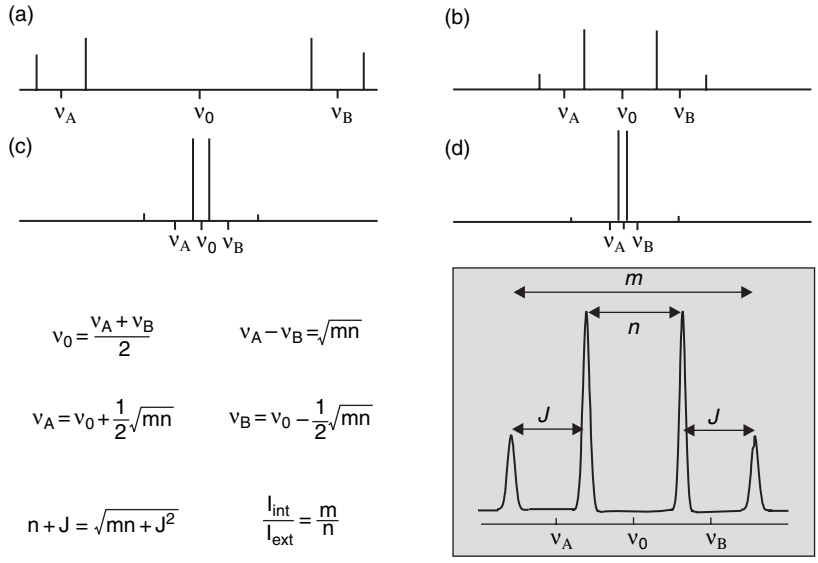


Figure 15.23 *Characteristics defining an AB system.* (a–d) Gradual modification of a two-proton coupled system as the ratio $\Delta\nu/J$ decreases. Typical appearance and formulae used to analyse an AB system.

15.15 Spin decoupling and particular pulse sequences

NMR instruments have accommodated numerous advances to facilitate spectral interpretation. Thus it is possible to cancel the effect of an existing spin-coupling between neighbouring nuclei. This *spin decoupling* technique is based on the statement that a nucleus in resonance does not always conserve the same spin state with time. This uninterrupted and fast transfer between different states induces a global effect upon the neighbouring nuclei.

In practice, a spin decoupling experiment begins with a record of the spectrum under normal conditions. Next, decoupling is achieved with the aid of a saturation pulse: a second spectrum is recorded while irradiating at the resonance frequency of the nucleus (or nuclei) that is (are) to be decoupled (Figure 15.24). This double resonance technique is used to identify nuclei which are coupled and which cause interpretation difficulties in the spectrum, in particular for superimposed signals.

Fourier transform NMR at high fields ($B_0 = 17.6\,\mathrm{T}$ for the study of $^1\mathrm{H}$ at 750 MHz) has considerably extended the potential of this method. This is due to an increase in acquisition speed and rapid computation of spectra.

A variety of experiments based upon particular pulse sequences can be applied to a sample to produce a specific form of NMR signals. For example, the 90-FID pulse sequence rotates the net magnetization down into the *xy*-plane, through which the relaxation times of the nuclei can be determined. Amongst the more

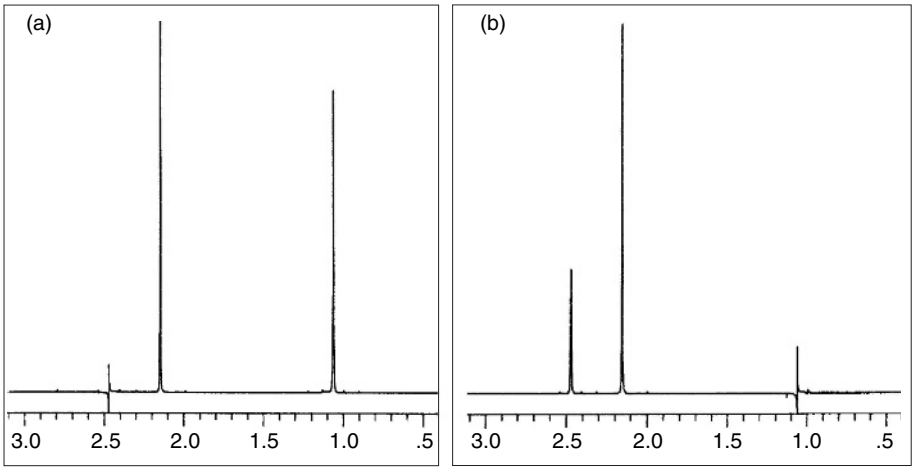


Figure 15.24 *Spin decoupling experiment on butanone.* Modification of the ^1H spectrum by (a) Irradiation of the CH_2 group at 2.47 ppm; (b) Irradiation of the CH_3 group (of ethyl) at 1.07 ppm, both for comparing with the spectrum of butanone, Figure 15.1. For such a simple compound such as this, the experiment is only of illustrative interest. On the other hand, a double resonance experiment would allow the precise determination of the different couplings in aspirin (Figure 15.22).

classical methods, multidimensional 2-D or 3-D NMR allow the deduction of very useful structural information. When analysing bio-polymers such techniques lead to results comparable with those obtained by radiocrystallography, permitting, amongst other things, the representation of molecular conformations in their natural medium.

■ ***Magnetic resonance imaging (MRI)*** Proton NMR can be exploited to obtain images of all objects containing hydrogen atoms, from living organisms to geological strata. This important development of NMR has been successfully applied to medical analysis under the name of MRI, a form of non-invasive physical observation, without penetration, adapted to soft tissues. The volume to be examined must be placed in a magnetic field, which requires the manufacture of very large supraconducting magnets for total body instruments. Proton signals are the easiest to observe since biological tissues contain around 90 per cent water (an individual of 70 kg comprises 50 kg of water compared to 13 kg of carbon). In other cases the signal of the CH_2 groups, present in lipids, are monitored. The final image is a cartographic presentation of the intensity distribution of the same type of proton signal. The contrast in the image is due to the variations in relaxation times for the protons of the selected plane. Amongst the specific technical complexities is the focusing, or spatial selectivity, needed to obtain a spectrum of a quasi-punctual volume within an object. 'Slices' of the patient are imaged as a matrix of small volumes of tissue (voxels) producing an individual signal. These 'slices' can then be reconstructed to provide images in any plane (Figure 15.25).

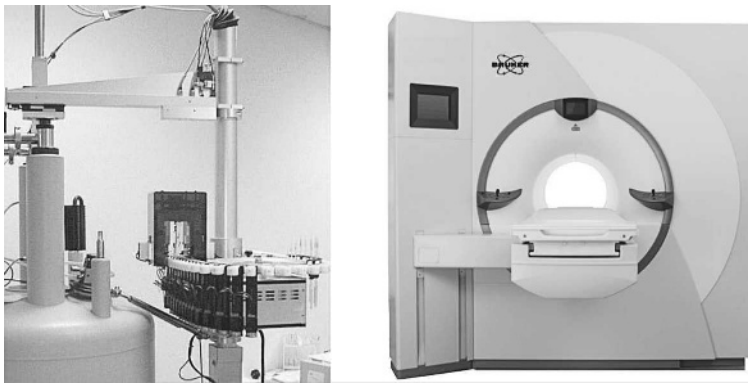


Figure 15.25 *Sample introduction for NMR spectrometers*. Left, automatic introduction of a sample placed in an 'NMR tube' within the magnetic field produced by a superconducting coil maintained at liquid helium temperature (reproduced courtesy of Varian). Right, a large magnet employed for the introduction of a particular kind of sample – the human body (part of a MRI instrument from SMIS).

15.16 HPLC-NMR coupling

The hyphenation of liquid chromatography with NMR spectroscopy is one of the most powerful and time-saving methods for the separation and structural elucidation of unknown compounds and mixtures (Figure 15.26). Unfortunately, HPLC-NMR spectroscopy is a relatively insensitive method requiring sufficient sample concentration in the NMR flow cell. Bearing in mind the tendency toward the miniaturization of HPLC and the consequent reduction of the quantities being chromatographed, on-line coupling of HPLC with NMR offered a real challenge. The use of deuterated organic solvents for mobile phase is generally expensive. The principle consists of passing the mobile phase from the exit of the detector of the chromatograph to a microcell placed in the NMR apparatus. The quantity of a compound in the probe depends about the detection volume.

There exist in principle two general methods for carrying out HPLC-NMR: *on-flow* and *stopped-flow* experiments.

With the on-flow mode of operation the output of the chromatographic separation is recorded simultaneous with ^{1}H NMR spectra. In most cases the flow of the mobile phase is decreased to allow a large number of NMR spectra to be recorded of one chromatographic peak.

However if the quantity of compound is very small then it may be necessary to interupt the flow of the mobile phase as soon as the maximum of the peak reaches the flow cell (indicated by UV detector). This stopped-flow mode is used in order to accumulate hundreds of scans to improve the signal/noise ratio of the resultant spectrum.

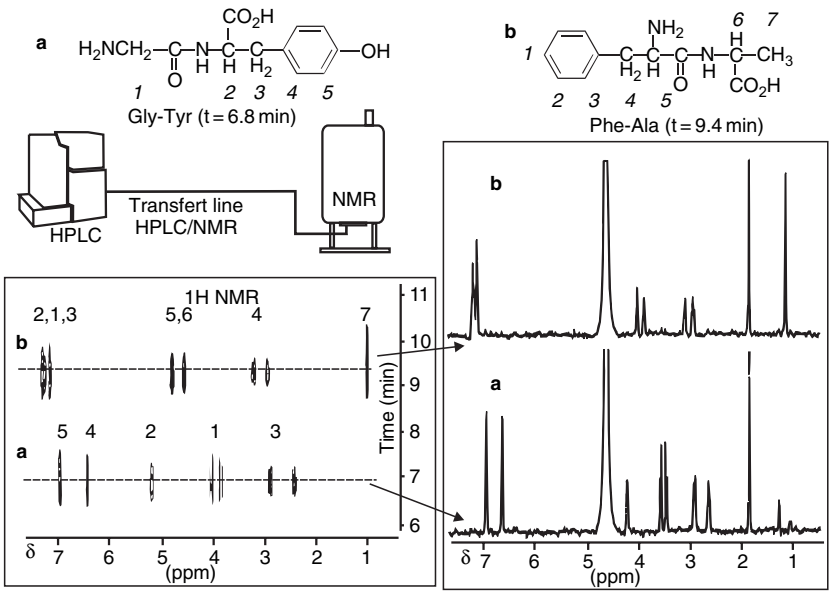


Figure 15.26 *An experimental result by HPLC-NMR coupling.* Separation of two dipeptides (5 μg each) with identification of the signals. For more clarity the peaks of the solvents around 2 and 5 ppm (acetonitrile and water) have been eliminated (Sweeder R.D. *et al. Anal. Chem.* 1999, 71(23), 5335).

15.17 Fluorine and phosphorus NMR

Fluorine and phosphorus are the two heteroatoms in organic chemistry most frequently studied by NMR after hydrogen and carbon.

Fluorine element, consisting of 100 per cent $^{19}F(I = 1/2)$, can be compared with ^{1}H for its sensitivity. Its electronegativity being greater than hydrogen (4 rather than 2.1), chemical shifts are distributed on a much greater range (Figure 15.27). As a consequence, in ^{19}F NMR, it becomes possible to distinguish between compounds that are chemically very similar. In particular, differences due to stereochemistry are significant with the coupling constants $J_{F\text{-}H}$ extended over a greater range compared to $J_{H\text{-}H}$ (Figure 15.17). On the other hand, relatively few molecules contain atoms of fluorine. Therefore, the fluorine NMR spectra are generally obtained from compounds to which a ^{19}F (or a group CF_3) has been intentionally introduced in a known position by chemical modification, in order to answer questions of structure from the comparison of spectra. The fluorine atom induces a chemical shift comparable to that of an hydroxyl (OH) group, but with minor modification in the stereochemistry of the molecule. The Van der Waals radius of fluorine is 1.35 Angstrom instead of 1.1 for the hydrogen atom.

Phosphorus (^{31}P, $I = 1/2$) another common monoisotopic element, has been studied since the beginnings of NMR. It has a great sensitivity and it is an important element in the composition of numerous biological compounds.

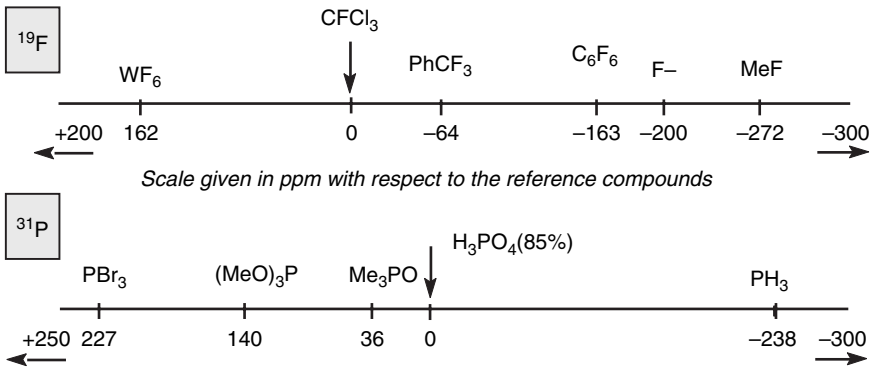


Figure 15.27 *Positions of some NMR signals due to fluorine and phosphorus.*

15.18 Quantitative NMR

Although NMR is a method essentially reserved for structural analysis, due to the quality of the information that it can yield, it can be used also to give the composition of mixtures. This application is possible if the signals chosen for locating each constituent have areas which can be measured independently. Although the sensitivity and the precision vary with the type of nucleus, this method presents interesting features such as the possibility to carry out analyses without complicated sample preparation and whithout destruction of the sample. There is no risk of polluting the instrument and in contrast to many other methods, such as chromatography, there is no need of a prior standardization step. From the molar ratios to which spectrum examination yields access, it is possible to deduce mass concentrations.

■ In organic synthesis, ^{1}H NMR permits to calculate the yields of a reaction. Finally, industry uses 'low resolution' analysers based upon ^{1}H NMR to quantitate water and fats in food industry, like in many other fields.

15.18.1 Measuring areas – application to simple analysis

Areas of peaks on spectra can be given in the form of numerical values, or for older apparatus, can be taken from the integration plots that are superimposed upon the spectrum (Figure 15.1). Yet it is difficult to measure signals to better than 2 per cent accuracy for various technical and physical reasons. NMR-lines are theoretically Lorentzian curves, which are quite broad at their bases, and bear substantial areas even under 1–2 per cent of peak intensity (usually in the noise region). In ^{13}C NMR, it is preferable to add a relaxation agent in order to avoid saturation, linked to the relaxation times, which alters the intensities of the signals. The delay between successive scans must be long enough to allow the magnetization to achieve equilibrium along the z-axis (approximately $5 \times T_1$ for the proton with the longest T_1).

Consider, for example, a sample comprising a mixture of acetone **A** and of benzene **B** diluted in $CDCl_3$ as solvent. On the ^1H spectrum of this mixture two signals are observed at $\delta = 2.1$ ppm (acetone) and at $\delta = 7.3$ ppm (benzene), with respect to TMS. This spectrum corresponds to the weighted superimposition of the two individual spectra (Figure 15.28).

In this particular case where the molecules of the two compounds each have six protons, the ratio of the areas of the two peaks is representative of the ratio n_A/n_B of the respective numbers of molecules of A and B (given that the response factors are equal for A and B). Assuming that the mixture only contains these two components, and designating S_A as the area of the acetone signal and S_B that of benzene, it follows that:

$$\frac{n_A}{n_B} = \frac{S_A}{S_B} \tag{15.11}$$

If C_A and C_B are the concentrations expressed in percentage mass of **A** and **B**, of which the molar masses are M_A and M_B respectively, then:

$$\begin{aligned} C_A + C_B &= 100 \\ \frac{C_A}{C_B} &= \frac{n_A \cdot M_A}{n_B \cdot M_B} \end{aligned} \tag{15.12}$$

Or, by substituing into expression 15.11:

$$\frac{C_A}{C_B} = \frac{S_A \cdot M_A}{S_B \cdot M_B} \tag{15.13}$$

Therefore,

$$C_A = 100 \cdot \frac{S_A M_A}{S_A M_A + S_B M_B} \quad \text{and} \quad C_B = 100 \cdot \frac{S_B M_B}{S_A M_A + S_B M_B} \tag{15.14}$$

15.18.2 Samples containing identifiable compounds

A more general case is that in which the signal (or signals) selected for each compound to be measured do not correspond globally to the same number of

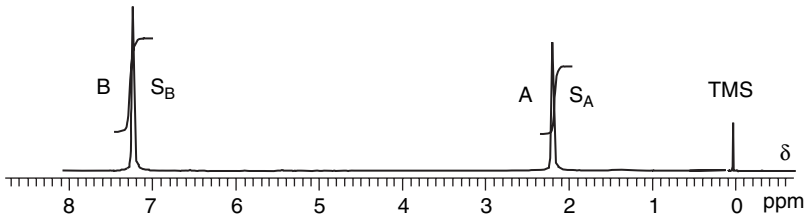


Figure 15.28 *^1H spectrum of a mixture of acetone (A) and benzene (B).* If $S_A = 111$ and $S_B = 153$ (arbitrary units), then knowing that $M_A = 58$ and $M_B = 78$ g/mol, $C_A = 35\%$ and $C_B = 65\%$ total mass.

protons, either because the molecules do not have the same total number of protons, or alternatively, if only a portion of the spectrum of each compound has been chosen in order to identify it.

Supposing for example, that the signal selected for compound A corresponds to a protons and the signal chosen for compound B corresponds to b protons (A and B do not represent acetone and benzene as in above section). When the spectrum of the mixture of A and B is recorded each molecule of B leads to a signal whose intensity is different to that of a molecule of A. The expressions in 15.15 will remain valid provided corrections are inserted to take into account these differences. Each area must be divided by the number of protons that are at the origin of the selected peak, in order to normalize the relative area to one proton of either A or B. Then substituing S_A and S_B by the corrected areas S_A/a and S_B/b, the two preceding expressions become:

$$C_A = 100 \cdot \frac{\frac{S_A}{a} M_A}{\frac{S_A}{a} M_A + \frac{S_B}{b} M_B} \quad \text{and} \quad C_B = 100 \cdot \frac{\frac{S_B}{b} M_B}{\frac{S_A}{a} M_A + \frac{S_B}{b} M_B} \tag{15.15}$$

Expression 15.16 can be transposed to the case where n constituents are visible on the spectrum. By using labels such as A, B, …, Z and in locating each constituant by a specific area, ca. S_i for i protons for the component I, the general equation 15.16 giving the mass per cent of each one can be formulated :

$$C_i = 100 \cdot \frac{(S_i/i) \cdot M_i}{(S_A/a) \cdot M_A + (S_B/b) \cdot M_B + \ldots + (S_i/i) \cdot Mi + \ldots + (S_Z/z) \cdot M_Z} \tag{15.16}$$

With this type of calculation, ^{1}H NMR is frequently used in organic chemistry as a method to find the yield of a reaction A → B. This is done by recording the spectrum of the coarse material after reaction and by identifying a signal belonging to the product formed (B) and a signal belonging to the remaining starting material (A). The yield of B with respect to A can be written using the previous notations :

$$R = 100 \cdot \left(\frac{S_B}{b}\right) / \left(\frac{S_A}{a} + \frac{S_B}{b}\right) \tag{15.17}$$

15.18.3 The internal standard method

A more general situation corresponds to the measure of a single compound in a sample. To do that, it is not indispensable to know the nature of all the other components that are present, or to identify *all* of the signals in the NMR spectrum. In fact, it is sufficient to find a signal belonging to the compound of interest.

In order to quantify compound X ($M = M_X$) in a mixture called E, a quantity p_R mg of reference compound R (with mass M_R) for use as internal standard, is

added to p_E mg of the sample E containing compound X, before recording the spectrum. The standard R is chosen such that the signal serving as indicator does not interfere with the signal chosen to quantify compound X (Figure 15.29). Next, on the NMR spectrum of the mixture containing the internal reference, a signal belonging to compound X (area S_X for x protons) is chosen as well as a signal belonging to standard R (area S_R for r protons).

By designating n_X/n_R the molar ratio of X and of R, as before, expression 15.19 can be obtained:

$$\frac{C_X}{C_R} = \frac{n_X \cdot M_X}{n_R \cdot M_R} \tag{15.18}$$

knowing that

$$\frac{n_X}{n_R} = \frac{S_X/x}{S_R/r} \tag{15.19}$$

The concentration C_R, expressed as a percentage mass of R, being equal to:

$$C_R = 100 \cdot \frac{p_R}{p_E + p_R} \tag{15.20}$$

the mass concentration (per cent) C_X of compound X can be deduced :

$$C_X = 100 \cdot \frac{p_R}{p_E + p_R} \cdot \frac{(S_X/x) \cdot M_X}{(S_R/r) \cdot M_R} \tag{15.21}$$

15.18.4 The standard addition method

The previous method can be improved by preparing a number of standard solutions that contain both the sample and increasing quantities of the same compound to be measured. From the corresponding NMR spectra of these solutions, a plot can be drawn representing the area of the signal selected for the measurement as a function of the quantity added. The intercept of the graph with the concentration axis will give the unknown concentration (Figure 15.30).

The difficulty of this method is in maintaining the stability of the NMR apparatus long enough to be able to undertake successive measurements of the standard solutions.

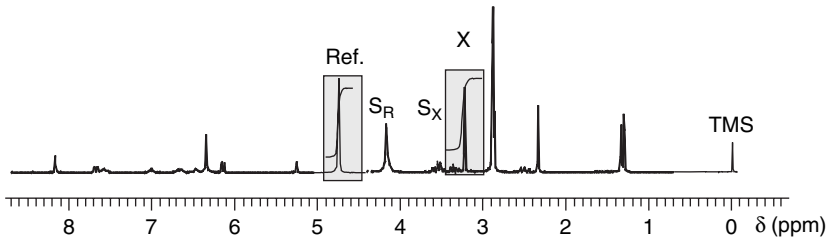


Figure 15.29 *Scheme displaying a spectrum of a sample into which a reference compound R has been added.* The signal X belongs to the compound to be measured and the signal S to the internal standard.

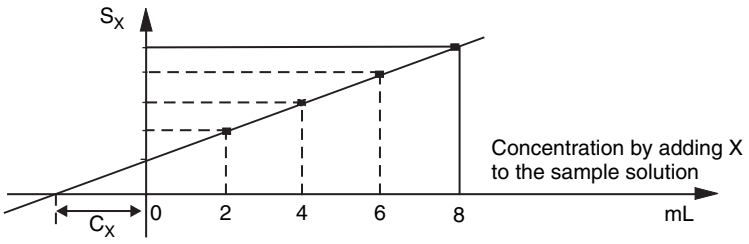


Figure 15.30 *Graph obtained for the standard addition method.* The unknown concentration corresponds to the abcissa segment between the origin of the axis and the intersection with the calibration curve.

15.19 Analysers using pulsed NMR

Pulsed wave Fourier transform NMR permits another form of routine measurement though rather yet underestimated by quality control analysists. Water and certain other organic compounds such as fats can be quantified by this technique from the hydrogen concentration in the sample. The corresponding instruments do not plot the usual NMR spectrum but give the global intensity of the FID immediately following the period of irradiation, as well as its time decay (Figures 15.31, 15.32 and 15.33). The rate with which the protons relax yields information about the environment of the hydrogen atoms. It thus becomes possible to distinguish the protons that are present in a solid compound and those that are part of a liquid. In food area, following standardization, this give access to the concentration of water and fats in a variety of samples.

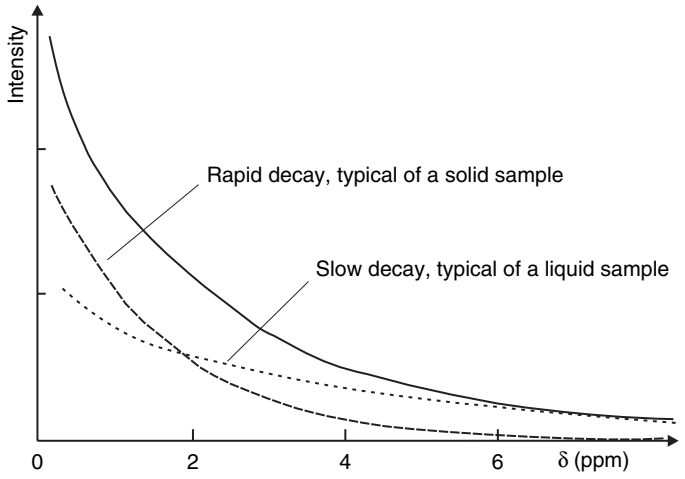


Figure 15.31 *FID for a solid/liquid mixture.* The amplitude of the FID which relates the quantity of protons as a percentage of the total mass, can be distinguished from relative measures based on T_2„ which permits the percentage of solid to be determined in the sample through deconvolution of the curve envelope and its constituents, as displayed on the graph.

Other applications follow the same principle and are based upon the detection of phosphorus or fluorine elements.

■ To determine the water resources of the subsoil (free water quantification and depth discrimination of water-tables), geologists can base their investigations upon ^{1}H NMR signals submittted to the low ambient Earth's field by using a suitably adapted equipment (the Larmor frequency is a few hundreds Hz).

Figure 15.32 *Low resolution ^{1}H NMR instrument for routine analyses.* The analyser operates on the principle of pulsed NMR and is used for quantifying water and fat in numerous food products (model minispec, reproduced courtesy of Bruker).

Figure 15.33 *NMR research instrument.* The typical appearance of these instruments corresponds, as here, to the combination of a superconducting magnet (at the bottom), of an electronic cabinet and a workstation for the analysis of results. Model Avance 400 (reproduced courtesy of Bruker).

Table 15.5 NMR chemical shifts of principal types of protons in organic molecules (reproduced by permission of Spectrometry Spin & Techniques)

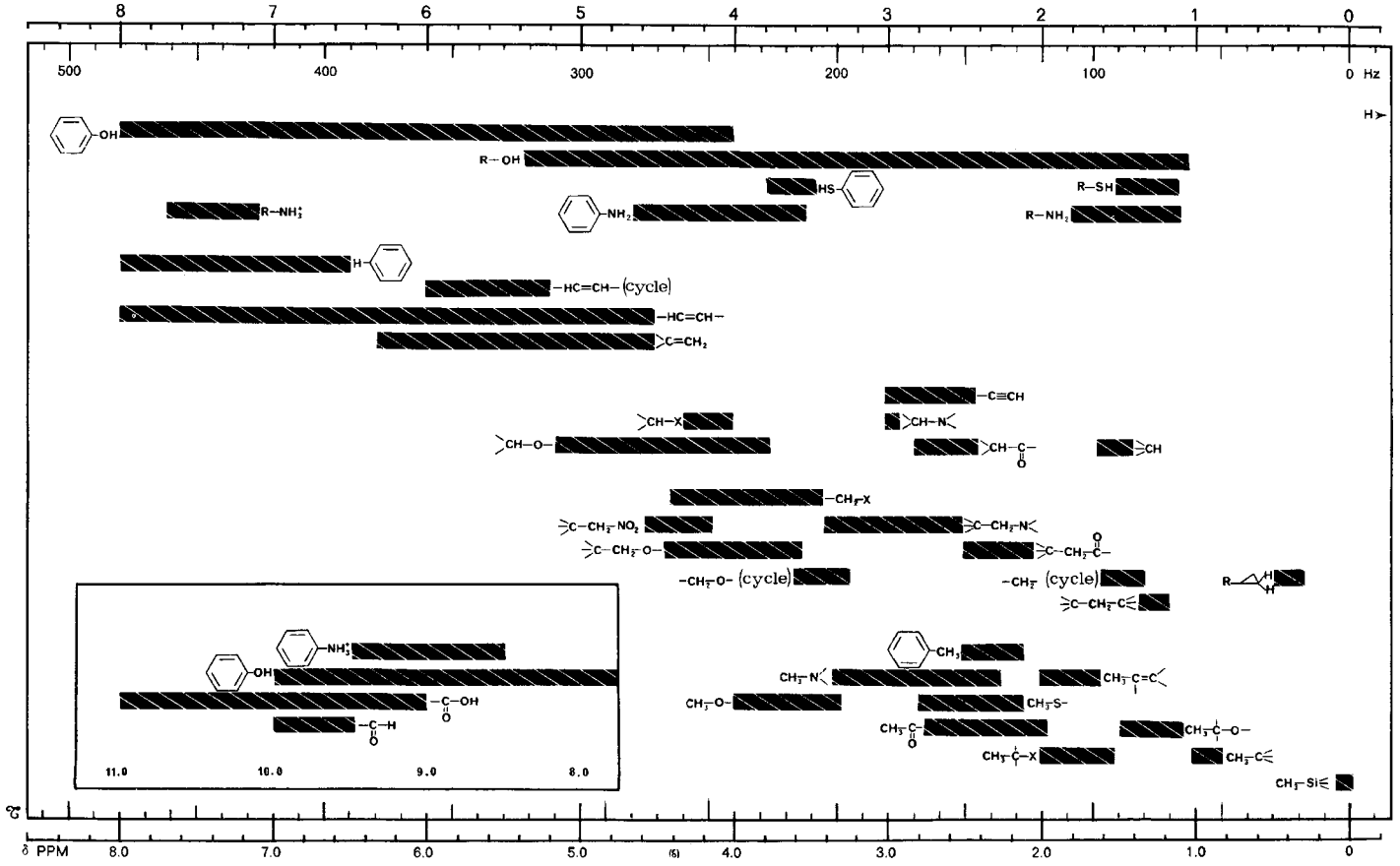
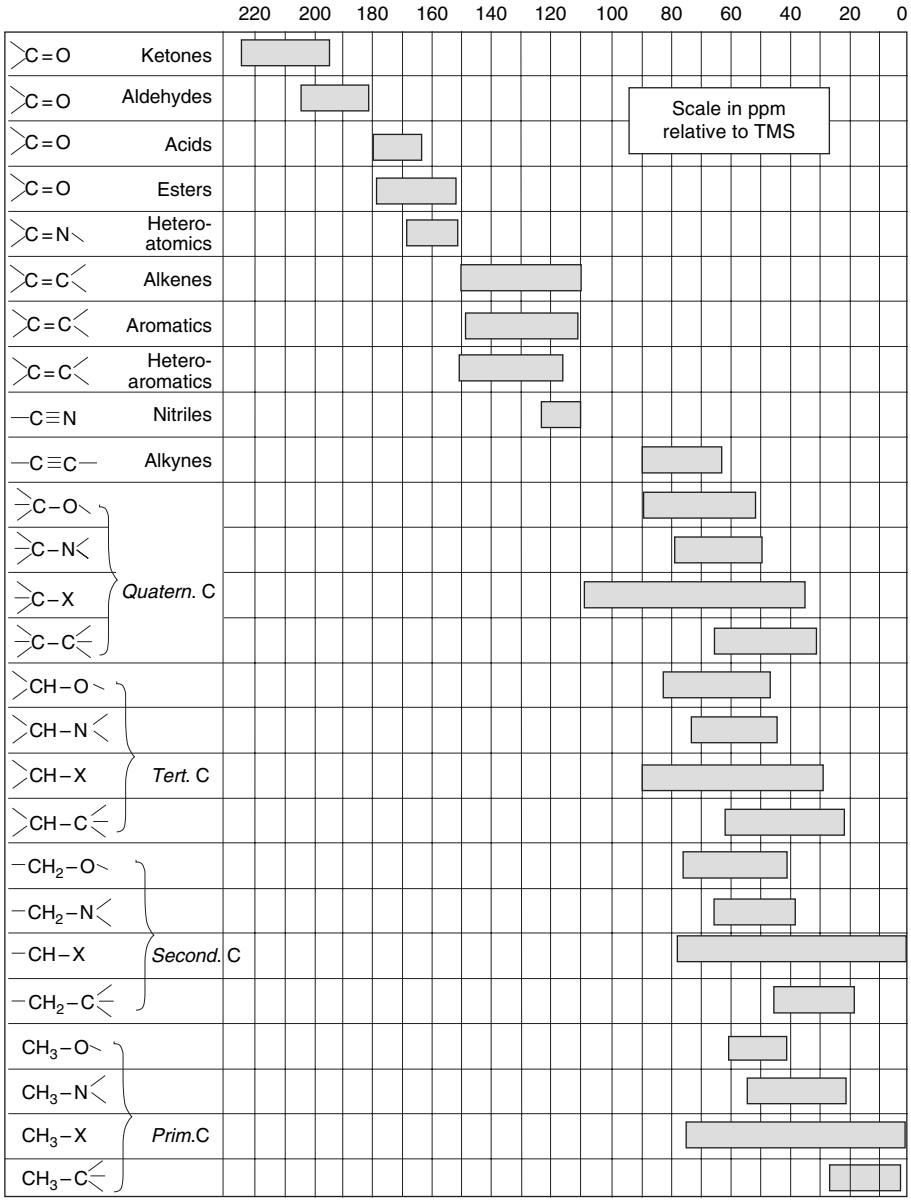


Table 15.6 ^{13}C NMR chemical shifts of principal types of carbons in organic molecules



Problems

15.1 Often in tables the values describing a nucleus' magnetic moment are represented as lying upon an axis parallel to $\vec{B}$, the applied magnetic field. Therefore, if for a proton, $\mu_z = 1.41 \times 10^{-26}\,\mathrm{J \cdot T^{-1}}$, calculate the constant γ for the proton.

15.2 If $T = 300\,\mathrm{K}$, calculate the ratio of the populations N_{E1}/N_{E2} for a proton in an NMR spectrometer where the applied magnetic field $B = 1.4\,\mathrm{T}$. Make the same calculation for the case where the field $B = 7\,\mathrm{T}$, given that $\gamma = 2.6752 \times 10^8\,\mathrm{rad\,T^{-1}\,s^{-1}}$.

15.3 In the ^{1}H NMR spectrum from a 200 MHz spectrometer the scale along the abscissa is represented by 1 ppm = cm.

1. What is the distance between two signals with a separation of 7 Hz?

2. If it is known that $\gamma\mathrm{H}/\gamma\mathrm{C} = 3.98$, what would happen to the distance calculated above if a ^{13}C spectrum were to be recorded on the same apparatus?

15.4

1. Calculate the chemical shift, δ, in ppm of a proton (^{1}H), whose NMR signal is displaced by 220 Hz with respect to TMS (the field of the spectrometer is 1.879 T).

2. The resonance signal for a proton is displaced by 90 Hz with respect to TMS when measured on a 60 MHz spectrometer. What would happen to this displacement if an apparatus of 200 MHz was employed?

3. What would be the corresponding chemical shift of the same proton with both of these spectrometers?

15.5 Two isomers A and B share the same molecular formula, $C_2HCl_3F_2$. What is the structure of each of these isomers when their proton NMR spectra, as recorded upon a 60 MHz apparatus, are the following:

The spectrum of isomer A comprises two doublets at 5.8 ppm and 6.6 ppm respectively ($J = 7$ Hz), while that of isomer B contains a triplet at 5.9 ppm ($J = 7$ Hz).

Consider now that a third isomer is present. Under the existing conditions, describe what its spectrum must be.

15.6 Numerous NMR experiments make use of the 'Magic Angle' technique which corresponds to the angle made by the spin vector and an axis parallel to the direction of the magnetic field in which it is held. What is the value of this angle in degrees?

15.7 25 mg of vanillin ($C_8H_8O_3$) is added to 100 mg of an unknown organic compound. The ^1H integration curve displayed upon the spectrum of the new mixture can be described as follows: one proton of the original compound corresponds to a value of 20 mm while a single proton of vanillin corresponds to 10 mm. What is the molecular weight of the unknown compound? Remember: H = 1, C = 12, O = 16 g/mole.

15.8 If the ratio of the magnetogyric constants γ_F/γ_H is 0.4913, calculate the distance which would separate the TMS signal from that originating from an atom of fluorine, given that the scale upon the spectrum is represented by 1 ppm = 2 cm. (The apparatus is a 200 MHz spectrometer.)

15.9 Following the reduction of acetone in isopropanol by means of a hydride the ^1H NMR spectrum of the crude, isolated product reveals that the reaction has not completed. The integration curve of the remaining acetone corresponds to a value of 24 (arbitrary units), while that of isopropanol is equivalent to 8. Calculate the molar yield of this transformation.

15.10 From the ^1H NMR spectrum below, which corresponds to a mixture of bromobenzene, dichloromethane and iodoethane, calculate the percentage composition of the three components. The values (without units) given in brackets on the integration curve are proportional to the areas of the corresponding signals.

Given: H = 1, C = 12, O = 16, Cl = 35.5, Br = 80 and I = 127 g/mol.