

Fundamentals of NMR spectroscopy

Ramakrishna V. Hosur

UM-DAE Centre for Excellence in Basic Sciences University of Mumbai, Kalina, Mumbai 400 098

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ABSTRACT

Nuclear Magnetic Resonance (NMR) discovered in 1946 has now become an indispensable tool for physicists, chemists, biologists and medical doctors alike. The technique and its applications are growing at alarming pace. The aim of this article is to present only the very fundamental concepts of NMR spectroscopy, pedagogically, and there will be no attempt to cover the various discoveries or the applications.

1. Nuclear spin and magnetic moment

Magnetic moment and spin angular momentum (or simply spin) of atomic nuclei are intrinsic properties. Spin angular momentum is a vector quantity denoted by I ; it has a magnitude, $\hbar\sqrt{I(I+1)}$, and I is a number called as 'nuclear spin' which takes discrete values.

The I value of a nucleus obeys the following rules.

- For even atomic number and even mass number I takes the value 0.
- For odd mass number $I = 1/2, 3/2, 5/2, \dots$ etc.,
- For odd atomic number and even mass number $I = 1, 2, 3, \dots$ etc.,

The intrinsic magnetic moment represented by the symbol, μ , is also a vector. The inter relation between magnetic moment and spin angular momentum is given in equation 1.

$$\mu = \gamma I \quad (1)$$

Here, γ = 'gyromagnetic ratio' or 'magnetogyric ratio', this is the characteristic feature of the given nucleus which may be positive or negative. The γ values are experimentally obtained by resonance absorption techniques.

The properties of nuclei used normally in chemistry and biology, are listed in Table 1.

The orientation of the magnetic moment in space is also quantized. For nuclear spin quantum number, I , there can be $(2I + 1)$ orientations, which will have different z-component of spin angular momentum. This is given by $m\hbar$ where m , called as the magnetic quantum number of the nucleus, can take $(2I +$

Table 1
Properties of some chosen nuclei

Nucleus	spin	Abundance	γ values rad s ⁻¹ T ⁻¹	Frequency of NMR at 11.7433 T (MHz)
¹ H	1/2	~100 %	267.522×10 ⁶	-500
² H	1	0.015 %	41.066×10 ⁶	-76.753
¹³ C	1/2	1.1 %	67.283×10 ⁶	-125.725
¹⁵ N	1/2	0.37 %	-27.126×10 ⁶	50.684
¹⁴ N	1	99.6 %	19.338×10 ⁶	-36.132
¹⁹ F	1/2	~100 %	251.815×10 ⁶	-470.470
³¹ P	1/2	~100 %	108.394×10 ⁶	-202.606

1) values: $I, (I-1), (I-2) \dots \dots -I$. All these orientations have identical energies.

2. Effect of magnetic field on the nuclear spins

An external magnetic field H_0 causes precessional motion of the spins around the direction of the magnetic field. The precessional frequency (ω_0) is given by equation 2,

$$\omega_0 = -\gamma H_0 \quad (2)$$

Further, the interaction between the magnetic moment and the magnetic field removes the energy degeneracy of the different orientations. For a spin $1/2$ nucleus, there will be two orientations: parallel and antiparallel with respect to the field. These states are represented by the symbols, α and β , respectively. Fig. 1 shows schematically, the energy separation of these two states, and the precessional motion executed by the spins in the

*Corresponding author: E-mail address: hosur@tifr.res.in (R. V. Hosur).

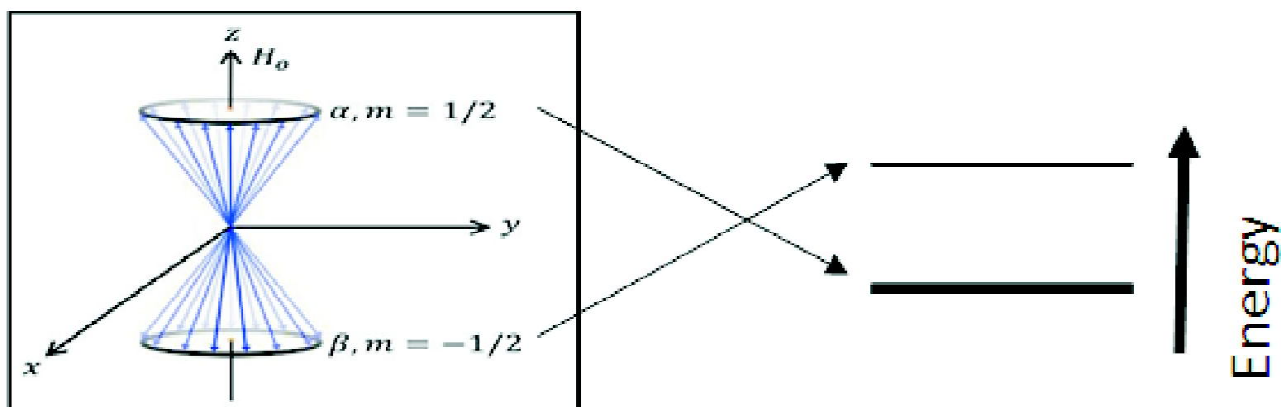


Fig. 1. In presence of magnetic field the precession of the spins in the two states α and β (left) and their energy levels (right). The α state is lower in energy than the β state. The populations are dictated by Boltzmann statistics. The α state is more populated than the β state.

two orientations. It is to be noted that in either orientation, the sense of rotation of the spins is the same. Further, in either case the spins in the ensemble will be distributed randomly on the surface of the cone and this is termed as 'hypothesis of random phases'.

The energy separation, ΔE , is given by,

$$\Delta E = \omega_0 \hbar \quad (3)$$

This equation holds good for energy separation between two neighbouring states ($\Delta m = \pm 1$) for any value of l , in general. Clearly, the energy separation and the frequency of precessional motion are directly related. In other words, the classical description represented by the precessional motion of the nuclear spins and the quantum mechanical description represented by the energy levels converge. When the system is in equilibrium, there will be more spins in parallel orientation than in antiparallel orientation. The population differences will be dictated by Boltzmann statistics. Thus, in the direction of the magnetic field there will be a net magnetization and zero magnetization in the transverse plane, by virtue of the 'hypothesis of random phases'; the term phase reflects upon the position of the precessing spin on the surface of the cone (see Fig. 1). The presence of phase coherence among the spins results in non-zero magnetization in the transverse plane.

3. Resonance absorption of energy

The magnetic moments of the nuclei can be determined by measuring the precessional frequencies. If an electromagnetic radiation whose frequency matches the precessional frequency is shone on the spin system, there will be absorption of energy which can be measured. This is indicated in Fig. 2 for a spin $\frac{1}{2}$ system.

This frequency lies in the Radio frequency regime indicating

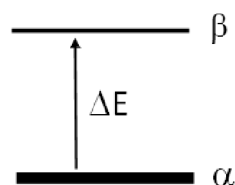


Fig. 2. Absorption of energy will take place when RF with frequency corresponding to the energy difference (ΔE) is applied.

thereby that the energy separations are very small compared to optical spectroscopy. While this may render NMR as a relatively insensitive technique, the many other aspects of NMR spectra make it an extremely powerful and useful technique for many applications in Chemistry and Biology. This arises from the fact that the absorption frequencies, depend not only on the magnitudes of the magnetic moments of the nuclei, but also on their electronic environments and the intramolecular interactions between the nuclei of the same type or of different types.

4. NMR spectral parameters

For any molecule, it is possible to observe resonances of individual nucleus types: ^1H -spectrum, ^{13}C -spectrum, ^{15}N spectrum etc. The structure and dynamics of the molecules can be assessed from a set of NMR parameters: (1) Chemical Shifts (2) Spin-Spin Coupling constants (3) Intensities of resonance lines and (4) Relaxation times and line widths. Three-dimensional structures of molecules are primarily assessed from the first three parameters and the dynamism in the molecules is evaluated from the last parameter.

4.1. Chemical shift

In a molecule an electron cloud encompasses every nucleus. Upon application of a magnetic field an induced current is gene-

rated in the electron cloud which creates a small magnetic field that generally opposes the externally applied field. Consequently, the magnetic field appears altered at the site of the nucleus. This is called as screening by the electron cloud. Equation 4 is used to calculate the effective field at the site of a nucleus,

$$H_{\text{loc}} = (1 - \sigma_{\text{loc}}) H_0 \quad (4)$$

In general, σ_{loc} , which is called the 'screening constant', can be positive or negative and has two components:

$$\sigma_{\text{loc}} = \sigma_{\text{d}} + \sigma_{\text{p}} \quad (5)$$

(σ_{d} and σ_{p} , represent diamagnetic and paramagnetic contributions, respectively). In case of proton, σ_{d} is the dominant contributor.

The change in the field due to screening is called as the 'chemical shift'. H_0 dependence of the chemical shift as seen in equation 4, is eliminated by defining it as a ratio (δ_i in equation 6).

$$\delta_i = \frac{(H_r - H_i)}{H_r} \times 10^6 \quad (6)$$

(where, H_r is the field at the site of a reference nucleus; H_i is the field at the nucleus of interest i). In terms of frequencies, this is given as,

$$\delta_i = \frac{(v_r - v_i)}{v_0} \times 10^6 \quad (7)$$

δ_i is expressed in parts per million (ppm) and it depends only on the screening constants. Thus, chemical shifts a dimensionless quantity. The reference frequency is always 0 ppm. The reference compounds should have the resonance frequency at the highest field. Fig. 3 shows the ranges of the chemical shifts for different nuclei depending upon the nature of the functional groups around them.

4.2. Spin-spin coupling

Spin-spin coupling, also denoted as J-coupling, arises out of the interaction of the electrons in the adjoining bonds. Pairing of nuclear and electron spins drives this process according to Pauli

Table 2
Ranges of J-values

Spin-pair	J(Hz)-range
$^3J: ^1H-^1H$	2–15
$^2J: ^1H-^1H$	(–12)–(–17)
$^1J: ^1H-^{13}C$	100–250
$^1J: ^1H-^{15}N$	80–100
$^1J: ^1H-^{31}P$	Few hundred Hz

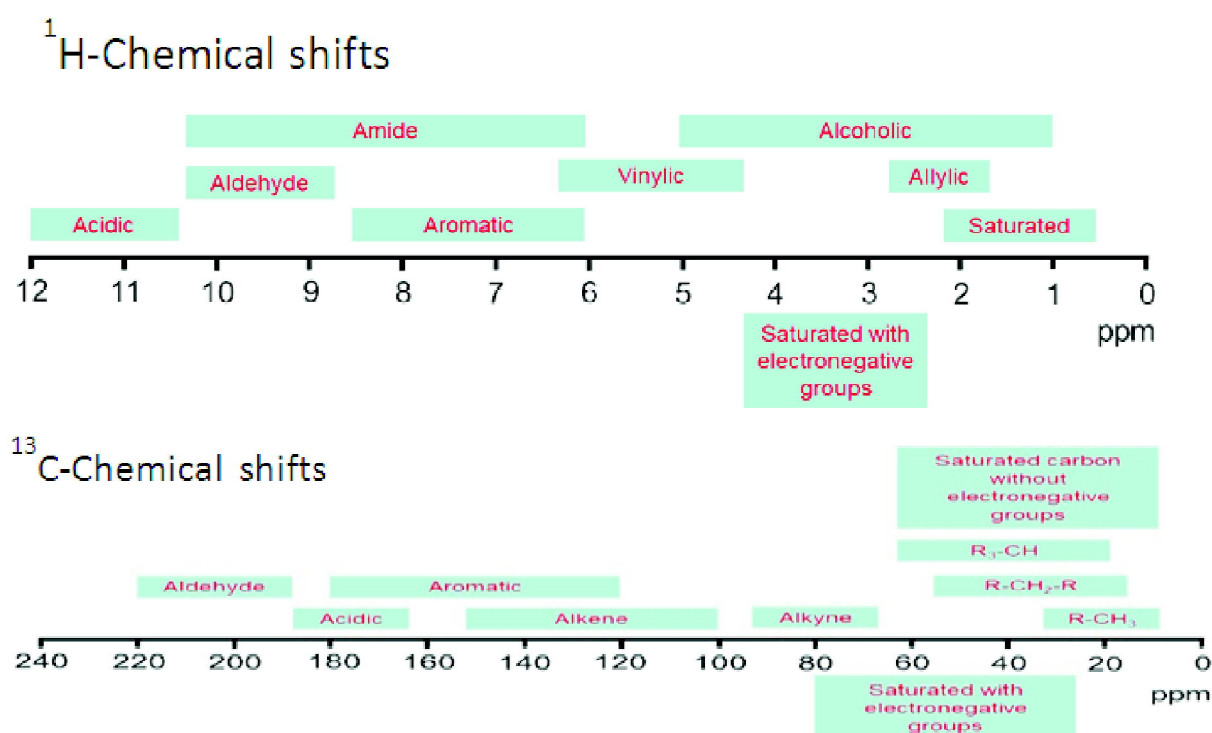


Fig. 3. Typical ranges of 1H (top) and ^{13}C (bottom) chemical shifts for different functional groups.

principles and this process continues as far as the spin order generated by such an interaction can be maintained. The spin-spin coupling can encompass up to 4-5 bonds depending on the nature of the bonds and the atoms considered. The 'coupling constant' is defined as the strength of the coupling and is denoted by the symbol nJ for two spins separated by n bonds. Table 2 shows the ranges of the different coupling constants.

4.3. Analysis of NMR spectra

The basic step of NMR analysis of a given molecule is to assign the individual peaks in the spectrum and calculate the chemical shifts and coupling constants. In all these reports, a certain nomenclature is followed for the nuclear spins. Uppercase alphabets, A, B, C etc. are used to mark the spins, and for systems with more than one spin the choice of the alphabets is based on the ratio of the pairwise difference in the resonance frequencies to the coupling constant between them. Thus, the spins A and X in an AX system, have large frequency separation compared to their J-coupling constant. In an ABX system, the frequency of spin X is far from those of both A and B, but spins A and B are themselves fairly close in relation to the coupling constant JAB. Spin systems like AX, AMX, AMQX, etc. are said to be weakly coupled while AB, ABC etc. are said to be strongly coupled. In most modern spectrometers with high magnetic fields, it would be mostly weak coupling situation. In well resolved spectra, this analysis is easy to perform and is referred to as First Order Analysis.

Following are key features of First Order analysis.

(i) The centres of the multiplets belonging to individual nuclei represent their chemical shifts.

(ii) J-coupling between magnetically equivalent nuclei (eg, A_2) does not lead to splitting of lines.

(iii) Total number of equivalent nuclei in a group is proportional to the integral of the multiplet of that group.

(iv) The coupling of a nucleus with n equivalent nuclei of another type of spinvalue I gets split into $(2nI + 1)$ lines. If the nucleus is J-coupled to two groups of equivalent nuclei having n and m nuclei with spins I_1 and I_2 respectively, then the total number of lines in the multiplet will be $(2nI_1 + 1).(2mI_2 + 1)$.

(v) Intensities of the lines of a particular nucleus, say A coupled with n equivalent nuclei with $I = 1/2$, are in the proportion of binomial coefficients in the expansion of $(a+b)^n$. Thus, in the spectrum of ethyl alcohol (Fig. 4), the CH_2 protons coupled to the three equivalent protons in the CH_3 group produce a 4 line multiplet pattern with intensities in the proportion 1:3:3:1. Similarly, the CH_3 protons produce a 1:2:1 multiplet pattern due to coupling to the two equivalent protons in the CH_2 group.

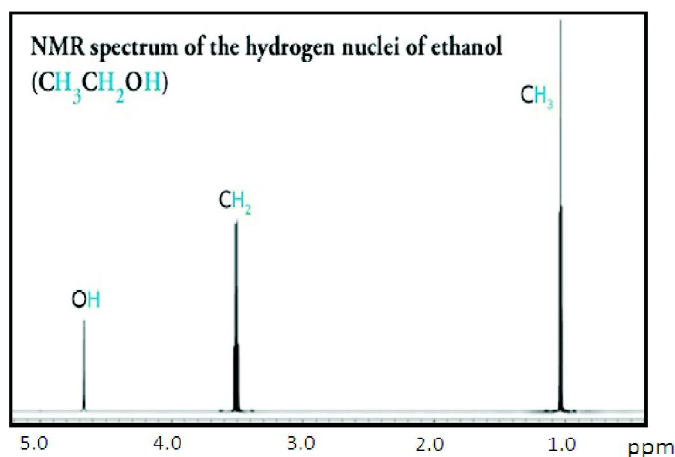


Fig. 4. NMR spectrum of ethyl alcohol.

In the most general case, energy levels of the spin system are calculated using quantum mechanics, and then transition frequencies and the intensities of the different lines are calculated. The basic interactions contributing to the energy levels in the case of solution state NMR are: (i) Chemical shift interaction and (ii) spin-spin interaction (J). Detailed discussion of these aspects is beyond the scope of this short article and can be found in many books listed at the end.

5. Relaxation times

On application of a magnetic field discrete nuclear energy levels are created and the nuclear spins are distributed according to the Boltzmann statistics. The spacing between the energy levels depends on the variation in the strength of the magnetic field and this in turn causes redistribution of spin population where some levels are losing populations and others are gaining. What brings about such a redistribution? Detailed theories indicate that this is achieved by transitions caused by fluctuations occurring in solutions due to rapid tumbling motions. Two types of characteristic times have been defined which are termed as (i) spin lattice relaxation time (T_1) and (ii) spin-spin relaxation time (T_2). The former relates to readjustments of the population differences or the magnetization along the z -axis (direction of the field) and the latter is related to decay of the magnetization in the transverse plane (x - y plane), which represents the phase coherence among the rotating spins, which in turn is related to the line widths in the spectrum.

6. Fourier transform NMR

NMR spectra were recorded in the early days by the so called 'slow passage' or 'continuous wave (CW)' spectroscopy. Either the field is swept maintaining the frequency constant or vice

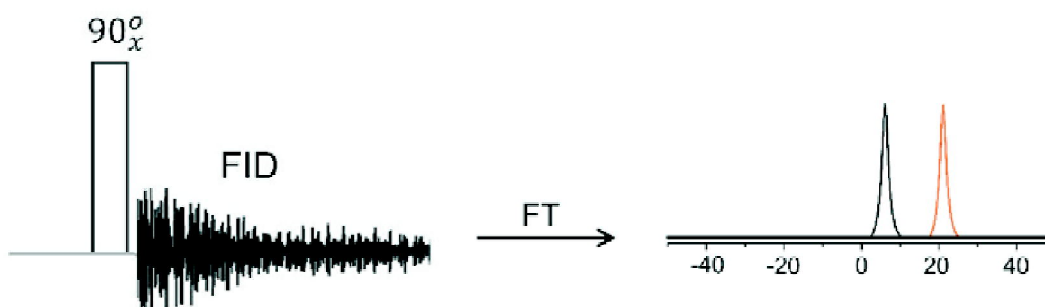


Fig. 5. Scheme of a typical FTNMR experiment. The magnetization rotates from the z-axis to the y-axis. The FID is then collected as a time domain signal by a detector placed in the transverse plane. Fourier transformation of the FID results in the NMR spectrum.

versa, to find the resonance situation. The field sweep had to be slow to ensure proper modification of the populations as dictated by the changing field. To enhance the signal-to-noise (S/N) ratios the 'signal averaging' technique is adopted. In this context, the development of Fourier Transform NMR constituted the greatest revolution in NMR methodology.

The idea is to simultaneously shine a large number of radio frequencies so that there will be a frequency to satisfy the resonance condition of every line in the spectrum. The 'RF pulse' is used to achieve this state; a single radio frequency is applied for a very short time (few microseconds). Such a time-domain input produces a wide range of frequencies in the frequency domain; this is a Fourier relation. Further, the effect of the RF pulse is also to rotate the net equilibrium z-magnetization into the transverse plane. When the RF is removed the magnetization components of the different spins rotate in the transverse plane with their characteristic precessional frequencies as they recover to the z-axis. Such a rotating magnetization induces a signal in the detector kept in the transverse plane and this is known as 'Free Induction Decay' or in short FID. Fig. 5 shows the scheme of a standard FTNMR experiment.

The FID represents a superposition of the contributions from all the spins and the different frequencies can be decoded by Fourier transformation. Mathematically, this is represented as in equation 8.

$$F(\omega) = \int_{-\infty}^{\infty} f(t)e^{-i\omega t} dt \quad (8)$$

Here, (ω) represents the NMR spectrum and $f(t)$ is the FID. Spectral lines can have two different line shapes depending upon the data acquisition schemes and these are called 'absorptive' and 'dispersive' lines shapes. These are shown in Fig. 6.

Absorptive line shapes are the most desirable because of the high resolution they yield in the spectra. With the arrival of

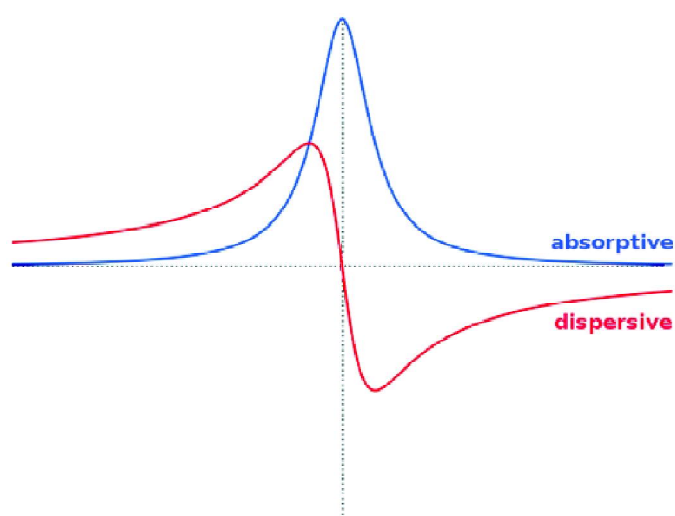


Fig. 6. Absorptive and Dispersive lines shapes in an NMR spectrum.

FTNMR concepts, the architecture of the NMR spectrometer changed completely. The data was collected digitally, computer became an integral part of the spectrometer for precise control of data acquisition parameters and data processing. This method of obtaining an NMR spectrum resulted in saving of recording time by several orders of magnitude and enabled substantial signal averaging for enhancing the S/N ratio in the spectrum. It also enabled recording spectra of lowly abundant nuclei and also of samples having very low concentrations. While FTNMR brought in many advantages, it also brought in some problems, such as mixed phases (which refers to admixture of absorptive and dispersive line shapes), dynamic range problems which relate to possible losing of small signals in the presence of huge signals (such as solvent signals), folding of signals etc. Of course, today, the technology has developed quite substantially to circumvent all these issues and one can routinely acquire high quality spectra of complex molecules. Fig. 7 shows a complex NMR spectrum of a protein in folded and unfolded states as an

Folded versus unfolded protein

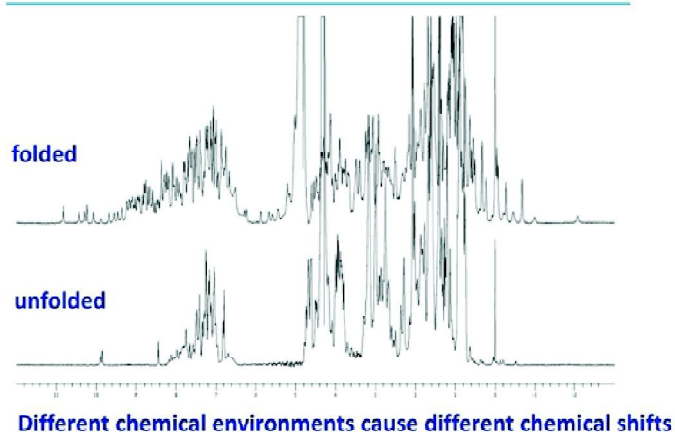


Fig. 7. FTNMR spectrum of a small protein.

illustration [1]. The excellent peak dispersion in the folded state demonstrates the potential of NMR in solving protein structures in the solution phase.

7. Multidimensional NMR

After FTNMR the most significant development is undoubtedly Multidimensional NMR Spectroscopy. In FTNMR, the excitation of the spins and detection of their response are separated in time. After excitation various kinds of spin manipulations can be introduced before the data is actually acquired. Schematically, a multidimensional NMR experiment can be represented as shown in Fig. 8. The time axis is segmented and various blocks of time periods are introduced for specific purposes.

Excitation--- t_1 --- M_1 --- t_2 --- M_2 --- t_3 --- M_3 ---|--- t_n ---acquisition(t_{n+1})

Fig. 8. Segmentation of the time axis.

The time variables, t_1, t_2, \dots, t_n are the so-called evolution periods during which magnetization components rotate with their characteristic frequencies; the data is actually acquired in the

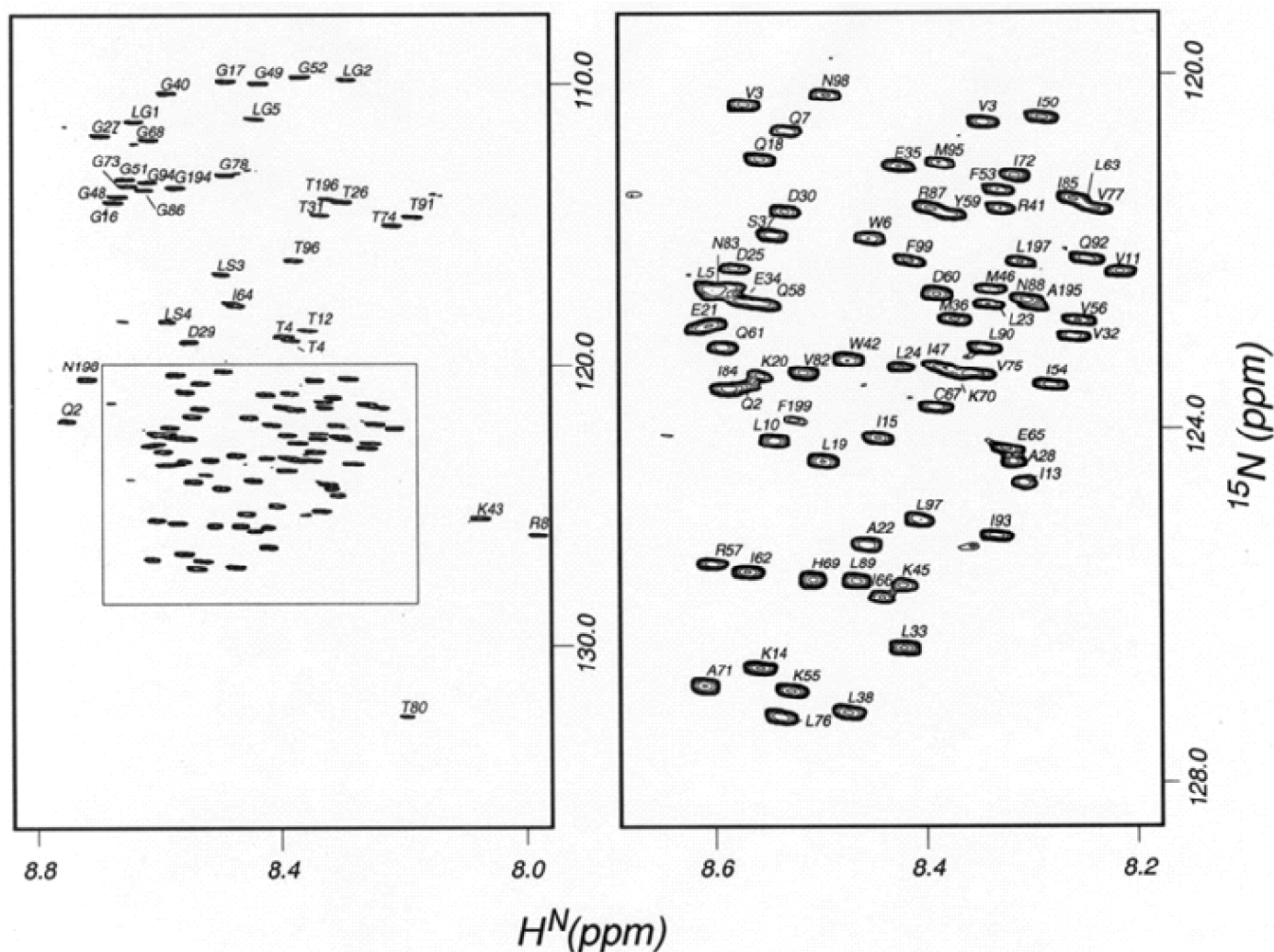


Fig. 9. An illustrative 2D spectrum of a protein HIV1-protease. The residue wise peak assignments are also indicated.

time period t_{n+1} . The evolution periods, also referred to as indirect detection periods, are systematically incremented to finally generate a $(n+1)$ dimensional time-domain data set, which after $(n+1)$ dimensional Fourier transformation yields the $(n+1)$ dimensional frequency-domain spectrum. The characteristic frequencies of the individual spins are marked by the evolution periods. Hence, numerous manipulations are possible with the frequencies during these periods.

M_n 's in Fig. 8 constitute the so-called mixing periods. It is the 'mixing' which establishes correlations between frequencies in adjacent evolution periods. These periods may consist of different combinations of pulses and delays as desired. The design of such a pulse sequence requires complete understanding of spin-dynamics under the influence of various Hamiltonians, pulses etc. In a 2D spectrum, there is one evolution period (t_1) and one mixing period (M_1). Different kinds of correlations between spins can be established and the two most common types of interactions exploited for this purpose are J-coupling interactions and through-space dipolar interactions. Till date, more than hundred of pulse sequences have been published. These developments allowed recording of high resolution spectra of most complex molecules and enable elucidation of structures of molecules such as proteins and nucleic acids in the solution phase. Fig. 9 shows an illustrative 2D NMR spectrum ($^1\text{H} - ^{15}\text{N}$ correlation spectrum) of a complex protein system [2].

The 2D spectrum shows correlation between amide proton (H^N) and ^{15}N chemical shifts. It is possible to have same nuclear types along both the axes and then they are called as homonuclear spectra.

8. Concluding Remarks

In this short article, designed to introduce NMR spectroscopy to the beginners, the fundamental concepts have been

described in a pedagogical manner. Theoretical rigour and technical aspects have been kept to the minimum. Students of chemistry will benefit most by grasping the basic concepts and then they can refer to extensive texts for detailed understanding of the various intricacies of the techniques and specific applications, which are indeed very wide ranging. While the basic concepts of nuclear magnetism, angular momentum, resonance absorption, Fourier transformation are common to all branches of NMR spectroscopy, the description here has been limited to solution state NMR. Solid state NMR is an extensive topic in itself and is mostly used in materials chemistry. It is useful to study anisotropic systems, such as powders, macromolecules, molecular assemblies etc. Students with such an inclination could refer to excellent texts on solid state NMR.

9. References

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10. Some texts for further reading

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Ramakrishna V. Hosur received Ph.D. (Chemistry) from TIFR in 1979. He was post-doctoral fellow at ETH Zurich (1981-83). He superannuated from TIFR in May 2018. He was also Director of the University of Mumbai–Department of Atomic Energy Centre for Excellence in Basic Sciences during December 2009–October 2017. He was the Convener of the National NMR Facility at TIFR during 2002–2018. His research has been in the area of NMR and its applications, which has been recognized by several awards. He was awarded ‘Padmashri’ by the President of India in 2014. He is an elected Fellow of International Society of Magnetic Resonance, The World Academy of Sciences, and all the Science Academies in India.