NMR spectrometry is an analytical technique that involves placement of organic [4], inorganic and organometallic [5] and natural products [6]. It has been a tool of great importance in identification and characterization of each family of protein sequence homologues [3]. NMR spectroscopy has contributed immensely to structural biology [3].

Keywords: NMR; COSY; 1D; 2D; Multi dimensional

Introduction

NMR spectroscopy was discovered after the second world war and was developed from experiments performed to accurately measure the nuclear magnetogeric ratio about seventy years ago [1]. Purcell et al. at Harvard and Bloch et al. at Stanford in 1945 reported the first observation of nuclear magnetic resonance in bulk matter for which they were jointly awarded with Nobel Prize for physics in 1952. Since then NMR applications to chemistry have been expanding continuously. The importance of NMR spectroscopy is paramount in the fields of organic, inorganic and analytical chemistry for the investigation of molecular structure and dynamics [2]. In the recent years, the applications of NMR have been extended to biology and medicine, so they have also become an integral part of life sciences. As the objective of life sciences is to understand the mechanisms of life at molecular level, so, it has inspired the investigators to study the interaction between and among biomolecules, also between biomolecules and ligands through NMR spectroscopy. NMR facilitates three-dimensional (3D) structure determination of biomolecules in high resolution and also provides information on their dynamics at an atomic level. In last two decades the tremendous technological and methodological advancements in NMR spectroscopy has proved it to be a powerful tool in structural biology [3].

The technical advancement in NMR is attributed to availability of large magnetic field strengths and increasingly refined probes, which resulted in increased sensitivity, resolution, reduction in amount of sample for data collection and development of procedures for isotope labeling and structure determination of proteins. The investigation of size of biomolecules through NMR in solution is steadily growing method. NMR spectroscopy has contributed immensely to structural genomics initiatives world-wide, which aim at exploring the protein 'fold space' and making available at least one experimental structure for each family of protein sequence homologues [3]. NMR spectroscopy has been a tool of great importance in identification and characterization of organic [4], inorganic and organometallic [5] and natural products [6]. NMR spectrometry is an analytical technique that involves placement of atomic nuclei in an external magnetic field, to cause generation of two spin states of unequal energy. Low energy spin state nuclei absorb energy of radiofrequency range to change their spin orientation with respect to applied magnetic field. Thereby the absorption frequencies peaks are plotted against peak intensities to offer an NMR spectrum [7].

NMR is a powerful technique that provides detailed information on topology, dynamics and 3D structure of molecules, in MRI [7]. Nowadays, NMR probably is the most important technique for structure elucidation, material characterization and studying molecular motion. Continuing efforts have been made to develop different NMR methods so as to obtain more information from NMR measurements, a number of experiments such as 1D-NMR (1H DEPT, 13C, 15N, 19F, 31P, etc.) generates good information about the structure of simple organic compounds, but in case of larger molecules the 1D-NMR spectra are generally overcrowded. Hence, the second approach of 2D-NMR (COSY, DQF COSY, MQF COSY, HETCOR, HSQC, HMOC, HMB, TOCSY, NOESY, EXSY, etc.) is used for the further larger molecules, but 2D-NMR spectra also becomes complex and overlapping when used for further very large molecules like proteins. Hence, so as to achieve high resolution and reduced overlapping in spectra of very large molecules, Multi Dimensional-NMR (Homonuclear and Heteronuclear) are generally used. This paper supports interpretation of structure of different organic compounds by different NMR techniques.

1D-NMR

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1D-NMR

1H-NMR: In 1H-NMR spectroscopy, spin transitions of only hydrogen nuclei are noticed. Interpretation of 1H-NMR spectra can be
well understood from data presented in table 1 representing different δ values, couplings, coupling constants and chemical shifts of ¹H nuclei processing in different chemical environments. Commonly, δ value scale of ¹H-NMR ranges from 0-10 ppm with respect to Tetra methyl Silane (TMS) as internal standard.

The chemical shift range of ¹H nuclei can also be understood from a chart given in figure 1.

For example ¹H-NMR spectrum of Ethyl-2-butenoate in CDCl₃, given in figure 2a, represents five different signals at 1.3, 1.7, 4.2, 5.8, and 6.9 ppm respectively corresponding to a, f, b, d and e hydrogen's respectively.

The multiplicity of peaks of ¹H-NMR spectrum of Ethyl-2-butenoate in CDCl₃ is confirmed by observing its decoupled peaks in ¹H-NMR decoupled spectrum given in figure 2b. Similarly, ¹H-NMR spectra of other compounds can be determined.

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In ¹C-NMR spectroscopy also transitions of only ¹C nuclei are noticed. ¹C-NMR spectral interpretation can be best understood from chart given in figure 3, representing different δ values (in ppm), couplings, coupling constants (in Hz) and chemical shifts of ¹C nuclei processing in different chemical environments. Usually, δ value scale of ¹C-NMR ranges from 0-220 ppm with respect to TMS as internal standard.

For example ¹C-NMR spectrum of Ethyl-2-butenoate in CDCl₃, given in figure 4, represents six different signals at 14.2, 18.6, 61.4, 122.6, and 144.4 and 166.5 ppm, corresponding to a, f, b, d, e and c carbons respectively.

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In ¹C-NMR spectroscopy also transitions of only ¹C nuclei are noticed. ¹C-NMR spectral interpretation can be best understood from chart given in figure 3, representing different δ values.
(in ppm), couplings, coupling constants (in Hz) and chemical shifts of $^{15}$N nuclei processing in different chemical environments. In practice, δ value scale of $^{15}$N-NMR ranges from 0-850 ppm using liquid ammonia as internal standard.

For example $^{15}$N-NMR spectrum of formamide in CDCl$_3$ exhibits a signal for nitrogen nuclei at 112.5 ppm. Whereas ethane 1,2 diamine in CDCl$_3$ exhibits signal for nitrogen nuclei at 12.2 ppm (Figure 6).

$^{31}$P-NMR: In $^{31}$P-nmr spectroscopy only $^{31}$P nuclei are observed. $^{31}$P-nmr spectral interpretation can be best understood from chart given in figure 7, representing different δ values, couplings, coupling constants and chemical shifts of $^{31}$P nuclei processing in different chemical environments. Usually, δ value scale of $^{31}$P-nmr ranges from -140-250 ppm with respect to 85% phosphoric acid as internal standard.

For example $^{31}$P-NMR spectrum of Diethyl Chloro Phosphate in CDCl$_3$ given in figure 8, shows a signal at 5 for $^{31}$P nuclei.

F-NMR: In F-NMR spectroscopy also transitions of only $^{19}$F nuclei are noticed. $^{19}$F-NMR spectral interpretation can be best understood from chart given in figure 9, representing different δ values, couplings, coupling constants and chemical shifts of $^{19}$F nuclei processing in different chemical environments.

Usually, δ value scale of $^{19}$F-NMR ranges from -300-50 ppm with respect to CFCl$_3$ (Tri Fluoro Methane) as standard. For example $^{19}$F-NMR spectrum of fluoroacetone in CDCl$_3$ given in figure 10, shows a signal at -226 ppm for $^{19}$F nuclei.
**17O-NMR:** In 17O-NMR spectroscopy involves transitions of only 17O nuclei. 17O-NMR spectral interpretation can be best understood from chart given in figure 11, representing different δ values, couplings, coupling constants and chemical shifts of 17O nuclei processing in different chemical environments. Commonly, δ value scale of 17O-NMR ranges from 0-1200 ppm using water or dioxane as internal standards.

For example 17O-NMR spectrum of primary, secondary and tertiary alcohol given in figure 12, shows a signal at 30, 50 and 70 ppm for 17O nuclei.

**DEPT:** DEPT is an advance technique to determine the number of hydrogens attached to a given carbon atom. DEPT employs introduction of a complex sequence of pulses in both 1H and 13C channel. In DEPT experiment pulses are selected on the basis of their flip angle such as of a complex sequence of pulses in both 1H and 13C channel.

DEPT-45 - carbon that possesses hydrogen (number 1 or 2 or 3) shows peak. Where as carbon that doesn’t possess hydrogens does not show any peak. In DEPT-90 - carbon that possesses only one hydrogen, exhibits peak in spectrum, whereas carbon with two or three hydrogens does not show any peak. In DEPT-135-carbon with one and three hydrogens shows positive peaks, whereas, carbon with two hydrogens shows negative peak. So, carbons with different number of hydrogens behave differently with different DEPT experiments. This variation indicates presence of number of hydrogen on each carbon. DEPT requires a Fourier transformed pulse spectrometer with a computer interface. The DEPT interpretation can be very well understood from DEPT spectrum of Ethyl-2-butenoate given in figure 13, which represents conventional spectrum at bottom. DEPT-45 exhibits shows coupled peaks for only those carbons that possess hydrogens, whereas nuclei like ‘c’ carbon does not exhibit any signal as it do not possess any hydrogen. The DEPT 90 exhibits peaks only for those carbon nuclei which possess only one hydrogen. The DEPT 135 spectrum shows two types of signals. One of the types is carbon having two hydrogens that exhibits signal downside. Another type is for carbon having one hydrogen or three hydrogens that exhibits signal upside [7-13].

**2D NMR**

**COSY:** COSY is a homonuclear 2D NMR correlation spectroscopy. COSY correlates chemical shift of two hydrogen nuclei located on two different carbons that are separated by a single bond via J coupling. Thereby detects the chemical shift for hydrogen's on both F1 and F2 axis. COSY experiment is categorized as follows:

- **Simple COSY**
- **DQF COSY**
- **TQF COSY**
- **MQF COSY**

**Simple COSY**

Simple COSY technique involves simple pulse sequence in which firstly a (π/2) pulse, is introduced in 1H channel to create an evolution phase. There after some time a second (π/2) pulse is introduced to create an acquisition phase. In 1H-1H COSY pulse sequence contains variable relaxation delay time (t1) and acquisition time (t2). Experiment is repeated with different values of t1 and t2. So that value of t1 is increased at regular intervals, to generate a series of different FID data during t2. COSY offers three bond coupling (J1,H). The COSY interpretation can be best understood from COSY spectrum of Ethyl-2-butenoate given in figure 14. The off diagonal peaks at point 1, 2, and 3 represents coupling of protons of hydrogens on ‘a’ with ‘b’, ‘c’ with ‘f’, and ‘e’ with ‘d’ protons of molecule of Ethyl-2-butenoate. There is no cross peak for c because it does not possess any hydrogens.

**DQFCOSY**

1H-1H DQF COSY is a modified technique of COSY that incorporates a typical pulse sequence, in which firstly a (π/2) pulse, is introduced in 1H channel. Nextly, after first pulse a second (π/2) pulse with just immediate third (π/2) pulse is introduced to eliminate singlet peaks. This simplifies the complex COSY spectrum. The DQF COSY interpretation can be best understood from Ethyl acetate (liquid) in CDCl3, simple COSY and DQF COSY spectra given in figure 15. In simple COSY spectrum of ethyl acetate the isolated ‘d’ hydrogen produces a singlet cross peak at spot ‘I’. But in case of 1H-1H DQF...
TQF COSY

$^1$H-$^1$H TQF COSY employs further typical pulse sequence, in which initially a $(\pi/2)_t$ pulse is introduced in 1H channel. Which is followed by introduction of a second $(\pi/2)_t$ pulse along with just immediate third $(\pi/2)_t$ and fourth $(\pi/2)_t$ pulse. This eliminates singlet and doublet peaks.

MQF COSY

$^1$H-$^1$H MQF COSY employs a pulse sequence, in which firstly a $(\pi/2)_t$ pulse is introduced in 1H channel. This initial pulse is followed by introduction of a second $(\pi/2)_t$ pulse along with a just immediate multiple number of $(\pi/2)_t$ pulse (as desired). This eliminates triplets or any unwanted multiple peaks and simplifies complex COSY spectrum.

HETCOR: HETCOR is a heteronuclear 2D NMR correlation experiment, which correlates two nuclei e.g. $^{13}$C or $^{15}$N with hydrogens separated by single bond. This detects chemical shift for hydrogen’s on F1 axis and hetero nucleus on F2 axis. No cross peak signifies no hydrogen present over carbon. One cross peak indicates there can be one, two or three hydrogens. Two cross peaks indicates presence of diastereotpe carbon (hydrogen). Interpretation of HETCOR spectra can be understood from HETCOR spectrum of ethyl-2-butenoate given in figure 16.

Peaks at point 1, 2, 3, 4 and 5 accounts for $a$, $b$, $d$, $e$ and $f$ carbons and attached hydrogens couplings. No cross peak for ‘c’ as does not contain any hydrogen so no coupling. Hence, with HETCOR, one can easily determine the single bonded hetero nuclear correlation.

HMQC and HSQC: HSQC is better than HMQC. This can be best supported from HSQC and HMQC spectrum of menthol given in figure 17. So, when high $^{13}$C resolution is required, then pulses should be calibrated on a well tuned and matched probe and HSQC should be run. But when high $^{13}$C resolution is not an essential requirement then HMQC can be run.

HMBC: HMBC is a heteronuclear 2D-NMR correlation spectroscopy. This technique correlates chemical shift of hetero nuclei e.g. $^{13}$C or $^{15}$N with hydrogens, through a multiple bond. So, detects chemical shift for hydrogen's nuclei on F2 axis and hetero nuclei on F1 axis. Interpretation of HMBC can be understood from HMBC spectrum given in figure 18.

Line drawn from 16.2 (C-2’) ppm, parallel to $^1$H axis, intersects three cross peaks of H-1’, which correlates C-2’ with two H attached on C-1’. Other cross peaks doesn’t show correlation. The line drawn from C-1’ at 29 ppm, intersects one cross peak correlates C-1’ to hydrogens...
Interpretation of EXSY spectrum can be understood from figure 20. regardless of whether there is a bond between them exists or not. The correlation between the nuclei which are physically close to each other for any given compound. Also, it be easily distinguished between two spectra of TOCSY and COSY between hydrogens 4-7 and 5-7 also. Similarly, other correlations can 5-6 and 6-7, but not between 4-7 and 5-7. TOCSY correlation occurs correlation, such as COSY correlations occurs among hydrogens 4-5, 4-6 and 5-6 also. Similarly, other correlations can also be easily distinguished between two spectra of TOCSY and COSY for any given compound.

NOESY: NOESY is a homonuclear correlation spectroscopy that correlates between the nuclei which are physically close to each other regardless of whether there is a bond between them exists or not. The interpretation can be understood from NOESY and COSY spectrum of a quinoline derivative given in figure 21. The two experiments differentiate on the diastereotopic protons of the CH2 group. NOESY spectrum, shows two NOE correlations at (4.29, 1.28) and (4.29, 3.13) ppm. There are no NOE's to the proton signal at 2.68 ppm. The DQF-COSY below shows J and J' correlations at (4.29,3.13), (4.29,1.28) and (3.13, 2.68) ppm. There are no four-bond correlations present as the J' coupling constants are close to zero. [7-14].

Multi dimensional NMR

As 1D-NMR spectroscopy possess one frequency axis with one intensity axis, which usually generates a very crowded and complex present on C-2'. Similarly, C-1 correlated with hydrogens of C-1', C-2' and C-3; C-2 with hydrogens of C-3, C-4, and C-of 1'; C-4 with hydrogens of C-3 and C-2; C-3 with hydrogens of C-2 and C-4; C-4 with hydrogens of C-3 and C-2.

TOCSY: 2D TOCSY (Total Correlation Spectroscopy) is a homonuclear experiment which produces a COSY-like plot. TOCSY possess two dimensions or axis of 'H-NMR. A 2D-TOCSY, gives correlations between all protons in a given spin system. Interpretation of TOCSY spectrum can be best learnt from the TOCSY and COSY spectrum of 3-heptanone given in figure 19. TOCSY and COSY spectrum of 3-heptanone can be easily distinguished on the basis of correlation, such as COSY correlations occurs among hydrogens 4-5, 5-6 and 6-7, but not between 4-7 and 5-7. TOCSY correlation occurs between hydrogens 4-7 and 5-7 also. Similarly, other correlations can also be easily distinguished between two spectra of TOCSY and COSY for any given compound.

EXSY: This is a homonuclear correlation spectroscopy that correlates between nuclei which are physically close to each other regardless of whether there is a bond between them exists or not. Interpretation of EXSY spectrum can be understood from figure 20.

1H EXSY spectrum of N,N-dimethyl acetamide is recorded at room temperature. At this temperature, the molecule exhibits slow rotation about the (CH3)N-C bond such that both methyl groups represented as 'a' and 'b' exchange with one another rotationally yet are distinct in the spectrum. This is evident by the cross peaks in the spectrum between the two methyl groups on the nitrogen.

Figure 18: HMBC spectrum of ethyl benzene.

Figure 19: TOCSY and COSY spectrum of 3-heptanone.

Figure 20: EXSY spectrum of N,N-dimethyl acetamide.

Figure 21: NOESY and COSY spectrum quinoline derivative.
ROESY-HMQC, 1H-15N HMQC-NOESY-HMQC can also be used. RF pulse sequence - Parallel execution of two or more magnetization transfer pathways in HSQC-TOCSY, 2D NOESY-HSQC, and CHα of amino acid unit 'A' (figure 22). Other experiments like 2D 'B' can be correlated with protons of NH group of amino acid unit 'C'. NOESY-TOCSY spectroscopy. Protons of NH group of amino acid unit combination of 2D NOESY and 2D TOCSY offers a new 3D NMR i.e. NMR experiments such as 4D, 5D and 6D NMR. For example, higher number of frequency axes generates other multi-dimensional and reduces the overlapping of peaks. A third dimension is applied so called 3D NMR spectroscopy that possess three frequency axis and one intensity axis [15-21].

Introduction of third dimension provides much better resolution and reduces the overlapping of peaks. Similarly introduction of further higher number of frequency axis generates other multi-dimensional NMR experiments such as 4D, 5D and 6D NMR. For example combination of 2D NOESY and 2D TOCSY offers a new 3D NMR i.e. NOESY-TOCSY spectroscopy. Protons of NH group of amino acid unit 'B' can be correlated with protons of NH group of amino acid unit 'C' and CHα of amino acid unit 'A' (figure 22). Other experiments like 2D HSQC-TOCSY, 2D NOESY-HSQC, 1H-15N ROESY-HMQQC, IH-13C ROESY-HMQQC, 1H-15N HMOC-NOESY-HMQQC can also be used. 5D NMR commonly makes use of four types of NMR pulse sequences such as sequence of HACA(N)CONH, HNCOCACB, (HACA)CON(CA) CONH and (H)NCO(NCA)CONH. Whereas for 6D NMR make use of a pulse sequences such as (H)NCO(N)CACONH. Usually, it takes several minutes to measure resonance in 2D NMR, several hours in 3D NMR, several days in 4D NMR, and several weeks in 5D and 6D NMR with conventional FTNMR methods. This is because of number of free inductions decays, that are number of data points sampled in the indirect dimensions, increases exponentially with increase in the number of dimensions. Such that to scan more than 15 complex points in each indirect dimension, when it takes one second per FID, then it takes about 30 minutes for 3D NMR, and 12 days for 5D NMR of measurement time. The time required for data collection is a rate limiting step for structural characterization or resonance measurements. In order to make the NMR measurements fast, following are the strategies that can be used such as [22-25]:

a. Ultrafast (single-scan) NMR Slice-selective excitation.
b. Non-linear sampling-Restricted time-domain sampling in the indirect dimension by omission of increments randomly or in a selective manner followed by spectral reconstruction.
   i. Maximum Entropy Reconstruction
   ii. Frequency Diagonolization Method
   iii. Three-way decomposition (Multiway decomposition)
   iv. Time-shared approach (Simultaneous Data Acquisition)
   - Parallel execution of two or more magnetization transfer pathways in the RF pulse sequence.
d. Projection NMR spectroscopy-Phase sensitive or non-phase sensitive joint sampling of two or more chemical shifts in the indirect dimension(s).
   i. RD-NMR (Relaxation Dispersion NMR)
   ii. GFT NMR (G-Matrix Fourier Transform)
   iii. GFT-based data collection followed by spectral re-construction
   c. Hadamard NMR spectroscopy- Elective excitation and encoding of phases of signals using Hadamard matrix.
   f. Fast pulsing methods-Reduction of relaxation delay period between scans.
   i. L-optimization
   ii. SO-FAST NMR (Selective Optimized Flip-Angle Short-Transient NMR)
   iii. Variable relaxation delay
   g. DNP-Dynamic nuclear polarization [26].

A drawback of NMR is the low sensitivity attributed to small magnetic moment of the nuclei (1H, 13C, 15N etc.) having small polarization and small signal intensity. So, requires extensive signal averaging to have a high signal-to-noise ratio. Whereas at same magnetic field and temperature, the polarization of electron spin reservoir (e.g. free, stable paramagnetic polarizing agent) is significantly larger due to the much higher magnetic moment of the electron spin. With Dynamic Nuclear Polarization (DNP) it is possible to transfer the large Boltzman polarization of electron spin reservoir to the nuclear spin reservoir, which causes enhancement in NMR signal intensities by several orders of magnitude; ultimately signal intensity and data acquisition rate are increased by DNP-NMR.

Conclusion

None of the analytical technique is good or bad. All the other analytical techniques of NMR play an equally important role in the structural elucidation individually or in combination.

References

2. Shaoxing W (2011) 1D and 2D NMR experiment methods. NMR Research Centre, Emory University, Atlanta, GA.