NMR Spectroscopy: Principles and Applications

Nagarajan Murali

Basic Concepts

Lecture 1
Scope and Syllabus
The aim of this course is to introduce the basic concepts of one and two-dimensional NMR spectroscopy to graduate students who have used NMR in their daily research to enable them to appreciate the workings of their analytical tool and enable them to run experiments with a deeper understanding of the subject. The students will be exposed to the close connection between theory and experiments in NMR. The basic quantum mechanical description and mathematical tools used to explain the concepts will be readily understandable for science students. A brief description of the working of a NMR spectrometer will be presented. Practical aspects in analysis of a small molecule will be presented with an example and the discussion will emphasize the reasons for the choice of particular experiments for the problem in hand.

Text Books:

Course Topics
Basic Concepts: What is NMR? The concept of spin and the interaction of spins with external magnetic field and other spins. Discussions on spectral features such as, chemical shift, multiplet structures (J coupling), and lineshapes.

Classical Description: Vector description to introduce the concept of free induction decay. Rotating reference frame and RF pulses and its effects. Time and Frequency domains, basic Fourier transform principles.

Advanced Tools: Energy levels and NMR spectra. Quantum mechanical approach based on product operators that are essential to understand the various experiments in one and two-dimensional NMR.

1D Techniques: One dimensional NMR such as one pulse experiment, spin-echo, and, inversion recovery experiments. Proton and Carbon 1Ds with proton decoupling.

Nuclear Magnetic Relaxation: Aspects of relaxation processes. The T_1, T_2, and nuclear Overhauser effect (nOe) stemming from relaxation. Aspects of dynamics and exchange.

Two Dimensional NMR: Homonuclear and heteronuclear two-dimensional NMR experiments such as COSY, DQFC, TOCSY, NOESY, ROESY, HSQC, HMQC, and HMBC.

Gradients in NMR: The use of pulsed field gradients in NMR experiments and the advantages.

NMR Spectrometer and Data Collection: A brief description of a NMR spectrometer and its working with attention to locking, shimming, tuning, and parameter optimizations.

Putting it all together: Analysis of small molecules and Bio Molecules by NMR – reasons for choice of suitable experiments to the problem in hand and merits of various experiments in different conditions.
Other Books

*Spin Dynamics Basics of Nuclear Magnetic Resonance*
Malcolm H. Levitt
John Wiley & Sons (2007)

*Principles of Nuclear Magnetism*
A. Abragam
ISBN- 0 19 852014 X

*Principles of Nuclear Magnetic Resonance in One and Two Dimensions*
Richard R. Ernst, G. Bodenhausan, and A. Wokaun

*Principles of Magnetic Resonance*  
(3rd Enlarged and updated edition)
C.P. Slichter
Springer-Verlog (1990)

*Protein NMR Spectroscopy*  
*Principles and Practise*  
Birth of NMR

1945 – Purcell, Torrey, and Pound (Harvard, Cambridge, Massachusetts) detected weak radio-frequency signals generated by the nuclei of atoms in about 1 kg of paraffin wax placed in a magnetic field.

Simultaneously, Bloch, Hansen, and Packard (Stanford, Palo Alto, California) independently observed radio signals from atomic nuclei in water in a magnetic field.

Birth of NMR

Purcell, Torrey, and Pound described NMR as observation of absorption by the nuclear spin system that produces an additional load that changes the quality factor $Q$ of the circuit that drives the resonance.

Bloch, Hansen, and Packard described NMR as forced precession of the nuclear magnetization in the applied radio frequency field and the induction of detectable electromotive force in a receiver coil.

The two approaches are distinct – the one by Purcell is quantum mechanical and that by Bloch is often termed as classical approach.
What is NMR?

**NMR** – *Nuclear Magnetic Resonance is a branch of spectroscopy that deals with the phenomenon found in assemblies of large number of nuclei of atoms that possess both “magnetic moments” and “angular momentum” is subjected to external magnetic field.*

**Resonance** – *Implies that we are in tune with a natural frequency of the nuclear magnetic system in the magnetic field.*
Nuclear Paramagnetism

The magnetic moment of a nucleus ($\mu$) arises from the non zero spin ($I$) angular momentum and the assemblies of such magnetic moments give rise to Nuclear Paramagnetism.

$$\mu = \gamma \hbar I$$

$\gamma$ – gyromagnetic ratio (can be positive or negative)
$\hbar$- Planck’s constant ($h$) divided by $2\pi$
What is Spin?

A rotating object possesses a quantity called angular momentum given by the right hand thumb rule.

Spin is a type of angular momentum that does not vanish even at absolute zero! So a physical motion to represent this type of angular momentum is not without error.

Spin is a fundamental property of electron and nucleus like mass, electric charge, and magnetism.
Some facts about Spin

Atoms with odd mass number have spin $I$ as integer multiples of one half (1/2, 3/2, 5/2, ....)

Atoms with even mass number but odd numbers of protons and neutrons have spin $I$ as integer numbers (1, 3, 4, 5, 7).

Atoms with even mass number and even atomic number have zero spin.

$I=2$ does not exist.

$I > \frac{1}{2}$ are known as quadrupolar nuclei.
Some facts about Spin

<table>
<thead>
<tr>
<th>Isotope</th>
<th>Spin</th>
<th>Natural Abundance %</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^1$H</td>
<td>1/2</td>
<td>100</td>
</tr>
<tr>
<td>$^2$H</td>
<td>1</td>
<td>0.015</td>
</tr>
<tr>
<td>$^3$H</td>
<td>1/2</td>
<td>0</td>
</tr>
<tr>
<td>$^{10}$B</td>
<td>3</td>
<td>19.9</td>
</tr>
<tr>
<td>$^{11}$B</td>
<td>3/2</td>
<td>80.1</td>
</tr>
<tr>
<td>$^{13}$C</td>
<td>1/2</td>
<td>1.1</td>
</tr>
<tr>
<td>$^{14}$N</td>
<td>1</td>
<td>99.6</td>
</tr>
<tr>
<td>$^{15}$N</td>
<td>1/2</td>
<td>0.37</td>
</tr>
<tr>
<td>$^{17}$O</td>
<td>5/2</td>
<td>0.04</td>
</tr>
<tr>
<td>$^{19}$F</td>
<td>1/2</td>
<td>100</td>
</tr>
<tr>
<td>$^{23}$Na</td>
<td>3/2</td>
<td>100</td>
</tr>
<tr>
<td>$^{27}$Al</td>
<td>5/2</td>
<td>100</td>
</tr>
<tr>
<td>$^{29}$Si</td>
<td>1/2</td>
<td>4.7</td>
</tr>
<tr>
<td>$^{31}$P</td>
<td>1/2</td>
<td>100</td>
</tr>
<tr>
<td>$^{35}$Cl</td>
<td>3/2</td>
<td>75.77</td>
</tr>
<tr>
<td>$^{37}$Cl</td>
<td>3/2</td>
<td>24.23</td>
</tr>
<tr>
<td>$^{63}$Cu</td>
<td>3/2</td>
<td>69.17</td>
</tr>
<tr>
<td>$^{65}$Cu</td>
<td>3/2</td>
<td>30.83</td>
</tr>
<tr>
<td>$^{107}$Ag</td>
<td>1/2</td>
<td>51.84</td>
</tr>
<tr>
<td>$^{109}$Ag</td>
<td>1/2</td>
<td>48.16</td>
</tr>
<tr>
<td>$^{129}$Xe</td>
<td>1/2</td>
<td>24.4</td>
</tr>
<tr>
<td>$^{207}$Pb</td>
<td>1/2</td>
<td>22.1</td>
</tr>
<tr>
<td>$^{12}$C</td>
<td>0</td>
<td>98.9</td>
</tr>
<tr>
<td>$^{16}$O</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>
Interaction of Spin with External Magnetic Field

The interaction of nuclear magnetic moment $\mu$ with external magnetic field $B_0$ is known as **Zeeman interaction** and the interaction energy known as **Zeeman energy** is given as:

$$E = -\mu \cdot B_0 = -\gamma \hbar I \cdot B_0$$

$$E = -\gamma \hbar B_0 m_I = \hbar \omega_0 m_I$$

where

$$\omega_0 = -\gamma B_0 = 2\pi \nu_0$$

$$m_I \rightarrow -I, -I + 1, ..., +I$$

NMR is a branch of spectroscopy and so it describes the nature of the energy levels of the material system and transitions induced between them through absorption or emission of electromagnetic radiation.
Resonance Method implies that the frequency of irradiation is same as that of the separation of energy levels in frequency unit.

Detection of Resonance is reduced to the measurement of frequency at which there is detectable change in the rate of transition of spin state.

\[ h\nu = E_{upper} - E_{lower} \]
Resonance of a single spin $I=\frac{1}{2}$

For $I=\frac{1}{2}$, $m_I$ can be $+\frac{1}{2}$ or $-\frac{1}{2}$. Usually the $\frac{1}{2}$ state is referred as $\alpha$ and the $-\frac{1}{2}$ state as $\beta$.

$$E = -\gamma \hbar B_0 m_I$$

$$E_\alpha = -\frac{1}{2} \gamma \hbar B_0$$

$$E_\beta = +\frac{1}{2} \gamma \hbar B_0$$

$$\Delta E_{\alpha \rightarrow \beta} = E_\beta - E_\alpha = \frac{1}{2} \gamma \hbar B_0 - \left( -\frac{1}{2} \gamma \hbar B_0 \right)$$

$$\Delta E_{\alpha \rightarrow \beta} = \gamma \hbar B_0 = -\hbar \omega_0 = -\hbar \nu_0$$
The characteristic frequency $\omega_0=2\pi\nu_0=\gamma B_0$ is known as **Larmor frequency**.

The Larmor or NMR frequency range from 2kHz to 1GHz, the former is the frequency of proton in earth magnetic field (0.5 Gauss) and the later is the proton frequency at a field strength of 25 Tesla (250000Gauss).
The effect of interaction of magnetic moment with the magnetic field is an alignment of the magnetic moment along the field and this competes with the thermal energy that disrupts such an alignment.

For a single Hydrogen nucleus (proton – spin 1/2) in a field $B_0=11.74$ T, the magnetic energy (Zeeman energy) is

$$\hbar \gamma B_0 = 3.3 \times 10^{-25} \text{ J}$$

The available thermal energy at room temperature is ($k_B$ is Boltzmann Constant)

$$k_B T = 4.1 \times 10^{-21} \text{ J}$$

Thermal energy is 4 orders of magnitude larger so there is only a very small bias for the spin to occupy the low energy state.
Bulk Magnetization

To observe NMR, we need a large collection of such nuclear magnetic moments so that a detectable change in the transition from low energy state to the higher energy state can be measured. For a spin $\frac{1}{2}$ system the population of spins in the two levels is

$$\frac{N_{\alpha}}{N_{\beta}} = e^{\frac{\Delta E}{k_BT}} = e^{\frac{3.3 \times 10^{-25}}{4.1 \times 10^{-21}}} = 1.00008049$$
Bulk Magnetization

Let’s take 0.7 ml of 10mM CHCl₃, the number of hydrogen atoms N is

\[ N \sim 0.01 \text{ moles/liter} \times 0.0007 \text{ liter} \times 6.0 \times 10^{23} \text{ per mole} = 4.2 \times 10^{18} \]

Then, the population difference is only about 4.0 \( \times 10^{13} \) protons and these many protons give rise to a net magnetization.
Alignment of Nuclear Magnetic Moments

No external magnetic field

Net magnetization is ‘zero’

External Field induces net magnetization

\[ M_0 = \mu_0 \frac{N\gamma^2 \hbar^2 I(I+1)B_0}{3k_B T} \]
Alignment of Nuclear Magnetic Moments

It takes a finite time to induce the magnetization by the external field and the time constant $T_1$ is known as longitudinal relaxation time.

External Field induces net magnetization

$$M_0 = \mu_0 \frac{N \gamma^2 \hbar^2 I(I+1)B_0}{3k_B T}$$
Setting the Context

So far, we have collected the very basic building blocks to understand NMR and have become familiar with the fundamental physical property that gives rise to such a phenomenon.

In this course we will focus mainly on NMR of samples in liquid state. It would be illustrative to collect more information as how a typical NMR spectrum would look like and familiarize various features.

It will then be easy to appreciate how every aspect of NMR can be clearly explained by theory and every theory can be tested by a suitable experiment.
Typical Proton NMR Spectrum

Let us look at the hydrogen NMR spectrum of a molecule called Quinine in $C_2HCl_3$ (also written as $CDCl_3$)

Aromatic ring protons

Protons on the double bond
The horizontal axis gives chemical shift in parts per million (ppm) unit. The vertical axis is intensity of the peak proportional to number of protons. Every peak has a characteristic line width. Some peaks show multiplet structure arising from interactions (through chemical bond) between protons and such interactions are known as J-coupling.
(a) Magnetic field is such that the two peaks resonate at 200.0002 and 200.004 MHz and the difference in frequency is 200 Hz.
(b) The same spectrum when the field is doubled the resonances appear at 400.0004 and 400.0008 MHz and difference frequency is 400 Hz.
Chemical Shift

The chemical shift is then defined with respect to a reference as

\[ \delta (\text{ppm}) = 10^6 \frac{\nu - \nu_{\text{ref}}}{\nu_{\text{ref}}} \]

The left peak has been chosen as reference and so appears at 0.0 ppm. But usually plotted as ppm increasing to the left and not as shown above.
With the definition of chemical shift in ppm scale the peaks in both the spectra are separated by just 1ppm. The magnetic field dependence of the difference in frequency is eliminated.
The basic line shape seen in a NMR spectrum is called \textit{absorption mode} line shape. The width of the line is given by the width at half height (h/2, left figure) and the area (integral) of the line is proportional to the number of spins contributing to the line. The two lines in the right hand side spectrum (a) and (b) have same area but different line widths and heights.
Scalar Coupling

Scalar or J coupling between nuclei is mediated by chemical bond between them. They are useful in establishing the bonding network of spins from NMR spectrum. The presence of scalar coupling is seen as a multiplet structure of NMR lines – for two spins each with I=1/2 the resonances split about the chemical shift into two lines called doublets. Spectrum (a) is from uncoupled and (b) is from coupled protons.
Scalar Coupling

More coupling partners split the line further and can be represented by a tree diagram. In Figure (a) the doublet appears due to the coupling to a second spin and in Figure (b) the doublet is further split by the coupling to the third spin giving a doublet of doublet structure.
Coupling Pattern vs Chemical Shift Difference

The multiplet structure and peak intensities depend on the relative size of the coupling constant and the chemical shift difference between the coupled spins. As the chemical shift difference reduce, the pattern displays features of strong coupling and finally a singlet appears when the chemical shift difference is zero – magnetic equivalence case.

(a) \((\nu_1 - \nu_2) = -90\text{Hz}; J_{12} = 5\text{Hz}\)

(b) \((\nu_1 - \nu_2) = -50\text{Hz}; J_{12} = 5\text{Hz}\)

(c) \((\nu_1 - \nu_2) = -20\text{Hz}; J_{12} = 5\text{Hz}\)

(d) \((\nu_1 - \nu_2) = -10\text{Hz}; J_{12} = 5\text{Hz}\)
Frequencies are often represented in NMR either $\omega$ radians/sec or as $\nu$Hz (per sec).

If a vector is rotating the rotational velocity (i.e radians swept per sec) is meaningful. Whereas, if a vector is oscillating then the number of oscillations per sec is in units of Hz.

$$\omega = \frac{2\pi}{T} \text{ rad} \cdot \text{sec}^{-1}$$

$$\nu = \frac{1}{T} \text{ sec}^{-1}$$

$$\omega = 2\pi\nu$$
Circular Motion vs Oscillations

The circular motion of the vector can be represented as oscillations of the x and y component of the vector. The x component varies as $\cos \theta$ and the y component varies as $\sin \theta$. The circular motion can be split into two orthogonal oscillations and vice versa.
Phase

Phase is a way of saying how far the vector has proceed around the circle. The position after a time \( t \) can be given by a phase factor (in radians)

\[ \phi = \omega t \]

The position of the vector at time \( t \) is given by the phase angle \( \phi \) measured from the x-axis. In the wave picture below, the angle shows how far in the wave has travelled in time \( t \).
Phase

Phase can also be used as a parameter to specify the starting position of the vector.

Starting position at time $t=0$ (a) phase $\phi = \pi/4$ radians or $45^0$; (b) phase $\phi = \pi/2$ radians or $90^0$; and (c) phase $\phi = \pi$ radians or $180^0$; The bottom row shows the time dependence of the x and y components as the vector moves from the starting position.

$$x(t) = r \cos(\omega t + \phi)$$
$$y(t) = r \sin(\omega t + \phi)$$
Complex Numbers

The relationship between circular motion and linear oscillations is useful to understand complex numbers. If we consider the x-axis as the real part and y-axis as the imaginary part then

$$\exp(i\theta) = \cos \theta + i\sin \theta$$

The position of the vector at time $t$ is given by the phase angle $\theta$ measured from the real-axis and is given by $r\exp(i\theta)=r\exp(i\omega t)$. 
Complex Numbers

A circular motion say counter-clock wise is expressed as

\[ \exp(i\theta) = \cos\theta + i\sin\theta \]

And one in the opposite sense (clock wise rotation) is then

\[ \exp(-i\theta) = \cos\theta - i\sin\theta \]

Thus the oscillation along real axis (x) can be written as sum of two rotations in opposite sense

\[ \exp(i\theta) + \exp(-i\theta) = 2\cos\theta \]

And the oscillation along the imaginary axis (y) can be written as the difference of two rotations in opposite sense

\[ \exp(i\theta) - \exp(-i\theta) = 2i\sin\theta \]
Photons

The photon energy in quantum mechanics is given as

\[ E = h \nu \]

where \( h \) is Planck’s constant. In terms of the angular frequency this expression is

\[ E = h \frac{\omega}{2\pi} = \hbar \omega \]

\( \hbar \) is \( h/2\pi \) is called \( h \) bar or \( h \) cross.
Some Important Constants

<table>
<thead>
<tr>
<th>Fundamental Quantity</th>
<th>SI Unit Symbol</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnetization</td>
<td>M</td>
<td>A/m</td>
</tr>
<tr>
<td>Permeability of free space</td>
<td>( \mu_0 = 4\pi \times 10^{-7} )</td>
<td>Wb/A m</td>
</tr>
<tr>
<td>Gyromagnetic ratio</td>
<td>( \gamma )</td>
<td>(s A/m)(^{-1})</td>
</tr>
<tr>
<td>Magnetic field strength</td>
<td>H=NI</td>
<td>A/m</td>
</tr>
<tr>
<td>Magnetic induction – Flux</td>
<td>B=( \mu_0 (H+M) )</td>
<td>Wb/m(^2) or Tesla (T)</td>
</tr>
<tr>
<td>Planck’s constant</td>
<td>h=6.62x10(^{-34})</td>
<td>J s</td>
</tr>
<tr>
<td>Planck’s constant/2( \pi )</td>
<td>( \hbar = 1.055 \times 10^{-34} )</td>
<td>J s</td>
</tr>
</tbody>
</table>

*A – Ampere; m – meter, s – second; Wb – Weber; J – Joule; T – Tesla; N – number of turns per meter; I – current in Ampere units.*
Extra Reading

Reading of Appendix A of Understanding NMR Spectroscopy By James Keeler is strongly encouraged.