NMR Spectroscopy
A Brief Introduction

Type of Spectroscopy

- IR Spectroscopy: Functional Groups
- UV Spectroscopy: Conjugation
- NMR Spectroscopy: Carbon-Hydrogen Framework
- Mass Spectroscopy: Molecular Size and Formula
- X-Ray Crystallography: Exact Structure
Energy Level of A Wave

<table>
<thead>
<tr>
<th>Wavelength (cm)</th>
<th>10^{-10}</th>
<th>10^{-8}</th>
<th>10^{-6}</th>
<th>10^{-4}</th>
<th>10^{-2}</th>
<th>10^{0}</th>
<th>10^{2}</th>
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<tbody>
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</table>

- Nuclear magnetic resonance (NMR) obtains the structure of molecules from their unique magnetic signatures of their component atoms.
- NMR is the spectroscopic study of the magnetic energy levels of nuclei.
- NMR is a valuable tool for the study of microstructures of polymer systems, especially for proteins.

Dr. Zerong Wang at UHCL
Why NMR Structure Determinations?

- $^{13}$C NMR yields site specific information, enabling analysis of individual atoms of the molecule.
- Enables spectral characterization of samples (confirm structures, etc).
- Can be used to follow processes such as polymerization without affecting the dynamics of that process.

Why NMR?

Advantages
- Highly specific
- Simple and clear interpretation of chemical structure

Disadvantages
- Low sensitivity per scan
- Long measurement time (signal averaging)
- Complex mixtures are difficult
- Solubility limitations of polymers
- Expensive equipment to operate and maintain
Typical Applications of NMR

- Structural (chemical) elucidation
  - Natural product chemistry
- Synthetic organic chemistry
  - Analytical tool of choice for synthetic chemists used in conjunction with MS and IR
- Study of dynamic processes
  - Reaction kinetics
  - Study of equilibrium (chemical or structural)
- Structural (three-dimensional) studies
  - Proteins, Protein-ligand complexes
  - DNA, RNA, Protein/DNA complexes
  - Polysaccharides
- Drug Design
  - Structure Activity Relationships by NMR
- Medicine - MRI

How NMR Works!

- Some atomic nuclei behave as tiny bar magnets when placed in a magnetic field and align. This tiny magnets rotates around the direction of the magnet at a characteristic frequency called Larmor frequency.

- In NMR, the Larmor frequency is disturbed slightly by an surrounding electric field of functional groups, which causes a slight deviation from the Larmor frequency of the nucleus.

- This characteristic deviation (chemical shift) is on the order of ppm of the Larmor frequency and can be used to identify particular atoms and their positions.
History of NMR

- **1937** Rabi predicts and observes nuclear magnetic resonance
- **1946** Bloch, Purcell: first nuclear magnetic resonance of bulk sample
- **1953** Overhauser: NOE (nuclear Overhauser effect)
- **1966** Ernst, Anderson: Fourier transform NMR
- **1975** Jeener, Ernst: 2D NMR
- **1985** Wüthrich: first solution structure of a small protein (BPTI) from NOE derived distance restraints
- **1987** 3D NMR + 13C, 15N isotope labeling of recombinant proteins (resolution)
- **1990** Pulsed field gradients (artifact suppression)
- **1996/7** New long range structural parameters:
  - residual dipolar couplings from partial alignment in liquid crystalline media
  - projection angle restraints from cross-correlated relaxation TROSY (molecular weight > 100 kDa)

**Nobel for Magnetic Resonance**

- **Isador I. Rabi**
  
  Nobel Prize in Physics, 1944
  For his resonance method for recording the magnetic properties of atomic nuclei.

- **Felix Bloch and Edward M. Purcell, USA**
  
  Nobel Prize in Physics, 1952
  The NMR phenomenon was demonstrated for protons in 1946.
Richard Ernst, Zurich,
Nobel Prize in Chemistry, 1991
For his fundamental contributions to
NMR methodology—Nuclear Magnetic
Resonance Fourier Transform
Spectroscopy

Kurt Wüthrich
Nobel Prize in Chemistry, 2002
NMR studies of structure and function
of biological macromolecules.

Paul C. Lauterbur (Urbana, IL) and Sir
Peter Mansfield (Nottingham, UK)
Nobel Prize in Physiology or
Medicine, 2003
For their pioneering contributions
which led to the application of
magnetic resonance in medical imaging.
Nobel for Magnetic Resonance

- Alexej A. Abrikosow (Argonne, IL) and Vitalij L. Ginzburg (Moscow)
  Nobel Prize in Physics, 2003

- For pioneering contributions to the theory of type-II superconductors, i.e., those alloys capable of withstanding the high magnetic fields that occur in MR applications.

First NMR Spectrum

NMR Signal of Water

Fig. 10. Photographic record of the proton signal in water. The four tracés from top to bottom correspond to the times $t_1, t_2, t_3, t_4$ of Fig. 9. In the text they are referred to as $a, b, c, d$, respectively.

First NMR Spectrum with Chemical Shift

NMR of Ethanol


Comparison with modern NMR spectrum

Basic Theory of NMR
A Basic Concept in Electromagnetic Theory

A moving perpendicular external magnetic field will induce an electric current in a closed loop.

For a single loop of wire, the magnetic field, $B$ through the center of the loop is:

$$B = \frac{\mu_0 I}{2R}$$

- $\mu_0$ – permeability of free space ($4\pi \times 10^{-7}$ T · m / A)
- $R$ – radius of the wire loop
- $I$ – current
Faraday’s Law of Induction

- If the magnetic flux ($F_B$) through an area bounded by a closed conducting loop changes with time, a current and an emf are produced in the loop. This process is called induction.
- The induced emf is:
  \[ \eta = \frac{dF_B}{dt} \]

![Simple AC generator](image)

Lenz’s Law

- An induced current has a direction such that the magnetic field of the current opposes the change in the magnetic flux that produces the current.
- The induced emf has the same direction as the induced current.
**Theory of NMR**

**Quantum Description**

- **Nuclear Spin (just like electron spin)**
- Nucleus rotates about its axis (spin)
- Nuclei with spin have angular momentum (p) or spin
  1) total magnitude

\[ \hbar \sqrt{I(I+1)} \]

2) quantized, spin quantum number \( I \)

3) \( 2I + 1 \) states: \( I, I-1, I-2, \ldots, -I \)

4) identical energies in absence of external magnetic field

---

**NMR Periodic Table**

<table>
<thead>
<tr>
<th>Element</th>
<th>Atomic Mass</th>
<th>Spin (I)</th>
<th>Natural Abundance (%)</th>
<th>Resonance Frequency (MHz)</th>
<th>Quadrupole Moment (mm^2)</th>
<th>Radioactivity</th>
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<td>137.33</td>
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</tbody>
</table>

**NMR “active” Nuclear Spin (I) = ½:**
- \( ^{1}H, \ ^{13}C, \ ^{15}N, \ ^{19}F, \ ^{31}P \)
- biological and chemical relevance
- Odd atomic mass \( I = \pm \frac{1}{2} \)

**NMR “inactive” Nuclear Spin (I) = 0:**
- \( ^{12}C, \ ^{16}O \)
- Even atomic mass & number

**Quadrupole Nuclei Nuclear Spin (I) > ½:**
- \( ^{14}N, \ ^{2}H, \ ^{10}B \)
- Even atomic mass & odd number
  \( I = +1, 0, -1 \)
**Magnetic Moment (μ)**

spinning charged nucleus creates a magnetic field

```
Magnetic moment
```

Similar to magnetic field created by electric current flowing in a coil

“Right Hand Rule”
determines the direction of the magnetic field around a current-carrying wire and vice-versa

\[ \vec{F} = q\vec{v} \times \vec{B} \]

**Gyromagnetic ratio (γ)**

- related to the relative sensitive of the NMR signal
- magnetic moment (μ) is created along axis of the nuclear spin

\[ \mu = \gamma p \quad \gamma = \frac{2\pi \nu}{\hbar} = \frac{\mu}{hI} \]

where:
- \( p \) – angular
- \( \gamma \) – gyromagnetic ratio (different value for each type of nucleus)

magnetic moment is quantized (m)

\[ m = 1, 1-1, 1-2, \ldots, -1 \]

for common nuclei of interest:

\[ m = +\frac{1}{2} \ & \ -\frac{1}{2} \]
### Important NMR Nuclei

<table>
<thead>
<tr>
<th>% Natural Abundance</th>
<th>I</th>
<th>M. Moment μ</th>
<th>γ *</th>
<th>ν MHz</th>
<th>B₀ Telsa</th>
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</thead>
<tbody>
<tr>
<td>³¹H</td>
<td>99.9844</td>
<td>1/2</td>
<td>2.7927</td>
<td>60.53</td>
<td>60</td>
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<td>²⁵H</td>
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<td>0.8574</td>
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<td>¹²C</td>
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<td>9.23</td>
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<td>0.7022</td>
<td>67.28</td>
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<tr>
<td>¹⁶O</td>
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<td></td>
<td>5.61</td>
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<td>1/2</td>
<td>2.6273</td>
<td>251.7</td>
<td>60</td>
</tr>
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<td>³¹P</td>
<td>100.0</td>
<td>1/2</td>
<td>1.1305</td>
<td>108.3</td>
<td>60</td>
</tr>
</tbody>
</table>

* Magnetogyric ratio γ unit: 10⁶ radians/(Telsa*sec)

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### Apply an external magnetic field

(i.e., put your sample in the magnet)

\[ \omega = \gamma B₀ = \nu/2\pi \]

- \( \omega \): resonance frequency in radians per second, also called Larmor frequency
- \( \nu \): resonance frequency in cycles per second, Hz
- \( \gamma \): gyromagnetic ratio
- \( B₀ \): external magnetic field (the magnet)

Spin 1/2 nuclei will have two orientations in a magnetic field +1/2 and -1/2.
**Net magnetic moment**

\[ \mathbf{B}_o = 0 \quad \mathbf{B}_o > 0 \]

**Ensemble of Nuclear Spins**

\[ \mathbf{B}_o = 0 \quad \text{Randomly oriented} \]

\[ \mathbf{B}_o > 0 \quad \text{Highly oriented} \]

Each nucleus behaves like a bar magnet.
The net magnetization vector

\[
\begin{align*}
\vec{M}_0 & \quad \text{net magnetization vector allows us to look at system as a whole} \\
\text{many nuclei} & \\
\omega & \\
\end{align*}
\]

\[
\begin{align*}
\text{one nucleus} & \\
\omega & \\
\end{align*}
\]

Allowed Energy States for a Spin 1/2 System

\[
\Delta E = \gamma \ h \ \vec{B}_o = h \ \nu
\]

\[
\begin{align*}
\vec{B}_o = 0 & \\
\Delta E = \nu & \\
\vec{B}_o > 0 & \\
\end{align*}
\]

Therefore, the nuclei will absorb light with energy \( \Delta E \) resulting in a change of the spin states.
Nuclear Spin Dynamics

Effect of a 90° x pulse

Nuclear Spin Evolution

RF receivers pick up the signals
Spin Orientation in a Magnetic Field (Energy Levels)

- Magnetic moment are no longer equivalent
- Magnetic moments are oriented in $2I+1$ directions in magnetic field

1. **Vector length is:**
   
   $\sqrt{I(I + 1)}$

2. **Angle ($\varphi$) given by:**
   
   $\cos \varphi = \frac{m}{\sqrt{I(I + 1)}}$

3. **Energy given by:**
   
   $E = -\frac{m\mu}{I}B_z$

   where,
   - $B_z$ – magnetic Field
   - $\mu$ – magnetic moment
   - $\hbar$ – Planck’s constant

Observing NMR Signal

- Need to perturb system from equilibrium.
  - $B_1$ field (radio frequency pulse) with $\gamma B_1/2\pi$ frequency
- Net magnetization ($M_o$) now precesses about $B_0$ and $B_1$
  - $M_x$ and $M_y$ are non-zero
  - $M_x$ and $M_y$ rotate at Larmor frequency
- System absorbs energy with transitions between aligned and unaligned states
  - Precession about $B_1$ stops when $B_1$ is turned off
Observing NMR Signal

- **Remember**: a moving magnetic field perpendicular to a coil will induce a current in the coil.
- The induced current monitors the nuclear precession in the X,Y plane.

\[ \nu = \gamma B \frac{\gamma}{2\pi} \]

- RF pulse along Y
- Detect signal along X
- To simplify the vector description, the X,Y axis rotates about the Z axis at the Larmor frequency (X', Y')
- \( B_1 \) is stationary in the rotating frame.
- Applying the $B_1$ field for a specified duration (Pulse length or width)
- Net Magnetization precesses about $B_1$ a defined angle (90°, 180°, etc)

$$\omega_1 = \gamma B_1$$

$90^\circ$ pulse

Net Magnetization precesses about $B_1$ a defined angle (90°, 180°, etc)

**NMR Transition**

Magnetic moments are oriented in one of two directions in magnetic field (for $I = 1/2$)

Difference in energy between the two states is given by

$$\Delta E = E_{1/2} - E_{1/2} = \gamma (h/2\pi) B_0$$

If $h \nu_{RF} = \Delta E_B \rightarrow$ NMR transition

For NMR: $B_0 = 1-20$ Tesla \,(B_{earth} \approx 10^{-4} \,T)

$\Delta E_{NMR} \approx 0.1 \,\text{cal/mole} \ll kT$ \,(\$E_{IR} \approx 1-10 \,\text{kcal/mole})

$B_0$ – external magnetic field
$h$ – Planck’s constant
$\gamma$ – gyromagnetic ratio
Magnetic Energy Levels for Nuclei of Spin 1/2 and 1

- NMR signal results from the transition of spins from the $\alpha$ to $\beta$ state
- Strength of the signal depends on the population difference between the $\alpha$ and $\beta$ spin
- The population ($N$) difference can be determined from the Boltzmann distribution and the energy separation between the $\alpha$ and $\beta$ spin states:
  \[
  \frac{N_\alpha}{N_\beta} = e^{\frac{\Delta E}{kT}}
  \]

NMR Signal Sensitivity

- NMR signal results from the transition of spins from the $\alpha$ to $\beta$ state
- Strength of the signal depends on the population difference between the $\alpha$ and $\beta$ spin
- The population ($N$) difference can be determined from the Boltzmann distribution and the energy separation between the $\alpha$ and $\beta$ spin states:
  \[
  \frac{N_\alpha}{N_\beta} = e^{\frac{\Delta E}{kT}}
  \]
Since: \[ \Delta E = \hbar \nu \]

and

\[ \nu = \frac{\gamma B_o}{2\pi} \]

then:

\[ \frac{N_\alpha}{N_\beta} = e^{\frac{\Delta E}{kT}} \]

\[ \frac{N_\alpha}{N_\beta} = e^{\left(\frac{\gamma \hbar B_o}{2\pi kT}\right)} \]

The \( \Delta E \) for \(^1\text{H} \) at 400 MHz \((B_o = 9.39 \text{ T})\) is \( 6 \times 10^{-5} \text{ Kcal} / \text{mol} \)

\[ \frac{N_\alpha}{N_\beta} = 1.000060 \]

\( \text{Very Small!} \)

\( \sim 60 \text{ excess spins per million in lower state} \)

---

**NMR Sensitivity**

NMR signal \((s)\) depends on: \( s \propto \gamma^4 B_o^2 N B g(\nu) / T \)

1. Number of Nuclei \((N)\) (limited to field homogeneity and filling factor)
2. Gyromagnetic ratio (in practice \(\gamma^2\))
3. Inversely to temperature \((T)\)
4. External magnetic field \((B_o)\) (in practice, homogeneity)
5. \(B_1^2\) exciting field strength (RF pulse)

\[ \frac{N_\alpha}{N_\beta} = e^{\frac{\Delta E}{kT}} \]

\[ \Delta E = \gamma \hbar B_o / 2\pi \]

Incease energy gap \(\rightarrow\) increase population difference \(\rightarrow\) increase NMR signal

\[ \uparrow \Delta E \quad \uparrow B_o \quad \uparrow \gamma \]
NMR Signal/Noise Ratio

\[ S/N = \gamma N I (I+1) \left( \frac{B_0}{T} \right)^{3/2} f \left( \frac{Q V_s}{b} \right)^{1/2} \]

Where \( \gamma \) is the magnetogyric ratio of nucleus
\( B_0 \) external magnetic field
\( N \) number of magnetically active nuclei
\( T \) sample temperature
\( Q \) quality factor of the resonant circuit
\( f \) filling factor
\( b \) bandwidth of detector
\( V_s \) volume of sample

Signal/Noise Enhancement

- S/N ratio improves along with the increasing of the strength of magnetic field, in a relationship of: \( (B_0)^{3/2} \)
- S/N ratio increases when number of scan increases, in a relationship of: \( \sqrt{N} \)
Effect of Magnetic Field on Population Differences

Increase in Magnet Strength is a Major Means to Increase Sensitivity
900 MHz NMR Spectrometer

- Analyze concentrations of 1 mM or less
- Characterize molecule with a molecular weight of 500,000
NMR Sensitivity

- Relative sensitivity of $^1H$, $^{13}C$, $^{15}N$ and other nuclei NMR spectra depend on
  - Gyromagnetic ratio ($\gamma$)
  - Natural abundance of the isotope

$\gamma$ - Intrinsic property of nucleus can not be changed.

$$\left(\frac{\gamma_H}{\gamma_C}\right)^3 \text{ for } ^{13}C \text{ is } 64x \ \left(\frac{\gamma_H}{\gamma_N}\right)^3 \text{ for } ^{15}N \text{ is } 1000x$$

$^1H$ is $\sim 64x$ as sensitive as $^{13}C$ and $1000x$ as sensitive as $^{15}N$!

Consider that the natural abundance of $^{13}C$ is $1.1\%$ and $^{15}N$ is $0.37\%$
relative sensitivity increases to $\sim 6,400x$ and $\sim 2.7 \times 10^5x$!!

---

$^1H$ NMR spectra of caffeine
8 scans ~12 secs

$^{13}C$ NMR spectra of caffeine
8 scans ~12 secs

$^{13}C$ NMR spectra of caffeine
10,000 scans ~4.2 hours
Increasing Magnetic Field Results in a Significant Cost!

Varian 900 MHz NMR Instrument
Improved Sensitivity using Cryoprobes (Bruker)

- In a cryogenic probe, the pick-up coils and some electronics are cooled to ~ 30 K drastically reducing the Johnson noise, which is generated by thermal agitation of electrons in a conductor.

- Increases sensitivity by a factor of 3.4-4.0
  - NMR signals are obtained by sequential averaging, this increase translates to 1/10-1/16 the number of measurements needed for averaging.
  - A one hour run is reduced to 6 min.
- A four-fold lower detection limit
  

Spin Relaxation

There are two primary causes of spin relaxation:

Spin - lattice relaxation, $T_1$, longitudinal relaxation.

Spin - spin relaxation, $T_2$, transverse relaxation.
Transition from the low energy to high energy spin state occurs through an absorption of a photon of radio-frequency (RF) energy.

\[ \nu = \frac{\gamma B_0}{2\pi} \]

Free Induction Decay

- The signals decay away due to interactions with the surroundings.
- A free induction decay, FID, is the result.
- Fourier transformation, FT, of this time domain signal produces a frequency domain signal.
CW NMR Experiments

The simplest NMR experiment is the continuous wave (CW) experiment. There are two ways of performing this experiment:

- a constant frequency RF emission probes the energy levels while the magnetic field is varied.
- a varying frequency RF emission probes the energy levels while the magnetic field remains constant.
The Fourier Transform

The Fourier transform (FT) is a mathematical technique for converting time domain data to frequency domain data, and vice versa. An FT is defined by the integral:

\[
f(\omega) = \int_{-\infty}^{\infty} f(t) e^{-i\omega t} dt = \int_{-\infty}^{\infty} f(t) [\cos(\omega t) - i \sin(\omega t)] dt
\]

### Observable

<table>
<thead>
<tr>
<th>Observable</th>
<th>Name</th>
<th>Quantitative</th>
<th>Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak position</td>
<td>Chemical shifts ((\delta))</td>
<td>(\delta = \nu_{\text{obs}} - \nu_{\text{ref}}/\nu_{\text{ref}} ) (Hz)</td>
<td>chemical (electronic) environment of nucleus</td>
</tr>
<tr>
<td>Peak Splitting</td>
<td>Coupling Constant (J) Hz</td>
<td>peak separation (intensity ratios)</td>
<td>neighboring nuclei (torsion angles)</td>
</tr>
<tr>
<td>Peak Intensity</td>
<td>Integral</td>
<td>unitless (ratio) relative height of integral curve</td>
<td>nuclear count (ratio) T_1 dependent</td>
</tr>
<tr>
<td>Peak Shape</td>
<td>Line width</td>
<td>(\Delta \nu = 1/\pi T_2) peak half-height</td>
<td>molecular motion chemical exchange uncertainty principal uncertainty in energy</td>
</tr>
</tbody>
</table>
The chemical shift

The valence electrons around the nucleus are caused to circulate by the applied magnetic field $B$. This circulation, termed a local diamagnetic current, induces a local magnetic field $dB$ that is oriented to oppose the applied field $B$. The net result is that the nucleus feels a reduced magnetic field $B_{loc}$; that is, the applied field has been shielded by the local diamagnetic current.

$\sigma$ is a dimensionless quantity called the shielding constant of the nucleus.

$$\delta B = -\sigma B$$

$$B_{loc} = B + \delta B = (1 - \sigma)B$$

$$\nu = \frac{\gamma B_{loc}}{2}\pi = \left(1 - \sigma\right)\frac{\gamma B}{2\pi}$$

Since the frequency at which resonance occurs is a direct function of the effective magnetic field $B_{loc}$, every nucleus that is in a distinct electronic environment will undergo resonance at a different applied frequency. Scanning the frequency gives a NMR spectrum.

The $\delta$-scale of chemical shift

The resonance frequency can be expressed in terms of chemical shift $\delta$, which is related to the difference between the resonance frequency, $\nu$, of the nucleus analyzed and that of a reference standard $\nu^o$:

$$\delta = \frac{\nu - \nu^o}{\nu^o} \times 10^6$$

For $^1H$ and $^{13}C$, the standard is tetramethylsilane, TMS: Si(CH$_3$)$_4$, for which $\delta=0$ ppm.

With the $\delta$-scale, shifts are independent of the applied field.

$$\delta = \frac{(1 - \sigma)B - (1 - \sigma^o)B}{(1 - \sigma^o)B} \times 10^6 = \frac{\sigma^o - \sigma}{1 - \sigma^o} \times 10^6 \approx (\sigma^o - \sigma) \times 10^6$$
Origin of the Shielding Constant

Electron density around the nucleus studied

Neighboring chemical groups in the molecule studied

\[ \sigma = \sigma_{\text{local}} + \sigma_{\text{neighbour}} + \sigma_{\text{solvent}} \]

- Diamagnetic contribution
- Paramagnetic contribution

**Note:** If the electron density is spherical or cylindrical around the nucleus, \( \sigma_{\text{local}} = \sigma_{d} \). For \(^1\)H NMR, the paramagnetic contribution is negligible.
**Diamagnetic Contribution $\sigma_d$**

- $B$ generates a circulation of charge in the ground-state electron distribution of the atom $\Rightarrow \delta B$ is created opposite to $B$, which shields the nucleus and gives rise to $\sigma_d$. $\sigma_d$ depends on the electron density around the protons.

**Lamb formula:**

$$\sigma_d = \frac{e^2 \mu_0}{3m_e} \int_0^\infty r \rho(r) \, dr$$

$\mu_0$ is vacuum permeability, $r$ is electron-nucleus distance, $\rho(r)$ is the electron density around the nucleus. Although the Lamb formula is strictly valid for spherical symmetry, when the diamagnetic contribution is dominant, it gives the right trend.

$\Rightarrow$ An electronegative atom $X$, directly bound to a proton (H-X) or with one carbon as intermediate (H-C-X), decreases the electron density around the proton and decreases the shielding $\sigma_d$ contribution. The transition appears at high frequency, a large $\delta$ is observed.

$\Rightarrow$ As the electronegativity of the neighbor atom increases, $\delta$ increases

<table>
<thead>
<tr>
<th>Group</th>
<th>C$_3$F$_7$</th>
<th>C$_3$H$_7$</th>
<th>C$_2$F$_2$</th>
<th>C$_2$H$_2$</th>
<th>C$_2$H$_4$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\delta$</td>
<td>0.13</td>
<td>2.04</td>
<td>2.45</td>
<td>1.98</td>
<td>0.15</td>
</tr>
<tr>
<td>Electron density</td>
<td>4.0</td>
<td>3.0</td>
<td>2.8</td>
<td>2.5</td>
<td>2.2</td>
</tr>
</tbody>
</table>

---

**Neighbouring Group Contribution $\sigma_{(neighbour)}$**

B generates current in the electron distribution of the neighboring group and gives rise to a magnetic moment $M$ proportional to $B$ via a constant vector $\chi$ called the magnetic susceptibility: $M = \chi B$.

For the sake of simplicity, let’s consider only **groups with axial symmetry** (linear). The anisotropy of the group is defined with $\Delta \chi = \chi_\parallel - \chi_\perp$. $M$ creates an anisotropic magnetic field $B_{\text{neighbour}}$ and a shielding constant, which are function of:

1) The distance, $r$, between the proton and the group.
2) The anisotropy $\Delta \chi$.
3) The position of the proton with respect to the group, which is given by the angle $\theta$.

**Mc Connell formula:**

$$\sigma_{(neighbour)} \propto (\chi_\parallel - \chi_\perp) \left( \frac{1 - 3 \cos^2 \theta}{r^3} \right)$$

$\sigma_{(neighbour)}$ can be positive or negative according to $\Delta \chi$ and the position of the proton ($\theta$). On the cone, $\delta = 0$.

---

**Figures and Diagrams**

- Diagram showing the circulation of charge and the shielding effect.
- Diagram illustrating the Mc Connell formula with the cone and the line $\delta = 0$. The cone angles are 55° and 125°.
All groups in a molecule which contain \( \pi \)-electrons generate anisotropic secondary fields in response to an external magnetic field. The lines-of-force in these fields can either shield or deshield hydrogens, depending on the exact shape of the field and the location of the particular hydrogen nucleus. In the diagrams below, protons within the (+) regions of space will be shielded (smaller \( \delta \)) and protons in the (-) regions will be deshielded (larger \( \delta \)).

**Special Case of the Aromatic Compounds**

The applied magnetic field causes the \( \pi \) electrons to circulate, inducing a ring current. The ring current, in turn, induces a small local magnetic field which increases the effective magnetic field felt by the ring hydrogens, causing an increase in chemical shift (\( \delta \)).
**[18]Annulene**

Note that [18]annulene has 12 hydrogens on the outside of the ring, and 6 hydrogens on the inside.

For the outer hydrogens, an applied magnetic field will augment the effective field, causing a shift to the “aromatic” region. The inner hydrogens, however, will experience the opposite effect, and should be shifted to a smaller δ value.

For the inner hydrogens, however, the induced field is anti-parallel to the applied field, making δ smaller.

The ring current induces a magnetic field for outer hydrogens which is parallel to the applied field, making δ larger.

---

The $^1$H NMR of [18]annulene shows two peaks. A singlet at δ 7.3 (12 hydrogens) and a singlet at δ -3.0 (6 hydrogens) consistent with the “ring current” model (magnetic anisotropy).
NMR Spectroscopy
Main Features ---- Integration

The area under the NMR resonance is proportional to the number of hydrogen which that resonance represents. In this way, by measuring or integrating the different NMR resonances, information regarding the relative numbers of chemically distinct hydrogens can be found.

Integration only gives information on the relative number of hydrogens different, not the absolute number.

\[ ^1H \text{ NMR Spectrum of 1-Butanol} \]
Number of Signals

- Protons that have different chemical shifts are chemically nonequivalent
- Exist in different molecular environment

Chemically Equivalent Protons

- are in identical environments
- have same chemical shift
- replacement test: replacement by some arbitrary "test group" generates same compound

\[ \text{ClCH}_2\text{CH}_2\text{CH}_3 \leftrightarrow \text{H}_3\text{CCH}_2\text{CH}_3 \leftrightarrow \text{CH}_3\text{CH}_2\text{CH}_2\text{Cl} \]

chemically equivalent
Diastereotopic Protons

- Replacement by some arbitrary test group generates diastereomers
- Diastereotopic protons can have different chemical shifts

\[
\begin{align*}
\text{Br} & \quad \text{H} & \quad \delta \ 5.3 \ ppm \\
\text{C} &= \text{C} \\
\text{H}_3\text{C} & \quad \text{H} & \quad \delta \ 5.5 \ ppm
\end{align*}
\]

Enantiotopic Protons

- are in mirror-image environments
- replacement by some arbitrary test group generates enantiomers
- enantiotopic protons have the same chemical shift
Nuclei with the same chemical environment or the same chemical shift are called equivalent. Nuclei with different environments or having different chemical shifts are nonequivalent.

Nuclei which are close to one another affect each other's effective magnetic field. This effect is observable for nonequivalent nuclei at a distance of three or less bond lengths from each other.

The effect is called spin-spin coupling or J coupling. The size of J is given in Hz unit, and is therefore dependent on the strength of the applied magnetic field.
Spin-Spin Coupling

- For two nuclei, A and B, apart from one another by three bonds in a molecule, there are a total of four possible configurations for the two nuclei in a magnetic field. The vertical lines in this diagram represent the allowed transitions between energy levels.

- An allowed NMR transition is one where the spin of one nucleus changes from spin up to spin down or vice versa. Absorptions of energy where two or more nuclei change spin at the same time are not allowed.

Scalar J Coupling

Electrons have a magnetic moment and are spin 1/2 particles.

J coupling is facilitated by the electrons in the bonds separating the two nuclei. This through-bond interaction results in splitting of the nuclei into $2I + 1$ states. Thus, for a spin 1/2 nucleus the NMR lines are split into $2(1/2) + 1 = 2$ states.

$$\text{Multiplet} = 2nI + 1$$

- $n$ - number of identical adjacent nuclei
- $I$ - spin quantum number
Scalar J Coupling

The magnitude of the J coupling is dictated by the torsion angle between the two coupling nuclei according to the Karplus equation.

\[
J = A + B \cos(\theta) + C \cos^2(\theta)
\]

Where:

- \(A = 1.9\)
- \(B = -1.4\)
- \(C = 6.4\)

A, B and C are dependent on the substituent electronegativity.

Torsion Angles

Coupling constants can be measured from NMR data. Therefore, from this experimental data we can use the Karplus relation to determine the torsion angles, \(\theta\).

Coupling constants can be measured between most spin 1/2 nuclei of biological importance,

\[ ^1H, \:^13C, \:^{15}N, \:^{31}P \]

The most significant limitation is usually sensitivity, S/N.
Two-bond and Three-bond Coupling

protons separated by two bonds (geminal relationship)
Large Coupling 10-15 Hz

protons separated by three bonds (vicinal relationship)
Smaller Coupling about 7 Hz

in order to observe splitting, protons cannot have same chemical shift
coupling constant ($J_2$ or $J_3$) is independent of field strength
Why do the methyl protons of 1,1-dichloroethane appear as a doublet?

To explain the splitting of the protons at C-2, we first focus on the two possible spin orientations of the proton at C-1.

There are two orientations of the nuclear spin for the proton at C-1. One orientation shields the protons at C-2; the other deshields the C-2 protons.
Why do the methyl protons of 1,1-dichloroethane appear as a doublet?

The protons at C-2 "feel" the effect of both the applied magnetic field and the local field resulting from the spin of the C-1 proton.

"true" chemical shift of methyl protons (no coupling)

this line corresponds to molecules in which the nuclear spin of the proton at C-1 reinforces the applied field

this line corresponds to molecules in which the nuclear spin of the proton at C-1 opposes the applied field
Why does the methine proton of 1,1-dichloroethane appear as a quartet?

The proton at C-1 "feels" the effect of the applied magnetic field and the local fields resulting from the spin states of the three methyl protons. The possible combinations are shown on the next slide.

There are eight combinations of nuclear spins for the three methyl protons. These 8 combinations split the signal into a 1:3:3:1 quartet.
The splitting rule for $^1$H NMR
A Much Simpler Rule

For simple cases, the multiplicity of a signal for a particular proton is equal to the number of equivalent vicinal protons + 1.

This is called the N+1 Rule: The number of proton neighbors plus itself gives the splitting number.

Pascal’s Triangle --- “True” For NMR

<table>
<thead>
<tr>
<th>Number of equivalent protons to which H is coupled</th>
<th>Appearance of multiplet</th>
<th>Intensities of lines in multiplet</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Doublet</td>
<td>1:1</td>
</tr>
<tr>
<td>2</td>
<td>Triplet</td>
<td>1:2:1</td>
</tr>
<tr>
<td>3</td>
<td>Quartet</td>
<td>1:3:3:1</td>
</tr>
<tr>
<td>4</td>
<td>Pentet</td>
<td>1:4:6:4:1</td>
</tr>
<tr>
<td>5</td>
<td>Sextet</td>
<td>1:5:10:10:5:1</td>
</tr>
</tbody>
</table>
Pairs of Doublets

H – C – C – H

Consider coupling between two vicinal protons.
If the protons have different chemical shifts, each will split the signal of the other into a doublet.
Let $\Delta \nu$ be the difference in chemical shift in Hz between the two hydrogens.

Let $J$ be the coupling constant between them in Hz.

When $\Delta \nu$ is much larger than $J$ the signal for each proton is a doublet, the doublet is symmetrical, and the spin system is called AX.

As $\Delta \nu/J$ decreases the signal for each proton remains a doublet, but becomes skewed. The outer lines decrease while the inner lines increase, causing the doublets to "lean" toward each other.
When $\Delta \nu$ and $J$ are similar, the spin system is called AB. Skewing is quite pronounced. It is easy to mistake an AB system of two doublets for a quartet.

When $\Delta \nu = 0$, the two protons have the same chemical shift and don't split each other. A single line is observed. The two doublets have collapsed to a singlet.
More Complicated or Simpler Than $^1$H Spectra?

Heteronuclear coupling: between different nuclei

$^1$H-$^1$C, $^1$H-$^3$P, $^1$C-$^19$F, etc.

$^1J_{CH} \approx 100$ to 250 Hz

$^1J_{CC} = 550$ s(i)s(j), e.g., CH$_2$=C=CH$_2$, $J = 98.7$ (1/2 * 1/3 = 1/6)

$^1$H-coupled $^1$C spectra often have overlapping resonances, so $^1$C spectra usually acquired in $^1$H-mode
Decoupling of $^1$H signal

Original

Decoupling at CH$_2$

Decoupling at CH

Decoupling at CH$_3$
Decoupling of $^1$H on $^{13}$C spectrum

The DEPT (distortionless enhanced polarization transfer) experiment is a useful 1D NMR experiment that provides information on the number of protons attached to the various $^{13}$C resonances in a carbon NMR spectrum. In the DEPT experiment we use two rf transmitters, one to apply pulses to the $^1$H spins and one to apply pulses to the $^{13}$C spins. We acquire the $^{13}$C signal during the FID time period. During the $^{13}$C acquisition, the $^1$H transmitter is used for broad-band (BB) decoupling to remove the splitting of $^{13}$C signals by attached protons. For the proton channel, it is important to note that we are using the transmitter that is normally used to do decoupling and not the one that is used for routine proton acquisitions.
A DEPT spectrum actually consists of several spectra, with the final data presentation depicting one spectrum for each type of carbon atom. Thus, CH, CH$_2$ and CH$_3$ carbons are each printed out on separate spectra, together with a $^{13}$C spectrum where all carbon types are shown. Each carbon type is thus identified unambiguously.

Results of the DEPT experiments (quaternary carbons are null in all spectra):

**DEPT 45**: CH, CH$_2$, CH$_3$ positive.

**DEPT 90**: CH positive, CH$_2$, CH$_3$ null (often a small residual signal is seen).

**DEPT 135**: CH, CH$_3$ positive, CH$_2$ negative.
pure (S)-carvone

CUMINALDEHYDE

13C NMR in CDCl3
Prediction of $^1$H NMR Signal
Shoolery chemical shift rules for $^1$H

- As we have seen, most of the different effects on $^1$H chemical shifts have been tabulated in one way or another.

- Furthermore, we also saw that most of the effects are additive, meaning that if we can estimate the different effects on the chemical shift of a certain $^1$H from different groups and bonds, we can in principle estimate its chemical shift by adding all the effects together.

- There are several empirical rules, derived mostly by Shoolery in the late 50s/early 60s.

- In order to use them, we first have to identify the type of proton we have, such as aliphatic CH$_3$, CH$_2$, CH, olefinic CH$_2$ or CH, aromatic, a or b to a ketone or alcohol, belonging to an a, b-unsaturated system, etc. They will have a base value.

- Then we look up the contributions from different groups attached to carbons in the surrounding of our system, and add them up to obtain the estimated chemical shift.

- We’ll analyze several cases to see how they work…

$$\delta H = \delta H_{\text{base}} + \sum \text{contributions}$$

Shoolery rules (continued)

- **Aliphatic compounds.** There are two approaches to the calculation of additive effects on the $^1$H chemical shifts.

- The first one is very simple. We just use two ‘skeletons’ with two base values, $R_1$-CH$_2$-$R_2$ or $R_1$-CH-($R_2$)-$R_3$, and add the effects from the $R_1$, $R_2$, or $R_3$ groups:

- So CH$_3$Br$_2$ would be $\delta = 1.25 + 1.9 + 1.9 = 5.05$ ppm, which compares pretty well with the experimental value of 4.94 ppm.

<table>
<thead>
<tr>
<th>Substituent</th>
<th>$\delta$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkyl</td>
<td>0.0</td>
</tr>
<tr>
<td>-C=C-</td>
<td>0.8</td>
</tr>
<tr>
<td>-C=C-</td>
<td>0.9</td>
</tr>
<tr>
<td>-C$_6$H$_5$</td>
<td>1.3</td>
</tr>
<tr>
<td>-CO-R</td>
<td>1.3</td>
</tr>
<tr>
<td>-OH</td>
<td>1.7</td>
</tr>
<tr>
<td>-O-R</td>
<td>1.5</td>
</tr>
<tr>
<td>-O-CO-R</td>
<td>2.7</td>
</tr>
<tr>
<td>-NH$_2$</td>
<td>1.0</td>
</tr>
<tr>
<td>-Br</td>
<td>1.9</td>
</tr>
<tr>
<td>-Cl</td>
<td>2.0</td>
</tr>
</tbody>
</table>
The second method is pretty more general. We start with methane ($\delta = 0.23$ ppm), and then we add substituent effects directly.

- Now, if instead of a substituent we have another carbon chain, we have to consider how many carbons it has, and each carbon will have an increment we need to add:

- Furthermore, if the carbons of these chains are substituted, we have to add increments according to their position in the carbon chain.

- It is a lot more complicated, but as we see, more general (and some say more accurate).

$$\delta = 0.23 + \sum S(\delta)$$

<table>
<thead>
<tr>
<th>Substituent</th>
<th>$\delta_{gem}$</th>
<th>$\delta_{cis}$</th>
<th>$\delta_{trans}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Alkyl-</td>
<td>0.45</td>
<td>-0.22</td>
<td>-0.28</td>
</tr>
<tr>
<td>-OR</td>
<td>1.21</td>
<td>-0.60</td>
<td>-1.00</td>
</tr>
<tr>
<td>-COOH</td>
<td>0.80</td>
<td>0.98</td>
<td>0.32</td>
</tr>
<tr>
<td>-Ar</td>
<td>1.38</td>
<td>0.36</td>
<td>-0.07</td>
</tr>
<tr>
<td>-C=O-C=O</td>
<td>1.24</td>
<td>0.02</td>
<td>-0.05</td>
</tr>
<tr>
<td>-OH</td>
<td>1.22</td>
<td>-1.07</td>
<td>-1.21</td>
</tr>
<tr>
<td>-Cl</td>
<td>1.08</td>
<td>-0.40</td>
<td>-1.02</td>
</tr>
</tbody>
</table>

**Shoolery rules (…)**

*Olefines.* For alkenes we change the tables for the base values, but we also have to consider the stereochemistry of the substituent (cis, trans, or gem):

So for cinnamic acid ($\text{trans Ph-CH} = \text{CH-COOH}$), we get that $\delta^\text{H} = 5.25 + 1.38 + 0 + 0.98 = 7.61$, and $\delta^\text{H} = 5.25 + 0.80 + 0 + 0.36 = 6.41$, pretty close to the reported values of 7.82 and 6.47 ppm.
Dr. Zerong Wang at UHCL

**Shoolery rules (…)**

- *Aromatics.* Finally, the Shoolery rules allow us to calculate the approximate chemical shifts in aromatic compounds. Again, we have a different base value of 7.26 (benzene…).

\[
\delta = 7.26 + R_{\text{ortho}} + R_{\text{meta}} + R_{\text{para}}
\]

<table>
<thead>
<tr>
<th>Substituent</th>
<th>(\delta_{\text{ortho}})</th>
<th>(\delta_{\text{meta}})</th>
<th>(\delta_{\text{para}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>CH(_3)-</td>
<td>-0.18</td>
<td>-0.10</td>
<td>-0.20</td>
</tr>
<tr>
<td>-NO(_2)</td>
<td>0.95</td>
<td>0.26</td>
<td>0.38</td>
</tr>
<tr>
<td>-COOH</td>
<td>0.85</td>
<td>0.18</td>
<td>0.25</td>
</tr>
<tr>
<td>-OCH(_3)</td>
<td>1.38</td>
<td>0.36</td>
<td>-0.07</td>
</tr>
<tr>
<td>-Cl</td>
<td>1.24</td>
<td>0.02</td>
<td>-0.05</td>
</tr>
<tr>
<td>-F</td>
<td>1.22</td>
<td>-1.07</td>
<td>-1.21</td>
</tr>
<tr>
<td>-CONH(_2)</td>
<td>1.38</td>
<td>0.36</td>
<td>-0.07</td>
</tr>
<tr>
<td>-CH=CH(_2)</td>
<td>1.24</td>
<td>0.02</td>
<td>-0.05</td>
</tr>
<tr>
<td>-SO(_3)H</td>
<td>1.22</td>
<td>-1.07</td>
<td>-1.21</td>
</tr>
</tbody>
</table>

• **For** p-Xylene:

\[
\delta_{H_a} = 7.26 - 0.18 - 0.10 = 6.98 \quad (6.97) \\
\delta_{H_b} = \delta_{H_a}
\]

• **For** 1-Chloro-4-nitrobenzene

\[
\delta_{H_a} = 7.26 + 0.95 - 0.02 = 8.19 \quad (8.17) \\
\delta_{H_b} = 7.26 + 0.03 + 0.26 = 7.55 \quad (7.52)
\]

• **For** mesitylene

\[
\delta_{H} = 7.26 - 2 \times 0.18 - 0.20 = 6.70 \quad (6.78)
\]

• **For** 2,4-dinitro-1-methoxybenzene

\[
\delta_{H_a} = 7.26 - 0.48 + 2 \times 0.26 = 7.30 \quad (7.28) \\
\delta_{H_b} = 7.26 + 0.95 + 0.38 - 0.09 = 8.50 \quad (8.47) \\
\delta_{H_c} = 7.26 + 2 \times 0.95 - 0.09 = 9.07 \quad (8.72)
\]

Dr. Zerong Wang at UHCL
NMR Results: 2-Butanol

NMR Results: 2-Methyl-1-propanol (Isobutanol)
NMR Results: 1-Pentanol

NMR Results: 2-Pentanol
NMR Results: 3-Pentanol

NMR Results: 3-Methyl-1-butanol (Isopentanol)
One chemical with molecular formula as \( \text{C}_4\text{H}_8\text{O}_2 \), its \(^1\text{H}\) NMR, \(^{13}\text{C}\) NMR, IR and MS spectra are given below. Please identify its structure.

Assign. Shift (ppm)

<table>
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<tr>
<th>ppm</th>
<th>Int.</th>
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</thead>
<tbody>
<tr>
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<td>527</td>
</tr>
<tr>
<td>60.44</td>
<td>1000</td>
</tr>
<tr>
<td>21.00</td>
<td>571</td>
</tr>
<tr>
<td>14.28</td>
<td>857</td>
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</tbody>
</table>

ppm | Int. |
<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
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<tr>
<td>15.0</td>
<td>6</td>
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<tr>
<td>18.0</td>
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<tr>
<td>26.0</td>
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<td>29.0</td>
<td>13</td>
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<tr>
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Structure Determination by NMR

Biological molecules such as proteins and nucleic acids can be large and complex. They can easily exceed 2000 atoms. Knowing their structure is critical in understanding the relationship between structure and function.

X-ray crystallography is an excellent method to determine detailed 3D structures of even some of the largest biological molecules. However, it has some significant difficulties. Getting crystals and is the structure biologically relevant.

NMR can be used to determine 3D structure and dynamics in solution! It’s limitation is molecular size. However, this is changing.

A good online book about basic NMR is at
http://www.cis.rit.edu/htbooks/nmr/
Nuclear Overhauser Effect

Caused by dipolar coupling between nuclei.

The local field at one nucleus is affected by the presence of another nucleus. The result is a mutual modulation of resonance frequencies.

\[
I = A \left( \frac{1}{r^6} \right)
\]

I - intensity
A - scaling constant
r - internuclear distance

Arrows denote cross relaxation pathways
\(r_{1,2}\) - distance between protons 1 and 2
\(r_{2,3}\) - distance between protons 2 and 3

The NOE provides a link between an experimentally measurable quantity, I, and internuclear distance.
NOE is only observed up to ~5Å.
Biomolecular NMR Experiments

- J Correlated Based Experiments
  - COSY - Correlated Spectroscopy
  - 2QF-COSY - Double Quantum Filtered Spectroscopy
  - HETCOR - Heteronuclear Correlated Spectroscopy
  - E.COSY - Exclusive COSY
  - HOHAHA - Homonuclear Hartmann Hahn (TOCSY)

- Nuclear Overhauser Based Experiments
  - NOESY - Nuclear Overhauser Effect Spectroscopy
  - ROESY - Rotating Frame Overhauser Effect Spectroscopy

- Three Dimensional Experiments Use a Combination
  - NOESY - TOCSY
  - NOESY - NOESY

Sample Factors Influencing NMR Sensitivity

- System should have short longitudinal $T_1$ to allow maximum number of scans
- Exhibit longest possible transverse $T_2$ to yield sharpest lines
- Be decoupled from other magnetically active centers so as to yield single line
- Be stable on NMR time scale
- Transfer of magnetization from adjacent scalar coupled nuclei to spin site under observation
- Isotopic enrichment of observed nucleus