

# Nuclear Magnetic Resonance (NMR) Spectroscopy

Chem 221  
Instrumental Analysis  
Spring 2005

## Overview

- Based on the interaction of *RF EMR* (up to ~800 MHz) with matter (in a magnetic field)
  - EMR interactions with *spin states of nuclei*
  - RF EMR*: much lower energy than optical EMR
- First demonstrated in 1946
- First commercial instrument: 1956
- We will be concerned with:
  - > origin of RF EMR interactions
  - > how these interactions are measured
  - > how chemical information can be obtained from NMR measurements

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## Theory: Quantum Treatment

- Energies of nuclear spin states are quantized:  
*Nuclear Spin Quantum Number (I)*  
where  $I = 0, \frac{1}{2}, 1, 1\frac{1}{2}, \text{etc.}$

### Three Groups of Nuclei:

- $I=0$   
-*non-spinning* nuclei, no magnetic moment, even # p<sup>+</sup> & n<sup>0</sup>  
-examples: <sup>12</sup>C, <sup>16</sup>O
- $I=\frac{1}{2}$   
-spherical spinning charge with magnetic moment  
-examples: <sup>13</sup>C, <sup>1</sup>H
- $I>\frac{1}{2}$   
-*non-spherical* spinning charge with magnetic moment  
-examples: <sup>2</sup>H, <sup>14</sup>N

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## More Quantum Numbers

- All nuclear spin states are *degenerate* unless in a uniform magnetic field
  - Where they *split* into  $2I + 1$  states
  - Defined by *Magnetic Quantum Numbers (m)*:  
 $m = I, I-1, I-2 \dots -I$

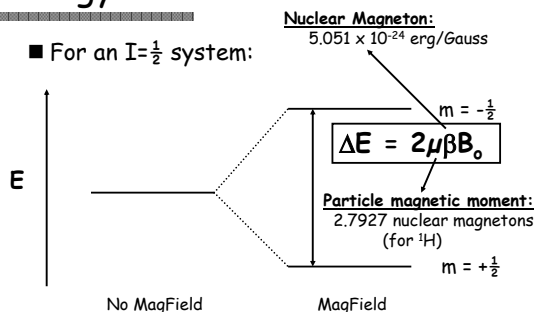
So, for  $I=0$ :  $m = 0$  (only 1 state) *NMR inactive*

for  $I=\frac{1}{2}$ :  $m = \pm\frac{1}{2}$  (2 states) *NMR ACTIVE*

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## Energy States

- For an  $I=\frac{1}{2}$  system:



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## In General:

- Selection Rule:**  $\Delta m = \pm 1$
- For *any* value of  $I$ :

$$h\nu = (\mu\beta B_0)/I$$

- So,  $\nu$  will vary with applied field strength ( $B_0$ )
- Example: for <sup>1</sup>H,  $\nu = 60$  MHz @  $B_0 = 14,092$  Gauss

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## A Classical Perspective

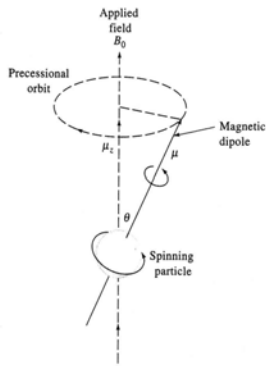
- A "classical" view will help us understand the measurement process.

Consider a spinning charged particle in a magnetic field:

- Particle will precess at a characteristic frequency (*Larmor Frequency*)  $\nu_0$ :

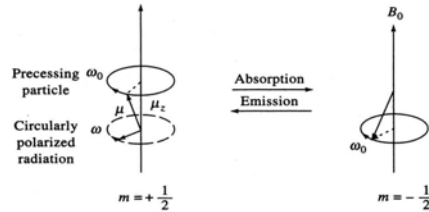
$$\nu_0 = \gamma B_0 / 2\pi$$

(where  $\gamma$  = magnetogyric ratio)



## "Classical" view of Absorption

- Application of *another magnetic field* ( $B_1$ ) **perpendicular to  $B_0$**  and at a frequency  $= \nu_0$  results in:
  - Absorption of applied EMR
  - Spin flip of particle to excited state



## Instrumental

### Continuous Wave (CW) NMR

original instruments used:

- ✓ Electromagnets (14 - 23 kG; 60 - 100 MHz)
- ✓ Fixed frequency RF source
- ✓ Swept (variable) magnetic field

-measure *absorption* of applied RF

⇒ *not very sensitive . . . Why?*

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## Back to Boltzmann!

- Using Boltzmann statistics, we can determine the relative populations of each of the two spin states:

$$N_2/N_1 = e^{-\Delta E/kT}$$

- $\Delta E$  is very small (relative to optical EMR) for RF EMR
- As  $\Delta E \downarrow$ ,  $N_2/N_1 \rightarrow 1$  (so,  $N_2 \approx N_1$ )
- BUT: for absorbance, we want  $N_1 \gg N_2$
- If absorption rate > decay rate, not much absorbance can occur before  $N_1 = N_2$  (saturation)
- When transition is saturated, **NO MORE ABSORPTION!**<sup>10</sup>

## Decay (relaxation) Processes

Two decay routes (non-radiative):

### 1. Spin-Lattice Relaxation ( $T_1$ )

-also called: *longitudinal relaxation*

-due to interactions between nuclear spin states and *magnetic micro environments* in the sample

-magnetic micro environments **must be at the Larmor Frequency** of the absorbing nuclei in order to couple

-what will affect coupling efficiency?

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## Coupling Efficiency: $T_1$

- $T_1$  is the *excited state lifetime* associated with spin-lattice relaxation (it is *inversely proportional* to the extent of spin-lattice relaxation)
- Temperature effects**
  - at some temperature, the frequency of molecular motion matches the Larmor frequency for a nucleus and coupling efficiency is at a *maximum* ( $T_1$  is at a *minimum*)
  - any change in temperature will result in an *increase* in  $T_1$  (decreased coupling efficiency)
- Any (e.g., viscosity) changes in *lattice mobility* will have a similar effect

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## More on $T_1$ Processes

- Other lattice components that can reduce  $T_1$ :
  - unpaired electrons (from radicals and paramagnetic species, like  $O_2$ )
  - nuclei with  $I \geq 1$
- Efficient Spin-Lattice Relaxation results in:
  - ✓ Decreased likelihood of saturation
  - ✓ Larger absorption signal (CW NMR)
  - ✓ Other effects?

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## Spin-Spin Relaxation: $T_2$

- Energy transfer with other magnetic nuclei
  - Nuclei must be in close proximity
  - Very efficient coupling in *solids* ( $T_2 \sim 10^{-4}$  sec)
  - Has no effect on *saturation*
  - Will cause **line broadening**:
    - $\Delta\nu = (2\pi\Delta t)^{-1}$  (according to Heisenberg)
    - so: linewidth  $\propto 1/T_2$
    - ( $T_2 \approx 10^{-4}$  sec  $\rightarrow \Delta\nu \approx 10^3$  Hz)
- In solutions: ( $< 1$  sec)  $T_2 < T_1$  (1-10 sec)
  - Controls linewidths ( $\sim 1$  Hz)
  - Affects saturation!

## How can S/N be increased?

- Increase  $B_0$ 
  - increases  $\Delta E$ , increasing population difference between spin states, so more nuclei can undergo transitions
  - How? Superconducting Magnets
- Multiplex Signal Measurement
  - small signal makes measurement limited by detector noise, so a multiplex measurement method should improve S/N
  - How? Fourier Transform

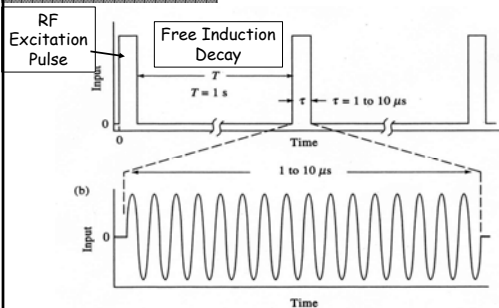
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## Pulsed FT-NMR

- At fixed  $B_0$ , irradiate sample with a *range* of RF EMR frequencies ... How?
  - by *pulsing* a fixed frequency ( $\nu_0$ ) RF source, a *range* ( $\Delta\nu$ ) of RF frequencies is generated.
  - the extent of the range is determined by the *pulse width*:
    - $\Delta\nu = 1/4\tau$  (according to Heisenberg)
    - where:  $\tau$  is the pulse width (seconds)

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## The Pulsed FT-NMR Measurement



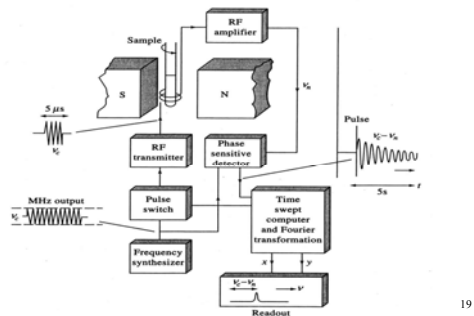
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## FT-NMR: Obtaining a Spectrum

- Obtain maximum number of excited nuclei during the RF pulse (saturation)
- Measure *RF Emission* (signal generated by nuclei spin flips back to ground state) when RF source is off - **FID**
- **FID** contains emission from all excited nuclei *all at their characteristic (Larmor) frequencies*
- Use Fourier Transform to convert *time domain* FID to **frequency domain** spectrum

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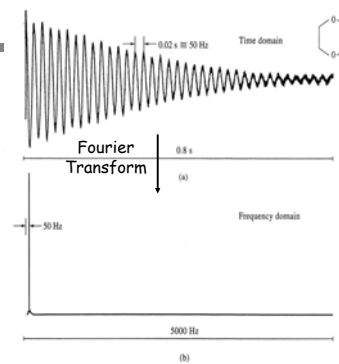
## FT-NMR: Instrumentation



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## FT-NMR: Example

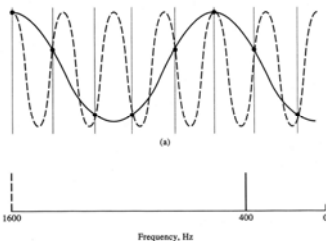
- Here, nuclei ( $^{13}\text{C}$ ) absorb at only one frequency
- The frequency of the time domain signal is the *difference* between the reference frequency and FID frequency



## Aliasing

- Why not measure the signal at the FID frequency?

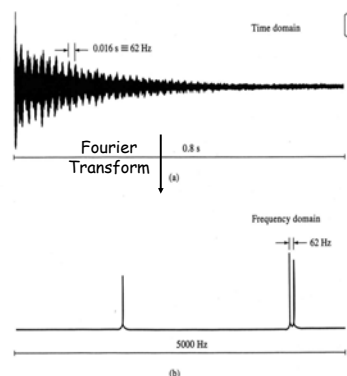
• If signal is undersampled, can detect signal at an erroneous lower frequency:



*Aliasing*

## Another Example

- Here,  $^{13}\text{C}$  nuclei absorb at *three* different frequencies
- Time domain signal shows all three FIDs simultaneously (*multiplex measurement*)



## Listen to your FID?

- Frequencies measured are in the audible sound range . . . hmmm
  - let your *brain* do the Fourier Transform:

<http://www.organik.uni-erlangen.de/research/NMR/music7.html>

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## Advantages of FT-NMR

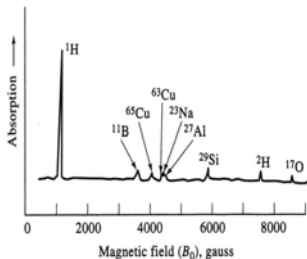
- **Multiplex Measurement gives:**
  - S/N enhancement
- **Increased  $B_0$  gives:**
  - increased signal
- Together:**
  - lower detection limits
    - Dilute samples
    - Lower abundance nuclei
  - decreased measurement time

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## Applications

### Qualitative Analysis

-seems to be an expensive, complex, relatively insensitive method of elemental analysis:



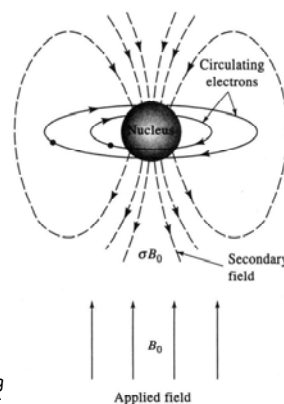
Get a single peak for each NMR active nucleus

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## The Chemical Shift

The Larmor frequency for nuclei will ALSO vary with the *chemical environment*:

Electrons *shield the nucleus* from the applied magnetic field:



$$B_o = B_{\text{appl}}(1 - \sigma)$$

Field experienced by nucleus

Shielding constant

Applied field

## The Chemical Shift: Example

So, as *shielding* ( $\sigma$ ) increases, resonance occurs at lower frequencies

Consider the  $^1\text{H}$  resonance for the following compounds:



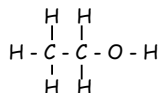
Decreasing electron density  $\longrightarrow$

$\longrightarrow$  Higher resonance frequency

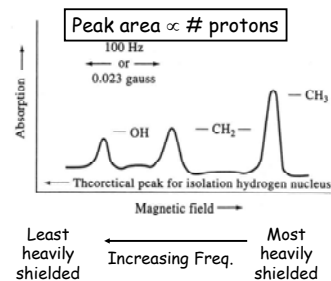
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## Example Spectrum: $^1\text{H}$

For Ethanol:



Increasing  $e^-$  density  $\longleftarrow$



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## The X-Axis: It's All Relative

- > In absolute units (freq. or magfield), x-axis values would vary from instrument to instrument
- > Calibrate relative to resonance of a standard
  - > For  $^1\text{H}$ , use *Tetramethylsilane (TMS)*
    - > Protons are more heavily shielded than most organic protons
    - > Resonance will be at *lowest* frequency
- > X-Axis calibrated with respect to relative shift from TMS:

$$\delta \text{ (ppm)} = \frac{\nu(\text{sample}) - \nu(\text{TMS})}{\nu(\text{TMS})} \times 10^6$$

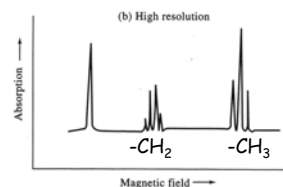
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## Spin-Spin Splitting

At higher resolution, we see that the  $-\text{CH}_2$  and  $-\text{CH}_3$  peaks are split:

Why?

-shielding is also affected by *nuclear spin* orientations of protons on adjacent carbons



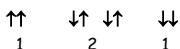
Let's look at this in more detail!

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## More Spin-Spin Splitting

### ■ Methyl Protons

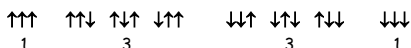
-split by the spins of the 2 adjacent *ethyl protons*:



**3 peaks**

### ■ Ethyl Protons

-split by the spins of the 3 adjacent *methyl protons*:



**4 peaks**

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## Quantifying Spin-Spin Splitting

- Spin-spin splitting is quantified by:

**J**: *Coupling Constant*

- J** is reported in *absolute units* (frequency, Hz) and is typically **1-20 Hz**
- J** depends only on the electronic and steric relationships between interacting nuclei
- Thus, **J** is *independent of spectrometer  $B_0$*

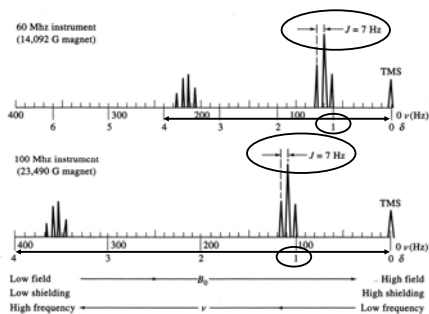
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## Effects of MagField on Spectrum?

**J**:  
unaffected

**$\delta$** :  
unaffected

**Resolution:**  
(Hz/ppm)  
 $\propto B_0$



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## Chemical Shift Trends

**$\delta$**  as an indicator of *e<sup>-</sup> density*  
-recall our first example:

	CH <sub>4</sub>	CH <sub>3</sub> Cl	CH <sub>2</sub> Cl <sub>2</sub>	CHCl <sub>3</sub>
<b><math>\delta</math></b> (ppm):	0.23	3.05	5.33	7.24

Decreasing e<sup>-</sup> density  $\rightarrow$

•But, chemical shifts do not only follow electron density ... Examples?

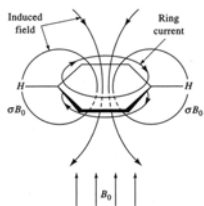
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## Other Chemical Shift Effects: Magnetic Anisotropy

### ■ Delocalized electrons

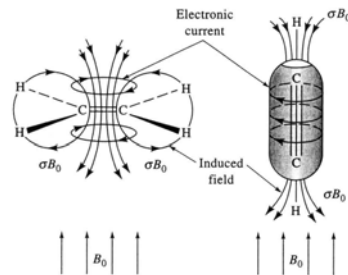
•“ring current” due to motion of electrons in delocalized  $\pi$ -orbitals can establish an *induced magnetic field* which adds to the applied field

•Aromatic protons shifted to *higher frequencies* (larger values of  $\delta$ )



## More Magnetic Anisotropy

Electrons circulate *in the plane of the bond*:  
**desields protons**



Electrons circulate *around the axis of the bond*:  
**shields protons**

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## Qualitative and Quantitative Methods

- Use *Correlation Table* to aid in assigning resonances to particular protons/functional groups
- Use spin-spin splitting patterns for structural elucidation
- For *quantitation*: integrate areas under peaks (proportional to # absorbing nuclei) and ratio to areas obtained from a sample with a *known concentration*

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## Nuclei other than $^1\text{H}$

- Must have  $I > 0$
  - Will have *decreased signal* relative to  $^1\text{H}$ 
    - Lower natural abundance
    - Lower magnetic moment ( $\mu$ )
- Examples:**
- $^{14}\text{N}$  - 99.6% N.A., but low  $\mu \rightarrow \sim 0.1\%$   $^1\text{H}$  signal
  - $^{13}\text{C}$  - 1.1% N.A., *plus* low  $\mu \rightarrow \sim 2\%$   $^1\text{H}$  signal
  - $^{19}\text{F}$  -  $\sim 100\%$  N.A., and high  $\mu \rightarrow \sim 83\%$   $^1\text{H}$  signal
- Will have *lower resonant frequencies*
    - Due to lower magnetic moments
  - Will have different chemical shift ranges and J-value magnitudes (usually larger than with  $^1\text{H}$ )

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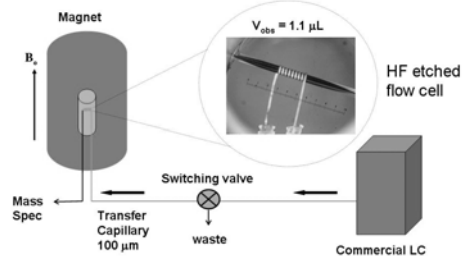
## NMR's Bleeding Edge?

### ➢ Microcoils!

- smaller coils = *improved S/N*
- Detn Limits in *ng-range*
- NMR detection for separations?
- NMR Microscopy?!

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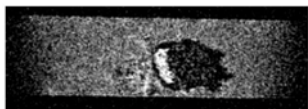
## HPLC - NMR



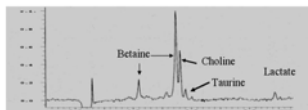
<http://mrel.bsg.uiuc.edu/microcoil/research/microcoils/>

## NMR Microscopy

• Image of L7 neuron of *Aplysia Californica*



• NMR spectrum of nucleus



<http://mrel.bsg.uiuc.edu/microcoil/research/microcoils/>

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