10: (N)MR spectroscopy

1. How can the Bloch equations be used to describe the effect of T_1 on the magnetization ?
2. How can sensitivity be optimized ?
3. What nuclear property allows to distinguish the signal from different molecules ?
4. How is chemical shift measured ?
5. What can MR spectroscopy measure ?

After this week you

1. can calculate the effect of multiple RF pulses on longitudinal magnetization
2. know the definition of Ernst angle
3. Understand the two basic mechanisms by which electrons influence the precession frequency of nuclear magnetization
4. Know the definition of chemical shift
5. Know how and under what molecular conditions NMR spectroscopy can provide non-invasive biochemical information

10-1. What is the effect of relaxation on M(t) ?

Bloch equations revisited

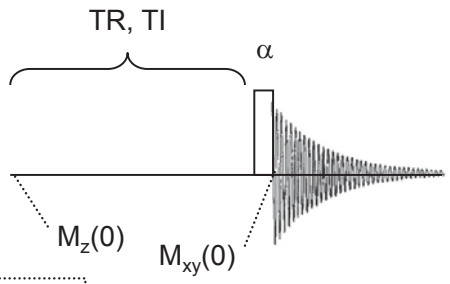
The effect of T_1 and T_2 on the signal :

T_1 T_2

flip angle $\alpha = \gamma B_1 \tau$:

$$M_z(t+\tau) = M_z(t)\cos\alpha$$

$$M_{xy}(0) = M_z(t)\sin\alpha$$



Effect of T_2 $\quad M_{xy}(t) = M_{xy}(0)e^{-\frac{t}{T_2}}$

Effect of T_1 $\quad \frac{dM_z(t)}{dt} = -\frac{M_z(t)-M_0}{T_1}$

Longitudinal coherence

$$M_z(t) = M_0(1-e^{-t/T_1}) + M_z(0)e^{-t/T_1}$$

$$M_z(0) = M_0\cos\alpha$$

$$M_z(t) = M_0(1-(1-\cos\alpha)e^{-t/T_1})$$

Longitudinal coherence:

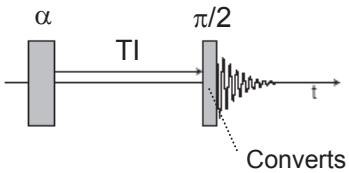
Effect of T_1 on signal depends on prior RF manipulations

What are the optimal conditions to measure T_1 ?

Inversion recovery

Inversion-Recovery

Multipulse experiment with two RF pulses
Usual experiment to measure T_1 **($\alpha=\pi$)**



Converts M_z to M_{xy} (signal)

Optimal choice of α for measuring T_1 ?

Use noise error propagation calculation (Lesson 1)

$$\frac{\partial M_z(t)}{\partial T_1} = \frac{t}{T_1^2}(1-\cos\alpha)e^{-t/T_1} \equiv F$$

$$\frac{dF}{d\alpha} = 0 = \frac{t}{T_1^2}\sin\alpha e^{-t/T_1}$$

$\Rightarrow \sin\alpha=0 \Rightarrow \alpha=\pi$

$$M_z(TI) = M_0(1-2e^{-TI/T_1})$$

Measured signal

$$M_z(TI) = M_0(1-e^{-TI/T_1}) + M_z(0)e^{-TI/T_1}$$

$$M_z(0) = M_0\cos\alpha$$

$$\rightarrow M_z(TI) = M_0(1-(1-\cos\alpha)e^{-TI/T_1})$$

Optimal t=TI to detect changes in T_1 ?

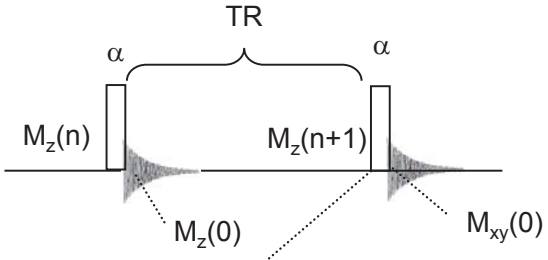
$$\frac{dF}{dt} = 0 = \frac{1}{T_1^2}(1-\cos\alpha)e^{-t/T_1} - \frac{t}{T_1^3}(1-\cos\alpha)e^{-t/T_1}$$

$$0 = \frac{1}{T_1^2}(1-\cos\alpha)e^{-t/T_1}\left(1-\frac{t}{T_1}\right)$$

TI = T_1

10-2. When is SNR (sensitivity) optimal ?

Situation: RF pulses α applied every TR seconds n times
Question: M_{xy} (=signal) maximal ?
$\Rightarrow$ Calculate the optimum ***flip angle*** α=f(TR)



$$M_z(t) = M_0(1-e^{-t/T_1}) + M_z(0)e^{-t/T_1}$$

Immediately after nth TR: $M_z = M_z(n)$

Assume $M_{xy}=M_\perp\sim0$ after TR seconds

After RF Flip α $M_z(0) = M_z(n)\cos\alpha$

After T_1 recovery $M_z(n+1) = M_0 - [M_0 - M_z(n)\cos\alpha]e^{-TR/T_1} = M_0[1-e^{-TR/T_1}] + M_z(n)\cos\alpha e^{-TR/T_1}$

In equilibrium (steady-state condition):
$M_z(n+1)=M_z(n)=M_z$

$$M_z(1-\cos\alpha\cdot e^{-TR/T_1}) = M_0(1-e^{-TR/T_1})$$

$$\Rightarrow M_z = \frac{M_0(1-e^{-TR/T_1})}{1-\cos\alpha\cdot e^{-TR/T_1}}$$

Equilibrium *transverse* Magnetization:
$M_{xy}(0)=M_z\sin\alpha$

$$M_{xy} = \frac{M_0(1-e^{-TR/T_1})\sin\alpha}{1-\cos\alpha\cdot e^{-TR/T_1}}$$

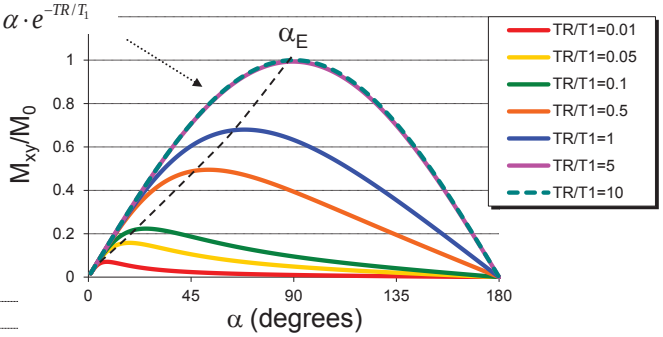
How does the signal depend on TR, T_1 and flip angle ?

Ernst Angle α_E

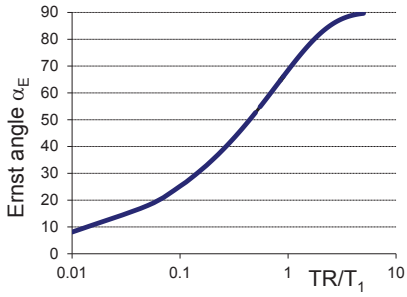
Richard Ernst
Physical Chemist
1991

$$M_{xy} = \frac{M_0\left(1-e^{-TR/T_1}\right)\sin\alpha}{1-\cos\alpha\cdot e^{-TR/T_1}}$$

Signal vs. Flip Angle α



Ernst Angle vs TR/T_1



M_{xy} (signal) → maximum at α_E

$dM_{xy}/d\alpha=0$: $\cos\alpha_E = e^{-TR/T_1}$

Where α_E = Ernst Angle

10-3. What role does the chemical environment play?

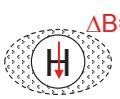
Chemical shift: Effect of B_0 on e-cloud

H
Proton: nucleus of ^{1}H

H
Proton + e cloud: ^{1}H atom

Resonance frequency of the pure proton: $\omega_L=\gamma B_0$ (Larmor frequency)

B_0



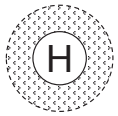
$$\frac{d}{dt}\vec{M} = \vec{M}\times\gamma(1+\delta)\vec{B}_0$$

Reorientation of e cloud in magnetic field:
e produce a small magnetic field ΔB at the proton: $\omega=\omega_L(1+\delta)$

Chemical shift δ

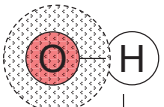
Nearby *electronegative* atoms (e.g. O, Cl):
attract electrons
→ lower electron density
→ *deshielding* of nearby H

H



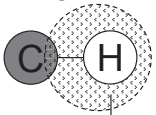
Hydrogen e$^-$

OH



Little shielding

CH



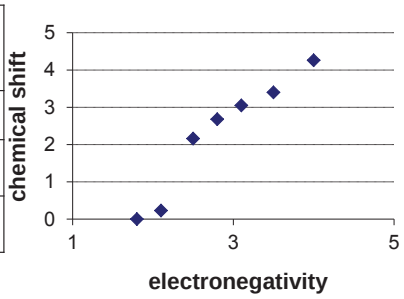
More shielding

→ Resonance frequency is higher in OH than CH

How is chemical shift δ linked to electronegativity ?

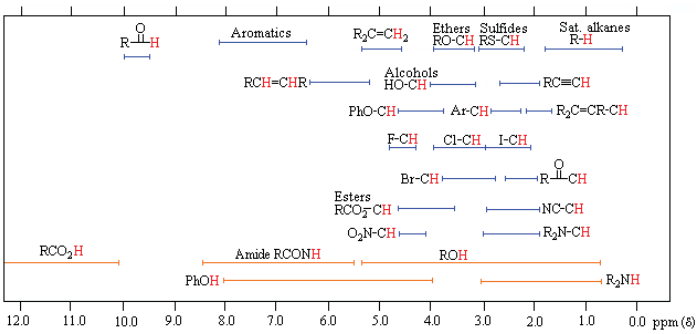
Example: Protons

Compound, CH_3X	CH_3F	CH_3OH	CH_3Cl	CH_3Br	CH_3I	CH_4	$(CH_3)_4Si$
X	F	O	Cl	Br	I	H	Si
Electronegativity of X	4.0	3.5	3.1	2.8	2.5	2.1	1.8
Chemical shift, δ / ppm	4.26	3.4	3.05	2.68	2.16	0.23	0



0 ppm is defined by resonance frequency of reference compound ω_L^{ref}
(e.g. tetramethylsilane (TMS) for ^1H)

$$\delta=(\omega-\omega_L^{ref})10^6/\omega_L^{ref}$$

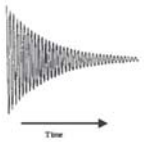


10-4. How can we measure chemical shift ?

MR spectroscopy

Free induction decay (FID) signal:

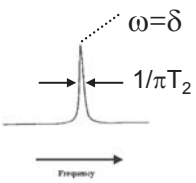
$$S(t) \propto M_{\perp}(0)e^{-i\omega t}e^{-t/T2}$$



distinguish resonance frequency
→ Fourier transformation (real part only):

$$G(\omega) \propto M_{\perp}(0)\frac{1}{(1+x^2)}$$

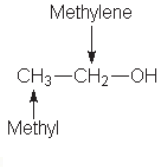
$$x \equiv (\omega-\delta)2\pi T_2$$

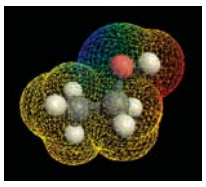


Area of resonance ∝ M(0) ∝ number of nuclei ∝ concentration (if relaxation can be neglected)

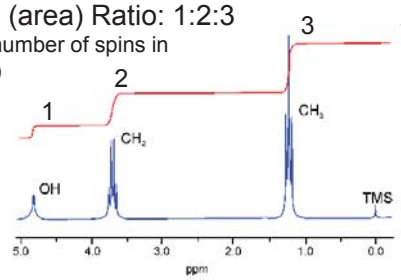
$$S(0) \propto \int_{-\infty}^{\infty} G(\omega)e^{i\omega t}d\omega\Big|_{t=0} = \int_{-\infty}^{\infty} G(\omega)d\omega \propto M_0$$

Example: Ethanol





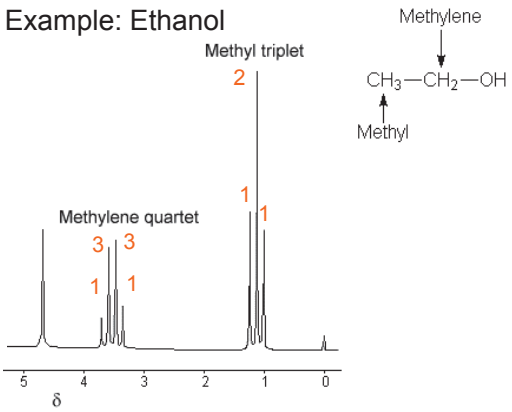
Integral (area) Ratio: 1:2:3
(relative number of spins in molecule)



Ex. illustration of chemical proximity

(triplet & quartet)

Example: Ethanol



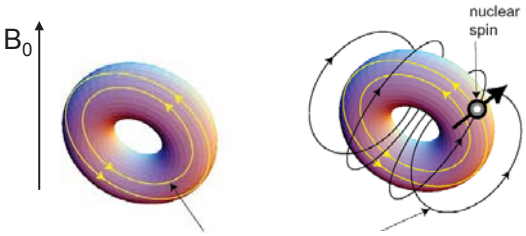
Nearby spin-1/2: ^{1}H resonance will split into two of equal magnitude (doublet)

Hyperfine splitting

nucleus ⇒ tiny magnetic field linked to its dipole:

changes polarity if spin is "up" or "down"

⇒ affects the e cloud in the molecule→ alters the magnetic field at a nearby nucleus:



CH_2 group → four combinations (with equal probability):

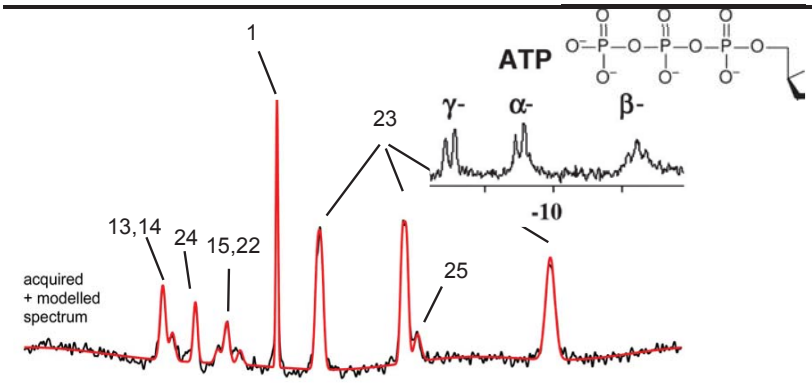
up-up, down-down, up-down, down-up

(The latter two produce the same magnetic field)

→ methyl **triplet** (relative intensity ratio **1:2:1**)[10-11]

Ex. ^{31}P NMR spectroscopy

Phosphate metabolism is at the heart of cellular energetics



1: phosphocreatine (PCr)
13: phospho-ethanolamine (PE)
14: phosphocholine (PC)
15: glycerophosphocholine (GPC)
22: glycerophosphoethanolamie(GPE)
23: ATP
24: inorganic phosphate (Pi)
25:dinucleotides (NAD(P)[H])

Also measured:
Intracellular pH
Creatine kinase activity
ATPase activity

10-5. What can MR spectroscopy measure ?

Concentration of biochemical compounds
- signal is proportional to the number of spins present, i.e. concentration
  After FT, integrate (measure the area of the peak).

Rules for a compound to be detectable:
1. Concentration > 1mM
2. Water-soluble compounds (mobile)
3. ^{1}H is most sensitive nucleus (gyromagnetic ratio)

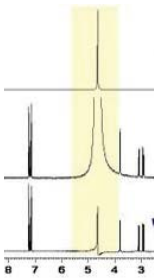
Spatial Resolution
Voxel volume ~ 1/Signal

Water (80M ^{1}H concentration)	Biochemical compounds (~mM concentration)
~1mm (human)	~ cm (human)
~50µm (rodent)	~ mm (rodent)

Why is spatial resolution better for rodent studies ?

Induced emf ζ depends on RF coil size (Lesson 9)

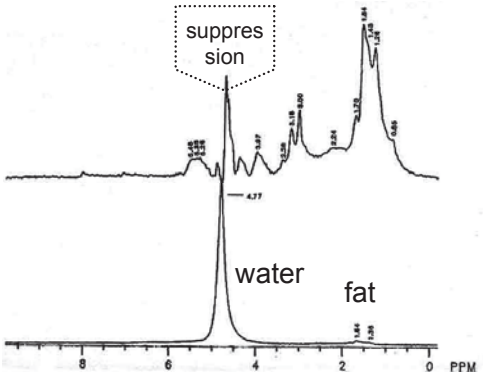
How can the huge water signal be suppressed in ^{1}H NMR ?



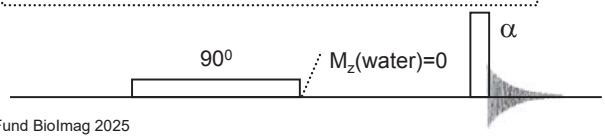
Full signal (no suppression)

Scaled signal (no suppression)

Scaled signal (with suppression)

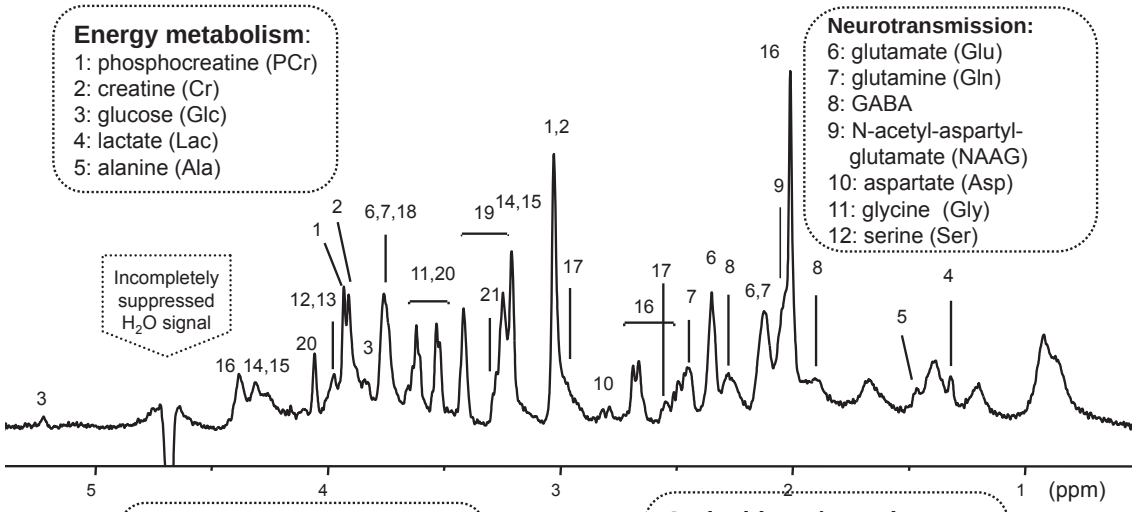


NB. Resonance suppression:
1. Minimize M_z : "selective" 90^0 pulse applied on-resonance on the signal to be suppressed.
2. Selectivity achieved by using weak B_1 (lecture 9), i.e. long RF pulse.
3. 90^0 (selective) followed by α^0 for excitation and detection [assume the suppressed signal is dephased (see Lecture 11)]



Ex. Proton spectroscopy of the brain

Biochemical compounds detectable in vivo



Membrane metabolism:
13: phospho-ethanolamine (PE)
14: phosphocholine (PC)
15: glycerophosphocholine (GPC)
16: N-acetyl-aspartate (NAA)

Antioxidants/osmolytes:
17: glutathione (GSH)
18: vitamin C (Asc)
19: taurine (Tau)
20: myo-inositol (Ins)
21: scyllo-inositol (s-Ins)

How can biochemical compounds be measured in vivo ?

Analysis of ^{1}H NMR spectroscopy of the brain