Introduction to 1D and 2D NMR Spectroscopy

(3) Diffusion experiments

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March 2023

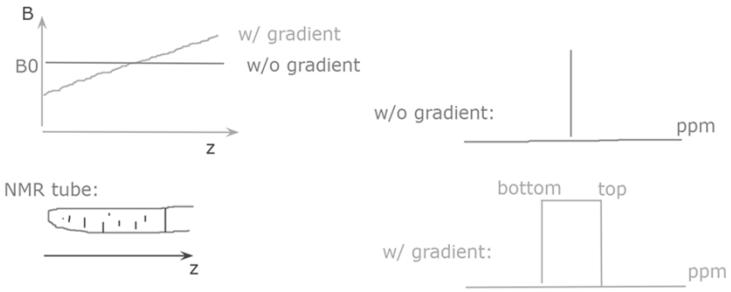
1

Objectives of this section

- Work with gradient pulses
- Work with a more complex experiment
- Learn ways to obtain high quality NMR data
  - Proper choice of acquisition parameters
  - Very careful phase and baseline corrections
- Learn a method to probe a physical attribute of your molecule/assembly

2

A Simple MRI



$$\omega = \gamma B$$

3

3

Pulsed Field Gradients (PFG) in Modern NMR

- Gradient is nothing but the “evil” that you are trying to get rid of during shimming.
- Gradient pulses: magnetic field gradients applied for short durations, usually 0.5 to 2.5 ms; used along with RF pulses.
- Applications:
  - MRI
  - Topshim: automated shimming by applying pulsed gradients
  - Remove artifacts in many modern 2D experiments
  - Detect diffusion of molecules/assemblies

4

4

Diffusion NMR

- NMR can detect the diffusion coefficients of molecules
  - The spatial location of the molecule is detected by applying a magnetic field gradient (Use Larmor Equation to explain why)
- Don't be overwhelmed by names – these are all the same thing:
  - DOSY (Diffusion Ordered SpectroscopY)
  - PFG (Pulsed Field Gradient) NMR
  - PGSE (Pulsed Gradient Stimulated Echo) NMR
  - Diffusion NMR
- Good at detecting diffusion of small molecules and aggregates (R_H <= 20 nm)
  - Complementary with dynamic light scattering (DLS)

5

5

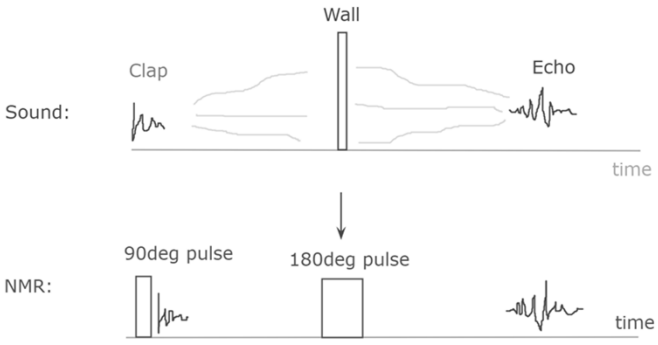
Principle of Diffusion NMR

- Gradient pulses
  - Positive and negative gradients
  - Marks the spatial location of molecules
- Radio frequency (RF) pulses
  - The "echo" technique
  - "Wraps up" the spatial location information so that molecules can carry it to different locations by diffusion

6

6

Echo



A sound echo

an NMR echo

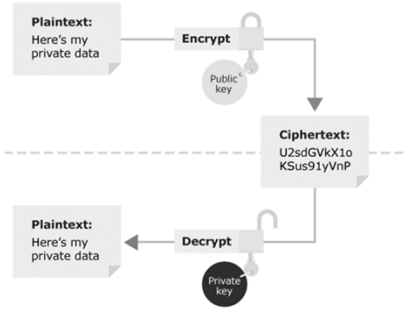
- A wall turns a sound wave by 180°, just like what a 180° pulse does to NMR signal

7

7

Using ***Echo*** to Carry Location Information to Different Places

- Echo: a signal that is scrambled in the middle but reassembled at a later point
- We can use echoes to carry certain information



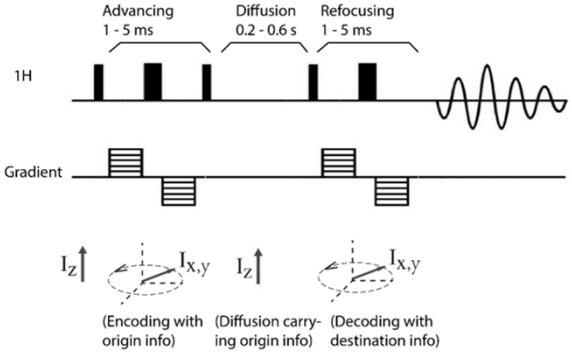
Credit: ico.org.uk

8

8

Principle of Diffusion NMR

- Advancing period: a coherence is <u>scrambled</u>/<u>encrypted</u> with location info as key
  - Location is defined by applied gradient
- The encrypted signal is then flipped back to z direction (by the 2nd 90deg pulse) to allow diffusion to occur
- Refocusing period: the coherence is <u>decrypted</u> with location info as key

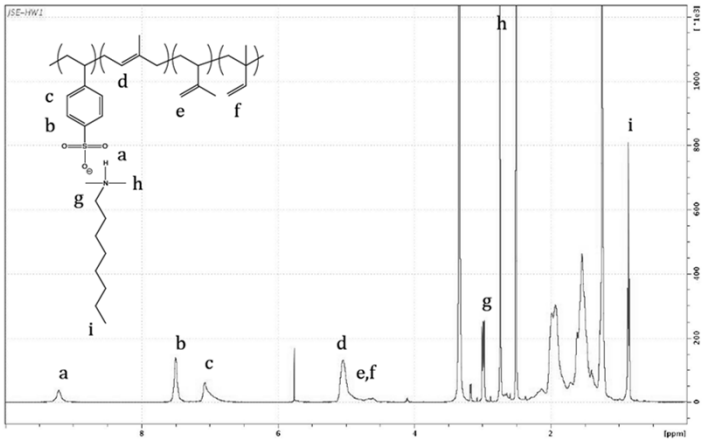


- If no gradient, same key for both periods ➔ *perfect echo (maximum intensity)*
  - regardless of molecules diffusing or not
- If the molecules do not diffuse, same key for both periods ➔ *perfect echo*
  - regardless of gradient
- If the molecules diffuse away and a gradient is present ➔ *<u>a smaller echo</u>*

9

9

Is the Ionic Complexation Tight or Loose?

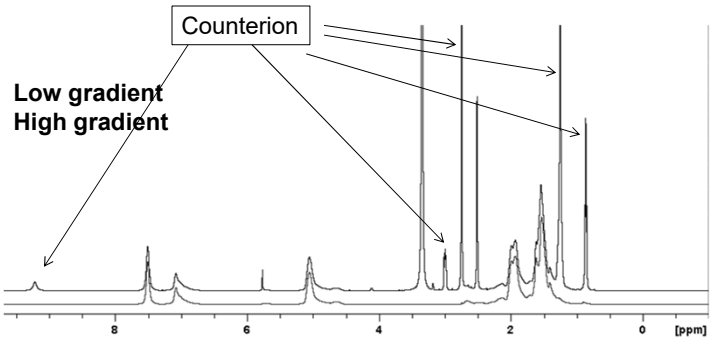


- Solvent: DMSO

10

10

Diffusion Behavior Can Tell!



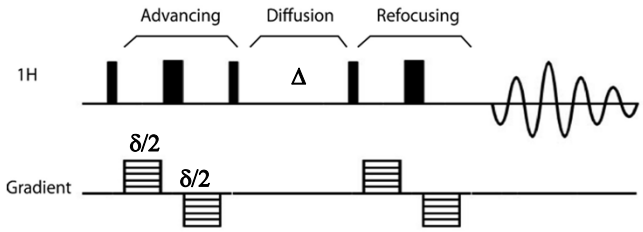
- Counterion diffuses much faster than the polymer
- The ionic association is quite loose even in DMSO
- You can easily tell whether a peak belongs to a large or a small molecule using the same technique

11

11

How to Run DOSY

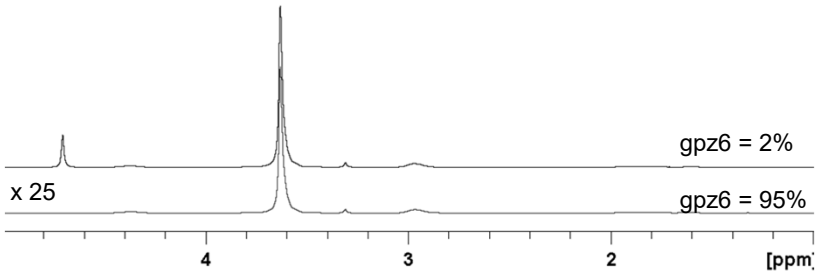
- Parameters to adjust/optimize before you run
  - Calibration of 90° pulse length.
  - Gradient pulse duration ("small delta, δ"): usually 500-2500 μs
  - Diffusion time ("big delta, Δ"): usually 0.2-0.6 s
  - Longer gradient pulse and longer diffusion time ➔ stronger attenuation
- Trial 1D runs with minimum (2-5%) and maximum (95%) gradient power
  - Goal: adjust small delta and big delta such that A_{max}/A_{min} = 1 – 5%
- Parameter to increment (variable) during the run
  - Gradient pulse amplitude (in unit of percentage; 100% is the maximum), to increment between min and max



12

12

Example: A 1D Trial for PEO in D_2O



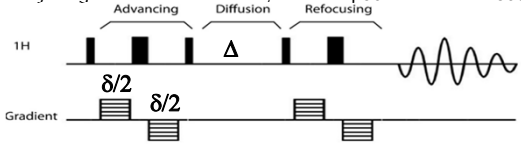
- PEO signal decays to ca. 4% of original at high gradient
- Water signal decays much faster than PEO
- Proper choice of a decay range for your experiments:
  - A single component decay: 1 – 1.5 decades (A_{max}/A_{min} = 1 – 5%)
  - A multi-component decay: 2 – 3 decades (A_{max}/A_{min} = 0.1 – 1%)
    - e.g. a polydisperse polymer

13

13

How to Choose Your Parameters

- Recycle delay (d1)
  - For quantitative 1D studies, should be 4 – 5 x T1
  - For diffusion studies, 3 x T1 is probably good enough
- Gradient Pulse duration (small delta)
  - Should be always <= 2500 μs
  - If you set it too long (> 2500 μs) by mistake, you might burn the amplifier!
- Diffusion time (big delta)
  - T1 relaxation occurs during diffusion period, decaying from full amp to 0
  - If diffusion time is set too long, you will get less signal due to T_1 relaxation
    - Big delta < ½ x T_1 is recommended
- Usually:
  - First, use default small delta (p30) and adjust big delta (d20) between 0.05 s and 0.5 s to achieve desired decay range
  - If desired decay range cannot be achieved, increase p30 to a max of 2500 us



14

14

Data Processing

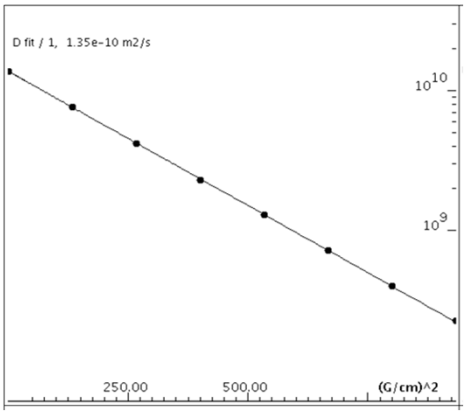
- For a monodisperse object:

$$I = I_0 \, e^{-D\gamma^2 g^2 \delta^2 (\Delta - \delta/3)}$$

- I: peak intensity;
- D: diffusion coefficient;
- g: gradient amplitude
- Log(I) vs g^2 curve would be a straight line for a single component

15

Example: A Monodisperse Polystyrene



2% 95%

- Relative stdev for this fit < 1%
  - Generally, relative stdev of < 2% can be easily achieved
- From D, hydrodynamic radius of your object can be calculated from the Stokes-Einstein Equation:

$$D = \frac{k_B T}{6\pi \eta r}$$

16

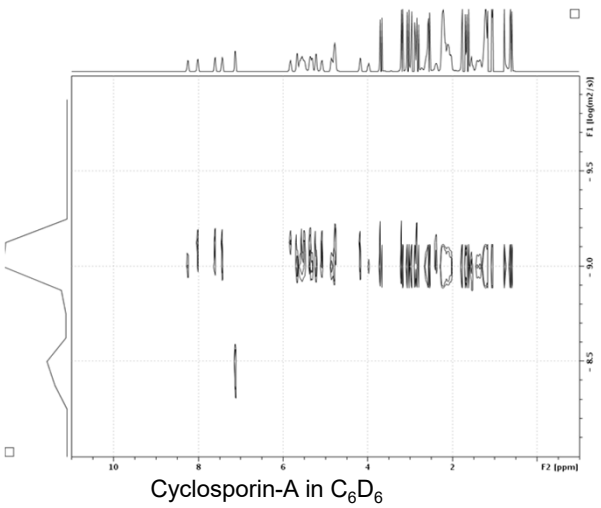
Convection

- gives the same effect to signal intensity as diffusion
- Factors that contribute to convection:
  - Low viscosity (acetone, chloroform)
  - higher T
  - wider tube cross section
  - Long sample height (which results in high temperature gradient)
    - 2.5cm sample is recommended for CDCl3 or acetone-d6
- Sign of convection:
  - increasing D with longer big delta
  - Polymers and large particles seem to “diffuse” as fast as small molecules

17

17

DOSY



Cyclosporin-A in C_6D_6

Vertical Axis is Diffusion Coeff.

Signals belonging to the same molecule are on the same row

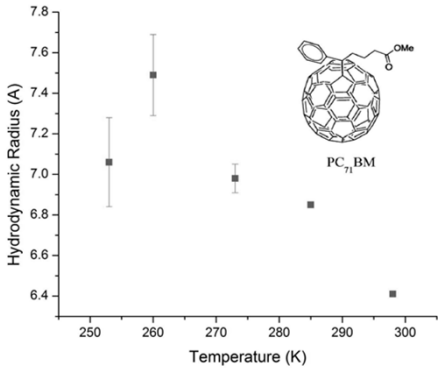
A good way to separate mixtures!

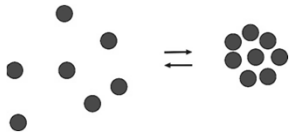
18

18

Transient Aggregation

- PCBM aggregates in solution?
- Diffusion NMR curve fit only finds one component
- R_H decreases with increasing T, why?
- Likely: PCBM spends time in both monomeric and aggregated states, and NMR detects a weighted average





19

19

Applications

- Measure D (and thus R_H (hydrodynamic radius)) of your target
  - Each peak on your ^1H spectrum can get its own D
- Determine molecular weight of polymers
  - Molecular weight distribution is possible
  - Any solvent is OK
- Separate mixture and remove impurity signals
- Probe interactions in solution: complexation; aggregation; dimerization; etc.
- Study single-molecule/assembly dynamic equilibrium

20

20