1D Qualitative Experiments: Various Nuclei, DEPT, INEPT

Rose Dykes, Aoife O'Donnell, Jennifer Lennox
Almac Sciences, 20 Seagoe Industrial Estate, Craigavon, Northern Ireland, BT63 5QD

Fundamentals of 1D Qualitative NMR

- In the presence of a magnetic field, nuclei with spins greater than zero have an overall nuclear magnetic dipole moment which allows for energy levels to be split. In NMR, a radiofrequency pulse is applied so that the nuclei will absorb energy to reach a higher energy level, flipping their spin to oppose the magnetic field.
- Identification (ID) tests can confirm the structure as well as the presence of impurities and/or residual solvents.
- IDs can also be used to support data for quantitative experiments and have many pharmaceutical applications such as API release, stability studies, reference standard testing, and early phase development.
- Most applications will require ^{1}H NMR and/or ^{13}C NMR to confirm structure due to the abundance of carbon and hydrogen in organic molecules, but these are far from the only nuclei that can be analysed using this technique.

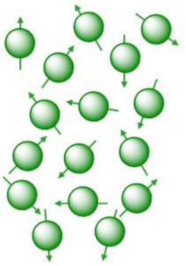

Figure 1: Spin-½ nuclei in the absence of an external magnetic field

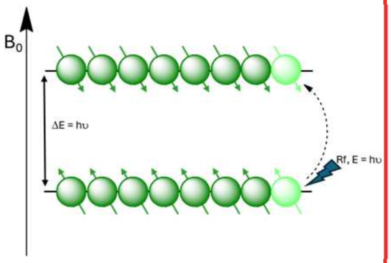

Figure 2: Spin-½ nuclei align either with (lower energy) or against (higher energy) an external magnetic field (B_0). When an Rf pulse is applied, it can induce the spin of a nucleus to flip orientation and move to a higher energy level.

Confirmation of Structure *via* ^{1}H NMR

Splitting pattern (multiplicity), integration and chemical shift can be assessed against a reference spectrum or chemical structure to confirm identity.

Integration

- The area under a peak is proportional to the relative number of nuclei giving rise to the signal.

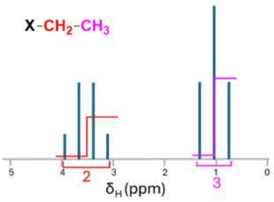

Figure 3: Integration of ^{1}H NMR spectrum

- The precise resonant frequency of the energy transition is dependent on the effective magnetic field at the nucleus.
- This field is affected by electron shielding which is in turn dependent on the chemical environment. This is called chemical shift (δ) and as a result, can give information about the chemical environment of the nucleus
- In general, the more electronegative the nucleus is, the higher the resonant frequency.

Chemical Shift

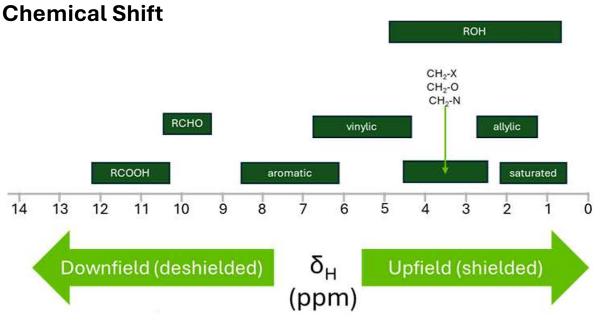

Figure 4: Diagram of ^{1}H chemical shift ranges for various functional groups

Multiplicity

- The effective magnetic field is also affected by the orientation of neighbouring nuclei that are chemically different.
  - This effect is known as spin-spin coupling which can cause splitting of the signal for each type of nucleus into two or more lines.
    - Peak splitting can give information on the connectivity of atoms in the molecule
- In general, neighbouring NMR active nuclei three or fewer bonds away lead to this splitting.
  - The splitting is described by the relationship where n neighbouring nuclei result in n+1 peaks

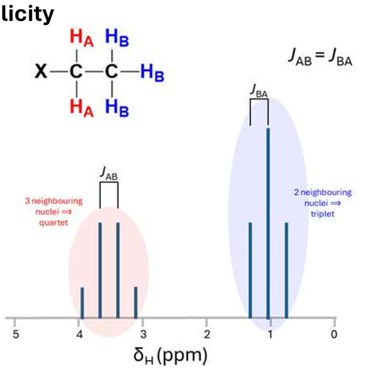

Figure 5: Multiplicity and coupling constants in ^{1}H NMR spectrum of an ethylhalide

- Coupling constants (J) are the difference (in Hz) between different peaks of a multiplet
- The coupling constant for many common pairs of nuclei are known can help when interpreting spectra.
- When two multiplets have the same coupling constant, the signals arise from two coupled nuclei.

DEPT - Distortionless Enhancement by Polarisation Transfer

- To aid in the assignment of ^{13}C NMR spectra, different DEPT experiments can be carried out
  - Differentiates carbons based on the number of protons bonded to it.
  - Signals are in-phase, out-of-phase, or absent depending on the type of DEPT experiment ran

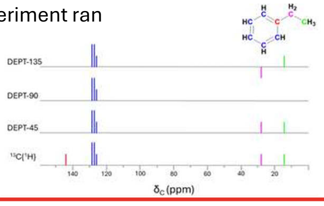

Figure 6: Diagram showing differences in signal patterns between DEPT-45, DEPT-90 and DEPT-135

INEPT – Insensitive Nuclei Enhanced by Polarisation Transfer

- Some spin-**½** nuclei, such as ^{13}C and ^{15}N, are less sensitive than ^{1}H due to lower natural abundance, and having a lower gyromagnetic ratio
- INEPT enhances the NMR signals for these insensitive nuclei by using J-coupling to pass polarisation from one more sensitive nucleus (e.g. ^{1}H) to s to another.

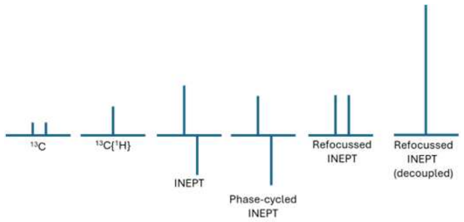

Figure 7: Diagram Showing Different Types of INEPT and the Expected Output