Subject: Analytical Instrumentation
Subject code: BT 808

Unit-5

Nuclear Magnetic Resonance Spectroscopy

3.4. Double Resonance Techniques

In Section 18.3.2.2 reference is made to the use of proton noise (or broadband) decoupling to simplify ^{13}C spectra and improve the signal-to-noise ratio. This is just one example of a large number of double resonance techniques which are available to either simplify spectra or as spectral interpretation tools to aid structure elucidation.

3.4.1. Homonuclear Spin Decoupling

The principles of homonuclear double resonance can be illustrated by considering two coupled protons. A and B, as shown in Figure 11. In the double resonance experiment nucleus A is observed in the usual way in a CW or FT experiment whilst simultaneously selectively irradiating nucleus B with a second much stronger RF field at

the "decoupling" frequency. This irradiation induces transitions between the two spin states of nucleus B. If sufficient irradiating power is applied, B flips between the α and β spin states so rapidly that nucleus A can no longer distinguish

between the two orientations of B and perceives an average orientation. The coupling J_{AB} disappears and the A signal doublet collapses to give a single line. In the same way irradiation of an arbitrary signal in a complex NMR spectrum results in the obvious collapsing of all multiplets which are coupled with the irradiated proton. At time before 2D NMR was feasible, this method was often used to prove the connectivities between different protons in a molecule.

3.4.2. Heteronuclear Spin Decoupling

In the case of heteronuclear spin decoupling the decoupled and observed nuclei are of different chemical types. The experiments are illustrated with examples in which protons are decoupled and the effect observed on the ^{13}C spectrum. However the methodologies are equally applicable to other heteronuclear spin systems.

3.4.3. NOE Difference Spectroscopy

The phenomenon of nuclear Overhauser enhancement was introduced in Section 18.3.2.3 to explain the increase in intensity of ^{13}C peaks when broadband proton decoupling is used. In general terms the NOE is defined as the change in signal intensity of a nucleus when a second nucleus is irradiated. It is beyond the scope of this article to give a theoretical treatment of the origin of the effect, which is caused by through space nuclear relaxation by dipolar interaction. Among other factors the magnitude of the enhancement has a $1/r^6$ dependence on the distance between the two nuclei. Therefore, homonuclear NOEs between protons have been used extensively to obtain information about internuclear distances and thereby to distinguish between possible structures.

The most convenient way of observing such enhancements, which rarely exceed 20% in the most favorable cases and are sometimes < 1%, is by a technique known as NOE difference spectroscopy. In such an experiment two spectra are recorded. The first is a conventional ^1H spec-trum, while in the second a chosen resonance is selectively irradiated during the interpulse delay of an FT experiment. The second spectrum is then subtracted from the first to give a "difference" spectrum.
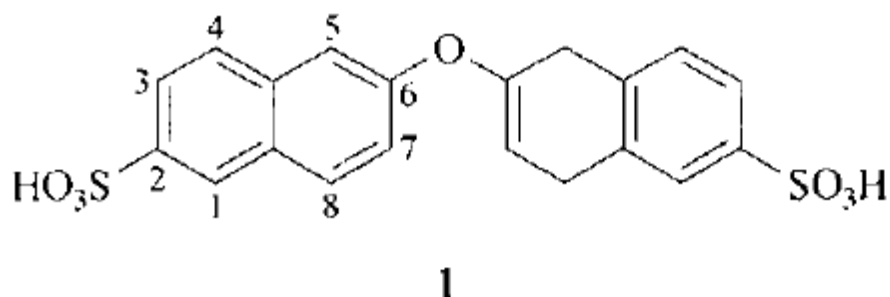
1

Figure 21 A shows the ^{1}H NMR spectrum for **1**. From this spectrum alone it is not obvious whether the ether linkage is at C-6 or C-7. The peak at 8.2 ppm is clearly due to H-1 whilst that at ca. 8.0 ppm is from H-5 or H-8, depending on which isomer is present. Figure 21 B shows the difference spectrum obtained when the resonance at 8.0 ppm is selectively irradiated. The irradiated resonance, which is partially or fully saturated, gives a negative signal in the difference spectrum. Positive signals are obtained from those resonances which exhibit an enhancement. Since a positive enhancement is observed for H-1 this is a good indication that H-1 and H-8 are close together. Measurable effects can be observed between atoms up to about 0.4 nm apart. Since the above description is rather simplistic it should be stressed that because dipolar relaxation of a particular nucleus may involve interaction with more than one other nucleus a basic understanding of the processes involved is a prerequisite to applying

the technique successfully. Also spin-spin coupling may complicate NOE measurements. If irradiation is applied to spin multiplets, NOE measurements may fail if selective population transfer is present and special precautions have to be taken in such cases. Furthermore, the NOE effect depends on the molecular correlation time τ. During slow molecular motions frequencies dominate the relaxation process. For macromolecules or small molecules in viscous solvents the NOE may completely vanish or become negative. A positive NOE effect is observable, therefore, only under condition $\omega\tau \ll 1$, which is always true for small
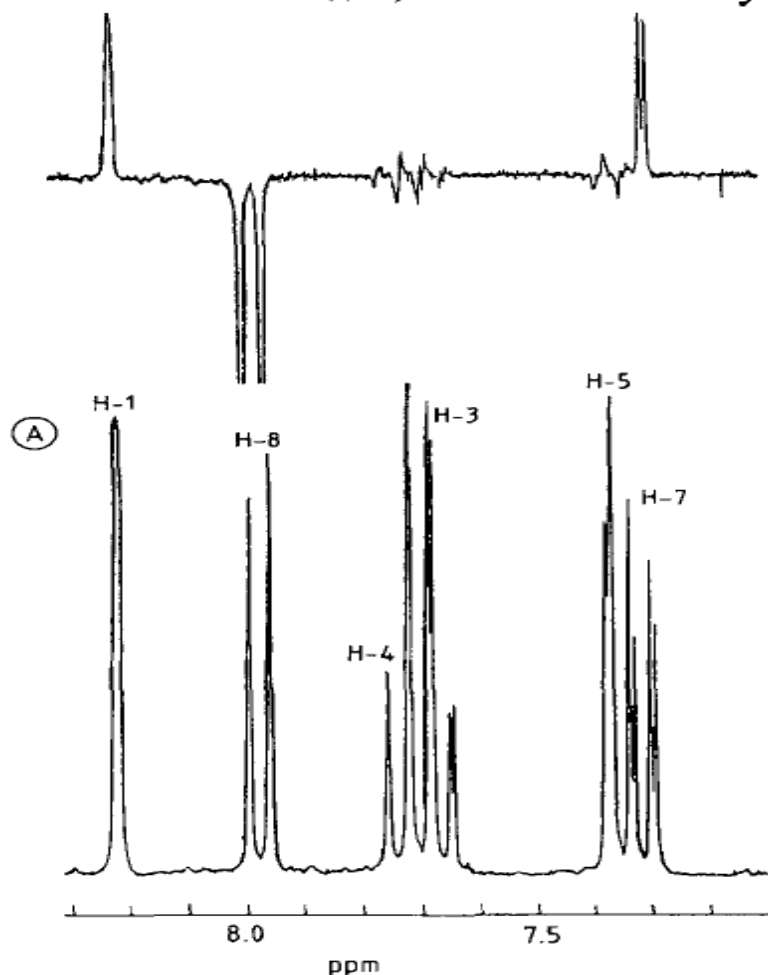

Figure 21. (A) 250 MHz ^{1}H NMR spectrum of 1; (B) NOE difference spectrum on irradiation of H-8

and isotropic molecules of low molecular weight compounds ($M_r < 500$) in solutions of low viscosity. In the last years the two-dimensional NMR technique has been used more frequently for measurement of the NOE effect (NOESY spectroscopy, see Section 18.3.6.5).

3.5. One-Dimensional Multi-Pulse FT Experiments

The FT experiment described in Section 18.3.1.2. and depicted in Figure 7 is a single-pulse experiment. This means that only one RF pulse is applied prior to recording the FID response. A large number of experiments have been developed, for a variety of purposes, in which more

than one pulse is applied prior to recording the FID. One of these, the inversion recovery method of determining T_1, is described below in some detail. Others will be mentioned only in so far as their application is concerned.

3.5.1. T_1 Measurement

The most common method of measuring T_1 is the inversion recovery experiment, which uses the pulse sequence: $[T_d - 180° - \tau - 90° \, (\text{FID})]_n$. The principle is pictured in Figure 22. Whereas a 90° pulse rotates the magnetization vector, which at equilibrium is M_{z0} and lies along the z-axis (Fig. 22 B), to the y-axis of the rotating frame, a 180° pulse which is twice as long inverts the magnetization to the $-z$-axis. Immediately after the pulse the magnetization vector M_z begins to relax back to its equilibrium value M_{z0} according to the following equation:

$$M_z = M_{z0}[1 - 2\exp(-\tau/T_1)] \qquad (2)$$

With time, M_z becomes less negative, passes through zero, and eventually relaxes back to M_{z0}. This process proceeds at a different rate for each peak in a spectrum. At a time τ after the 180° pulse, a 90° pulse is applied which tips the magnetization vector onto the $-y$- or $+y$-axis, depending on whether M_z was negative or positive. The delay T_d required before the sequence can be repeated should be at least five times the longest T_1 being measured to enable the system to relax completely. As in the case of the single pulse experiment the sequence is repeated n times and the FID added together. The resulting FID can then be transformed in the usual way. The sign and the amplitude of the resulting peaks depends on the length of the interpulse delay τ and T_1 for each
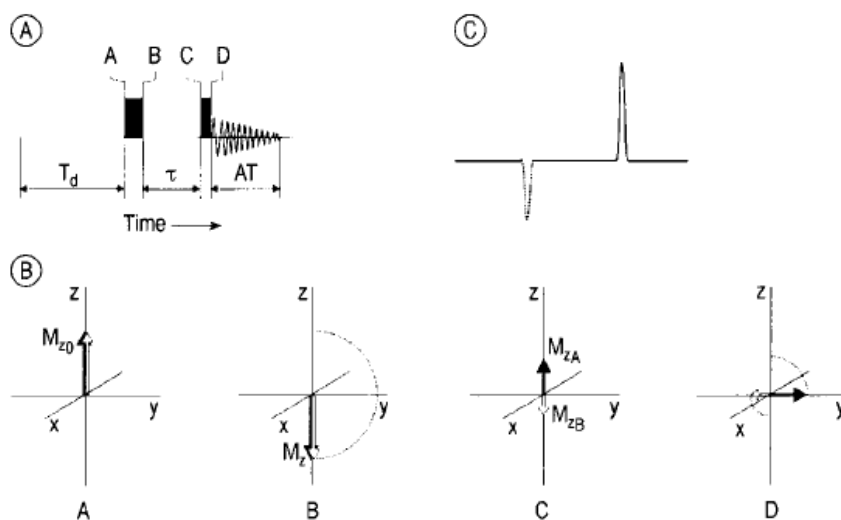

Figure 22. The principle of the inversion-recovery experiment for T_1 measurement: Puls sequence (A) and position of the macroscopic magnetization M_z (B) during the experiment at four selected points (A-D), shown for two nuclei A and B with different T_1 values, resulting in the spectrum (C)
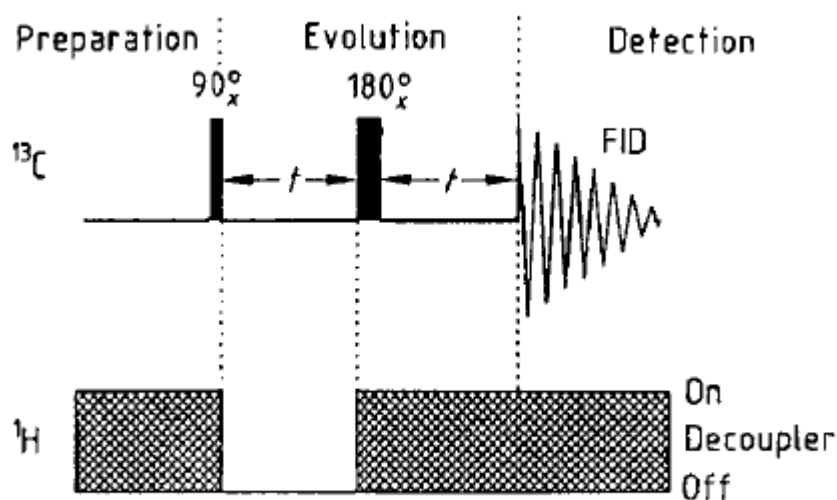
Figure 23. J-modulated spin-echo pulse sequence

3.5.2. T_2 Measurement

It is much more difficult to measure T_2 than T_1. One of the main reasons is that imhomogeneities in the magnetic field can make a significant contribution to the apparent spin–spin relaxation time. Complex, so-called spin-echo pulse sequences [27], [28] have been developed for such measurements.