5.2: Basic NMR Excite-Record Experiment and Related Spectrum

In this Chapter, we will consider the most basic type of NMR experiment: acquiring a 1D data for a spin-½ sample pre-equilibrated in an external, static magnetic field. We will see how the properties of the NMR setup (e.g., static magnet power B_o) and of the target system (e.g. the gyromagnetic ratio value γ) are affecting the obtained spectra. Finally, we will briefly explain why we are seeing spectra at all, that is why nuclei of the same chemical type (and thus γ value) report different NMR frequencies.

Learning Objectives

- Distinguish the three basic stages of a 1D NMR experiment (equilibration, excitation pulse, recording FID) and understand their purpose
- Get the basic idea of an excitation pulse of alternating B_1 field and of its roles in NMR experiments (system excitation)
- Develop understanding between the NMR resonance frequency of a spin-½ nuclei of a certain chemical type and magnetic field strength $\boldsymbol{B_0}$
- Understand why we are getting a spectrum with multiple lines instead of just one single peak from all the nuclei of the same type (the concept of shielding/de-shielding and B_{eff})

The Basic One-Dimensional NMR experimental scheme for a spin-½ system

Figure V.2.1 shows the three main stages of a basic NMR experiment used to record a one-dimensional or 1D spectrum for a spin-½ sample (e.g. ^{1}H, ^{13}C, ^{31}P, ^{19}F etc). The three main stages of the experiment are (1) equilibration, (2) excitation and (3) recording. Let's consider each one in some detail.

During the first stage, the sample (spin-½) system is operating solely under the influence of a constant magnetic field B_o, oriented along the long axis of the main magnet (Z axis). As described in the previous section (Chapter V.1), the spin population is split into two subgroups (the Zeeman splitting) with their distinct energy values. According to the Boltzmann distribution formula (Chapter I.5 , Chapter V.1), the population of the lower-energy state is greater than the population of the higher energy state, which creates an overall excess of the lower-energy state spins and population imbalance.

Stage (2) is designed to perturb the spin system out of the Boltzmann equilibrium established during the initial stage of the experiment. This is typically achieved by applying the second magnetic field (called B_1) oriented in the direction perpendicular to B_o (magnetic fields are vector quantities, they have a direction!). The parameters of B_1 field (e.g. its orientation, power frequency and duration of acting) are chosen in such a way as to "excite" a substantial fraction of the lower-energy spins to the higher energy level. **Figure V.2.1** demonstrates this by showing more spin arrows located on the higher energy level at the expense of the lower-energy state. Once "excited", the system has a new, non-Boltzmann distribution of populations at the two energy levels.

The last, third stage of the experiment starts at the moment when B_1 field is turned off. At this stage, the spin system is operating again under the influence of B_o only, just like during stage (1), but the system is out of equilibrium at the start. Thus the spins start "flipping" (changing energy levels stochastically) until the system restores its Boltzmann populations at the two energy levels. During this process of equilibration and spin "flipping" the magnetic field of the sample is changing ("flipping" spins are little "flipping" magnets!). According to the laws of electricity and magnetism, changing magnetic field will create alternating electric current in the nearby electric circuits. This very current is being detected as the raw NMR "signal" or Free Induction Decay (FID).

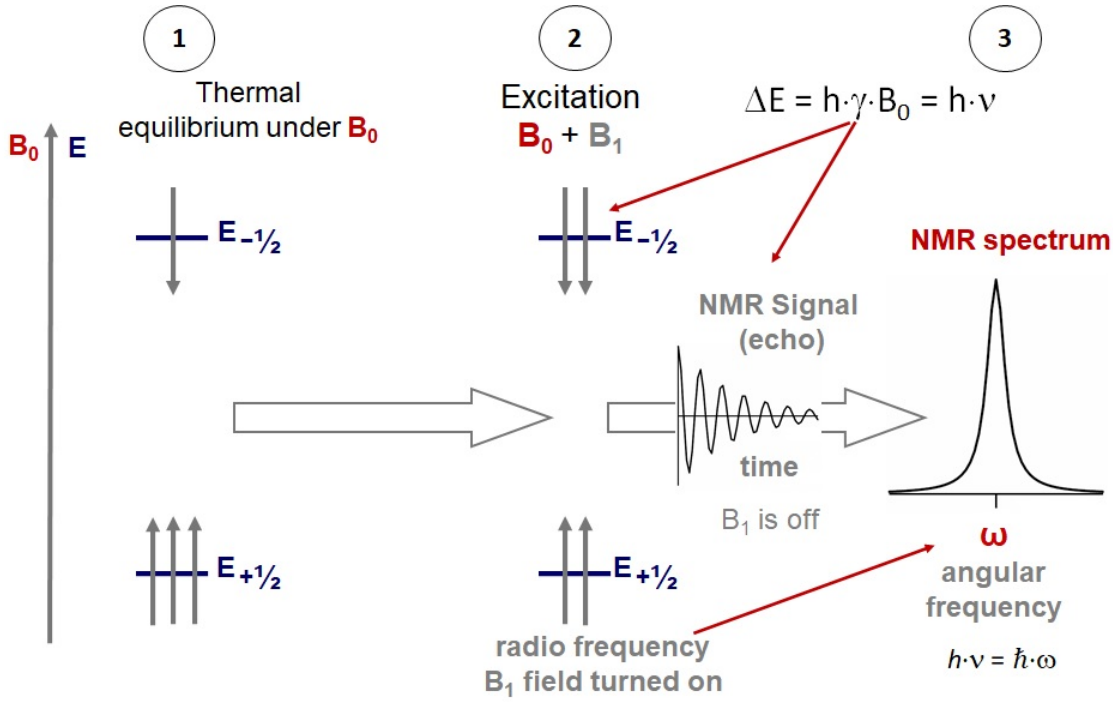

Figure V.2.1 Basic 1D NMR experiment. The three basic stages are labeled with numbers in circles as follows: (1) Thermal equilibration of the sample under constant magnetic field B_o; (2) perturbing the sample out of equilibrium (excitation) with an alternating magnetic field B_1 ; (3) Recording the "echo" (FID) from the perturbed system as it relaxes back to the equilibrium while field B1 is turned off (field B0 is always on). Constant magnetic field strength value B_o (stages 1 and 2) and angular frequency $\omega = 2 \cdot \pi \cdot \nu$ (stage 3) are shown red text color to highlight the fact that these values are strongly interconnected: B_o value defines the properties of the spectrum including the angular frequency or the position of the peak along the X-axis.

Proton NMR: a Spectrum with Multiple Peaks or a Single Peak for All the Protons ?

From the description above, it appears that for all the spin-½ nuclei with the same gyromagnetic ratio value γ (e.g., for all ^{1}H in the sample) the energy gap **Δ*E*** between the spin-up (spin projection +½) and spin-down (spin projection -½) states should be the same since this value is defined by the product $\gamma \cdot B_o$, [Chapter V.1, equation (2)]. It is correct that the γ value is the same for all the nuclei of the same type in the sample (and in the Universe for that matter) but the intensity of the magnetic field is not equal to exactly B_o for every nucleus.

Here is why. A biological molecule (e.g. a protein or DNA) contains protons (^{1}H) existing in a variety of local electronic environments, e.g. a methyl group protons (CH_3) are sharing electrons with a single carbon atom, whereas aromatic protons in a phenylalanine side chain "share" multiple electrons of the aromatic ring. Under the influence of a combination of external magnetic fields B_o (constant) and B_1 (alternating), these electron groups react differently and thus produce different local "current effects", which in turn generate different local (small but still relevant!) magnetic fields of their own. Thus, the methyl group protons and aromatic protons experience different effective magnetic fields $\mathbf{B_{eff}}$, which in most cases will be *not* exactly equal to B_o.

Equation V.2.???

$$\Delta E_{eff} = h \cdot \gamma \cdot B_{eff} \quad (5.2.1)$$

$$\Delta E_{eff} = h \cdot \nu_{eff} \quad (5.2.2)$$

Equation V.2.1 establishes that the magnitude of Zeeman energy splitting is defined by the gyromagnetic ratio γ of the specific nucleus type and the effective value of the magnetic field B_{eff}. To see how this logic extends even further, let us consider backbone amide protons (^1H_N) in a protein sample. Depending on the chemical type of each amino acid residue, its amide proton will have slightly different chemical and thus electronic environment. Moreover, for the residues positioned inside a folded protein structure local non-covalent neighbors also differ and would influence the local magnetic field through their local electric currents. Thus, for all the amide protons (which are chemically the same type of nuclei but structurally are different), the local magnetic fields B_{eff} may be different for each individual amide proton ^1H_N. The same logic applies to al other types of protons or other spin-½ nuclei in a protein molecule. Combined, the local electron effects or currents may lead to B_{eff} be lower than the main

magnet field B_o (so-called "shielding effect") or greater than B_o ("de-shielding"). The quantitative shielding effect is often described explicitly as a unitless quantity σ of relatively small absolute value and the overall algebraic relationships between B_o, B_{eff} and σ are as follows:

Equation V.2.5.2.7

$$\Delta E_{eff} = h \cdot \nu_{eff} \tag{5.2.3}$$

$$\Delta E_{eff} = \hbar \cdot \omega_{eff} \tag{5.2.4}$$

$$\nu_{eff} = \gamma \cdot B_{eff} \tag{5.2.5}$$

$$\omega_{ref} = 2 \cdot \pi \cdot \nu_{ref} \tag{5.2.6}$$

$$B_{eff} = B_0 \cdot (1 - \sigma) \tag{5.2.7}$$

In Equation V.2.2, we introduced another flavor of frequency, angular frequency ω, which is reported in units radians/sec (instead of oscillations/second as for the oscillation frequency ν). With the angular frequency, we also had to introduce the so-called reduced Plank constant $\hbar$, which is the Plank's constant divided by 2π. This seems a bit redundant (it is!) but the radial frequency can be simply more convenient to use for us later on, so stay tuned.

The frequency ν_{eff} value defined in this way is called *Larmor frequency*. So, why does a protein sample report a spectrum of ^{1}H signals with different frequencies instead of a single "proton peak"? This is because each type of proton experiences a slightly different effective magnetic field B_{eff} due to local field shielding and thus operates under a slightly different energy gap $\Delta E_{eff} = h\gamma \cdot B_{eff}$ as in equation (1) above. In turn, $\Delta E_{eff} = h \cdot \nu_{eff} = \hbar \cdot \omega_{eff}$ (the frequency of the transition across an energy gap is directly proportional to the energy gap value) and thus $\nu_{eff} = \gamma \cdot B_{eff}$. So, the registered frequency of transition (resonance frequency!) would be different for different protons because each one of them operates under a different effective magnetic field. **Figure V.2.1** shows an ^{1}H NMR spectrum of a modest size proteins sample.

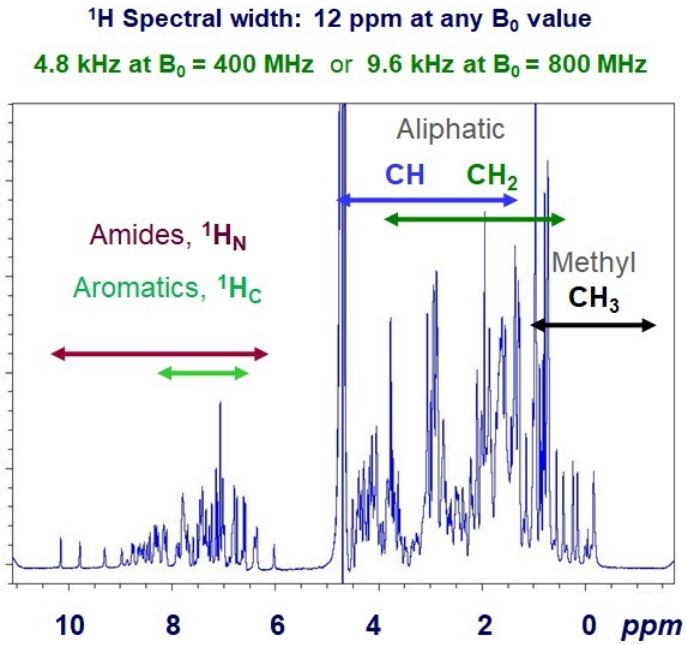

Figure V.2.2 A 1D ^{1}H spectrum of a protein sample (S.L. Smirnov, WWU, Bellingham, WA). Note that the spectrum width of the spectrum expressed in units of Hz does depend on the power of the magnet B_o (expressed in units of Hz).

In **Figure V2.2**, the central, strongest resonance (at 4.8 ppm; the one which goes way above the range of the Y-axis shown) comes from protons in water ([^1H_2O] ≈ 55 M !) and this position approximately corresponds to the B_o magnetic field. With respect to the water spectral line, ^{1}H nuclei from aliphatic groups (CH, CH_2 and CH_3) are di-shielded and thus experience B_{eff} fields exceeding B_o ($B_{eff} > B_o$, "up-field shift"). On the other side of the spectrum, amide and aromatic protons are experiencing B_{eff} lower than B_o ($B_{eff} < B_o$, such nuclei are shifted "down-field"). Fundamentally, the difference between the resonance frequency $\Delta\nu$ between any two nuclei a and b (of the same chemical type, e.g. ^{1}H) reports a respective difference between their respective B_{eff} values:

Equation V.2.5.2.8

$$\nu_{eff,a} - \nu_{eff,b} = \gamma \cdot (B_{eff,a} - B_{eff,b}) \tag{5.2.8}$$

Example 5.2.1

Determine the approximate value of resonance frequency ν of protons in an NMR spectrometer equipped with a magnet with a field strength of B_o=11.7 Tesla.

Solution

According to formula (1) above $\boldsymbol{\nu = \gamma \cdot B_0}$= 42.58 {MHz/Tesla} · 11.7 {Tesla} = 498.2 MHz.

Such a unit would be referred to (and labeled as) a 500 MHz magnet.

Example 5.2.2

It is stated in **Figure V.2.2** that a proton spectrum of a certain protein sample covers frequencies within the range of 4.8 kHz (4800 Hz) if the data recorded on a 400 MHz magnet. Calculate the field strength B_o of such a magnet and the difference in local fields B_{eff} between the two extreme frequency points (not peaks!) within the spectrum ($\boldsymbol{B_{ff,\ rightmost} - B_{eff,\ leftmost}}$). Calculate both requested values in units of Tesla. Compare the two numerical values.

Solution

$B_o = \boldsymbol{\nu} / \boldsymbol{\gamma}$ = 400 {MHz} / 42.58 {MHz/Tesla} = 9.39 Tesla

$\boldsymbol{B_{ff,\ rightmost} - B_{eff,\ leftmost}} = (\boldsymbol{\nu_{rightmost} - \nu_{leftmost}})/\boldsymbol{\gamma}$ = (***spectral width***) / $\boldsymbol{\gamma}$ = 4800 {Hz}· (1 {MHz}/10^6 {Hz}) / 42.58 {MHz/Tesla} = 1.13×10^{-4} Tesla

We see here that the biggest difference in B_{eff} is much, much smaller than the overall magnetic field B_o generated by the main magnet: $\boldsymbol{\Delta B_{eff}} << B_o$

This is a very general phenomenon in biomolecular NMR today.

Practice Problems

Problem 1. Why is the signal from water proton in Figure V.2.2 the strongest among all the resonances present? In other words, why is the water resonance intensity exceeds any signal originating from other ^{1}H nuclei in the protein sample?

Problem 2. For the magnet operating at $\mathbf{B_0}$ of 11.7 Tesla, calculate resonance base frequency for ^{1}H, ^{13}C, ^{15}N, and ^{31}P nuclei. Do these numbers tell you why the NMR magnet strength is represented as the ^{1}H frequency?

Problem 3. Looking at the spectrum in **Figure V.2.2**, compare the intensity values of various peaks: are the peak intensities uniform throughout the spectrum or not? What can be the reason(s) for this intensity (non?)-uniformity?

Problem 4. Example 2 above shows that the difference in resonance frequency between any two nuclei of the same type (e.g. protons) depends on the magnet strength B_o. This is not very convenient because such a measure is equipment-specific. Can you propose a resonance frequency unit, which would show such a difference as numerically the same quantity for any value of B_o? Hint: proportionating (normalizing) for B_o might be helpful.
