TEJASVI NAVADHITAMASTU

"Let our (the teacher and the taught) learning be radiant"

Let our efforts at learning be luminous and filled with joy, and endowed with the force of purpose

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NUCLEAR MAGNETIC RESONANCE

- Nuclear magnetic resonance (NMR) is a physical phenomenon in which nuclei in a magnetic field absorb and re-emit electromagnetic radiation. This energy is at a specific resonance frequency which depends on the strength of the magnetic field and the magnetic properties of the isotope of the atoms.

- Nuclear magnetic resonance(NMR) spectroscopy is a powerful analytical technique with a wide variety of applications used for structural studies, or as a simple quality assay for which structural information is important.

- It is nondestructive, and high-quality data may be obtained from milligram, even microgram, quantities of sample.

- NMR spectroscopy can provide the data necessary to determine the complete structure of a molecule.

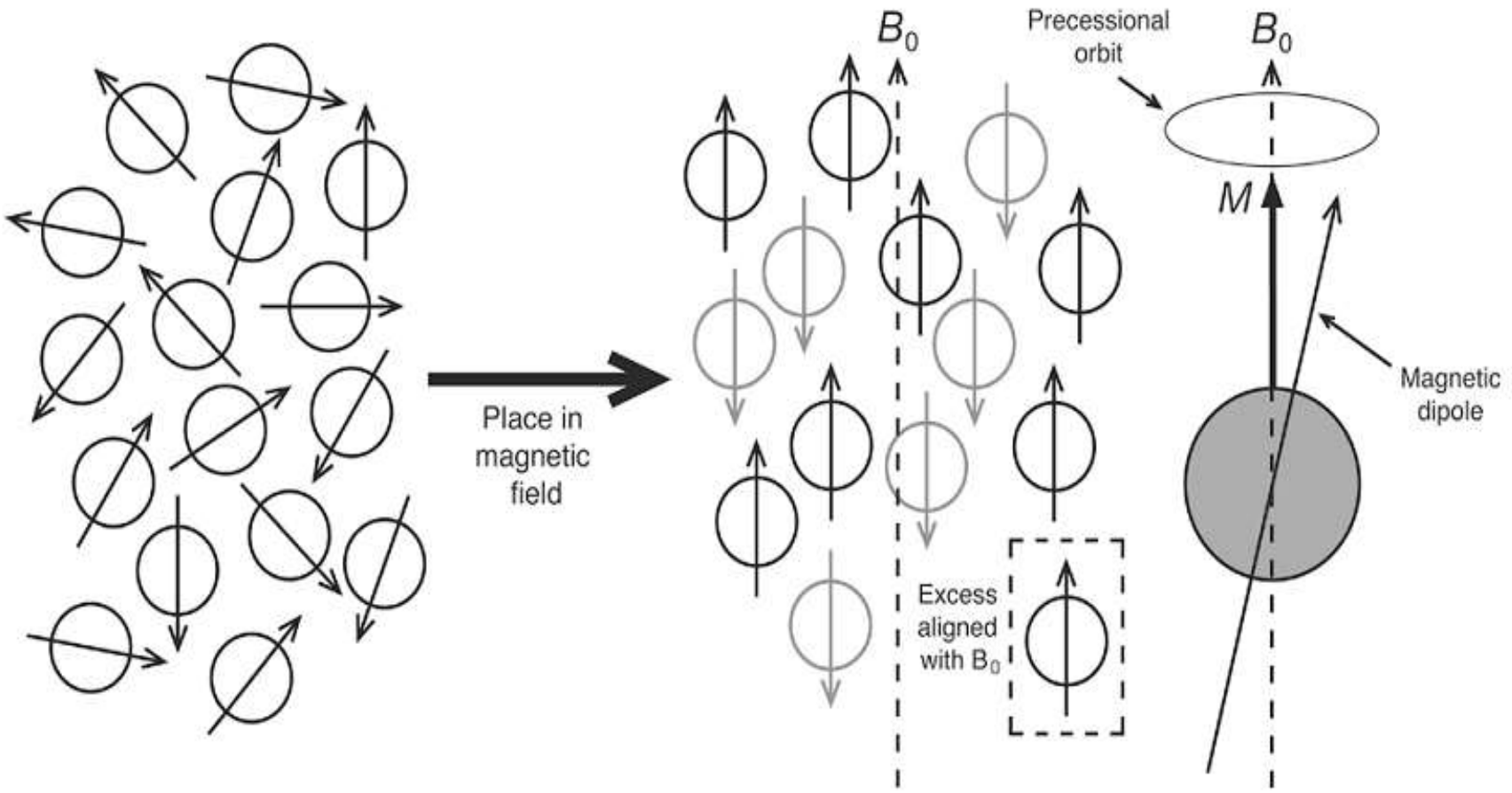
PRINCIPLES OF NMR SPECTROSCOPY

NMR differs from most other forms of spectroscopy in that it is the atomic nuclei that are the subject of study, and the measured energy is in the radio-frequency range.

Many nuclei possess an angular momentum, which means that they have a characteristic spin quantum number (I) and may be analyzed using NMR. These nuclei are charged, and a spinning charge generates a magnetic field. Simply the nuclei behave like tiny magnets that interact with an applied, external magnetic field. The most common nuclei analyzed by NMR are the proton (H) and the ^{13}C isotope of carbon, as well as ^{19}F and ^{31}P, all of which have a spin I= 1/2.

Once the nuclei are placed within a strong external magnetic field(B_0), the spin of the nuclei will align with that field. There are only two orientations that the spin 1/2 nuclei can adopt: either aligned with the applied magnetic field (parallel or spin +1/2) or aligned against the field (antiparallel or spin −1/2).

The parallel orientation has a slightly lower energy associated with it and, therefore, has a slightly higher population. It is this excess of nuclei in the spin +1/2 state that produces the net magnetization that is manipulated and measured during an NMR experiment.



Nuclear spin and magnetic vectors are randomly ordered outside of the NMR magnet. However, once placed in an applied magnetic field, the NMR magnet and the nuclei align either with the applied field, B0, (parallel) or against it (antiparallel). There is a slight excess in the population aligned parallel to B_0. Although the magnetic dipole tracks a precessional orbit, the net magnetization (M) is aligned with B_0

Radio-Frequency Pulse and Relaxation

Early NMR instruments relied on electromagnets and a simple radio-frequency(RF) transmitter, and the analyses were performed by a sweep through the frequency range of the instrument.

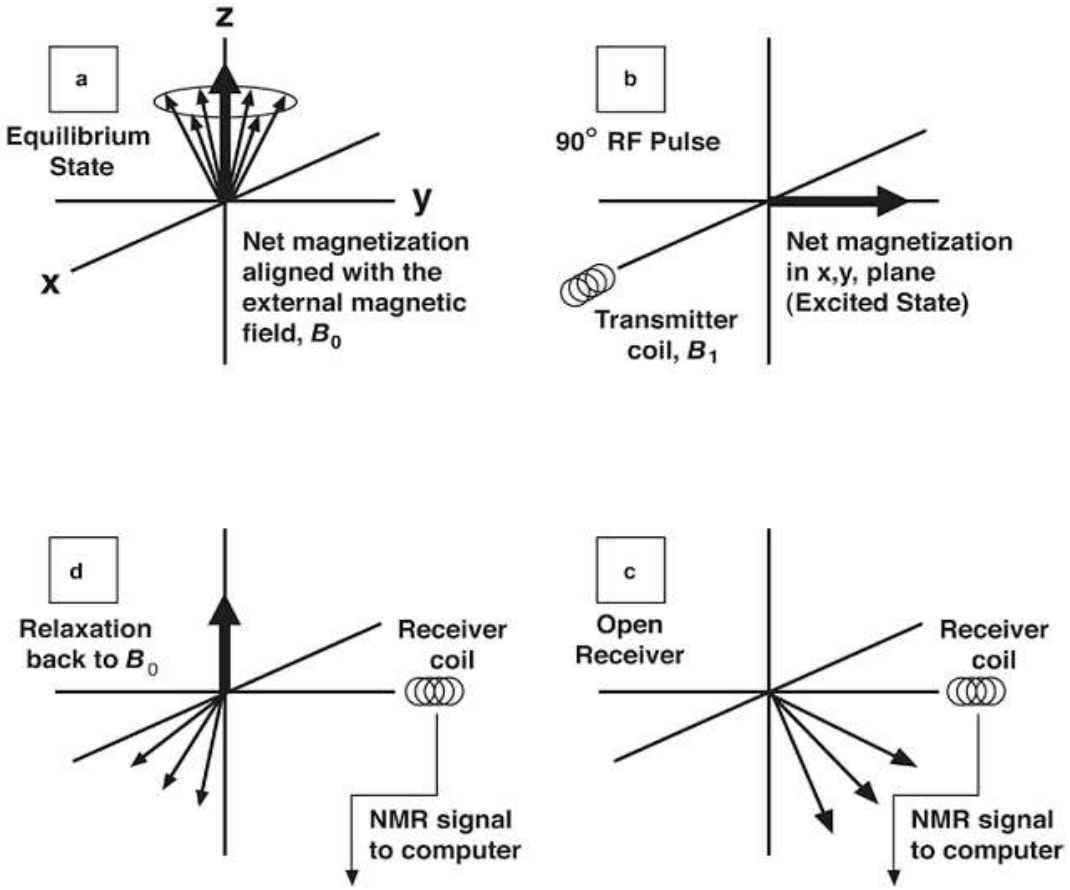
The collected spectra contain the frequency information; hence, it is termed frequency-domain NMR. Although this enabled the development of NMR spectroscopy, it was not sufficient to facilitate the modern NMR experiment.

One of the major developments in NMR technology was the RF pulse, in which a large range of frequencies is excited by a short pulse of RF energy around a centered carrier frequency, which is at resonance frequency of the nuclei under study. This pulse simultaneously excites all of the protons in the sample, and the NMR data for all the protons is collected during a short time after the pulse is applied.

In NMR, the carrier frequency, transmitter power, and duration of the RF pulse determine the frequency range of the pulse.

(a) Priyor to the RF energy pulse, the net magnetization (M) composed of all the component vectors is in the equilibrium state, aligned with B0.

(b) The 90° RF pulse, which covers the resonance frequencies of all relevant nuclei in the sample and originates perpendicular to the z-axis (B1), causes the nuclei to move to a higher energy state, and the net magnetization rotates into the xy-plane.



(c) Once in the xy-plane, the net magnetic vector separates into the component vectors for each unique population of nuclei. As these oscillate in the xy-plane, they emit RF signals that are detected by the NMR instrument after passing through the receiver coil, which is located perpendicular to both B0and the transmitter coil.

(d) As the component vectors continue to oscillate in the xy-plane (and emit RF signals), the nuclei begin to relax back to the equilibrium state. The NMR instrument may be set up to repeat this process, with additional pulses, numerous times; the collected data are then added together to improve the signal/noise ratio and resolution

Once the sample is placed in the magnet, the protons align parallel or antiparallel to the applied, external magnetic field, B0, with an excess in parallel orientation. The net magnetization of the nuclei in the parallel orientation is aligned with the z-axis in an xyz graphical representation of the system .

After a pulse of RF energy is applied to the system, the nuclei precess coherently and individual nuclei absorb energy and shift to a higher energy state. The pulse, which is applied by a transmitter coil perpendicular to the z-axis (B1), tilts the net magnetization vector away from the z-axis and toward the xy- plane

Once the net magnetization has been tilted into the xy-plane by a 90° pulse, the magnetization begins to decay back to the z-axis. This process is termed **NMR relaxation**, and it involves both **spin-lattice(T1)** and **spin-spin(T2) relaxation**. T1 relaxation is associated with the interaction of the magnetic fields of the excited-state nuclei with the magnetic fields of other nuclei within the “lattice” of the total sample. T2 relaxation involves the interactions of neighboring nuclei that lead to a diminishment in the energy state of the excited-state nuclei and the loss of phase coherence

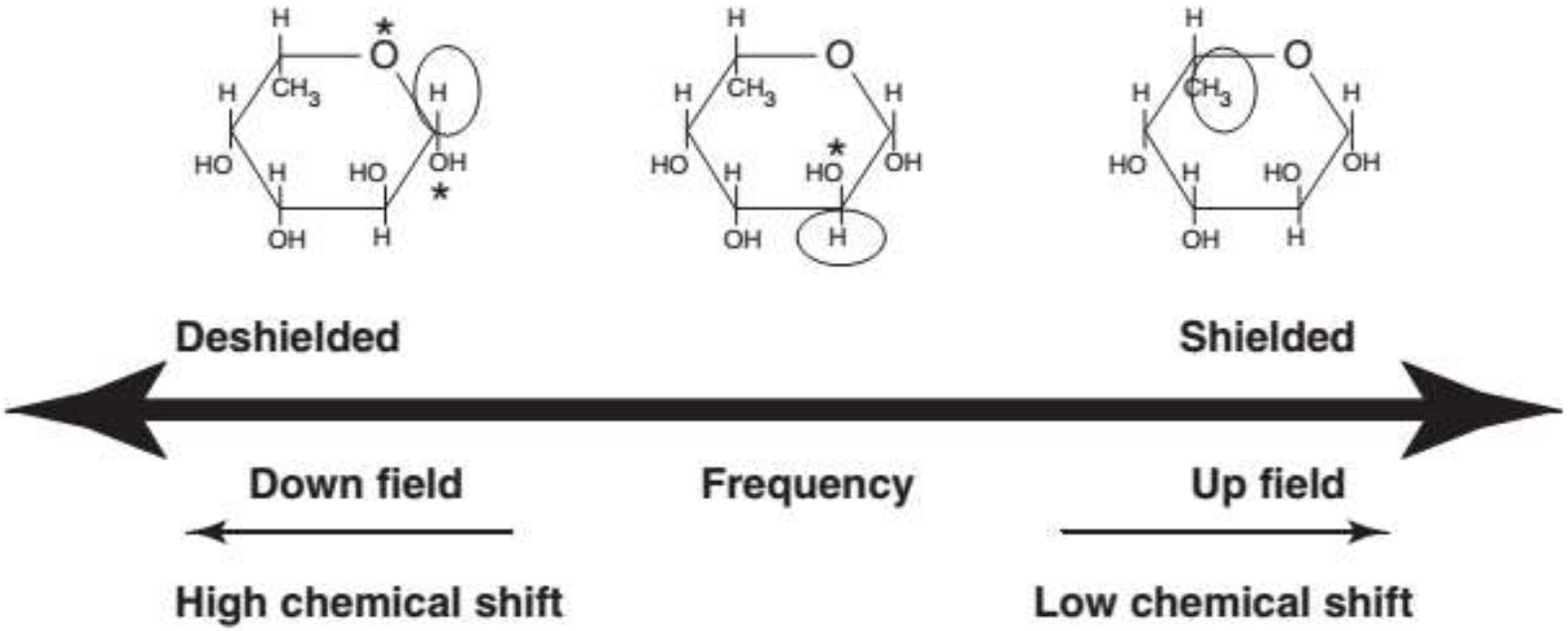
Chemical Shift and Shielding

The total H population in the sample determines the net magnetization of the system in the external magnetic field, B0. The exact frequency of a unique population of protons (i.e., all protons in a specific chemical configuration in the molecule), however, is also dependent on the immediate environment of the nucleus, principally the density of the electron cloud surrounding the nuclei, which determines the electronic environment of the nuclei. This is referred to as the "shielding" effect, because the electrons create a secondary, induced magnetic field that opposes the applied field, shielding the nuclei from the applied field.

The resulting frequency differences are so small, relative to the Larmor frequency, that they are commonly reported in parts per million (ppm). However, they are large enough to be clearly detected and resolved during an NMR experiment. The frequency differences that result from the differences in the electronic environments yield the chemical shift of the nuclei.

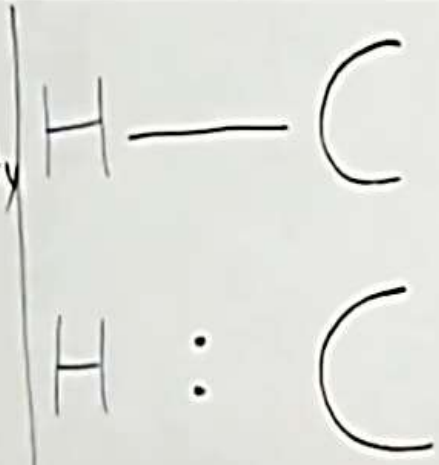
Those protons that have a relatively dense electron cloud are considered shielded, since the electron cloud works in opposition to the external magnetic field, and the resonances will be found on the right, or upfield, side of the spectrum, at a lower chemical shift. As deshielding increases, the resonances are shifted further to the left, or downfield, at a progressively higher chemical shift

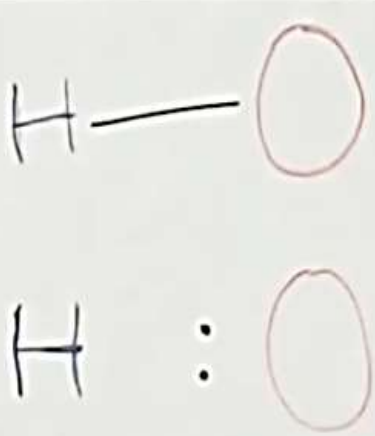
Just as **per** cent means out of a hundred, so **parts per million** or **ppm** means out of a **million**. Usually describes the concentration of something in water or soil. One **ppm** is equivalent to 1 milligram of something **per** liter of water (mg/l) or 1 milligram of something **per** kilogram solid.

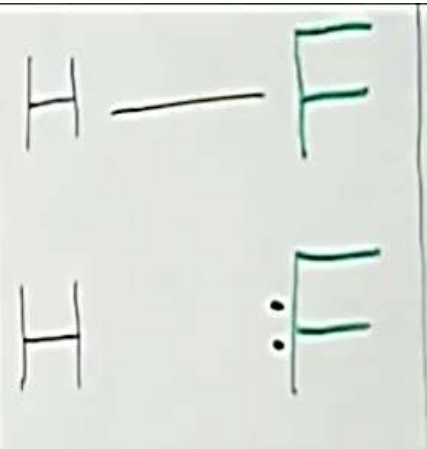


The shielding effect is responsible for the small, but detectable, differences in the resonance frequencies of nuclei such as protons. Protons that are not close to an electronegative group in the molecule, such as the protons in the methyl group in fucose (top right), a common 6-deoxy sugar, will be shielded by the electrons surrounding it, and it will have a low chemical shift, upfield in the NMR spectrum. Protons near one oxygen atom (indicated by an asterisk), such as the ring protons in sugars (top middle), will be intermediately shielded and have a chemical shift toward the middle of the spectrum. And a proton that is near two oxygen atoms, such as the anomeric proton in sugars (top left), will be relatively deshielded and have a high chemical shift downfield in the spectrum.

Electronegativity

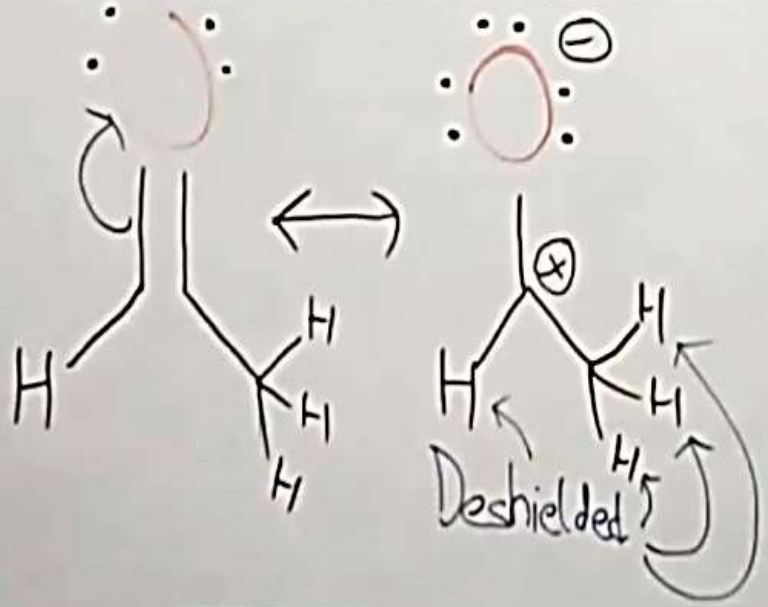


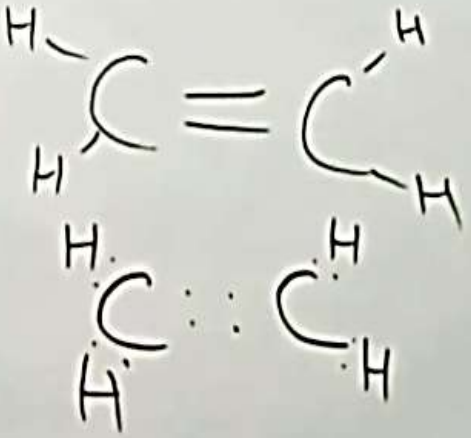




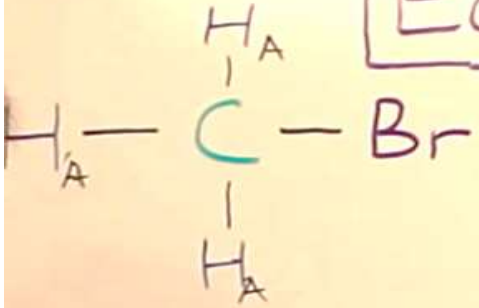
Shielded | Deshielded | More Deshielded

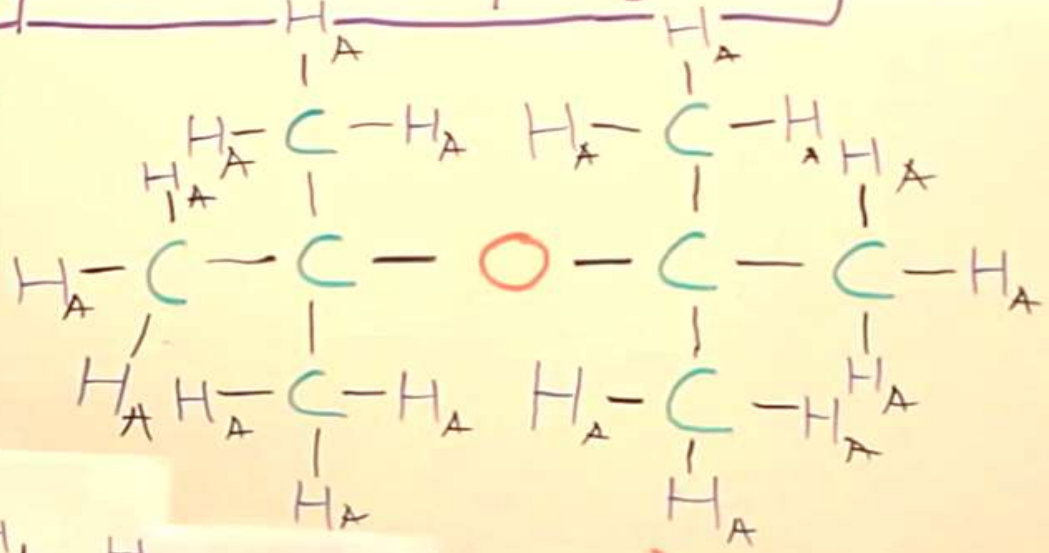
Resonance

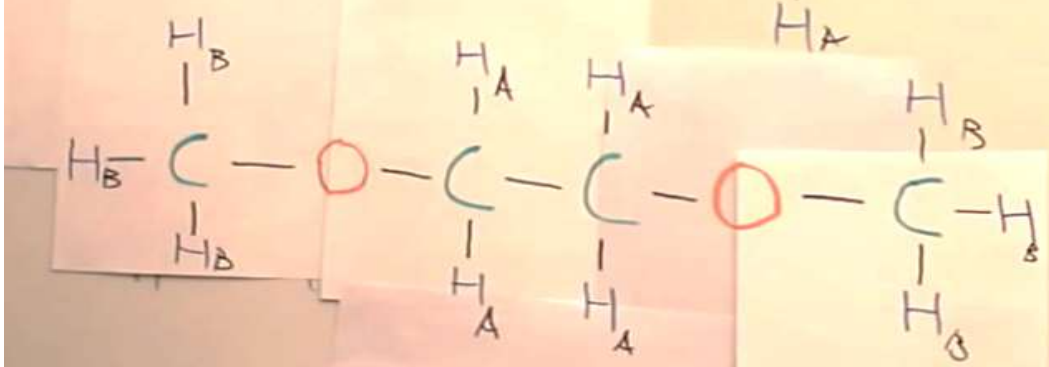




Equivalent Hydrogens







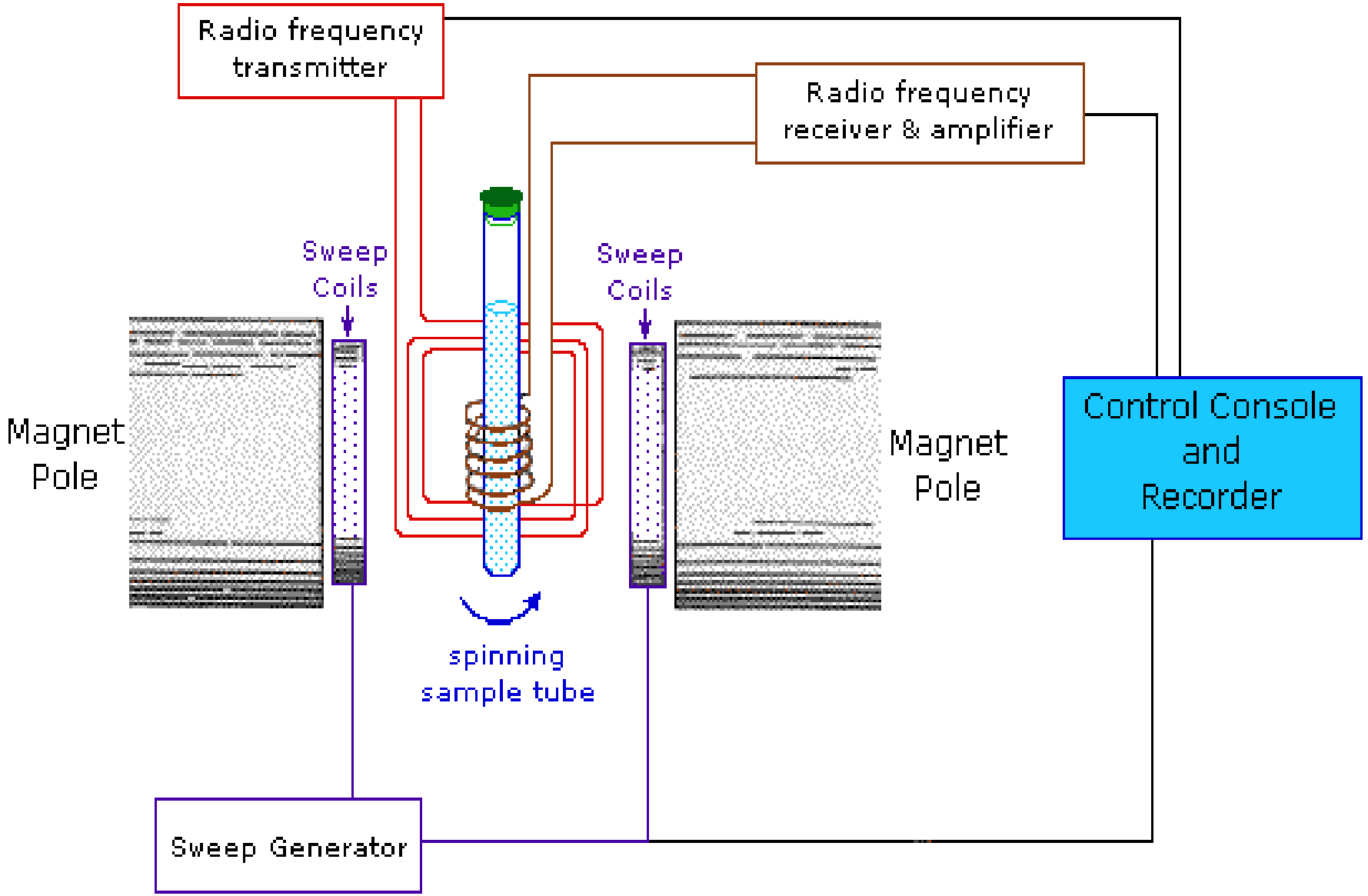
1). Distance from
2). # of Electron Withdrawing groups.
(Electronegative)

Instrumentation

To begin with, the NMR spectrometer must be tuned to a specific nucleus, in this case the proton. The actual procedure for obtaining the spectrum varies, but the simplest is referred to as the continuous wave (CW) method.

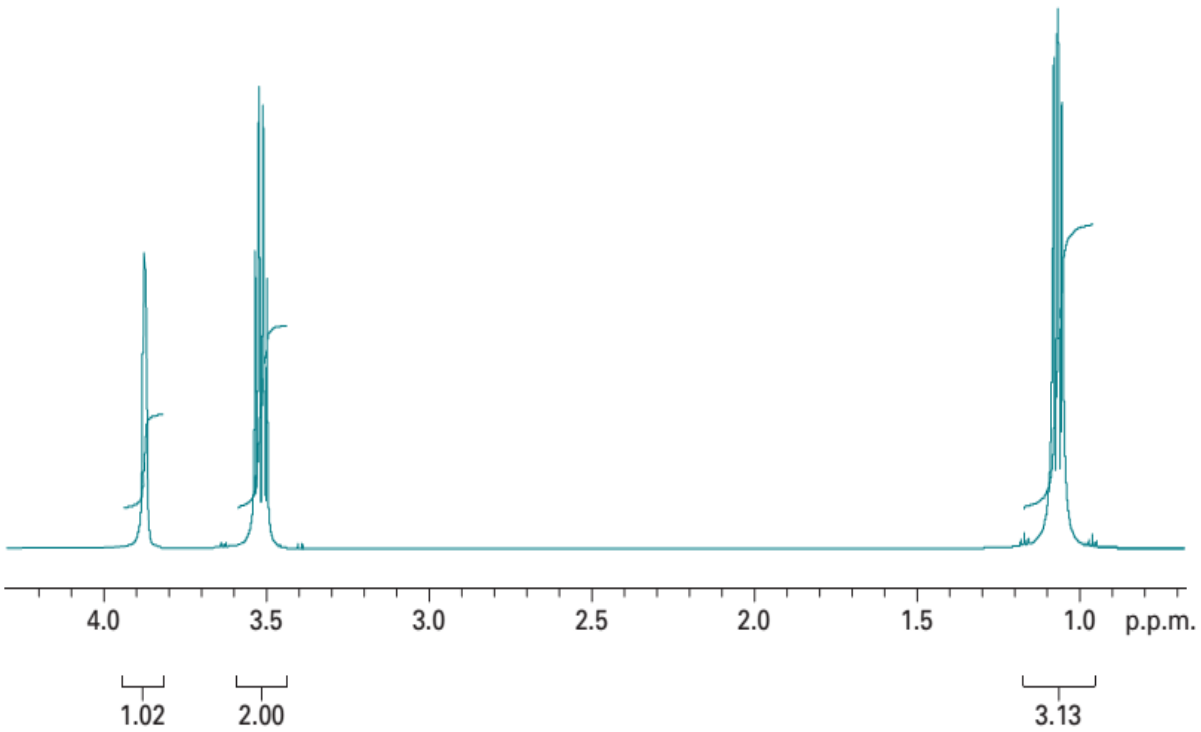
A solution of the sample in a uniform 5 mm glass tube is oriented between the poles of a powerful magnet, and is spun to average any magnetic field variations, as well as tube imperfections. Radio frequency (Rf) radiation of appropriate energy is broadcast into the sample from an antenna coil (colored red). A receiver coil surrounds the sample tube, and emission of absorbed rf energy is monitored by dedicated electronic devices and a computer. An NMR spectrum is acquired by varying or sweeping the magnetic field over a small range while observing the Rf signal from the sample. An equally effective technique is to vary the frequency of the Rf radiation while holding the external field constant.

As an example, consider a sample of water in a 2.3487 T external magnetic field, irradiated by 100 MHz radiation. If the magnetic field is smoothly increased to 2.3488 T, the hydrogen nuclei of the water molecules will at some point absorb Rf energy and a resonance signal will appear. An animation showing this may be activated by clicking the Show Field Sweep button. The field sweep will be repeated three times, and the resulting resonance trace is colored red. For visibility, the water proton signal displayed in the animation is much broader than it would be in an actual experiment.


Radio frequency transmitter
Radio frequency receiver & amplifier
Sweep Coils
Sweep Coils
Magnet Pole
Magnet Pole
Control Console and Recorder
spinning sample tube
Sweep Generator

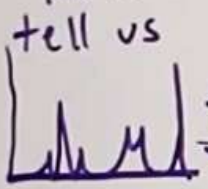
1H NMR spectrum of ethyl alcohol (H3C–CH2 –OH) with integrated peaks

The chemical shift is plotted along thex-axis, and measured in p.p.m. instead of the actual magnetic field strengths. This conversion makes the recorded spectrum independent of the magnetic field used. The signal of the internal standard TMS appears at chemical shift = 0 p.p.m. The type of proton giving rise to a particular band may thus be identified by the resonance peak position, i.e. its chemical shift, and the area under each peak is proportional to the number of protons of that particular type.



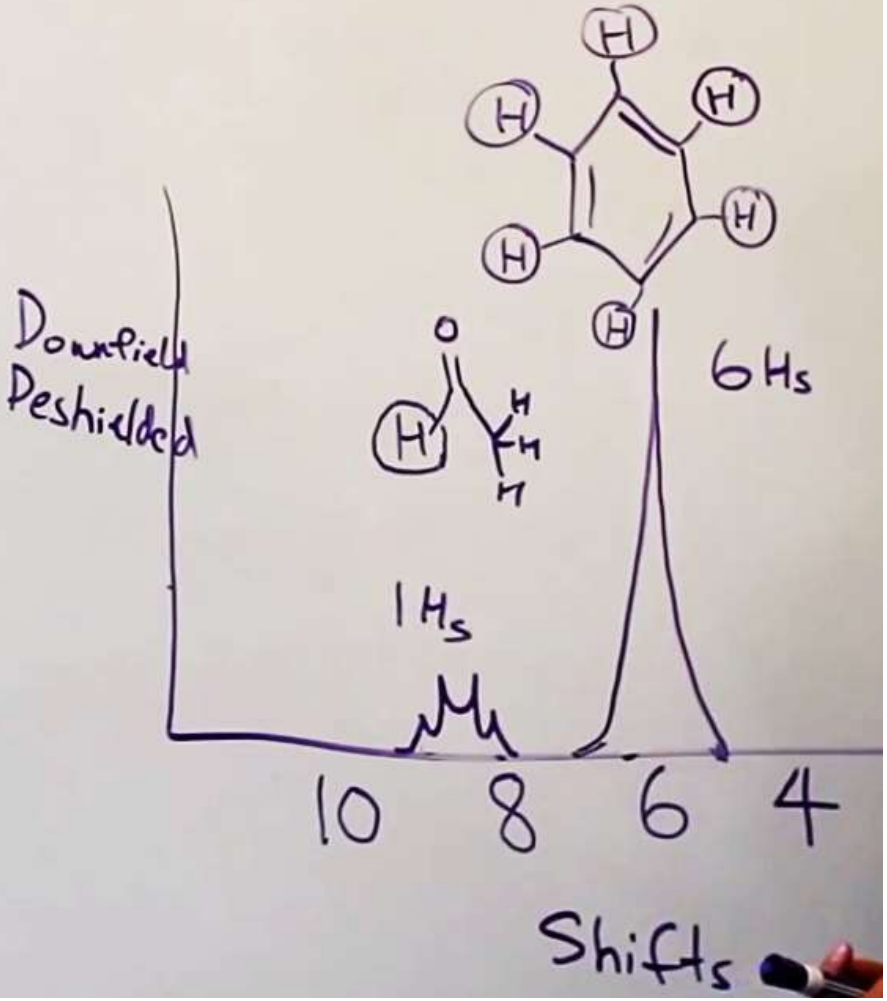
1H NMR spectrum of ethyl alcohol, in which there are three methyl, two methylene and one alcohol group protons. The peak areas are integrated, and show the proportions 3 : 2 : 1. Owing to the interaction of bonding electrons with like or different spins, a phenomenon called **spin-spin coupling** (also termed **scalar** or **J-coupling**) arises that can extend to nuclei four or five bonds apart. This results in the splitting of the three bands into several finer bands (hyperfine splitting). The hyperfine splitting yields valuable information about the near-neighbour environment of a nucleus.

NMRs tell us

1).  the Structure of molecules based on Hs environment

2). Height of Peaks ≈ # of Hs

3). Splitting tells us # of unequiv. Hs within 3 Bond distance (n+1 rule)



Applications

1. **Molecular structure determination**; NMR spectroscopy is the main method of structure determination for organic compounds. The chemical shift provides a clue about the environment of a particular proton or carbon, and thus allows conclusions as to the nature of functional groups.

2. **Solution structure of proteins and peptides:** The structures of proteins up to a mass of about 50 kDa can be determined with biomolecular NMR spectroscopy.

3. **Magnetic resonance imaging:** The basic principles of NMR can be applied to imaging of live samples. Because the proton is one of the more sensitive nuclides and is present in all biological systems abundantly, 1H resonance is used almost exclusively in the clinical environment. The most important compound in biological samples in this context is water.

ELECTRON PARAMAGNETIC RESONANCE SPECTROSCOPY: EPRS

In both EPR and NMR techniques, two possible energy states exist for either electronic or nuclear magnetism in the presence of an external magnetic field.

In the low-energy state, the field generated by the spinning charged particle is parallel to the external field. Conversely, in the high-energy state, the field generated by the spinning charged particle is antiparallel to the external field.

When enough energy is input into the system to cause a transition from the low- to the high-energy state, the condition of resonance is satisfied. Energy must be absorbed as a discrete dose (quantum) hv, where h is the Planck constant and v is the frequency. The quantum energy required to fulfil the resonance condition and thus enable transition between the low- and high-energy states may be quantified as

$$h\nu = g\beta B$$

Where g is a constant called spectroscopic splitting factor, b is the magnetic moment of the electron (termed the Bohr magneton), and B is the strength of the applied external magnetic field. The frequency v of the absorbed radiation is a function of the paramagnetic species b and the applied magnetic field B. Thus, either v or B maybe varied to the same effect.

With appropriate external magnetic fields, the frequency of applied radiation for EPR is in the microwave region, and for NMR in the region of radio frequencies. In both techniques, two possibilities exist for determining the absorption of electromagnetic energy (i.e. enabling the resonance phenomenon):

1. Constant frequency v is applied and the external magnetic field B is swept; or
2. Constant external magnetic field B is applied and the appropriate frequency v is selected by sweeping through the spectrum

Principles

The absorption of energy is recorded in the EPR spectrum as a function of the magnetic induction measured in Tesla (T) which is proportional to the magnetic field Strength applied. The area under the absorption peak is proportional to the number of unpaired electron spins. Most commonly, the first derivative of the absorption peak is the signal that is actually recorded.

Instrumentation

The magnetic fields generated by the electromagnets are of the order of 50 to 500 mT, and variations of less than 10^{-6} are required for highest accuracy.

The monochromatic microwave radiation is produced in a **klystron oscillator** with wavelengths around 3 cm (9 GHz).

The samples are required to be in the solid state; hence biological samples are usually frozen in liquid nitrogen.

The technique is also ideal for investigation of membranes and membrane proteins.

Instead of plotting the absorption A versus B, it is the first-order differential (dA/dB) that is usually plotted against B. Such a shape is called a ‘line’ in EPR spectroscopy.

Generally, there are relatively few unpaired electrons in a molecule, resulting in fewer than 10 lines, which are not closely spaced.

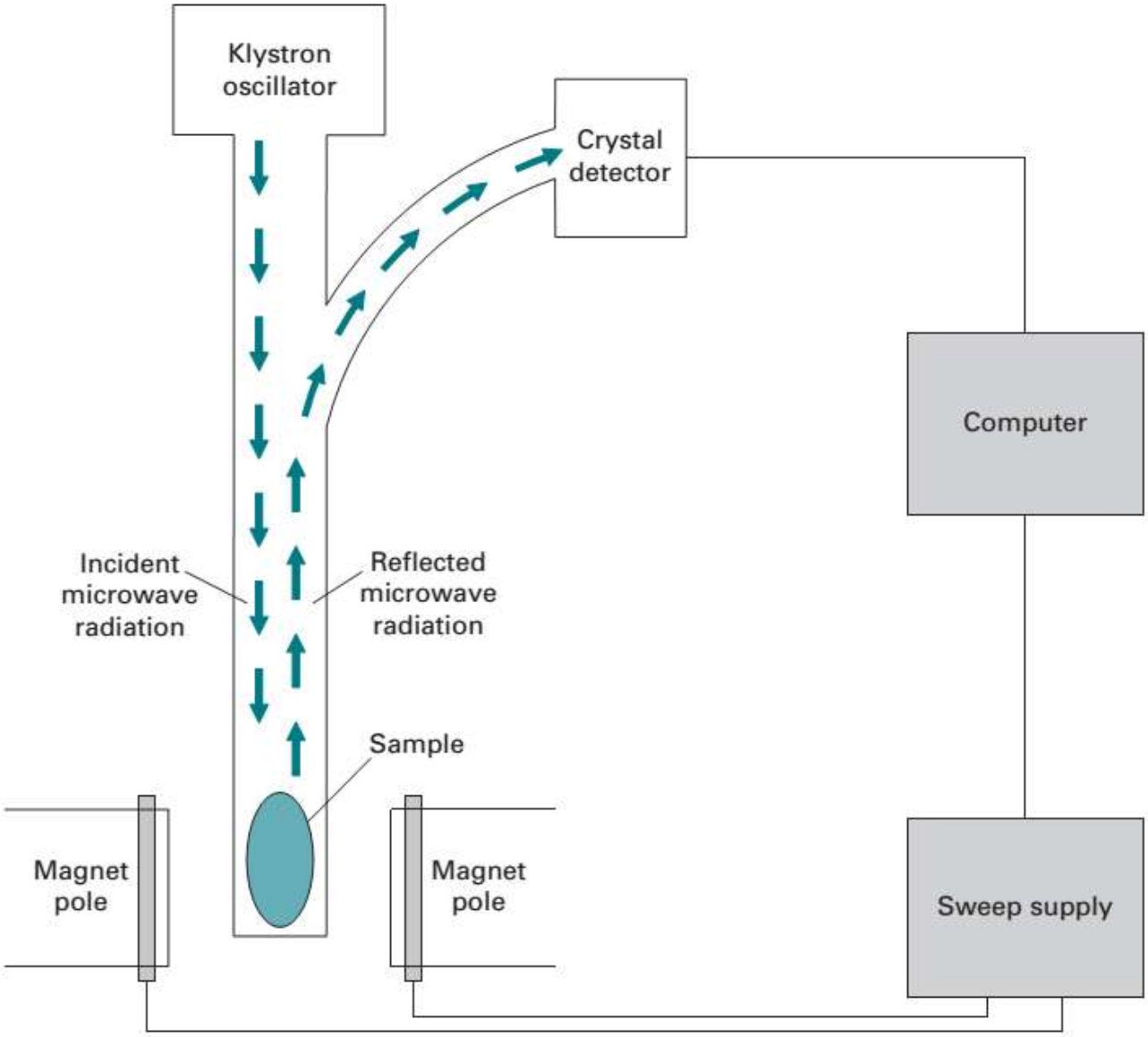
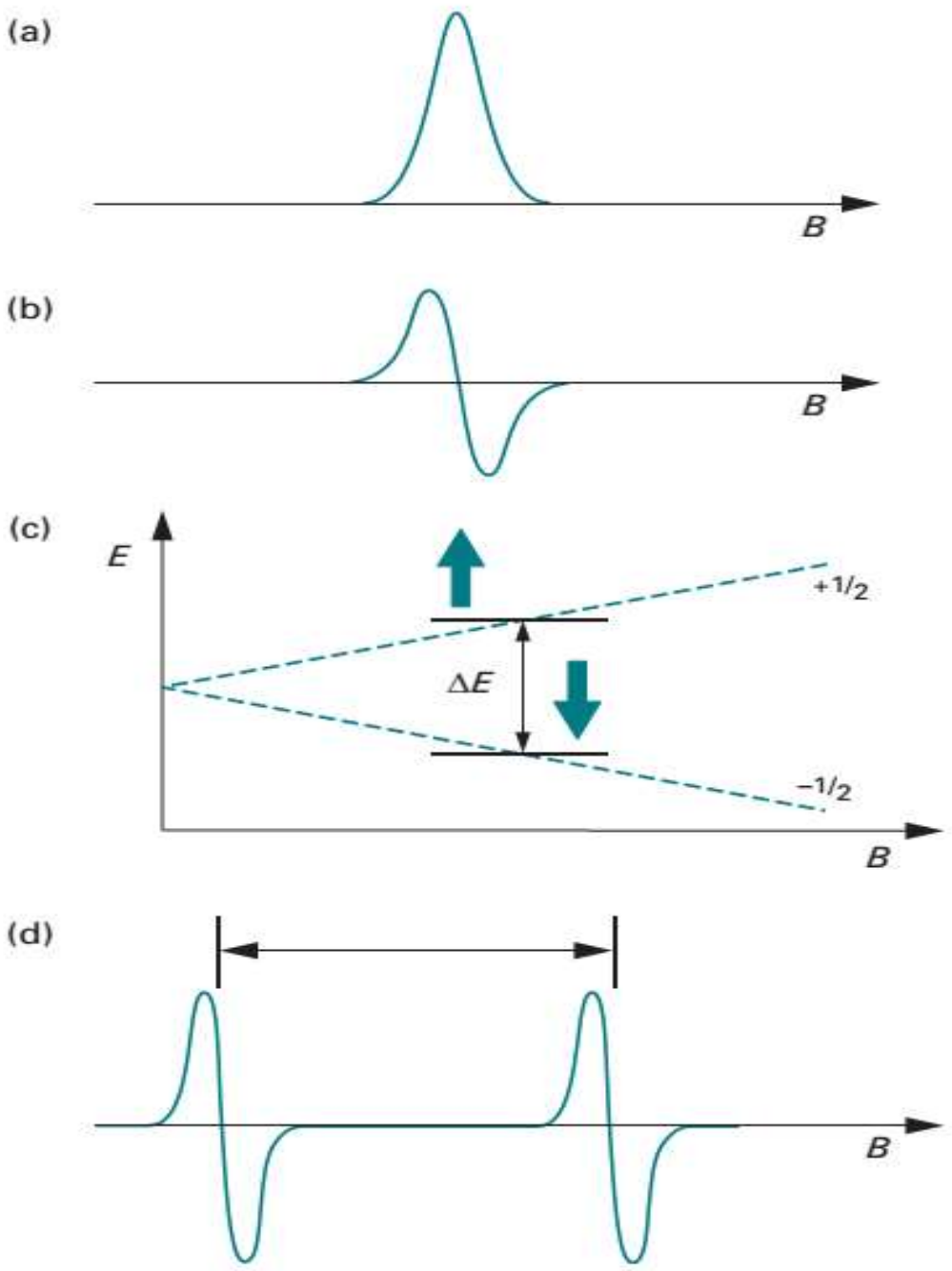


Diagram of an EPR spectrometer

Instead of the absorption signal (a), EPR spectrometry records its first derivative (b).

(c) The energy of the two spin states of a free electron is shown as a function of the external magnetic field B. Resonance happens when the energy of the applied microwave radiation is the same as the energy difference DE.

(d) Hyperfine splitting due to coupling of an unpaired electron with a nuclear spin of 1/2. For the hydrogen atom, the distance between the two signals is 50.7 mT



Applications

1. **Metalloproteins:** EPR spectroscopy is one of the main methods to study metalloproteins, particularly those containing molybdenum (xanthine oxidase), copper (cytochrome oxidase, copper blue enzymes) and iron (cytochrome, ferredoxin). Both copper and non-haem iron, which do not absorb in the UV/Vis region, possess EPR absorption peaks in one of their oxidised states.

2. **Free radicals:** Molecules in their triplet states have unpaired electrons and thus are amenable to EPR spectroscopy. Such molecules possess the property of phosphorescence and EPR may deliver data complementary to the UV/Vis region of the spectrum. For instance, free radicals due to the triplet state of tryptophan have been observed in cataractuous lenses. Carcinogenesis is also an area where free radicals have been implicated.

3. **Irradiated foodstuffs:** Another major application for EPR is the examination of irradiated foodstuffs for residual free radicals, and it is mostly used to establish whether packed food has been irradiated.

FOOD APPLICATIONS OF MAGNETIC RESONANCE SPECTROSCOPY AND RELATED TECHNIQUES

NMR method	*Food*	*Analysis*
MRI	Whole wheat bread	Water migration between arabinoxylan and gluten
^{1}H-MRI	Avocado	Nondestructive assessment of bruising
^{1}H-MRI	Honey	Authenticity screening
HR ^{1}H NMR	Pork meat	Quantitative fatty acid chain composition
qHNMR	Processed foods	Quantification of benzoic acid
^{1}H and spin-spin relaxation	Rice and potato starches	Impact of hydration levels on starch gelatinization
2D NMR	Green coffee bean extract	Analysis of organic compounds
^{1}H NMR, TOCSY, HSQC and HMBC	Hazelnut	Metabolic profiling of hazelnut cultivars
DOSY-NMR	Beverages	Sucrose quantification
^{1}H, 1H-DPFGSE, and F2-DPFGSE band-selective HSQC	Olive oil	Detection of aldehydes
^{13}C qNMR	Wine	In situ determination of fructose isomer concentration
Solid-state ^{13}C NMR	Milk protein concentrate	Change in molecular structure and dynamics of protein
UF iSQC NMR	Viscous liquid foods	Sugar content, quality testing, and determination of adulteration
TD-NMR	Mayonnaise and salad dressing	Through-package fat determination
TD-NMR	Biscuit dough	Influence of fiber on proton mobility
TD-NMR	Beef	Meat quality parameters
TD-NMR (SMART Trac™)	Organogels in cream cheese	Fat content
HR-MAS-NMR	Tomato	Metabolic profiling, tissue differentiation, and fruit ripening
^{1}H HR-MAS	Fish	Rapid assessment of freshness and quality
CP-MAS-NMR	Wheat bran	Hydration, plasticization, and disulfide bonds
CP-MAS-NMR	Starch	Chemicophysical properties

NMR nuclear magnetic resonance spectroscopy, *MRI* magnetic resonance imaging, *CP-MAS* cross polarization magic-angle-spinning NMR, *HR-MAS* high-resolution magic-angle spinning, *DOSY-NMR* diffusion-ordered ^{1}H NMR, *qHNMR*, quantitative proton NMR, *TD-NMR* time-domain NMR, *DPFGSE* double pulsed field gradient spin echo, *UF iSQC NMR* ultrafast intermolecular single-quantum coherence

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