CSIR NET Life Science Unit 13

Nuclear Magnetic Resonance

NMR is a spectroscopic technique that exploits the magnetic properties of nuclei. It detects the change in nuclear spin energy in the presence of an external magnetic field as a result of absorption of electromagnetic radiation in the radio-frequency region. Its prominence as a biophysical technique lies in its ability to reveal the atomic structure of macromolecules in solution, provided that highly concentrated solutions (1 mM, or 15 mg ml-1 for a 15-kD protein) can be obtained. NMR depends on the fact that certain atomic nuclei are intrinsically magnetic; only a limited number of isotopes relevant to biochemistry display this unique property, known as *spin* (^{1}H, ^{13}C, ^{14}N, ^{31}P etc.). Protons, electrons and neutrons possess a property called spin. Spin is expressed in multiples of 1/2 and can be + or –. Some atomic nuclei also have spin. If a particular nucleus is composed of p protons and n neutrons, its total mass is $p + n$, its total charge is +p and its total spin will be a vector combination of p + n spins each of magnitude 1/2. If the number of both the protons and neutrons in a nucleus is even, then there is no overall spin. All nuclei with an even mass number (total number of protons and neutrons in the nucleus) and an even atomic number (number of protons in the nucleus) have thus a nuclear spin of zero. Any atomic nucleus that possesses either odd mass number, odd atomic number or both will possess a spin value.

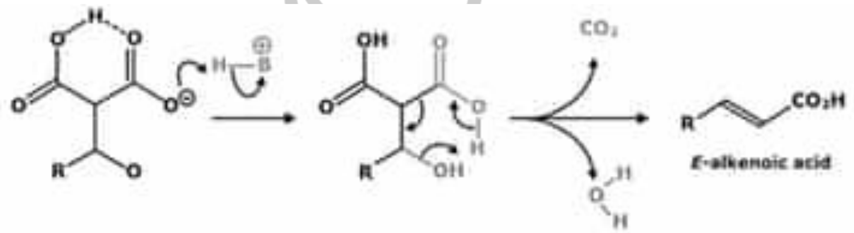
Table 1. The atomic number, number of neutrons and spin quantum number of some elements

Element	^{1}H	^{12}C	^{13}C	^{14}N	^{15}N	^{16}O	^{19}F	^{31}P
Atomic number	1	6	6	7	7	8	9	15
Number of neutrons	0	6	7	7	8	8	10	16
Spin quantum number	½	0	½	1	½	0	½	½

The spinning of a proton generates a magnetic moment. This moment can take either of two orientations or spin states (called α and β), upon application of an external magnetic field. The energy difference between these states is proportional to the strength of the imposed magnetic field. The α state has slightly lower energy and hence is slightly more populated because it is aligned with the field. A spinning proton in the α state can be raised to an excited state (β state) by applying a pulse of electromagnetic radiation of radio wave frequency (RF, pulse), provided the frequency corresponds to the energy difference between the α and the β states. Radiowaves flick the nucleus from the lower energy state to the higher state; the spin orientation will change from α to β; i.e., resonance will be obtained. The nucleus is now eager to return to the lower, more stable energy state; in doing so energy will be dissipated again and this is what is detected by the spectrometer. A resonance spectrum for a molecule can be obtained by varying the magnetic field at a constant frequency of electromagnetic radiation or by keeping the magnetic field constant and varying electromagnetic radiation.

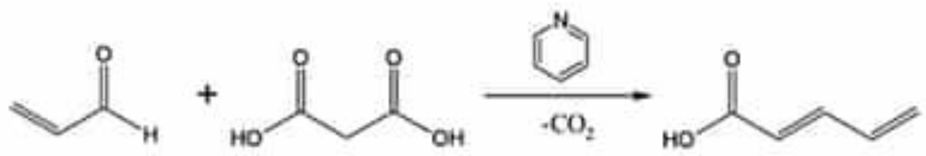
Table 2. Fermentation by genetically engineered organisms

PRODUCT	APPLICATION	ORGANISM
B. growth hormone	Milk production(cows)	*Escherichia coli* (E. coli)
Cellulase	Cellulose	*E. coli*
H. growth hormone	Growth deficiencies	*E.coli*
Human insulin	Diabetics	*E. coli*
Monoclonal antibodies	Therapeutics	Mammalian cell culture
Ice-minus	Prevents ice on plants	*Pseudomonas syringae*
Sno-max	Makes snow	*Pseudomonas syringae*
t-PA	Blood clots	Mammalian cell culture
Tumor necrosis factor	Dissolves tumor cells	*E.coli*



(IV) (V) (VI)

Example-



Now in an applied magnetic field, not all atoms like for e, g. hydrogens and carbons in an ethanol or propanol molecule resonate at exactly the same frequency. This variability exists due to the fact that the hydrogens and carbons in a molecule are surrounded by electrons and exist in minutely different electronic environments from one another. The flow of electrons around a magnetic nucleus generates a small local magnetic field that opposes the applied field (diamagnetic anisotropy). Each nucleus is surrounded by electrons, and in a magnetic field these will set up a tiny electric current. This current will set, up its own magnetic field, which will oppose the magnetic field that we apply. The electrons are said to shield the nucleus from the external magnetic field. If the electron distribution varies from say, ^{13}C atom to ^{13}C atom in an ethanol molecule, so does the local magnetic field, and so does the resonating frequency of the ^{13}C nuclei. Thus, the changes in the distribution of electrons around a nucleus effect:

1. The local magnetic field that the nucleus experiences.
2. The frequency at which the nucleus resonates.
3. The chemistry of the molecule at that atom.

This variation in frequency is known as the *chemical shift* and it is denoted by δ. The chemical shift of a nucleus depends on many factors, but the surrounding electron density is often the dominant one. A high electron density causes a large shielding effect. Let us consider ethanol; the carbon attached to the -OH group will have relatively fewer electrons around it compared to the other carbon (the oxygen atom is more electronegative and draws electrons towards it, away from the carbon atom). The external magnetic field that this carbon nucleus feels will, therefore, be slightly greater than the felt by the other carbon with more electrons. Since this carbon is less shielded (deshielded) from the applied external magnetic field, it feels a stronger magnetic field and there will be a greater energy difference between the two energy states of the nucleus. The greater the energy difference, the higher the resonance frequency. So, for ethanol, it is expected the carbon with the OH group attached to resonate at a higher frequency than the other carbon; the same is revealed by the ^{13}C NMR spectrum.