Analytical methods for confirming the structure of synthesized drugs: instrumental analysis

Instrumental methods of analysis

- Instrumental (physical and physico-chemical) methods of analysis are based on the use of the relationship between the measured physical properties of substances and their qualitative and quantitative composition.
- In analytical chemistry, elemental, functional, molecular, and phase analysis of a substance is performed.

Elemental analysis

- Elemental analysis is the qualitative detection and quantitative determination of the content of elements and the elemental composition of substances, materials and various objects. These can be liquids, solid materials, gases and air.

Elemental analysis

- Elemental analysis allows you to answer the question of which atoms (elements) the analyzed substance consists of.

Elemental analysis

- Qualitative elemental analysis makes it possible to determine from which atoms of the elements the molecules of organic matter are built.
- Quantitative elemental analysis establishes the elemental composition of the compound and the simplest formula of the compound.

Functional analysis

- Functional analysis (chemical), a set of chemical and physical methods of analysis (mainly of organic substances) based on the determination of reactive groups of atoms (individual atoms) in molecules - functional groups. Such groups are hydroxyl, carboxyl, nitro group, amino group.

Functional analysis

- Functional analysis is used to confirm the intended structure of a substance or reaction mechanism, to establish the percentage of individual compounds of a known structure in a mixture. The chemical methods use characteristic reactions of functional groups

Molecular analysis

- Molecular analysis is the discovery of molecules and the determination of the molecular composition of the analyzed substance, i.e., finding out which molecules and in what quantitative ratios this analyzed object consists of.

Phase analysis

- Phase analysis is a method of determining the quantity and chemical composition of individual phases in heterogeneous systems.
- Phase analysis is carried out only in relation to solids.

Phase analysis

- Physical methods such as X-ray phase analysis, spectral and electron probe analyses, electronography, and nuclear magnetic resonance are used to perform phase analysis.

NMR spectroscopy

- NMR spectroscopy, commonly known as Magnetic Resonance Spectroscopy (MRS), is a strong analytical method used to analyze the local magnetic fields around atomic nuclei. It is based on the absorption of electromagnetic radiation by atom nuclei in the radiofrequency area, which generally ranges from 4 to 900 MHz.

NMR spectroscopy

- Over the last several decades, NMR spectroscopy has emerged as a major tool for identifying the structure of organic molecules. It is uncommon among spectroscopic approaches in that it is normally required to analyze and interpret the full spectrum.

The NMR spectroscopy concept consists of three main steps:

- **Magnetic nuclear spin alignment:** The sample is immersed in a constant magnetic field (B0), which aligns the magnetic nuclear spins.
- **Spin alignment perturbation:** A weak oscillating magnetic field, known as a radio-frequency (RF) pulse, is used to disturb the nuclear spin alignment.
- **Detection and analysis of released electromagnetic waves:** As a result of the disturbance, the sample produces electromagnetic waves, which are detected and evaluated.

NMR spectroscopy

- NMR spectroscopy is a reliable approach for detecting monomolecular organic molecules in organic science. It provides distinct and tractable spectra capable of distinguishing different functional groups and providing vital insights into molecule structure and interactions. NMR spectroscopy of protons and carbon-13 is among the most regularly used methods, however NMR may be applied to any substance containing nuclei with spin.

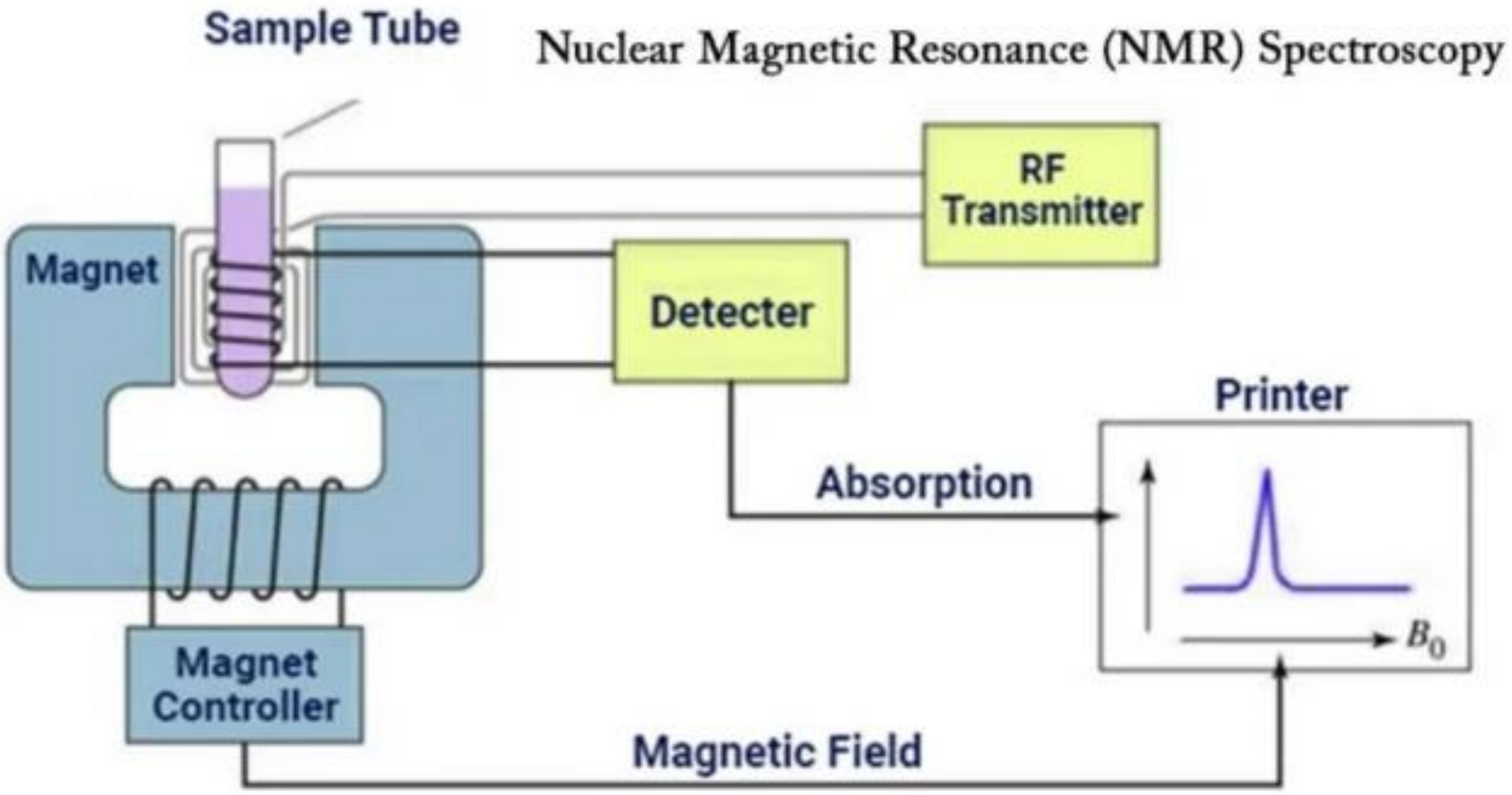
NMR spectroscopy

- NMR spectra are well-resolved, predictable, and provide useful information for even tiny compounds. For chemical identification, they have virtually supplanted traditional wet chemistry experiments. NMR spectroscopy, on the other hand, takes a rather substantial amount of purified chemical, often 2-50 mg, however the sample is frequently recovered. Because solid-state NMR analysis needs specialist equipment, it is better to dissolve the sample in a solvent.

NMR spectroscopy

- Correlation spectroscopy, such as two-dimensional (2D) NMR, allows for the study of correlated resonances and makes it easier to identify nearby substituents. More advanced 3D and 4D NMR technologies are also available to enhance or suppress certain resonances. NOE spectroscopy, which observes the relaxation of resonances, permits the creation of three-dimensional models of molecules based on nuclei closeness.

NMR spectroscopy



Basis of NMR Spectroscopy

- NMR spectroscopy is extremely useful in organic chemistry. It has transformed organic lab research by delivering critical information on the structure, composition, and purity of molecules. One of the most frequent NMR methods in organic chemistry is proton (1H) NMR.
- The behavior of protons (nuclei) in a molecule is investigated using NMR spectroscopy. Protons behave differently depending on their chemical surroundings, such as the existence of nearby atoms or functional groups. This behavioral variance enables the understanding of the molecular structure.

Basis of NMR Spectroscopy

- When a magnetic field is applied to a sample, the protons in the molecule align with the field. The protons can be disturbed and identified by using radiofrequency pulses. Protons' absorption and emission of energy at certain frequencies, known as resonance frequencies, give important information about their chemical environment and interactions.
- Researchers may discover the structural properties of the molecule by analyzing the NMR signals collected from the sample, including the connectivity of atoms and the types of functional groups present. Furthermore, NMR spectroscopy allows for the measurement of component concentrations as well as the evaluation of sample quality.

Principle of NMR Spectroscopy

- The behavior of atomic nuclei with spin and electric charge in the presence of an external magnetic field is the basis for Nuclear Magnetic Resonance (NMR) spectroscopy. Energy transfers between lower energy states (base energy) and higher energy levels can occur when a sample is put in a magnetic field.
- The sample's nuclei can absorb energy from the external magnetic field and move to higher energy levels. This energy transfer occurs at a certain wavelength associated with radio frequencies. When the nuclei return to their base energy level, they radiate energy at the same frequency that was absorbed.

Principle of NMR Spectroscopy

- The radiated energy, or signal, is measured and processed to provide an NMR spectrum tailored to the nucleus under investigation. The spectrum reveals information about the chemical environment and nuclear interactions in the sample.
- In summary, the absorption and emission of energy by atomic nuclei with spin and electric charge when exposed to an external magnetic field is the basis of NMR spectroscopy. NMR spectroscopy gives significant information on the structure, dynamics, and chemical characteristics of molecules by studying the resultant signals.

Working of Nuclear Magnetic Resonance (NMR) Spectroscopy

- Sample Preparation: The sample of interest is prepared, which is commonly an organic substance dissolved in a suitable solvent. Atomic nuclei having spin, such as protons (1H) or carbon-13 (13C), should be present in the sample.
- Magnetic Field Application: A strong magnetic field created by a superconducting magnet is applied to the prepared sample. The magnetic field in the sample aligns the atomic nuclei.

Working of Nuclear Magnetic Resonance (NMR) Spectroscopy

- Radiofrequency Excitation: The sample is excited using radiofrequency (RF) pulses. These RF pulses have frequencies that correspond to the resonance frequencies of atomic nuclei in a magnetic field. The radiofrequency pulses are generally in the hundreds of megahertz (MHz) to gigahertz (GHz) range.

Working of Nuclear Magnetic Resonance (NMR) Spectroscopy

- Nuclear Magnetic Resonance and Signal Detection: Nuclear magnetic resonance occurs when RF pulses induce atomic nuclei to move from a lower energy state to a higher energy state. When the RF pulse is terminated, the nuclei revert to their original condition and release energy at the same resonance frequency. This released energy is recognized as an NMR signal by sensitive radio receivers.

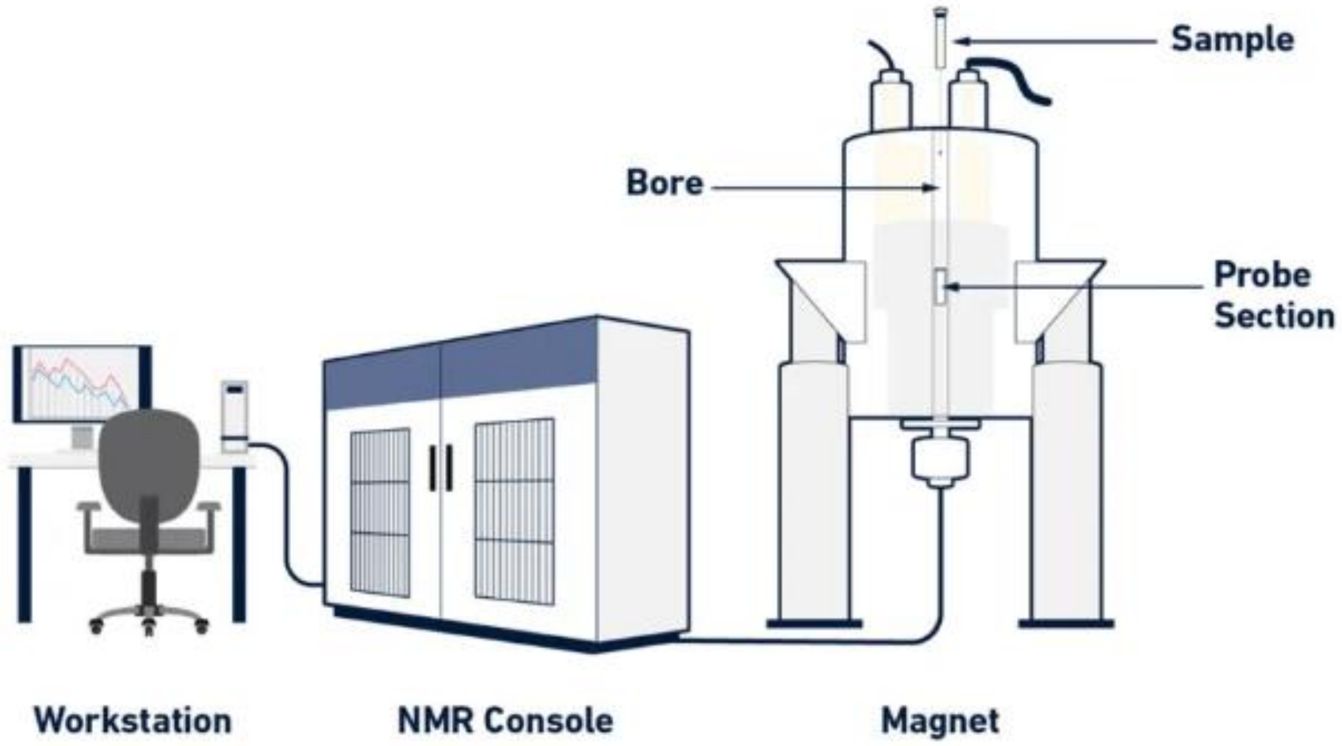
Working of Nuclear Magnetic Resonance (NMR) Spectroscopy

- **Signal Analysis:** The obtained NMR signals are processed and analyzed to retrieve useful sample information. The signal's resonance frequencies and intensities reveal information on the electronic structure of the molecules and their distinct functional groups. Chemical changes caused by intramolecular magnetic field effects are utilized to detect and differentiate distinct molecules. NMR spectroscopy may also offer extensive information on a molecule's structure, dynamics, reaction state, and chemical environment.

Working of Nuclear Magnetic Resonance (NMR) Spectroscopy

- **Types of NMR:** Proton (1H) NMR and carbon-13 (13C) NMR spectroscopy are the two most frequent forms of NMR spectroscopy. These procedures are aimed at analyzing hydrogen and carbon nuclei, respectively. NMR spectroscopy, on the other hand, is applicable to any sample containing nuclei with spin, allowing the investigation of elements such as nitrogen, phosphorus, and fluorine.

General design of an NMR spectrometer with its principal components.



Instrumentation of Nuclear Magnetic Resonance (NMR) Spectroscopy

- **Sample Holder:** The sample under analysis is held in a glass tube that is generally 8.5 cm long and 0.3 cm in diameter.
- **Magnetic Coils:** When an electric current flows through these coils, it creates a magnetic field. The coils' magnetic field interacts with the atomic nuclei in the sample.
- **Permanent Magnet:** A permanent magnet creates a uniform magnetic field within the NMR instrument. The magnetic field strength is normally in the 60-100 MHz range.

Instrumentation of Nuclear Magnetic Resonance (NMR) Spectroscopy

- **Sweep Generator:** The sweep generator changes the intensity of the magnetic field supplied to the sample. It enables fine-tuning and modifications to guarantee that the necessary magnetic field strength is achieved.
- **Radiofrequency Transmitter:** This component is made up of a coil that generates a brief yet strong radio wave pulse. To excite the atomic nuclei, a radiofrequency pulse is delivered to the sample.
- **Radiofrequency Receiver:** The radiofrequency receiver coil detects the radio frequencies released by the atomic nuclei as they relax to a lower energy level. It records the NMR signals produced by the material.

Instrumentation of Nuclear Magnetic Resonance (NMR) Spectroscopy

- **RF Detector:** The RF detector aids in determining the unabsorbed radio frequencies after the sample has been excited. It gives information about the NMR signals' resonance frequencies and intensities.
- **Recorder**: The NMR signals received by the RF detector are recorded by a recorder. It collects and saves data for further study.
- **Readout System:** The NMR data is analyzed, processed, and interpreted using a computer-based readout system. It includes tools for spectral analysis, data management, and NMR spectrum display.

NMR Spectroscopy Techniques

- **Resonant Frequency:** The exact frequency at which NMR active nuclei absorb electromagnetic radiation when put in a magnetic field is referred to as the resonant frequency in NMR spectroscopy. The resonance frequency is directly connected to the energy of absorption and is unique to the isotope under investigation. The strength of the magnetic field is related to the intensity of the NMR signal.

NMR Spectroscopy Techniques

- **Spectra Acquisition:** Acquiring NMR spectra involves igniting the sample with a radiofrequency pulse. The nuclei in the sample undergo nuclear magnetic resonance and generate a response signal as a result of this pulse. The NMR signal, on the other hand, is often quite faint, necessitating the use of sensitive radio receivers to identify and amplify the signal for further investigation.

Chemical Shift in NMR Spectroscopy

- Chemical shift refers to the difference in resonant frequency between the spinning protons (or other nuclei) in a molecule and a reference molecule. It is a crucial property used for determining the molecular structure in nuclear magnetic resonance. NMR spectroscopy can detect various nuclei, including 1H (proton), 13C (carbon-13), 15N (nitrogen-15), 19F (fluorine-19), and many more. Among them, 1H and 13C are the most commonly used nuclei.

Nuclear Magnetic Resonance (NMR) spectroscopy is founded on quantum mechanics concepts and the behavior of atomic nuclei in a magnetic field.

- **Magnet Generation:** NMR spectrometers create a strong and homogenous magnetic field using a superconducting magnet. To produce superconductivity, these magnets are often cooled to very low temperatures (about 4 K) using liquid helium and liquid nitrogen.
- **The Probe:** The sample to be studied is put within the superconducting magnet’s cylindrical chamber known as the probe. Magnetic coils surround the sample in the probe. These coils perform a variety of functions, including irradiating the sample with radiofrequency (RF) pulses and detecting the NMR signals released by the sample.

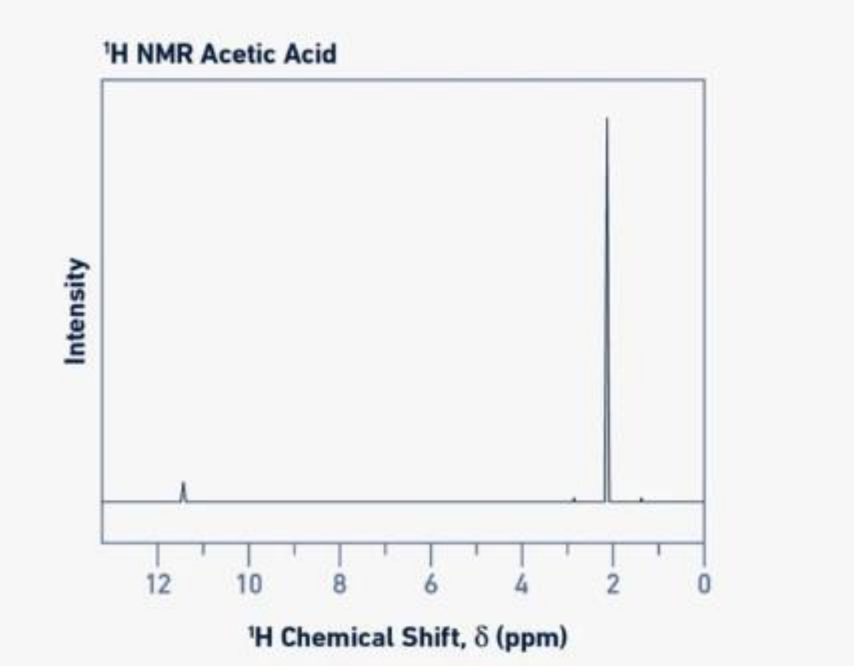
Nuclear Magnetic Resonance (NMR) spectroscopy is founded on quantum mechanics concepts and the behavior of atomic nuclei in a magnetic field.

- **Sample Alignment:** When a sample is put in a magnetic field, the nuclear spins of the atoms inside the sample align with the magnetic field. Nuclear spin alignment is critical for NMR analysis.
- **Excitation by RF Pulse:** To acquire NMR signals, the sample is exposed to a brief and intense RF pulse. This pulse causes the aligned nuclear spins to precess around the magnetic field.
- **Detection of NMR Signals:** The magnetic coils in the probe also serve as radio receivers. After the RF pulse stimulation, they detect the NMR signals released by the precessing nuclear spins. These signals are extremely faint, necessitating the use of sensitive detecting devices.

Nuclear Magnetic Resonance (NMR) spectroscopy is founded on quantum mechanics concepts and the behavior of atomic nuclei in a magnetic field.

- **Electronic System and Data Analysis:** The NMR spectrometer's electronic system controls the experimental settings and allows the operator to set up and alter numerous NMR experiment parameters. It also makes data collecting and mathematical processing of obtained data into an NMR spectrum easier.
- **NMR Spectrum:** A graphical depiction of the NMR signals acquired from the sample is the NMR spectrum. It is made up of a succession of peaks with varying strengths that correspond to distinct atomic nuclei in the sample. The chemical shift, which is related to the Larmor frequency of the nuclei in the magnetic field, determines the position of these peaks.

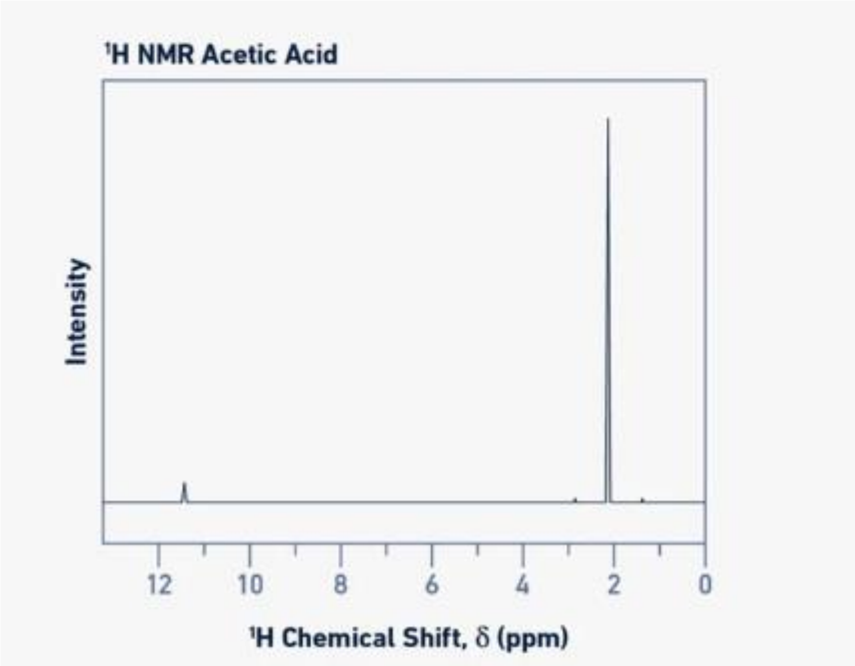
NMR spectrum



H solution NMR spectrum of acetic acid. The signals correspond to the molecule's two distinct H nuclei, and their areas are proportional to the number of nuclei contributing to the signal.

- **Chemical Shift:** The spectrometer's measured NMR signal, known as the Free Induction Decay (FID), is processed to yield a relative magnitude known as chemical shift (). This magnitude is independent of the magnetic field and may be compared between equipment. Chemical shift is measured in parts per million (ppm) and is calculated by the observed Larmor frequency (vL) in relation to the Larmor frequency (L0) of a reference nucleus.
- **Reference Compound**: To set the zero value of the chemical shift scale, a reference substance, such as tetramethylsilane (TMS) or sodium trimethylsilylpropanesulfonate (DSS) for 1H NMR, is utilized.
- **Spectral Interpretation**: An NMR spectrum may tell you a lot about the chemicals in a sample. The chemical shift values of chemical groups inside a molecule can be used to identify them. In a proton (1H) NMR spectrum, for example, various protons in the molecule will produce unique signals at different chemical shifts.

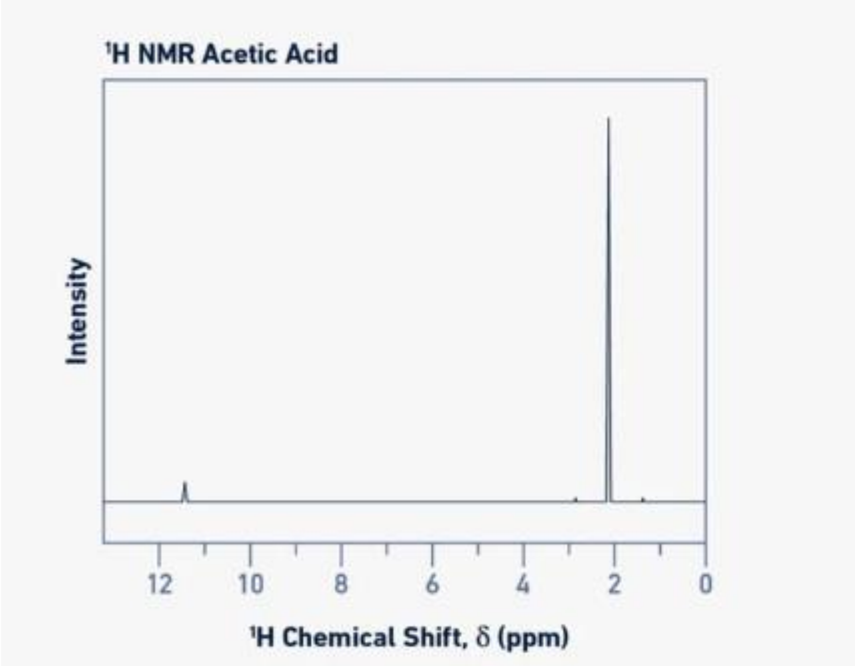
NMR spectrum



H solution NMR spectrum of acetic acid. The signals correspond to the molecule's two distinct H nuclei, and their areas are proportional to the number of nuclei contributing to the signal.

- **Signal Intensity:** The area beneath a signal in 1H-NMR spectra is proportional to the number of protons (atomic nuclei) creating that signal. As a result, the signal regions may be utilized to calculate the relative abundance of various protons inside the molecule. This intensity connection, however, does not apply to 13C-NMR spectra.
- **Signal Splitting (Multiplicity)**: When two nuclei are joined by a few chemical bonds, their spins might interact, resulting in signal splitting or multiplicity. The pattern of splitting is governed by the number of connected nuclei and the coupling constant (J), which is dictated by the kind of nuclei and the number of chemical bonds that separate them. The N+1 rule and Pascal's triangle can be used to anticipate the number of split peaks and their respective intensities.
- **Scalar Coupling:** Scalar Coupling happens when the number of chemical bonds separating two nuclei is less than four. It gives extra information on the structure and connectivity of the molecule. Scalar coupling can induce signal splitting and peak intensity decrease.

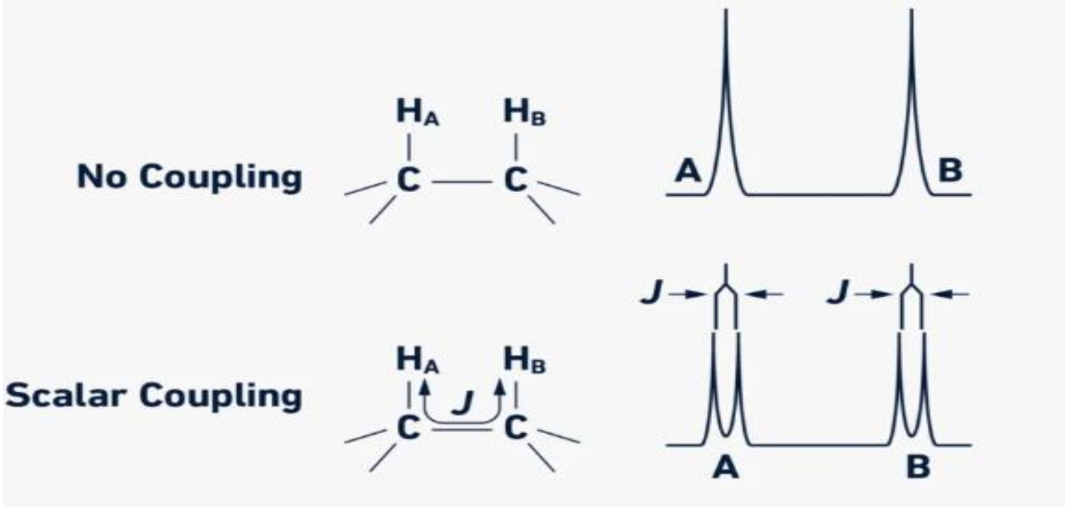
NMR spectrum



H solution NMR spectrum of acetic acid. The signals correspond to the molecule's two distinct H nuclei, and their areas are proportional to the number of nuclei contributing to the signal.

- **Nuclear Overhauser Effect (NOE):** The nuclear Overhauser effect (NOE) is found in NMR spectra and is caused by the interaction of nuclear spins between atoms that are spatially close yet distant in the molecular sequence. It is required for macromolecule structural determination.
- **Spectral Assignment:** When interpreting an NMR spectrum, each observable signal is assigned to the relevant atomic nucleus in the molecule(s) present in the sample. This technique, known as spectrum assignment, can be difficult for complicated compounds. To properly describe a material, many types of NMR investigations with diverse and complimentary information are frequently performed.

Example of a scalar coupling.



NMR signals from HA and HB emerge as simple peaks when there is no scalar coupling (top). The signals will divide if the two neighboring protons HA and HB exhibit scalar coupling with a constant J (bottom). Because both protons HA and HB are associated with one proton bound to a contiguous carbon nucleus, each proton signal will split into two signals, generating a doublet, with the split distance equal to the coupling constant, J.

Applications of NMR Spectroscopy

- **Quality Control:** NMR spectroscopy is widely used as an analytical technique in chemistry for quality control purposes. It helps in determining the composition and purity of a substance, ensuring its adherence to specific standards.

Applications of NMR Spectroscopy

- **Research and Structural Analysis:** NMR spectroscopy plays a crucial role in research for determining the content, purity, and molecular structure of a sample. It is used to quantitatively analyze mixtures containing known compounds. Chemists rely on one-dimensional NMR techniques for studying chemical structures, while two-dimensional techniques are employed for more complex molecules. NMR methods have also become a substitute for X-ray crystallography in determining protein structures.

Applications of NMR Spectroscopy

- **Molecular Dynamics:** Time domain NMR spectroscopy techniques are employed to study molecular dynamics in solution. These techniques provide insights into the behavior, conformational changes, phase transitions, solubility, and diffusion of molecules at the molecular level.
- **Solid-State Analysis:** Solid-state NMR spectroscopy is utilized to investigate the molecular structure of solids. It provides valuable information about the arrangement, interactions, and dynamics of molecules in solid materials.
- **Diffusion Measurements**: NMR spectroscopy offers methods for measuring diffusion coefficients, which are important in understanding the movement and distribution of molecules in various systems.