

SOLID STATE NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY – A REVIEW

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ABSTRACT

Solid state NMR is a kind of (NMR) spectroscopy characterized by the presence of anisotropic (directionally dependent) interactions. It is a powerful method for the characterization of solid materials and to determine the molecular structure of solids, particularly useful if a sample is insoluble, exhibits polymorphism or if there is a molecular change when it is dissolved. It is also applicable to heterogeneous samples. In this review, we reveal that the principle, instrumentation, and applications of solid state NMR.

Keywords: Nuclear magnetic resonance, Spectroscopy.

INTRODUCTION

Nuclear Magnetic Resonance (NMR) spectroscopy^{1,2} in the pharmaceutical research has been used primarily in a classical, organic chemistry frame work. Typical studies have been included the structure elucidation of compounds, investigating the chirality of drug substances, the determination of cellular metabolism and protein studies to name but a few. In each case, solution phase NMR has been utilized. Although 90 % of pharmaceutical products on the market exist in the solid form, solid state NMR (SSNMR) is in its infancy as applied to pharmaceutical problem solving and methods development.

Principle

The principle behind SSNMR³ is that many nuclei have spin and all nuclei are electrically charged. If an external magnetic field is applied, an energy transfer is possible between the base energy to a higher energy level (generally a single energy gap). The energy transfer takes place at a wavelength that corresponds to radio frequencies (Rf) and when the spin returns to its base level, energy is emitted at the same frequency. The signal that matches this transfer is measured in many ways and processed in order to yield an NMR spectrum for the nucleus concerned. Following figure shows the basic principle of NMR.

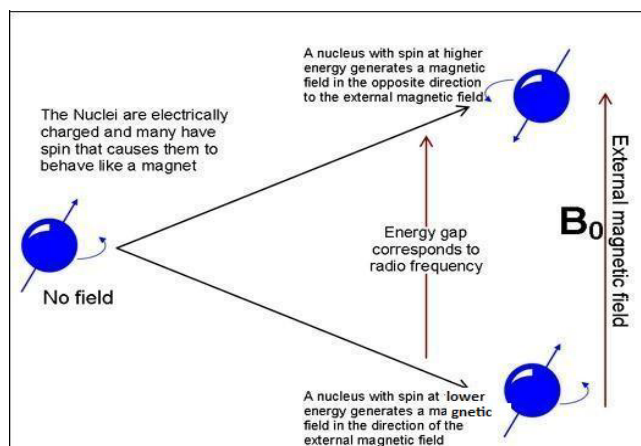


Fig. 1: SSNMR principle [Available at: <http://chem.ch.huji.ac.il/nmr/whatis-nmr/whatisnmr.html>]

In solution NMR, spectra consist of a series of very sharp transitions, due to averaging of Anisotropic NMR interactions⁴ by rapid random tumbling. By contrast, solid-state NMR spectra are very broad, as the full effects of Anisotropic or Orientation-dependent⁴ interactions are observed in the spectrum. Following

figure shows the differences between solution state NMR spectra and solid State NMR spectra of ^{13}C . In figure 2, The broad nature of the signal is primarily due to Dipolar interactions, which do not average out to zero in the solid state, and Chemical Shift Anisotropy (CSA).

Interactions in SSNMR

These interactions are orientation dependent. In media with no or little mobility (e.g. crystals, powders, large membrane vesicles, molecular aggregates), anisotropic interactions⁴ have a substantial influence on the behaviour of a system of nuclear spins⁵. Various Interactions in the SSNMR are: Dipolar interactions, Chemical shift Anisotropy, Quadrupolar interactions, Zeeman Interactions, and Spin-Spin Coupling or J-Coupling.

Dipolar Interactions or Dipolar Coupling

This coupling can be either Homo Nuclear or Heteronuclear. The dipolar interaction⁶ results from interaction of one nuclear spin with a magnetic field generated by another nuclear spin, and vice versa [Fig. 3]. Dipolar interactions are directly proportional to the magnetogyric ratios of the nuclei and inversely proportional (for r^3) to the distance between them.

Thus the Dipolar coupling constant of the j, k nuclei can be written as: $R_{jk} = (\mu_0 \gamma_j \gamma_k) / 4\pi r^3$ where as R_{jk} = interaction between j & k , μ_0 = magnetic moment. In organic solids, the hetero nuclear dipolar coupling between ^{13}C nucleus and bonded proton can be 40 kHz. In order to remove the hetero nuclear dipolar coupling, a strong Rf field equal to or greater than the interaction energy must be applied at the proton resonance frequency. The following figure shows the "Hetero Nuclear Dipolar Interaction" between hydrogen and carbon atom.

This line broadening factor arises from asymmetry in the electron density surrounding a given nucleus. In the magnetic field electrons that surround a nucleus produces a secondary field. This secondary field contributes to the total field felt at the nucleus, and therefore has the potential to change the resonance frequency of the nucleus. This interaction of the secondary field produced by the electrons with the nucleus is the shielding interaction. The frequency shift that this interaction causes in an NMR spectrum is the chemical shift. The observed spread of the chemical shifts is called the "Chemical Shift Anisotropy"⁷. The chemical shift anisotropy $\Delta\sigma$ is related to the term $(3\cos^2\theta - 1)$ This term could be zero when the angle θ is equal to $54^\circ 44'$. This angle is called magic angle $\Delta\sigma \propto ((3\cos^2\theta - 1)/r^3)$. In other words, the chemical shift of a nucleus is the difference between the resonance frequency of the nucleus and a standard, relative to the standard. This quantity is reported in ppm and given the symbol δ . $\delta = (\nu - \nu_{\text{REF}}) \times 10^6 / \nu_{\text{REF}}$. In NMR spectroscopy, this standard is often Tetramethylsilane, $\text{Si}(\text{CH}_3)_4$, abbreviated TMS. The chemical shift is a very precise metric of the chemical environment around a nucleus.

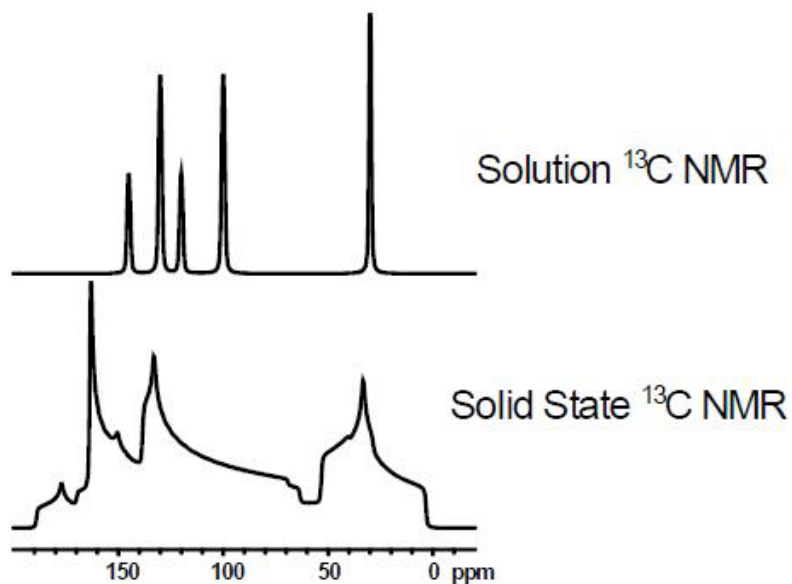


Fig. 2: Comparison between spectra of solution ^{13}C NMR and solid state ^{13}C NMR [Available at: http://mutuslab.cs.uwindsor.ca/schurko/ssnmr/ssnmr_schurko.pdf]

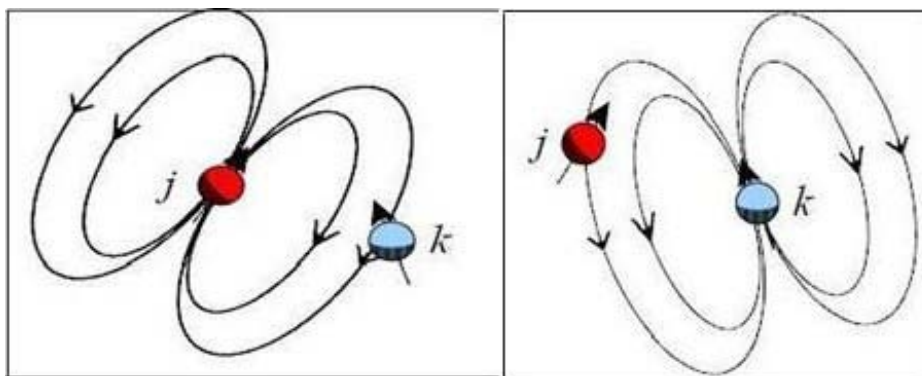


Fig. 3: Dipolar coupling between Nuclei J and K. [Available at: http://mutu-slab.cs.uwindsor.ca/schurko/ssnmr/ssnmr_schurko.pdf]

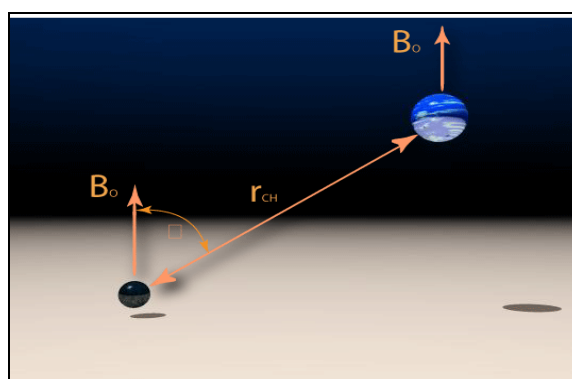


Fig. 4: Hetero nuclear dipolar coupling between ^1H and ^{13}C in the magnetic field (B_0). [Available at: http://www.emory.edu/NMR/Hall/solid/Theory_basic.htm]

Chemical Shift Anisotropy (CSA) ⁷ :

Quadrupolar Interactions⁸

Quadrupole nuclei have spin $> \frac{1}{2}$ and have an "Electric Quadrupole Moment"; can be characterized by asymmetric distribution of nucleons and Non spherical positive electric charge distribution; Interacts with electric field gradients. Size of the quadrupolar interaction depends on Nucleus and symmetry of site. Quadrupolar interactions can be as large as several MHz. For nonintegral spin quadrupolar nuclei, the central transition is much narrower (about 10 kHz) and therefore can be narrowed to a single, narrow line by magic angle spinning. The

residual (second order) line width of the central transition is inversely proportional to the applied magnetic field.

Zeeman Interaction⁹

It is the interaction between the magnetic moment of the nucleus and the externally applied magnetic field. The nuclear magnetic moment, μ (ampere meter²), is proportional to the nuclear spin quantum number (I) and the magnetogyric ratio (γ : radian tesla⁻¹ second⁻¹). $\mu = \gamma I \hbar / 2\pi$. Thus the Zeeman interaction occurs only with

nuclei which possess a spin greater than zero and yields $2I + 1$ energy levels of separation. Since the Zeeman interaction incorporates the magnetogyric ratio, a constant for each particular nucleus, the frequency for each nucleus is different at a specific applied magnetic field.

Spin-Spin Coupling or J coupling^{10,11}

Nuclei experiencing the same chemical environment or chemical shift are called equivalent. Those nuclei experiencing different environment or having different chemical shifts are non equivalent. Nuclei which are close to one another exert an influence on each other's effective magnetic field. This effect shows up in the NMR spectrum when the nuclei are non equivalent. If the distance between non-equivalent nuclei is less than or equal to three bond lengths, this effect is observable. This effect is called spin-spin coupling or J coupling.

Most Important Methods of SSNMR

A number of methods have been developed and considered in order to minimize large anisotropic interactions between nuclei and to increase S/N in rare spin (e.g., ^{13}C , ^{15}N) NMR spectra. Those are: Magic angle spinning, Cross polarization, Dilution, Multiple Pulse Sequence, CP-MAS (Cross Polarization and Magic angle Spinning), CRAMPS (Combined Rotation And Multiple Pulse Sequence)

Magic angle spinning (Sample rotation)¹²

This technique was discovered independently by both Andrew and Lowe. Magic-angle spinning introduces artificial motion by placing the axis of the sample rotor at the magic angle (54.74°) with respect to B_0 . It is used to average the chemical shift anisotropy (ω). $\omega \propto \frac{1}{2}(3\cos^2\theta - 1)$. The term $3\cos^2\theta - 1 = 0$ when $\theta = 54.74^\circ$. The rate of MAS must be greater than or equal to the magnitude of the anisotropic interaction to average it to zero.

Cross polarization (CP)

Cross polarization¹³ is one of the most important techniques in Solid State NMR and provides a four-fold signal enhancement for ^{13}C experiments. This concept was implemented by Pines et al. In this technique, polarization from abundant spins such as ^1H or ^{19}F is transferred to dilute spins such as ^{13}C or ^{15}N . The overall effect is to enhance S/N (signal to noise ratio) and to induce rapid spin-lattice relaxation at the nuclei. CP consists of bringing the heteronuclei into a dipolar thermal contact by adjusting their precession rate (Hartman-

Hahn match), during a time called "contact time", during this time the low frequency nuclei gets polarized.

Dilution¹³

This technique is used to minimize anisotropic interactions and to increase S/N ratio of rare spin NMR spectra. This only leads to "high-resolution" spectra if there are no heteronuclear dipolar interactions (i.e., with protons, fluorine).

Multiple-Pulse Sequences

Pulse sequences¹⁴ can impose artificial motion on the spin operators (leaving the spatial operators, *vide infra*) intact. Multiple pulse sequences are used for both heteronuclear (very common) and homonuclear (less common) decoupling.

SSNMR Instrumentation¹⁵

Sample holder

The sample holders generally used are glass tubes, which are 7.5. Cms long and has 0.3 cms diameter. They must be chemically inert, durable and transparent to radio frequency radiation¹⁵.

Magnet

- Electromagnet or Permanent magnets for spectrometers operating up to 100 MHz.
- Super Conducting magnets for spectrometers operating at 230 MHz.
- The strength and direction of the magnet should not change from point to point. Strength must be 20,000 gauss.

Sweep Generator

For obtaining a signal, the nucleus must undergo resonance. In order to make the nucleus resonate, the precession frequency of the nucleus should become equal to the frequency of the applied radio frequency field H_0 . If the frequency of the field is kept constant then, precession frequency of the nucleus must be changed by varying the strength of the magnetic field in order to get the resonance of Nucleus. This method is known as Field Sweep method. The instrument used for this method is known as Sweep generator. The variation in the strength of the magnetic field is obtained by fixing a pair of Helmholtz coil in the pole faces of the main magnet¹⁵.

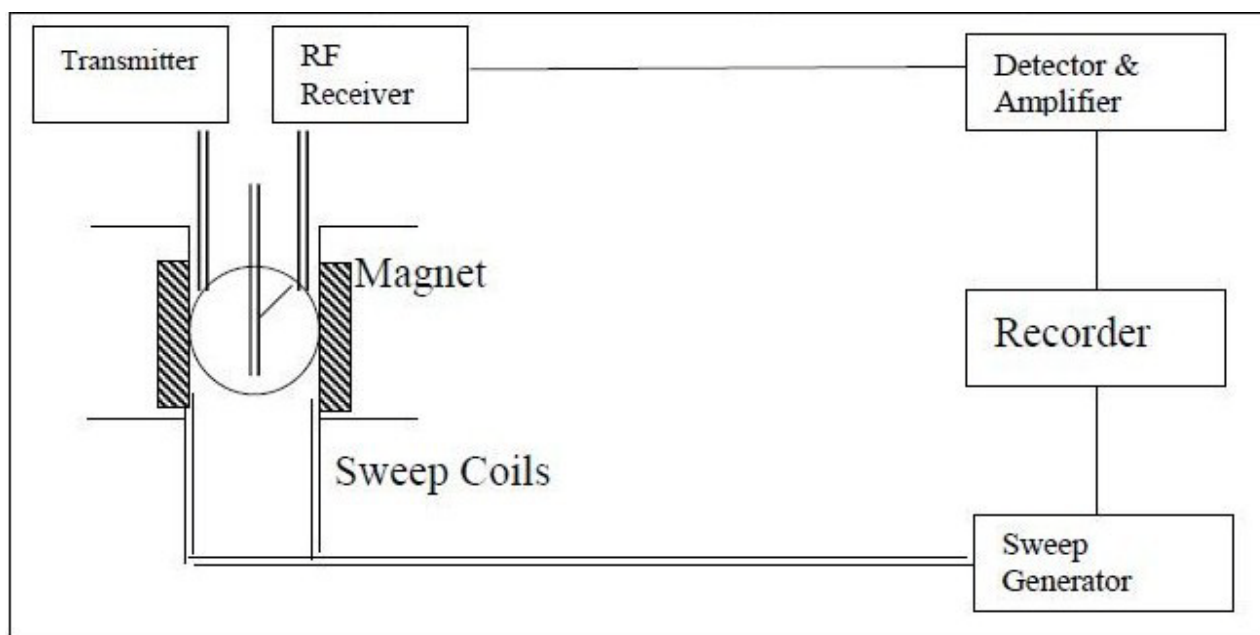


Fig. 6: Schematic diagram of the SSNMR spectrometer [Ref: Gurdeep R.Chatwal, ShamK.Anand, "Instrumental Methods of Chemical analysis" 2008, page no.2.205]

Radio Frequency Receiver

When the Radio frequency is passed through the sample, absorption and dispersion takes place. RF Receiver receives both absorption and dispersion signals.

Detector

Detector separates absorption signal from dispersion signal by one of the following two methods. Those are Radio frequency bridge method and Nuclear induction method

Readout system

The absorption signal received from radio frequency receiver and Detector, is then amplified and fed in to a chart recorder.

Other specific Hardware ¹⁶ used for high resolution in SSNMR:

High-Power Amplifiers

Due to the large spectral widths often encountered in SSNMR spectra, intense radiofrequency (rf) pulses are required on the spectrometer observe channel to excite uniformly the entire spectral range and produce distortion-free spectra.

High-Speed Digitizer

Since the NMR spectrum arising from nuclear spins in a powder sample often spans a very large frequency range, instruments for solids are generally equipped with high-speed digitizers capable of much faster sampling rates than spectrometers designed specifically to examine liquids.

Magic-Angle Spinning (MAS) Hardware and Equipment

Sample spinning at a precisely defined speed and angle places further stringent design requirements on a solid-state NMR instrument. A critical component is the MAS speed controller ¹⁶, which supplies the spinning gases as well as monitors and controls the spinning speed. Quite often the gas is compressed dry air; although, for low and high temperature experiments, nitrogen gas should be used, especially to avoid oxidation at high temperatures.

High-Power Probes

In recent years, High-power probes for standard bore magnets (54 mm) have become available. The most common probe head found in a solid-state NMR lab is a double-resonance probe capable of cross-polarization and MAS experiments in conjunction with high-power proton decoupling.

SSNMR Applications

In biology

Solid-state NMR is one of the most useful techniques for probing the structure and dynamics of DNA. Since it can measure orientational as well as distance information, it is a useful complement to liquid-state NMR and crystallographic methods. ¹⁷

- Solid State NMR of DNA Fibres: Orientation of phosphate groups and Molecular conformation of different forms of DNA can be deduced by using ³¹P Solid state NMR techniques ¹⁸.
- SSNMR technique is helpful in Identifying active compounds, such as enzyme inhibitors, receptor antagonists and peptide agents, that prevent the aggregation of amyloid proteins involved in neurodegenerative diseases.
- Resolving drug structures at the site of action : Solid State NMR in combination with site directed mutagenesis (SDM) and bioinformatics approaches is used for resolving binding sites. The intramolecular drug structures at the target site can be resolved with high resolution as can the electronic environment.
- NMR Spectra of Specifically Labelled Membrane Proteins: Solid-state NMR is a uniquely suited technique to determine the structure of specifically labelled membrane peptides and proteins.

- Biological Membrane Structure By Solid-State NMR: Nuclear magnetic resonance (NMR) spectroscopy, and particularly solid-state NMR spectroscopy, is a method of choice to study the structure and dynamics of both the lipid and the protein components of model and biological membranes ¹⁹.
- Quantification (with site specificity and atomic resolution), Orientation and dynamics of side chains in synthetic model peptides adsorbed onto polystyrene (PS) surfaces can be studied by using SSNMR. Ex: The 14 amino acid leucine-lysine peptide is known to form an α -helical secondary structure at liquid-solid interfaces ²⁰.
- Solid-State NMR Spectroscopy of Membrane-Associated Myelin Basic Protein—Myelin basic protein (MBP) maintains the tight multi lamellar compaction of the myelin sheath in the central nervous system through peripheral binding of adjacent lipid bilayers of oligo dendrocytes. Myelin instability in multiple sclerosis (MS) is associated with the loss of positive charge in MBP as a result of post translational enzymatic deimination. A highly conserved central membrane-binding fragment (murine N81-PVVHFFKNIVTPRTPPP-S99, identical to human N83-S101) represents a primary immune dominant epitope in MS. Magic-angle spinning solid-State NMR spectroscopy can be used to characterize the molecular conformation and dynamics of this central immune dominant epitope of MBP in a lipid milieu ²¹.
- Human α -defensins [human neutrophil peptides (HNPs)] are immune defence mini-proteins that act by disrupting microbial cell membranes. Elucidating the three-dimensional (3D) structures of HNPs in lipid membranes is important for understanding their mechanism of action. Solid-state NMR (SSNMR), can be used to determine the 3D structure of HNP-1 in a microcrystalline state outside the lipid membrane, which provides benchmarks for structure determination and comparison with the membrane-bound state ²².
- Cell Walls, Biomaterials, and More: An ultimate goal in biomolecular spectroscopy and microscopy is to bridge the gap between structural and cell biology. Solid-state NMR spectroscopy is a method that is capable of providing atomic information under such in situ conditions and has already been utilized in this direction for more than two decades.
- Amyloids and Other Aggregates: SSNMR spectroscopy has become the premier structural method to study amyloid fibrils. Three-dimensional structures have not only been obtained for several amyloid proteins of medium size but high-resolution studies were also possible for significantly larger proteins, such as those related to α -synuclein and even the full-length HET-s prion.

In drug design

- SSNMR (solid-state NMR) is proving to be a powerful tool for the structural analysis of membrane proteins and amyloid fibrils and is attracting attention as a potentially valuable counterpart to the conventional methods for struct. based drug design ²³.
- Mapping the binding sites for ligands in membrane-embedded receptors using a combination of CP-MAS NMR and site-directed mutagenesis: A convenient starting point in struct. based drug discovery is to identify where the native ligand or an unoptimized lead compound interacts with its target and to produce a three-dimensional model of the ligand within the binding pocket. The SSNMR method of CP-MAS (cross-polarization magic-angle spinning) can be used to obtain high-resolution spectra from which to detect weak interactions of ligands with receptors embedded in native membrane or isolated and incorporated into defined lipid bilayers ²⁴.
- Structures of bound ligands: SSNMR can provide information about the molecular conformations of native ligands, lead compounds and drugs bound to target receptors. Such

information can be exploited to refine the structures of known ligands to improve binding affinity or efficacy²⁵.

- Struct. Based design of amyloid Inhibitors: All amyloid fibrils contain repeat arrays of β -strands arranged with inter strand hydrogen bonds parallel to the fibril long axis. The three-dimensional architecture of amyloid fibrils varies from protein to protein, but involves a complex network of electrostatic, vander Waals and π - π interactions between amino acid side groups, which bring together laminae of β -sheets arranged with water-exposed faces and water excluded cores. SSNMR is the method of choice for probing polypeptide structures and intermolecular contacts in intact amyloid fibrils and could therefore be useful in drug design.
- An attractive therapeutic strategy for preventing amyloid deposition in brain and other tissues is to identify agents that interfere with the fibril growth process by arresting the onset or propagation of fibril growth. Information about the structural features of an amyloid species could help to identify inhibitors of amyloidogenic proteins. SSNMR can assist in this process by identifying specific interactions between amino acid side groups that could be targeted by agents aimed at destabilizing the growing fibrils or preventing the lateral association of β -sheet layers.
- To determine the structural changes after binding a small molecule at their site of action.
- To intimate dynamic information and electronic details for bound molecules.

- To investigate the details of the binding sites.
- NMR can reveal important details about the major secondary structure elements of receptor molecule²⁵⁻²⁷.

SSNMR Pharmaceutical Applications

- Solid-state nuclear magnetic resonance (SSNMR) has recently attracted growing interest for the characterization of pharmaceutical compounds in the solid state. This is primarily due to considerable improvements in the instrumentation and to the fact that about 80% of the drugs currently available on the market are present in the solid form. SSNMR allows not only the chemical structure but also the physical properties (polymorphism, multiple molecules per asymmetric unit cell, disorder) of the pharmaceutical substances to be investigated²⁸.
- Solid-state NMR spectroscopy (SSNMR) is an extremely powerful technique for the analysis of pharmaceutical dosage forms. The most widely used application of solid state NMR in the pharmaceutical industry as in the area of polymorphism or pseudopolymorphism. Solid state NMR is an important tool in conjunction with thermal analysis, optical microscopy, IR spectroscopy, and powder and single-crystal X-ray crystallographic techniques for the study of polymorphism. The investigation of polymorphism by solid-state NMR can be performed at either the bulk drug or the dosage form level. This ability provides a significant tool for the investigation of polymorphic conversion under various processing techniques (e.g. various fluidized-bed granulation, dry blending, lyophilisation, and tableting conditions)²⁹.

Table 1: Various types of pharmaceutical samples investigated by solid-state NMR spectroscopy

S. No.	Sample	NMR Nucleus	Brief description of studies
1.	Bulk drug	¹³ C, ³¹ P, ¹⁵ N, ²⁵ Mg, ²³ Na	Solid state structure elucidation, Drug excipient interaction studies (variable temperatures), (Pseudo) Polymorphic characterization at qualitative and quantitative level, Investigation of hydrogen bonding with salt compounds.
2.	Excipients	¹³ C, ²⁵ Mg, ²³ Na	Determination of the degree of cross linking for polymer systems, Drug excipient interaction studies (variable temperatures), (Pseudo) Polymorphic characterization at qualitative and quantitative level.
3.	Capsules	¹³ C, ³¹ P	Identifying the residue from the dissolution baths, Drug capsule interaction studies.
4.	Tablets	¹³ C, ³¹ P,	Drug excipient interaction studies, (Pseudo) Polymorphic characterization at qualitative and quantitative level

The SSNMR of following drugs have been performed

- The crystalline forms of prednisolone t-butyl acetate.
- The pseudopolymorphic forms of testosterone, Androstanolone.
- Analgesic drugs, Methionine, Acetaminophen, Cyclopenthiiazide, Cortisone acetate.
- Polymorphic forms of 4-methyl, 2- nitro acetanilide.
- The conformational analysis of pharmaceutical compounds [e.g. Three different forms of Tartaric acid (+ isomer, Racemic mixture, meso form)].
- Drug excipient interactions have been studied by using various aspirin samples.
- The ³¹P MAS NMR spectra of N-[(diphosono)methyl]-3-isobutyl thio-morpholine.
- Differences in the SSNMR spectra for different polymorphic forms of benoxaprofen and nabilone and pseudopolymorphic forms of cefazolin.
- Quantitative ¹³C CP/MAS NMR has been used to determine the relative amounts of carbamazepine anhydride and carbamazepine di hydrate in mixtures

- Quantitative solid state ³¹P NMR has been applied to enantiomeric purity of organo phosphorus compounds.
- Quantification of amorphous content in a binary mixture of crystalline and amorphous phase.
- Quantification of amorphous Trehalose.
- The SSNMR results suggested that this method would be useful for comparing solid and solution state conformations of molecules.
- The solid dispersion approach is a well-known method to increase the bioavailability of poorly water- soluble drugs, and solid-state nuclear magnetic resonance (SSNMR) is a valuable tool for characterizing both molecular structure and mobility of such dispersions. POLYOX™ WSP, polyethylene oxide, has been previously evaluated as a matrix for solid dispersion applications³⁰.
- It is a non-destructive, non-invasive technique that can be employed to simultaneously examine the physical and chemical states of both the active pharmaceutical ingredient (API) and the excipients present as they exist within the formulation³¹.
- Other applications of SSNMR:

- Analysis of ceramic fibres: There has been considerable interest in the use of ceramic fibres as reinforcement in high temperature composite materials. Characterization of these materials, is often difficult by traditional methods since they are insoluble, predominantly amorphous and often present experimental difficulties due to their sample state. Solid state NMR is being widely used to probe these materials³².
- Structure of SiAlON ceramics: "SiAlON" ceramics are materials composed of three dimensional arrangements of (SiAl)(ON)₄ tetrahedral and occasionally contain aluminium in a six fold octahedral coordination. These ceramics are of interest because of their thermal and mechanical properties. Properties such as the glass transition temperature, hardness and elastic modulus are dependent on the substitution of nitrogen for oxygen in the local structure of oxynitride glasses. Solid state NMR is a new and powerful method for probing the structure and chemistry of these materials³³.
- Determination of phase composition in silicon nitride powders: Silicon nitride (Si₃N₄) is an important ceramic, both for its own use and as starting material in the production of other ceramics and composites. In typical Si₃N₄ precursor powders, all three phases as well as impurities such as silicon oxy nitrides and silicates may be present. The physical properties of Si₃N₄ ceramics are very sensitive to the composition of the precursor powder. For this reason, SSNMR can be used to determine the composition of batches of Si₃N₄ powder before carrying out final product formation and sintering³⁴.
- Solid state NMR has been applied to: Organic complexes, Inorganic complexes, Zeolites, Mesoporous solids, Microporous solids, Aluminosilicates/Phosphates, minerals, Biological molecules, Glasses, Cements, Food products, Bones, Semiconductors, Metals and Alloys, Archaeological specimens, Polymers, Resins^{35,36}.

CONCLUSION

SSNMR is a powerful method for the characterization of solid materials, particularly useful if a sample is insoluble, exhibits polymorphism or if there is a molecular change when it is dissolved. It is also an important and useful technique for identification and characterization of APIs (Active Pharmaceutical Ingredients) in the pharmaceutical industry. It is sensitive even for small amounts of active ingredient present in the formulations and can be used as a complementary tool for the study of polymorphism in such materials. It is applicable to heterogeneous samples and there are experiments to discriminate between different components.

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