NMR as unique and complementary tool for studying structure and dynamics

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Nuclear magnetic resonance (NMR) spectroscopy is a powerful tool providing atomic-level structural information that finds applications in almost all fields of natural science, from chemistry to biochemistry and pharmacy, materials science, geology and physics. NMR is a technique based on the absorption of radiofrequency radiation by atomic nuclei in the presence of a strong external magnetic field. Solid-state NMR has become established as an important method for structural characterization in chemistry, biology and the materials sciences. Although not as routinely utilized as solution NMR, solid-state NMR has found wide applicability, especially in the study of inorganic materials (*e.g.* zeolites, polymers) and also in the field of biological systems such as tissues, membrane proteins, fibres and protein aggregates. Solid-state NMR is able to provide relevant information regarding structure, morphology, heterogeneity or dynamic processes of these samples. The recent applications of solid-state NMR in areas such as biosolids, heterogeneous catalysis, and nanotechnology have shown the promise of obtaining detailed information in these nonclassical systems such as its use in polymeric and supramolecular systems, on its use in organic and inorganic multicomponent materials and on its use in biomolecular systems.

One of the most powerful attributes of NMR as a tool for investigating chemical structure is the ability to access many different nuclides and hence to study a wide variety of elements (small selection presented in Table 1). Indeed, all elements with non-radioactive isotopes have at least one magnetic nuclide. Moreover, NMR is isotope-specific in that each nuclide has its own resonance range in a given magnetic field and these overlap only in extremely rare cases. Thus, the different elements contained in a given compound may all be suitable for NMR study, the separate spectra providing complementary information.

Table 1. Some relevant properties of spin-1/2 nuclei

Isotope	Natural abundance (%)	Gyromagnetic ratio γ (10^7 rad s^{-1} T^{-1})	Frequency ratio	Relative receptivity$
^{1}H	99,9885	26,7522128	100,000000	1,000
^{29}Si	4,6832	-5,3190	19,867187	$3{,}68\times10^{-4}$
^{13}C	1,07	6,728284	25,145020	$1{,}70\times10^{-4}$
^{31}P	100	10,8394	40,480742	$6{,}65\times10^{-2}$
^{19}F	100	25,18148	94,094011	0,834
^{15}N	0,368	-2,71261804	10,136767	$3{,}84\times10^{-6}$

[$] Receptivity of a nucleus at natural abundance relative to that of ^{1}H, proportional to $[\gamma^3 I(I + 1)]$, where γ is the gyromagnetic ratio of the nucleus and I the nuclear spin quantum number.

The molecular Brownian motions in solution have the effect of averaging to zero several intrinsic properties of the nuclei, thus cancelling out any possible effects in the NMR spectra. In the solid-state the orientation of molecules is not random but ordered, and different parameters have to be taken into account for a full comprehension of the NMR data. For instance, the NMR spectra of any molecule acquired both in solution and in the solid-state will show apparent differences in terms of signal resolution. The most conspicuous is the very broad signals observed in the solid state occupying the whole chemical shift range of the nuclei and without a clear baseline. This broadening is caused by anisotropic spin interactions that are not averaged to zero in the solid state. There are different physical mechanisms that contribute to the broadening, the dominant one being the dipole–dipole interaction affecting the local field seen by the nuclei I that can be expressed as $B_{loc} = \pm\mu_S r_{IS} - (3\cos^2\theta_{IS} - 1)$, where μ_S is the magnetic moment of nuclei S, r is the internuclear distance between nuclei I and S and θ_{IS} is the angle between the internuclear vector and the local field (Figure 1). The angular dependence is not averaged in solids and leads to the broad signals commonly observed. Also, depending on the values of the magnitude described above, the splitting caused by the dipole–dipole interaction can reach figures in the range of tens of kilohertz. Relaxation via the chemical shift anisotropy (CSA) mechanism also contributes to broadening, although to a lesser extent, which also presents a variation with the θ angle with the same $(3\cos^2\theta_{IS} - 1)$ dependence. One of the main implementations to eliminate the angle dependence in solids NMR is via the application of the magic angle spinning or MAS. The NMR sample rotates at an angle of 54°44' resulting in the factor $(3\cos^2\theta_{IS} - 1)$ taking a zero value, thus 'magically' removing the angle parameter from both the dipole–dipole and CSA broadening mechanisms and producing much sharper NMR signals.

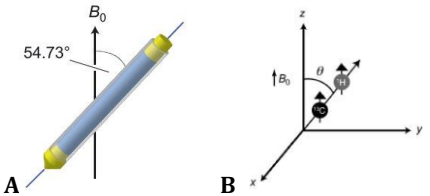


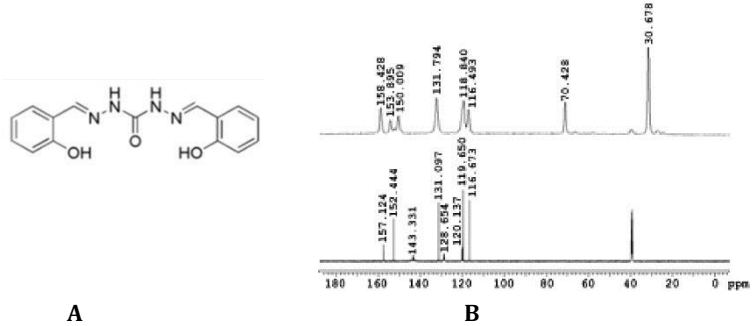
Figure 1. Magic angle. (A) Sample is span at the magic angle with respect to external magnetic field. (B) θ is the angle between the ^{1}H-^{13}C bond vector and the direction of the direction of the external magnetic field B_0.

In practical terms, for the magic angle spinning to produce good results the rotation frequency must be of the order of the CSA linewidth (*i.e.* a few kilohertz, 2–100 kHz). Dipolar broadening also benefits from the MAS application, although it is commonly necessary to include a decoupling block at the end of the pulse sequences to achieve complete elimination. Because of the large dipolar interaction, the power needed for the decoupling is much higher than the values used normally in solution-state NMR and different methods are used to avoid overheating the sample.

Solid-state NMR suffers from the same insensitivity as solution NMR experiments, and several approaches are applied in order to enhance the NMR signal in solids. One of the most popular is the cross polarization (CP) method, which achieves the transfer of

polarization from a high γ nucleus to a low γ one using the dipolar coupling between them. The source of polarization is typically the very sensitive ^{1}H spin, and by using the CP the sensitivity of the nuclei S to which it is dipolar coupled can be enhanced by a factor of up to γ_H/γ_S. The CP pulses extend for several milliseconds in contrast to the microsecond duration used in solution state experiments. During these CP pulses, cross relaxation is transferred between the coupled nuclei such as ^{1}H and ^{13}C or ^{15}N, with the effect of increasing the magnetization of the heteronuclei. Because the T_1 relaxation time of the ^{1}H is generally much shorter than that of the S nucleus, the pulse sequence can be repeated much faster (more accumulations within a given experiment time) with the consequent improvement in sensitivity. For the CP to work experimentally, a match has to be reached between the radiofrequency fields of the ^{1}H and the S nucleus (the called Hartmann-Hahn condition): $\gamma_H B_H = \gamma_S B_S$. Additional sensitivity enhancements can be obtained by including dipolar decoupling on the ^{1}H frequency channel during FID recording as well as applying MAS to sharpen the NMR signals.

Application of the NMR methods to solids provides much more information than for liquids or gases (Figure 2). The matter is that interactions, which determine NMR spectra of solids, in contrast to interactions in liquids, keep anisotropic properties. The main interactions are dipole–dipole interactions, quadrupole interactions, and interactions of nuclear magnetic moments with magnetic moments of electrons or paramagnetic particles.



A **B**

Figure 2. 1,5-Bis(salicylidene)carbohydrazide (A) and (B) ^{13}C CP-MAS spectrum of its form V (top) and ^{13}C spectrum in DMSO-d6 solution (bottom). (M. Rubčić, 2014)

Dipole–dipole interactions between nuclear magnetic moments create additional local magnetic fields at the nucleus sites that result in splitting and broadening of NMR lines. These interactions usually are the most important in solids and play the key role in structural studies. However, they also can mask other interactions that prevent extraction of additional information. If this is a case, high resolution NMR techniques should be applied.

Quadrupole interactions, which are the interactions between nucleus quadrupole moments and electric field gradients, are observed for nuclei with spins $I > 1/2$ situated in noncubic lattice sites. These interactions can be both greater and smaller the dipole–dipole ones. Moreover, in several cases they can be much greater the Zeeman interactions of nucleus magnetic moments with an external magnetic field. Quadrupole interactions lead to resonance NMR lines split and shift. The latter is the most important difference between the quadrupole and dipole–dipole interactions.

Solid pharmaceutical products like tablets and capsules represent almost 90% of all pharmaceutical dosage forms on the market since they possess several advantages in comparison to other formulations. First, active pharmaceutical ingredient (API) and other compounds, excipients, which are included in a formulation, are most stable as dry solids. Second, compact forms make them more convenient for oral administration that results in better patient compliance. Third, the manufacturing process, like packing, transportation and storing is easier for solid pharmaceutical products. In addition, controlled release of drug is facilitating by solid than with liquid dosage forms. The inner structure of the tablet in terms of the particle arrangement in the three dimensional space and their interstices influence tablet's properties and depends on characteristics of API, excipients and on manufacturing process. Sometimes the extreme conditions of processing the formulation into the dosage form can alter the API or increase its interactions with excipients. API can exist in different solid forms. For instance, it may crystallize in more than one polymorphic form. Different inter- and intramolecular interactions such as van der Waals interactions and hydrogen bonds can be present in different polymorphs, which alter solubility, bioavailability, chemical stability, etc. NMR studies can be performed in solid state not only at the bulk level, but also in the dosage form (Figure 3).

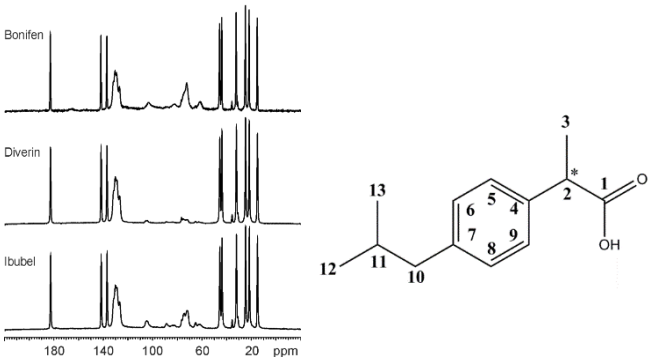


Figure 3. (A) ^{13}C CP-MAS spectra of three different tablet samples of ibuprofen. Sample identify is marked on the left of each spectrum. (B) Structure of ibuprofen, (RS)-2-(4-(2-methylpropyl) phenyl)propanoic acid, commonly used nonsteroidal anti-inflammatory drug, which is used in the treatment of pain, fever and rheumatic disorders. (A. Kotar, 2015)

Solid-state NMR spectroscopy is a powerful tool widely applied to nearly all aspects of pharmaceutical chemistry: characterization of API, the drug products and excipients in solid dispersions as well as exploration of interactions between API and excipients in solid dispersions and between API and membrane proteins. Solid-state NMR is a non-destructive, non-invasive, multinuclear technique that can probe the local chemical environment of specific nuclei within molecules. Different shielding/deshielding of NMR signals arising for instance from (carbon) atoms of API and excipients allowing survey of polymorphism determination within the formulation. This technique provides not only the chemical structure but also physical properties such as polymorphism, co-crystallization, solvation, multiple molecules per asymmetric unit cell, disorder, intra- and intermolecular hydrogen bonding, tautomerism and local conformation changes. Solid-state NMR can provide information about chemical stability of API and excipients, which is of great

importance in evaluation of degradation processes of pharmaceuticals in the solid state. Water molecules that are adsorbed at surface of tablet or localized in the interior of crystal lattice can be also defined by solid-state NMR. In addition, insight into interactions of water with API and excipients provide information on its residence times and molecular mobility. The NMR spectra obtained by solid-state NMR contain qualitative and quantitative information about investigated samples. The gained information is useful in pharmaceutical industry during the quality control and stability studies. Solid-state NMR affords information at the atomic level. Complementary methods that offer microscopic insights regarding the morphology, size and shape in the process solid-state characterization include differential scanning calorimetry, thermogravimetric analysis, FT-IR, Raman spectroscopy, powder X-ray diffraction and optical and electron microscopies. The advantages of using solid-state NMR experiments in characterization of pharmaceuticals over traditional techniques are the following:

- non-destructive and non-invasive analysis of bulk drugs and formulations
- quantitation of forms
- investigation of low levels of drugs in formulations with isotopic labeling
- determination of the conformation and the arrangement of molecules
- study of molecular dynamics.

Studies of protein structure are important in understanding protein function. It is well known that structural differences are expected between liquids and solids. Many biological membranes are thermotropic and lyotropic liquid crystals. The membrane, composed of lipids and proteins, is the site of many crucial biological mechanisms, such as cell recognition, signal propagation, cell fusion, cancer, Alzheimer and prion processes. Many techniques are inoperative in such anisotropic media and solid-state NMR has demonstrated its potential and fascinating applications for example in the field of membrane proteins, or even in tackling complex systems such as the structure of whole intact virus.

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