### Identification Techniques I

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Contemporary procedures for spectra evaluation can be subdivided into two
groups. Unsupervised procedures are employed for explorative data analysis or for
empirical investigation of samples with no additional information available, e.g.
cluster analysis (CA) and principal component analysis (PCA) (Fig. 3.40).
Supervised procedures are used to assign new objects to already established
classes (available additional knowledge), e.g. discriminant analysis (DA) and
SIMCA. All these procedures nowadays are absolutely essential, in particular
when large amounts of data like in spectroscopic imaging have to be dealt with.

3.4 Solid State NMR

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3.4.1 Introduction

Nuclear magnetic resonance (NMR) is one of the most powerful and versatile
techniques in many scientific fields. It can be applied to a wide variety of samples,
both in liquid and in solid phase. Recently NMR technique has also been de-
veloped to investigate soft matter.

In this chapter the physical principles of NMR, a brief description of the
instrument features and an evaluation of NMR technique in the field of cultural
heritage, will be presented.

As conservation science requires nondestructive and minimally invasive
investigation methods, only solid state NMR (ss-NMR) technique will be deep-
ened. This because liquid state NMR, even if more established, is a destructive
technique in which the sample must be dissolved in a solvent.

The NMR phenomenon consists in the absorption of energy in the range of
radio frequencies by atomic nuclei subjected to a static magnetic field $B_0$.
The analysis of an NMR spectrum can provide many types of information such
as the number and type of chemical species in a molecule, rather as infrared
3.4.2 Principles and Concepts

The physical NMR phenomenon depends on the fact that many nuclei possess an angular momentum called nuclear spin. When a sample is placed in a magnet, nuclear spins generate a bulk macroscopic magnetisation. The simplest experiment consists in perturbing the system from the equilibrium applying a proper radio-frequency pulse and then monitoring the response of the system to the applied perturbation. The so-called NMR-active nuclei are those nuclei having a nuclear spin $I \neq 0$. The number $N$ of energy levels generated by Zeeman effect for a certain nucleus in the presence of a static magnetic field $B_0$ depends on the nuclear spin $I$ and can be calculated using the following equation:

$$N = 2I + 1$$  \hspace{1cm} (3.1)

Eq. 3.1. The number of nuclear energy levels calculated from the nuclear spin $I$.

Because NMR signals are observed when transitions among energy levels occur, it is evident that nuclei with $I = 0$ do not give rise to any NMR signal. To simplify the discussion, in the following only nuclei with $I = \frac{1}{2}$ will be considered. These nuclei generate only two energy levels, namely $m_I = + \frac{1}{2}$ and $m_I = - \frac{1}{2}$, where $m_I$ is the spin quantum number. The energy difference $\Delta E$ between the two levels depends on the applied magnetic field $B_0$ and it is described by Eq. 3.2:

$$\Delta E = \gamma h B_0 = h v_L$$  \hspace{1cm} (3.2)

Eq. 3.2. Energy difference between the two levels generated by Zeeman effect for a nucleus with $I = \frac{1}{2}$. $\gamma$ is the gyromagnetic ratio and it is a constant characteristic for each nucleus, $h$ is the Plank constant divided by $2\pi$, and $v_L$ is the Larmor frequency specific to each nuclear species.

The frequency $v_L$ known as Larmor frequency, is specific to each nuclear species. In the absence of magnetic field, all nuclear magnetic moments $\mu_i$ are randomly oriented. Since nuclei possess a magnetic moment, when a strong external magnetic field is applied along the $z$-axis, they align either against or with it, with a small excess of nuclei (population excess) aligned with the field.

As a result a bulk magnetisation $M = \sum_{i=1}^{n} \mu_i$ aligned with the applied field is generated. However, the only presence of $B_0$ is not sufficient to observe the NMR signal. This point may be easily explained calculating the ratio between the populations $n_1$ and $n_2$ of the two energy levels ($E_1$ and $E_2$) which is determined by the Boltzmann distribution. This ratio is obtained from Eq. 3.3:

$$\frac{n_1}{n_2} = e^{-\frac{\Delta E}{kT}} \approx 1 + \frac{\mu B_0}{kT}$$  \hspace{1cm} (3.3)

Eq. 3.3. The Boltzmann distribution: $n_1/n_2$ is the ratio between the population of the two levels. $\Delta E = E_1 - E_2$ is the energy difference between the two levels, $T$ is the absolute temperature, $\mu$ is the nuclear magnetic moment, $k$ is the Boltzmann constant and $B_0$ is the static applied magnetic field.
Because the energy difference between the two levels $\Delta E = -\mu B_0$ at normal temperature is much lower than the thermal energy $kT$, it is evident that the ratio $n_1/n_2$ is just slightly higher than 1. Under these circumstances the population difference between the two energy levels is too low, thus preventing the observation of the NMR signal. In order to observe the signal the population difference must be increased applying a proper radio frequency pulse, in other words the system must be perturbed from the equilibrium.

In a semi-classical description, nuclear magnetic moments in a static magnetic field $B_0$ applied along the $z$-axis, precess at the Larmor frequency $\nu_L$ which is proportional to $B_0$, see Eq. 3.4.

$$\nu_L = \gamma B_0$$

Eq. 3.4: The Larmor frequency dependence from the magnetic field strength $B_0$; $\gamma$ is the gyromagnetic ratio.

The system is perturbed from equilibrium applying along $x$- or $y$-axis, a radio-frequency field $B_1$, which oscillates at the proper radio-frequency $\nu_1$. When $\nu_1$ is equal to the Larmor frequency, the resonance condition occurs. This is the reason why the NMR spectrum gives information for a single nuclear species at once. At the resonance the field $B_1$ interacts with the magnetisation $M$ to produce a torque which moves the magnetisation towards the $xy$ plane. Since the precession frequency about the static magnetic field $B_0$ is equal to the rotating $B_1$ frequency, the magnetisation will remain perpendicular to the $B_1$ field component. To summarise, the magnetisation will precess about $B_1$ and, at the same time, it will precess about $B_0$. The $B_1$ field is applied as a pulse duration $t_p$ which usually lasts for a few microseconds. The angle $\theta$ (flip or tip angle) through which the magnetisation is tipped from the $z$-axis, is easily calculated: $\theta = \gamma B_1 t_p$. Each duration $t_p$ of the radio-frequency pulse produces a particular tip angle. The intensity of the detected signal depends on the chosen tip angle. As an example, the signal will be maximum for a tip angle of $\pi/2$, or it will be null at $\pi$.

As the radiofrequency pulse is switched off, nuclei relax back to equilibrium generating a signal called free induction decay (FID). The frequency domain spectrum is obtained by applying a Fourier transformation to the FID, see Fig. 3.41.

It is worth noticing that, after perturbing a system with a radio-frequency pulse sequence, the system will return back to equilibrium through a process called “relaxation” characterised by a time constant known as “relaxation time”. Relaxation times are parameters tightly related to the properties of the investigated material.

The main source of chemical information in high-resolution NMR spectroscopy is the chemical shift which is a very sensitive probe of the chemical environment of each resonating nucleus. This property is due to the fact that, in presence of $B_0$, the electrons surrounding a nucleus, generate a small magnetic field opposite to $B_0$ which shields the nucleus, the higher the electron density surrounding the nucleus, the higher the shielding effect. Thus, $B_0$ is not the effective magnetic field at which nuclei are subjected but it is slightly changed by the additional magnetic field generated by the electron currents. As a consequence, nuclei of the same species in different chemical environments resonate at slightly different frequencies. This allows one to obtain structural information from the position of the signals in the spectrum. As an example, the $^{13}C$ NMR spectrum of ethylbenzene is reported in Fig. 3.42. Note that different carbon nuclei in the molecule give signals at different positions, i.e. different chemical shifts.

Other important structural information can be obtained by investigating the coupling constants accounting for the coupling between nuclei through 1, 2 and 3 bonds. In addition to one-dimensional NMR spectroscopy, two dimensional approaches have been developed for the determination of the structure of complex molecules like proteins. A detailed discussion of these techniques that goes beyond the scope of this chapter can be found in specialised books [23, 24].

In liquid state, the Brownian motion of molecules averages to zero the dipolar interactions among nuclei, besides the position of lines in the spectrum does not depend on the orientation of the molecules on respect to the applied magnetic field. Therefore, spectra of liquid samples show sharp and well-resolved signals.

In the solid state, the dipolar interactions among nuclei are not averaged to zero, causing a marked enlargement of signals. Furthermore, in the solid state the position of signals depends on the orientation of molecules on respect to $B_0$. As a consequence, each single crystal gives rise to a distribution of lines (powder spectrum) which are very close to each other, causing a further enlargement of the line width (chemical shift anisotropy). In the case of nuclei with spin higher than $\frac{1}{2}$, the interaction between the nuclear quadrupolar electric moment and the electric field gradient generated by electrons, is another source of line broadening, however, the case of quadrupolar nuclei will not be discussed. Another problem is that experiments on solid samples may be very time-consuming as nuclear relaxation times may be very long. To overcome these difficulties, three techniques
of acquisition have been developed, namely magic angle spinning, dipolar decoupling and cross polarisation. The first two techniques average the dipolar interactions among nuclei and the chemical shift anisotropy effect, while the third one allows the obtainment of spectra of low abundant (dilute) nuclei in a reasonable time.

Many terms of Hamiltonian which cause line broadening in solid samples, involve the geometric factor \(3\cos^2\theta-1\) which accounts for the orientation of the shielding/dipolar tensor on respect to the applied magnetic field \(B_0\). It is easy to demonstrate that by spinning the sample at the magic angle, i.e. 54.7°, the geometric factor \(3\cos^2\theta-1\) is averaged to zero on the NMR timescale. Therefore, this technique is routinely used to remove the effects of chemical shift anisotropy and to assist in the removal of dipolar coupling.

The magic angle spinning technique involves the high speed spinning of the powdered solid sample packed in a rotor at the magic angle with respect to the direction of the static magnetic field, see Fig. 3.43.

The dipolar decoupling technique involves the irradiation of abundant nuclei (such as protons) having dipolar interaction with dilute nuclei (such as carbons) that must be observed. The high power decoupling thus allows the removal of the dipolar broadening to obtain a spectrum with much more sharp lines.

The cross polarisation technique is applied to dilute nuclei such as \(^13\)C or \(^{29}\)Si characterised by a low natural abundance (1.1 % for \(^13\)C and 4.7 % for \(^{29}\)Si) and low gyromagnetic ratio, and thus having a very weak observable net magnetisation. The NMR experiments on dilute nuclei are very time-consuming since they require a high number of scans. The CP technique consists in transferring the polarisation from abundant to dilute nuclei. The overall effect of the CP technique is to enhance the signal to noise ratio (S/N).

The polarisation transfer between nuclei with different Larmor frequencies is obtained by varying the radio-frequency pulses \(B_1\) of the two nuclear species allowing the matching of their energy levels. This matching is called Hartman-Hahn condition [25]. As an example, in the case of proton and carbon systems, the Hartman-Hahn condition is: \(\gamma_B B_{1C} = \gamma_H B_{1H}\), where \(\gamma_C\) and \(\gamma_H\) are the carbon and proton gyromagnetic ratios, respectively. Since \(\gamma_H\) is four times \(\gamma_C\) the match occurs when the strength of the applied radio-frequency field \(B_{1C}\) is four times the strength of the applied proton field \(B_{1H}\). As a consequence, there is an enhancement of the dilute spin signal intensity by as much as the ratio of gyromagnetic ratios of the abundant and dilute spin. In the case of proton and carbon nuclei, because \(\gamma_H/\gamma_C = 4\), the enhancement factor is 4. It is important to note that the Hartman-Hahn condition (i.e. the contact between the two nuclear species) must be maintained for a time (contact time \(\tau_c\)) that allows the maximisation of the NMR signal. On the other hand, \(\tau_c\) cannot exceed a certain value, depending on the analysed sample, above which relaxation processes occur thus causing a loss of the signal intensity. A representation of the CP pulse sequence is reported in Fig. 3.44.

In the presence of cross-polarisation, because polarisation is being transferred from protons to carbons, the shorter \(T_1\) relaxation time of protons dictates the recycle delay for signal averaging. As a result, the time of acquisition of carbon spectra is markedly shortened on respect to carbon spectra acquired without cross-polarising.

### 3.4.3 The NMR Spectrometer

In modern high field NMR spectrometers the static magnetic field \(B_0\) is generated by a superconducting magnet (Fig. 3.45).

The magnet is constituted by an outer stainless steel or aluminium dewar which contains liquid nitrogen and an inner dewar containing the superconducting coil (1) immersed in liquid helium in order to reach the very low temperature needed to make
the material constituting the coils superconducting. A set of shim coils providing a room-temperature homogeneity adjustment is inside a room-temperature bore (2), a spinner assembly is equipped with the system for spinning the NMR sample holder (3). The probe-head (4) is introduced into the magnet from the bottom and is connected to at least three cables providing the $^2$H lock, $^1$H and $^3$X frequencies. In the case of solid state dedicated probeheads the lock channel is not necessary. The probehead transmits the radio-frequency energy to the sample and also detects the signal from the sample which is positioned within the rf coil of the probehead itself. The rf coil needs to be tuned for the specific sample to be analysed. Another important part of the spectrometer is the cabinet which is shown in Fig. 3.46.

The computer controls the radio-frequency and pulses generation. Typically three radiofrequency channels, namely the observed, the lock and the decoupling channel, are present inside the cabinet. The frequencies, after the amplification are transmitted to the probehead. As the output from the sample is small the output stage consists of a radio receiver able to amplify the signal. Then the signal is digitised and fed into the computer memory.

3.4.4 Evaluation of ss-NMR as Used in Conservation Science

A chemical characterisation of materials associated with archaeological, historical and artistic objects, completes the information obtained through the study of historical documents, supplying information on their nature and the modifications that might have occurred due to manufacturing processes and/or natural ageing.

Recently, in this field the ss-NMR technique started having an important role even if some drawbacks must be taken into consideration. The main drawback is that 50–80 mg of material is required to perform the analysis when using the standard 4 mm rotor to contain the sample. Nevertheless, the method is non-destructive and the sample can be recovered after the NMR experiment and investigated with different techniques. This is possible because the sample does not require any treatment before the acquisition. Furthermore, the quantity of information obtained from the same sample by means of an NMR experiment is often a sufficient reason for planning this kind of analyses. Besides, the use of 4 mm rotors with an internal insert to reduce the volume, or the use of 2.5 mm rotors, permits to lower the quantity of sample needed for the analysis (10–12 mg).

Solid state NMR is a powerful technique in the field of conservation science. First of all the NMR observables are specific for the chemical element under investigation and are sensitive to distances in short and medium ranges. Besides, even in the solid state, the study of the cross-polarisation dynamics process in many cases allows the obtention of quantitative results.

A very interesting aspect is that ss-NMR can be used in the investigation of amorphous materials such as glasses and amorphous polymers used as conservation materials. As an example, the network structure in fluoride-containing aluminosilicate glasses has been studied through a quantitative determination of the coordination aluminum sites using $^{27}$Al MAS NMR and the triple-quantum magic angle spinning (3QMAS) technique [26].

Some examples of ss-NMR application in the field of cultural heritage are reported in the following in order to highlight its potentiality.

The characterisation of stone material coming from the Cheops pyramid was performed by $^{29}$Si and the $^{27}$Al ss-NMR spectroscopy [27]. In particular, the hypothesis that a clay binder was added to stone material in order to obtain some sort of concrete was studied.

ss-NMR technique was applied in the UNESCO World Heritage site of Guanajuato City Main Church (Central Mexico) in which building stones from seventeenth century were analysed by $^{29}$Si and $^{27}$Al MAS NMR in order to determine the degradation degree and to plan preservation and restoration interventions [28].
An interesting s-nmr application is represented by a rubber analysis. This natural polymeric material is slightly soluble in slightly volatile, so that techniques such as gas chromatography (GC) or liquid chromatography (LC) cannot be used. It was possible to demonstrate the presence of a sample of a modern rubber [20]. Recently a 2nd Na and H solid state NMR has been used to study the degradation of rubber [21]. By quantitative (Q-NMR) measurement, the amount of both Na and H ions in the rubber was determined. Furthermore, information on the presence of rubber in the sample could be obtained by Na-23 NMR and H-1 NMR experiment [20].

The study of the interactions between the rubber and the sample requires the use of a technique that is sensitive to the polarization properties of the rubber. The method used was based on the use of polarized light and the analysis of the fluorescence properties of the rubber. In addition, the method was able to detect the presence of the rubber even when it was not in close contact with the sample.

As the s-nmr spectra are used, the techniques can be used to fingerprint the samples consisting of a complex mixture of rubber and other materials. The method is useful for the identification of the rubber used in a sample, the determination of the rubber's origin, and the study of the rubber's composition. The method can be used to detect the presence of rubber even when it is not in close contact with the sample.

The method is particularly useful for the detection of rubber in samples that are not easily accessible, such as those that are buried or contained in a matrix. The method can be used to detect the presence of rubber in samples that are not easily accessible, such as those that are buried or contained in a matrix.

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