Electronics And Instrumentation

8th Semester

Subject: Analytical Instrumentation
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Unit-5

Nuclear Magnetic Resonance Spectroscopy

3.8. Applications

Solution NMR spectra usually contain a wealth of information. Previous sections have provided an overview of experimental techniques, the parameters which can be determined from NMR spectra

and techniques which can be used for the assignment of signals in spectra. Here some of the applications of solution NMR spectroscopy are briefly summarized.

3.8.1. Chemical Structure Determination

NMR spectroscopy is the most powerful tool available for the unambiguous determination of molecular structures of compounds in solution. In a modern chemical research laboratory the principal tools for structure determination are usually

mass spectrometry, to obtain molecular mass data, and NMR spectroscopy which provides detailed information about the groups present and how they are assembled. The abundant nuclei ^{1}H, ^{19}F, and ^{31}P have been used for over 40 years to study the structures of organic and inorganic chemicals. In the early 1970s the advent of FT methodology enabled a much wider range of nuclei to be studied and in particular resulted in an explosive growth in the use of ^{13}C NMR as a complementary technique to ^{1}H NMR in the identification of organic compounds. The most useful NMR parameters are, in general, chemical shifts, coupling constants, and integrals from which structural information can be deduced.

In addition to the study of monomeric species, NMR is a long established tool for studying the structures of synthetic polymers [41], [138]. In the case of copolymers the various groups present can be identified, their relative concentrations determined, and often information about sequence distributions obtained. The latter provides a measure of the random/block character of the polymer. Whilst ^{1}H and ^{13}C are the most commonly studied nuclei for such work several others such as ^{19}F and ^{29}Si have been used where appropriate. For vinyl polymers ^{13}C and ^{1}H NMR spectra provide information on tacticity. Chain branching and end groups can also be identified from NMR spectra of polymers.

During the last decade NMR has developed as a powerful tool for biochemical structure determination. The most impressive applications are in the determination of protein structures with molecular masses up to the 25 000 – 30 000 range [42], [139]. Such work has been made possible by the development of multidimensional NMR techniques. 2 D and 3 D techniques are widely used for such work and 4 D experiments are being developed. 3 D and 4 D methodologies are used in conjunction with ^{15}N and/or ^{13}C labelling of the amino acid residues to reduce resonance overlap.

To date NMR protein structures have usually been calculated on the basis of interproton distances and dihedral angles derived from NOE and *J* coupling measurements, respectively. Each structure determination necessitates a large number of sophisticated experiments, so that data acquisition and subsequent analysis may take several months. The value of the solution structure lies in the fact that it provides a starting point for the study of protein/substrate interactions, a knowledge of which may then aid drug design.

3.8.2. Quantiative Chemical Analysis by NMR

NMR spectroscopy offers several important advantages for quantitative analysis over other techniques, including LC and GC chromatography. Firstly a single technique can be used to unambiguously confirm the identity of the components and quantify them. For chromatographic analysis spectroscopic techniques such as MS and NMR are generally required initially to identify components. Secondly and most importantly pure samples of the compounds of interest are not required to calibrate the response of the instrument in an NMR experiment. This is a result of the fact that, given due attention to the experimental NMR conditions, the integrated resonance intensity is directly proportional to the concentration of nuclei giving rise to the resonance. Relative concentrations can be obtained directly from relative resonance intensities and absolute concentrations by adding a known amount of another compound as an internal intensity standard. The nondestructive nature of the NMR experiment offers an additional advantage. On the negative side, the NMR experiment is inherently much less sensitive than other spectroscopic and chromatographic methods. Therefore the technique is rarely suitable for quantifying components present at very low concentrations even when the more sensitive nuclei such as ^{1}H, ^{19}F, or ^{31}P are used.

In a modern NMR laboratory, pulsed FT techniques are normally used for quantitative analysis. For such work careful selection of the experimental parameters is required to obtain accurate intensity relationships between the resonances in a spectrum. Whilst ^{1}H NMR spectra are normally integrated for structural identification work, the accuracy required to identify the relative numbers of different types of protons in a molecule is much less than for quantitative analysis.

For proton NMR the main consideration is ensuring complete relaxation between successive pulses for all the different types of hydrogen atoms present. This requires the interpulse delay to be at least 5 times the longest T_1. In addition, for nuclei such as ^{13}C, where broadband decoupling is usually required, the inverse gated technique (Section 18.3.4.2) should be used to prevent the occurrence of NOE effects. A further consideration in the case of spectra from nuclei such as ^{13}C and ^{19}F, which may have very wide spectral widths, is whether the RF pulse has sufficient power to irradiate all the nuclei equally effectively. The digital resolution and data processing requirements for a particular application also require careful selection.

The wide range of chemical, biochemical, and clinical applications include strength determinations, mixture analyses, polymer analyses, and reaction monitoring. Choice of nucleus obviously depends on the individual application. For organic chemical applications, the proton would normally be the preferred nucleus because of its relatively high sensitivity. However, proton spectra of mixtures are often highly congested and the greater dispersion afforded by ^{13}C NMR may make it the nucleus of choice. Two specialist applications are described briefly below.

Isotope Content Determination. A highly developed example of isotope content determination is the site-specific natural isotope fractionation (SNIF) NMR of deuterium [44]. The natural abundance of deuterium at different sites in a natural product can vary significantly depending on its biochemical origin. This pattern of natural abundances of deuterium is often quite characteristic of the source of the material, as is the case for alcohol in wine. By using a standard with a known deuterium content, ^{2}H NMR can be used to determine the deuteration levels in the alcohol. This data provides information about the origin of the wine and is a means of detecting watering down or artificial enrichment.

Enantiomeric Purity Determination. NMR has become an increasingly important tool for the determination of enantiomeric purity [45]. Many pharmaceuticals and agrochemicals are chiral, with only one enantiomer having the required effect. At best the other is inactive, at worst it may have undesirable properties. This situation has led to a surge in enantioselective synthesis, the products of which require analysis. Chromatographic methods which use chiral columns are usually used for quality control purposes with an established process, but at the research/development stage NMR is often the method of choice. In an achiral medium, enantiomers cannot be distinguished by NMR because their spectra are identical. Diastereoisomers, however, which have different physical properties, can be distinguished by NMR. Therefore the determination of enantiomeric purity by NMR requires the use of a chiral auxiliary that converts a mixture enantiomers into a mixture of diastereoisomers. Chiral lanthanide shift reagents [46] and chiral solvating agents form diastereomeric complexes in situ and can be used directly for NMR analysis. A third method involves reaction with chiral derivatising agents prior to NMR examination.

Lanthanide shift reagents (LSR) are *tris* (β-diketonate) complexes. Further complexation with organic compounds may result in large shifts in the NMR resonances of the latter. This is caused by the magnetic properties of the lanthanide ions. Compounds containing a wide range of functional groups, including alcohols, amines, and ketones, form complexes with LSRs. Prior to the widespread introduction of high-field instruments such materials were widely used to increase chemical shift dispersion in spectra. If an LSR chelate containing an optically pure ligand interacts with a pair of optical isomers, two diastereoisomers result which can be distinguished by NMR. Many of the ligands used in such reagents are derivatives of camphor.

Chiral solvating agents (CSA) form diastereoisomeric solvation complexes with solute enantiomers via rapidly reversible equilibria in competition with the bulk solvent.

Where quantitative chiral purity determinations are required, the minimum level of detection for the minor component and accuracy achieved depend on factors which include the resolution between the resonances from the diastereoisomers formed and the signal-to-noise ratio in the spectrum.

3.8.3. Rate Processes and NMR Spectra

NMR is an important tool for the study of certain types of rate processes. In deciding whether a process is amenable to study by NMR the rate must be compared with the "NMR timescale," which refers to lifetimes of the order of 1 s to 10^{-6} s. An example of a process which can be studied in this way is rotation about the C–N bond in an amide such as *N*,*N*-dimethylacetamide (3).

Rotation causes the two *N*-methyl groups to exchange positions. In the room-temperature ^{1}H NMR spectrum separate peaks are observed for the two *N*-methyl groups. If the temperature is raised, the rate of rotation increases and the *N*-methyl resonances broaden until a temperature is reached when the two peaks coalesce into a single broad resonance. This peak sharpens as the temperature is raised further until finally the fast exchange limit is reached, above which no further change is detected. The temperatures at which slow exchange, coalescence, and fast exchange are observed for a particular process depend on the rate of the process and the frequency difference between the exchanging lines. Thus at higher field coalescence will take place at a higher tem-

perature. Analysis of such spectra enables exchange rates to be determined over a range of temperatures. Examples of other processes which have been studied in this way include keto–enol tautomerism, ring inversion, and proton-exchange equilibria.

3.8.4. NMR Methods Utilized in Combinatorial Chemistry and Biochemistry

Combinatorial chemistry provides a powerful means of rapidly generating the large numbers of structurally diverse compounds necessary for the biological screening required by drug discovery. Thus, combinatorial chemistry has become an indispensable tool in pharmaceutical research. The diversity of techniques being employed has caused the adaptation of a number of NMR methods for combinatorial chemistry purposes. These methods include the NMR analysis of reaction intermediates and products in solution or in the gel state and the analysis of ligands interacting with their receptors. High-throughput screening strategies were developed which involve the acquisition of 2D spectra of small organic molecules in a few min-

utes. Libraries of more than 200 000 compound can be tested in less than one month. There are many advantages of high-throughput NMR-based screening compared to conventional assays, such as the ability to identify high-affinity ligands for protein targets. This suggests that the method will be extremely useful for screening the large number of targets derived from genomics research [145].

SHUKER et al. described a NMR method in which small organic molecules that bind to proximal subsites of a protein and produce high-affinity ligands are identified. This approach was called "SAR by NMR" because structure-activity relationships (SAR) are obtained here from NMR. This technique involves a series of 2D spectra of a labeled receptor protein in the presence and absence of potential ligands. The method reduces the amount of chemical syntheses and time required for the discovery of high-affinity ligands and appears particularly useful in target-directed drug research [141].

NMR has become established also as a valuable technique in clinical biochemistry where ^{1}H NMR in particular is used to study complex biochemical fluids such as plasma, urine, and bile. The major problem associated with such work is

the removal or reduction of the very large water signal. One method involves freeze-drying the sample and redissolving it in deuterated water. NMR techniques are also available for selective suppression of the water signal. These methods, such as presaturation of the water, are required to reduce the water signal such that acceptable suppression can be attained from phase cycling. The same effect can be accomplished with magnetic field gradients alone because the removal of the undesirable resonances is accomplished in a single scan.

Under the conditions used, only low molecular mass constituents are observable, the signals from proteins and other large molecules are not resolved. Applications include screening and monitoring for metabolic diseases, the study of biochemical mechanisms associated with disease processes, and the identification of drug metabolites.