State of the art in spectroscopic instrumentation (STAG)

Mid infrared sampling methods (MISM)

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State-of-the-art in high resolution NMR instrumentation

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Introduction

Food products are heterogeneous, fragile and structurally complex materials. Therefore Nuclear Magnetic Resonance (NMR) techniques, which can non-invasively acquire information about the constituents, the structure and the textural properties of a food sample, are particularly useful both for the food scientist and the industrialist. NMR spectroscopy also has the advantage of using electromagnetic radiation in a frequency range at which food products are entirely transparent so that the signal is acquired from the complete sample, not just a superficial layer.

The first paper concerning an application of NMR spectroscopy in food science, for studying hydration in foodstuffs, was published in 1950, thus being almost as old as the method itself, and older than the first commercially available NMR spectrometer. NMR spectroscopy has been widely applied to the analysis and the quality control of food products since the early 1960s, mainly in its low resolution version. However, with the advent of new NMR technologies, the range of applications and of types of information available has greatly increased.

The term NMR now actually covers a large diversity of structural and analytical techniques, which produce signals in the time domain (Low Resolution Pulse NMR), the frequency domain (High Resolution Continuous Wave, and Fourier Transform NMR spectroscopy) and the spatial domain (Magnetic Resonance Imaging). To be accurate, one should define LR as producing signals in the time domain, HR in the time and frequency domains and MRI in the time, frequency and spatial domains.

Depending on the particular instrumental technique used, it is possible to have structural information concerning a molecule, determine the concentrations of either major or minor constituents in solids and liquids, obtain information about the proportions of an isotope at individual sites of a component molecule, or analyse the spatial distribution and mobility of nuclei within a sample.

The cost and constraints associated with the instrumentation required to obtain each type of information are very different and determine whether a particular technique may be applied to everyday quality control measurements, or for authenticity control of high added-value products, or must be limited to food research applications.

Some definitions of parameters and instrumental factors

Only those parameters relevant to instrumental quality, and which could be checked by a potential purchaser, are reviewed. In addition to these parameters, experimental factors important for the everyday user are also highlighted. Thus, for instance the Sensitivity is included whereas Chemical Shift is omitted. Moreover, the influence of Nuclear Overhauser Effect (NOE) on sensitivity is mentioned (the operator could alter these effects), but the influence of the Magnetogyric ratio (a physical constant of each nucleus that cannot be altered by the operator) is omitted.

Sensitivity

In NMR, the Sensitivity is commonly (but, strictly speaking, incorrectly) defined as the minimum concentration of test material required to produce a detectable signal. Therefore, signal-to-noise ratio (S/N) is the usual way to measure the Sensitivity in NMR. Signal-to-noise is defined as the ratio of the amplitude of the signal (A) to the twice the root-mean-square (rms) of the noise amplitude. Alternatively, the peak to peak noise (Npp) could be used instead of rms. The Npp is calculated as the height between the greatest positive and the greatest negative noise peaks within the chosen noise domain of the spectrum.

Thus the final definition of this Sensitivity is given by:

$$S/N = \frac{2.5 \times A}{Npp}$$

In order to compare Sensitivity of different instruments, the same conditions must be employed. Unfortunately, the criteria of Sensitivity are not chosen in the same way by different manufacturers. The best suggestion for a purchaser would be to use his own standard sample and parameters in order to compare the performances of various spectrometers.

The common ^{1}H test sample for Sensitivity is 0.1% ethylbenzene in deuterochloroform. For ^{13}C the common test sample is 40% para-dioxane in deuterobenzene. For ^{19}F, ^{31}P and ^{15}N Sensitivity tests 0.05% trifluorotoluene in deuterobenzene, 3% triphenylphosphate in deuterochloroform and 45% or 90% formamide in deuterated dimethylsulfoxide respectively, are good examples of test samples.

There are various ways the sensitivity may be enhanced. Increasing the concentration of the compound analysed is the best choice when this is possible. When enough sample is available, larger diameter sample tubes should also be used.

In the case of NMR of nuclei other than the proton, artificial enrichment with the particular isotope, although rather tedious, is a very efficient technique for signal enhancement. Since Sensitivity increases with the magnetic field strength, it is often advisable to use more powerful (higher frequency) spectrometers. The Nuclear Overhauser Effect (NOE) can be used in order to spectacularly improve the Sensitivity.

Once the FID is recorded, mathematical data treatment is commonly performed in order to enhance Sensitivity. The FID is often multiplied by a window function called "matched filter" of type E :

$$E = \exp(-t/a); \text{ with } a > 0$$

However, a broadening of the line widths also results from multiplying the FID by the above function.

Resolution

Resolution could be defined as the minimum distance between two lines in a spectrum at which they can be still distinguished. There are several criteria to specify the resolution. The most common way to express the resolution is the width at half-height of a line measured in Hz.

An even better criterion for resolution is the difference between the experimental FID and the theoretical exponential, or alternatively the difference between the experimental line in the spectrum and the ideal Lorentzian. Although more important, the latter criterion is far from common.

The sample most often used to determine the resolution for the 1H nuclei is 1% o-dichlorobenzene in deuterated acetone. Alternatively, especially for fields below 200 MHz, a 10% solution is employed. For other nuclei, the usual samples are those used for the Sensitivity tests. For uncommon nuclei or even for the nuclei listed above for Sensitivity tests, it is common to prepare test samples in the laboratory. It is worth mentioning that even the quality of sample tube affects the measurement. Another type of measurement is made on a sample of 20% $CHCl_3$ in deuterated acetone. This sample is used to check the lineshape by measuring the linewidth at 0.55% and 0.11% of the maximum height.

Resolution enhancement can be had by very careful shimming (fine magnetic field adjustment) as the homogeneity of the magnetic field is critical.

The homogeneity of the sample is another straightforward parameter to be carefully checked. Very good mixing and filtration of the sample are required. The resolution is also improved by the use of diluted samples in lower viscosity solvents.

The type and quality of the probe is also critical, although it is not as straightforward to check and improve as the first two parameters. Upgrading the probe is the most important action for improving the performances of a spectrometer.

Minimisation of the sample volume theoretically enhances the resolution. However, owing to discontinuities in magnetic susceptibility, at the solvent-air and solvent-tube wall interfaces, field gradients appear, resulting in broadening of the lines. Thus an optimum sample volume can always be experimentally determined for each combination of magnet and probe. As a general rule one can remember that a 5 mm probe leads to better results in terms of resolution than a 10 mm one. An alternative to reducing the sample volume is to reduce the volume where the resonance takes place by applying field gradients. This latter technique is the cornerstone of NMR Imaging.

Acquisition time is a parameter that should also be set sufficiently long to achieve high resolution. Once the FID has been recorded, mathematical treatment of the data can further improve the resolution. There are several common treatments of the FID data which enhance the resolution. Popular examples are the reverse of the matched filter for sensitivity enhancement E_1 and the Lorentz-Gauss transformation G:

$$E_1 = \exp(-t/a); \text{ with } a < 0$$

$$G = \exp(-t/a)\exp(-t^2/b); \text{ with } a < 0 \text{ and } b > 0$$

However, there is no way to improve both resolution and Sensitivity at the same time, and a compromise must be made between the two. Another mathematical treatment of the raw data which improves the resolution is the so called zero filling. This is particularly useful when the acquisition time was too short and the FID was truncated. Doubling the data table by adding zero values at the end leads to better resolution.

Dynamic Range

The dynamic range represents the maximum ratio of amplitudes that can be detected. No amount of time and number of scans can recover a signal that is smaller than the lower limit of the dynamic range. Both the dynamic range and the maximum range of frequencies that can be detected are limited by the analog-to-digital converter (ADC). This problem occurs in Pulse NMR, where all frequencies are acquired simultaneously, but not with continuous wave (CW) spectrometers, where the signal at each peak is acquired separately.

Although the manufacturers of the latest generations of spectrometers have pushed the limit of the dynamic range to figures as high as 10,000 : 1, the problem is still present. For the common high field spectrometers built only a few years ago the dynamic range is only of the order of a few thousands to one.

Various signal suppression techniques, usually employed to eliminate the solvent signal, can help to improve the sensitivity for small signals. The result of these techniques is the detection of weak signals, but at the cost of cancelling or reduction of the strong signal and often with the introduction of artefacts.

Decoupling

Decoupling is performed by irradiation at the resonance frequency of a particular signal or group of signals in order to remove the spin-spin interactions of this signal with other signals under observation.

Various techniques and sequences have been developed to improve the decoupling effect. The techniques differ depending on whether selective or broadband decoupling, homo- or heteronuclear decoupling is required.

As well as the simplification of a spectrum by reduction or suppression of multiplicity of signals, other more subtle, but very important effects can be obtained. The NOE effects are common examples of additional effects of the decoupling. Decoupling times are part of all modern multipulse techniques leading to uni- or multidimensional spectra correlated via coupling constants.

Both the technical characteristics of the instrument and the degree of calibration of various decoupling parameters are very important to obtain both qualitative and quantitative results. The latest generation of spectrometers have a multitude of facilities for decoupling. Thus several channels can be used in order to decouple practically any nucleus. Also, recent pulse sequences produce very efficient broadband decoupling over a wide range of frequencies. However, most of the high field spectrometers in use to date have very clear limitations in their decoupling performances. For instance, it is still very common to find high field spectrometers that only decouple the hydrogen nuclei.

Fourier Transform

Fourier Transform is the usual mathematical treatment applied to the FID (evolution of the magnetisation as function of time) in order to obtain the spectrum (a function of frequency). All modern spectrometers perform this operation automatically and in a way transparent to the user. However, when a scientist is approaching the limits of the

application of NMR this operation as well as many other aspects related to instrumentation become crucial. For instance the speed of the transformation could be of concern. If the FT transformation of a routinely recorded FID takes a few seconds on a modern spectrometer, and a two dimensional set of data is transformed in no more than a few minutes, for multidimensional spectra the situation is quite different. Thus, the first full 4D spectrum recorded in 1990 required about 60 hours of processing on a workstation. Both the computing power and the software are major problems in such cases.

3D spectra have been commonly recorded since about 1989, and since 1993 commercial state-of-the-art instruments have had 4D facilities.

Fourier transform is the only method employed at the moment on commercial instruments, but the future might be quite different. The Maximum Entropy Method (MEM) is another powerful example of a data processing method that has been intensively explored during the last decade.

Solvents

Usually the modern routine NMR spectrometers lock the magnetic field on the deuterium frequency of the solvent. Although other techniques exist, the use of deuterated solvents is the best choice, when possible, as it gives better quality spectra.

Apart from the solubility of the sample, other factors should be considered when choosing a solvent. The price plays an important role as it varies greatly from one solvent to another. The viscosity of the solvent has an important effect on the resolution of the spectrum. Viscous solvents will usually produce lower resolution spectra than non-viscous ones. The usual non-viscous deuterated solvents are: acetone, acetonitrile, chloroform, dichloromethane and methanol. On the other hand, viscous solvents will be more suitable for NOE experiments. Also, the higher the strength of the deuterium signal from the solvent, the better it is for NOE experiments, allowing the gain of the lock signal to be reduced. The usual viscous deuterated solvents are: benzene, dimethylsulfoxide, dimethylformamide, pyridine, toluene and water. The boiling and melting points have to be considered when variable temperature experiments are planned.

The position of the residual undeuterated solvent signals as well as the deuterium content have to be considered when spectra of samples in very small quantities (less than few mg) are to be done. The presence or absence of water traces in a solvent may also be relevant in some cases.

Sample Tubes

The quality of the tube (e.g. concentricity, wall thickness) are very important when very good resolution is required. On the other hand, each type of spectrometer has a lowest recommended quality sample tube and the use of lower quality sample tubes can lead to damage to the probe. Each manufacturer specifies the quality parameters in terms of diameter, wall thickness, concentricity, inner and outer diameters. Each product (part number) has a specified tolerance for these parameters.

Apart from the catalogue quality, the history of the tube is also very important (e.g. how it was cleaned, dried, stored, even what samples were previously analysed in it) as these operations might lower its quality.

Sample Handling

The homogeneity of the sample is very important at high magnetic fields. Thus, filtration of the solution and mixing are conditions required for very high resolution spectra. For very dilute samples, other contamination problems should be considered (e.g. acetone from the washing process, or sweat from the fingertips).

Sample Volume

Each probe has a specific detection coil that receives signals from only a finite volume in the core of the magnet. Sample volumes greater than this detection coil volume should be used in order to obtain an optimum volume that minimises field distortions caused by the solution/air interface. When enough sample is available this optimum volume of solution should be used. For samples in small quantities, other strategies can be used (e.g. capillary tubes, vortex suppressers, microprobes, etc).

Sample Concentration

When enough sample is available, the concentration is chosen, depending on the information desired. Thus high concentrations are desired for good signal-to-noise ratios (e.g. nuclei with low natural abundance), for short recording times and for some two dimensional experiments e.g. correlated spectrsocopy (H-C COSY). On the other hand, very dilute solutions are preferable for high resolution spectra, NOE experiments, and other two dimensional experiments (e.g. H-H COSY) .

Special conditions pertaining to solid state NMR

Recent developments in instrumentation now make it possible for high-resolution NMR spectra to be obtained for solid-state samples. Although the basic principle is the same (i.e. excitation of nuclei by pulses followed by detection of the evolution of magnetization in time) there are differences between solid- and liquid-state NMR.

Three factors affect the NMR response of solid samples which do not arise or are averaged to zero in liquid phase. These are as follows:

(i) Dipole-dipole interactions. They are due to the direct influence of neighbouring dipoles on the magnetic field experienced by the nucleus under investigation. They are several orders of magnitude greater than the indirect interactions mediated by electron clouds (J-coupling).

(ii) Chemical shift anisotropy. The electron shielding at the nucleus depends on orientation, and for a solid sample all possible orientations are present. This results in broad peaks with characteristic line shapes. Both these interactions occur in solution but are generally averaged to zero as a result of the random motion of the molecules.

(iii) Extremely long relaxation times (T_1). This results in a considerable amount of time being required to obtain a spectrum with a reasonable signal-to-noise ratio. This problem is normally not encountered with liquid samples.

In order to obtain HR solid-state NMR spectra each of these factors has to be overcome:

(i) Dipolar interactions can be minimised by using high-power decoupling fields. If the decoupling power usually employed for liquids is about 5 W, typically 50 - 100 W are required for solids.

(ii) Broadening due to chemical shift anisotropy can be reduced using the magic-angle-spinning (MAS) technique, MAS involves fast spinning of the sample at an angle of 54.7^0 to the applied magnetic field. In order to have an idea of what fast spinning means, one should note that the normal spinning rate for liquid samples is 10 - 20 Hz, whereas for solids it is of the order of 10 KHz, representing a supersonic speed.

(iii) Relaxation times may be reduced by the technique of cross-polarization (CP), which results in polarization transfer between highly polarizable ^{1}H-nuclei and the observed nuclei (usually ^{13}C).

Nowadays, ^{13}C-CPMAS NMR spectra are routinely recorded and most I=1/2 spin nuclei are accessible with the exception of ^{1}H and ^{19}F. Although not routine practice, the difficulties in recording the latter two nuclei can be overcome.

It is also worth mentioning that instead of classical sample tubes, small ceramic rotors are used in solid state NMR.

The Spectrometer

Continuous Wave NMR Spectrometers

Continuous Wave (CW) NMR spectrometers were the most commonly used instruments until the mid 1970s. Both permanent and electromagnets were employed in CW spectrometers. Electromagnets are lighter than permanent magnets but the disadvantage is the necessity to have water-cooled temperature control and an extra power supply system.

The advantages of pulse spectrometers over the CW instruments are numerous. However, two advantages of the CW spectrometer should be mentioned. Firstly the absence of dynamic range problems, and secondly the greater facility for recording spectra of nuclei spread over a very large frequency range. For this reason, ^{19}F spectra are still recorded on CW spectrometers.

Electromagnet Pulse FT-NMR Spectrometers

The pulse FT-NMR spectrometers became commercially available at the beginning of the 1970s. Practically all the FT spectrometers have either electromagnets or superconducting magnets. The basic difference between pulse and CW irradiation is that the nuclear spins are excited in pulse spectrometers by applying a short radio frequency pulse covering the whole range of frequencies in the spectrum, whereas in CW-NMR, the spins are excited successively, as the spectrometer frequency passes through each spin's resonance condition. Although the pulse technique was suggested from the beginning of NMR as an alternative to CW irradiation, the difficulty in treating the resulting data (FID) considerably delayed its practical application.

FT spectrometers with field strengths of between 80 - 100 MHz are very common as they are relatively cheap, and easy to service and maintain.

The highest achievable magnetic field strength for an electromagnet is 100 MHz. This is due to the fact that iron saturates at 2.35 T.

Superconducting Magnet NMR Spectrometers

All superconducting spectrometers are pulse FT-NMR instruments. Commercially available field strengths range from 100 to 750 MHz. The latest generations of superconducting spectrometers offer many facilities, apart from the advantages due to the wider spread of signals at high field. They attain extremely high field stability, resolution and sensitivity, and have extensive pulse programming and fast data processing facilities for multidimensional experiments. They are also modular, which means that each instrument can be customized for the particular needs of a laboratory and can be easily upgraded. The same instrument can perform liquid, solid and imaging NMR experiments.

A major constraint introduced with the use of superconducting magnet instruments comes from the need to cool the magnet coil with liquid helium and liquid nitrogen. Therefore, an important characteristic of a superconducting magnet is the time between refills of liquid nitrogen and liquid helium. The usual rate for nitrogen is every 2 weeks, whereas for liquid helium it varies between 2 and 9 months, depending on the type of magnet.

The world's leading NMR manufacturers are Varian and Bruker, while the leading magnet manufacturer is Oxford Instruments. Other important NMR instrument makers are General Electric and JEOL.

Brief History of the Advancements in Instrumentation

1939 - Rabi et al. reported the first observation of the NMR phenomenon in gas phase;

1946 - First successful experiments in liquid (Block, Hansen and Packard) and in solid phase (Purcell, Torrey and Pound);

1949 - Discovery of the chemical shift in liquids;
- Practical demonstration of pulse NMR by Hahn;

1950 - The first reported application of NMR in food science.
- Commercial instruments were pioneered by Varian Associates.
- Hahn discovered spin-echoes.

1951 - The first NMR spectrometer that could plot a spectrum on a chart recorder was built at Caltech.

1952 - The first commercial spectrometer was produced by Varian. The field strength was 30 MHz.

1954 - The first high pressure experiments were performed.
- Carr and Purcell modified the Hahn pulse sequence in order to more easily visualise the echoes.

1955 - A 40 MHz spectrometer was introduced by Varian, together with the first sample spinning device.

1957 - The first ^{13}C-NMR spectra were reported by Lauterbur and Holm.

1958 - Golay developed the concept of improving the homogeneity of a field by introducing extra fields that cancel out unwanted harmonics.
- A 60 MHz spectrometer was developed by Varian. The electromagnet weighed 2,500 kilograms.
- An important modification of the Carr-Purcell multiple-echo sequence was developed by Meiboom and Gill (CPMG).

1959 - Magic angle spinning was discovered, but for some time it had very limited applications.
- A 100 MHz electromagnet spectrometer was developed by Varian.

1960 - The integrator device was developed and introduced as an upgrade for old spectrometers.

1960s - The first high quality ^{13}C-NMR spectra were published.

1961 - The user-friendly concept was incorporated in the Varian A-60 spectrometer.
- The decoupler unit was developed and introduced as an upgrade for old spectrometers.

1962 - The spin-locking concept was developed.
- The first superconducting spectrometer was made by Varian.

1966 - The equation for the optimum tip angle to obtain the best signal-to-noise ratio was published by Ernst.
- Ernst published classical papers on applications of Fourier Transform to NMR. Varian delayed in applying this revolutionary discovery.

1966 - The first commercial superconducting NMR was introduced as the Varian HR 220.

c.a.
1970 - The first FT-NMR spectrometers were introduced by Bruker.

1970s - The limit of detection of NOE effects was about 5%.
- Oxford University, Oxford Instruments and Bruker increased the field strength in steps of 270, 360 and 400 MHz. Other manufacturers followed them.
- In addition to Bruker and Varian, other manufacturers like JEOL and General Electric become strong in the NMR market. Other smaller manufacturers were absorbed by these world leaders.

1971 - J. Jeener suggested two-dimensional NMR by describing the H-H COSY experiment.

1973 - Lauterbur demonstrated the use of magnetic resonance as an imaging technique.

1975 - Theoretical extension of dimensionality to more than two dimensions by R. Ernst; Ernst's group showed that 2D NMR provides a more efficient way of obtaining an image.

1978 - A commercial 500 MHz instrument was introduced.
- A 600 MHz non-persistent magnet was developed superconducting magnets became widely used.
- Commercial development of NMR imaging.

early 1980s - The first MRI instruments were purchased by major hospitals.

1981 - G. J. Martin and M. L. Martin developed SNIF-NMR techniques.

ca. 1985 - Techniques for integrating imaging and spectroscopy were developed.

1987 - The 600 MHz instruments were commercially introduced.
- The first 3D NMR spectrum was published.

1989 - High temperature NMR experiments were carried out at over 2,000 C.

end of 1980s - NOE enhancements of less than 1% were easily detectable.

1990 - 3D NMR experiments were widely performed.
- The first 4D experiment was reported.

1991 - Echo-planar imaging was shown to be a very fast alternative to the classical imaging techniques.

1993 - 750 MHz spectrometers became commercially available.
- A rough estimate shows that there are about 15,000 NMR instruments in use worldwide.

Present limits

Samples can be investigated in all states: gaseous, liquid as dispersions in liquids, or solid. NMR spectra of practically all isotopes with nuclear spin quantum numbers other than zero have been recorded.

The highest intensity magnetic field available to date is 750 MHz. The highest temperature reached in an NMR probe is more than 2,000 ^{o}C using a laser heating system. The highest pressure reached in an NMR experiment was 10,000 atmospheres.

The dynamic range has been pushed beyond 10,000 : 1. The NOE enhancements of less than 1% are easily detectable. 3D experiments are routine and 4D experiments are often performed.

Future trends

There is every reason to believe that both the instrumental and theoretical aspects of NMR will continue to progress as they have over the last 10 years. New pulse sequences will be invented, computer power will continue to grow and field strength and field homogeneity will increase. These improvements will have repercussions on all the NMR techniques and will lead to new applications in food analysis and an extension of the role of NMR in industry.

4D experiments will become routine and the increase in dimensionality will probably continue.

Further improvements in software are also to be expected. This will on the one hand facilitate the use of the instrument, and on the other hand data processing and spectrometer operation will be possible using PC type computers.

A new revolution in instrumentation is also to be expected once the new materials with superconducting properties at the temperature of liquid nitrogen are introduced.

So far, little has been done to introduce NMR instruments into the factory. One of the major factors limiting the on-line use of NMR is the influence of external magnetic fields. New instruments are now becoming available based on completely closed magnets which are much less sensitive to the environment, and one should expect to see the technique being applied more widely for on-line quality control. At the same time, the on-line analysis of many products would be greatly facilitated by the use of open or horseshoe shaped magnets similar to those which have already been tested in the coal and mining industries. The introduction of such instruments would require

more research on optimal magnet configurations, field stabilisation systems, and computer algorithms to correct for peak broadening due to field inhomogeneity.

Many of the homonuclear techniques applied in High Resolution NMR will percolate down to Low Resolution NMR. T_1 and T_2 dispersion, and cross relaxation, for example, give information about the rotational and translational movements within molecules and so could be valuable for the study or monitoring of drying, gelatinisation and dissolution processes.

^{31}P, ^{14}N, ^{17}O, and ^{23}Na nuclei will be used more often in High Resolution NMR studies, despite the evident problems of sensitivity. The interpretation of certain processes is simplified by the selectivity of these spectra which contain information only about particular food components, such as proteins, phospholipids, soluble salts or water.

Liquid-phase NMR will continue to be important, but the use of solid-state NMR will become more frequent as it adds the valuable possibility of observing the as is biomolecule, without lengthy extraction procedures which can denature the sample.

SNIF-NMR will be applied more extensively for both product authentication and identification of origin. The extension of SNIF-NMR to include ^{13}C would greatly improve the discriminating power of the technique.

The NMR spectroscopy on localised volume, using both NMR and Magnetic Resonance Imaging techniques will probably became routine. This would allow spectroscopic investigation of different regions of a foodstuff and not simply averaging over the whole sample as is currently the case.

Applications to food chemistry

The following applications are presented rather as examples of the power of the technique and are by no mean exhaustive.

High resolution liquid-phase spectroscopy

Quantitative and structural information can be obtained from High Resolution NMR spectroscopy by interpreting the chemical shifts and spin-spin couplings, and by following changes in the relative proportions and chemical shifts of the resonance peaks. This information can be used for the determination of molecular structure and stereochemistry, the quantitative analysis of complex mixtures and the monitoring of chemical and biochemical transformations of food products.

Structural identifications

High-resolution solution-state NMR is mainly used for structure identification of pure or nearly pure species isolated from food products by chemical and physical procedures. Much has been done on the stereochemistry of saccharides. Several NMR experiments may be used to identify compounds present in raw materials, in processed food or during food processing. Recently, high-field proton NMR was used to determine the structure of polyunsaturated fatty acids from several fish lipid samples, and the composition of fats in several foods was analysed by high-field ^{1}H- and ^{13}C-NMR. Although the most powerful technique for structure elucidation at the moment is X-ray analysis, NMR is unsurpassed for compounds which cannot be isolated as crystals.

Quantitative determinations

High-resolution solution-state NMR offers the advantage of simultaneously providing information on the proportion and the structure of the various components. NMR methods were reported as being more reliable than classical ones. For example, sucrose in sugar beet juices was quantitatively determined both by NMR and by the classical polarimetric method, revealing the superiority of the first method.

Fatty acids in various edible oils can be quantitatively determined by ^{13}C spectra recorded on narrow spectral widths with very high resolution, without prior hydrolysis of the triglycerides.

Proton FT-NMR has been used to study the relationship between Iodine Number and degree of unsaturation of butterfat, to quantify ethanol in wine and spirits, and to determine the structure of fatty acids in oils. In these cases, limited sample preparation is required as the solids in the samples do not interfere with the liquid signal and the different liquid constituents can be distinguished by their chemical shifts. It is worth noting that some of these simple determinations may be performed with equal precision and less cost using Low Resolution Pulse NMR instruments.

Seed oils may be studied by ^{1}H-NMR and the functional groups giving rise to resonance peaks determined. Using these simple attributes, it is possible to estimate the degree of unsaturation and the mean molecular mass of the oils. ^{1}H-NMR can be used to estimate the oxidative deterioration of oils directly from the ratio of olefinic to aliphatic protons.

^{13}C-NMR can furnish much information about the composition of complex mixtures as it covers a chemical shift range 20 times greater than that of ^{1}H-NMR. In a mixture of glucose, fructose, sucrose, maltose and raffinose, it is possible to quantify each sugar as the peaks from the C-1 carbons of the aldoses and the C- 2 carbons of the ketoses

are clearly resolved and situated in an uncluttered region of the ^{13}C-NMR spectrum. However, sample preparation is long, usually involving grinding of solid samples, then drying, extraction, evaporation, filtration and dissolution in D_2O.

^{31}P-NMR permits the resolution and precise quantification of the various phospholipid families and their natural derivatives. The technique has been shown to be a viable method for the routine analysis of phospholipids of biological origin, but only after a very long sample preparation, involving the extraction of phospholipids from the lipid fraction of the tissues.

Mechanistic Studies

One can follow the rate of in-vivo or in-vitro biosynthesis of labelled substances. In this way, a mechanism of biosynthesis of rishitin, a stress metabolite of potato, was proposed. ^{31}P-NMR spectra have been used for the study of metabolism *in vivo*, as well as in a novel use that can be described as an in vivo pH meter. The position of the resonance peak from the inorganic phosphate present in biological materials is sensitive to local pH, which allows ^{31}P-NMR to be used to monitor both the intracellular and the extracellular pH in a non-invasive manner.

^{17}O-NMR is often preferable to ^{1}H- or ^{2}H-NMR for studying water in food systems as the latter techniques are subject to errors due to cross-relaxation between the water protons and those of the solid matrix, and chemical exchange of the ^{2}H on the water molecules with the ^{1}H of the matrix. The very low natural abundance of ^{17}O means that the samples may need to be treated with enriched water to attain optimal sensitivity. Studies of the T_2 relaxation times of ^{17}O have been useful, for example, to obtain a better understanding of the changes in water mobility that occur in bread during staling, due to starch recrystallisation and water binding. Mobility of water in various sugar-water systems was studied by measuring ^{17}O relaxation times. Recent studies reported the detection of internal motions in oligosaccharides by measurement of T_1 relaxation times of protons at different magnetic fields (300 and 500 MHz).

^{43}Ca and ^{25}Mg-NMR studies have been performed on milk fractions and cation binding to milk proteins has been investigated.

Analysis of flowing systems

Analysis of flowing systems can be achieved by implementing appropriate circulation systems and ensuring pre-polarization of the observed sample in the magnetic field.

Characterisation and authentication

The conventional methods of detecting adulteration of expensive commodities such as alcoholic beverages and flavouring materials have been supplemented with techniques based on the analysis of carbon and hydrogen isotopes in low molecular weight molecules such as water, ethanol or flavour molecules. The most specific of these techniques, site-specific natural isotope fractionation (SNIF-NMR) uses NMR spectroscopy to determine site-specific isotope ratios. The ^{2}H / ^{1}H and ^{13}C / ^{12}C ratios can be used to discriminate between synthetic and fermentation ethanols in beverages and spirits, between ethanol from different plant sources and to detect the chaptalization of wine. The method can also be used for the authentication of acetic, propionic and lactic acids, which are commonly used in the food industry as preservatives and seasonings. SNIF-NMR (^{2}H-NMR) is at present the only method that can unambiguously determine the origin (botanical, semisynthetic or synthetic) of some aromas and flavourings such us vanillin, anethol, benzaldehyde and linalool. As the simple sugars are not yet all accessible to routine site-specific deuterium quantitation, they must first be fermented to form ethanol which is then analysed by SNIF-NMR. Precautions must be taken in applying SNIF-NMR as many of the standard physical and chemical procedures used to prepare samples for analysis may introduce significant variations in the proportions of the various isotopomers.

High-resolution solution-state NMR spectra (^{1}H- and ^{13}C-) are also used for authentication and regulatory/legislative purposes.

High resolution solid-state NMR spectroscopy

High Resolution solid-state NMR has obvious advantages over standard liquid-phase High Resolution NMR: it gives access to quantitative information concerning the less mobile constituents of a sample *in situ*, without lengthy sample preparation and without denaturing the molecules. However, the instrumental constraints as well as the time and costs required for NMR experiments preclude its use in the routine analysis of food products. Despite this limitation, much information has been acquired recently concerning the chemical composition of "as is" food samples, the structure of molecules in whole samples and the behaviour of various food components during processing and storage.

Composition and chemical structure elucidation

Both ^{1}H and ^{13}C-MAS NMR spectroscopy have been applied to the quantitative analysis of triglycerides in individual oilseeds, such as rape and sunflower. The global composition in various polysaccharides and phenolics of woody food components and of cell walls has been partly elucidated from their solid-state ^{13}C CPMAS-NMR spectra.

Structural conformation of solid materials

Amorphous or differently ordered conformations of the same biopolymer give very different solid state ^{13}C-NMR spectra. This provides excellent fingerprinting spectra for the identification of ordered states in other environments. The strategy is to obtain solid-state ^{13}C-NMR spectra for crystalline samples exhibiting X-ray diffraction and then to use these spectra to identify and quantify molecularly ordered, but non-crystalline, forms. This method can be applied in the analysis of granular starches and provides the ratio of double helical (ordered) to single-chain (amorphous) populations, and this can be compared with quantitative estimates of crystallinity obtained from X-ray diffraction studies.

Spectra of native cellulose have similarly been interpreted as a composite of crystalline and amorphous features.

Efforts are being made to understand the relationship between chemical shift and conformation. There is a significant amount of experimental data available for particular forms of polysaccharides and proteins with simple repeating structures.

Crystalline polymorphism can be probed by solid-state NMR with the rule of the thumb that a separate resonance should be observed for each non-equivalent atomic site within the crystalline unit cell. This can mean multiple resonances for single molecular sites due to asymmetries of molecular packing within the crystal. Determination of crystalline polymorphism in trigycerides is important as triglycerides are part of many fat-structured foods.

Solid-state ^{13}C-NMR can also be used to detect structural changes due to hydration of macromolecules. This technique has been used to directly observe starch and protein in both dry flours and hydrated dough. The changes in the spectra of hard and soft wheat flours after hydration have been interpreted in terms of the plasticising effect of water. A study of the relaxation rates of the different components has also indicated spatial phase separation of starch and proteins molecules within the flours and dough, whereas the lipids would appear to be mainly present complexed with amylose.

Microdynamic studies

Information on the relative mobility of individual structural parts have been obtained for some gluten fractions and polysaccharide gels.

Mechanisms of flour and dough hydration have also been studied in order to understand the effects of varietal flours on baking characteristics.

Frozen systems

An early practical demonstration of high-resolution solid-state ^{13}C-NMR involved low-temperature observation of room temperature liquids. Chain segments within polysaccharide gels were immobilised by freezing so that all of the polymer was detected in a cross-polarization experiment.

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Selected Food-Based Applications

This list of applications is not intended to be comprehensive. The object is merely to provide illustrations of the potential applications of each technique. The reader is also referred to other publications in the QUEST series as this list is not intended to reproduce work from these documents.

The following codes are used at the end of each reference to indicate the method used:

NMR	Nuclear magnetic resonance
M	Mid-infrared / FTIR
N	Near infrared
R	Raman

Dairy

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Meat

Spray, M.J., Scotter, C.N.G. and Hall, M.N. (1990). Technical Memorandum No. 585, Campden Food and Drink Research Association, Chipping Campden, UK *N*

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Confectionery

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Grain/cereals

Baianu, I.C. (1989). In Nuclear Magnetic Resonance in Agriculture. Ed. Pfeffer, P.E. and Gerasimowicz, W.V. CRC press, Boca Raton *NMR*

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