Accred Qual Assur (2006) 11: 130–137
DOI 10.1007/s00769-005-0006-7

GENERAL PAPER

Analytical Methods Committee

Report by the analytical methods committee: evaluation of analytical instrumentation Part XVI Evaluation of general user NMR spectrometers

Received: 1 November 2004
Accepted: 31 March 2005
Published online: 5 April 2006

Electronic Supplementary Material Supplementary material is available for this article at http://dx.doi.org/10.1007/s00769-005-0006-7

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The Analytical Methods Committee has received and approved the following report from the Instrument Criteria Sub-Committee.

Introduction

This report was compiled by the above Sub-Committee of the AMC which consisted of Professor S Greenfield (Chairman), Dr M Barnard, Dr C Burgess, Professor S J Hill, Dr K E Jarvis, Dr M Sargent and Mr D C M Squirrell with Mr C A Watson as Honorary Secretary. The initial input of the features for consideration and the reasons for their consideration was undertaken by a working party of Dr R Fletton, Dr P Sidebottom and Dr A Kenwright to whom the committee express their thanks.

The purchase of analytical instrumentation is an important function of many laboratory managers, who may be called upon to choose between a wide variety of competing systems which are not always easily comparable. The objectives of the Instrumental Criteria Sub-Committee are to tabulate a number of features of analytical instruments which should be considered when making a comparison between various systems. As is explained below, it is then possible to score these features in a rational manner, which allows a scientific comparison to be made between instruments and as an aid to equipment qualification.

The over-all object is to assist purchasers in obtaining the best instrument for their analytical requirements. It is hoped that this evaluation will, to some extent, also help manufacturers to supply the instrument best suited to their customer's needs. It is perhaps pertinent to note that a number of teachers have found the reports of use as teaching aids.

No attempt has been made to lay down a specification. In fact, the Committee considers that it would be invidious to do so: rather it has tried to encourage the purchasers to make up their own minds as to the importance of the various features of the equipment that is on offer by the manufacturers.

An overview of general user NMR

Principles of NMR spectroscopy

Nuclear spin and nuclear magnetism

Within the Periodic Table about 100 nucleides possess a spin angular momentum which is described by a *spin quantum number* I. Depending on the nuclear species I may have an integral or half-integral value (i.e. 1/2, 1, 3/2 etc.) and the spin angular momentum is

$$[I(I+1)]^{1/2} \cdot h/2\pi \quad h = \text{Planck}'s \text{ constant}$$

I=1/2 nuclei include ^{1}H, ^{3}H, ^{13}C, ^{15}N, ^{19}F and ^{31}P while ^{2}H and ^{14}N have I=1. Nuclei with I=0, such as ^{12}C and ^{16}O, have no spin angular momentum and do not give NMR spectra. The great majority of NMR studies relate to spin half nuclei (very largely ^{1}H, ^{13}C, ^{19}F and ^{31}P).

Nuclei with $I>1/2$ in addition possess a *nuclear quadrupole moment*. In many cases this causes severe line broadening through its effect on relaxation processes (see below) and signals are consequently difficult to observe.

Since they are charged particles, nuclei with a spin angular momentum also possess a *nuclear magnetic moment*, colinear with the spin axis, which has the value

$$\gamma \cdot [I(I+1)]^{1/2} \cdot h/2\pi$$

γ, the *magnetogyric ratio* (and thus the magnetic moment) is a constant unique to the nuclear species.

Nuclei in a magnetic field

Nuclear spin angular momentum exhibits quantised behaviour: the nucleus can adopt only $2I+1$ orientations with respect to an axis defined in space.

Where this axis is provided by an external magnetic field the different orientations differ in energy. Thus for ^{1}H (and other I=1/2 nuclei) two orientations are allowed: that with the nuclear magnetic moment oriented (roughly) in the field direction being of lower energy than that oriented the other way. There are thus two energy levels for the system.

In a similar manner there are three energy levels for an I=1 nucleus in a magnetic field, four for an I=3/2 nucleus and so on.

If a large assembly of identical spin 1/2 nuclei (e.g. protons) is exposed to a magnetic field a Boltzmann distribution of population is established between the energy levels.This occurs through *relaxation processes* involving interaction of the nuclear magnetic moments with fluctuating magnetic fields generated in the environment by molecular motion.

This process is characterised by a *longitudinal relaxation time* T_1 which for protons is typically about 1 s.

Nuclear magnetic resonance

If electromagnetic radiation with a frequency matching the separation of the energy levels is input to the system nuclei in the lower energy state reorient to the higher with absorption of energy while nuclei in the upper state change to the lower with emission of radiation ("stimulated emission"). This is the phenomenon of *Nuclear Magnetic Resonance* and, for the spectrometers described here, this will involve frequencies from about 30–500 MHz.

The frequency of magnetic resonance depends on both the strength of the applied field and the magnetic moment of the nuclear species involved. Thus in a field of 4.7 Tesla (T) ^{1}H nuclei resonate at 200 MHz and ^{13}C nuclei at 50 MHz, while in a 9.4 T field the resonance frequencies are 400 MHz and 100 MHz respectively.

The net absorption of radiation depends on the excess of nuclei in the lower energy level; at all attainable field-strengths the excess is very small (ca 1 in 10^5) owing to the smallness of the nuclear magnetic moment.

NMR signals are thus very weak in comparison with infrared and uv/visible absorptions. Methods of alleviating this problem are described later.

The absorption of radiation disturbs the energy level populations while relaxation processes attempt to restore them to their Boltzmann values. In modern spectrometers it is usual to repeatedly excite the sample with a pulse of radiation and sum the NMR responses to improve sensitivity. In such experiments the time between pulses must be sufficient to allow recovery of the population difference through relaxation. If inappropriate conditions are used there may be a complete loss of signal through equalisation of energy level populations (*saturation*).

The chemical shift

Within a molecule a nucleus does not experience the full applied magnetic field since it is slightly shielded by the surrounding electrons to an extent which is characteristic of the local chemical environment. Thus in a molecule which has the same nuclear species in several chemically distinct locations, for example ethanol, the protons of the CH_3, CH_2 and OH groups experience slightly different magnetic fields and consequently resonate at different frequencies. This is the phenomenon of the *chemical shift*.

The value of NMR in the elucidation of molecular structure arises from the fact that the frequency of an NMR signal is indicative of the chemical grouping in which the nucleus is located. It is therefore possible to infer the presence or absence of a particular chemical unit in an unkown molecule from the appearance or non-appearance of signals in the appropriate region of its spectrum. Characteristic chemical shifts therefore serve the same purpose as characteristic group frequencies in infrared spectroscopy.

In contrast to most types of spectroscopy the frequency of an NMR signal is dependent on the instrument used since it is determined by the magnet fieldstrength. To avoid this problem it is usual to describe chemical shifts with respect to the signal of a reference compound (tetramethylsilane - TMS - for ^{1}H and ^{13}C) in dimensionless units of ppm (parts per million).

For spin 1/2 nuclei the chemical shift range is 10^3–10^5 times the normal linewidth; NMR is therefore a very precise probe of chemical structure.

Nuclear spin coupling

Where several groups of magnetic nuclei are proximate in a chemical structure (as is the case with protons in most organic molecules) they may interact through the intervening electrons – an effect called *spin-spin coupling* or *indirect coupling* and characterised by a *spin coupling constant*. The result of this interaction is the splitting of the coupled

signals into multiplets with structures dependent on the number and spatial disposition of the interacting nuclei. For an unknown molecular structure this effect provides valuable insight into the relative locations of chemical groups which have been identified from their chemical shifts.

Magnetic nuclei also interact directly through space (like two bar magnets) – an effect called *direct* or *dipolar coupling*. These interactions may be large but in solution they are not directly observable in the spectrum since on the NMR timescale they are averaged to zero by molecular motions. The fluctuating magnetic field generated by the modulation of dipolar interactions through molecular motion is however a major contributor to relaxation processes (*dipolar relaxation*).

In the solid state, where molecular motions are much reduced, this averaging does not occur; dipolar interactions are then a major obstacle to the observation of spectra and must be overcome by special techniques.

Spin decoupling

Since ^{13}C is only about 1% abundant ^{13}C NMR signals are usually split only by spin couplings to ^{1}H (or other abundant magnetic nuclei) and are difficult to observe. It is therefore usual to remove this effect by simultaneously exciting proton resonances while observing ^{13}C (*heteronuclear spin decoupling*). This improves detection limits through collapse of the coupled multiplets to singlets but information helpful in the assignment of spectra is thereby lost. Several specialised experiments are available for recovery of this.

As an aid to assignment in the spectra of abundant nuclei *homonuclear spin decoupling* has long been employed. In this one multiplet in a spin coupled spectrum is subjected to specific excitation while rest of the spectrum is recorded in the usual manner. If a signal has a splitting due to spin coupling in common with the irradiated group this will be removed in the decoupled spectrum and the signal multiplicity reduced. This is of value in simplifying complicated spectra and, particularly, in resolving ambiguities as to which signals exhibit a common spin splitting.

In practice information on homonuclear coupling is usually obtained on modern instruments by *COSY* experiments (see below).

Nuclear overhauser effect

Where two protons are in spatial proximity in a molecule the fluctuation in their magnetic dipolar interaction caused by molecular tumbling provides an efficient mechanism for nuclear relaxation (see above). In these circumstances if the signal of one nucleus is irradiated with a low power while the other signal is observed there will be a change in the intensity of the latter. This effect is termed the *Nuclear Overhauser Effect* and it is brought about by a transfer among the energy levels of the population disturbance caused by the irradiation.

For typical organic molecules this effect is positive and the maximum change possible is 50%. The utility of the NOE arises from the fact that its initial rate of build up with irradiation time is strongly dependent on the separation of the nuclei involved. By running a spectrum with irradiation of one signal it is possible to determine which signals arise from close nuclei and get an estimate of their relative separations.

This is of great value in distinguishing between isomers and determining molecular conformations. The actual size of the nuclear Overhauser enhancement is dependent on molecular mobility and declines with increasing molecular weight. Thus for molecules of about 1000 MW NOEs are commonly near zero while at molecular weights of a few thousand NOEs become negative. To get over this difficulty experiments have been devised to measure *rotating frame NOEs* (*ROEs*) which do not suffer from this sign variation with molecular weight. Such experiments are mainly of interest for specialized protein and peptide studies.

Quantitative aspects of NMR

If NMR spectra are recorded under conditions which avoid saturation the intensity of a signal is directly related to the number of nuclei giving rise to it. Thus in the ^{1}H spectrum of ethanol the signals due to CH_3, CH_2 and OH have relative intensities of 3:2:1.

In addition the signal intensity is independent of the molecular environment; thus, for example, a CH_3 group will give a signal of the same intensity whether it is part of an alkane, an ether or an ester.

This quantitative aspect when combined with chemical shifts to identify molecular sub-units and spin coupling to deduce their connectivity gives NMR particular power as a tool for elucidating molecular structures.

In the special case of ^{13}C NMR, which is almost always used for the purpose of structural elucidation, it is usual to shorten experiment times by recording spectra under conditions where signal intensities are not strictly quantitative.

Pulsed fourier transform NMR (PFTNMR)

Scanning a spectrum to observe signals sequentially (*continuous wave spectroscopy*) is an inefficient method for spin 1/2 NMR, where linewidths are usually much smaller than the total spectral width. It is also totally unsuitable for recording spectra of nuclei like ^{13}C which, by reason of its low natural abundance and smaller nuclear magnetic moment, i*s ca.* 6000 times less sensitive than ^{1}H.

All modern NMR spectrometers therefore utilise *pulsed excitation* and *Fourier transformation*. In this method electromagnetic radiation with a frequency appropriate to the nucleide under observation is applied as a pulse of short duration (typically around 10 μsec.). The effect of the pulse is to generate a range of frequency components sufficient to excite all nuclei simultaneously.

The response of the sample to the pulse is a *free induction decay (FID)*, a composite signal containing contributions from all the excited resonances and decaying away through relaxation processes. This is sampled and digitised for computer processing.

To convert this *time domain* signal to a conventional (*frequency domain*) spectrum the digitised FID is subjected to *Fourier transformation,* a mathematical process which in effect picks out the individual frequency components from the FID. Commonly the FID may be multiplied by appropriate weighting functions to enhance spectral resolution or signal-to-noise ratio.

In almost all NMR spectroscopy the process of inputting a pulse of radiation and sampling the FID is repeated many times and Fourier transformation is applied to the summed FIDs. In this way the signal-to-noise of the resulting spectrum (as compared with that of the spectrum from a single pulse) is increased as the square root of the number of FIDs acquired.

By this means ^{13}C spectra on 10 mg samples of most organic molecules can be obtained in about 1 h and signals of sensitive nuclei like ^{1}H and ^{19}F observed from microgramme size samples.

Dynamic range and solvent suppression

The detected FID is converted to digital form for computer processing using an A/D converter. For ^{1}H NMR, in particular, this may present a problem of *dynamic range* since both large and small signals may be present in the spectrum and must both be detected. It is therefore important that the A/D converter has sufficient resolution. In practice most modern instruments use 16 bit A/D converters although lower resolution converters may be encountered on old spectrometers.

A problem may still arise, however, where the FID is dominated by a very strong signal. This is most commonly met with in ^{1}H NMR where the solution has a substantial content of H_2O (it is also sometimes necessary to record spectra in a solution which is largely H_2O).

It is not permissible for the FID to overflow the A/D converter since this causes a distortion of all of the resulting spectrum. Modern spectrometers therefore adjust the gain to fit the FID into the digitiser but, in this case, the scaling may be such that background noise is insufficient to trigger the least significant bit of the digitiser. It then becomes impossible to recover a small signal buried in the noise by the accumulation of many FIDs.

To get over this difficulty *solvent (or signal) suppression* techniques are employed - these are often used in 2D as well as 1D spectroscopy (see below).

The simplest and most widely used of these methods *presaturation* can be implemented on all FT spectrometers. In this technique an irradiating field (rather like spin decoupling) is applied at the appropriate frequency for several seconds to saturate the solvent response. The irradiation is then gated off before application of the observing pulse and detection of the FID. Since the solvent response takes several seconds to recover from saturation through relaxation processes its contribution to the FID is much reduced. The sequence is then repeated as many times as necessary.

It should be noted that any signals in the vicinity of the solvent signal will also be lost or attenuated by this method. If, however, a signal in another part of the spectrum is undergoing exchange with the solvent (this is not uncommon with H_2O) it may be possible to saturate this and suppress the solvent response without loss of adjacent signals.

A number of other methods for suppressing or not exciting the solvent have been developed but may require more advanced instrumentation (e.g. selective excitation or pulsed field gradients).

NOE measurements by FT methods

In most cases the measurement of steady state NOEs, where one signal in the spectrum is continuously irradiated, is not very useful for structural purposes since static NOE values cannot be directly related to internuclear distances.

A much more useful experiment (*Truncated Driven NOE*) involves irradiating selected signals for a series of short periods and thus studying NOE buildup rates. Results are usually presented as difference spectra obtained by subtracting a spectrum with irradiation outside the spectral range from the on-resonance spectrum (*NOEDIFF*). Difference spectra ideally show signals only for the nuclei affected by the irradiation. The advantage of this technique is that it can be implemented on all modern NMR instruments.

On instruments with pulsed field gradient and shaped pulse facilities more sophisticated experiments giving higher quality results may be undertaken. An important recent development is the *DPFGSE-NOE* experiment which gives results similar to a difference experiment but free of subtraction artefacts. By this method very small enhancements can be reliably measured.

Distortionless enhanced polarization transfer (DEPT)

^{13}C spectra recorded with ^{1}H decoupling consist of a series of single peaks with no evidence for which arise from CH_3, which from CH_2 etc. This information can be recovered while retaining the clarity of spectra without the multiplicity due to coupling by the use of DEPT, a pulse sequence involving excitation on both ^{1}H and ^{13}C channels. With appropriate conditions nuclei respond to the DEPT sequence according the number of directly bonded protons. In practice it is usual to record DEPT spectra under several sets of conditions.

With suitable addition and subtraction of these spectra it is possible to produce *edited spectra* showing only methyl groups, only methylene groups and so on. This technique is of particular value where spectra are complicated or structures totally unknown.

Note, however, that two dimensional techniques such HMQC (see below) provide the same information and are tending to supersede DEPT.

Two dimensional NMR

Pulsed excitation offers much scope for experimental innovation. Thus it is possible to disturb the nuclear spins before applying the observe pulse and sampling the FID and to use this to study various internuclear interactions within the molecule (*two dimensional* and *multidimensional NMR*).

Multidimensional NMR is used almost exclusively to investigate biopolymers and commonly requires isotopically enriched samples and advanced instrument configurations but two dimensional methods are widely used on spectrometers of the General User type.

The most widely used 2DNMR methods involve detection of homonuclear spin coupling (*Correlation Spectroscopy – COSY)* or heteronuclear spin coupling (*Heteronuclear Correlation Spectroscopy – HETCOR*). Several variants offering advantages over these basic experiments have been developed e.g. *COSY45*, *phase-sensitive COSY*, *double quantum filtered COSY* and *COLOC*, *HMQC* and *HMBC*.

NOESY and *ROESY* are two dimensional methods for detecting, respectively, conventional and rotating frame NOEs. The latter experiment is of value under circumstances where the conventional NOE is near zero (see above).

In these experiments the system of nuclear spins is first disturbed by a sequence of pulses and the development of the disturbance under the influence of spin coupling interactions observed as a function of time; in essence a 2D experiment is a series of 1D acquisitions differing in the delay between the disturbance and the observing pulse used to generate the FID. This delay provides a second time domain which is converted by Fourier transformation to a second frequency axis in the resulting 2D spectrum.

A 2D spectrum is normally represented as a map with the proton chemical shifts along both axes (COSY) or with the proton shifts along one axis and the heteronucleus shifts along the other (HETCOR). The presence of a spin coupling interaction between two signals is indicated by a *cross peak* with the coordinates of the two chemical shifts.

Since 2D NMR involves the acquisition of many FIDs experiments tend to be time consuming; methods have therefore been developed to ease this problem.

In the case of COSY the time needed by an experiment is often determined not by poor signal-to-noise but by the need to sequentially cycle signal phases in the detection system to eliminate unwanted components of the NMR response. The use of *pulsed field gradients* removes these components without the need for phase cycling and allows the length of experiments to be determined only by sensitivity requirements. With typical sample quantities (a few mg.) a several fold reduction in experiment times is commonly achieved. Pulsed field gradients require additional equipment but are widely applicable in advanced NMR experiments.

The basic HETCOR experiment involves the detection of the heteronucleus; since in most instances this is ^{13}C experiment times are necessarily long. Experiments which detect heteronuclear spin coupling by observation of the sensitive nucleus (usually ^{1}H) have therefore been developed. These are termed *inverse mode* or *indirect detection* methods. HMBC (Heteronuclear Multiple Bond Correlation) and HMQC (Heteronuclear Multiple Quantum Coherence) are experiments of this type. Most modern spectrometers can undertake these experiments without any modification but, for reasons of sensitivity, an *indirect detection probe* is desirable.

It should however be noted that for the acquisition of normal 1D spectra the sensitivity of indirect detection probes is inferior to that of normal geometry probes. If a single probe is to be employed to routinely run both 1D and 2D spectra a decision has to be made as to which experiment is the more important.

Definition of a general user NMR spectrometer

In this report this term is used to refer to an instrument operating for ^{1}H at a frequency of 200–500 MHz and used routinely to acquire spectra of ^{1}H, ^{13}C and the easier heteronuclei (typically ^{19}F and ^{31}P) for conventional solution samples.

A General User Spectrometer is characterised by intensive use and a high throughput of diverse samples: commonly a single instrument may fulfil both routine service and research oriented functions.

Instruments of this type are typically employed to solve problems of chemical structure and purity using a standard range of NMR methods, often by a spectroscopist and/or technician providing all NMR support to a laboratory, or to record spectra on their own samples by a group of preparative chemists without special spectroscopic expertise.

Excluded from this category are high field systems, spectrometers used for the study of solids and low resolution instruments designed for the assay of water or fats in industry. Also excluded are instruments of the General User type dedicated to a single specialised function (e.g. instruments used for monitoring processes in chemical production) Accessories such as HPLC flow probes (typicallyused with HPLC equipment to examine drug metabolites or natural products) and flow injection multisamplers (for analysis of combinatorial chemistry products) are not easily accommodated in a General User environment and will not be considered here.

Scope and comparison of NMR with other techniques

As a technique providing information on molecular structure NMR is to be compared with IR and uv/visible spectroscopy, mass spectrometry and single crystal X-ray diffraction. High resolution NMR is essentially a method for the study of solutions; the observation of solids requires additional equipment. Provided there is sufficient molecular mobility it is also possible to study molecules bound to surfaces using a high resolution magic angle spinning probe Unlike, for example, mass spectrometry, NMR is

non-destructive: the total sample is recoverable with more or less difficulty depending on the NMR solvent used.

Owing to the smallness of the nuclear magnetic moment NMR is at least an order of magnitude less sensitive than IR spectroscopy and several orders of magnitude less sensitive than uv/visible spectroscopy and mass spectrometry. Sample sizes routinely used are around 1 mg for ^{1}H and around 10 mg for ^{13}CNMR. Smaller samples may be run at the expense of longer acquisition times.

Provided an NMR acquisition is run under suitable conditions signals will be present in the resulting spectrum for all magnetic nuclei of the observed species present in the sample and the intensity of each signal will be directly proportional to the number of nuclei giving rise to it. These features give the technique a unique advantage over other common forms of spectroscopy.

For spin 1/2 nuclei the spectral dispersion is similar that of high resolution mass spectrometry and increases with the strength of the magnetic field. Most NMR spectra therefore provide a high level of accessible information and spectra can commonly be fully interpreted. NMR is the most versatile of the common spectroscopic methods and may offer several approaches for the solution of a problem.

For example it may be possible to record the spectra of several different nucleides on a particular sample and thereby gain complementary information. Where more than one spectrometer is available it may be helpful to record spectra on a higher fieldstrength instrument to increase spectral dispersion.

With the pulsed excitation used on all modern instruments many experimental variants are available providing information such as connectivity in molecules (for structural elucidation), proximity of atoms (for molecular conformation), conformational and chemical exchange, molecular diffusion etc. Much of this work cannot be easily undertaken by other techniques.

In consequence NMR spectroscopy with General User instruments, often but not necessarily in combination with mass spectrometry and IR spectroscopy, has been, for many years, the main method for determining the structures of organic molecules of synthetic or natural origin. If samples are adequately soluble and contain suitable nuclear species the method is equally applicable to covalent inorganic compounds.

In contrast to X-ray diffractometry, the definitive method of structural determination, NMR does not require a pure or crystallisable sample and is thus more widely applicable. Variable temperature NMR is widely employed for the study of processes like tautomerism and conformational exchange.

Through its interpretability and quantitativeness NMR is of particular value in chemical development where the products of a reaction and the major solvents and impurities can often be identified and the relative amounts approximately estimated from inspection of a simple 1D spectrum.

NMR methods have considerable potential for monitoring processes in chemical production but, for reasons of low instrumental sensitivity, expense and the environmental sensitivity of the magnet, have so far been little exploited. With the introduction of actively shielded magnets and the recent large advances in sensitivity achieved with cryogenic probes this situation may change.

In recent years advances in instrumentation have extended the use of NMR well beyond small molecule chemistry. The use of NMR methods to determine the structure of small proteins utilises sophisticated high field instruments but spectrometers of the General User type are often employed in the study of cell suspensions (e.g. for following cell metabolism using labelled substrates) and the investigation of ligand-to-protein binding (by observing changes in the spectrum of the small ligand molecule or in the protein if suitably isotopically labelled).

New methods of sample introduction requiring modifications to conventional probe geometry have been developed for particular purposes e.g. HPLC probes for on-line analysis of chromatographic fractions and flow injection probes for use with a multisampler for mass screening of combinatorial chemistry products. High resolution MAS probes (already mentioned) have been much used in combinatorial chemistry for the on-bead analysis of bound products.Of these three types of probe only the latter is easily accommodated in a General User environment.

NMR spectroscopy, which separates the signals of nuclei according to chemical shift, is to be distiguished from Magnetic Resonance Imaging (MRI), a technique with important medical and non-medical applications. In MRI the combined NMR signal of all nuclei of a particular species (in medical imaging normally ^{1}H) is detected from a restricted volume in the intact sample, which is selected by applying an appropriately shaped external magnetic field gradient. An image of the total sample is built up from the signals of different volumes selected sequentially by varying the field gradient.

True NMR spectroscopy of living samples requires specialised equipment falling outside the General User category.

General instrument description

The major components of a modern General User instrument are

1. a superconducting magnet (4.7–11.8 Tesla) – note the Earth's field is about 0.00005T. After installation this consumes no electrical power but it is environmentally sensitive and requires regular liquid nitrogen and helium refills to maintain superconductivity. With proper servicing such magnets are stable indefinitely.
2. an electronics console generating the frequencies for the observation and/or decoupling of all nucleides of interest and, in addition, a deuterium frequency to provide a control signal (*lock)* from the deuterated solvent in which the sample is dissolved. Commonly the system consists of separate high frequency (^{1}H and ^{19}F) and broadband (^{13}C, ^{31}P etc.) channels, though more elaborate multichannel configurations may be available.

Further functions of the unit include the amplification of the observation frequency, its gating into a sequence of short duration pulses for input to the sample and the detection of the NMR response. In current instruments these functions are often under the control of one or more dedicated processor units.

3. a probe which holds the sample within the bore of the magnet. This contains coils for the excitation and detection of both the NMR response and the deuterium lock signal and for heteronuclear decoupling. A turbine assembly for spinning the sample tube and a facility for controlling the sample temperature are also provided. Probes are interchangeable and it is common to have several probes with complementary capabilities.
4. a data system which is used for overall spectrometer control, including the sample temperature and spinning rate, the optimisation of magnet resolution and the operation of an autosampler. The other major functions of the data system are the accumulation of NMR responses (free induction decays) to improve sensitivity and their Fourier transformation to generate conventional NMR spectra. Data systems are typically Unix workstations or powerful PCs and may be networked for remote recovery or processing of NMR data.

For all models of spectrometer a considerable range of accessories is available to enhance instrument capabilities. Within a General User environment the most useful accessories are likely to be a sample changer and additional probes. Two important recent developments, *actively shielded superconducting magnets* and *cryogenically cooled probes*, are likely to have a major impact on General User spectrometers and widen the applicability of NMR in general. Conventional superconducting magnets produce large stray fields beyond the confines of the magnet casing and are thus very sensitive to environmental disturbance. In actively shielded magnets the use of additional superconducting coils greatly reduces the stray field and, in consequence, allows instruments to be installed in less favourable environments.

At the time of writing actively shielded magnets are available for most instruments in the General User category and it is likely they will be standard on all spectrometers in the future. With correct handling superconducting magnets have an indefinite lifetime. Laboratories acquiring used equipment or relocating existing instruments are therefore likely to be faced with the problems associated with conventional magnets for many years to come. The environmental needs of conventional magnets are therefore described later.

In cryogenically cooled probes the operating temperatures of the observing coil and the preamplifier are reduced by cooling with liquid helium. This results in improved coil efficiency and a reduction of random noise in the coil and the preamplifier. Large increases in probe sensitivity are thereby achieved: current cryoprobes have up to four times the sensitivity of conventional probes. Since this is equivalent to a 16 fold reduction in spectrum acquisition times it represents a major advance in the power of the technique.

At the time of writing cryoprobes are available only for 500 MHz instruments but it is to be anticipated that they will eventually become available for the range of General User instruments.

Evaluation of overall instrument performance

Since General User NMR spectrometers are employed for a wide range of structural and semi-quantitative purposes it is not feasible to devise a simple set of tests to unequivocally demonstrate the superiority of one instrument over another.

In the first instance the performance of a spectrometer is determined by the qualities of the magnet, the electronics and the probe, while for some experiments the performance of accessories (e.g. variable temperature and pulsed field gradient systems etc.) may also be important. It is quite possible that one manufacturer's spectrometer will have a performance advantage over a competing instrument with one probe while being inferior with another.

Where different manufacturers' probes are directly comparable sensitivity differences are likely to be small and of little practical significance in a General User environment. For example for routine samples a difference in signal-to-noise of (say) 5% between two instruments is unimportant while the resolution is largely dependent on the properties of the sample solution (viscosity, paramagnetic impurities, insoluble particles etc.) rather than ultimate instrumental capabilities.

Note that manufacturer's signal-to-noise figures on test samples may not be an adequate guide to performance since higher sensitivity may have been achieved by detecting the signal from a larger volume of solution and resolution will have been fully optimised. This does not reflect the common situation in routine operation where sample is limited and the time devoted to shimming is restricted by the needs of sample throughput.

In evaluating instruments of the General User type factors such as the availability of desirable features and ease of use are likely to outweigh minor differences in performance.

For first time purchasers of NMR equipment financial considerations and available locations usually determine the selection of magnet fieldstrength.

It is then necessary to decide which nuclei are expected to be observed, how much sample is likely to be available and which experiments will be undertaken. These factors may affect the choice of electronics consoles and will determine the choice of probes.

The other major consideration is whether to purchase an autosampler and, if so, what sample capacity will be needed.

Most intending purchasers then base instrument evaluations on the type of samples they expect to run. The following list, which is neither comprehensive nor relevant to all cases, is intended to indicate the sort of experiments and factors which might be considered in an evaluation :

(1) signal-to-noise of ^{13}C and/or other heteronuclear spectra obtained from normal 1DNMR acquisitions.

(2) effectiveness of solvent suppression in recording ^{1}HNMR spectra of aqueous solutions
(3) effectiveness of autosampler - test by running a number of samples in a variety of solvents and look for any mechanical problems, the ease with which field/frequency lock is established, the time required to adjust resolution and the quality of the resulting spectrum in terms of signal-to-noise, lineshape and phase correction.
(4) if ^{19}FNMR spectra are likely to be required only occasionally this facility can often be provided by retuning the coil of a ^{1}H probe. If this feature is likely to be of interest check for the quality of the results obtained, in particular for the presence of a broad background signal from fluoropolymers used in the probe construction. Is this acceptable and, if so, does it vary between the probes of competing manufactures?
(5) quality of results obtained in NOE difference spectra in terms sensitivity and cancellation of unwanted signals and artefacts. This experiment is a good test of spectrometer stability.
(6) quality of results obtained in 2D experiments in terms of signal-to-noise and absence of spurious peaks in the resulting spectra. Experiments such as phase sensitive COSY and HMBC or HMQC are likely to be of most interest. Intending purchasers may find it instructive to observe the results of COSY with and without pulsed field gradients and HMBC and HMQC with both inverse and normal geometry probes.

The conditions used to acquire the spectra should be carefully defined. For 1D spectra this may involve specifying the pulse repetition time and the number of FIDs acquired. More parameters are involved in 2D acquisitions and persons unfamiliar with NMR may wish to specify only the total length of the acquisition leaving the instrument operator to choose appropriate conditions.

In all cases experiments should be undertaken in the intending purchaser's presence and the ease of setting up, acquiring and processing data noted.

The list of Instrumental Criteria is available as Electronic Supplementary Material.