Please read the highlighted text

Nuclear Magnetic Resonance Instrumentation

Shi Bai
University of Delaware, Newark, DE, USA

The nuclear magnetic resonance (NMR) spectrometer consists of a superconducting magnet, a sample probe, a radio-frequency (RF) transmitter, a receiver, and a computer for instrument control and data processing. The superconducting magnet provides an ultrastable magnetic field in the central volume of the magnet. To maintain the current, the solenoid that produces the field is immersed in liquid helium to maintain the superconducting state. Surrounding the magnet volume is a set of vacuum jackets and a liquid nitrogen–filled volume to isolate the low-temperature regions from room temperature. The magnet has a bore to house the sample probe and a room-temperature shim (RTS) coil assembly to reduce inhomogeneity of the magnetic field across the active sample volume. The sample to be analyzed is usually dissolved in a deuterated solvent and is contained in a specially ground glass tube that is inserted into the volume of the probe coil. RF pulses, from one or a set of high-power transmitters, are applied to the sample through the RF coil that surrounds the active sample area to perturb the sample. The RF pulses are controlled to ensure precise frequency, phase, duration, and amplitude of the RF voltage. The response of the sample is gathered by recording the induced voltage across the RF coil. The response (the signal) is digitized as a so-called free induction decay (FID) with a high-performance RF receiver including a preamplifier, an amplifier, and an analog-to-digital converter (ADC). By using Fourier transformation (FT), the time-domain FID is converted into the frequency-domain spectrum, often known as the NMR spectrum.

Encyclopedia of Analytical Chemistry R.A. Meyers (Ed.)

1 INTRODUCTION

NMR spectroscopy has a wide range of applications in chemistry, pharmaceuticals, proteomics, structural biology, structural genomics, metabolomics, and materials science and engineering, where it is used to identify structural features of the molecules in a sample. It is a powerful analytical tool for quantitative and qualitative characterization of these molecules. Because of the wide range of problems that analysis by NMR can address, NMR spectrometers are found in industrial, academic, and government laboratories around the world, wherever identification and quantification of the components of a sample are important.

The NMR signal arises from interactions between the magnetic moments of nuclear spins and the applied external magnetic field (*see* **Zeeman Interaction in Nuclear Magnetic Resonance**). The NMR spectrometer is used to detect these NMR signals, the interpretation of which yields important structural and dynamic information. The traditional information obtained from the NMR spectrum is the various interactions of nuclear spins with environment, such as chemical shifts, scalar coupling, and relaxation rates. For certain nuclei with nuclear spin $I > 1/2$ the magnitude of the quadrupolar coupling can also give information on the local electronic state (*see* **Quadrupole Couplings in Nuclear Magnetic Resonance, General**).

When a collection of NMR-active nuclear spins (which we consider here to have $I = 1/2$) is placed in a strong static magnetic field B_0, the magnetic moments, μ, associated with the nuclear spins precess about the direction of the magnetic field. The precession frequency is known as the *Larmor frequency*, ω_0, of the nucleus. If the magnetic moment is not directed along the magnetic field, it has two components, the projection along the field and the projection along the plane perpendicular to the field. The precession about the field causes this latter component to be time dependent.

After a sample has remained in a field for a time sufficiently long to establish equilibrium, (*see* **Relaxation in Nuclear Magnetic Resonance, General**) the collection of spins shows the following two qualities: (i) there is a finite magnetization aligned with the magnetic field and (ii) there is no net magnetization perpendicular to the field. The first of these phenomena is understood by the fact that there are two states with projection along the field, either parallel (α) or antiparallel (β). The population of α nuclear spins is slightly greater than that of β spins because of a slightly lower energy associated with α nuclear spins. The second is a result of the fact that the precession of each of the individual moments occurs in such a way that the various spin moments have incoherent phase. The Larmor frequency, ω_0, which is the nuclear precession frequency in the applied external magnetic field expressed in radians per second, is proportional to the strength of the external field:

$$\omega_0 = \gamma B_0 \tag{1}$$

where γ is the magnetogyric ratio of the nucleus being observed, and B_0 is the magnetic-field strength in tesla. When the Larmor frequency is expressed in units of cycles per second (Hz), we have $\upsilon_0 = \gamma B_0/2\pi$. For a magnet with a field of 14.09 T, the nominal Larmor frequencies of ^{1}H and ^{13}C are 600.0 and 150.9 MHz, respectively. For this reason, besides quoting the magnetic-field strength in tesla, it is common parlance to specify the field strength of an NMR spectrometer in terms of the ^{1}H Larmor frequency. It also happens that the Larmor frequency is directly proportional to the energy difference between the two states:

$$\Delta E = \hbar\omega_0 \tag{2}$$

where $\hbar$ is Planck's constant divided by 2π. Thus, a measure of the Larmor frequency is a measure of the energy spacing between the two stationary states of a spin. For example, for a 600 MHz magnet at 300 K, the energy difference, ΔE, is 3.97×10^{-25} J for ^{1}H nuclei. This small energy difference results in a very small population difference between the two levels at room temperature. The induced voltages (the NMR signal) are therefore quite small, even at the highest achievable magnetic fields. This fact makes NMR spectroscopy a relatively insensitive technique, when compared to other spectroscopic methods such as infrared or optical spectroscopy. Because of this issue, the majority of developments in NMR instrumentation focus on various means to increase the sensitivity of NMR measurements.

Since the Boltzmann factor that determines the magnitude of the NMR signal is proportional to the externally applied field B_0, higher magnetic-field strength leads to higher sensitivity in the NMR experiment. The additional benefit of higher field is that, at higher frequencies, the natural noise in the detection circuits (including the NMR probe, receiver, and amplifiers) is lower, so that the signal-to-noise ratio (S/N) increases faster than linearly with the magnetic field. In recent decades, we have seen a continual development of higher and higher magnetic fields for use in NMR spectroscopy.

A second practical reason for the use of higher magnetic fields follows directly from quantum mechanical consequences of the direct dependence of the magnitudes of various couplings on magnetic field. The two couplings that are the "bread and butter" of NMR are the chemical shift and the scalar, or J, coupling (*see* **Chemical Shifts in Nuclear Magnetic Resonance**; **Scalar Couplings in Nuclear Magnetic Resonance, General**). What makes NMR such an important technique is that nuclei in different local environments have precession frequencies (and hence energy spacings) slightly different from the nominal Larmor frequency. The measurement of all of these frequencies, reported as chemical shifts, specifies the local structure of the molecule in which the spins are embedded. The chemical shift range expressed in hertz is proportional to the applied magnetic-field strength, B_0. Thus, at higher fields, two resonances are separated by a larger frequency difference than they would be at lower field. On the other hand, the spin–spin coupling is independent of field. Thus, at higher field, the chemical shift difference between two resonances becomes relatively larger than the J coupling. The form of the NMR spectrum depends on the relative size of the J coupling and chemical shift difference between the two resonances. In particular, when the J coupling is much smaller than the chemical shift difference, the form of the spectrum becomes quite simple. Thus, evaluating a spectrum at high field guarantees a simpler spectrum than one acquired at lower field.

For spins other than 1/2 there is an additional effect, the quadrupolar coupling, to electric field gradients that can affect the spectrum. This interaction is independent of field strength, so carrying out experiments at higher magnetic field provides, in some cases, a simplification of the NMR spectrum. Therefore, there are several reasons to develop higher magnetic fields for NMR spectroscopy or to use the highest possible magnetic field for studies.

As mentioned above, the equilibrium condition is such that the sample has a macroscopic magnetization that lies along the magnetic field, with no macroscopic magnetization perpendicular to the magnetic field. A common way to measure the precession frequencies is to place magnetic moments perpendicular to the main magnetic field so that they precess about its direction. This strategy employing the transverse magnetization allows one to record magnetic fields in the sample that are many orders of magnitude smaller than the main static fields. One may create this transverse magnetization by applying appropriate bursts of RF energy to the sample, after which the sample acquires a macroscopic magnetization perpendicular to the main field direction for a finite amount of time. The RF burst (called a *pulse*) must have a carrier frequency close to the frequency of precession of the nuclear spins; it must be reasonably strong; and it must produce a coherent time-dependent magnetic field, B_1. An RF current through a solenoidal coil (or coils in some geometries) produces a B_1 field along the axis of the solenoid; if the current is time dependent, the magnitude of the field is also time dependent. The application of such a field (oriented perpendicular to the main field) to a system initially at equilibrium in the main field results in the creation of magnetization perpendicular to the main field. If the time of excitation is sufficiently short (i.e. the excitation is a short pulse), the transverse magnetization at the end of the RF pulse will be coherent and will subsequently precess about the main field at a frequency characteristic of its particular environment, as determined by interactions such as the chemical shift and J coupling. The RF pulse may produce various effects, depending on the amplitude, the RF phase, and the carrier frequency, so these parameters must be precisely controlled to make the greatest use of a modern NMR spectrometer.

Let us introduce a bit of nomenclature. If the magnetization is allowed to rotate 90° from its equilibrium position along the external magnetic field (or z axis) during the pulse, the RF excitation is referred to as a 90° pulse. If the magnetization is allowed to rotate 180° during the RF excitation, the pulse is said to be a 180° pulse, and so on. In addition, the relative phase of the RF excitation is important. Relative to some coordinate system, the B_1 field may fall along the x axis or the y axis, or any other axis in the transverse plane. Generally, the x and y directions (and sometimes the $-x$ and $-y$ directions) are the most important, and so the pulse is called an *x pulse* or a *y pulse*, with symbols 90°_x and 90°_y, respectively. The phase of the pulse indicates the Cartesian axis around which the magnetization is rotated and, hence, where the magnetization ends after the 90° pulse. An x pulse puts the magnetization initially parallel to the y axis, whereas a y pulse puts the magnetization along the $-x$ axis. It is important to realize that the sense of rotation is arbitrary; by convention a right-hand Cartesian axis system is chosen; however, the rules can be reformulated for left-hand rotations, with equivalent results.

Following a 90° RF pulse, the net magnetization, being perpendicular to the field, precesses at the Larmor frequency around the direction of external magnetic field. This motion of the magnetization induces a time-dependent voltage in the probe coil, which can be detected and digitized as a function of time, which is the FID. FT of the time-domain FID produces a frequency-domain NMR spectrum that contains all the frequency components of the magnetization. From the frequency spectrum, the analyst can determine the presence or absence of certain types of nuclei and certain types of chemical environment. Aside from this simplest NMR experiment, the control of pulses allows one to perturb the magnetization's evolution by a variety of other experimental protocols to provide selective information on couplings and kinetic constants.

In an NMR spectrometer designed for detection of nuclei in liquid samples, there are usually several RF systems operating simultaneously, generally at different frequencies. These are termed *channels* of the spectrometer. In addition to the channel employed for detection of the desired nucleus (the *observe channel*), one channel is usually dedicated to the detection of the deuterium signal from the deuterated solvent (e.g. $CDCl_3$) in which the sample is dissolved. This channel is often referred to as the *lock channel* because the corresponding signal is used for the purpose of controlling the long-term stability of the magnetic field. The number of additional RF channels depends on the configuration of the spectrometer, and that in turn is dictated by the kinds of experiments being done on the spectrometer. For most routine analytical NMR spectrometers, besides the lock channel, there are two observe RF channels, one for high-frequency ^1H detection and another for multinuclear detection usually at lower frequencies, with the capability of providing any frequency over a wide range. This channel is often labeled the *broadband channel*. In addition, many experiments require decoupling of some nuclei (often protons), so the high-frequency channel may also be known as the *decoupling channel*. For high-end biomolecular NMR research spectrometers, there are at least three observe channels (^1H, ^{13}C, and ^{15}N) because of the need to carry out highly sophisticated multiple-frequency experiments. Modern instruments usually have equivalent broadband transmitters for each channel, while the amplifiers dedicated to different nuclei may be different (e.g. ^1H amplifier has a unique high-frequency configuration, with the low-frequency broadband amplifiers used for the remaining channels being the same).

It should be obvious from the short discussion above that the NMR spectrometer requires the following: (i) a strong, stable, and highly homogeneous magnet, (ii) a

probe circuit that houses the sample and has a coil, often a solenoid, to which RF voltage is applied to produce the perturbing field, (iii) a set of RF amplifiers to generate the RF voltages (pulses) applied to the coil, (iv) an RF receiver capable of detecting the tiny voltages generated by the precession of the nuclear magnetization, (v) a system to perform the detection (which usually involves some demodulation, amplification at a lower frequency, the necessary devices to transform the analog voltages into digital data, and some means of storage), and (vi) a number of computers with special software to control all aspects of spectrometer RF pulsing, data acquisition, and processing.

After a brief discussion of the history of, and the current state of the art in, NMR instrumentation, each part of the NMR spectrometer is described in more detail. Since our laboratory is mostly equipped with Bruker BioSpin instrumentation, the Bruker NMR spectrometer is used as an example to illustrate the concepts and architecture of the NMR instrumentation in the following sections. Similar descriptions could be made of laboratories employing other NMR spectrometers (e.g. Varian, JEOL, or Tecmag), although the details of analysis, processing, and setup may be slightly different. A general procedure to operate a solution NMR spectrometer and to acquire an NMR spectrum is given. Finally, the future perspective in the development of NMR instrumentation is discussed.

2 BRIEF HISTORY OF NUCLEAR MAGNETIC RESONANCE INSTRUMENTATION

Since the first detection of NMR signals in bulk materials independently by Bloch et al.[1] and Purcell et al.[2] in 1946, NMR instrumentation has evolved rapidly and continuously for more than 60 years. Early NMR experiments were carried out with permanent magnets or electromagnets. In those days, the magnetic field was slowly swept over a region with an RF field constantly impinging on the sample. When the resonance condition of Equation (1) was encountered, the response was observed as a change in the quality (Q) factor of the coil. Thus, early spectrometers (known as *continuous-wave (CW) spectrometers*) produced a plot of the Q factor of the coil as a function of the field, the Q factor of the coil being a measure of the absorption of energy by the sample. In principle, one could sweep the frequency while holding the field constant, and that was indeed done by some groups. It was, however, more difficult to sweep the frequency of the excitation field, so the majority of spectrometers at that time were of the field-swept variety.

The first commercial NMR spectrometer with a permanent magnet running at the ^{1}H resonance frequency of 30 MHz was pioneered by Varian in 1952. It was a field-swept spectrometer. Already, the trend toward building magnets with higher fields was evident. By the early 1960s, electromagnets with field strengths up to 100 MHz were commercially available. In general, these spectrometers could detect only the resonance of a single nuclear species, usually the proton.

In the mid-1960s, the first superconducting magnet was introduced by Varian.[3] The superconducting solenoid magnet, much more stable than electromagnets, provided magnetic-field strengths unattainable with electromagnets. This resulted in an overall increase in sensitivity. In addition, because at higher fields one could still attain the same magnetic-field homogeneity, the resolution of the NMR measurements was greatly enhanced by the use of the superconducting magnets.

At about the same time that superconducting magnets became available, the demonstration[4] of the Fourier transform relationship between the FID and the frequency spectrum and the development of fast Fourier transform (FFT) paved a new path for NMR spectroscopy. The combination of the detection of the time response and the superconducting magnet added another dimension to the NMR experiment. Because of the ultrastability of superconducting magnets and the exact repeatability of the time response, signal averaging (which had been done only occasionally in swept-mode systems) became commonplace. One year after the demonstration of the technique, the first FT NMR with a superconducting magnet was marketed.[5] The FT experiment is generally repeated in a few tens of seconds, rather than the many minutes it took for acquisition of a single CW spectrum, and allowed a dramatic increase in the S/N within a given time. This added the real possibility of detecting nuclei like ^{13}C with routine spectroscopy, and soon multinuclear spectroscopy became a part of the analytical toolbox.

Of equal importance has been the fact that excitation sequences were developed to parse or factor the information in a spectrum. For example, the utilization of RF-pulse sequences has resulted in multidimensional NMR techniques, from which rich structural information is easily extracted.[6,7] These multidimensional NMR experiments have become part of routine experimental protocols and are now offered as a standard component of any NMR spectrometer software package.

Since its introduction, the superconducting magnet has been one of the driving forces behind modern NMR spectroscopy. The magnetic-field strength attainable by the highest-field commercial superconducting magnet has increased roughly linearly from a Varian 200 MHz spectrometer in 1966 to a Bruker BioSpin 950 MHz spectrometer in 2006.[3]

Developments in probe technology also greatly expanded the utility of NMR over the years. In early

studies, the probe circuit was tuned for a single nucleus (usually ^{1}H) and the response of the ^{1}H was monitored with all the interactions that naturally affect the proton being seen in the structure of the spectrum. Over the years, probes have evolved from a single-channel type to those that contain multiple RF channels to accommodate the excitation and detection of several nuclei simultaneously. Early multichannel probes had to be tuned manually, which is a time-consuming operation. Modern probe design and computer control allow fully automatic tuning and matching (ATM) of several different simultaneous circuits. This automatic operation has produced an open-access laboratory environment, where the experimenter does not have to delve into the eccentricities of RF electronics to obtain usable NMR spectra. Recently, the development of cryogenically cooled probes has led to a leap in sensitivity owing to noise reduction in the probe electronics.

In the wake of the rapid development of digital electronics, extensive digital control of the spectrometer is common nowadays. This also simplifies the adjustments that were necessarily made by operators with older analog (or partially analog) spectrometers. Needless to say, the rapid developments in computer technology quickly become incorporated into NMR spectrometers.

3 CURRENT STATE OF THE ART IN NUCLEAR MAGNETIC RESONANCE INSTRUMENTATION

A typical twenty-first-century state-of-the-art NMR spectrometer is shown in Figure 1. An actively shielded superconducting solenoid produces a 600 MHz magnetic field (A). The magnet is mounted on antivibration posts (B) to isolate low-frequency (<25 Hz) building floor vibrations that might affect the NMR spectroscopy. Floor vibrations can be easily observed as satellite peaks appearing around the main resonance peaks in a high-resolution NMR spectrum. With reasonable magnetic-field homogeneity, the intensities of floor vibration peaks can be reduced to less than 0.55% of the main peak intensity.

The RF generators, amplifiers, receivers, lock channel, pulsed-field-gradient (PFG) amplifiers, and other spectrometer control units are mounted inside the spectrometer console (E). In a basic configuration, there are three RF channels including one for ^{1}H, a second for other (sometimes called X) nuclei (i.e. ^{13}C, ^{15}N, ^{31}P...), and the third for the deuterium lock that regulates the magnetic field. The RF pulses are created by a computer-controlled pulse program. This computer program controls precisely the frequencies and the timing

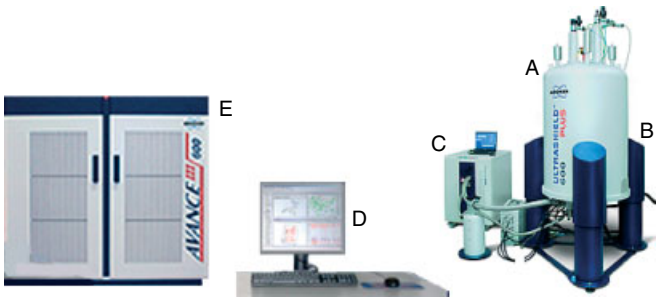

Figure 1 Modern NMR spectrometer: A, actively shielded superconducting magnet; B, antivibration posts; C, cryogenic cooling unit; D, primary computer; E, spectrometer console.

of the RF pulses. Frequency generation and timing of RF pulses are carried out digitally to produce pure frequencies. The digital pulse instructions are converted into analog waveforms that are brought to the appropriate power level by the RF amplifiers, which are linear over a wide range (<120 dB) and capable of producing several hundred watts of RF power. Modern receivers are completely digital and use oversampling technology to eliminate artifacts related to the digitization process.

The ^{2}H lock channel is also digitally controlled by its own microprocessor to compensate for any field drift and to maintain field homogeneity through adjustment of the RTS coils. The primary computer (D) provides a graphic user interface (GUI) that allows the spectrometer operator to set up a pulse program, control experimental progress, and process the acquired data.

The type of probe in a particular system depends on the type of research for which the spectrometer is used or the role that the NMR spectrometer serves. In Figure 1, the cryogenically cooled probe (mounted inside the magnet) is controlled by a cryocooling unit (C). Other multiple probe configurations are available commercially. The most popular configuration for routine use in analytical solution NMR is a 5-mm inverse broadband probe with PFG capability. Another useful probe for routine service is the so-called quad nuclei probe, which is pretuned for four different nuclei, with ease of switching among the channels by digital commands. These probes are readily used for acquiring data in one-dimensional (1-D), two-dimensional (2-D), and three-dimensional (3-D) NMR experiments without the necessity of changing cabling or retuning the probe.

The architecture of modern NMR spectrometers is modular, which makes expansion of the spectrometer functionality relatively simple. The addition of a sample changer or other automation accessories and the associated control software makes a routine open-access spectrometer that can be easily operated by users having only had a brief basic training. An additional RF amplifier and transmitter (e.g. ^{15}N) can be added

to the basic spectrometer to extend the capability for analyzing biological samples. In addition, many types of cryogenically cooled probes are available for the enhanced sensitivity required in experiments such as the detection of natural-abundance ^{15}N, the ^{13}C 2-D INADEQUATE (incredible natural-abundance double quantum transfer experiment), the ^{13}C measurement of polymeric materials at high temperature, and triple-resonance (^{1}H, ^{13}C, ^{15}N) experiments that are essential to analysis of biological macromolecular samples. When equipped with a high-power amplifier, a solid-state probe, and a magic-angle spinning (MAS) controller, the modern NMR spectrometer can readily analyze solid materials.

NMR spectroscopy has also become an integral part of many hyphenated analytical techniques such as liquid chromatography/nuclear magnetic resonance (LC-NMR) (*see* **High-performance Liquid Chromatography Nuclear Magnetic Resonance**). These techniques find a great deal of use in the pharmaceutical and chemical industries, where effective separation and structural elucidations may be carried out in a single apparatus. The mixture separated by liquid chromatography (LC) is routed to the NMR spectrometer where the individual pure components of the mixture are injected individually into a flow NMR probe to be analyzed.

Further developments even combine LC, NMR, and mass spectrometry into a liquid chromatography/nuclear magnetic resonance/mass spectrometry (LC-NMR/MS) system. Such systems are valuable because it is often desirable to have multiple means of analyzing some samples. A commercially available integrated system allows the use of an NMR spectrometer and a time-of-flight (TOF) mass spectrometer to give both analyses of a sample. This technology is an ideal platform for metabolomic studies. The use of solid phase extraction (SPE) provides an efficient interface between LC and NMR when demanded for special flow probes. The liquid chromatography-mass spectrometry (LC-MS) provides the exact mass of the analyzed sample and access to the sum formula. The combination of these three powerful techniques often gives the answer to a question in a matter of minutes.

The ubiquity of nuclear magnetic resonance spectroscopy, especially its wide applications to analysis of chemicals in the laboratory or in the factory, has created an industry catering to the needs of NMR spectroscopy. Companies specialize in the production of NMR solvents, or in specifically isotopically enriched materials for use in studies that focus on a particular site (such as in studies of enzymes). Some companies specialize in consumables such as NMR tubes. Finally, the development of NMR software for specific applications is yet another niche market that is serviced by a number of companies. Readers are referred to the website http://www.ebyte.it/library/NmrMriCompanies.html for a constantly updated directory of NMR and magnetic resonance imaging (MRI) companies.

4 DESCRIPTION OF NUCLEAR MAGNETIC RESONANCE SPECTROMETER

A modern NMR spectrometer consists of a superconducting magnet, including a cryoshim coil assembly to compensate for the inhomogeneity of magnetic field, an RTS coil assembly to adjust the magnetic homogeneity variations between the different samples, a sample probe, transmitter channels including a lock channel, an RF receiver system, and a computer. In addition, there may be a subsidiary slave acquisition computer (AC) for controlling the RF transmitters, which is addressed by the primary computer. A schematic diagram of an NMR spectrometer is shown in Figure 2. The primary computer (A) serves as an interface between the spectrometer operator and all the components of the spectrometer. The operator's instructions are interpreted by the primary computer and are transferred to an AC through an Ethernet cable (B). From the AC, all incoming information is routed to appropriate devices through a data bus or through RS-232 or RS-485 serial lines. The information returned to the AC, such as variable temperature-control output, lock signal, shimming responses, and other control signals are transferred back to the primary computer through the Ethernet cable (B). The time-critical information such as pulse timing and frequency control are sent back and forth from the AC to the appropriate control boards through a high-speed data bus. Signals that are not time critical (shim, lock, and variable temperature control) are transferred through an RS-232 or RS-485 cable. Most recently, the RS-485, which is faster than RS-232 and can be used to control several units simultaneously, has been used for communication to the preamplifiers and signal-generation units.

All NMR experiments start with a sequence of instructions for RF pulsing, delays, and data acquisition. The pulse sequence, entered by the operator at the primary computer, is transferred to the AC as the instructions for the synthesizer to produce RF signals with a specific frequency near the Larmor frequencies of the nucleus being studied. All RF events are synchronized to the same clock. The digital RF signal instructions are converted into analog commands (voltages) that can drive the RF amplifiers. The output voltage sequence of the RF amplifiers (ranging from milliwatts for decoupling to hundreds of watts for excitation) is applied across the probe coil to produce the RF magnetic field that excites the sample.

At appropriate times, the probe coil is also used as a detector of the NMR signal. The resulting induced

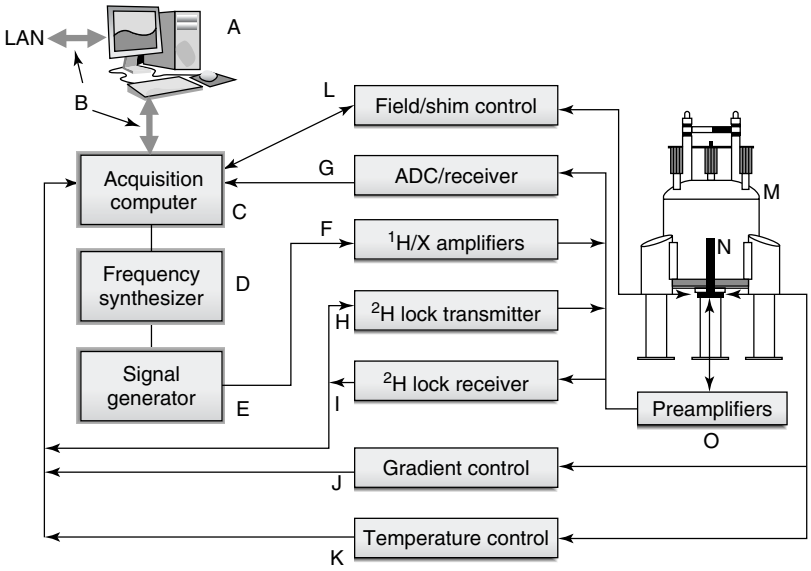

Figure 2 Schematic diagram of an NMR spectrometer: A, primary computer; B, Ethernet connections; C, acquisition computer; D, frequency synthesizer; E, signal generator; F, RF amplifiers; G, RF receivers and analog-to-digital convert; H, ^{2}H lock transmitter; I, ^{2}H lock receiver; J, gradient control and amplifier; K, temperature-control unit; L, room-temperature field and shim control and power supply; M, superconducting solenoid magnet; N, NMR probe; O, preamplifiers.

voltage is amplified by an RF preamplifier. The amplified signal is fed to the receiver section, which includes a reference mixer to reduce the frequency of NMR signal from the RF region to the audio frequency region. At this point, the signal is often amplified with an audio amplifier. The analog voltage is then digitized. Prior to digitization, a digital noise reduction filter is often used to remove aliased signals and noise. The digitized signal is then stored in the principal computer. When the experiment is repeated, the data are coadded and stored in the principal computer.

If the spectrometer is equipped with a gradient accessory for destruction of undesired magnetization remaining between repeated scans, gradient-pulsing control is carried out by the same computer, so that RF and gradient control are synchronized. As shown in Figure 2, the lock channel (F and H) can be considered as an independent ^{2}H spectrometer parallel to the *observe* and *decoupling* channels.

Subsequent to data collection, the digitized signal (the FID) is transferred to a frequency-domain spectrum using the FFT algorithm by the primary computer. At this point, digital manipulation of the FID is possible, for example, with various apodization procedures. An example of FFT is given in Figure 3, where the time-domain FID is on the left and the frequency-domain spectrum is on the right.

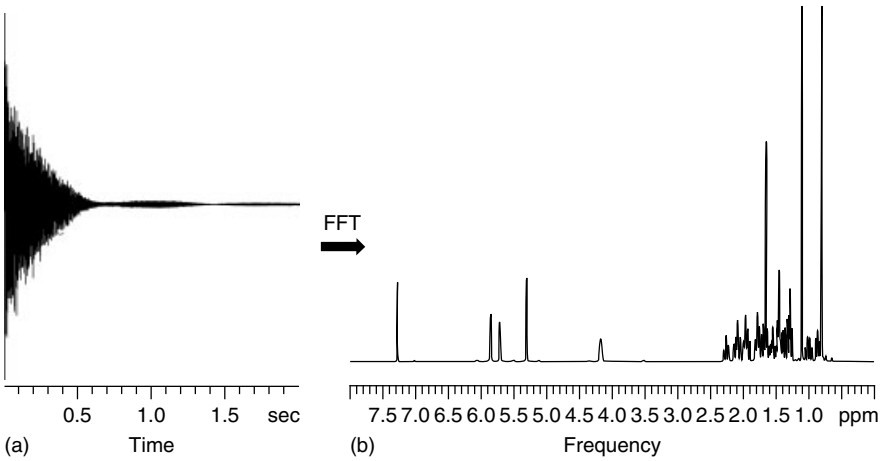

Figure 3 ^{1}H NMR spectra of gramicidine-S in DMSO-d6: (a), the FID in time domain and (b), spectrum in frequency domain. The Fourier transform is used to convert the time-domain FID to frequency-domain spectrum.

The exponential decay of the FID signal results from nuclear relaxation processes.

4.1 Superconducting Magnet

An ultrastable strong magnet field in the active sample volume is provided by a solenoid made of alloys generally containing niobium, which are superconducting at the temperature of liquid helium. A schematic diagram of a superconducting magnet is given in Figure 4. The intense static magnetic field is the result of the constant current in a coil of superconducting niobium-alloy wire that comprises the magnet (F). The wire must be immersed in a bath of liquid helium (<6 K) (D) to keep it in superconducting state. In the superconducting state, the electric current running through the magnet coil is said to be *persistent*, in that there are no losses of current due to resistive effects. Besides the main coil, a set of cryoshim coils also in the superconducting state is provided to create small fields to offset inhomogeneities in the main field. Once set, the currents in all the superconducting coils remain constant and are not generally changed by the user. To reduce the boil-off of liquid helium, it is encased in a vacuum jacket packed with materials that limit radiative losses. This setup is surrounded by a further jacket (E) filled with liquid nitrogen (77 K) and another vacuum jacket (C) to isolate it as much as possible from the room-temperature environment. The central bore of the magnet, which houses the sample probe, is at room temperature.

At the time of installation of the NMR spectrometer, the jackets are pumped down to $\sim 1 \times 10^{-5}$ Pa to remove as much air and other ''contaminants'' (if present) as possible before the cryogenic liquids are added. The helium jacket is generally precooled with liquid nitrogen at this stage, with the liquid nitrogen being removed before the liquid helium is added. After the cool down, the magnet is charged with electric current by connecting it to a special power supply. For an actively shielded 600 MHz magnet, a 134-A current is slowly introduced into the magnet coil until the magnetic-field strength reaches 14.09 T. Following this procedure, the cryoshim coils are also given appropriate currents. The optimal set of currents is determined by observation of the line shape of a water sample. Generally, one should be able to achieve a line width of less than 0.5 ppm with a reasonable line shape using only the cryoshim system. Once the magnet field strength is reached and the cryoshim currents have been optimized, a set of heaters that has kept some parts of the wire nonsuperconducting to allow connection to the power supply is carefully turned off to produce a self-contained superconducting magnet with persistent currents. The final step is the complete removal of the power supply from the magnet. The magnet is not connected to any source of power during operation and will continue to be persistent almost indefinitely, as long as it is kept at temperatures low enough to maintain the wires in the superconducting state.

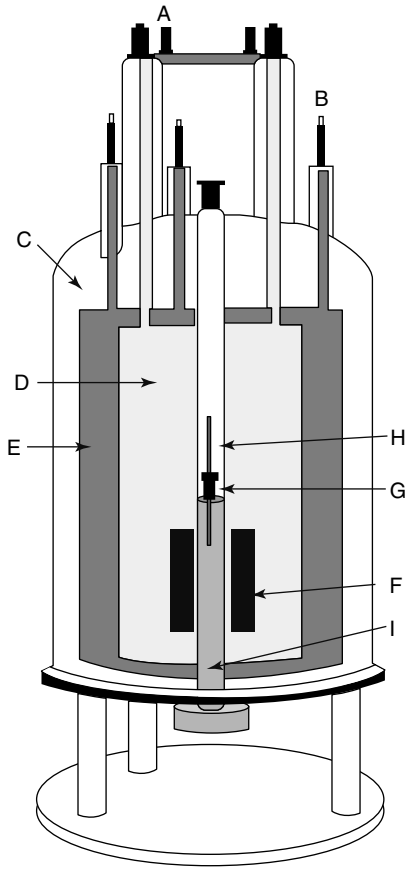

Figure 4 Schematic diagram of a superconducting solenoid magnet: A, liquid helium refill port; B, liquid nitrogen refill port; C, outer vacuum chamber; D, liquid helium bath; E, liquid nitrogen bath; F, superconducting solenoidal magnet; G, sample spinner; H, sample tube; I, NMR probe.

Any coil produces a continuous field, both inside the coil and outside. The components of the field outside the coil affect the space around the magnet and could potentially present safety hazards for those working around the magnet. Magnets produced most recently are *actively shielded*. In actively shielded magnets, the field components outside the magnet housing are compensated with additional coils and special windings to minimize the intrusion of the magnetic field into that space. Active shielding allows much more effective use of laboratory space.

Magnets in the range of 300–500 MHz (proton frequency) are currently very popular in the analytical NMR laboratory. The use of magnets in the range of 600–750 MHz is becoming increasingly accessible as the technology becomes more stable and the costs of these

systems decrease. As of 2007, a 950 MHz magnet has been successfully tested in a manufacturing facility and will be relocated to a customer's laboratory. Thus, one should expect the trend toward the use of ever-higher magnetic fields to continue for some time.

As we have mentioned, magnets are generally (if somewhat colloquially) specified by the proton resonance frequency and the diameter of the room-temperature bore. Thus, a 500/SB MHz magnet means that the proton resonance frequency is 500 MHz and the room-temperature bore diameter is 54 mm (''standard bore''). There are also wide-bore (WB) systems, and these are similarly expressed as 500/WB, typically meaning a bore of 89 mm.

To maintain the magnet in the superconducting state, a weekly refill of the liquid nitrogen reservoir and a periodic refilling of the liquid helium reservoir (approximately every 2–5 months, depending on the magnet design) are required.

4.2 Signal Generation and Radio-frequency Amplifiers

In a modern NMR spectrometer, a set of RF transmitters produces the voltage that creates the perturbing time-dependent magnetic field. Usually, an RF transmitter consists of an RF source, a phase modulator, and an amplifier to generate the voltage of the desired amplitude, frequency, and phase. The phase modulator determines the phases of the pulses during the experiment, such that one may demand an x, a y, or any other pulse of RF magnetic field. The amplifier provides the RF voltage that impinges on the coil. It must be capable of providing outputs of a few watts (for a solution-state NMR spectrometer) to a few hundreds of watts or even kilowatts (for a solid-state NMR spectrometer).

The RF system provides the means to produce the voltages that excite the nuclei in the sample. The RF waveforms are generally produced at low power with various gating devices that allow the passage of RF current when an appropriate voltage is applied. The RF signal from a digital synthesizer passes through this stage, and from the output comes the appropriately modulated RF signal. The modulators in this stage are all connected to the AC that controls timing, frequency, shapes, phase, and amplitudes of the RF pulses according to the pulse program loaded into it from the primary computer. In modern NMR experiments, there are often excitations at several different frequencies for the various nuclei in a sample, so there are several channels, each similar to the others in function but working at a different frequency. When multiple RF channels are involved, all channels must be able to operate independently, as well as being synchronized with each other through their control by the AC. In a modern NMR spectrometer, the timing controllers are able to control more than 60 events with a timing resolution of 12 ns. The modulated output drives the high-power amplifiers that produce voltages corresponding to radiation up to several kilowatts across the sample coil.

All NMR experiments start from the operator's instructions, a computer-programmed pulse sequence. In a pulse program, the frequencies, timing, phase, and amplitude of pulses in the channels are specified by the experimenter. This is usually done at the primary computer. Execution of the pulse program presents a series of digital instructions to the NMR spectrometer interface (usually through the intermediacy of the AC), containing a DAC that produces the appropriate analog waveform from the digital instructions of the pulse program. All ''run-time'' decisions, such as conditional loops and phase and frequency shifts, are made by the AC to control counters, gates, and electronic phase shifters in the RF signal-generation unit. The signal-generation unit creates the analog RF pulses and delays that drive the sample to make a response. A typical signal-generation unit has a frequency range of 3–1100 MHz, a frequency resolution less than 0.005 Hz, a switching time less than 300 ns, and phase resolution less than 0.006°. An additional function of a signal-generation unit is to produce reference signals, gating, and the dwell clock for the receivers.

The output of the signal-generation unit is passed to high-power RF amplifiers for further amplification. The output of the amplifiers is passed directly to the sample coil, where it perturbs the sample in a manner determined by the pulse program written by the experimenter. The high-power amplifier has a wide linear range (120 dB or more) so that it can accurately reproduce the RF signal passed to it. The RF output of the amplifier ranges from a few hundred watts for a solution-state spectrometer to a kilowatt for a solid-state spectrometer.

The pulse has a limited region of frequencies around the carrier frequency that it affects. The bandwidth of the excitation by an RF pulse is determined by the RF field strength, B_1, which also determines the 90° pulse width. The relationship between pulse width and the RF field strength is straightforward:

$$\frac{\gamma B_1}{2\pi} = \frac{1}{PW_{360}} = \frac{1}{4PW_{90}}\text{Hz} \tag{3}$$

where PW_{360} and PW_{90} are the pulse widths for 360° and 90°, respectively. For example, a 90° pulse width of 10 μs corresponds to a B_1 field strength of 25 kHz, a typical value for pulse excitation on a modern solution NMR spectrometer. This pulse width is generally acceptable to excite the full range of possible nuclear frequencies for nuclei such as ^{1}H and ^{13}C. This type of pulse is sometimes

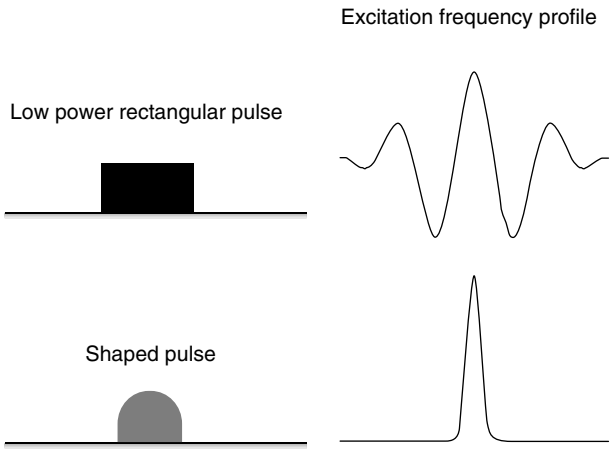

Figure 5 Schematic excitation profiles of a low-power rectangular pulse and a shaped pulse.

called a *hard pulse*. For some nuclei, a uniform excitation is not always possible using the conventional solution NMR hardware because of the large range of frequencies the excitation must span. As mentioned above, pulses also have phase properties that determine along which transverse axis, x, y, $-x$, or $-y$, the magnetization lies after the RF pulse. This pulse phase is defined relative to an internal reference signal shared with all transmitter channels and receivers.

Pulse shapes also affect the excitation bandwidth. The common hard pulse described above is rectangular, with a finite width, constant amplitude, and phase. Sometimes it is necessary to excite a narrow region of the spectrum for selective transfer experiments, solvent suppression, and reduction of the dimensionality of multiple-dimension pulse sequences. In principle, the simple selective pulse can be a long, weak rectangular pulse. However, as shown in Figure 5, the excitation profile of a rectangular pulse often involves extended side lobes in the frequency excitation profile, which often create a sinc-like oscillation in the resulting spectrum. Also shown in Figure 5, the selective pulse, sometimes called a *soft pulse*, is mostly a shaped pulse, which is often used to smooth out the side lobes in the excitation profile caused by the rectangular pulse shape. The most commonly used shaped pulses have a profile of a ramp, a sine, a trapezoid, or a Gaussian. The selective shaped pulses, characterized by their duration, frequency profile, and phase behavior, are typically 1–100 ms in length, three orders of magnitude longer than the hard pulses.

4.3 Detection and Radio-frequency Receiver

Each RF channel is equipped with high-performance preamplifiers and RF receivers to detect and amplify the signal. Subsequent to excitation, the detected sample response is an analog voltage across the coil. The signal is ultimately converted to a digital form by a highly sophisticated ADC. The weak NMR signal (approximately microvolts) goes through preamplification while it is still a radio-frequency signal. This preamplification occurs in a unit separated from the console and located near the magnet to avoid loss in transmission through the cable. With an actively shielded magnet, the spectrometer console can be located much closer to the magnet than with a conventional magnet. In this case, the preamplifiers may be housed inside the spectrometer console to maximize the usage of laboratory space. The preamplified signal is usually then transformed into a lower-frequency signal by demodulation against a reference signal. After that, the lower-frequency signal is amplified again (often to filter high-frequency noise) to the level of several volts and finally digitized for storage and further processing.

A receiver must be able to amplify the signal over all frequencies within the spectral width (SW) linearly; otherwise, the amplitudes in the spectra cannot be used for quantitative results. In addition, the receiver must retain all phase information of the input signal. A large dynamic range is desirable for a receiver so that small signals in the spectrum are detected with appropriate ratios so that they can be quantitated. The receivers in modern spectrometers have variable gain under the control of the experimenter. It is very important to set the receiver gain properly. If the signal is overamplified (i.e. the gain is set very high so that receiver output is greater than 5 V), the digitizer is saturated by the largest signals, resulting in some signals seeming to have the same amplitude. A ''clipped'' FID is the result of saturating the digitizer. If the gain is too small, only a part of the dynamic range of a digitizer is used and poor reproduction of the signal results.

The chemical shift ranges (kilohertz) are much smaller than Larmor frequencies (typically tens or hundreds of megahertz). For example, the frequency range of a proton spectrum at 600 MHz is between 7 and 8 kHz, and is about 20 kHz for a ^{13}C spectrum obtained on the same spectrometer. Often, one is only interested in chemical shift differences, instead of the absolute Larmor frequency. Therefore, it is a common approach to demodulate the signal to a lower frequency, which is equivalent to subtracting a reference frequency from the detected signal frequency before digitization. The reference frequency signal is usually chosen to be the carrier frequency of the exciting pulse. In addition, the RF signal is frequently referenced against a waveform with a phase reference differing by 90°. This gives the so-called in-phase and out-of-phase components of the signal, which are simultaneously digitized and stored together. This data detection protocol, referred to as *quadrature*

detection, is widely used. It has the advantage that the Fourier transform of the quadrature signal distinguishes between positive and negative frequencies (relative to the reference).

The digitizer works on the ''sample and hold'' principle, in which the signal is sampled repetitively with a specified interval. The time between samples is called the *dwell time* (*DW*). The minimum DW one may use is dictated by the Nyquist theorem, which states that the digitization rate must be at least twice as large as the highest frequency one wishes to observe. If the Nyquist theorem is violated during digitization, spectral aliasing or folding may occur, in which peaks that have frequencies outside of the spectral window are detected as peaks at frequencies within the spectral window, often with a weaker intensity or a phase shift. To improve the performance of digitization, one may use an analog or digital filter to remove signals at frequencies outside of the spectral window. Digital filtering has replaced analog filtering done with passive analog electronic circuits. Employing oversampling, where many more points than required by the Nyquist theorem are collected, has helped the performance of digital filter programs and is common in most modern spectrometers.

In quadrature detection, the NMR signal consists of two time series related by the phase between the two reference signals. Because there are twice as many data in a quadrature set, the S/N in a spectrum collected in the quadrature mode is increased by a factor of $\sqrt{2}$ over that of a spectrum that comes from a single time series. In an experiment using quadrature detection, the RF-pulse frequency is at the center of the spectrum to assure a uniform excitation across the spectrum. Since it is almost impossible to make the two channels in the quadrature detection exactly identical, there are sometimes quadrature-detection-related artifacts that appear in the Fourier spectrum. Phase cycling of the RF excitation and the detection in a repeated experiment is often required to eliminate such artifacts.

4.4 The Spectrometer Field-frequency Lock System

The magnetic field and the frequency must both remain constant to within at least one part in 10^{10} during the course of an NMR experiment. Some experiments such as ^{13}C acquisition, 2-D, and 3-D experiments may take several hours to several days. Magnetic fields of a superconducting magnet drift more than this (one part in 10^{10}) over a period of time, which leads to a loss of resolution in the NMR spectrum. To overcome the field drift, a field-frequency lock system is widely used in modern solution NMR spectrometers. The frequency of the deuterium (^2H) signal of the deuterated solvent of the sample is constantly monitored and reports on the magnetic-field drift. The value of the deuterium frequency is then used to ''correct'' for the field drift by applying a small additional field parallel to the main field using an RTS coil. This feedback system, which can be considered a parallel spectrometer, is often referred to as *the lock channel*. The lock channel contains a dedicated transmitter, receiver, and compensation circuit.

The choice of the ^2H signal to be that of the deuterated solvent (D_2O, $CDCl_3$, CD_3CN, DMSO, etc.) is convenient since the material is present in the sample and does not generally interfere with the spectroscopy of the commonly observed nuclei. If the nucleus of detection is ^2H, then one usually uses the NMR spectrum of some other nucleus (such as ^{19}F or ^1H) as the basis for the field-frequency lock system.

The lock system works in the following way. The absorption component of the ^2H NMR signal of the deuterated solvent is generally displayed by the computer. The dispersion component is used to form the compensation voltage that regulates the field, as shown in Figure 6. When the magnetic-field shifts, there is a change in the deuterium resonance frequency. The error signal that occurs depends on the magnitude of the frequency shift and on the direction of drift. This error signal controls a feedback circuit that adjusts the current in the B_0 shim coil in the RTS assembly to compensate for the field drift. In most cases, the deuterium NMR signal is detected by the CW method, because the CW method is simple and provides sufficient sensitivity for monitoring the frequency change of a single and strong resonance.

Since the lock channel is virtually a separate ^2H NMR spectrometer, its operation relies on careful settings of the ^2H transmitter power, the ^2H receiver gain, and the receiver phase. The highest possible lock transmitter

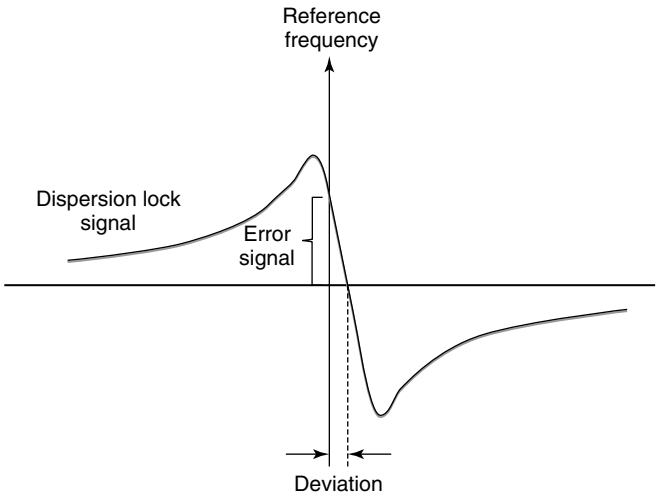

Figure 6 The sign and magnitude of error signal from deuterated solvent is used in a frequency-lock feedback circuit to compensate the field drift.

power (without saturation) ensures the highest sensitivity in the lock signal. Saturation of the ^2H through use of too high a RF power will limit the ability to detect a frequency change. The amplifier gain controls the magnitude of the lock signal. If the gain is too low, the error signal is low and the amount of signal is not sufficient. On the other hand, excessively high amplification introduces unnecessary noise that may cause the lock to suffer random jumps. The error signal also depends on the proper setting of the lock phase.

4.5 The Shim System

The typical solution-station NMR resonance line widths in solution range from a few tenths of hertz to a few hertz. To maintain a magnetic field over an active volume of about 0.3–0.7 cm^3 and over a period of hours is extremely demanding. For example, in order to resolve fine structure with a splitting of the order of 0.5 Hz in a 600 MHz spectrometer, a relative field homogeneity $\Delta B/B_0$ of better than 10^{-9} is required. Inhomogeneous magnetic fields across the sample volume cause various distortions of the line shape, which leads to poor resolution and loss of sensitivity. Besides the careful design and construction of the superconducting solenoid, the magnetic field's homogeneity is achieved by adjusting currents by two methods: cryoshimming and room-temperature shimming. The shim coils are a set of coils wound in various configurations that carry electric currents to generate small magnetic-field gradients to compensate or cancel out small gradients in the magnetic field of the large solenoid. The cryoshims are superconducting coils that are adjusted when the magnet is energized. The cryoshims remove gross inhomogeneities in the magnetic field.

It is important to remember at this juncture that the static field of the typical superconducting magnet and cryoshim system is already extremely homogeneous, often with inhomogeneities of no more than 10^{-8} over the volume of the sample. That high quality notwithstanding, to obtain the maximum performance required for high-resolution NMR, further optimization of the field homogeneity is needed. This second procedure is carried out with the RTS system. The set of room-temperature coils is mounted in the magnet bore surrounding the sample probe. The current in these coils is adjusted for each sample to compensate any remaining field gradients, specifically including gradients caused by differences from sample to sample.

The process of preparing the system to measure an NMR spectrum requires one to carry out what is called *shimming*, adjustment of the currents through the RTS coils to optimize the magnetic-field homogeneity across the sample volume, which allows one to maximize the sensitivity and resolution. Some people enjoy this iterative process, and some people find it an uncomfortable part of the setup to carry out the NMR experiment; it must be done with care to achieve a good NMR spectrum. To monitor homogeneity improvement while shimming, one uses the increase in the deuterium lock signal, or perhaps the time extent of an FID, to indicate approach to the optimal conditions. In addition, one sometimes examines the line shape of some resonance to ensure a combination of narrowness of the resonance band with a symmetric line shape. As discussed below, modern instruments are often equipped with triple-axis pulse field gradients, and shimming can be performed automatically by first mapping the magnetic-filed profile in the sample and then compensating for the field inhomogeneity with adjustments of the appropriate RTS coils.

There are two types of shim coils, axial and transverse, which are also known as *spinning* and *nonspinning* shims, respectively, to indicate the condition under which each is adjusted. Each shim coil is designed to produce a gradient profile near the center of the coil that varies as a specific mathematical function of the coordinates. The basis set functions corresponding to the individual coil geometries in almost all spectrometers are the spherical harmonic functions, the solutions of Laplace's equation.[8] The coils are therefore labeled by the Cartesian representation of the appropriate spherical harmonic function. Table 1 lists 17 of the RTSs and their corresponding labels. In some spectrometers, there may be as many as 64 RTSs that can be adjusted. The spinning shims have orientations that produce a gradient along the axis of sample rotation. They are best adjusted when the sample is spinning. The nonspinning shims are adjusted without sample rotation because they produce gradients perpendicular to the direction of the main field. Rotation would average these out, and one wishes to compensate them with the gradient coils, so the adjustment is done without spinning.

One of the most exciting developments in the NMR instrumentation in recent years is the gradient-shimming method,[9,10] which completely changes the conventional method of magnet shimming described above. Gradient shimming utilizes a gradient-echo sequence. The result of this process is a resonance phase map, which directly correlates to the field inhomogeneity across the sample. This profile is obtained experimentally and is compared with a reference phase map previously recorded on the basis of the known electric current settings of each individual shim coil, so that all required changes of current in each shim coil can be calculated. The gradient-shimming method was first applied to NMR spectroscopy of biomolecular samples that had a strong solvent (90% H_2O) signal. The strong solvent signal is used to map the inhomogeneity across the active sample volume in three dimensions. The gradient-shimming method is now

Table 1 Common room-temperature shims and their Cartesian representations

Shim	Order	Orientation		Function
Z^1	1	Axial	Spinning	z
Z^2	2	Axial	Spinning	$2z^2 - (x^2 - y^2)$
Z^3	3	Axial	Spinning	$z[2z^2 - 3(x^2 + y^2)]$
Z^4	4	Axial	Spinning	$8z^2[z^2 - 3(x^2 + y^2)] + 3(x^2 + y^2)^2$
Z^5	5	Axial	Spinning	$48z^3[z^2 - 5(x^2 + y^2)] + 90z(x^2 + y^2)^2$
X	1	Transverse	Nonspinning	x
Y	1	Transverse	Nonspinning	y
XZ	2	Transverse	Nonspinning	zx
YZ	2	Transverse	Nonspinning	zy
XY	2	Transverse	Nonspinning	$x[4z^2 - (x^2 + y^2)]$
X^2-Y^2	2	Transverse	Nonspinning	$y[4z^2 - (x^2 + y^2)]$
Z^2X	3	Transverse	Nonspinning	$x^2 - y^2$
Z^2Y	3	Transverse	Nonspinning	xy
ZXY	3	Transverse	Nonspinning	$z(x^2 - y^2)$
$Z(X^2-Y^2)$	3	Transverse	Nonspinning	xyz
X^3	3	Transverse	Nonspinning	$x(x^2 - 3y^2)$
Y^3	3	Transverse	Nonspinning	$y(3x^2 - y^2)$

available for samples in organic solvents where the ^{2}H resonance of a deuterated solvent is observed through the lock channel for inhomogeneity mapping. The accessory required for ^{2}H gradient shimming is now a standard part of a commercial spectrometer. An example of gradient shimming is shown in Figure 7, where spectrum A is taken with the current in all RTSs set to zero and spectrum B is obtained after a two-minute ^{2}H gradient shimming with only one iteration. The high efficiency of shimming demonstrated here is almost impossible to achieve by the manual iterative technique, even for experienced NMR spectroscopists.

4.6 The Pulsed-field-gradient Amplifier

The use of the PFG method has become very popular because of its applications in a wide variety of experiments, including coherence selection, solvent suppression, gradient shimming, and its use as a spoil pulse to dephase magnetization. The technique works by transiently applying a magnetic-field gradient to the sample through a gradient coil in the probe, along the z axis (single-axis gradient) or along x, y, and z axes (three-axis gradients). The gradient amplifier is operated independently from, but is always in synchrony with, the RF channels. Usually a gradient-control unit generates the gradient pulse on the basis of commands from the pulse program. Since the gradient pulse involves application of a high direct current across the gradient coil, residual eddy currents are unavoidable. Most commercial systems have self-screening to minimize the influence of these eddy currents. The shape of the gradient can be linear or rectangular, but other shapes can be programmed as well. For reduction of eddy currents, the gradient pulse shape may be a sinusoidal waveform. Like an RF pulse, a gradient pulse is specified by duration, a shape, an amplitude, and a polarity.

4.7 The Computer and Software

There are at least two computers and a number of microprocessors in a spectrometer. The primary computer provides the GUI to the spectroscopist. The computer controls spectrometer action, processes and displays, and data, and performs other tasks necessary to obtain a spectrum. The primary computer usually is a Linux workstation running Enterprise WS4 or WS5, or a PC running Windows Vista or XP. A typical workstation configuration includes 1 GB of internal memory, a 200 GB hard drive, and a 19'' (or larger) flat panel monitor, a DVD/CD-R drive, and two network cards for spectrometer and local area network (LAN) connections. With rapid developments in computer information technology, the specific characteristics of the primary computer change almost on a monthly basis. Using the GUI of the primary computer, the NMR spectroscopist selects an experiment from a list of experimental protocols, changes the parameters if necessary, and commands the computer to start the experiment. The spectroscopist's instructions are sent from the primary computer to an AC located in the console through an Ethernet connection. The AC works as an interface between the transmitter, receiver, lock channel, gradient amplifier, probe, and the primary computer. This computer does not perform onboard data manipulation and only transmits data between various spectrometer components. The communication to timing-critical units such as receiver, transmitter, digitizer, and lock channel is carried out through a high-speed virtual memory expansion (VME) data bus. The Ethernet link

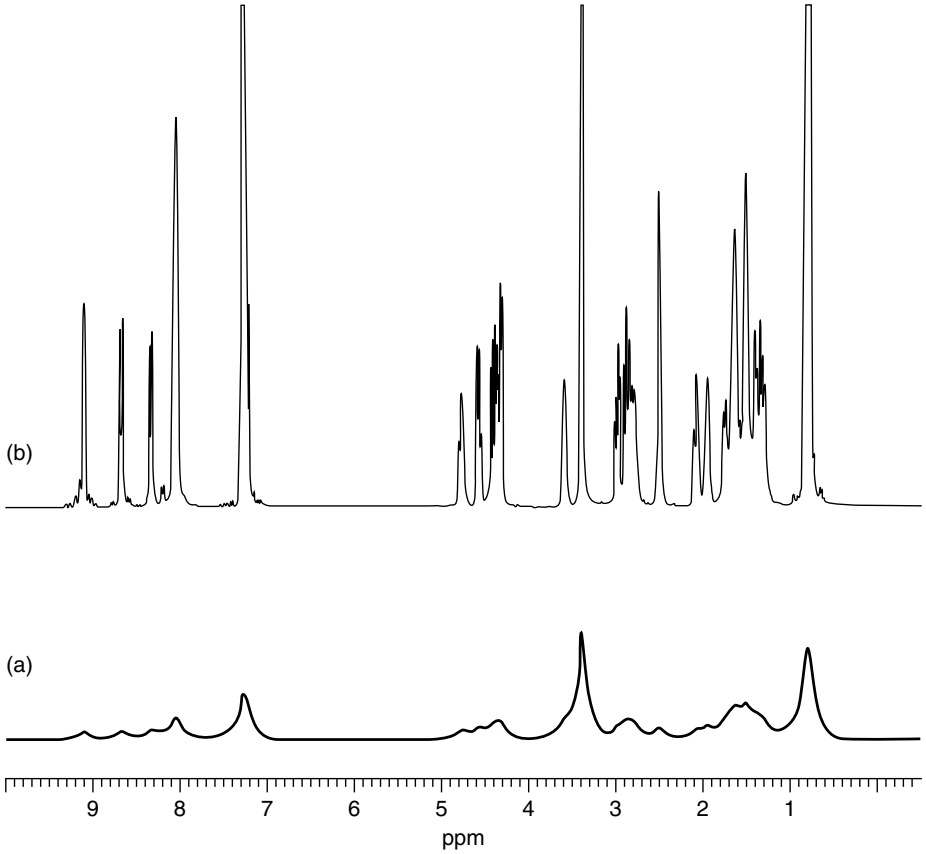

Figure 7 The gradient-shimming method: ^{1}H spectrum of gramicidine-S in DMSO-d6. The bottom trace (a) was obtained by setting all on-axis shims to zero. After a deuterated gradient shimming of z1–z5 for a few minutes, a high-resolution spectrum (b) was obtained.

to the primary computer provides an effective connection by which the commands entered at the operator's desk are transmitted to the AC and hence to various spectrometer components. Non-timing-critical units such as the variable temperature control, the pneumatic unit for probe control, and the automatic sample changer are connected to the spectrometer computer through an RS-232 or RS-485 bus.

Acquired and digitized data are transferred to the primary computer for storage and further processing. Data are saved on a hard drive and may be transferred to a file server through an Ethernet connection. The time-domain data are generally transformed into a frequency-domain spectrum by NMR software resident in the primary computer. A local printer or a network printer can be used to plot the spectrum obtained through this process.

NMR software has several major functions: spectrometer control, data acquisition, and data processing. Most NMR software code is written in the C and C++ programming languages, but may include recent Java and Tcl/Tk add-ons on a Linux- or a Windows-based PC computer. The software packages have an extensive microprogramming language for operators to develop automated operations, to control the spectrometer, and to process the data. The NMR software package is usually accessed through the graphical interface. NMR software usually presents a large display of the current NMR data in either the time or the frequency domain. The program subroutines can be accessed through drop-down menus with many levels of commands that handle tasks from data acquisition to processing. Most commands can also be entered through a command line. There are also control icons for quick access to many routinely used procedures. Interactive operations, such as spectral display, phasing, peak picking, and integration are done by convenient mouse operations.

Automation of operations is an important convenient feature of recently released NMR software. This is especially useful for laboratories where minimally trained users submit samples. The user places the NMR sample on a sample changer carousel and selects the NMR experiments to be carried out through the GUI of the spectrometer's primary computer. The sample is automatically moved, at the appropriate time, from the carousel to the NMR probe by the sample changer, and

the experiment preparation and execution are carried out by the NMR software with basically no intervention from the user. After data acquisition and reduction, a notification of the experiment, usually including the processed NMR spectrum as a portable document format (PDF) file, is sent to the user through an email. The system administrator may choose to archive acquired data to a network data server through a network file system (NFS) protocol or other Internet file-transfer protocols.

A database is used to manage all pulses and other experimental parameters, including durations and power levels for RF pulses for each probe. These parameters can be easily accessed when a new experimental protocol is to be parameterized. As a part of the software package, there are many modules to perform specific functions, such as nuclear relaxation analysis, analysis of shaped pulses, spectral simulation, and experimental simulation, as well as graphic display of the pulse program. A molecular drawing program is even included in some NMR packages for users to document molecular structures for each spectrum. Recently released software also provides web access, which allows users to check the status of data acquisition and to control the spectrometer through the Internet. In addition, there are frequently subroutines for calculation of theoretical spectra or fitting the spectra to some form. NMR software continues to be a fast-developing component of most modern NMR spectrometers, a result of the rapid advances in computer technology and programming.

4.8 The Nuclear Magnetic Resonance Probe

The NMR probe, housing the sample for analysis, transmits RF radiation to the sample and detects the weak NMR response. Most solution NMR probes are mounted in the magnet bore from the bottom of the magnet. For insertion of the sample, a spinner loaded with the NMR sample tube is placed into the probe from the top of the magnet bore. A pneumatic device supplies a cushion of air or nitrogen gas that buoys the spinner/sample combination, allows it to be slowly positioned into the probe as the pressure is reduced, and finally ejects the spinner when the experiment is finished. The sample tube, surrounded by the RF coils, is usually spun at 15–20 Hz to reduce residual magnetic-field inhomogeneities. A simplified schematic diagram of the NMR probe and sample spinner is given in Figure 8.

In general, the sample temperature in the NMR probe can be controlled from -100 to $+150\,^{\circ}\mathrm{C}$ to within $\pm 0.1\,^{\circ}\mathrm{C}$. If the desired sample temperature is higher than room temperature, heated air or nitrogen gas is passed over the sample tube. Cold nitrogen gas generated by a heater immersed in a liquid nitrogen dewar is used as a thermally controlled bath for the sample if the desired sample temperature is below room temperature. Recently, various gas refrigeration devices have become available commercially for cooling nitrogen gas before it is introduced into the probe for temperature regulation at or slightly below room temperature.

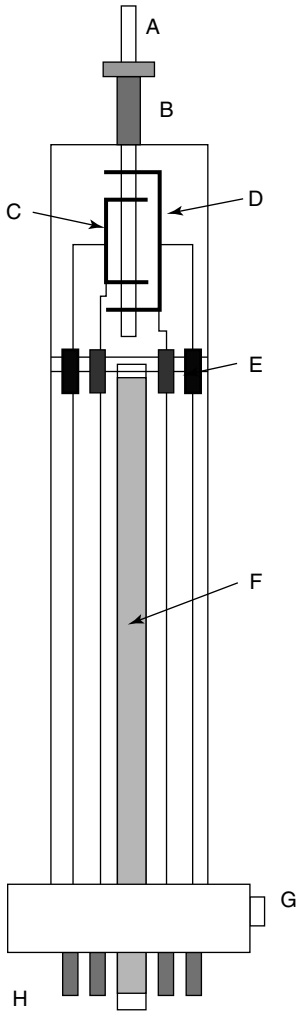

Figure 8 Schematic diagram of NMR probe: A, sample tube; B, sample spinner; C, inner RF coil; D, outer RF coil; E, variable capacitors for probe LC circuit frequency tuning; F, variable temperature dewar and heater; G, RF connectors for transmitting RF pulses and receiving NMR signals; H, knobs for probe tuning.

A simplified probe (LC) circuit, which consists of an RF coil having inductance L and two variable capacitors, is given in Figure 9. Optimal performance of the NMR spectrometer depends critically on detection sensitivity, which is achieved by adjusting the tuning capacitor C_{T} and matching capacitor C_{M} such that the resonance frequency of the probe LC circuit is the nuclear Larmor frequency and the impedance of the circuit is matched to 50 Ω.

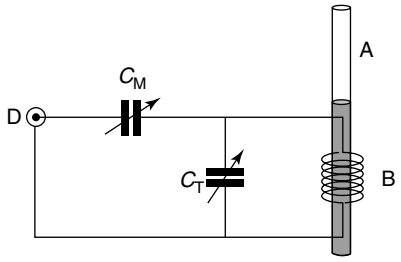

Figure 9 Simplified probe LC circuit: A, NMR sample tube; B, RF coil; C_T, variable capacitor for tuning resonance frequency; C_M, variable capacitor for matching the impedance of the LC circuit; D, BNC coaxial connector to the transmitter and receiver.

In general, insertion of a sample changes the tuning of the probe circuit. Samples containing a high-dielectric material (such as water) may particularly cause a change in probe tuning. Samples that are electrically conducting cause significant changes in both matching and tuning. Therefore, it is imperative to tune and match the probe for each sample. As shown in Figure 8, conventional probe tuning and matching knobs that control the capacitor settings are located at the bottom of the probe. To tune the probe circuit at the appropriate frequency, the response of an RF channel to an imposed RF voltage is compared to the response of a purely resistive 50 Ω load. The RF voltage is generally swept through a range of frequencies as it originates from a sweep generator. Adjustment of the two capacitors, usually by iterative changes, produces a situation in which the RF response of the circuit appears identical to that of the purely resistive load. To aid in this tuning step, the reflected voltage from the circuit is usually displayed through a GUI at the primary computer display. Sometimes, the response is sent to an array of light-emitting diodes (LED), which report the reflected voltage as a string of lit diodes. Whatever the device for detection, the object of the tuning step is to minimize reflected RF voltage from the probe RF circuit.

Tuning the probe circuit requires a set of skills of the user. It can be a time-consuming and frustrating process for one who has not experienced the process. A recent development in the NMR probe technology is ATM by a device that carries out this procedure automatically under software control. The advantage of ATM is especially obvious in an open-access NMR laboratory, where many users are untrained or minimally trained in tuning RF circuits.

Various high-resolution probes for study of solution-state samples are commercially available. Each type of probe meets some requirements for specific applications in chemistry, biochemistry, and material sciences. However, many features are common to all probes, which makes it possible to classify probes into well-defined groups according to the particular values of certain parameters. For example, commercial probes are determined by the sample tube diameter. Currently, one may purchase probes that accept tubes with diameters of 1.7, 2.5, 5, 8, 10, and 20 mm. The choice of diameter depends on the application. Probes with a diameter of less than 3 mm are called *microsample probes* and are used in situations where the amount of sample is limited. The high efficiency of a microsample probe is a result of a high filling factor due to the limited volume of the sample tube. The most commonly used NMR probes accept 5-mm-diameter NMR tubes. Probes for larger tubes can be employed for special applications, where the sensitivity of the measurement is limited.

Besides the sample tube diameter, solution NMR probes can be classified as *standard* or *inverse* based on the configuration for signal detection and for decoupling. A typical standard high-resolution probe has at least two RF coils. The inner coil is for the X-nucleus detection, while the outer coil is mainly used for ^{1}H decoupling. The outer coil is sometimes used for observation of a nuclear signal as well, but this almost always involves a lower sensitivity. The outer (or ^{1}H) coil is usually doubly tuned to allow it to be used to detect the ^{2}H resonance for use in the field-frequency lock system. In some probes, the X channel is tuned only at a fixed frequency (e.g. ^{13}C). However, there are so-called broadband probes in which the frequency may be tuned over a range to allow detection of a variety of nuclei. A typical configuration for a broadband probe might allow detection of nuclei that have frequencies in the range from ^{109}Ag to ^{31}P. The standard probe is generally optimized to observe an X nucleus, normally ^{13}C, while allowing decoupling of protons. Other standard probes may be tuned for multiple resonances in the X channel, for example by throwing a switch. One such probe is the so-called quad nuclei probe that has an inner coil tuned to predefined nuclei, usually ^{31}P, ^{13}C, and ^{15}N, or ^{19}F, ^{31}P, and ^{13}C. In such probes, the outer coil is doubly tuned to ^{1}H and ^{2}H.

An inverse probe reverses the uses of the inner and outer coils. The inner coil is tuned to the ^{1}H resonance frequency and the outer coil is used for the X-nucleus frequency (^{13}C, ^{15}N, ^{31}P, etc.). Such probes are often used when one wishes to detect the ^{1}H resonance while applying decoupling power to the X nucleus. Because of the high sensitivity of ^{1}H detection, inverse probes have been widely used for indirect-detection 2-D experiments such as heteronuclear multiple quantum correlation (HMQC) and heteronuclear multiple band correlation (HMBC). Inverse probes are also optimized for 2-D ^{1}H–^{1}H correlation experiments such as the correlated spectroscopy (COSY) experiment and the nuclear Overhauser effect spectroscopy (NOESY) experiment. Most triple-resonance probes (that can be used for detection or decoupling of three different nuclei in an experiment) are of the inverse design, with an inner coil

tuned to ^{1}H resonance frequency and an outer coil doubly tuned for simultaneous decoupling two nuclei, often ^{13}C and ^{15}N.

Another aspect of probe technology is the incorporation of coils that allow the generation of PFGs. For most of multiple-dimensional experiments it is necessary to apply a gradient to the main field for a short time, which is accommodated by these coils. The gradient coils are actively shielded, with stray magnetic fields screened by a second coil located around the principal gradient coil. Some probes contain a single z-gradient coil that provides a linear gradient in the direction of the main magnetic field. For more complex experiments, one may also purchase probes that contain an assembly of gradient coils that independently generate linear gradients in each of the three orthogonal directions x, y, and z to allow a succession of different gradients that are required in some sophisticated experiments.

For special experimental situations such as LC-NMR, the probe has to be designed to accommodate the needs of the other procedure. In particular, in LC-NMR the sample flows through a capillary tube, the active volume of which is only microliters. The inverse mode is often used for LC probe to provide enhanced sensitivity for ^{1}H observation. Inverse operation is necessitated because of the small amount of sample. Another kind of probe used widely in industrial laboratories is a high-throughput flow probe, similar in concept to the probe used in LC-NMR. In these probes, a capillary tube is used as a sample tube with a volume of a few hundred microliters.

One of the most exiting developments in probe technology in recent years is the cryogenically cooled probe. Such a probe was proposed decades ago[11,12] but it has only recently become commercially available. The cryogenically cooled probe offers a dramatic increase in the S/N by reducing the operating temperature of the NMR coil assembly and preamplifiers to 25–30 and 77 K, respectively, which reduces the inherent noise in the RF circuits. The probe coils, a few millimeters away from the sample tube, which is usually near room temperature, are cooled with cold helium gas that is controlled by an automatic closed-cycle cooling system. The cryogenically cooled probe can be configured as a standard probe where the inner coil is for X detection or as an inverse probe. In the standard configuration, the cooled probe can be used for collecting natural-abundance ^{15}N spectra and even for acquiring ^{13}C spectra of high-molecular-weight polymers where the sample is maintained at high temperature. The ability to insulate the region of the RF coils from a very different temperature at the sample (which is only millimeters away) is one of the triumphs of the design of NMR technology. Triple-resonance cryogenic probes operated in the inverse mode have been extensively used for analysis of proteins, as well as for other biomolecular analyses. It appears that, the high cost and demanding requirements for laboratory infrastructure notwithstanding, cryogenically cooled probes are becoming mainstays of industrial, academic, and government NMR laboratories.

4.9 Automation and Sample Changers

In the modern NMR laboratory, more and more work is performed automatically. While all of the operations of the spectroscopy have been under computer control for many years, it is only recently that the initial step of selecting and inserting a sample has become a fully automated part of the process of acquiring an NMR spectrum. The sample changer is, at its heart, a robot that selects a specific sample by command of the computer and places it in the NMR spectrometer; at the end of an acquisition, it reverses this process by ejecting the sample. The choice of a particular sample changer depends on the number of samples to be analyzed. A small sample changer that can handle from 8 to 24 samples is typically mounted on top of the magnet and samples are analyzed sequentially. A larger sample changer (handling up to 120 samples) is often floor-mounted and uses pneumatic or magnet-mounted arms to move the sample between the magnet and sample carousel. NMR software controls the queuing system and determines when a sample is analyzed on the basis of queuing rules preprogrammed by the system administrator.

5 PRACTICAL ASPECTS OF NUCLEAR MAGNETIC RESONANCE SPECTROMETER OPERATION

To obtain a high-quality spectrum, the sample must be prepared carefully. The appropriate NMR probe and experiments must be chosen for the specific application. The standard experimental procedures must be followed to assure reproducibility and accuracy of measurements. As with any experimental procedure, safety precautions must be observed. In this section, practical details of procedures and precautions are discussed.

5.1 Safety Precautions

Extreme caution must be taken while working around magnets, particularly when magnetic materials such as gas cylinders and tools are used in the vicinity of the magnets. Persons with medical metal implants must consult a physician before working around magnets. Credit cards and other magnetic recording materials must be kept away from the magnets.

5.2 Sample Preparation

Sample preparation is the first step, and a critical one, if one is to obtain a high-quality NMR spectrum. Usually the sample is dissolved in a deuterated solvent that serves at the same time as the origin of the lock signal. A list of common NMR solvents is given in Table 2, along with chemical shift references and other physical properties. Nonviscous solvents provide a sharp NMR resonance and are always the preferred choice for use in preparing a sample. If a solvent is viscous but necessary to the chemistry, NMR spectra may be acquired at an elevated temperature to increase the mobility of the solute and solvent molecules to improve the resolution. It is preferable to avoid the overlap of NMR signals from the residual protonated solvent molecule and the sample signal, if possible. If temperature is a variable in the NMR measurement, boiling point and melting point of the solvent must be taken into consideration. If a large amount of the solvent is to be used for NMR analysis, then cost may also be a factor in the solvent selection. Deuterated chloroform is, by far, the most widely used solvent for studies involving organic molecules. Deuterated acetone, because of its low viscosity and very sharp resonance, is often used as the standard sample for establishing homogeneity of the magnetic field. Because of its ability to dissolve many organic materials, deuterated dimethylsulfoxide (DMSO) has also become popular as a solvent for NMR studies. In studies of biological macromolecular systems, detection of amide and imido protons is critical to structure determination with NMR. A mixture of 90% H_2O with 10% D_2O is often used as the solvent in these studies to limit deuterium exchange from D_2O to backbone amide NH protons in peptides and proteins or to imido protons of DNA and RNA base pairs. Because H_2O gives a large signal in the proton spectrum of these solutions, solvent-suppression techniques to reduce the intensity of the water signal have been developed.

For obtaining a ^1H NMR spectrum, the sample concentration should be kept at moderate or low levels to reduce spectral artifacts, such as spinning sidebands. For a ^{13}C analysis in which the natural abundance limits the signal strength, a high sample concentration is preferable.

Table 2 Common NMR solvents[13]

Solvent (formula)	^1H chemical shift (ppm) (multiplicity)	^{13}C chemical shift (ppm) (multiplicity)	Melting point (°C)	Boiling point (°C)
Acetic acid-d_4 (CD_3COOD)	11.65(1) 2.04(5)	178.99(1) 20.0(7)	16.7	118
Acetone-d_6 (CD_3COCD_3)	2.05(5)	206.68(1) 29.9(7)	−94	56.5
Acetonitrile-d_3 (CD_3CN)	1.94(5)	118.69(1) 1.39(7)	−45	81.6
Benzene-d_6 (C_6D_6)	7.16(1)	128.39(3)	5.5	80.1
Chloroform-d_1 ($CDCl_3$)	7.24(1)	77.23(3)	−63.5	61.62
Deuterium oxide-d_2 (D_2O)	4.80(1)		3.81	101.42
Dichloromethane-d_2 (CD_2Cl_2)	5.32(3)	54.0(5)	−95	39.75
N, *N*-Dimethyl formamide-d_7 ($(CD_3)_2NCDO$)	8.03(1) 2.92(5) 2.75(5)	163.15(3) 34.89(7) 29.76(7)	−61	153
Dimethylsulfoxide-d_6 (CD_3SOCD_3)	2.50(5)	39.51(7)	18.45	189
Methanol-d_4 (CD_3OD)	4.78(1) 3.31(5)	49.15(7)	−97.8	64.7
Pyridine-d_5 (C_5D_5N)	8.74(1) 7.58(1) 7.22(1)	150.35(3) 135.91(3) 123.87(3)	−42	115 −116
Tetrahydrofuran-d_8 (C_4D_8O)	3.58(1) 1.73(1)	67.57(5) 25.37(5)	−108.5	66
Toluene-d_8 ($C_6D_5CD_3$)	7.09(m) 7.00(1) 6.98(5) 2.09(5)	137.86(1) 129.24(3) 128.33(3) 125.49(3) 20.4(7)	−95	110.6
Trifluoroacetic acid-d_1 (CF_3COOD)	11.50(1)	164.2(4) 116.6(4)	−15.4	72.4
Trifluoroethanol-d_3 (CF_3CD_2OD)	5.02(1) 3.88(4 × 3)	126.3(4) 61.5(4 × 5)	−43.3	75

There are several features of solution preparation that are necessary to ensure a high-quality spectrum. Insoluble particles suspended in the solution to be analyzed lead to a situation in which the NMR resonances are often poorly resolved. Generally, careful filtration of the solution is recommended. Gases, especially oxygen, dissolved in the solution provide additional nuclear spin relaxation pathways for the spins in the analyte, with the net effect that the resonances may be broader than one might expect. The result is that the resonances are less well resolved. Degassing the sample by the freeze-pump-thaw method can generally reduce the amount of dissolved gas in a sample. It is especially important to carry out this removal of dissolved oxygen if the experiment involves measuring the intrinsic relaxation rate for the analyte. As an internal standard of the chemical shift, a trace amount of tetramethylsilane (TMS, 0.0 ppm) is added to a sample as an internal chemical shift reference. Sometimes, the residual proton signal of a deuterated solvent may also be used as a secondary chemical shift reference, rather than adding TMS. The ^{1}H and ^{13}C chemical shifts with respect to TMS of some common NMR solvent are listed in Table 2.

5.3 Data Acquisition

After a sample is loaded into a probe, acquisition parameters, including observe and decoupling frequencies, are loaded into the data directory of the current experiment by a command to the NMR-control software. If the sample is to be examined at a temperature other than room temperature, the temperature controller is adjusted and a sufficient time for temperature equilibration must be allowed. It is important to note that the temperature that is reported by the hardware is not the temperature at the sample, and the temperature must be determined by some previous calibration of the system.

The probe must be tuned to the frequencies specified by the acquisition parameters. In addition to sample properties, temperature is a variable that may affect the tuning, so it is important to allow the probe to equilibrate before carrying out tuning. The tuning and matching capacitors of the probe LC circuit are adjusted iteratively until the circuit is resonant at the appropriate frequency and its impedance is matched to that of the spectrometer's RF output.

To average out inhomogeneities across the active volume, the sample is spun at 15–20 Hz. To begin, the ^{2}H signal of the deuterated NMR solvent is located by adjusting magnetic-field offset while keeping the deuterium frequency constant. Once the lock signal is found, the magnetic field is locked with the solvent deuterium signal on resonance. After the frequency lock has been established, the magnetic-field homogeneity in the active sample volume is optimized by shimming. Shimming is carried out iteratively. The procedure begins by adjusting the spinning shims iteratively until no improvement in the lock level is possible. The sample spinning is stopped, and the nonspinning shims are adjusted iteratively until the optimal condition is achieved. Although these two steps sound straightforward, the iteration among a large number of shims takes some time, unless performed automatically. With proper room-temperature shimming, the ^{1}H line width of typical small organic molecules such as $CHCl_3$ can be improved from a few hundred hertz in a cryoshimmed magnet to a few tenths of a hertz. For a routine operation in a properly maintained spectrometer, shimming generally only involves adjusting the first- and/or the second-order shims when the sample is changed. However, for a new probe or spectrometer, shimming to a proper line resolution and line shape may take hours or even days.

Before beginning the acquisition of data, one must choose the appropriate pulse program and enter the appropriate acquisition parameters, including pulse widths, related power levels, SW, number of data points to be acquired (time domain, TD), DW, and number of scans (NS) to be coadded. In some cases, one may obtain the appropriate set of acquisition parameters from files saved on the computer, particularly for routine ^{1}H and ^{13}C experiments. Calibration of pulse widths and amplitudes must be done routinely and properly documented.

Here we discuss some of the parameters that must be adjusted before beginning an experiment with quadrature detection. In quadrature detection, the transmitter frequency should be placed at the center of the SW such that only a frequency range of $\pm$SW/2 needs to be digitized. The data-sampling rate, dictated by the Nyquist criterion, must be larger than or equal to SW (i.e. $2 \times (SW/2)$). By this requirement, the relation of the sweep width to the data-sampling time interval, or DW, is as follows:

$$DW = \frac{1}{SW} \tag{4}$$

The total acquisition time (AT) is simply the product of the dwell time and half of the time-domain data points (TD/2), which provides the following relation:

$$AT = DW\frac{TD}{2} = \frac{1}{SW}\frac{TD}{2} \tag{5}$$

The digital resolution (DR) of the spectrum is defined as the ratio of total SW in hertz to the total number of points in the spectrum. If zero filling is not applied to the time-domain data set before FT, the number of points in the frequency-domain spectrum is one half of TD or

TD/2. The DR can then be expressed as

$$DR = \frac{SW}{(TD/2)} = \frac{1}{AT} \tag{6}$$

As shown in Equation (6), digital resolution is simply the reciprocal of the total acquisition time. DR should always be smaller than the natural line width of the NMR resonances of the sample to assure the ability to resolve fine structure. For a typical ^{1}H spectrum, the resonance line width ranges from a few tenths of a hertz to a few hertz, and the AT must be set to a few seconds or longer. Prior to data acquisition, the receiver gain should be adjusted to prevent the signal from being too large or too small for the detection; it is particularly important that the gain be not so big as to produce signals that saturate the ADC converter, a situation that introduces artifacts into the spectrum. The acquisition is generally terminated when the number of FIDs requested by the operator have been added together, given the symbol NS or NA. Most NMR-control programs allow the spectrometer operator to terminate acquisition for any reason at any time before NS is reached.

5.4 Data Processing and Reduction

Upon the completion of data acquisition, the FID is generally apodized by multiplication with a function defined by the operator. This action is usually done to improve either the S/N or the resolution. To ensure that zero-frequency artifacts are minimized at this point, the average of the data over some region is subtracted from each point to ensure that the oscillation is about the zero of the data. After manipulation of the data by these procedures, the data are ready to analyze. The FFT is used to convert the time-domain FID to a frequency-domain spectrum. Subsequent to this procedure, the NMR spectrum may require a phase correction, which can be carried out with a subroutine. In addition, the spectrum may be further treated to ''flatten the baseline''. Here a standard protocol is used to determine the low-frequency broad components of the spectrum, often by fitting certain parts of the spectrum and subtracting, in a point-by-point fashion, to ''remove'' these components from the spectrum.

An important part of presenting a spectrum is ensuring that the data are properly referenced. This referencing is frequently done by choosing the peak representative of a particular chemical shift (0.0 ppm for TMS) and issuing a command to the computer to set all frequencies relative to its frequency, the reference position. If the TMS signal is absent from the spectrum, one sometimes uses the residual ^{1}H signals of the deuterated solvent as a secondary chemical shift reference.

At this point, one usually wishes to identify all of the resonances in the spectrum by specifying their positions relative to the reference. This can be done manually, but there are computer programs that can also perform this operation. In the semiautomatic mode, the spectrometer operator sets the minimum and maximum limits for the program to accept as a peak. The process of identifying the resonances in this manner is called *peak picking*. Most NMR software automatically labels the chemical shift of each peak found between the limits defined by user with its chemical shift.

An important quality is the relative amount of signal corresponding to each peak. This is found by integration of the peak. The spectrometer operator may use a software function to select spectral regions for automatic calculation of the peak integrals. These are displayed on the graphical presentation of the spectrum and are frequently listed in a table generated by the software.

At this point, the only remaining procedure is to make a hard-copy version of the spectrum. Most NMR software provides a plotting facility to do this. Depending on the needs of the spectroscopist, one may plot different regions of the spectrum to demonstrate some particularly important aspect of the data. The format of the plot depends on the kind of experiment. For multidimensional experiments, the plotting may be quite complicated. For example, for 2-D experiments, the ''forest'' style plot that was prevalent in early versions of NMR software has now been replaced almost completely by the contour plot.

The operating procedures discussed above are summarized in Figure 10. All steps can be automated in an

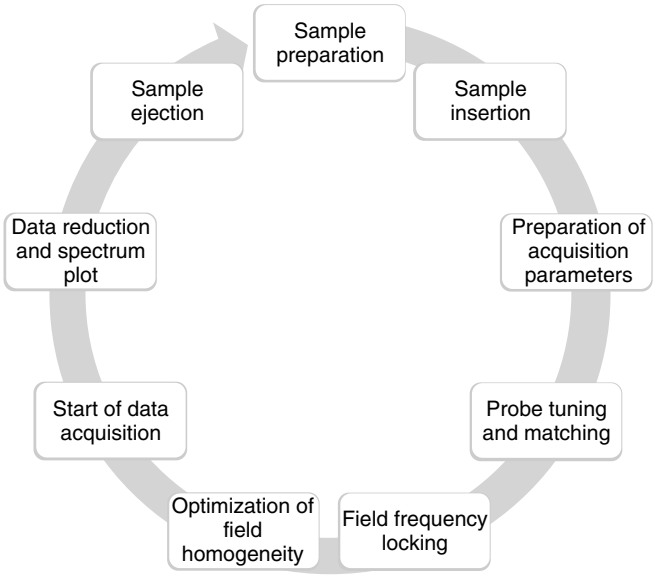

Figure 10 A typical flow chart for a routine NMR operation.

open-access NMR laboratory. It is worthwhile to mention that the automation procedures are suitable to a direct-injection autosampler system. Samples are extracted from vials or well plates with a needle that can be injected into a capillary tube. The capillary tube allows flow into the NMR-active region, where the sample is analyzed. The typical probe used in this flow injection autosampler system is an ATM capillary-flow probe.

6 FUTURE PERSPECTIVES IN THE DEVELOPMENT OF NUCLEAR MAGNETIC RESONANCE SPECTROMETERS

As the NMR spectrometer is one of the critical tools used in industrial, academic, and government laboratories, each development that improves the NMR spectrometer has made a significant impact on the chemical, pharmaceutical, and biotechnology industries. There is no doubt that this trend will continue. Newly introduced methods and techniques have been quickly applied to all areas of NMR instrumentation, whether they be developments affecting the magnet, the probe, the console, the computer hardware, or the NMR software. In general, the developments have focused on enhancement of sensitivity and resolution, automation of the spectrometer operation, reduction of space required for the NMR system, enhancing the reliability of components, mating NMR with other technologies to provide synergy, and ease of operation by less-experienced users.

The driving force to produce ever-higher magnetic field is the enhancement of sensitivity and resolution, especially for the analysis of biological macromolecular samples in solution or in solids. In the solid state, quadrupolar coupling, which broadens the NMR line of nuclei possessing a spin greater than 1/2 (two-thirds of the elements in the periodic table), can be made nearly first order in sufficient high fields. Thus, there is a tremendous emphasis in both solid- and solution-state NMR to provide reliable magnets that have higher fields, such as the recently introduced 950 MHz spectrometer. This trend will not slow down in the future.

The introduction of actively shielded magnets has led to less need for laboratory space. For example, the 5-G line of an actively shielded Bruker BioSpin 800 MHz magnet is about 1.5 m, which is comparable to the stray field footprint of an unshielded 300 MHz magnet. This means a great reduction in the space required for NMR spectrometers. It is certain that there will be continual pressure to replace older magnets with modern shielded magnets.

The conservation of cryogenic liquids (nitrogen and helium) is another driving force in the development of magnet technology, particularly as these liquids become harder to obtain and therefore more expensive. Several commercial systems exist for this purpose. For example, using the excess cooling power of a cooling unit for a cryogenically cooled probe, nitrogen gas can be condensed and introduced back into the nitrogen chamber of the magnet for reuse. With this technique, it is possible to eliminate or reduce the number of liquid nitrogen refills, reduce cryogen costs, and increase the flexibility for performing long-term experiments by eliminating interruptions for nitrogen refills.

Considering that helium is a nonrenewable resource, it is anticipated, not too far in the future, that similar techniques will be employed to cool down helium gas from the boil-off to help maintain the temperature of the superconducting solenoid. Even now, it has become economically feasible and socially important to collect helium boil-off gas and return the gas, rather than allow it to escape into space.

A great potential saving may accrue if the new technologies in high-temperature superconducting materials can be used to replace the current superconducting materials that require cooling to liquid helium temperatures with materials that only require cooling to liquid nitrogen temperatures. There are still many technological hurdles to overcome before this becomes reality, and there might be some technological reasons that it cannot be done, but if such a magnet could be developed, it would have a tremendous impact on the way in which NMR spectroscopy is done.

With the rapid development of digital electronics, much of the spectrometer control and operation have been consolidated onto a single computer motherboard. For example, identical peripheral component interconnect (PCI) cards perform individual functionalities, such as timing, frequency, and gradient controlling, depending on a preloaded firmware. The communication backbone of the NMR spectrometer now consists of two data paths, a LAN-based approach (Ethernet) for non-time-critical operations (diagnostics, firmware upgrades, and configuration parameters) and an ultrafast low-voltage differential signaling (LVDS) bus for time-critical (real-time) applications (pulse timing, loop, and other real-time decisions). The resolutions of frequency and timing control have been greatly improved with LVDS communications. Special ADC architectures have been developed in which the dynamic range of the digitizer is expanded from the previous 16 to 22 bits. In addition, receiver bandwidth has been expanded to as much as 5 MHz. Both of these developments benefit areas such as polymer analysis, solid-state applications, and impurity detection in mixtures.

With wide-bandwidth receivers, a recent technique of parallel detection of multiple nuclei[14] allows simultaneous recording of multiple-dimension NMR spectra for more than one nucleus. This method reduces the total time required for data collection per sample. A further development by Varian of a receiver with a single 80 MHz ADC to digitize the NMR signal directly at the intermediate frequency provides spectra quickly with fewer steps: these spectra have flat baselines and are free of quadrature detection–related artifacts. This new capability allows single-scan operation with the associated higher throughput when sensitivity is not an issue. It is anticipated that more and more ultrafast ADC will become available and be used in an NMR spectrometer.

To obtain information on the absolute concentration of individual components of a mixture with ^{1}H NMR spectroscopy requires the addition of an external reference compound that may overlap some of the resonance of the material to be analyzed. A new technique[15] employs an artificial electronically synthesized reference signal, which can be tuned in terms of chemical shift displacement and amplitude to suit the particular constraints of the experiments. This effectively replaces the external reference and avoids contaminating the sample.

The most active area in NMR instrumentation development in recent years has been probe development. In particular, cryogenically cooled probes have begun to be utilized in a wide variety of applications, not only in complex biological systems but also in small molecule structure elucidation in organic and inorganic chemistry. The principal advantage of a cryogenically cooled probe is the tremendous increase in S/N, which makes possible a substantial reduction in total time required for data collection, in some cases by up to a factor of 16 when compared with a conventional probe. The cryogenically cooled probes also allow the detection of ^{1}H and ^{13}C NMR spectra of extremely small ($\sim$30 μL) samples often found in studies in drug research in the pharmaceutical industry. For the same reason, cryogenically cooled probes have a great potential in the NMR microimaging probes.

In solid-state NMR, similar probe developments have provided new arenas for experimentalists. The development of the ultrafast MAS probes that spin up to 70 kHz has been offered improved spectral resolutions for ^{1}H and has eliminated the need of high-power decoupling. In biosolid NMR applications, RF heating in the sample chamber due to electric fields (E-fields) of the RF pulses severely affects the lifetime of the biosolid sample under MAS conditions.[16,17] New coil designs from Bruker and Varian reduce or eliminate RF heating caused by the E-field.

NMR software packages have been continuously updated to be consistent with advances in hardware. Most NMR software is designed for operation under Windows and Linux operating systems. The software generally has a straightforward interface that takes advantage of widespread PC standards that are commonly used in word processing, graphics, and presentation programs. Recent versions of NMR software keep track of NMR experimental parameters for each probe by retrieving information stored in a microchip in the probe. With these new versions, the RF power is monitored under software control to ensure that power does not exceed the limit for each probe; if this happens, the software terminates the experiment to prevent damage to the probe.

Another innovation is web-based access to the spectrometer through a new generation of software. With this software, one may monitor the status of an experiment or even start and terminate an experiment from a remote computer. With further development of this sort of software control, an operator will soon be able to access and control the NMR spectrometer through a cellular phone or other mobile communication device. A recent software development is the integration of accounting programs into the software to track usage for administrative purposes.

The developments in software, from remote control to automation, have changed the concept of spectrometer operation. For example, the tedious work associated with magnetic-field shimming has been greatly reduced with the utilization the gradient-echo technique. Using a new algorithm available in the most recently released software by Bruker, it takes less than 1 h for a spectrometer operator to produce a homogeneous magnetic field that meets the resolution and line-shape requirements, started from a newly installed magnet. Without this spectrometer automation, the same process may have taken a few days for an experienced console engineer to accomplish when this was done manually.

One should expect that integrated analytical systems involving hyphenated techniques that involve NMR will continue to grow in importance. The combinations of LC with NMR and of LC-MS with NMR are now popular options for an analytical chemist. Integrated software that interprets NMR spectra and correlates the structural features from various analytical techniques is the next logical point for development as these hyphenated techniques become more common in the analytical laboratory.

ACKNOWLEDGMENTS

Cecil Dybowski has helped by reading the initial manuscript and providing suggestions. Mark Chaykowski of Bruker BioSpin and Daniel Barabino of Varian, Inc. have provided NMR pictures and copyrighted materials that have greatly helped in the development of this article.

ABBREVIATIONS AND ACRONYMS

AC	Acquisition Computer
ADC	Analog-to-Digital Converter
AT	Acquisition Time
ATM	Automatic Tuning and Matching
BNC	Bayonet-Neill-Concelman Connector
COSY	Correlated Spectroscopy
CW	Continuous Wave
DAC	Digital-to-Analog Converter
DR	Digital Resolution
DW	Dwell Time
DMSO	Dimethylsulfoxide
E-Field	Electric Field
FFT	Fast Fourier Transform
FID	Free Induction Decay
FT	Fourier Transform
GUI	Graphic User Interface
HMBC	Heteronuclear Multiple Band Correlation
HMQC	Heteronuclear Multiple Quantum Correlation
INADEQUATE	Incredible Natural Abundance Double Quantum Transfer Experiment
LAN	Local Area Network
LC	Liquid Chromatography
LC-MS	Liquid Chromatography/Mass Spectrometry
LC-NMR	Liquid Chromatography/Nuclear Magnetic Resonance
LC-NMR/MS	Liquid Chromatography/Nuclear Magnetic Resonance/ Mass Spectrometry
LED	Light-emitting Diode
LVDS	Low-voltage Differential Signaling
MAS	Magic-angle Spinning
MRI	Magnetic Resonance Image
MS	Mass Spectrometry
NFS	Network File System
NMR	Nuclear Magnetic Resonance
NOESY	Nuclear Overhauser Effect Spectroscopy
NS	Number of Scans
PC	Personal Computer
PCI	Peripheral Component Interconnect
PDF	Portable Document Format
PFG	Pulsed Field Gradient
Q	Quality
RF	Radio Frequency
RTS	Room-temperature Shim
S/N	Signal-to-noise ratio
SPE	Solid Phase Extraction
SW	Spectral Width
TD	Time Domain
TMS	Tetramethylsilane
TOF	Time-of-flight
VME	Virtual Memory Extension
WB	Wide bore
1-D	One-dimensional
2-D	Two-dimensional

RELATED ARTICLES

Biomedical Analysis **(Volume 1)**
Nuclear Magnetic Resonance of Biomolecules

Carbohydrate Analysis **(Volume 1)**
Glycoprotein Analysis: Using Nuclear Magnetic Resonance

Verification of Chemicals Related to the Chemical Weapons Convention **(Volume 2)**
Nuclear Magnetic Resonance Spectroscopy in Analysis of Chemicals Related to the Chemical Weapons Convention

Coating Analysis **(Volume 2)**
Nuclear Magnetic Resonance of Coating and Adhesive Systems

Environmental Analysis of Water and Waste **(Volume 4)**
Nuclear Magnetic Resonance for Environmental Monitoring

Food Analysis Techniques **(Volume 5)**
Nuclear Magnetic Resonance in Analysis of Plant Soil Environments • Nuclear Magnetic Resonance in the Analysis of Foodstuffs and Plant Materials

Forensic Science **(Volume 5)**
Nuclear Magnetic Resonance Spectroscopy for the Detection and Quantification of Abused Drugs

Nucleic Acids Structure and Mapping **(Volume 6)**
Nuclear Magnetic Resonance and Nucleic Acid Structures

Hydrocarbons Analysis **(Volume 8)**
Nuclear Magnetic Resonance Characterization of Petroleum

Pharmaceuticals and Drugs **(Volume 8)**
Nuclear Magnetic Resonance Spectroscopy in Pharmaceutical Analysis

Polymers and Rubbers **(Volume 9)**
Nuclear Magnetic Resonance, Solid State in Analysis of Polymers and Rubbers

Process Analysis **(Volume 9)**
Nuclear Magnetic Resonance and Magnetic Resonance Imaging for Process Analysis

Steel and Related Materials **(Volume 10)**
Nuclear Magnetic Resonance in Metals Analysis

Nuclear Magnetic Resonance and Electron Spin Resonance Spectroscopy **(Volume 13)**
Carbon-13 Nuclear Magnetic Resonance Spectroscopy

Nuclear Magnetic Resonance and Electron Spin Resonance Spectroscopy **(Volume 14)**
Nuclear Magnetic Resonance of Geological Materials and Glasses • Parameters, Calculation of Nuclear Magnetic Resonance • Quadrupolar Nuclei in Solid-state Nuclear Magnetic Resonance • Solid-state Nuclear Magnetic Resonance • Solid-state Nuclear Magnetic Resonance: Spin-1/2 Nuclei Other than Carbon and Proton • Solution Nuclear Magnetic Resonance: Spin-1/2 Nuclei Other than Carbon and Proton • Two-dimensional Nuclear Magnetic Resonance of Small Molecules • Two-, Three- and Four-dimensional Nuclear Magnetic Resonance of Biomolecules

REFERENCES

1. F. Bloch, W.W. Hansen, M. Packard, 'Nuclear Induction', *Phys. Rev.*, **69**, 127 (1946).
2. E.M. Purcell, H.C. Torrey, R.V. Pound, 'Resonance Absorption by Nuclear Magnetic Moments in a Solid', *Phys. Rev.*, **69**, 37–38 (1946).
3. J.W. Emsley, J. Feeney, 'Forty Years of Progress in Nuclear Magnetic Resonance Spectroscopy', *Prog. in NMR Spectrosc.*, **50**, 179–198 (2007).
4. I.J. Lowe, R.E. Norberg, 'Free-Induction Decays in Solids', *Phys. Rev.*, **107**, 46–61 (1957).
5. E.D. Becker, 'A Brief History of Nuclear Magnetic Resonance', *Anal. Chem.*, **65**, A A 295–302 (1993).
6. L. Mueller, A. Kumar, R.R. Ernst, 'Two-dimensional Carbon-13 NMR spectroscopy', *J. Chem. Phys.*, **63**, 5490–5491 (1975).
7. W.P. Aue, E. Baryholdi, R.R. Ernst, 'Two-dimensional Spectroscopy. Application to Nuclear Magnetic Resonance', *J. Chem. Phys.*, **64**, 2229–2246 (1976).
8. G.N. Chmurny, D.I. Hoult, 'The Ancient and Honorable Art of Shimming', *Concepts Magn. Reson.*, **2**, 131–149 (1990).
9. P.C.M. Van Zijl, S. Sukumar, M. Johnson, P. Webb, R.E. Hurd, 'Optimized shimming for high-resolution NMR using three-dimensional image-based filed mapping', *J. Magn. Reson. A*, **111**, 203–207 (1994).
10. S. Sukumar, M.O. Johnson, R.E. Hurd, P.C.M. Van Zijl, 'Automated shimming for deuterated solvents using filed mapping', *J. Magn. Reson.*, **125**, 159–162 (1997).
11. D.I. Hoult, R.R. Richards, 'Signal-to-noise ratio of nuclear magnetic resonance experiments', *J. Magn. Reson.*, **24**, 71–85 (1976).
12. P. Styles, N.F. Soffe, C.A. Scott, D.A. Cragg, D.J. White, P.C.J. White, 'A high-resolution NMR probe in which the coil and preamplifier are cooled with liquid helium', *J. Magn. Reson.*, **60**, 397–404 (1984).
13. S. Budavari, M.J. O'Neil, A. Smith, P.E. Heckelman, *The Merck Index, an Encyclopedia of Chemicals, Drugs, and Biologicals*, Eleventh Edition, Merck Co., Inc., Rahway, NJ, 1989.
14. E. Kupce, R. Freeman, B.K. John, 'Parallel acquisition of two-dimensional NMR spectra of several nuclear species', *J. Am. Chem. Soc.*, **128**, 9606–9607 (2006).
15. S. Akota, L. Barantin, M. Trieweiler, 'Concentration measurement by proton NMR using the ERTIC method', *Anal. Chem.*, **71**, 2554–2557 (1999).
16. J.B. d'Espinose de Lacailleriea, B. Jarry, O. Pascui, D. Reichert, 'Cooking the sample: Radiofrequency induced heating during solid-state NMR experiments', *Solid State NMR*, **28**, 225–232 (2005).
17. A. Krahn, U. Priller, L. Emsley, F. Engelke, 'Resonator with reduced sample heating and increased homogeneity for solid-state NMR', *J. Magn. Reson.*, **191**, 78–92 (2008).