

# NMR Spectroscopy in India

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Most branches of spectroscopy are dedicated to elucidating structures of compounds. Nuclear Magnetic Resonance (NMR) is a very powerful spectroscopic technique not only for structure elucidation of organic, inorganic and biomolecules but has also evolved as a leading medical diagnostic tool (MRI) due to its non-invasive character.

In India, till most part of 1960's, commercially available cw (Continuous Wave) NMR spectroscopy (typically  $^1\text{H}$  resonance at 60 MHz) was used for structure studies employing permanent magnets with field/frequency sweep to scan the various chemical shifts. The slow sweep employed yielded  $^1\text{H}$  spectra in single scan and taking a few seconds to minutes for completion/recording. Each NMR line was scanned individually and hence was time consuming. These were rugged but the sensitivity (Signal to Noise or S/N ratio) was small. The chemical shift dispersion was also small (about 600 Hz for 10 ppm) which resulted in severe overlap of lines when the complexity of the molecule increased. Even then, such NMR systems were used successfully in Organic Chemistry Depts.

During this period, due to paucity of funds and import restrictions, some advanced Research Institutes in India also built home-designed wideline NMR systems at frequencies of few MHz. These systems used small electromagnets and studied solid samples which do not put strain on the stability and homogeneity of the magnets. These were therefore confined to Physics labs to study for example, phase transitions at variable temperatures which show linewidth variations. Chemical shifts and J couplings could not be measured since these are small interactions swamped by  $^1\text{H}$  dipole-dipole couplings.

In 70's, a handful of electromagnet NMR systems were available commercially but again with cw technology. They also used  $^2\text{H}$  lock to stabilize the magnetic field. This was found necessary since electromagnets operate from electrical mains and voltage fluctuations have an adverse impact on the stability of the magnetic field ( $B_0$ ). An unstable  $B_0$  spoils the sharpness of NMR lines.  $^2\text{H}$  lock circuit employs an additional  $^2\text{H}$  frequency rf channel with the sample having deuterated solvent to yield  $^2\text{H}$  resonance. Any shift in the frequency of  $^2\text{H}$  resonance (due to

## Market Dynamics in India

- ⇒ About 230 NMR spectrometers are presently in India.
- ⇒ Pharmaceutical and Chemical companies are the major users with nearly 50% share.
- ⇒ There are one each 800MHz & 700 MHz, 6 Nos. 600 MHz and 24 Nos. 500 MHz NMR units.
- ⇒ Four NMR systems equipped with High Resolution – MAS( HRMAS) are in use.
- ⇒ Presently 20 NMR systems are added every year.

instability in  $B_0$ ) gives a negative feedback signal to correct  $B_0$ .  $B_0$  stability of the order of one part in a million was achieved.  $^2\text{H}$  lock technology is used to stabilize any drift in superconducting magnets even today. Electromagnets were bulky and needed water cooling of the magnet. Even then, they quickly replaced permanent magnet systems mainly because NMR frequencies could be deployed upto 100 MHz. At 100MHz, not only the chemical dispersion increased by 1.66 times compared to 60MHz but further, sensitivity (S/N) increased by 2.2 times compared to 60 MHz NMR since S/N increases as  $(B_0)^{3/2}$ . Second order effects were also prominent in low frequency NMR systems and was undesirable.

One of the main drawbacks of cw technology was that it did not permit study of nuclei other than a few abundant, high gyromagnetic ratio nuclei such as  $^1\text{H}$ ,  $^{19}\text{F}$  etc. Organic chemists were equally interested in the study of well dispersed  $^{13}\text{C}$  nucleus whose chemical shift range is in excess of 200 ppm. Unfortunately, due to the low 1.1% abundance of  $^{13}\text{C}$  and a gyromagnetic ratio  $1/4$  of  $^1\text{H}$ ,  $^{13}\text{C}$  lines are very weak and cannot be differentiated from electronically generated noise.

By early 70's, a way out for this was already in the offing. These were named Fourier Transform NMR (FT-NMR). The highest frequency was still limited to 100 MHz but then study of less abundant and low gyromagnetic ratio nuclei were amenable for study since multiple scans could be acquired and co-added in a short time and in a coherent manner. As a result, S/N increased with every scan (signal increasing as square root of number of

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Dr. C.V.Manjunath was born in December 1951 in Tumkur, Karnataka. He obtained his B.Sc (Hons,Physics) degree from Central College, Bangalore in 1970. He post-graduated with M.Sc (Physics, Electronics) in 1972 from Central College. The same year, he joined for Ph.D programme in the Physics Dept of Indian Institute of Science, Bangalore to work in the field of Electron Paramagnetic Resonance (EPR) and obtained his Ph.D in 1978. Dr.Manjunath worked in the same Dept, for a year, in a project on ENDOR. In 1979, he started working for BRUKER group of companies in Systronics, Ahmedabad and later for Bruker India Scientific Pvt.Ltd, Bangalore where he served in various disciplines of sales, applications, installation and service support of BRUKER products. Since 2002, Dr.Manjunath is serving as Managing Director of Bruker India Scientific Pvt Ltd.



scans). In FTNMR, the experiment is performed in a few msec unlike secs of cw NMR. This is possible since instead of a slow field/frequency sweep, a rf rectangular pulse is applied whose envelope covers all frequencies (as an inverse of the pulse width). Shorter the pulse width, larger the frequency coverage. The widths are usually a few microseconds. Under ideal conditions, this superposition of sinusoidal NMR signals will yield a beat pattern over an infinite time. Due to the dispersive nature of spin-spin interactions, there is a loss of coherence (over a time period  $T_2$  or spin-spin relaxation time or transverse relaxation time) and the resulting exponential decay of the NMR signal is called Free Induction Decay (FID) extending to msec or more depending on the nucleus and its coupling to nearby nuclei. A time to frequency domain Fourier transformation of the FID yields a NMR spectrum similar to that obtained by cw technique but in a much shorter time. NMR thus spread from Organic to Inorganic and materials chemistry. Nearly 25 electromagnet FTNMR spectrometers in the frequency range 80-100 MHz were installed till late 1980's.

The quest for higher frequency NMR systems continued and by mid 70's, FTNMR spectrometers working at 200, 270 and even 300 MHz were commercially available. These employed superconducting magnets which yielded much higher magnetic fields. They were very stable, with relatively small drift rates, and worked independent of mains power. Long solenoid coil of Superconducting wire made of NbTi or NbSn was immersed in liquid helium and a current of the order of 100 amps produced a magnetic field in the vertical direction. The current flows for years without much current dissipation and hence the conductor is said to be superconducting. In order to preserve the expensive liquid helium in the dewar, an outer jacket of liquid nitrogen was provided (thermal barrier) along with high vacuum and superinsulation (radiation shield). Liquid helium could be stored in the magnet dewar for a few weeks in 1970's. Till 1975, due to the expense involved in obtaining liquid helium and liquid nitrogen, transport problems and scarcity of supply, there was not a single NMR with superconducting magnet in India. In 1976, the first supercon NMR at 270 MHz was made operational in IISc Bangalore since IISc had its own cryogenic facility. Helium gas evaporating from the magnet was recovered and liquefied. This was a national facility and samples, submitted from all corners of India, were analysed. The magnet required liquid helium refilling once in a week and was therefore difficult to maintain in other parts of the country.

During early 70's, NMR manufacturers were able to design larger magnets with longer liquid helium re-filling intervals. Four more supercon NMR systems in the frequency range 300-500 MHz were added in 1983 which were again supported either by in-house or close-by cryogenic facilities.

By mid-1980's, liquid helium was being imported by a few private entrepreneurs in India and the distribution was streamlined. At the same time, a few NMR vendors started selling 200, 300 and 400 MHz NMR magnets with liquid helium hold time of one year. This encouraged not only Government Research Institutes to go in for high field NMR spectrometers but also Private Research Institutes spread far and wide in the country. Nearly 20 supercon NMR spectrometers were added in the country between 1985-1995.

Worldwide, interest in NMR also leap-frogged with the introduction of DEPT, 2 Dimensional NMR such as Correlation Spectroscopy (COSY), Nuclear Overhauser Spectroscopy (NOESY), C-H correlation, Total Correlation Spectroscopy (TOCSY) which spread out coupled nuclei as contours along two dimensions thus assisting in rapid structure determination. 2D NMR became possible since the magnetic field was rendered more stable with supercon magnets.

By mid-1990, the first 600 MHz NMR spectrometer was installed in India in TIFR, Mumbai. This heralded the beginning of use of NMR for Biological applications including peptides, proteins etc. 3D NMR of liquid samples with triple resonance probes involving nuclei such as  $^1\text{H}$ ,  $^{13}\text{C}$ ,  $^{15}\text{N}$ , three/four channel NMR consoles with  $^2\text{H}$  decoupling of labeled compounds etc became possible. In heteronuclear correlation expts, there was also a shift towards use of inverse detection ( $^1\text{H}$  detection) which speeded up the experiments. Heteronuclear correlations such as  $^{13}\text{C}$ - $^1\text{H}$ ,  $^{15}\text{N}$ - $^1\text{H}$  etc were carried out even with 300MHz NMR spectrometers in the form of HSQC, HMBC. With increase in sensitivity of the NMR spectrometers due to higher frequency of operation, advancement in electronics, possibility of inverse detection, in addition to other factors, the number of scans per t1 increment in 2D NMR was reduced. In order to achieve quadrature detection in F1 (indirect dimension) without involving phase cycling schemes, z Gradient pulse schemes were introduced along B0. The so called Gradient Accelerated Spectroscopy also gave artefact-free spectra in one scan by doing away with phase cycling (involving multiples of 8 scans per t1 increment). Nowadays, nearly all systems installed carry the Gradient spectroscopy electronics.

By 2005, the highest frequency NMR at 800 MHz (with the superconducting wire maintained at 2K with ultra-stabilised technology) was made available in TIFR and this further boosted NMR applications to biological applications. For proteins, NMR is now seen as an alternative to structure determination by X ray since NMR, in general, requires less tedious sample preparation.

Biological samples also required tricky and sophisticated water suppression to assist in the analysis of sample signals close to water resonance and also to prevent saturation of the receiver. This required excellent homogeneity of the magnet to achieve narrow water resonance. Gradient shimming was made available which speeded up accurate shimming procedures. In addition, NMR systems are now having larger number of room temperature shims to ease the tedium of shimming.

The number of samples to be run per day is ever increasing, especially in Pharmaceutical companies. In the last few years, autosamplers with carousels having capacity ranging from 8 to 120 are available and are being routinely bought with 300-500 MHz NMR spectrometers. Multiple expts with the same sample can be automated for overnight runs and since NMR systems are never switched off even during night times (because of use of supercon magnets) all multinuclear and multidimensional expts can be finished on a number of samples overnight. In order to have the flexibility of running not only  $^1\text{H}$  but also all nuclei in an automated run, instrument



manufacturers have introduced multinuclear probes (including  $^1\text{H}\{^{19}\text{F}\}$  and vice versa) with automatic tuning and matching facility so that the NMR software can switch over from one nucleus to another during an autosampler usage, with nil operator interface.

Many of the biological samples (and also any long 2D run) need temperature to be controlled accurately. Samples can be controlled between  $-150$  to  $+150$  deg C ( $\pm 0.1$  deg C). Temperatures below ambient are achieved with liquid nitrogen evaporators or mains operated refrigeration units.

For processing large amounts of data collected during a 2D NMR experiment entailed the use of fast and state-of-art computers. Till early 90's, some of the NMR suppliers designed their own computers which were quickly replaced by commercial ones such as Silicon Graphics, Sun etc systems. These were found to be rather expensive to maintain and used to run on the not-so-familiar Unix platform. Since late 90's, PC data stations have taken over NMR processing/ acquisition and are either Windows XP or Linux based.

Eversince 2D and 3D NMR became routine in laboratories worldwide (from early 90's), there was a need for the electronics to keep pace not only for reducing electronically generated noise but also improve the stability of frequency and field. For example, to do homodecoupling expts, long term 2D, water suppression, selective decoupling in 3D, 1D NOE etc, an excellent stability of  $B_0$  and very fine frequency setting resolution and stability are required. Similarly, during an automated run overnight, auto-2H-locking for different solvents is a must. Completely digital design of 2Hlock electronics with independent field and frequency variability was introduced in 1994 and has become the norm today with unsurpassed stability and immunity from outside magnetic disturbances.

In addition, to reduce noise in the receivers, receivers with DDS electronics and with digital filter capability were introduced in 1985 by some vendors. These also employed oversampling technology at full digitizer speed ( $>5$  MHz) with decimation thrown in to get better dynamic range extending even upto 21 bits for  $^1\text{H}$  work. By late 1990, some NMR spectrometers were equipped with receivers with offsets of 20 kHz (more than 500 kHz of late) which eliminated dc and quad image artifacts in  $^1\text{H}$  spectra when only a few scans were used (for example concentrated samples/gradient expts etc). Speeding up acquisition of 2D NMR is of paramount importance and dual receiver systems have now been developed which, for example, can acquire not only  $^1\text{H}$  in a scan but also  $^{13}\text{C}$  during the mixing time of a TOCSY sequence.

More advancements were made available for biologists and natural product chemists working on 2D/3D experiments with limited sample quantities. Cryoprobe/chilliprobe is one such innovation. Radiofrequency coil and  $^1\text{H}, ^2\text{H}$  preamplifiers were cooled to 20K thereby reducing shot noise in the electronics. This resulted in improving sensitivity by 4 times and reducing the time of expt by a factor of 16. Although these probes were designed with  $^1\text{H}$  nucleus in mind, of late, cryoprobes are available with cooled  $^{13}\text{C}$  preamplifiers also. These inverse probes (excellent for  $^1\text{H}$ ) can now be used for DEPT and

multidimensional NMR expts also, with  $^{13}\text{C}$  as the observe nucleus. This has some advantages wrt to evolution times, coupling constants, linewidths etc. Cryoprobes have also been designed with Q-factor immunity for samples with buffers and also the use of shaped sample tubes. Since 2003, there are 8 NMR spectrometers (500-800 MHz) in India which are equipped with cryo/chilli probes. Although such probes can now be equipped even with 400 MHz NMR systems, unfortunately, they have not been popular with routine users, due to their cost, power requirements, expensive yearly replacement of cold heads, pumps etc.

With the demands of natural product chemists for inexpensive NMR systems with excellent sensitivity for study of micro gram sample quantities, NMR suppliers came up with solutions such as 1mm, 1.7mm, 2.5mm and even 3mm probes. They needed deuterated solvent quantities ranging from 5ul to about 150 ul. For the same amount of sample, these probes offered much better sensitivity compared to 5mm samples. Further, for example on the 1mm probe, there was virtually no solvent signal seen and excellent solvent suppressed spectra could be obtained with these probes using the existing solvent suppression pulse sequences. The sample tubes are toploading and compatible with autosamplers.

Analysis of mixtures, drug metabolite studies, impurity profiling etc needed a technique different from the simple sample preparation used so far. NMR coupling to HPLC took root and since 2004, there are half a dozen LC-NMR systems in India. 3mm flow-probe is common. NMR is used as a detector in parallel with DAD/UV and sometimes even MS. Elutes could be analysed on line (for 1D NMR of essentially  $^1\text{H}$ ) or stopped HPLC flow (1D and 2D). For more involved multidimensional, multinuclear expts, peak trapping units (after HPLC) are available. The drawback of these methods is that they use large quantities of expensive deuterated solvent at the HPLC stage and further, the sensitivity is low due to minute quantities of sample eluting in single HPLC run. There was also a problem of back mixing, probe contamination due to the previous elute etc. HPLC-SPE-NMR with Solid Phase Extraction (SPE) introduced after the HPLC stage, resulted in better sensitivity since sample accumulation was possible with repeated HPLC runs. The SPE cartridges could be of size suitable for the probe in question ranging from 1mm to 3mm. HPLC could be run with normal solvents and deuterated solvent was added into the SPE cartridge which does not consume more than 100 ul. One can work with the normal flow probe or even the microprobe such as the 1mm probe described above (essentially for off-line LC-NMR expts).

Superconducting magnets, unlike permanent or electromagnets which have yokes, used to have large radial and axial stray fields with the 5G line extending even upto 4 m or more (for 500 MHz and above). By mid 1990's large laboratory space also became a luxury since more NMR systems were being housed under the same roof for ease of operation, cryogen refills etc. Further, LC-NMR/MS expts were envisaged which needed HPLC, MS etc systems to be close to the NMR probe so as to reduce the length of capillary on way to the flow LC-NMR probe. All electronics had to be pushed beyond the 5G line and



NMR manufacturers were forced to reduce the radial stray field. Various called Ultrashield, active shield etc, supercon magnets were designed with an outercoil of superconducting wire carrying a current in the opposite direction. This curtailed the 5G lines drastically. Of late, Ultrashield plus or premium shield magnets are available at 400 MHz and above, with the 5 G line ending on the legs of the magnet.

By late 1990's pharmaceutical research Institutes started analyzing compounds grown on substrates/beads without the capability to separate and dissolve in deuterated solvents for NMR analysis. High-Resolution-MAS (HRMAS) probes were made available for such use with the samples loaded with D<sub>2</sub>O and being rotated at around 4KHz at magic angle (about 54 deg wrt B<sub>0</sub>) to reduce heteronuclear CSA, dipolar broadening and at the same time get excellent lineshape with the use of 2H lock electronics. There are four NMR systems in the country equipped with HRMAS.

NMR of solid samples was practiced in parallel with liquid state NMR and the first dedicated solid state NMR facility at 300 MHz, with widebore magnet, started operation in 1985 in IISc. Since then, at least six more similar systems (with WB magnets) in the frequency range 300-500 MHz have been added in our country. In addition, there are about a dozen NMR systems in the country which can do both liquid and solid state samples with 54 mm magnet bore (standard bore). Such solid state probes have slightly lower temperature and power handling capabilities compared to the wide bore ones but then these are found to be adequate for routine X-observe CPMAS studies. Such systems are now routinely sought after for polymorphic studies of solid samples in pharmaceutical industries, complementing results from X ray, IR etc.

Study of Quadrupolar nuclei, 2D NMR and 1H observation in solid samples requires use of advanced pulse sequences with fast frequency, phase switching and power capabilities. These put strain on the existing hardware and since beginning 2000, NMR manufacturers have modified the design of synthesizers which has resulted in improvement of phase and frequency resolution by at least two orders of magnitude. Simultaneous frequency, phase and amplitude switching times are now better than 50 ns thanks to embedded software in chips and faster communication links between various subunits of a NMR spectrometer. Receivers now are equipped with digitizers better than 20 MHz, which can cover spectral widths of more than 10 MHz and is found to be adequate even for nuclei with quadrupole moments ( $I > 1$ ). There are some NMR instruments now available with receiver Intermediate Frequencies (IF) in excess of 700 MHz which satisfies the demands of solid state chemists. The first generation of CPMAS probes operated on the single air bearing technology and with the advent of double bearing design from later half of 1980's, 4mm sample rotors could be spun even upto 15 kHz and nowadays smaller rotors are available with spinning speeds reaching more than 50 kHz. Such spinning speeds offer excellent narrowing of broad lines of solids and also yield cleaner spectra with well dispersed spinning sidebands. Probes are now available with not only small adjustments around the magic angle but also over a wide range.

Microimaging is another field of NMR which is fast catching up in the country. The first microimaging NMR in IISc was installed in 1990 on a vertical 400 wide bore magnet capable of handling samples (such as mice) for sizes upto about 4cms. There are now half a dozen such systems in the country with one of them using a 500 MHz narrow bore magnet and can take sample sizes upto 1 cm. The samples can be temperature controlled, and various sensors can not only monitor the health of the animal during the experiment but also trigger various NMR acquisitions. One of the microimaging setups in India was used mainly for material research. One can reach resolutions down to a few tens of microns with these systems and can not only do nearly all NMR imaging experiments (similar to MRI) but also spectroscopy. There are also two miniimaging NMR systems in the country and one of them was established way back in 1993 in AIIMS. These are horizontal bore magnets handling bigger animals. One of these magnets requires only a yearly top-up of liquid helium and requires no liquid nitrogen filling.

About 230 NMR spectrometers were bought and installed in the country (since mid 1970). A rough breakup of these, in various disciplines, is as follows:

<i>Distribution of NMR spectrometers</i>	<i>Total 230</i>
Pharma & Chemical companies:	110
Universities:	40
Research Institutes:	
IISc, IIT, TIFR, Defence Space Research etc	40
National Laboratories	40

Frequency-wise breakup of NMR bought/installed since mid 1970 is as given in the following table:

<i>Frequency of NMR</i>	<i>No. of units</i>
800 MHz	1
700 MHz	1
600 MHz	6
500 MHz	24
400 MHz	86
300 MHz	88
270 MHz	1
200 MHz	23

In the last 5 years, at least 20 NMR systems are being added per year in India.

Although in 1960's NMR started as a subject of research interest, confined to a few Departments in India, it has steadily grown as an invaluable tool for organic chemists. Even though this segment of science, in research labs and pharmaceutical industries, is still one of the major investors in NMR, in the last decade, NMR in India is diversifying to other areas such as solid state NMR of polymorphic samples, LC-NMR of drug metabolites, mixtures, NMR micro and mini imaging, and multidimensional NMR of biological samples. ■