APPLICATIONS OF NUCLEAR MAGNETIC RESONANCE AND HIGH RESOLUTION RADIO-FREQUENCY SPECTROSCOPY

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1. INTRODUCTION

THE discovery of the tuning in of the spinning nuclei of atoms in magnetic fields was announced, as early as in 1946, simultaneously by Felix Bloch of Stanford University and Edward Purcell of the Harvard University who used rather different techniques—one being of induction type and the other of absorption. Within a short period the subject grew with no bounds and like many other discoveries of physics spread into distinctly separate fields of research, such as, chemistry, metallurgy, geology and even biology. It was not surprising that the Nobel Prize was awarded to this discovery in 1952 just after 6 years of its first announcement—a recognition of the importance of the subject even before it unfolded all its potentialities. An earlier article on the subject in this journal (*Curr. Sci.*, 1959, 28, 183), dealt chiefly with the principles of nuclear magnetic resonance (n.m.r.) and in the present article it is proposed to lay emphasis on some of the more important applications of this versatile technique.

The n.m.r. method mainly depends on the fact that many isotopes display a finite nuclear magnetism and possess a gyromagnetic ratio γ, given by,

$$\gamma = \frac{\mu}{I\hbar},$$

where

$$\hbar = \frac{h}{2\pi}.$$

By nature these gyromagnetic ratios are such that for no two nuclei they are identical. Hence it is possible to label each nucleus with a definite value of γ and the n.m.r. method can then distinguish between isotopes by their differing gyromagnetic ratios. It has been shown in the previous article (*loc. cit.*) that the precession frequency (ν) is given by the relation,

$$\omega = 2\pi\nu = \gamma H,$$

and can be measured very accurately in a given magnetic field. This enables one to calculate γ for a nucleus.

2. NUCLEAR MAGNETIC MOMENTS AND NUCLEAR SPINS

The use of this phenomenon, immediately after its discovery, was made for the accurate determination of the nuclear magnetic moments and spins of several stable isotopes with spin number $I > 0$. It is obvious from the n.m.r. condition mentioned above that

$$\nu = \frac{\gamma H}{2\pi} = \frac{\mu}{Ih} \cdot H.$$

This relation can be used to determine the magnetic moment μ of a nucleus (whose spin number I is known) by measuring its magnetic resonance frequency in a given field H. It must be noted here that the radio-frequency ν can be measured with an absolute accuracy of one part in 10^6 or even better. However, there are difficulties in measuring the magnetic field H to the same accuracy. The Planck's constant h is known to an accuracy of the order of one part in 10^4. It is, therefore, considered advisable to compare experimentally the resonance frequencies of two nuclei of known I in the same magnetic field H. Then using the relation

$$\frac{\gamma_n}{\gamma_{un}} = \frac{\mu_n}{\mu_{un}} \cdot \frac{I_{un}}{I_n},$$

the unknown magnetic moment μ_{un} can be determined by selecting a reference nucleus whose magnetic moment μ_n has been very accurately determined earlier by precision methods. Usually proton has been used as a reference nucleus since its μ has been measured precisely to a desirable accuracy.

One special feature of the nuclear induction method or the Bloch or cross-coil technique is that it can determine the sign of the nuclear magnetic moment. The phase of the nuclear induction signal relative to the leakage signal depends on the sign of the nuclear magnetic moment. Under identical leakage conditions, the signal traces of the reference and the other nucleus, the sign of whose magnetic moment is to be determined, can be compared. In this way the signs of several nuclear magnetic moments have been either determined or confirmed.

A suitable expression can be derived for the n.m.r. signal strength involving the spin number I, the gyromagnetic ratio and other quantities which can easily be evaluated. This expression can then be used to compare the signal strengths since the ratio of γs can be obtained by the ratio of frequencies at which the two resonances are observed in the same field H. It is then

possible to determine the spin number for a nucleus from the known spin number of the nucleus chosen for comparison. There are other methods too, such as the measurement of absolute signal strength, electric quadrupole splitting and the fine structure of the resonance line. However, it is very convenient to find an unknown spin number by comparing the signal strengths as mentioned above.

Thus the n.m.r. technique has helped considerably in obtaining the nuclear properties such as the precise measurements of magnetic moments, their signs and the spin number which are essential data needed for the elucidation of the nuclear structure, the most vexed problem of the day.

3. Nuclear Relaxation Phenomena

The n.m.r. technique has been successfully utilised for the study of nuclear relaxation phenomena. Through the observation of the nuclear magnetic resonances, it is possible to study the details of molecular motions even in complex systems. For this purpose one distinguishes between two relaxation times—one called the spin lattice or longitudinal relaxation time T_1 which has something to do with the establishment of thermal equilibrium between nuclear spins and their surroundings. The coupling mechanism depends upon the nature and properties of the system and the nucleus. For instance, lattice vibration may be a dominant factor in pure solids, large electronic magnetic moment in solids impregnated with paramagnetic impurities, random Brownian motions in liquids and gases, and interaction of unpaired conduction electrons near the top of Fermi band with nuclear moments in metals. The measurement of T_1 by n.m.r. technique has thrown sufficient light on some of the mechanisms so far proposed.

A second relaxation time T_2, often called the spin-spin or transverse relaxation time, plays an equally important role in the n.m.r. phenomena. It describes the processes by which the nuclei in a given system tend to lose their phase coherence and thereby bring about changes in the strength of a precessing macroscopic magnetic moment vector of the nuclear spins. Several factors have been proposed, which contribute to the transverse relaxation, such as the intrinsic nature of the sample, homogeneity of the applied magnetic field, non-homogeneous magnetic fields within the sample, viscous media, dipole-dipole interactions or spin-spin collisions. The measurement of T_2 by n.m.r. technique has provided a means of understanding these various processes. Both T_1 and T_2 are vitally important in understanding the character of the nuclear resonance signals and several workers have been busy in the measurement of these relaxation times.

4. Chemical Shift

In the n.m.r. technique, all magnetic nuclei should obey the fundamental resonance condition $\omega = \gamma H$. However, one should not forget the facts that the nuclei used in n.m.r. experiments are embedded in bulk samples such as solid, liquid or even gas at high pressure. In these varied samples of different phases there are likely to be internal molecular magnetic fields and on application of steady external magnetic field an induced internal magnetic field can also arise. In terms of these effects, it is necessary to modify the above fundamental n.m.r. condition as

$$\omega = \gamma \left(H_{ex} + H_{local} \right) = \gamma H_{ex} \left(1 + \frac{H_{local}}{H_{ex}} \right),$$

or

$$\omega = \gamma H_{ex} \left(1 + \sigma \right)$$

where H_{ex} is the externally applied steady field, and σ is usually defined as the internal electron distribution susceptibility. This improved resonance condition clearly indicates a shift in the resonance field or the frequency, the magnitude of which depends on the local field produced at the nucleus by the internal electron distribution in the sample. These shifts in the resonance field or frequency are called 'chemical shifts' and are often extremely small in comparison with the applied steady field, say, a few parts in 10^6 or more in some cases. For instance, the shift in methyl alcohol for protons is of the order of 16 milligauss in 10 kilogauss. The chemical shifts are field-dependent, and obviously, to observe these very small but significant shifts in nuclear magnetic resonance spectrometer, the steady magnetic field should be extremely homogeneous, say 1 part in 10^6 or even more ; the stability of both the magnetic field and the frequency of the oscillator producing the transverse oscillating field H_1 should be very high.

The chemical shifts are conveniently measured by the n.m.r. technique with respect to an external standard, preferably in coaxial tubes, one containing the standard and the other the substance under investigation. The chemical shift parameter δ is usually expressed as

$$\delta = \frac{H_{sample} - H_{standard}}{H_{standard}}.$$

where H_{sample} is the observed resonance field for the sample under investigation and $H_{standard}$ that for the standard sample.

The chemical shift parameter δ is generally a function of electron density around the nucleus in question since the electrons are involved in the diamagnetic shielding. Attempts have also been made to correlate δ with electronegativities as electronegativity is related to electron density. Some investigators have tried to establish a relation between δ and the Hammetts' constants σ for compounds in which a substituent is located in a *para* or *meta* position. But the situation is not quite so simple as one expects. Ramsey has developed a general theory for the magnetic shielding of nuclei in molecules and has shown that there is a contribution of both diamagnetic and paramagnetic terms. This theory of Ramsey has been simplified further by Saika and Slichter who have introduced three terms: (*i*) the diamagnetic contribution from electrons associated with atoms in question; (*ii*) the paramagnetic contribution from the orbital motions of the valence electrons and (*iii*) the contributions from other atoms. This theory has been able to explain qualitatively the correlation of chemical shifts with ionic character in many fluorine compounds. Although it is difficult in general to calculate an accurate theoretical value, it cannot be denied that the shift has something to do with the nature of the chemical binding in the atom. The chemists have, therefore, always made attempts in their own way in correlating the observed shifts with the electronic structures of molecules and the nature of the chemical binding.

In some cases the chemical shifts are extremely high, of the order of $1·5\%$. They are attributed to the effect of mixing of the ground and low-lying excited states of electrons as in the case of UF_6 for which the second order paramagnetic term is nearly twice as large as that for F_2. Considerable amount of work in this connection has been done with respect to cobalt complexes where such low-lying states are expected and the shifts obtained are of the order 100 gauss in 10 kilogauss. Recently, Orgel has worked out the 'Ligand Field' theory which seems to fit well with nuclear magnetic resonance shifts observed in our laboratory in solutions of cobalt complexes.

The chemical shifts are of great aid in the investigation of structural problems involving nuclei in different locations. When several identical nuclei are embedded in a molecule but in different electronic environments, it is obvious that each group of identical nuclei has a different chemical shift and consequently a different resonant frequency. In such a case in an extremely homogeneous magnetic field and with a liquid specimen the n.m.r. should show a fine structure. The well-known examples are the methyl and ethyl alcohols where the peaks of proton resonance in CH_3, CH_2 and OH are well separated out and the areas under the peak stand roughly in the ratio $3 : 2 : 1$, as is expected if each peak corresponded to the chemically different CH_3, CH_2 and OH protons. Thus the n.m.r. spectrum becomes the finger-print of the molecules identifying the exact locations of the nuclei in a molecule. Structures of several organic compounds have been studied in this way and the n.m.r. technique has thus become a powerful tool in the hands of chemist for the structural study, identification and chemical analysis.

5. N.M.R. SHIFTS IN METALS

The n.m.r. shift of any nucleus observed in a metal occurs at considerably lower fields than in compounds for a fixed frequency. This is called the "Knight shift" and has been attributed to the paramagnetism of conduction electrons since the shifts are too large to be accounted for by a simple difference in magnetic susceptibility of the materials, or by differences in the diamagnetic correction for the metallic and non-metallic atoms. Korringa has shown theoretically that Knight shifts and the spin lattice relaxation time T_1 are interdependent. Knight shifts have also been measured in several metals over temperature ranges and the results do not support the assumption of an appreciable degree of electron and lattice interaction. There are several phenomena of metallic state which need sensitive checks on the validity of wave functions proposed for conduction electrons and the nuclear magnetic resonance shifts may prove fruitful in resolving some of these problems.

6. MULTIPLET STRUCTURES IN N.M.R. SPECTRA

Subsequent to the discovery of the chemical shift, while working with spin-echo experiments Hahn and Maxwell discovered the existence of two parameters, one field dependent and the other field independent. The chemical shift is the field-dependent parameter while the field-independent one is called the spin-spin interaction, and was then explained by introducing a rotationally invariant interaction between nuclear spins. The spin-spin interaction is a sort of indirect coupling of the nuclei through the electrons and is responsible for the

multiplet structure observed in the resonance. The fine structure is also independent of temperature and the splittings are of the order of 10^{-3} gauss to 2 gauss, much smaller than the chemical shifts. Since the effect is due to the coupling of non-equivalent sets of magnetic nuclei by the bonding electrons, the intensity of the split components depends on the statistical weights of the different spin combinations. The number of components in the splitting or the multiplet are equal to $2nI+1$, where n is the number of equivalent nuclei which split the resonance and I the spin number. Such splittings have been observed by n.m.r. technique and the number of components have been verified.

The line widths in such structures are of the order of a few cycles/sec. To obtain a spectrum of this nature, the n.m.r. spectrometer needs a high resolution and much more so in a complex spectra where the chemical shift is superimposed by the spin-spin interactions. A resolution of the order of 1 in 10^8 or at least 10^7 is expected for the study of such spectra where it is possible to distinguish the chemical shift from the spin-spin interaction by the field dependence of the former.

7. Experimental Arrangements

One has now to ask the question as to what are the essential features of a high resolution spectrometer. The field homogeneity of the magnet in this spectrometer should be of the order of 1 in 10^8. The magnet may be a permanent one or an electromagnet. Electromagnets are used for this purpose and the current and the voltage are both regulated and stabilized in order to maintain the desired high stability. The field is locked up by a proton signal or by a superstabilizer. A superstabilizer consists of two electronically controlled coils put on the large pole-pieces of the magnet. One of the coils senses even the small changes in the magnetic field and the other one *corrects* it. Sometimes the magnets are well shimmed for higher homogeneity. A permanent magnet can also be used which certainly does not need the current or voltage stabilizer, but its pole-pieces must be extremely polished and aligned perfectly parallel. Usually the pole-pieces are about 12″ in diameter. The bigger the diameter, the better is the homogeneity at the centre. The residual inhomogeneity, if any, can also be corrected by current shimming.

In addition to the homogeneity of the magnet, the oscillator frequency has to be stable to an order of 1 in 10^8. Thermally controlled crystal oscillators are used for this purpose and the receiver should introduce as little noise as possible. The nuclear induction head which contains the sample is a tricky part of the spectrometer and the details appear in the literature.

In a high resolution spectrometer there is an arrangement to spin the sample. The inhomogeneities over the sample volume are averaged out by spinning the sample with rotational frequencies of a few hundred revolutions per minute. In this process of spinning each nucleus is carried through the entire distribution of fields in a time short compared to T_2.

The possibility of distinguishing the chemical shift by its field dependence has already been mentioned. But with a permanent magnet, field cannot be varied. Even with an electromagnet the field variation may be limited within attainable field strengths to identify the chemical shifts. Under these circumstances a new technique known as the 'double resonance' can be used with the help of which the spin-spin multiplets can be collapsed and a simplified spectrum easily amenable for interpretation can be obtained. The principle of the double resonance is to agitate the spin orientation of the interacting nuclei very frequently so that the perturbing field on the observed nuclei is, averaged out. This is best done by using another oscillator and transmitting coil at right angles to the already existing transmitting coil. With the help of this, we can use a strong radio-frequency field of the right frequency to introduce transitions between the various spin states of the interacting nuclei.

8. Some Applications

The first complete analysis of the complex spectra of ethanol was made by Arnold in which the predictions of the chemical shift and spin-spin interactions were verified. The spectra clearly showed the three peaks of proton chemical shifts in OH, CH_2 and CH_3 along with the spin-spin interaction which gave three splitting components in OH and CH_3 and eight in CH_2. Later Anderson studied nuclear magnetic resonance spectra of several intricate hydrocarbons, using double resonance. This technique has been further extended by Shoolery to the interesting study of several boranes. Many other investigators have studied controversial structural problems which they have successfully solved by the application of high resolution n.m.r. spectroscopy, and this new technique is already becoming a cherished tool of the organic chemist.

This does not exhaust the chemical applications of n.m.r. spectroscopy. Reaction kinetics can be followed by measuring rates of increase or decrease of n.m.r. signals due to reactants and products. Chemical exchange rates can be studied since there is a gradual change in the appearance of n.m.r. in a particular chemical environment as the mean life-time in that environment decreases. Proton exchange in alcohol and water mixtures and in ammonia and ammonium ion has been studied in this way. Rates of rotation around single bonds in general and the rate of inversion of non-planar nitrogen of cyclic imines have been the subject of n.m.r. investigation. Chemical analyses both qualitative and quantitative of certain isotopes containing magnetic nuclei can be easily carried out by n.m.r. method. This technique is well suited where fast analysis is desired and the sample under study if costly and rare can be preserved during the process of analysis. Deuterium in natural water has been analysed with ease and sufficient accuracy.

The radio-frequency spectroscopy is quite useful for the study of crystal structures containing light atoms which are weak scatterers of X-rays. The line shapes and widths give very important information in the crystal study. Line shapes are usually determined by the interaction between the nearest dipole neighbours. The location of the nuclei with respect to the crystal axes, in a single crystal, can be determined from the nuclear magnetic resonance line shape as a function of the crystal orientation in the magnetic field. In crystal powders the nuclear magnetic resonance line shape is determined by averaging over a sphere the angular dependence. The average line shapes are considerably broadened but still give the proton-proton distance though with less accuracy. Line shapes for three spins at corners of an equilateral triangle and four tetrahedral spin $(I = \frac{1}{2})$ have been calculated. For more general systems in which the nuclei are not localized in small groups the calculation of the line shape is a difficult task. However, Van Vleck has shown that the second moment of the n.m.r. line can be related to given structural model. This is a powerful method of determining the inter-nuclear distance.

The technique of n.m.r. has also been used to understand the quadrupole interactions. Nuclei with $I > \frac{1}{2}$ have electric quadrupole moments and in a rigid crystal lattice, the quadrupole interactions split the magnetic resonance line. Since in a single crystal the magnetic resonance is split into $2I$ components

it is posssible to determine the spin number. From the number and spacing of lines one can determine the strength of interaction e^2qQ (the coupling constant) and the asymmetry parameter η. Several boron and sodium compounds have been investigated in this way.

Nuclear magnetic resonance has even invaded the fields like geology and biology. The earth's magnetic field can be scanned with great precision by the proton precessional magnetometer, a sensitive device to measure the variations in the earth's magnetic field. Rockets shot out of the earth's atmosphere to bring in the upper air information carry this type of magnetometer to measure the intensity of the earth's magnetic field at various heights. It is also used for prospecting minerals in geological survey. In the field of biology, n.m.r. has been used to examine the water content in proteins, carbohydrates and vegetable tissues. Water-structure changes have been examined in Hemocyanin, soluble starch and egg albumin. Proton resonance study with muscle stretching has been made. Analysis of biochemicals can be made without destroying the material. Structure of giant molecules can also be investigated.

Before concluding it is fascinating to know two remarkable effects which n.m.r. can detect. Direct experimental evidence has been obtained from the n.m.r. of protons in antiferromagnetic crystals regarding the formation of sub-lattices due to the alternate arrangement of spins. X-rays would not have detected this since X-ray scattering is independent of spin. The only other way of detecting it is by neutron diffraction. Another fantastic concept is that of negative temperature. In this concise article, space does not permit to go into the details. However, it may be mentioned that the n.m.r. studies of crystals of long relaxation times have established the state of negative temperature. It is a state which, instead of being cold, is very hot and can give up energy to a system in contact with it at a positive temperature.

Although n.m.r. spectroscopy had its birth quite recently, it has already become a powerful tool in the hands of scientists belonging to different disciplines. The increasing spate of publications on the subject speaks for the popularity of the n.m.r. technique as a tool in investigating physico-chemical problems. At the present moment the cost of instrumentation of a high resolution n.m.r. spectroscope unfortunately restricts the number of incumbents in this field which impediment we may hope in the coming years may disappear.

1. Andrew, E. R., *Nuclear Magnetic Resonance*, Cambridge University Press, New York, 1955.
2. Pople, J. A., Schneider, W. G. and Bernstein, H. J., *High Resolution Nuclear Magnetic Resonance*, McGraw-Hill Book Company, Inc., New York, 1959.
3. Ramsey, N. F., *Nuclear Moments*, Wiley, New York, 1953.
4. Roberts, J. D., *Nuclear Magnetic Resonance*, McGraw-Hill Book Company, Inc., New York, 1959.
5. Gutowsky, H. S., "Nuclear Magnetic Resonance," *Ann. Rev. Phys. Chem.*, 1954, **5**, 333.
6. Pake, G. E., "Magnetic Resonance", *Scientific American*, 1958, 1.
7. Shoolery, J. N. and Weaver, H. E., *Ann. Rev. Phys. Chem.*, 1955, **6**, 433.
8. Smith, J. A. S., "Nuclear Magnetic Rosonance Absorption," *Quart. Rev. (London)*, 1953, **7**, 279.

PROFESSOR T. R. SESHADRI: 60TH BIRTHDAY CELEBRATIONS

THE Sixtieth Birthday of Prof. T. R. Seshadri, Head of the Department of Chemistry, University of Delhi, was celebrated with *eclat* on February 3, 1960, in the Old Library Hall of the University. The large gathering included many of his old students and friends. Felicitous speeches were made on the occasion and messages of greetings were received from Sir Robert Robinson, F.R.S., N.L., and Sir C. V. Raman, F.R.S., N.L., amongst others.

Prof. Seshadri is well known for his original studies of the chemistry of naturally occurring compounds, particularly flavonoids. To mark the occasion, his past and present students brought out a souvenir which contains 21 review articles covering the different branches of the research work of Prof. Seshadri and his pupils.

We are happy to note that his 60th Birthday Celebrations have almost synchronized with his election to the Fellowship of the Royal Society.

SYMPOSIA ON 'WAVE PROPAGATION' AND 'ELECTRON DEVICES'

IN connection with the Golden Jubilee Year (1959-60) of the Indian Institute of Science. Bangalore, the Department of Electrical Communication Engineering arranged two symposia, one on "Wave Propagation" and the other on "Electron Devices". The symposia were held on November 23-25, 1959, and were inaugurated by Dr. S. Bhagavantam, Director of the Institute.

About 100 delegates representing various organisations of Government and industry. academic institutions, etc., took part in the presentation of papers and the discussions.

The Symposium on "Wave Propagation" opened with an address by Dr. K. R. Ramanathan, Director, Physical Research Laboratory, Ahmedabad, on "The Earth's Outer Atmosphere and Interplanetary Space".

There were three technical sessions under the Chairmanships respectively of Sri. P. N. Agerwala, Chief Engineer (Planning), Posts & Telegraphs, Government of India, Col. B. M. Chakravarthi, Superintendent, Electronic Research and Development Establishment, Bangalore, and Col. K. K. Mehta, Chief Inspector, Inspectorate of Electronic Equipment, Bangalore. Thirteen papers were presented and discussed in these Technical Sessions.

Sri. B. V. Baliga, Managing Director, Bharat Electronics Ltd., Bangalore, who presided over the Symposium on "Electron Devices" and was also Chairman of the first Technical Session, presented a review of "Progress in the Field of Electron Devices". The other two Technical Sessions were held under the Chairmanships of Prof. A. I. Vishnevsky, Indian Institute of Technology, Bombay, and Sri. Jagdeesh Prasad, Managing Director, Indian Telephone Industries, Bangalore. Sixteen papers were read and discussed in the Technical Sessions.

Two General Discussions were arranged one at the end of each Symposium. The first was on "The Interdependence of Research in the Physics and Engineering of Wave Propagation" and the second was on "The Impact of the Development of a New Electron Device on the Components Industry and on Communication Systems". Prof. S. V. Chandrasekhara Aiya, Head of the Department of Electrical Communication Engineering, Indian Institute of Science, Bangalore, initiated these discussions.

The symposia were a success especially as they helped in bringing together persons actively engaged in these fields in research, development planning, manufacture and maintenance. The group discussions, held outside the Technical Sessions, of selected papers evoked considerable interest and were of great value to the participants. Mr. S. Sampath, Assistant Professor of the Department, was the Convener of the Symposium.