HIGH-SENSITIVITY METHODS FOR THE DETECTION OF QUADRUPOLE RESONANCE

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ABSTRACT

Nuclear double resonance techniques have extended the frequency range in which it is possible to detect pure quadrupole resonance in polycrystalline samples down to 50 kHz. It is now possible to detect with ease the n.q.r. of ^{17}O, ^{14}N and ^{2}D.

The technique is briefly described and its advantages and limitations pointed out. A brief review of the work published to date is given which includes the n.q.r. of ^{14}N in amino acids, purines, pyrimidines and nucleosides and tetrahedral model compounds; the n.q.r. of ^{17}O in quinones, xanthenes, KH_2PO_4 and ice; and the n.q.r. of ^{2}D in deuterated acetamide and deuterated amino acids.

INTRODUCTION

Most methods for the sensitive detection of pure quadrupole resonance (n.q.r.) and indeed for nuclear magnetic resonance (n.m.r.) rely upon the presence of two spin species. The first, the A spins, are abundant and their magnetization is easily measured. The A spins are usually protons. The second species, the B spins, are rare or otherwise difficult to detect directly. The experimental cycle consists of first preparing the A spins in a polarized state and then polarizing the B spins through contact with the partially aligned A spins. A radio-frequency field is applied to the sample which may induce transitions in the B spin system, thus detecting a B spin resonance. The transitions in the B spin system so caused are then detected either directly or through their effect upon the A spin bath when the two spin systems are caused once again to cross-couple.

The high sensitivity of the methods is largely due to the pre-polarization of the B spin system prior to inducing the resonance. In some methods it is also due to the fact that it is possible to repetitively irradiate the B spin system and put it in thermal contact with the A spin bath before examining the A spin system to detect the cumulative effect of many such energy transfers.

In the initial experiments the polarization of the A spins was achieved along a powerful applied radio-frequency field by spin locking in the rotating frame. The cross-coupling between the A and B spin systems was achieved by ensuring that the Larmor frequencies of the two spin systems, each referred to its own rotating frame, were equal. This powerful method is clearly described in the initial paper of Hartmann and Hahn[1] and is usually referred to as double

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resonance in the rotating frame (d.r.r.f.). Although it was in fact used to detect the n.q.r. of sodium and chlorine near defects in sodium chloride, it is of very limited applicability to n.q.r. as it requires a sample in the form of a single crystal. It is still much used in various modifications for the sensitive detection of n.m.r.

A major advance in the sensitive detection of n.q.r. in powdered samples was made by Slusher and Hahn[2], who introduced the method of double resonance with spin mixing in the laboratory frame (d.r.l.f.). In this method the sample containing the A and B spins is polarized in a large d.c. applied magnetic field. The sample is then moved bodily to a region of zero d.c. magnetic field. During the removal from the magnetic field the A spins are adiabatically demagnetized and also come into thermal contact with the B spins when the energy level splitting of the A spins, in a diminished applied d.c. magnetic field, is coincident with an energy level splitting of the B spins due predominantly to the quadrupolar interaction. The pre-polarization of the B spins is brought about simply by removing the sample from the applied magnetic field!

While in the region of zero applied d.c. magnetic field a strong radio-frequency field of amplitude $2H_{1B}$ is applied at a frequency ν_B. If this frequency corresponds to an energy level splitting in the B spin system, power is resonantly absorbed by that system. Further, if

$$\gamma_B H_{1B} = \gamma_A H_L$$

where the γs are the two magnetogyric ratios and H_L represents a typical local field at an A spin site, the two spin systems come into thermal contact if there is a dipolar coupling between them. Power is thus transferred through the B spin system to the A spin system if ν_B corresponds to an allowed transition in the B spin system. Finally, the sample is remagnetized by returning it to the region of the d.c. applied magnetic field and the magnetization remaining in the A system is monitored by sampling the free induction decay that follows a 90° pulse. The spectrum of the B spins is swept, a different value of ν_B being used in each cycle, and an absorption is indicated by a drop in the remanent magnetization in the A system at the completion of a cycle.

This method has two drawbacks. The first is that the large value of H_{1B} necessary broadens the resonance lines and also makes detection at low frequencies impossible owing to direct absorption by the proton bath. The second and more important limitation is that this coupling is not effective with integer spin nuclei. The expectation value of the angular momentum in any singlet state produced by the application of an electric field is zero, as can be readily deduced by invoking the invariance of an electric field under time reversal. The associated magnetic moment is said to be 'quenched'. The energy levels of half-integer spins retain at least twofold degeneracy in an electric field (Kramer's Theorem) but those of integer spins do not, so that the magnetic moment associated with these states is zero. This suppresses the dipolar coupling between the two spin systems in small magnetic fields which is necessary in d.r.l.f. experiments.

These limitations are removed in a further development of the method which may be called double resonance with spin mixing by level crossing (d.r.l.c.), which was first described in the unpublished Ph.D. thesis of J. Koo,

a student of Prof. E. L. Hahn. In this method the B spins are pre-polarized by level crossing during the removal of the sample from the magnetic field exactly as in the previously described method. Again the B system is irradiated at a frequency v_B, and if v_B corresponds to an energy level splitting, power is absorbed. However, during the time spent in zero applied d.c. magnetic field no attempt is made to couple the A and B spin systems. The coupling is achieved by the second level crossing that occurs when the sample is being remagnetized on being returned to the large applied d.c. magnetic field. Once in the large field, a 90° pulse is applied and the free induction decay of the A spin system is sampled as before. The sensitivity of the method can be increased by irradiating the B spins and level crossing with the A spins many times before sampling the magnetization of the A spins at the completion of the cycle.

The method is applicable to either half-integer or integer spins because the cross-coupling occurs in a finite applied d.c. magnetic field which is effective in 'unquenching' the B system. The effective magnetic moment associated with a given energy level of energy E in a magnetic field B is given by $-\partial E/\partial B$. This is finite in a finite field B but, for integer spins, tends to zero as B tends to zero if the levels are not degenerate.

In this thesis Koo describes the detection of the pure quadrupole resonance of ^{14}N in phenothiazine and in 4-chloro-3-nitrobenzene sulphonic acid potassium salt. He also describes a successful attempt to detect the n.q.r. of deuterium.

Within my group at Oxford we have further refined the technique. We have shown that the large values of H_{1B} used by Koo (~ 10 G) lead to excessive line broadening. Using a single second-level crossing and $H_{1B} \sim 10^{-1}$ G, the n.q.r. of ^{14}N can be detected with high sensitivity and with linewidths less by a factor of 10 to 100 than those found by Koo. Furthermore the use of low values of H_{1B} removes the danger of direct absorption by the A spins, so that n.q.r. can be detected at frequencies as low as 50 kHz. We have shown that the method is ideal for the detection of the n.q.r. of deuterium. The resulting combination of high sensitivity and narrow linewidths has enabled us to study ^{14}N, ^{17}O and ^2D n.q.r. in amino acids, purines, pyrimidines and nucleosides and many other organic solids, as will be described.

A CRITIQUE OF THE DRLC METHOD

As we have described the details of our technique in considerable detail in a recent publication[3], I will not repeat it here; rather I will attempt to point out its chief advantages and drawbacks.

The method is capable of detecting with high sensitivity and narrow linewidths the pure quadrupole resonance of many nuclei in polycrystalline organic solids. The sensitivity is largely independent of the frequency of the transition detected, so that transitions can be detected with ease down to 50 kHz. The apparatus is simple, consisting of a permanent magnet of no great homogeneity, a fixed-frequency spectrometer which is capable of detecting the free induction decay of protons following a single 90° pulse, a sample moving apparatus and a Dewar system. The equipment is easily

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automated by means of a frequency synthesizer and a simple digital programmer for advancing ν_B and runs unattended for periods of about 12 h.

The only serious limitation of the method lies in the required range in values of the relaxation times of the spins involved. Let the longitudinal relaxation times of the protons and the quadrupolar nuclei in the applied d.c. magnetic field H and in zero field be $T_{1p}(H)$, $T_{1p}(0)$, $T_{1q}(H)$, $T_{1q}(0)$, respectively.

(i) Clearly, if $T_{1q}(0)$ is very short, then the energy resonantly given to the B spins will decay to the lattice and will not be passed to the A spins during the second-level crossing. With dwell times τ in the zero d.c. magnetic field region of 1–10 s we need $T_{1q}(0)$ greater than a few seconds. In the diamagnetic organic solids we have studied we have only seldom found this condition a limitation.

(ii) If $T_{1p}(H)$ is short compared with the sample transit time, then the magnetization built up by the d.c. polarizing field will decay before the first-level crossing. This will prevent the prepolarization of the B spins and the sensitivity of the method is lost. This is the most serious limitation at 77 K and prohibits the study of many interesting samples such as long-chain polypeptides. We are currently designing an apparatus capable of attaining temperatures as low as 4 K.

(iii) It is often supposed that the method requires $T_{1p}(0) \gg \tau$ but this is not so. We have previously[3] discussed this point in some detail and have shown that, while the final proton signal detected depends strongly upon $T_{1p}(0)$, the *change* in this signal which indicates a B spin system resonance does not. Briefly, if $T_{1p}(0) \gg \tau$, then the final free induction decay signal is large, but only a proportion of it (depending upon the relative abundance of protons and quadrupolar nuclei) can be effected by saturating a B spin transition. If $T_{1p}(0) \ll \tau$, then during the time τ the proton magnetization decays to zero, but it is partially restored by the B spin system during the second-level crossing. The extent of the remagnetization depends upon the magnetization remaining in the B system after irradiation. The total *change* in the final magnetization of the A spin system resulting from the saturation of a transition of the B spins is largely independent of $T_{1p}(0)$.

A SURVEY OF PUBLISHED WORK TO DATE

Amino Acids

Our initial study[4] of the n.q.r. of ^{14}N in some amino acids proved that these previously undetected resonances could be measured by the d.r.l.c. method. A comparison of the resonances of glycine, diglycine and triglycine showed that the three nitrogen sites in triglycine had characteristic and easily distinguishable electric field gradients. The amino nitrogen in the N^+H_3 group has an almost identical spectrum in all three peptides. Similarly, the second nitrogen site in diglycine has a spectrum very different from that in the terminal amino group but very similar to the 'second' nitrogen in triglycine.

A study[5] of the n.q.r. of ^{14}N in model compounds, in which the nitrogen atom lies in a tetrahedral environment, was carried out to gain insight into the amino site in amino acids. The value of the electric field gradient anisotropy

constant η derived from the measured spectra was shown to accurately reflect the symmetry of the site in the expected manner.

A second, more thorough investigation[6] of the n.q.r. of ^{14}N in seven amino acids was performed and both 'backbone' and side chain nitrogen sites were investigated. An attempt was made to correlate the quadrupolar coupling constant of nitrogen in the N^+H_3 site with the structure of the group as revealed by x-ray diffraction.

An independent study of some of these same amino acids, using the same method was published[7] by another group of workers based in Ljubljana and Stockholm. Unfortunately, the spectra they obtained are complicated by the presence of satellite lines of strength comparable with that of the n.q.r. lines. Satellite lines result from the simultaneous flip of a nitrogen nuclear spin and that of a nearby proton, and are an example of the solid state effect. They are discussed in the thesis of Koo, and their field dependence and use as a structure probe has been described by us[5]. Allowing for these extra lines and for the presence of the small d.c. magnetic field applied during the time τ by these workers, the results published by the two groups agree.

Finally, the n.q.r. of both ^{14}N and ^{2}D has been measured in deuterated amino acids[8]. The change in the electric field gradient at the nitrogen site on substituting deuterium for hydrogen is remarkably similar throughout the substances studied. It is shown that the quadrupole coupling constant of deuterium correlates linearly with the inverse third power of the hydrogen bond distance R in both $N—D\cdots O$ and $O—D\cdots O$ groups. R represents the distance $D\cdots O$ in each case. A similar correlation had previously been deduced[9] for the $O—D\cdots O$ group from single crystal n.m.r. studies. Such correlations should be of value in structure determination and also allow of extrapolation of the deuterium coupling constant to the limit of no hydrogen bonding $(R \to \infty)$.

Purines, Pyrimidines and Nucleosides

We have performed a study of the ^{14}N n.q.r. in various purines, pyrimidines and their nucleosides[10]. An attempt at assigning the spectral lines observed to the various nitrogen sites was made and a simple orbital population analysis was carried out. However, much remains to be done in this direction, as the spectra are complicated and the technique has advanced a great deal in the interval. Particularly interesting would be a study of spectra of crystals containing paired bases such as exist in the nucleic acids.

Deuterium Resonances

The detection[11] of the n.q.r. of deuterium in a powdered sample was performed in deuterated acetamide $(CH_3 \cdot CO \cdot ND_2)$ by the d.r.l.c. method. Each line in the spectrum displays a sixfold degeneracy which can be shown to be the characteristic spectrum of two deuterium nuclei in almost identical environments and close enough to interact. Since that time we have observed even better-resolved sixfold multiplets from D_2O included as a water of crystallization. The study of deuterated amino acids[8] has already been mentioned.

Oxygen Resonances

^{17}O has a half-integral spin and so its n.q.r. may be detected in powdered samples by d.r.l.f. or by d.r.l.c. methods. Hsieh, Koo and Hahn[12], using the d.r.l.f. technique, have studied the n.q.r. of naturally abundant ^{17}O in some quinone- and xanthene-like compounds. They are able clearly to distinguish C=O, C—O—H and C—O—C sites. Blinc *et al.*[12], again using d.r.l.f. techniques, have studied the n.q.r. of ^{17}O in paraelectric and ferroelectric KH_2PO_4 and deduce the presence of a double minimum O—H$\cdots$H hydrogen bond. Finally, Edmonds and Zussman[14] have used d.r.l.c. to detect the n.q.r. of ^{17}O in isotopically enriched ice. The results are consistent with single-crystal n.m.r. studies and should lead to a better understanding of hydrogen bonding in ice.

Some studies of ^{17}O have been performed on single-crystal samples by the d.r.r.f. method, which is outside the scope of this revue; however, mention of them may be found in reference 7.

CONCLUSION

Although the techniques of d.r.l.f. and d.r.l.c. have been vigorously pursued for only 3 years, much of value has already been discovered. The techniques should find increasing application in the study of n.q.r., particularly at frequencies below 3 MHz, where the gain in sensitivity over conventional methods is so great. This should open the door to the study of the n.q.r. of ^2D, ^7Li, ^9Be, ^{10}B, ^{11}B, ^{14}N, ^{17}O, ^{23}Na, ^{33}S and other light nuclei.

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