



# Field-cycling method with central transition readout for pure quadrupole resonance detection in dilute systems<sup>☆</sup>

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## Abstract

We present a modification of a field-cycling method which uses the NMR signal of the central transition at high field to indirectly detect zero-field quadrupole transitions. The quadrupole transitions at zero-field are detected as changes in the overall intensity of the central transition signal after the field cycle, and the method is relatively immune to lineshape distortions of the central transition caused by receiver dead time, frequency response of the probe, longer pulse lengths, etc. Cross-polarization with protons is used to enhance the central-transition signal and to increase the recycling rate of the experiment. The technique is especially useful when mixtures of several species are present. In a frozen solution of phenylboronic acid, <sup>11</sup>B quadrupole signals of the tetrahedral species at 600 kHz and planar-trigonal species at 1450 kHz are clearly resolved. The field-cycling approach allows high-sensitivity detection of low-frequency quadrupole transitions; the experiment is sensitive enough to study boronic-acid protease inhibitors bound to proteins and may possibly be extended to lower sensitivity nuclei. The experiments are performed using a low-temperature field-cycling apparatus, operated at 10–30 K, capable of pneumatically moving the sample from the high field of a commercial 500 MHz magnet to the area above the top of the magnet where the low field is controlled by a pair of Helmholtz coils.

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## 1. Introduction

The number of available experimental methods to study quadrupolar nuclei is steadily increasing. Extending applicability of these methods to dilute systems still poses a great challenge given the sensitivity limitations caused by low gyromagnetic ratios of the nuclei of interest and/or significant quadrupole broadening. Quadrupole coupling parameters provide a valuable experimental handle on coordination geometry, bond hybridization, or partial ionic character of bonding in the vicinity of the quadrupolar nuclei in a variety of

systems. Metal centers in proteins can serve as an example of a system of great interest and difficulty. A more commonly used experimental approach to determining quadrupolar parameters is solid-state high-field NMR spectroscopy. In dilute, non-ordered samples which are typical for biomolecular NMR, the quadrupole coupling constant and the asymmetry parameter of half-integer nuclei can be determined from the positions of singularities in the second-order quadrupole-broadened lineshape of the central transition. A detailed review of the typical challenges of central-transition spectroscopy of half-integer nuclei (severely broadened lines, overlapping lineshapes of several species, etc.) as well as recent experimental advances can be found elsewhere [1]. A landmark achievement of the high-field central transition NMR methodology is a recent study of <sup>67</sup>Zn bound to a small DNA-binding protein domain [2].

We do not discuss integer-spin nuclei in this article. They present a special case in both high-field and low-field NMR and often require specialized experimental

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techniques due to the absence of a central transition, and magnetic moment quenching at low field [3].

An alternative experimental approach to determine quadrupole parameters, as outlined in the pioneering paper by Pound [4], is based on the observation of pure electric quadrupole spectra in the absence of externally applied magnetic field. Since the original observation by Dehmelt and Kruger, nuclear quadrupole resonance (NQR), also known as pure quadrupole resonance (PQR), was used to accumulate quadrupole coupling data for a vast collection of chemical substances [5]. For nuclei with spin  $>3/2$  the quadrupole coupling constant and the asymmetry parameter could be deduced directly from the frequencies of pure quadrupole transitions at zero-field without having to use single crystals. The situation is more complicated for spin  $3/2$  nuclei which have a single transition at zero magnetic field, and for which specialized methods are required to estimate the asymmetry parameter [6].

Detection sensitivity of pure quadrupole transitions is, however, limited, especially for low-frequency transitions. A field-cycling approach was developed by one of us [7], and independently by Slusher and Hahn [8], to overcome the low sensitivity of direct pure quadrupole resonance detection. In these field-cycling experiments a strong high-field NMR transition is used to indirectly detect weaker zero-field quadrupole transitions and can in principle result in very high detection sensitivity [9]. The quadrupole resonance is detected when the search RF pulse applied at zero-field creates population changes of the energy levels, which are subsequently detected as a change in the readout signal at the high field after the field cycle. The zero-field spectrum is acquired by incrementing the frequency of the search RF pulse to cover the desired spectral region. An alternative zero-field detection approach was developed by Pines and co-workers [10,11], in which zero-field coherence phenomena are initiated by a rapid change in magnetic field and the zero-field spectra are generated by Fourier transformation of the time-domain points.

In hydrogen-containing substances, proton high-field resonance is commonly used as the readout signal accompanied by a magnetization transfer between the quadrupolar nuclei and the protons via dipole–dipole interactions. This strategy was used to study quadrupolar coupling in numerous small molecule compounds [12]. Our attempts to extend this method to biological macromolecules [13] were impeded by fast proton relaxation at low fields observed in biomolecular samples even at temperatures below 30 K. Such fast relaxation was observed both in lyophilized protein samples, as well as in frozen solutions of proteins in commonly used cryo-protective buffers. Rapid relaxation creates severe sensitivity problems in the proton readout approach because the quadrupolar transitions are detected as small changes in the strong proton NMR signal, and

such strategy is intrinsically prone to signal-to-noise limitations. Furthermore, for low gyromagnetic species of biological interest, such as  $^{55}\text{Mg}$  or  $^{67}\text{Zn}$ , the contact time for each energy transfer must be very long, and the optimum RF field is excessive. Several of our attempts to alleviate the problem, such as use of multiple level-crossing methods and sample deuteration, did not produce the high sensitivity required for biological samples [14].

Here we describe an alternative field-cycling strategy, in which the central transition of the quadrupolar nucleus itself is used as the readout signal at high field. The utility of this approach for protein samples was demonstrated in a study of boronic acid inhibitors bound to serine proteases [15].

Overlapping spectral lines of distinct spectral species can also create a significant complication for lineshape analysis in high-field central-transition spectroscopy. A number of methods such as MQ-MAS, nutation spectroscopy, and dynamic angle spinning have been developed to separate the overlapping central-transition signals in an extra dimension. In a field-cycling experiment one can correlate high-field and zero-field signals to achieve line separation in two dimensions [11]. Our technique can produce the equivalent of two-dimensional spectra by correlating the high-field central transition signals with the zero-field pure quadrupole resonance.

The technique described here was used to investigate pure quadrupole resonance of  $\text{B}^{11}$ , which has a fairly high gyromagnetic ratio, but the method can possibly be extended to other lower sensitivity half-integer quadrupolar nuclei. We did not succeed in observing  $^{67}\text{Zn}$  or  $^{55}\text{Mg}$  signals, but this could be due to the shortcomings of our locally tuned probe which was designed to fit in a severely limited space inside the dewar inside the narrow-bore 500 MHz magnet. Recent observation of a  $^{67}\text{Zn}$  central transition resonance in a protein using an alternative low-temperature detection scheme [16], which uses a transmission line probe and a low-temperature preamplifier, demonstrates the feasibility of such detection and may possibly be combined with our field-cycling strategy.

## 2. Experimental method

The field-cycling technique described here follows the general strategy of previously described field-cycling methods in which a relatively strong NMR signal at high field is used to indirectly detect weaker transitions at low field. The key requirement of this approach is that the spin order which is used as a readout signal must survive through the field cycle. The experiment is performed at low temperature to reduce thermal relaxation due to fast molecular motion. The utility of the proton

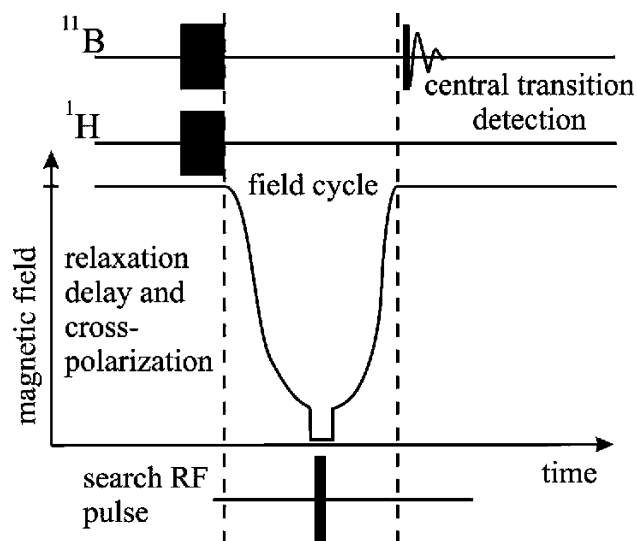


Fig. 1. The timing diagram of the field-cycling method with boron central transition detection. The field-cycling part of the sequence is simplified; see Fig. 3 for a more detailed field-cycling diagram.

NMR signal as the readout is limited by the short proton  $T_1$  at low field which we observed in biomolecular samples even below 30 K. Instead, we observe the central transition of the quadrupolar nucleus, which in general has a much longer  $T_1$  than protons and may be a better readout signal for biological applications. Proton magnetization is used to enhance the signal of the central transition before the field cycle by Hartmann–Hahn cross-polarization, and paramagnetic doping ( $\sim 1$  mM  $\text{Ni}^{2+}$ ) is used to reduce the proton  $T_1$  to about 10 s without significant effect on the relaxation properties of the quadrupole central transition at low field.

Fig. 1 shows a simplified diagram of the experiment. Before the field cycle the protons are polarized in high magnetic field (11.7 T) and then the magnetization is transferred to the central transition of the quadrupolar nucleus using Hartmann–Hahn cross-polarization. After that the sample is physically moved to low or zero magnetic field where a short search RF pulse is applied. Finally, the sample is moved back to high field and the central transition of the quadrupolar nucleus is observed after a  $90^\circ$  pulse. The low-field search frequency is incremented from cycle to cycle and, when it is close to quadrupole resonance, a change in the readout signal intensity is observed. The resulting quadrupole spectra are shown in the results section.

### 2.1. Field-cycling apparatus

Our field-cycling instrument was based on a pneumatic shuttle system that moves the sample between two points of different field strength. In the mechanical field-cycling approach one can use well-developed superconducting magnet technology to produce a homogenous

high field for the field-cycling system. The high magnetic field of a superconducting magnet allows higher sensitivity of the NMR signal detection than that which can be achieved with a switched electromagnet, but the field-cycling time is relatively long and samples with short proton  $T_1$  pose a serious problem.

We designed our field-cycling system based on a 11.7 T 43 mm microbore Oxford magnet. The field-cycling apparatus (Fig. 2) was a pneumatic shuttle tube with an RF probe at the high-field end and an RF solenoid at the low-field point. The sample was shuttled a distance of about 1 m to the top of the magnet where the low field can be controlled by a pair of Helmholtz coils. The transfer system was placed in a variable temperature liquid-helium flow dewar (Cryo Industries, Manchester, NH), and maintained at low temperature by a constant helium flow. The high-field probe had to fit into the narrow leg of the flow dewar (23 mm inside diameter), which was inserted into the 43 mm bore of the magnet, while the low-field assembly was located in the wider part of the flow dewar (61 mm inside diameter) above the exterior of the 11.7 T magnet. This assembly consisted of the low-field RF search coil (see Fig. 2), which was wound as a solenoid on a short length of Teflon tube, producing a vertical (longitudinal) RF field. The sample was moved pneumatically up the thin-walled transfer tube by applying partial vacuum from the top end, and slid up into the Teflon tube which is mated to the brass tube. An upper mechanical stop arrested it in the center of this tube.

After the search RF irradiation was applied, the sample was pushed back down by a slight overpressure

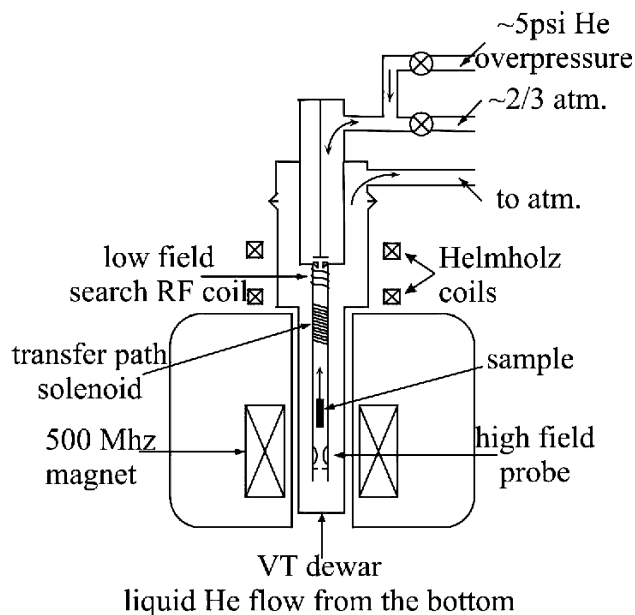


Fig. 2. Low-temperature field-cycling apparatus. The tail of the variable temperature flow dewar is placed inside the bore of the commercial 500 MHz superconducting magnet dewar.

of helium gas. The sample moved to low field in about 300 ms. Moving the sample back to high field required a 600 ms delay before the central transition signal could be read to insure that the sample came to rest in the probe after bouncing off the lower stop. This was good enough for our purposes, although it can probably be improved with a higher pushdown pressure and a more sophisticated stop assembly.

The tube that supports the apparatus was larger in diameter above the upper, low-field, assembly, to lessen pressure drop along this tube during shuttling (Fig. 2). An adapter tube was temporarily inserted into this section to facilitate insertion and removal of the sample, when needed.

The sample holder was a plastic cylinder with a screw-on cap, made out of Delrin or Teflon. The cylinder, which held about 0.5 ml frozen sample, was about 30 mm long and 9.2 mm in outside diameter. Samples were either solid, or were rapidly frozen in a small open glass liquid nitrogen dewar outside the system, and dropped into the shuttle tube after the latter was already at or below 77 K, taking care not to form solid nitrogen in the tube.

The low temperature was produced and regulated in the flow dewar. The temperature difference between the high-field point and the low-field point, which are about 1 m apart, depended on the helium flow and the field-cycle repetition time. For most of the boron-readout experiments the repetition time was about 6 s, the temperature at the bottom was set for 10 K, and the helium consumption was about 1.5 L/h from a slightly pressurized (9 kPa) liquid helium storage tank. At these operating conditions the temperature at the low-field point, about a meter above the center of the superconducting magnet, was about 30 K. The temperature of the sample up at this point, during the brief time the sample stays there, is unknown, but is likely to have been close to 10 K because of the very short time (150 ms) the sample spent at the low-field point, and in transit. The temperature at the low-field point could be reduced to about 20 K, but at considerably greater cost in liquid helium consumption, and no improvement in performance.

It is important that after cross-polarization the protons and the quadrupolar nuclei do not come into thermal contact at any time during the field cycle. Such cross-relaxation can occur when the external magnetic field passes the level-crossing field, which is the field value at which proton Zeeman splitting matches the splitting of boron quadrupolar levels [17,18]. Although level-crossing can be used for indirect detection of quadrupole resonance when using the proton signal as the readout [12], it has a detrimental effect for our approach because the short proton  $T_1$  in biological samples destroys a considerable part of the proton polarization during the field cycle, and cross-relaxation

of the boron central transition with the depolarized protons will destroy the boron signal. Mechanical movement of the sample from the superconducting magnet into the low field coils produces rather slow variation of the field and could result in cross-relaxation at the level-crossing condition. Indeed, if the field-cycling is done by simple mechanical movement from high to low field, the boron signal disappears after the field cycle. To avoid that, we had to maintain the external magnetic field, seen by the sample during the mechanical movement, above the level crossing condition. It is also important to pass through the level-crossing value of the magnetic field rather quickly after the sample has arrived at the upper position.

These problems were largely avoided by arranging the Helmholtz coils so that, instead of bucking the fringe field of the main magnet, they initially increased it to above the level-crossing value, as the sample arrives. We also added a transfer-path solenoid coil (Fig. 2) wound around the sample transfer tube to maintain the sample above the level-crossing value all the way along the sample transfer path. Once the mechanical transfer of the sample is completed, the cycling to zero field can be done by switching the currents through the coils, which is intrinsically faster than the mechanical field-cycle.

The exact value of the applied magnetic field at which cross-polarization occurs is hard to predict. The solution for the energy levels of the Hamiltonian is complicated when the Zeeman and the quadrupolar interactions are of the same order of magnitude. The energy levels are orientation-dependent, producing powder patterns, and cross-polarization can occur with different pairs of energy levels of the quadrupolar system, because no strict selection rules apply. Experimentally it was found that during the mechanical shuttle of the sample the field should be above 600 G to prevent polarization loss.

We observed that boron magnetization disappears in a matter of milliseconds at zero-field, perhaps because of fast relaxation or cross-polarization processes of some kind. Therefore, cycling to zero-field has to be faster than one millisecond, but reversal of the current through the Helmholtz coils requires too much time because of the relatively high coil inductance, eddy currents in the dewar, and the limitations of the power supplies. Instead, we used DC pulses through the low-field RF solenoid to produce relatively fast field changes. Fig. 3 shows a diagram of the field cycle. Once the mechanical sample transfer was completed, DC through the RF solenoid was turned on, generating an additional field of about 450 G. After that the current through the Helmholtz coils was reversed, which takes about 40 msec. When the current through the Helmholtz coils reaches the desired value, the DC through the RF coil was turned off momentarily (1 ms) and the search RF irradiation was performed. The rate of the current change through the RF solenoid was controlled in part by series

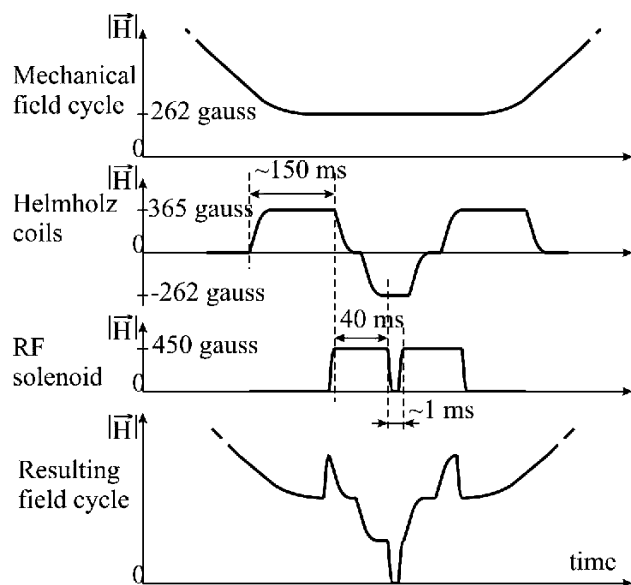


Fig. 3. Timing diagram of the field cycle part of the sequence. The time and field axes are not to scale.

inductance and resistance of the circuit (not shown). The rate has to be fast enough to minimize time at zero-field. However, it has to be slow enough to be adiabatic [19] (slower than any resonance frequency of the system), in order to preserve the energy level populations. Straightforward low frequency filtering allowed the RF search power (see Fig. 1) to be applied to the same coil when the field at the sample is nearly zero. After the end of the search RF irradiation pulse, all the currents were turned off in the reversed order, and, finally, the sample was shuttled back to the high field of the main magnet. Our field-cycling strategy appears similar to the one developed in the Pines lab [20], with one very important difference: our “fast” field step, generated by running DC through the RF solenoid, is slow enough to be adiabatic, while theirs has to be non-adiabatically fast in order to initiate transient phenomena at zero-field [10,11].

## 2.2. High-field RF probe. Cross-polarization and central transition detection

The high-field RF probe, at the bottom of the flow dewar (see Fig. 2), was based on a short hollow tubular Teflon “coil” form (or RF assembly) attached to the lower end of the brass transfer tube. Below this short RF assembly was a simple short stop assembly based on a thick piece of felt, to help avoid breaking the plastic sample holder. The sample was thus stopped in the middle of the hollow teflon tube of the RF assembly. The tuning capacitors were just above the RF assembly. The RF assembly was unusual mainly in that the local “coils” for protons (at 500 MHz) and for the  $^{11}\text{B}$  central

transition (at about 160.4 MHz) were both each single-turn, grounded to a lower ground plane at the center; and six of the eight tuning and matching capacitors were home-made tubular ones that extended for some centimeters above the short RF assembly. We now describe the probe in more detail.

A complication of operation at low temperature is that the voltage of dielectric breakdown of helium gas is low, which limits the maximum RF power we can apply to the sample. This is why we used single-turn center-tapped coils, to reduce electric fields as far as possible, and avoided commercial variable capacitors. Four longitudinal copper resonator foils 3 mm wide and 0.25 mm thick were held with glass tape on the Teflon tube extension (inside diameter 10.5 mm) of the brass shuttle. The Teflon tube had four longitudinal grooves milled on its outside surface, to mechanically hold the four longitudinal resonator foils as close to the sample as possible. These foils formed two orthogonal push-pull “coils,” each coil comprising in part a pair of these foils separated radially by 13 mm, and on directly opposite sides of the sample. The grooved tube and foils fit inside a ground cage consisting of the two annular radial copper ground-planes, whose planes were perpendicular to the axis. They were separated longitudinally by 12 mm, and held together mechanically and electrically by four heavy copper longitudinal support wires soldered to them. All four resonator foils were soldered to the lower ground plane, as a center-tap. The lower ground plane also supported the lower sample-stop assembly at the bottom, and the upper one was connected to the long brass shuttle tube. The holes in the center of the ground planes were needed to let the sample through. The support wires were displaced by  $45^\circ$  from the resonator foils, to allow RF flux to pass. They were as close as possible to the inside wall of the flow Dewar.

The resonator foils extended through larger slots in the top ground plane. The  $^{11}\text{B}$  (160 MHz) resonator foils were each connected to a pair of parallel 62 pf ceramic tuning capacitors (American Technical Ceramic 700B series) in parallel, which were grounded to the upper ground plane, and located just below it. All other capacitors were made of copper wire inside Teflon tubing that was, in turn, inside copper tubing, having capacitance between the wire and the copper tubing of  $\sim 2$  pf/cm. For the 160 MHz resonators there were two such fine-tuning capacitors, which were about 20 cm long, extending above the sample region. For one of these, the wire was connected at one end to the upper end of each of the resonator foils, in parallel with the 62 pf capacitors, and at the other end it was not connected at all, but projected into the Teflon tubing that was in turn inside a thin-walled copper tube that was grounded by being soldered to the outside of the brass transfer tube. The other one of these two tubular capacitors was similar but was made variable and adjustable from the top of

the Dewar, by having the upper part of its shield movable. It could be moved up longitudinally (leaving a section of inner wire and insulating tube exposed), or down, by a rod and linkage from the top of the Dewar, for fine tuning. The upper end of this movable shield tube slid inside another shorter thin-walled tube that was soldered to the brass transfer tube, and served to ground the movable tube. For proton tuning a pair of short ( $\sim 2$  cm) versions of the above was used. Matching to a  $50\ \Omega$  transmission line was performed with similar ungrounded wire/tube capacitors that could not be varied from the top, but were changed by “cut and try” at room temperature. Tuning these coils was tedious and not straightforward because the two half-turn foils are each individually resonated by the capacitors connected to them, and are not strongly coupled to each other. This is an unavoidable consequence of their close spacing to the inner wall of the flow dewar. Each pair of foils thus had two resonances rather close together in frequency, observed as usual with a variable-frequency signal generator and directional coupler, and the correct, lower frequency one, had to be tuned to the desired resonance frequency. For setup at room temperature, and checking in the dewar, it was useful to have a small ( $\sim 1.5$  mm) one turn pickup coil connected eccentrically to a long small diameter coax line, that could be passed into the sample hole and rotated to verify the intensity of the RF field around the edge of the sample region.

Our high-field RF assembly produced a  $90^\circ$   $^{11}\text{B}$  pulse of  $10\ \mu\text{s}$  with about  $300\ \text{W}$  excitation, and a  $10\ \mu\text{s}$  proton  $90^\circ$  pulse at  $17\ \text{W}$ . Due to dielectric breakdown, the available proton power was not enough to do proton decoupling, which would have been advantageous if CPMG detection were to be used (see below). It may be possible to achieve higher RF power at low temperature using a transmission-line probe design [16], which was hard to implement in our case given the space limitations inside the flow dewar. Our assembly is not a transmission-line probe because all coaxial elements are short compared to the relevant RF wavelengths.

Use of cross-polarization (CP) with protons as a way to enhance the NMR signals of nuclei with low gyromagnetic ratio has become a standard approach in solid-state NMR [21]. For non-spinning samples, cross-polarization of the central transition with protons is fairly straightforward. To avoid dielectric breakdown during the cross-polarization pulse we use lower-power off-resonance irradiation during cross-polarization. In their original experiments, Hartmann and Hahn [22] demonstrated that at low RF power the cross-polarization is most efficient when the irradiation frequency is off resonance by the amount necessary to fulfill the Hartmann–Hahn condition for the effective field in the rotating frame:  $\gamma_a H_{ea} = \gamma_b H_{eb}$ . The effective field  $H_e$  experienced by the nucleus in the rotating frame is

$$\gamma_a H_{ea} = \sqrt{(\omega_a - \omega_{0a})^2 + \gamma_a^2 H_{1a}^2},$$

where  $H_{1a}$  is the RF strength and  $\omega_a$ ,  $\omega_{0a}$  are the irradiation frequency and the resonance frequency for nucleus  $a$ , respectively. In our case  $\gamma H_1/2\pi$  was about  $10\ \text{kHz}$  and the resonance offset  $(\omega - \omega_0)/2\pi$  was  $30\ \text{kHz}$  for both nuclei. The cross-polarization rate for off-resonance cross-polarization is theoretically lower by a factor of  $\sin^2 \theta$ , where  $\theta$  is the angle the effective field makes with the  $z$ -axis or  $\tan \theta = \gamma H_1/(\omega - \omega_0)$ , but it was fast enough for useful cross-polarization in our samples. Since the orientation of the effective field was at a fairly small angle with the  $z$ -axis, there was no need for a  $90^\circ$  pulse on the proton channel before the spin-locking cross-polarization pulse, which is required to lock the spins along the effective field direction when the irradiation is on resonance [23]. The cross-polarization was done simply by applying two simultaneous initial RF pulses at the proton and the boron channels (Fig. 1), both  $30\ \text{kHz}$  off-resonance. The cross-polarization pulses were  $50\ \text{ms}$  long. The combination of cross-polarization and shorter proton  $T_1$ , which was manipulated using paramagnetic doping with  $1\ \text{mM Ni}^{2+}$ , resulted in at least 3-fold gain in signal when the field cycle was performed every  $6\ \text{s}$ .

The NMR signal of the central transition at high field is used as the readout signal after the field cycle. The undistorted lineshape is not absolutely crucial for this method, and the requirements for the instrumentation are not very stringent. In most of the experiments presented here the signal was acquired as a free induction decay after a  $90^\circ$  pulse. The linewidth of the boron central transition in our static samples is relatively small ( $12\ \text{kHz}$ ), owing to relatively low quadrupolar interaction and the high gyromagnetic ratio of boron; thus the whole line can be excited using a  $90^\circ$  pulse of moderate RF power.

If enough RF power is available it is advantageous to use spin-echoes for detection. The Carr–Purcell–Meiboom–Gill (CPMG) [24,25] multiple spin-echo pulse train can be adapted to remove the inhomogeneous second-order quadrupole broadening of the central transition resulting in higher detection sensitivity [26]. The linewidth of the boron central transition is about  $12\ \text{kHz}$  with the corresponding  $T_2$  of  $26\ \mu\text{s}$ . In our case the CPMG sequence produced no echo for an inter-pulse time  $\tau > T_2$ , indicating that the dominant broadening mechanism is not the inhomogeneous second-order quadrupole broadening, but rather the dipole–dipole interactions with the protons which acts to effect homogeneous broadening due to rapid spin flips of the protons. In conventional solid-state NMR experiments, proton decoupling [27,28] is normally used to remove dipolar broadening of hetero-nuclei by protons. In our low temperature experiments, however, it was not

possible to generate high enough RF field at 500 MHz for proton decoupling because the field in our coil was limited by the dielectric breakdown of helium gas.

### 3. Results and discussion

The method was successfully applied to study boronic acid protease inhibitors bound to serine proteases [15]. Here we present results obtained with simple solutions of phenylboronic acid, which we used as model system. Boronic acids act as Lewis acids forming covalent adducts with electron donors. Phenylboronic acid with planar boron coordination can bind a hydroxide ion forming an adduct in which boron coordination geometry becomes close to tetrahedral (Fig. 4). Figs. 5 and 6 show spectra of a frozen 100 mM solution of phenylboronic acid in a water/glycerol buffer to which 50 mM NaOH was added to produce roughly equimolar concentrations of trigonal-planar and tetrahedral coordination geometries.

The signal of the central transition (Fig. 5A) is observed after a  $90^\circ$  pulse with no proton decoupling. The spectrum shows a rather featureless asymmetric line, which does not allow a precise estimate of the quadrupole interaction but only sets a higher limit on its magnitude. The second-order lineshape singularities, which are rather sensitive to different broadening mechanisms, do not appear in the spectrum. The asymmetry of the line could be the result of the quadrupolar broadening or of the presence of more than one species, which would be hard to disentangle without field cycling.

In a field-cycling PQR experiment the readout signals can be stacked into a matrix where the second dimension is the search RF frequency at zero-field (Fig. 5B). The changes in the intensity of the readout signal vs. the search RF irradiation frequency reveal the pure quadrupole transitions at zero-field. These transitions can be seen if a slice through the central transition peak is taken along the irradiation frequency axis. In the Fig. 5C the higher frequency peak at 1450 kHz is due to planar boron species of neutral phenylboronic acid and the 600 kHz peak corresponds to four-coordinate anion.

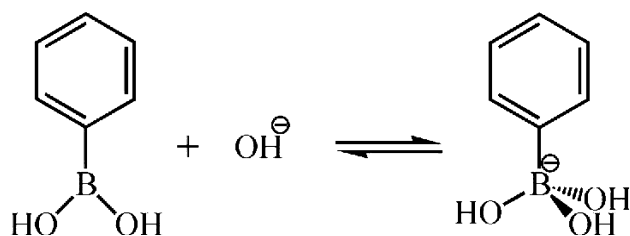


Fig. 4. Phenylboronic acid forms a covalent adduct with hydroxide ion. Boron coordination is planar in the neutral acid and is close to tetrahedral in the anion.

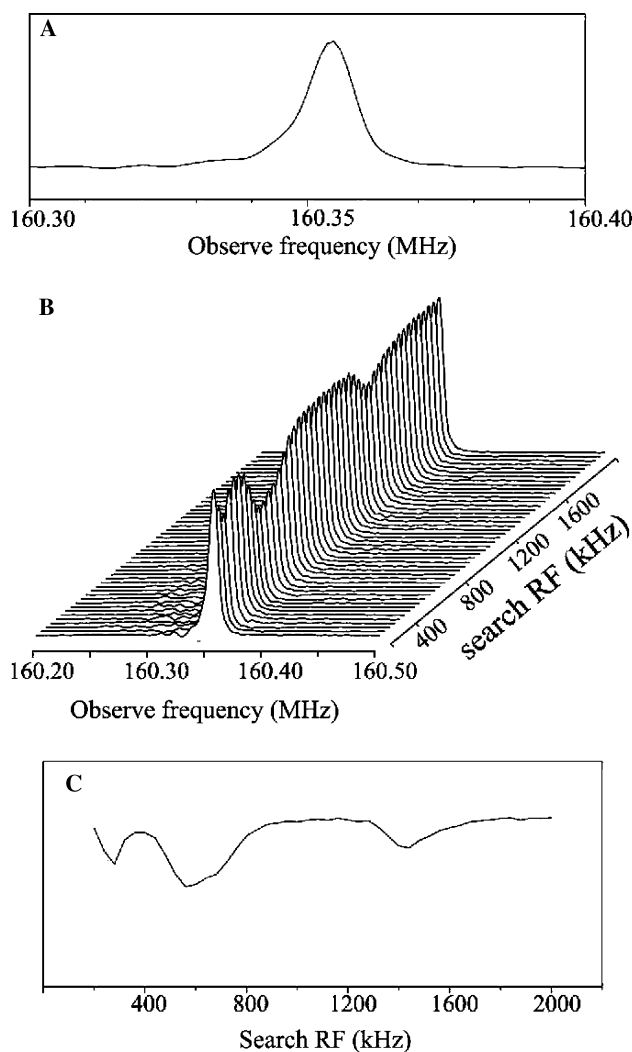


Fig. 5. The spectra of the 100 mM phenylboronic acid solution titrated with NaOH. (A) The  $^{11}\text{B}$  central transition observed after a  $90^\circ$  pulse at high field. The line demonstrates slight asymmetry, but no second order lineshape singularities are visible. (B) A stack plot of a field-cycling experiment. The readout signals of the central transition are stacked in a matrix where the second dimension is the search RF frequency. The spectrum was collected using a  $200\ \mu\text{s}$  long  $\sim 20\ \text{G}$  peak search RF pulse. (C) A quadrupole spectrum is obtained as a slice through the central transition ridge of the matrix. The low frequency peak at  $\sim 280\ \text{kHz}$  is not a pure quadrupole resonance, but a multiple-spin transition which appears at high RF power.

The low frequency peak at 280 kHz is not a pure quadrupole transition, but a multiple-spin transition which we did not completely understand. It only appeared at high RF powers and is probably the result of the boron-proton dipole-dipole perturbation of the boron's pure quadrupole Hamiltonian at zero-field.

It is convenient to convert such data into a more conventional-looking 2D spectrum. That can be done by subtracting the readout signals of a PQR run from a readout signal with no zero-field irradiation. Practically, one can just use a readout FID from a PQR run at an irradiation frequency where no PQR lines are present

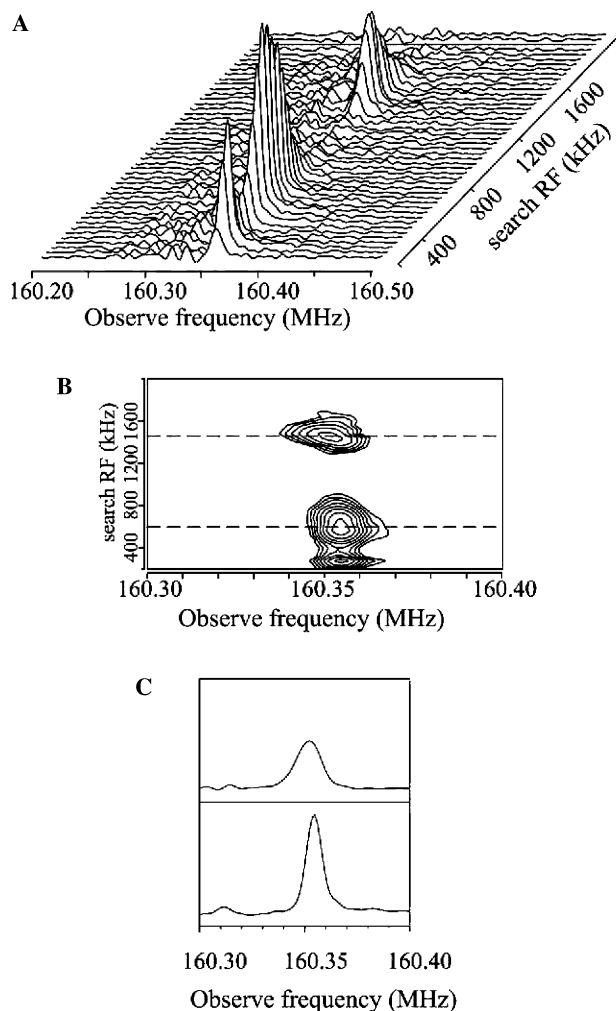


Fig. 6. (A) Stack plot of the difference spectra, obtained from the spectra in Fig. 5B, by subtracting all readout spectra from one readout spectrum, which is the spectrum at the high search frequency end of the series of one-dimensional spectra.  $^{11}\text{B}$  transitions appear as two-dimensional peaks. (B) Contour plot of the same spectra. The peaks appear to have a slightly different chemical shift value and a different linewidth in the observe dimension. (C) Peak cross-sections along the observe dimension (dashed lines in spectrum (B)) showing different linewidths of the two peaks.

and subtract all other FIDs from it. The resulting spectrum is shown in Fig. 6A. In the contour plot (Fig. 6B) one can more easily see that the two peaks have different linewidths and have slightly different chemical shifts in the observed dimension. The slices through the peaks (Fig. 6C) clearly show the different linewidths of the central transitions of the two species.

Poor resolution of multiple species in high-field central-transition spectroscopy, which is probably exaggerated in our non-spinning samples with no proton decoupling, remains a great challenge for the NMR spectroscopy of quadrupolar nuclei. Similar to MQ-MAS, nutation spectroscopy and dynamic angle spinning methods, our field-cycling 2D approach utilizes

quadrupole interactions to resolve the overlapping spectral species in an extra dimension. The frequency of the pure quadrupole resonance detected at zero-field is a convenient experimental approach to determine nuclear quadrupole parameters. The strategy of cryogenic temperatures, combined with cross-polarization, allows high detection sensitivity as is evident from our study of inhibition of serine proteases by peptide boronic acids [15]. It may be applicable to other quadrupolar nuclei in a variety of biological or non-biological dilute systems. Protonated samples have the advantage of a significant sensitivity gain when cross-polarization is used. The field-cycling approach is especially advantageous in the systems where several sites with distinct quadrupole parameters are present.

#### 4. Conclusions

The method described here is a modification of a field-cycling strategy for pure quadrupole resonance detection. The field-cycling quadrupole resonance techniques [7,8] allow high-sensitivity indirect detection of pure quadrupole resonance, but the high detection sensitivity allowed by using proton resonance as the readout signal cannot be easily extended to study biological samples due to fast proton relaxation at low field even at temperatures below 30 K. The described technique overcomes this difficulty by using the central transition of the quadrupolar nucleus as the readout signal after the field cycle. The detection sensitivity of the central transition signal is enhanced by using initial low temperature cross-polarization with the protons, while the proton high-field relaxation is manipulated by paramagnetic doping, allowing faster recycling time for the experiment. The method can be used with frozen solutions or lyophilized powders of biomolecules, as well as in a variety of other non-biological systems.

This field-cycling approach allows high-sensitivity detection of low-frequency pure quadrupole transitions, which adds an extra dimension to the high-field spectra and separates overlapping central transition signals of multiple species with distinct quadrupole coupling parameters. The method also demonstrates the feasibility of pneumatic field-cycling at low temperature (10–30 K) with good temperature stability and reasonable liquid helium consumption. Our results [15] on proteins at concentrations of around 1 mM are high in sensitivity for this type of experiment, but were difficult to obtain.

The experiment we describe has many novel features, but by no means represents the best way to perform these experiments. A wider-bore high-field magnet would obviously be very helpful. A different method of temperature maintenance with the possibility of use of a higher-pressure or different thermal exchange gas, perhaps using a higher temperature at the bottom of the



apparatus, would be desirable to allow high-power proton decoupling. Recent advances in NMR methodology [16,29,30] may further increase the utility of this or related methods and may extend the power of NMR spectroscopy to a wider selection of nuclei and systems.

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### References

- [1] M.E. Smith, E.R.H. van Eck, Recent advances in experimental solid state NMR methodology for half-integer spin quadrupolar nuclei, *Prog. Nucl. Magn. Reson. Spectrosc.* 34 (1999) 159–201.
- [2] A.S. Lipton, G.W. Buchko, J.A. Sears, M.A. Kennedy, P.D. Ellis,  $^{67}\text{Zn}$  solid-state NMR spectroscopy of the minimal DNA binding domain of human nucleotide excision repair protein XPA, *J. Am. Chem. Soc.* 123 (2001) 992–993.
- [3] G.W. Leppelmeyer, E.L. Hahn, Nuclear dipole field quenching of integer spins, *Phys. Rev.* 141 (1966) 724–731.
- [4] R.V. Pound, Nuclear electric quadrupole interactions in crystals, *Phys. Rev.* 79 (1950) 685–702.
- [5] H. Chihara, N. Nakamura, Nuclear Quadrupole Resonance Spectroscopy Data, in: *Landolt-Bornstein*, New Series, Group III, vol. 20, Springer, Berlin, 1988.
- [6] G.S. Harbison, A. Slokenbergs, 2-Dimensional nutation echo nuclear-quadrupole resonance spectroscopy, *Z. Naturforsch.* 45A (1990) 575–580.
- [7] A.G. Redfield, Pure nuclear electric quadrupole resonance in impure copper, *Phys. Rev.* 130 (1963) 589–595.
- [8] R.E. Slusher, E.L. Hahn, Sensitive detection of nuclear quadrupole interactions in solids, *Phys. Rev.* 166 (1968) 332–347.
- [9] Y. Hsieh, E.L. Hahn, J.C. Koo, Pure nuclear quadrupole-resonance of naturally abundant O-17 in organic solids, *Chem. Phys. Lett.* 13 (1972) 563–566.
- [10] A. Bielecki, J.B. Murdoch, D.P. Weitekamp, D.B. Zax, K.W. Zilm, H. Zimmermann, A. Pines, Fourier-transform pure nuclear-quadrupole resonance by pulsed field cycling, *J. Chem. Phys.* 80 (1984) 2232–2234.
- [11] D.B. Zax, A. Bielecki, K.W. Zilm, A. Pines, D.P. Weitekamp, Zero-field NMR and NQR, *J. Chem. Phys.* 83 (1985) 4877–4905.
- [12] D.T. Edmonds, Nuclear-quadrupole double-resonance, *Phys. Rep.* 29 (1977) 234–290.
- [13] D. Ivanov, A. Redfield, Development of a field cycling NMR system for PQR detection in biopolymers, *Z. Naturforsch.* 53A (1998) 269–272.
- [14] D. Ivanov, Development of a Field-Cycling NMR Method to Study Quadrupolar Nuclei in Biological Systems, Ph.D. Thesis, Brandeis University, UMI Number: 3004961, UMI Dissertation Services, Ann Arbor, Michigan, 2001.
- [15] D. Ivanov, W.W. Bachovchin, A.G. Redfield, Boron-11 pure quadrupole resonance investigation of peptide boronic acid inhibitors bound to alpha-lytic protease, *Biochemistry* 41 (2002) 1587–1590.
- [16] A.S. Lipton, J.A. Sears, P.D. Ellis, A general strategy for the NMR observation of half-integer quadrupolar nuclei in dilute environments, *J. Magn. Reson.* 151 (2001) 48–59.
- [17] J. Koo, Y.N. Hsieh, N-14 Nuclear quadrupole coupling constants in imidazole, *Chem. Phys. Lett.* 9 (1971) 238–241.
- [18] D.T. Edmonds, P.A. Speight, Nitrogen quadrupole resonance in amino acids, *Phys. Lett. A* 34 (1971) 325–326.
- [19] E.M. Purcell, R.V. Pound, A nuclear spin system at negative temperature, *Phys. Rev.* 81 (1951) 279–280.
- [20] A. Bielecki, D.B. Zax, K.W. Zilm, A. Pines, Zero-field NMR and NQR spectrometer, *Rev. Sci. Instrum.* 57 (1986) 393–403.
- [21] A. Pines, M.G. Gibby, J.S. Waugh, Proton-enhanced NMR of dilute spins in solids, *J. Chem. Phys.* 59 (1973) 569–590.
- [22] S.R. Hartmann, E.L. Hahn, Nuclear double resonance in rotating frame, *Phys. Rev.* 128 (1962) 2042–2053.
- [23] I. Solomon, Etude de la relaxation en resonance magnetique par la methode de precession forcee transitoire, *Compt. Rend. Hebd. Acad. Sci.* 248 (1959) 92–94.
- [24] H.Y. Carr, E.M. Purcell, Effects of diffusion on free precession in nuclear magnetic resonance experiments, *Phys. Rev.* 94 (1954) 630–638.
- [25] S. Meiboom, D. Gill, Modified spin-echo method for measuring nuclear relaxation times, *Rev. Sci. Instrum.* 29 (1958) 688–691.
- [26] F.H. Larsen, H.J. Jakobsen, P.D. Ellis, N.C. Nielsen, Sensitivity-enhanced quadrupolar-echo NMR of half-integer quadrupolar nuclei. Magnitudes and relative orientation of chemical shielding and quadrupolar coupling tensors, *J. Phys. Chem. A* 101 (1997) 8597–8606.
- [27] L.R. Sarles, R.M. Cotts, Double nuclear magnetic resonance and the dipole interaction in solids, *Phys. Rev.* 111 (1958) 853–859.
- [28] M. Mehring, A. Pines, W.K. Rhim, J.S. Waugh, Spin-decoupling in resolution of chemical shifts in solids by pulsed NMR, *J. Chem. Phys.* 54 (1971) 3239–3240.
- [29] K.L. Ivanov, N.N. Lukzen, H.-M. Vieth, S. Grosse, A.V. Yurokovskaya, R.Z. Sagdeev, Investigation of the magnetic field dependence of CICNP in multinuclear radical pairs. 1. Photoreaction of Histidine and comparison of model calculation with experimental data, *Molec. Phys.* 100 (2002) 1197–1208.
- [30] D. Rovnyak, C. Filip, B. Itin, A.S. Stern, G. Wagner, R.G. Griffin, J.C. Hoch, Multiple quantum magic-angle spinning spectroscopy using nonlinear sampling, *J. Magn. Reson.* 161 (2002) 43–55.