

cularly polarized $n\pi^*$ in the $\parallel b$ spectrum.

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Zero-Field NMR of Small-Amplitude Motions in a Polycrystalline Solid

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Abstract: The librational motions of the water molecules in polycrystalline barium chlorate monohydrate have been studied by using proton and deuterium zero-field NMR. In contrast to high-field NMR, subtle molecular motions produce readily observable changes in the zero-field spectrum. Computer simulations and application of a novel-pulsed zero-field technique confirm that the splitting observed in the zero-field spectrum of the hydrate results from the motionally induced asymmetry of the magnetic dipole-dipole coupling tensor.

NMR has been an excellent tool for the study of motion in condensed matter since one observes a time average over the motion resulting in an average chemical shift, quadrupolar coupling, or dipolar interaction. Because powder patterns¹ are insensitive to the relatively small perturbations of these motions one often resorts to single-crystal measurements² or oriented liquid crystal measurements.³ Zero-field NMR offers an approach to this problem which is useful for polycrystalline or otherwise disordered materials since it has been demonstrated to provide sharp dipolar⁴⁻⁶ and quadrupolar information⁷⁻⁹ from such systems down to very low frequencies. Zero-field NMR should be sensitive to small amplitude motions which result in splittings or extra lines in the frequency spectrum. Such motions typically do not result in observable changes in the high-field NMR spectrum. In this report we present the first experimental results for the study of libration in a polycrystalline hydrate using proton and deuterium zero-field experiments.

The proton zero-field spectrum of a static water molecule would consist of lines at zero frequency and at $\pm\nu_d = 3\gamma^2 h/8\pi^2 r^3$, where r is the internuclear distance of the two protons.⁴ The characteristic motion of the waters in a typical hydrate are rapid 180° flips about their C_2 axes¹⁰ and librations about three axes.^{11,12} To a good approximation the librational modes correspond to rotations about the x , y , and z axes¹² of the molecular coordinate system shown in Figure 1 and are commonly referred to as rocking, waving, and twisting, respectively. The influence of the motion on the proton zero-field spectrum is treated by calculation of its effect on the dipolar Hamiltonian, \mathcal{H}_d , which is responsible for the zero-field spectrum. The rapid 180° flips have no effect since they merely exchange the two protons. Waving has no effect since it leaves the orientation of the internuclear vector r invariant. The dipolar Hamiltonian is therefore motionally averaged by only two of the librational modes. The resulting motionally averaged Hamiltonian, \mathcal{H}_d' , is given in the molecular frame by

$$\mathcal{H}_d' = \langle R_z(\theta_z)R_x(\theta_x)\mathcal{H}_dR_x(\theta_x)^{-1}R_z(\theta_z)^{-1} \rangle = \bar{I}_1(R_z(\theta_z)R_x(\theta_x)DR_x(\theta_x)^{-1}R_z(\theta_z)^{-1})\bar{I}_2 \equiv \bar{I}_1\bar{D}'\bar{I}_2 \quad (1)$$

where θ_x and θ_z are the librational angles about the x and z axes,

respectively, and the brackets signify a time average over the librational motion. To second order in the angles θ_i characterizing the libration, we can write the motionally averaged tensor, \mathbf{D}' , in angular frequency units as^{11,13}

$$\mathbf{D}' = d \begin{bmatrix} 1 - 3\langle\theta_z^2\rangle & 0 & 0 \\ 0 & -2 + 3\langle\theta_z^2\rangle + 3\langle\theta_x^2\rangle & 0 \\ 0 & 0 & 1 - 3\langle\theta_x^2\rangle \end{bmatrix} \quad (2)$$

where $d = \gamma^2 h/2\pi r^3$. Application of the rotations in the reverse order of eq 1 produces the same expression for \mathbf{D}' to this order of approximation. An unequal intensity in the amplitudes of the two librational modes produces a nonaxially symmetric average dipolar tensor. This is made more clear by defining $\Delta = \mathbf{D}'_{22}$ and $\eta = (\mathbf{D}'_{11} - \mathbf{D}'_{33})/\mathbf{D}'_{22}$ and rewriting eq 2 as

$$\mathbf{D}' = \begin{bmatrix} -\Delta(1-\eta)/2 & 0 & 0 \\ 0 & \Delta & 0 \\ 0 & 0 & -\Delta(1+\eta)/2 \end{bmatrix} \quad (3)$$

Calculation of the sudden experiment zero-field spectrum for this case proceeds in a manner analogous to that described pre-

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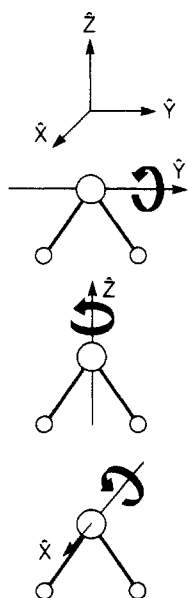


Figure 1. The three librational modes of the water molecules in barium chlorate monohydrate. In this molecular coordinate system the H_2O lies in the plane of the paper with its C_2 axis parallel to the z axis. From top to bottom these modes are referred to as waving, twisting, and rocking. Waving does not produce a reorientation of the internuclear vector, thus only twisting and rocking have an averaging effect on the dipolar tensor.

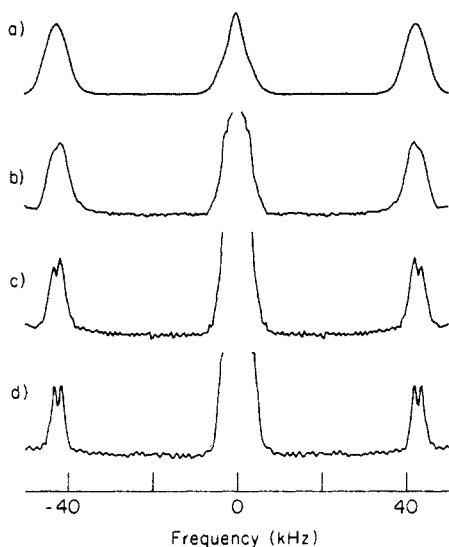


Figure 2. Proton sudden zero field spectra of barium chlorate monohydrate as a function of isotopic dilution by deuterium; (a) isotopic abundance, (b) 60% protons, (c) 31% protons, (d) 10% protons. Structure due to the asymmetric dipolar tensor of dilute water molecules is observed as the intermolecular contribution to the line width is reduced. Unpaired protons in the dilute samples contribute to the line centered at zero frequency.

viously.^{4,5} The normalized high-field signal expected for a powder sample is given by

$$S(t_1) = \cos \left\{ \frac{\Delta}{4} (3 + \eta) t_1 \right\} + \cos \left\{ \frac{\Delta}{4} (3 - \eta) t_1 \right\} + \cos \left\{ \frac{\Delta}{2} \eta t_1 \right\} \quad (4)$$

where t_1 is the evolution time in zero field. The effect of the motion is to split the lines of the static spectrum by an amount proportional to the asymmetry of the dipolar tensor. These motionally produced splittings or additional lines in the zero-field spectrum are in sharp contrast with the shoulders on broad powder patterns which occur in the high-field case.

The zero-field spectrum of a motionally averaged spin one nucleus follows from a treatment similar to that above. Explicit expressions for the dependence of the quadrupole coupling constants

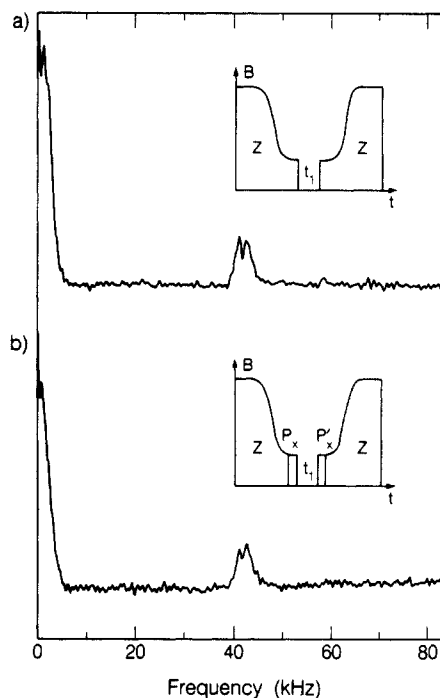


Figure 3. (a) Proton zero field spectrum of 90% deuterated $\text{Ba}(\text{ClO}_3)_2 \cdot \text{H}_2\text{O}$ obtained with the sudden experiment field cycle. The applied field, B_z , is shown schematically in the inset as a function of time. Zero-field evolution is initiated by sudden switch-off of the field. After the t_1 evolution period the field is switched on suddenly and the sample returned to high field where the magnetization M_z is measured as a function of t_1 . Here only the positive frequency portion of the spectrum is displayed. All three lines characteristic of the motionally averaged nonaxially symmetric dipolar tensor are resolved, appearing at 1.37, 41.8, and 43.4 kHz with line widths of approximately 2 kHz, considerably narrower than that obtained with the fully protonated material. (b) Zero-field spectrum from the sudden field cycle with dc pulses. This cycle, shown in the inset, is identical with the sudden experiment except for the application of 90° dc magnetic field pulses, P_x and P_x' , at the initiation and termination of the zero-field evolution period. These experiments employed a dc field of 0.010 T oriented orthogonal to B_0 . The spectrum obtained is essentially identical with that of the sudden experiment.

and asymmetry parameter on the librational amplitudes have been calculated.^{11,13} Both the quadrupole coupling constant and asymmetry parameter depend on all three librational modes as well as the exchange frequency characterizing the 180° flips. In barium chlorate at room temperature, however, the frequency is sufficiently high that one need only consider an average over the two orientations.¹⁰ The 180° flips average the static quadrupole tensor, which has its principal axis along the O-D bond, to one with its principal component either along the C_2 axis or perpendicular to the molecular plane of the water molecule.¹⁴ The asymmetry parameter is also effected; its value near unity is a consequence of the motion.¹⁵ One notes however that librational amplitudes are a function of the reduced mass of the molecule, hence the amplitudes and NQR frequencies will differ slightly in HDO and D_2O .

Zero-Field Experiments

Proton Zero-Field Spectra. All spectra reported here were obtained at room temperature by using a homebuilt 180 MHz proton frequency instrument that has been modified for the zero-field experiments.¹⁶

The proton zero-field spectrum of isotopic abundance barium chlorate has been published before.⁴ Intermolecular dipolar couplings produce line widths of approximately 7 kHz thus ob-

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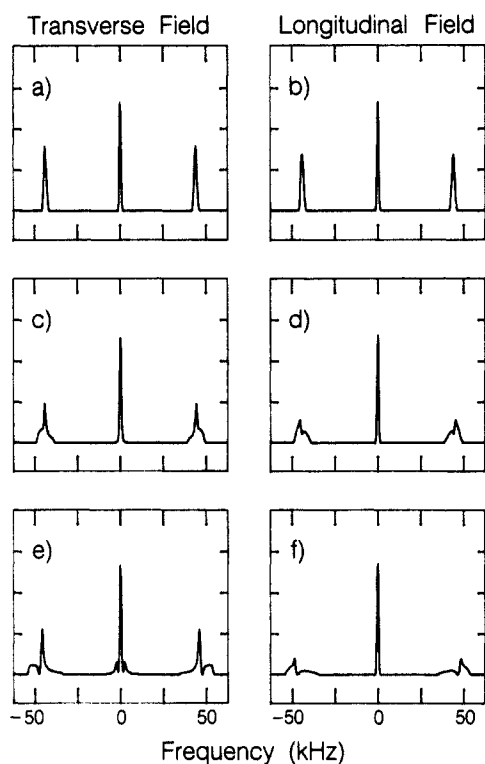


Figure 4. Simulations of zero-field spectra were obtained by using the sudden field cycle in the presence of variable residual fields for a powder sample of isolated pairs of protons where $r = 1.6 \text{ \AA}$. The sudden experiment utilizes a field along the z direction which is suddenly switched off to initiate evolution. Simulations on the right correspond to the residual field aligned along the z axis and those on the left the x axis of the lab frame. Magnitudes of the residual fields used in the simulations from top to bottom are as follows: (a,b) 0.35 G; (c,d) 1.2 G; and (e,f) 2.4 G. Additional simulations for general orientations of the residual field in the x - z plane are in qualitative agreement with those shown. In all cases quite large residual fields are required to produce splittings in the spectrum.

scuring the splitting due to the motion. The effect of isotopic dilution by deuterium on the line width of the proton zero-field spectrum is shown for a series of dilution levels in Figure 2. An increase in the amount of structure in the spectrum is seen as the level of protonation decreases. The spectrum from a 10% protonated sample, Figure 3, shows all three lines predicted by eq 4 for the asymmetric dipolar tensor.

By combining eq 2 through 4 one can use the experimental splittings to obtain the difference $\langle \theta_z^2 \rangle - \langle \theta_x^2 \rangle \simeq 0.024$. Using $r = 1.52 \text{ \AA}$, a value obtained from neutron diffraction measurements,¹⁷ one can calculate $\langle \theta_x^2 \rangle = 0.044$ and $\langle \theta_z^2 \rangle = 0.070 \text{ (rad}^2\text{)}$. Ideally for the zero-field calculations one would like to use the value r' given by $r' = \langle 1/r^3 \rangle^{-1/3}$ where the brackets signify averaging over the librational and vibrational modes. A precise determination of this quantity requires a precise knowledge of the molecular potential. In the absence of this information the neutron diffraction data seem reasonable, however, as Pedersen's¹⁸ calculations have found the internuclear distances, r_e , varying from 1.52 to 1.55 \AA and $\langle 1/r^3 \rangle = 0.98(1/r_e^3)$ which is a rather negligible difference. A detailed treatment of this subject is beyond the scope of this paper; however, it is clear that corrections due to differences in $\langle 1/r^3 \rangle$, $1/r_e^3$, and $\langle 1/r \rangle^3$ will have little effect on the calculated $\langle \theta^2 \rangle$'s.

A second experiment was performed to determine if the observed splittings could be due to residual magnetic fields present during the zero-field evolution period. The field cycle is shown in the inset of Figure 3b. In this experiment a dc pulse calibrated to rotate the initial magnetization from the z axis to the x - y plane⁸

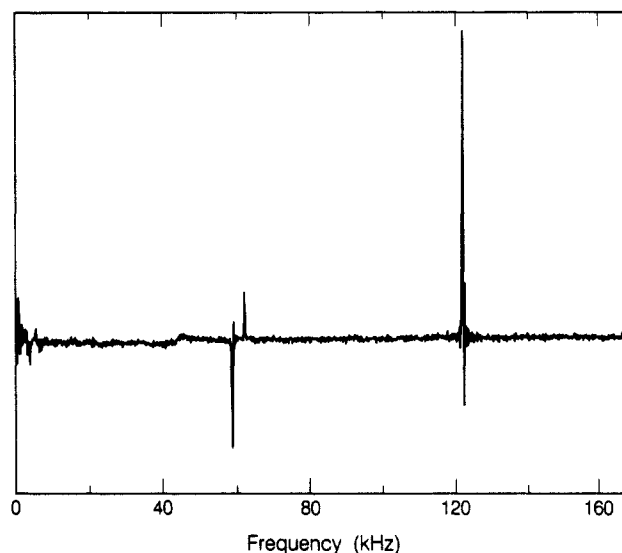


Figure 5. Indirect detection zero field deuterium NQR spectrum of 50% deuterated barium chlorate monohydrate: all three lines expected are resolved from which one calculates $e^2qQ/h = 122.7 \text{ kHz}$ and $\eta = 0.96$ in reasonable agreement with single crystal measurements of the perdeuterated material which obtained $e^2qQ/h = 121.5 \pm 0.4 \text{ kHz}$ and $\eta = 0.976 \pm 0.007$.¹¹ The intensities and phases of the peaks are a complicated function of the level crossing dynamics, initial zero-field condition, and dc pulse angles and are not completely understood. In all cases encountered thus far, the frequencies of the sudden transition experiments (which are well understood^{5,8}) and dc pulsed experiments are identical. The bump at approximately 40 kHz is due to proton pairs. Its small relative size gives an indication of the deuterium selectivity of the indirect experiment.

was given immediately after the sudden switch-off of the intermediate field. In addition, an identical dc pulse was given at the conclusion of the t_1 period to store the effect of the zero-field evolution. This sequence being identical with the sudden experiment in every other detail has the effect of simply changing the relative orientation of the stray field with the initial condition of the magnetization. The spectrum obtained with this sequence, Figure 3b, is essentially identical with that of the sudden experiment.

Effect of Stray Fields. Computer simulations of the zero-field spectrum of a powder distribution of static pairs of protons in a stray nonzero field were performed as a means of further probing the effects of stray fields. The simulations assumed an internuclear distance of 1.6 \AA and stray magnetic fields of varying strengths and directions. In the sudden experiment the magnetization at $t_1 = 0$ is along the lab z direction. The symmetry of a powder then requires only examination of stray fields with components along the z axis and a perpendicular axis which we define as the x axis. The results of these simulations, some of which are shown in Figure 4, bear little or no resemblance to the experimental spectra and indicate that residual fields $>1 \text{ G}$ are required to produce splittings comparable to those seen in Figure 1. Experimental measurements typically place an upper limit of 0.025 G on the magnitude of the stray field.

Deuterium Spectra. Although the rapid C_2 flips do not manifest themselves in the proton spectrum, they are readily observable via their effect on the deuterium quadrupole spectrum.^{11,14,15} The deuterium zero-field NQR spectrum of a 50% deuterated sample of barium chlorate was obtained at room temperature by using the indirect detection method which is described in detail elsewhere.⁸ Since room temperature deuterium low field T_1 's are of the order of ms, an indirect detection method is necessary to observe those deuterons in the HDO molecules. The indirect method is selective for the deuterons in that ideally only they are induced to evolve during the zero-field evolution period and hence little or no signal is observed due to remaining proton pairs. In the spectrum, shown in Figure 5, the ν_+ , ν_- , and ν_0 lines are all clearly resolved, and from their frequencies one calculates $e^2qQ/h = 122.7 \text{ kHz}$ and $\eta = 0.960$ which is in good agreement with

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earlier work.¹¹ Combining the zero-field proton and deuterium data with the quadrupole coupling constants of the static molecule found by Chiba,¹¹ one can calculate $\langle \theta_y^2 \rangle$ for the H₂O molecule. In brief this is done by (1) calculating $\langle \theta_x^2 \rangle_{\text{HDO}}$ and $\langle \theta_z^2 \rangle_{\text{HDO}}$ using the formulas in reference 18 to correct for the reduced masses, (2) using these expressions to calculate $\langle \theta_y^2 \rangle$ from the zero-field HDO data, and (3) calculating $\langle \theta_y^2 \rangle_{\text{H}_2\text{O}}$ by the reverse procedure in step 1. Using the explicit expressions for the field gradient tensor averaged by libration and the C₂ flipping, one obtains $\langle \theta_y^2 \rangle = 0.123(\text{rad}^2)$. We note the librations have a relatively minor effect on the quadrupole spectrum, and the value of η near unity is primarily a consequence of the C₂ flips.¹⁵ An advantage of the dipolar measurements is that the static dipole interaction is inherently axially symmetric and any asymmetry is the direct result of motion.

Discussion

The nonaxially symmetric dipolar tensor produced by libration is readily observable via the proton zero-field spectrum. The excellent agreement between the results of the two versions of the zero-field experiment as well as the results of computer simulations rules out the possibility of splittings due to residual fields. Our results for the mean square amplitudes of the librational modes are in fair agreement with earlier data,^{11,18} especially when one considers that the exact librational modes might differ slightly from the inertial rotations assumed.¹² With the high resolution possible in the dipolar zero-field experiment one has a sensitive measure of relatively small changes in the dipolar tensor.

The zero-field NQR results for HDO demonstrate the high resolution of the experiment and the precision with which it can measure the asymmetry parameter. The parameters relating to

the motion are underdetermined with a single NQR experiment since the quadrupolar frequencies are a function of the three librational modes, the rate of the 180° flips, as well as the values of $(e^2qQ/h)_0$ and η_0 , the parameters of the static molecule. We note that room temperature deuterium NQR measurements of a hydrate are usually inaccessible to frequency domain techniques because of their relatively short T_1 's and low quadrupolar frequencies. In contrast to typical frequency domain NQR methods there is no power broadening¹³ and ideally no signal due to protons. This fact generally allows resolution of the ν_0 lines, a tremendous aid in the assignment of the spectra.

In summary the zero-field experiment has been demonstrated to provide information about the dipolar and electric field gradient tensors in a two spin system and thus provide information about its motional characteristics. In general these should provide complementary information since they possess unique principal axis systems and hence are affected differently by the different motions which occur in a system. The zero-field measurements have the significant advantage of being made with a powder sample whereas the earlier measurements required a single crystal.¹¹ This aspect should allow study of subtle motions in systems inaccessible to single crystal measurements including amorphous and polycrystalline materials as well as biological samples.

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Deuterium Transfer in the Bioconversion of Glucose to Ethanol Studied by Specific Isotope Labeling at the Natural Abundance Level

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Abstract: Site-specific natural isotope fractionation measured by deuterium NMR (SNIF-NMR) was used for investigating the deuterium transfers occurring in the fermentation of sugars into ethanol. In contrast to carbon-13, which is usually assumed to be randomly distributed within the glucose skeleton, very large deviations with respect to a statistical repartition are determined for deuterium. By transforming glucose samples from different origins into acetates and nitrates, the absolute values of the D/H ratios in the nonexchangeable sites were obtained. The hydroxyl sites were considered to contribute to the isotope content of the starting water medium. The site-specific isotope parameters were determined for more than 50 ethanol samples obtained by fermenting glucose samples issued from different starches and sucroses in water media with different isotope ratios. Several concentrations of sugar, several strains of the same yeast, and different temperatures of fermentation were considered. The whole set of isotope parameters characterizing the nonexchangeable sites of glucose, the starting and end water media, and the methyl and methylene sites of ethanol was submitted to factor analysis. The results are satisfactorily reproduced on the basis of two main factors which may be associated with the independent contributions of the isotope ratios of glucose and water. The isotope parameters of the end products and of the starting materials were then considered to be related by a set of linear equations and the coefficients of the redistribution matrix were calculated by multidimensional analysis. Thus the isotope ratio of the methyl site of ethanol exhibits a strong sensitivity toward the nonexchangeable sites of glucose and depends to a lesser extent on the isotope content of the starting water medium. By contrast, no direct connection is found between glucose and the methylene site which is only sensitive, with a strong discriminating effect against deuterium, to the isotope content of water. A redistribution coefficient slightly less than unity (0.96 for a concentration of sugar of 100 g L⁻¹) is found between the isotope ratio of the end and starting water media. Although the bioconversion of glucose into ethanol occurs with high fractionation effects which are the source of high deuterium depletions in the methyl and methylene sites of ethanol as compared to glucose, constant redistribution parameters can be obtained in appropriate conditions and the isotope parameters measured in alcohols may be used as a fingerprint for characterizing the sugar and aqueous juice from which they have been produced. The site-specific natural isotope parameters of ethanol therefore constitute a faithful and powerful probe for investigating the physiological biochemical and climatological effects which have governed the photosynthesis of sugars in natural conditions.

The first issue of this journal, which appeared in 1879, began with a paper by D. P. Ricketts¹ which was devoted to the char-

acterization of sugars from different origins. More than a hundred years later, the problem is still a challenge since the distinction