

# High Magnetic Field Consequences on the NMR Hyperfine Shifts in Solution

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**Pseudocontact shifts arise from the isotropic reorientational average of the dipolar coupling between unpaired electron and nuclei, in the presence of magnetic susceptibility anisotropy. The effect of residual orientation due to high magnetic fields on pseudocontact shifts is evaluated here. The effect is found to be smaller and of opposite sign with respect to another novel effect of high magnetic fields on hyperfine shifts due to saturation of the electron spin magnetic moment as described by the Brillouin equation.** © 1998 Academic Press

As higher and higher magnetic fields have become available, partial orientation of molecules in a magnetic field has been detected through NMR, from noncomplete averaging to zero of quadrupolar and dipolar interactions, both in diamagnetic (1–8) and paramagnetic (9, 10) small molecules. Recently, because of their potential in containing structural information, residual dipolar couplings in solution have attracted the attention of several researchers, leading to the accurate measurement of a magnetic field dependence of the  $^{15}\text{N}$ – $^1\text{H}$  splitting in diamagnetic (11) and paramagnetic (12, 13) proteins and to their use in structure determination (14–16). Also, the chemical shift in solution, being the average of a tensorial interaction, should be sensitive to partial orientation of a molecule in solution, and, although predicted (8), a magnetic field dependence of the chemical shift has only very recently (17) been detected on a diamagnetic protein. The effects observed are of the order of parts per billion. Paramagnetic compounds with large magnetic anisotropy are expected to give rise to a magnetic field dependence of hyperfine shifts, which should be much larger than parts per billion. The hyperfine shift is due to the coupling between the unpaired electrons and the resonating nuclei (see Appendix). We report here an investigation of the effect of the magnetic field on the hyperfine shift experienced by paramagnetic molecules with high magnetic anisotropy. We are going to show that indeed there is an orientation effect on the dipolar contribution to the hyper-

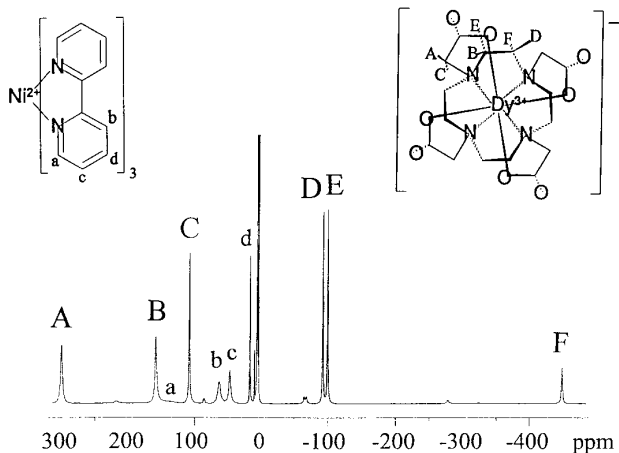
fine shift which, however, is cancelled or overwhelmed by an effect of spin saturation which is known in molecular magnetism.

We have investigated the magnetic field dependence (4.7, 11.7, 18.8 T, corresponding to a proton resonance frequency of 200, 500, 800 MHz) of the hyperfine shifts of a molecule containing the  $\text{Dy}^{3+}$  ion ( $\text{DyDOTA}^-$  (18)), which has a very large magnetic anisotropy (19), and compared it to that of  $\text{Ni}(\text{bipy})_3^{2+}$ , which is expected to be essentially isotropic (20) (small spin–orbit coupling parameter  $\lambda$  and orbitally nondegenerate ground state). The shifts of the dysprosium complex are mainly pseudocontact in origin, with  $\chi_{\parallel}$  smaller than  $\chi_{\perp}$  (19), those of the nickel complex are basically only contact in origin (20). A schematic picture of the complexes and of their NMR spectrum is shown in Fig. 1. The temperature control units of the various instruments were accurately calibrated using a standard ethylene glycol “NMR thermometer” (21). As an internal reference, *tert*-butyl alcohol was used. Differences in hyperfine shifts with field as large as 6 ppm are indeed observed.

The hyperfine shift data at the 500 and 800 MHz fields are reported in Fig. 2 as a function of temperature (the shifts at 200 MHz are affected by an error comparable to the difference in shift). A field effect is clearly present for  $\text{DyDOTA}^-$  and absent, or small below detection, for  $\text{Ni}(\text{bipy})_3^{2+}$ . The effect on  $\text{DyDOTA}^-$  signals is proportional to the magnitude of the hyperfine shift, as shown in the inset of Fig. 2. The hyperfine shifts *decrease* in absolute value with increasing field.

The pseudocontact shifts are the result of an average of the dipolar interaction between the electrons and the nucleus over all the possible orientations (22–24), and therefore they should also be able to monitor the presence of a partial alignment of the molecule in a magnetic field. We present here the proper equation for an axial system, which is appropriate for  $\text{DyDOTA}^-$ , derived by evaluating the expectation value of the pseudocontact shift weighted by an orientational population function that in turn depends on the magnetic anisotropy of the molecule:

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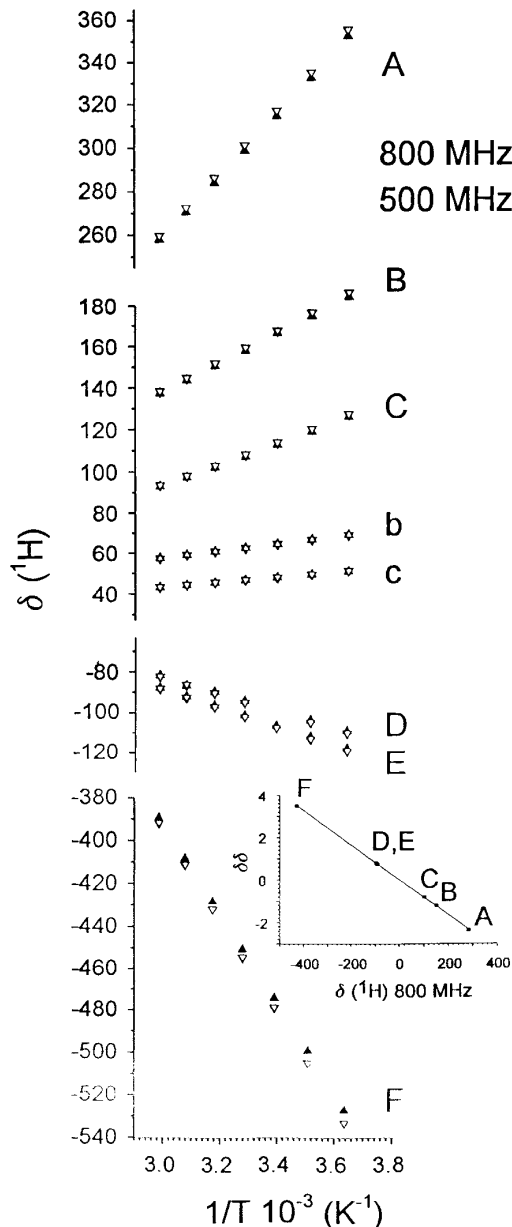
**FIG. 1.** 200 MHz  $^1\text{H}$  NMR spectrum of  $\text{DyDOTA}^-$  in water solution at 303 K. The sample also contains  $\text{Ni}(\text{bipy})_3^{2+}$ . The schematic structures of the two complexes and the signal assignment are also shown (18, 29). The signals of fractional intensity arise from a less abundant diastereoisomer of  $\text{DyDOTA}^-$  (18).

$$\delta^{pc,or} = \frac{1}{12\pi r^3} (\chi_{\parallel} - \chi_{\perp}) (3 \cos^2\theta - 1) \times \left\{ 1 + \frac{B_0^2}{15\mu_0 kT} (2\chi_{\parallel} + \chi_{\perp}) \right\} \quad [1]$$

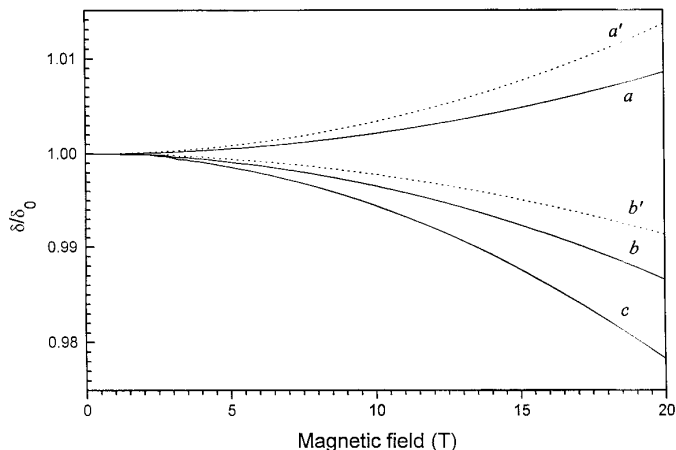
The derivation of this equation is outlined in the appendix. The symbols have the following meaning:  $r$  is the metal–nucleus distance,  $\chi_{\parallel}$  and  $\chi_{\perp}$  are the parallel and orthogonal components of the magnetic susceptibility tensor  $\chi$ ,  $\theta$  is the angle between the metal–nucleus vector and the principal axis of  $\chi$ ,  $B_0$  is the external magnetic field,  $\mu_0$  is the magnetic permeability of a vacuum,  $k$  the Boltzmann constant, and  $T$  the absolute temperature. The term in curly brackets is the orientation-dependent term. Note that this term does not correspond to the “degree of magnetic alignment” term that describes orientation effects on  $J$  couplings and diamagnetic shifts (1–14, 17). For paramagnetic systems with relatively high magnetic anisotropy and with the current high fields available, the term in curly brackets should be measurably larger than 1 (up to a few percent for a high magnetic susceptibility metal ion such as  $\text{Dy}^{3+}$ ). However, contrary to observation, this equation predicts an *increase* in the absolute value of the hyperfine shift with increasing magnetic field.

An implicit assumption in the derivation of all equations for the hyperfine shift is that the electron Zeeman energy is negligible with respect to  $kT$ . In this limit, the difference in population of the Zeeman levels, and therefore of the sample magnetization,  $M$ , increases linearly with field. As a consequence, the magnetic susceptibility  $\chi = M/B_0$  is field-independent. For the 18.8 T field of the 800 MHz spectrometer, the electron Zeeman energy is about  $20 \text{ cm}^{-1}$ , that is, one-tenth of  $kT$  at room temperature. Furthermore, for a system with  $n$

unpaired electrons, the overall splitting of the Zeeman levels amounts to  $n$  times the Zeeman energy. When the Zeeman splitting is not negligible with respect to  $kT$ , differential population of the levels according to Boltzmann law makes the increase in magnetization of the sample with field less pronounced, and the magnetic susceptibility decreases. This phe-



**FIG. 2.** Temperature dependence of the chemical shifts of the hyperfine shifted  $^1\text{H}$  NMR signals of  $\text{DyDOTA}^-$  (A–F) and  $\text{Ni}(\text{bipy})_3^{2+}$  (b, c) at 500 (open symbols) and 800 (filled symbols) MHz. The assignment is shown in Fig. 1. The inset shows the linear relationship between the difference in chemical shift  $\delta\delta = (\delta(^1\text{H}, 800 \text{ MHz}) - \delta(^1\text{H}, 500 \text{ MHz}))$  and the shifts of the  $\text{DyDOTA}^-$  signals at 315 K. Similar plots are obtained at all temperatures.



**FIG. 3.** Sample calculations of the relative changes in hyperfine shifts ( $\delta/\delta_0$ ) with field due to orientation and saturation effects. The parameters used for the calculations were  $S = 1/2$ ,  $L = 5$ , and an anisotropy comparable to that observed for the DyDOTA<sup>-</sup> complex. Dashed curves represent the case where  $\chi_{\parallel} > \chi_{\perp}$ , whereas continuous lines are calculated with the same parameters but with  $\chi_{\parallel} < \chi_{\perp}$  as in the case of the DyDOTA<sup>-</sup> complex. Curves  $a$  and  $a'$  represent a pure orientation effect and are calculated using Eq. [1] and field-independent  $\chi$  values. Pure saturation effects are shown by curve  $c$ , which is calculated inserting into the equation for pseudocontact shifts (16) the  $\chi$  values given by the Brillouin equation (Eq. [2]); this is the same for the two cases of  $\chi_{\parallel} > \chi_{\perp}$  and  $\chi_{\parallel} < \chi_{\perp}$  and the same behavior should also be followed by contact shift (16). Curves  $b$  and  $b'$  represent the simultaneous effect of orientation and saturation and are calculated by inserting into Eq. [1] the  $\chi$  values given by the Brillouin equation (Eq. [2]). The size of the effect depends on the relative values of  $\chi_{\parallel}$ ,  $\chi_{\perp}$ , and  $\chi$ .

nomenon, named saturation, is described by the well-known Brillouin equation (25, 26):

$$\chi = \mu_0 \frac{g_e \mu_B}{2B_0} \left[ (2S + 1) \coth\left(\frac{2S + 1}{2S} y\right) - \coth\left(\frac{1}{2S} y\right) \right], \quad [2]$$

where  $y = g_e \mu_B B_0 S / kT$ . Saturation would thus predict a *decrease* in the absolute value of both contact and pseudocontact shifts with increasing field, which would be in agreement with the experimental finding. Figure 3 shows the expected field dependence of the pseudocontact shift calculated according to orientation effects alone (curve  $a$ ,  $a'$ , Eq. [1]), saturation effects alone on pseudocontact shifts (curve  $c$ ,  $\chi$  values calculated through Eq. [2] and the equation for pseudocontact shifts, see Appendix), and orientation and saturation effects simultaneously present (curve  $b$ ,  $b'$ ,  $\chi$  values calculated through Eq. [2] and inserted into Eq. [1]). Orientation and saturation play opposite roles, and saturation effects appear to overwhelm those arising from orientation of the molecule in high magnetic fields. In any case, Eq. [1] should be of general validity provided that it incorporates the appropriate field dependence of magnetic susceptibility. In this respect, we note that saturation effects

may be stronger and may affect  $\chi_{\parallel}$  and  $\chi_{\perp}$  to a different extent when zero field splitting (ZFS) is present, as it is the case for lanthanides. Indeed, ZFS contributes sizably to the spreading of the electron Zeeman levels, and therefore increases saturation. However, this does not affect the qualitative behavior presented in Fig. 3.

The phenomenon of saturation derived from Eq. [2] occurs also for an isotropic system. Therefore, a saturation effect is also to be expected for contact shifts. For the Ni(bipy)<sub>3</sub><sup>2+</sup> complex the effect is predicted to be of the order of 0.1% for an increase in field from 500 to 800 MHz, i.e., too small to be detected outside the uncertainty on the hyperfine shift reading because of the relatively large linewidths of the Ni(bipy)<sub>3</sub><sup>2+</sup> signals.

## CONCLUDING REMARKS

We have shown that magnetic field effects on hyperfine shifts can be measured and understood in terms of (i) partial orientation of the molecule in the external magnetic field and (ii) saturation effects. We have provided an equation (Eq. [1]) for axially anisotropic systems that adequately describes the pseudocontact shift including the effects of orientation and is of general validity. This effect at 18.8 T (800 MHz for <sup>1</sup>H Larmor frequency) may be as large as a few percent of the total pseudocontact shift. The effect of saturation is of opposite sign and is in general larger, especially if there is ZFS. Under the present  $B_0$  values, the pseudocontact shift can still be safely used as structural constraint for solution structure determination as the corresponding field effect is presently well below the tolerance dictated by other sources of uncertainty (27–29). Contact shifts are also predicted to be field dependent due to saturation effects, according to Eq. [2].

## APPENDIX

There is a contact contribution to the hyperfine shift due to the presence of unpaired electron density on the resonating nucleus. In the case of a single  $S$  multiplet interacting with nuclei, the contact shift is expressed in terms of the principal components of the  $\chi$  and  $g$  tensors and in a more approximate form, in terms of  $S(S + 1)$  (22, 30),

$$\begin{aligned} \delta^{con} &= \frac{1}{\mu_0} \frac{A}{\hbar} \frac{1}{3\gamma_I \mu_B} \left( \frac{\chi_{xx}}{g_{xx}} + \frac{\chi_{yy}}{g_{yy}} + \frac{\chi_{zz}}{g_{zz}} \right) \\ &\cong \frac{1}{\mu_0} \frac{A}{\hbar} \frac{\chi}{\gamma_I g_e \mu_B} \\ &= \frac{A}{\hbar} \frac{g_e \mu_B S(S + 1)}{3\gamma_I kT}, \end{aligned} \quad [A1]$$

where  $\mu_0$  is the magnetic permeability of a vacuum,  $A/\hbar$  is the Fermi contact coupling constant,  $\chi$  is the magnetic suscepti-

bility,  $\gamma_I$  is the nuclear magnetogyric ratio,  $g_e$  is the electron Landé factor,  $\mu_B$  the electron Bohr magneton,  $k$  the Boltzmann constant, and  $T$  the absolute temperature.

Then there is a pseudocontact contribution (22, 31, 32) to the hyperfine shift that is due to the dipolar coupling between the electron and the resonating nucleus, and is different from zero when there is magnetic susceptibility anisotropy. For axial symmetry, the equation is

$$\delta^{pc} = \frac{1}{12\pi} \frac{1}{r^3} (\chi_{\parallel} - \chi_{\perp})(3 \cos^2\theta - 1), \quad [\text{A2}]$$

where the vector connecting the metal center and the nucleus has module  $r$  and forms an angle  $\theta$  with the principal axis (which is also the axis of symmetry) of the  $\chi$  tensor.

We derive hereafter the equation to evaluate the additional contribution to the pseudocontact shift arising from partial orientation in a high magnetic field of a molecule characterized by an anisotropic magnetic susceptibility tensor with axial symmetry.

The dipolar interaction between a nuclear spin and an electronic spin gives rise, in the solid state, to a contribution to the nuclear shift given by

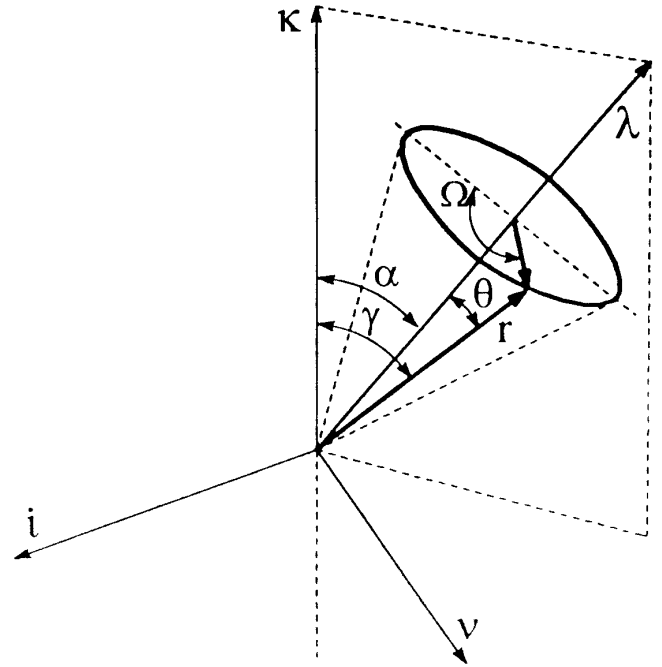
$$\begin{aligned} \delta^{dip} = \frac{1}{4\pi r^3} \left[ \chi_{\parallel} \cos^2\alpha (3 \cos^2\theta - 1) \right. \\ \left. + \chi_{\perp} \sin^2\alpha (3 \sin^2\theta \cos^2\Omega - 1) \right. \\ \left. + \frac{3}{4} (\chi_{\parallel} + \chi_{\perp}) \sin 2\alpha \sin 2\theta \cos \Omega \right], \quad [\text{A3}] \end{aligned}$$

where  $\chi_{\parallel}$  and  $\chi_{\perp}$  are the two components of the axially symmetric magnetic susceptibility tensor  $\chi$  and, as shown in Fig. 4,  $r$  is the electron–nucleus distance,  $\theta$  and  $\Omega$  define the position of the electron–nucleus vector in the system of principal axis of the magnetic susceptibility tensor, and  $\alpha$  is the angle between the direction of the principal axis of the magnetic susceptibility tensor and the external magnetic field axis. The familiar equation for pseudocontact shifts in solution (Eq. [A2]) can be derived by taking the rotational average of Eq. [A3] by integrating in  $d\alpha$  and  $d\Omega$ .

If the molecule assumes preferential orientations with respect to the static magnetic field, then the pseudocontact shift should be evaluated by weighting each orientation according to its population. Calling  $E(\alpha)$  the energy of a specific orientation, and assuming a Boltzmann distribution, we obtain:

$$\delta^{pc,or} = \frac{\int_0^{2\pi} \int_0^{\pi} \delta^{dip} e^{-\frac{E(\alpha)}{kT}} \sin \alpha \, d\alpha \, d\Omega}{\int_0^{2\pi} \int_0^{\pi} e^{-\frac{E(\alpha)}{kT}} \sin \alpha \, d\alpha \, d\Omega}. \quad [\text{A4}]$$

One of the possible factors inducing a preferential orienta-



**FIG. 4.** A molecule possessing magnetic anisotropy, with the  $z$  axis oriented along a generic  $\lambda$  direction.  $\alpha$  is the angle of the  $z$  axis with the external field direction  $k$ ;  $\theta$  is the angle between the metal–nucleus vector  $r$  and the  $z$  axis;  $\gamma$  is the angle between the metal–nucleus vector and the external field;  $\Omega$  defines the position of  $r$  on the surface of the cone about  $\lambda$ .

tion of the molecule in a high magnetic field is the anisotropy of its magnetic susceptibility tensor. Indeed, the energy of a molecule is

$$E(\alpha) = -\frac{B \cdot \chi \cdot B}{2\mu_0}, \quad [\text{A5}]$$

which depends on  $\alpha$  through

$$E(\alpha) = -\frac{\chi_{\perp} B^2}{2\mu_0} - \frac{(\chi_{\parallel} - \chi_{\perp}) B^2 \cos^2\alpha}{2\mu_0}. \quad [\text{A6}]$$

The constant part of the interaction energy  $E(\alpha)$  can be factored out and eliminated, and if

$$\frac{(\chi_{\parallel} - \chi_{\perp}) B^2 \cos^2\alpha}{2\mu_0 kT} \ll 1$$

in a first-order approximation, we obtain

$$\delta^{pc,or} = \frac{\int_0^{2\pi} \int_0^\pi \delta^{dip} \left( 1 + \frac{(\chi_{\parallel} - \chi_{\perp}) B^2 \cos^2 \alpha}{2\mu_0 kT} \right) \sin \alpha \, d\alpha \, d\Omega}{\int_0^{2\pi} \int_0^\pi \left( 1 + \frac{(\chi_{\parallel} - \chi_{\perp}) B^2 \cos^2 \alpha}{2\mu_0 kT} \right) \sin \alpha \, d\alpha \, d\Omega}. \quad [A7]$$

Indicating  $(\chi_{\parallel} - \chi_{\perp}) B^2 / 2\mu_0 kT = a$ , we obtain

$$\delta^{pc,or} = \frac{1}{4\pi r^3} (3 \cos^2 \theta - 1) \times \frac{\left[ -\frac{2}{3} (\chi_{\parallel} - \chi_{\perp}) + a \left( -\frac{2}{5} \chi_{\parallel} + \frac{4}{15} \chi_{\perp} \right) \right]}{-2 - \frac{2a}{3}}. \quad [A8]$$

Again, in a first-order approximation, this yields,

$$\delta^{pc,or} = \frac{1}{12\pi r^3} (3 \cos^2 \theta - 1) (\chi_{\parallel} - \chi_{\perp}) \times \left[ 1 + \frac{1}{15} \frac{B^2}{\mu_0 kT} (2\chi_{\parallel} + \chi_{\perp}) \right], \quad [A9]$$

which is the final equation for pseudocontact shifts including orientation effects.

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