THE EFFECT OF PARTIAL ORIENTATION: RESIDUAL DIPOLAR COUPLINGS

Sharp proton NMR lines are associated to short electron relaxation times, which in turn depend on the availability of low lying excited states (Chapter 4). Such systems also provide magnetic susceptibility anisotropy and pseudocontact shifts (Chapter 2). Magnetic anisotropy induces partial orientation (self-orientation) in high magnetic fields. Under these circumstances, the dipolar coupling energies average to values different from zero and residual dipolar couplings arise. Hyperfine shifts are also expected to change under partial orientation. The correction of the pseudocontact shifts in system partially oriented due to magnetic susceptibility anisotropy is negligible with respect to the isotropic values, but may become detectable at the highest magnetic fields available today.

3.1 PARTIAL ORIENTATION EFFECTS ON NUCLEUS-NUCLEUS DIPOLAR COUPLING

The network of the interactions among nuclear magnetic moments, between magnetic moments of nuclei and unpaired electrons, and between nuclear magnetic moments and the field generated by electron currents in orbitals, is responsible for the prohibitively broad NMR spectra of static samples in the solid state (Chapter 5). In solution, the fast isotropic reorientations of molecules average out the contributions corresponding to different nuclear positions with respect to the external magnetic field. This allows for a substantial simplification of the NMR spectrum, leaving only sharp lines. In diamagnetic systems the latter are centered at an average value of the chemical shielding interactions; in paramagnetic systems they are shifted with respect to this average value by the pseudocontact shift described in Chapter 2 (Table 3.1). In both cases they are split by the scalar coupling with covalently bound spins ($J$-splitting).

This simplification of the spectra implies a substantial loss of relevant structural and dynamical information. This information can be partially recovered by making anisotropic the distribution of the molecular orientations. Anisotropic reorientations can be achieved either when molecules interact with strongly aligned objects or when the molecules themselves have some preferred orientations in a high magnetic field because their magnetic susceptibility is anisotropic. In the latter case, partial self-alignment of the molecules occurs. Many structured nucleic acid fragments have this feature, as well as several molecules containing paramagnetic metal ions.

Partial alignment, either due to external media or to self-alignment, reintroduces some effects related to the anisotropy of the nuclear interactions (Table 3.1). The most widely known and exploited effect is the so-called residual dipolar coupling, measured as a perturbation to the $J$-splitting (Section 12.4) observed for alignments as small as 1/1000, with respect to the value measured for unaligned sample (Fig. 3.1). Another effect related to paramagnetism is the residual dipolar shift, discussed in Section 3.5.
Figure 3.1 Coupled peaks in $^1$H-$^1$N IPAP HSQC experiments for (A) a diamagnetic sample and (B) a paramagnetic sample with sizable magnetic susceptibility anisotropy. The difference in the frequency distance between the two peaks is due to the (positive) residual dipolar coupling; the mean of the peak frequencies is shifted due to the pseudocontact shift.

**Table 3.1 Summary of Phenomena Involving Nuclear Spins and Their Manifestations in Isotropic and Anisotropic Solutions**

<table>
<thead>
<tr>
<th>Effect</th>
<th>Due to Interaction of the Nuclear Spin with</th>
<th>Isotropic Average</th>
<th>Result in the Presence of Partial Alignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical shielding</td>
<td>Electron currents in orbitals</td>
<td>Isotropic chemical shift</td>
<td>Residual chemical shift anisotropy [1]</td>
</tr>
<tr>
<td>Dipolar shielding</td>
<td>Average electron magnetic moment</td>
<td>Pseudocontact shift</td>
<td>Residual dipolar shift [2]</td>
</tr>
<tr>
<td>Quadrupolar coupling</td>
<td>Electric field gradients</td>
<td>(average to zero)</td>
<td>Residual quadrupolar coupling [3]</td>
</tr>
<tr>
<td>Dipole–dipole coupling</td>
<td>Magnetic moments of the neighboring nuclei</td>
<td>(average to zero)</td>
<td>Residual dipolar coupling [4]</td>
</tr>
</tbody>
</table>
Partial alignment of a molecule causes the occurrence of residual dipolar couplings because when not all orientations have the same probability, the dipole-dipole interaction between nuclear magnetic moments does not average to zero. From Eqs. (1.1), (1.4), and $\mu = h \gamma I$, the residual dipolar coupling for the coupled nuclei A and B, at distance $r_{AB}$, is (Fig. 3.2)

$$
\Delta \nu^{\text{rdc}} = \frac{< E_{\text{dip}} >}{h} = -\frac{\mu_0 h \gamma_A \gamma_B}{8\pi^2 r_{AB}^3} \langle 3\cos^2 \gamma - 1 \rangle 
$$

(3.1)

where $\gamma$ is the angle between the direction of $B_0$ and that of the AB vector (i.e., of the vector connecting the coupled nuclei A and B). The equation describing the residual dipolar couplings can be written as a function of the Saupe tensor (or order tensor) $S$ [5], which describes the degree of alignment of a molecule [2],

$$
\Delta \nu^{\text{rdc}} = -\frac{\mu_0 h \gamma_A \gamma_B}{8\pi^2 r_{AB}^3} \left[ S_{zz} \left( 3\cos^2 \alpha - 1 \right) + \left( S_{xx} - S_{yy} \right) \sin^2 \alpha \cos 2\beta \right] 
$$

(3.2)

where $\alpha$ is the angle between the AB vector and the $z$-axis of the $S$ tensor, $\beta$ is the angle which describes the position of the projection of the AB vector on the $xy$ plane of the $S$ tensor, relative to the $x$-axis, when $x$, $y$, and $z$ are the principal directions of the traceless order tensor $S$, and

$$
S_{ii} = \frac{1}{2} \int_0^\pi (3\cos^2 \theta_{ji} - 1) P(\theta_{ji}) \sin \theta_{ji} \, d\theta_{ji}
$$

(3.3)

where $\theta_{ji}$ is the angle between the $i$ direction and the magnetic field direction, and $P(\theta_{ji})$ is the probability for that orientation. According to the typical convention, the axes of the reference frame are defined so that the magnitudes of the principal components are $|S_{zz}| > |S_{yy}| > |S_{xx}|$. As shown in Appendix II [Eq. (II.3)], when $P(\theta_{ji})$ is constant, $S_{ii} = 0$ and thus $\Delta \nu^{\text{rdc}} = 0$. 

**FIGURE 3.2** The angle $\gamma$ describes the orientation of the vector connecting the two coupled nuclei A and B with respect to the external magnetic field, along which the magnetic moments of the nuclei are oriented. The angle $\gamma$ changes due to molecular reorientation in solution and, if present, due to internal molecular dynamics.
Eq. (3.2) is often written [6] as

\[
\Delta \nu^{\text{rdc}} = -\frac{\mu_0 \hbar \gamma_A Y_B S_{LS}}{8 \pi^2 r_{AB}^3} A_{ax} \left[ (3 \cos^2 \alpha - 1) + \frac{3}{2} R \sin^2 \alpha \cos 2\beta \right]
\]  

(3.4)

where the alignment (traceless) tensor \( A \) is related to the Saupe matrix through the relationship \( A = \frac{2}{3} S \), so that, since \( S_{xx} + S_{yy} + S_{zz} = 0 \),

\[
A_{ax} = \frac{2}{3} \left( S_{zz} - \frac{S_{xx} + S_{yy}}{2} \right) = S_{zz}
\]

(3.5)

and \( R = \frac{A_{ax}}{A_{ax}} \). In Eq. (3.4) \( S_{LS} \) is the generalized Lipari-Szabo order parameter [7], not to be confused with the Saupe matrix. \( S_{LS} (0 \leq S_{LS} \leq 1) \) accounts for the effect of internal motions with correlation times shorter than the molecular reorientation time. It corresponds to the spin relaxation order parameter, but it is sensitive to motions extending up to the millisecond time scale [8]. In fact, the averaging in Eq. (3.1) extends to times as long as \( 10^{-2} \) s because \( J \)-splitting values are of the order of 1–100 Hz; therefore, the orientation of the AB vector with respect to the magnetic field depends on the superposition of the internal molecular motions up to the milliseconds time scale and of the orientational distribution of the molecule as a rigid object (described by the \( A \), or \( S \), tensors). Equations are also available for the case of internal motions not axially symmetric about the mean orientation, so that residual dipolar couplings depend on the generalized order parameters \( S_{zz,\text{int}} \) and \( S_{xx,\text{int}} - S_{yy,\text{int}} \) instead of a single \( S_{LS} \) Lipari-Szabo parameter [9]. The case of extensive internal molecular mobility will be discussed in Section 9.6.

### 3.2 THE MAGNETIC FIELD INDUCED ORDER

As seen in Section 1.6, the magnetic susceptibility describes the proportionality between the induced magnetic moment in a molecule and the applied magnetic field [Eq. (1.27)], i.e., the extent of the magnetization of a substance in a magnetic field. It is represented by a tensor, indicated as \( \chi^{\text{mol}} \) [Eq. (1.45)], because \( \langle \mu \rangle \) can be different depending on the orientation of the molecule with respect to the magnetic field. Anisotropy in the magnetic susceptibility tensor of a molecule causes partial orientation of the molecule itself.

In diamagnetic molecules the anisotropy of \( \langle \mu \rangle \), and thus of \( \chi^{\text{dia}} \), is due to the interaction of the magnetic field with the motion of the electrons in their orbitals (contributions from paramagnetic excited states should also be considered). It is mainly related to the anisotropy of the electron currents, for instance obtained in the presence of aromatic rings. It is usually rather small in biomolecules, except for instance in the case of heme proteins or when multiple aromatic planes are stacked together, as in the case of double stranded nucleic acids.

Even if the local contributions to the overall susceptibility are small, they can become very relevant when they are summed over several repetitions in one or two dimensions. This is the case of bicelles and filamentous phages: such macroscopic objects can align completely in a magnetic field. Thus, they provide an anisotropic environment, which induces partial alignment also on the biomolecules in solution.
In the presence of a paramagnetic metal, the unpaired electron spin(s) provide a paramagnetic contribution \[ \chi \text{, see Eq. (1.36)} \] to the \( \chi^{\text{mol}} \) tensor \( \chi^{\text{mol}} = \chi^{\text{dia}} + \chi \). As described in Section 1.6, in electron configurations with a nonzero orbital magnetic moment, the average induced electron magnetic moment \( \langle \mu \rangle \) is anisotropic. In brief, in paramagnetic systems, an anisotropic \( \chi \) tensor arises due to the orbital contribution to the electron magnetic moment, and this induces partial self-orientation of the molecule.

In all cases where the molecular magnetic susceptibility tensor, \( \chi^{\text{mol}} \), is anisotropic, since the average magnetic moment per particle \( \langle \mu \rangle \) induced by a magnetic field \( B_0 \) is defined as

\[
\langle \mu \rangle = \frac{\chi^{\text{mol}} \cdot B_0}{\mu_0},
\]

the change in the energy of the system resulting from the placement of the molecule in the magnetic field

\[
E = -\frac{B_0 \cdot \langle \mu \rangle}{2},
\]

is anisotropic, depending on the orientation and magnitude of \( \langle \mu \rangle \) with respect to \( B_0 \). The different energy for the different molecular orientations relative to \( B_0 \) causes a partial alignment favoring the lowest energy states (Fig. 3.3). This alignment can be expressed by the components of the order tensor [see Eq. (3.3)]

\[
S_{ii} = \frac{\int 3 \cos^2 \alpha - 1 \exp[-E(\alpha, \beta)/(kT)] \cos \alpha \cos \beta}{\int \exp[-E(\alpha, \beta)/(kT)] \cos \alpha \cos \beta}
\]

where \( \alpha \) and \( \beta \) are the spherical angles describing the direction of \( B_0 \) in the frame where the \( \chi \) tensor is diagonal. When the anisotropic energy \( E(\alpha, \beta) \), described by Eq. (3.7), is much smaller for a subset

![Figure 3.3](image_url)

**Figure 3.3** The energy of the system differs depending on the molecular orientations with respect to the external magnetic field due to anisotropy of the magnetic susceptibility tensor. This causes partial self-orientation of the molecule, and thus occurrence of self-orientation residual dipolar couplings.
of orientations than for all others, so much so that the latter are not thermally accessible, the molecular alignment is complete: this is the case of bicelles and other orienting devices that are totally aligned by the magnetic field. On the other hand, if the differences in energy among the different orientations are small, the alignment is only partial. Therefore,

\[
S_\alpha = \frac{\int 3\cos^2 \alpha - 1}{2} \exp \left[ \frac{B_0^2}{2\mu_0 kT} \left( \chi_{\alpha \alpha}^{\text{mol}} \cos^2 \alpha + \chi_{\beta \beta}^{\text{mol}} \sin^2 \alpha \cos^2 \beta + \chi_{kk}^{\text{mol}} \sin^2 \alpha \sin^2 \beta \right) \right] d \cos \alpha d \beta
\]

(3.9)

as obtained from Eqs. (3.6)–(3.8), with \( \chi^{\text{mol}} \) representing the whole molecular magnetic susceptibility tensor, defined in Eq. (1.45), and thus containing both the diamagnetic and the paramagnetic contributions. The exponential can be approximated to first order, so that

\[
S_\alpha = \frac{B_0^2}{15\mu_0 kT} \left( \chi_\alpha - \frac{\chi_\beta + \chi_{kk}}{2} \right) = \frac{3}{2} \frac{B_0^2}{15\mu_0 kT} (\chi_\alpha^{\text{mol}} - \chi^{\text{mol}})
\]

(3.10)

where

\[
\chi^{\text{mol}} = \chi_{xx}^{\text{mol}} + \chi_{yy}^{\text{mol}} + \chi_{zz}^{\text{mol}}.
\]

(3.11)

### 3.3 PARAMAGNETIC METAL IONS AND RESIDUAL DIPOlar COUPLINGS

Substituting the \( S_{xx}, S_{yy}, \) and \( S_{zz} \) components provided by Eq. (3.10) into Eq. (3.2), the equation for self-orientation residual dipolar couplings (in Hz) is obtained:

\[
\Delta V_{\text{dir}} = -\frac{1}{4\pi} \frac{B_0^2}{15kT} \frac{\gamma_A \gamma_B \hbar}{2\pi} \left[ \Delta \chi_{xx}^{\text{mol}} (3\cos^2 \alpha - 1) + \frac{\Delta \chi_{xy}^{\text{mol}}}{2} \sin^2 \alpha \cos 2\beta \right]
\]

(3.12)

where \( \alpha \) is the angle between the AB vector and the z-axis of the \( \chi^{\text{mol}} \) tensor, \( \beta \) is the angle which describes the position of the projection of the AB vector on the xy plane of the \( \chi^{\text{mol}} \) tensor, relative to the x-axis (Fig. 3.4), and

\[
\Delta \chi_{xx}^{\text{mol}} = \chi_{xx}^{\text{mol}} - \frac{\chi_{xx}^{\text{mol}} + \chi_{yy}^{\text{mol}}}{2} = \frac{3}{2} (\chi_{xx}^{\text{mol}} - \chi^{\text{mol}})
\]

(3.13)

analogously to the definition of axial and rhombic anisotropies provided in Eq. (2.47). As in that case, the conventional definition of the x-, y-, and z-axis of the \( \chi^{\text{mol}} \) tensor is such that \( |\Delta \chi_{xx}^{\text{mol}}| \leq \frac{2}{3} |\Delta \chi_{xy}^{\text{mol}}| \). To be noted that whereas pseudocontact shifts depend on the anisotropy of the magnetic susceptibility tensor of the paramagnetic metal, \( \chi \), only, residual dipolar couplings depend on the anisotropy of the whole molecular magnetic susceptibility tensor, including the diamagnetic contribution. Of course, when the magnetic susceptibility tensor is isotropic, \( (\chi_{xx}^{\text{mol}} = \chi_{yy}^{\text{mol}} = \chi_{zz}^{\text{mol}} = \chi^{\text{mol}}) \) the residual dipolar couplings are zero. Therefore, it is common to express the residual dipolar couplings (as also the pseudocontact
673.3

PARARAMETRIC METAL IONS AND RESIDUAL DIPOLAR COUPLINGS

shifts) as a function of the magnetic susceptibility anisotropy tensor \((\Delta \chi_{\text{mol}})\), defined as the difference between \(\chi_{\text{mol}}\) and \(\bar{\chi}_{\text{mol}}\).

When the difference is calculated between the splitting of coupled nuclei observed for the paramagnetic molecule and a diamagnetic analog (Section 12.4), the contribution from the paramagnetic metal can be isolated and the equation to be used to analyze the residual dipolar coupling data becomes

\[
\Delta \nu = -\frac{1}{4\pi 15kT} \frac{B_0^2}{\gamma_A \gamma_B h} S_{LS} \left[ \Delta \chi_{\text{ax}} (3 \cos^2 \alpha - 1) + \frac{3}{2} \Delta \chi_{\text{rh}} \sin^2 \alpha \cos 2\beta \right]
\]

(3.14)

where \(\Delta \chi_{\text{ax}}\) and \(\Delta \chi_{\text{rh}}\) coincide with those defined as in Eq. (2.47) and the generalized Lipari-Szabo order parameter \(S_{LS}\) is introduced [analogously to Eq. (3.4)].

The equation describing self-orientation residual dipolar couplings is thus just a specialized case of the Eq. (3.4) used, when orientation is induced by external orienting media. For both external and internal orienting media, the residual dipolar couplings globally depend on five parameters, the two anisotropy values (\(A_{\text{ax}}\) and \(R\) for residual dipolar couplings induced by external orienting media, and \(\Delta \chi_{\text{ax}}\) and \(\Delta \chi_{\text{rh}}\) for self-orientation residual dipolar couplings) and the three angles defining the orientation of the tensor principal components. Once these five parameters have been determined, the residual dipolar coupling of any specific nuclear pair depends on the orientation of the nuclear pair vector in the common reference frame where the tensor is diagonal.

Eq. (3.14) describes the residual dipolar couplings in the frame where the \(\chi\) tensor is diagonal, in the sense that the angles \(\alpha\) and \(\beta\) are spherical coordinates providing the orientation of the AB vector in such frame. Residual dipolar couplings can be written in the same frame in Cartesian coordinates (Section 2.2.2.1)

\[
\Delta \nu = -\frac{1}{4\pi 15kT} \frac{B_0^2}{\gamma_A \gamma_B h} S_{LS} \left[ \Delta \chi_{\text{ax}} \frac{2z_{\text{AB}}^2 - x_{\text{AB}}^2 - y_{\text{AB}}^2}{r_{\text{AB}}^2} + \frac{3}{2} \Delta \chi_{\text{rh}} \frac{x_{\text{AB}}^2 - y_{\text{AB}}^2}{r_{\text{AB}}^2} \right]
\]

(3.15)

where \(x_{\text{AB}} = (x_A - x_B)^2\), \(y_{\text{AB}} = (y_A - y_B)^2\), and \(z_{\text{AB}} = (z_A - z_B)^2\), or using the direction cosines (\(\cos \varphi_i\) are the direction cosines relating a vector to the \(i = x, y, z\) axes of the reference frame).
\[
\Delta V^{rdc} = -\frac{3}{4\pi} \frac{B_0^2}{15kT} \frac{\gamma_a \gamma_b h S_{LS} }{2\pi r_{AB}^3} \sum_i \chi_{ii} \cos^2 \varphi_i
\]  \hspace{1cm} (3.16)

where the index \(i\) runs on the three principal directions of the \(\chi\) tensor and \(\varphi_i\) are the angles between the AB vector and each of these principal directions.

Analogously to pseudocontact shifts, also for the residual dipolar couplings the equations can be written in any reference frame, different from that defined by the principal directions of the magnetic susceptibility anisotropy tensor, in spherical coordinates

\[
\Delta V^{rdc} = -\frac{3}{4\pi} \frac{B_0^2}{15kT} \frac{\gamma_a \gamma_b h S_{LS} }{2\pi r_{AB}^3} \left[ \chi_{zz} \left(\frac{3\cos^2 \alpha - 1}{2}\right) \frac{\sin^2 \alpha \cos 2\beta}{2} + \left(\chi_{xy} - \chi_{yy}\right) \frac{\sin^2 \alpha \cos 2\beta}{2} \right]
\]
\[
+ \chi_{xy} \sin^2 \alpha \sin 2\beta + \chi_{yz} \sin 2\alpha \cos \beta + \chi_{zx} \cos 2\alpha \sin \beta \right]
\]  \hspace{1cm} (3.17)

where \(\alpha\) and \(\beta\) are the angles between the AB vector and the \(z\)-axis of the chosen reference frame and between the projection of the AB vector on the \(xy\) plane and the \(x\)-axis of the chosen frame, respectively; in Cartesian coordinates

\[
\Delta V^{rdc} = -\frac{3}{4\pi} \frac{B_0^2}{15kT} \frac{\gamma_a \gamma_b h S_{LS} }{2\pi r_{AB}^3} \left[ \chi_{zz} \frac{2z_{AB}^2 - x_{AB}^2 - y_{AB}^2}{2r_{AB}^2} + \left(\chi_{yy} - \chi_{yy}\right) \frac{x_{AB}^2 - y_{AB}^2}{2r_{AB}^2} \right]
\]
\[
+ \chi_{xy} \frac{2x_{AB} z_{AB}}{r_{AB}^2} + \chi_{yz} \frac{2x_{AB} z_{AB}}{r_{AB}^2} + \chi_{zx} \frac{2x_{AB} z_{AB}}{r_{AB}^2} \right]
\]  \hspace{1cm} (3.18)

or using the direction cosines

\[
\Delta V^{rdc} = -\frac{3}{4\pi} \frac{B_0^2}{15kT} \frac{\gamma_a \gamma_b h S_{LS} }{2\pi r_{AB}^3} \sum_{i,j} \chi_{ij} \cos \varphi_i \cos \varphi_j
\]  \hspace{1cm} (3.19)

where the indices \(i\) and \(j\) run over the three axes \(x, y,\) and \(z,\) and \(\varphi_i\) are the angles between the AB vector and each of the three axes. In these cases, the \(\chi\) tensor is not diagonal, and the five unknowns are the 5 components defining such symmetric tensor. The sixth component (one of the three diagonal elements, for instance \(\chi_{zz}\)) can be set to the value making the tensor traceless (i.e., \(\chi_{zz} = -\chi_{yy} - \chi_{xx}\)) because, as it should be clear from Eq. (3.14) and the definition of \(\Delta \chi_{av}\) and \(\Delta \chi_{rb},\) the residual dipolar couplings do not depend on the trace of the \(\chi\) tensor.

From Eqs. (3.16) and (3.19), the residual dipolar couplings can also be written as

\[
\Delta V^{rdc} = -\frac{3}{4\pi} \frac{B_0^2}{15kT} \frac{\gamma_a \gamma_b h S_{LS} }{2\pi r_{AB}^3} \mathbf{P}^T \mathbf{\chi} \mathbf{P}
\]  \hspace{1cm} (3.20)

where \(\mathbf{P}\) represents the coupled nuclei vector with components \(x_{AB}, y_{AB},\) and \(z_{AB},\) written in the same frame of the \(\chi\) tensor (and \(\mathbf{P}^T\) is the transposed vector).

Despite the same functional form of the equations describing pseudocontact shifts [Eq. (2.46)] and residual dipolar couplings [Eqs. (3.12) and (3.14)], the spherical coordinates are differently defined: the angles in the equation for the pseudocontact shifts describe the position of the nuclei in the frame defined by the \(\chi\) tensor, with origin on the paramagnetic metal ion position (Fig. 2.6), while those in the equations for the residual dipolar couplings describe the orientation of the vector connecting the
coupled nuclei (Fig. 3.4) in the frame defined by the magnetic susceptibility anisotropy tensor (either \( \chi^{\text{mol}} \) or \( \chi \) depending on the measurement procedure, see Section 3.4); the distance corresponds to the metal-nuclear distance \( r \) for pseudocontact shifts and to the fixed distance between coupled nuclei \( r_{AB} \) for residual dipolar couplings. Therefore, the residual dipolar couplings do not depend at all on the distance of the observed nuclei from the paramagnetic center, as well as on the position of each nucleus with respect to the \( \chi \) tensor. Furthermore, the residual dipolar couplings increase quadratically with the \( \gamma \) of the coupled nuclei.

As for pseudocontact shifts, residual dipolar couplings can be predicted by knowing nuclear coordinates and tensor components. On the other hand, the tensor components can be calculated if at least 5 residual dipolar couplings are known (in rigid systems), because the anisotropy (traceless part) of the \( \chi \) tensor is determined by 5 terms (\( \Delta \chi_{ax}, \Delta \chi_{ay}, \Delta \chi_{az}, \Delta \chi_{xy}, \Delta \chi_{xz}, \Delta \chi_{yz}, \Delta \chi_{zz} \)), depending on the measurement procedure, see Section 3.4.

From Eq. (3.19), it is possible to write a residual dipolar coupling, after normalization to a prefactor, as a product between an AB vector with respect to the coordinate axes and one vector containing the 5 independent components of the \( \chi \) tensor:

\[
\Delta \nu^{\text{rdc,norm}} = \frac{3}{4\pi} \frac{B_0^2 S_L S_S}{15kT} \frac{\gamma \alpha^2 h^2}{2\pi r_{AB}^3} \left( \begin{array}{c} \chi_{yy} \\ \chi_{xy} \\ \chi_{xz} \\ \chi_{yz} \\ \chi_{zz} \end{array} \right) = v \cdot \left( \begin{array}{c} \chi_{yy} \\ \chi_{xy} \\ \chi_{xz} \\ \chi_{yz} \\ \chi_{zz} \end{array} \right)
\]

\[
(3.21)
\]

The vector containing the components of the \( \chi \) tensor is the same for all nuclear pairs, whereas the vector \( v \) depends on the orientation of the pair in the reference frame. Therefore, if residual dipolar couplings are measured for several pairs \( n \geq 5 \), it is possible to write the vector \( \mathbf{D} \) containing the normalized residual dipolar couplings as a product between an \( n \times 5 \) matrix, where the rows are the 5-component \( v_i \) vectors, and the vector containing the components of the \( \chi \) tensor:

\[
\mathbf{D} = \left( \begin{array}{c} \Delta \nu_1^{\text{rdc,norm}} \\ \Delta \nu_2^{\text{rdc,norm}} \\ \vdots \\ \Delta \nu_n^{\text{rdc,norm}} \end{array} \right) = \left( \begin{array}{c} v_1 \\ v_2 \\ \vdots \\ v_n \end{array} \right) \mathbf{\chi} \]

\[
(3.22)
\]
FIGURE 3.5 Possible angular positions within the $xz$ and $yz$ planes of a nucleus B coupled to the nucleus A positioned in the origin of the main frame of a magnetic susceptibility tensor with $\Delta \chi_{ab} = 0$ (A), $|\Delta \chi_{ab}| = |\Delta \chi_{ab}|/3$ (B), $|\Delta \chi_{ab}| = 2|\Delta \chi_{ab}|/3$ (C), and with residual dipolar coupling $\Delta \nu$ equal to $1/3$ of the maximum value, $D_{\text{max}}$. The value $D_{\text{max}}$ is experienced when the nucleus is along the $z$-axis (B'). $B^\circ$ indicate the nuclear positions with residual dipolar coupling equal to zero; the value of the residual dipolar coupling in the plane perpendicular to the $z$-axis depends on the $\Delta \chi_{ab}$ value and on the angular position. The positions of the nucleus B with residual dipolar coupling equal to $-D_{\text{max}}/3$ are also shown, in red.
If the molecular structure is known, the $v_i$ vectors can be calculated, so that it is possible to determine the $\chi$ tensor by determining the Moore–Penrose inverse of the $V$ matrix:

$$\chi = V^{-1}D$$

(3.23)

using the singular value decomposition (SVD) method [10]. Alternatively, the 5 independent components of the $\chi$ tensor can be determined through best fit calculations, performed in order to minimize the quadratic difference between experimental and calculated residual dipolar couplings, again with the elements of the $V$ matrix [or with the nuclear coordinates if Eq. (3.18) is used] calculated from the known molecular structure. Due to the presence of experimental errors, the accuracy of the approach requires a number of experimental residual dipolar couplings substantially larger than five and well distributed within the range of values permitted by the tensor.

### 3.4 Residual Dipolar Couplings in the Presence of Diamagnetic and Paramagnetic Anisotropy

We have seen that in paramagnetic molecules, residual dipolar couplings must be calculated by taking into account the presence of both a paramagnetic and a diamagnetic contribution to the magnetic susceptibility anisotropy (when the diamagnetic susceptibility anisotropy is not negligible with respect to paramagnetic susceptibility). If a diamagnetic analog of the investigated molecule is available, the corresponding residual dipolar couplings depend only on the diamagnetic contribution to the magnetic susceptibility anisotropy.

In this case, residual dipolar couplings can be obtained by subtracting the experimental $^1J$ couplings measured for a diamagnetic analog from the corresponding values measured for the paramagnetic sample (assuming that cross-correlations effects are negligible, see Section 4.9). In this way the residual dipolar couplings depend only on the paramagnetic $\chi$ tensor only, i.e., on the same tensor responsible for the pseudocontact shifts. Alternatively, the differences between residual dipolar couplings at two different magnetic fields ($B_{01}$ and $B_{02}$) can be obtained by subtracting the $^1J$ couplings measured at these fields, and in this case we obtain

$$\Delta(\Delta \nu)^{de} = -\frac{1}{4\pi} \frac{B_{01}^3 - B_{02}^3}{15kT} \frac{\gamma_A \gamma_B}{2\pi r_{AB}^3} \left[ \Delta\chi_{\text{mol}}^\text{ax} (3\cos^2 \alpha - 1) + \frac{3}{2} \Delta\chi_{\text{mol}}^\text{rh} \sin^2 \alpha \cos 2\beta \right].$$

(3.24)
These values thus depend on the overall $\chi^{\text{mol}}$ tensor. Since the diamagnetic and paramagnetic susceptibility tensors are diagonal, in general, in different frames, the residual dipolar couplings measured in paramagnetic samples depend on their tensorial sum (Fig. 3.7) [11].

As an example, in cytochrome $b_5$ [11], a heme protein containing an iron ion, $^1$H-$^1$N $^1$J couplings have been measured at two different fields (500 and 800 MHz) for the protein in both the oxidized (paramagnetic, low spin iron(III)) and reduced (diamagnetic, low spin iron(II)) states. From the analysis of the difference between the $^1$J couplings at the two fields measured for the oxidized protein against the protein structure, the overall $\chi^{\text{mol}}$ tensor can be determined. The corresponding anisotropy values are $\Delta\chi_{\text{ax}} = 6$ and $\Delta\chi_{\text{rh}} = 0$. If the same analysis is performed for the reduced protein, the $\chi^{\text{dia}}$ tensor can be determined, in this case with anisotropy values $\Delta\chi_{\text{ax}} = 3$ and $\Delta\chi_{\text{rh}} = 0$. The paramagnetic $\chi^{\text{mol}}$ tensor can be evaluated from the pseudocontact shifts for the protein in the oxidized form, and its anisotropy values are $\Delta\chi_{\text{ax}} = 2.20 \pm 0.05 \times 10^{-32} \text{ m}^3$ and $\Delta\chi_{\text{rh}} = -1.34 \pm 0.04 \times 10^{-32} \text{ m}^3$. If the same analysis is performed, in this case with anisotropy values $\Delta\chi_{\text{ax}} = -0.8 \pm 0.1 \times 10^{-32} \text{ m}^3$ and $\Delta\chi_{\text{rh}} = 0.1 \pm 0.3 \times 10^{-32} \text{ m}^3$. The paramagnetic $\chi^{\text{mol}}$ tensor can be determined from the pseudocontact shifts for the protein in the oxidized form, and its anisotropy values are $\Delta\chi_{\text{ax}} = 2.8 \pm 0.1 \times 10^{-32} \text{ m}^3$ and $\Delta\chi_{\text{rh}} = -1.1 \pm 0.2 \times 10^{-32} \text{ m}^3$, in good agreement with what expected from the tensorial sum $\chi^{\text{mol}} = \chi^{\text{dia}} + \chi$. A similar analysis is available in the literature for oxidized and reduced cytochrome $b_{562}$ from $E. \text{coli}$ [12]. In this case, the diamagnetic and paramagnetic contributions to the molecular magnetic susceptibility anisotropy are of the same magnitude, but the z-axis of $\chi^{\text{dia}}$ tensor is almost perpendicular to the z-axis of the $\chi$ tensor. The resulting diamagnetic, paramagnetic and total magnetic susceptibility anisotropy values are $\Delta\chi_{\text{ax}} = 1.6 \pm 0.1$, $-2.0 \pm 0.4$, $1.8 \pm 0.2 \times 10^{-32} \text{ m}^3$, respectively, and $\Delta\chi_{\text{rh}} = -0.4 \pm 0.3$, $0.5 \pm 0.3$, $-0.4 \pm 0.2 \times 10^{-32} \text{ m}^3$, respectively. Again, the tensors sum up as expected.
3.5 PARTIAL ORIENTATION EFFECTS ON THE DIPOLAR AND CONTACT SHIFTS

In Chapter 2 it is shown that the anisotropy of $\chi$ is responsible for pseudocontact shifts. We have seen in the previous sections that the anisotropy of $\chi$ can also induce partial orientation. The effect of partial orientation was however not taken into account when the equations for the pseudocontact shifts, as well as for the contact shifts, were derived. Therefore, Eqs. (2.34)–(2.39), (2.46), (2.48), and (2.49), provided to calculate the pseudocontact shifts, may need to be corrected for the fact that magnetic anisotropy induces partial orientation. Changes in contact shifts [Eq. (2.7)] may as well be expected in all cases that $<S_z>$ is not isotropic (Section 2.2.1) [13].

3.5.1 PARTIAL ORIENTATION EFFECTS ON THE PSEUDOCONTACT SHIFTS

Pseudocontact shifts occur when the metal magnetic susceptibility is anisotropic and they are derived (Chapter 2) under the assumption that every orientation of the molecule is equally probable. However, the magnetic susceptibility tensor axes are fixed within the molecule and the magnetic anisotropy introduces a dependence on orientation of the interaction energy between the paramagnetic ion and the magnetic field. If the energy of the interaction between the molecule and the magnetic field is orientation dependent, it follows that not all molecular orientations are equally probable (Fig. 3.3). As a consequence, changes in the observed pseudocontact shifts should be expected. Actually, it is not the metal magnetic susceptibility, the only cause of the orientation dependence of the molecular energy, but the overall molecular magnetic anisotropy, which includes the diamagnetic part [Eqs. (3.6) and (3.7)]. As we have seen, the latter is likely to increase with the size of the molecule, and it has been indeed found relevant in biological macromolecules.

We have seen in Section 2.2.2.1 that the contribution of the pseudocontact shift to the overall nuclear chemical shift can be calculated from the dipolar shielding tensor [Eqs. (2.28), (2.29), and (2.32)] as

$$\delta_{\text{pcs}} = -\frac{1}{3}(e_x \cdot \sigma \cdot e_x + e_y \cdot \sigma \cdot e_y + e_z \cdot \sigma \cdot e_z) = -\frac{1}{3} \text{Tr}(\sigma)$$

(3.25)

where $e_x$, $e_y$, and $e_z$ are the three principal directions of a reference frame. In the presence of partial orientation, the chemical shifts average to values, which depend on the components of the shielding tensor $\sigma$ along the main directions of the order tensor $S$ [14]:

$$\delta_{\text{pcs}} = -\left[\frac{\text{Tr}(\sigma)}{3} + \frac{2}{3} (\sigma_{xx}S_{xx} + \sigma_{yy}S_{yy} + \sigma_{zz}S_{zz})\right].$$

(3.26)

Therefore, if $\chi^\text{mol} \equiv \chi$, by substituting the $S$ components defined in Eq. (3.10) into Eq. (3.26) (the $\chi$ and $S$ tensors are diagonal in the same frame) Eq. (3.27) is obtained

$$\delta_{\text{pcs}} = -\frac{\text{Tr}(\sigma)}{3} - \frac{2}{3} \frac{B_0^2}{15 \mu_0 kT} \left[\sigma_{xx} \left(\chi_{xx} - \frac{\chi_{yy} + \chi_{zz}}{2}\right) + \sigma_{yy} \left(\chi_{yy} - \frac{\chi_{xx} + \chi_{zz}}{2}\right) + \sigma_{zz} \left(\chi_{zz} - \frac{\chi_{xx} + \chi_{yy}}{2}\right)\right].$$

(3.27)
CHAPTER 3 THE EFFECT OF PARTIAL ORIENTATION

Since \( \sigma_x = -(3x^2 - r^2)\chi_{x}(4\pi r^5), \sigma_y = -(3y^2 - r^2)\chi_{y}(4\pi r^5), \) and \( \sigma_z = -(3z^2 - r^2)\chi_{z}(4\pi r^5) \) [\( x, y, \) and \( z \) are the coordinates of the nucleus in the frame where \( \chi \) is diagonal, see Eq. (2.40)], using the relationships \( \cos^2 \varphi = (\cos 2\varphi + 1)/2, \) \( 3 \sin^2 \theta \cos^2 \varphi = 1 = (3 \sin^2 \theta \cos 2\varphi)/2 - (3 \cos^2 \theta - 1)/2, \) \( \sin^2 \theta = -(\cos 2\varphi - 1)/2, \) \( 3 \sin^2 \theta \sin^2 \varphi - 1 = -3 \sin^2 \theta \cos 2\varphi)/2 - (3 \cos^2 \theta - 1)/2, \) the equation for the pseudocontact shifts can be determined taking into account the effect of partial orientation due to the anisotropy of the \( \chi \) tensor (to be compared with Eq. (2.46) describing the pseudocontact shifts as determined for the case of isotropic reorientation),

\[
\delta^{\text{pos}} = \frac{1}{12\pi r^3} \left\{ \Delta \chi_{xx} (3 \cos^2 \theta - 1) \right\} \left[ 1 + \frac{B_0^2}{15\mu_b kT} \left( 2\chi_{zz} + \chi_{yy} \right) - \frac{3}{4} \Delta \chi_{ab} \right] \\
+ \frac{3}{2} \Delta \chi_{ab} \sin^2 \theta \cos 2\varphi \left[ 1 + \frac{B_0^2}{15\mu_b kT} \left( 2\chi_{xx} + \chi_{yy} - \chi_{zz} \right) \right]
\]

(3.28)

where \( \theta \) is the angle between the metal–nucleus vector \( r \) and the \( z \)-axis of the diagonal \( \chi \) tensor, and \( \varphi \) is an angle related to the projection of \( r \) on the \( xy \) plane of the tensor. For systems with axial symmetry \( (\chi_{xx} = \chi_{yy} = \chi_{zz}) \) Eq. (3.28) becomes [15]

\[
\delta^{\text{pos}} = \frac{(3 \cos^2 \theta - 1)(\chi_{0} - \chi_{\perp})}{12\pi r^3} \left[ 1 + \frac{B_0^2}{15\mu_b kT} (2\chi_{1} + \chi_{\perp}) \right].
\]

(3.29)

This correction of the pseudocontact shifts due to partial orientation is however minor and can be neglected in most practical applications [15]. It is predicted to increase with the square of the magnetic field. Up to magnetic fields of the order of 20 T, this effect is hard to observe in practice because it is of the same order and of opposite sign with respect to the difference in pseudocontact shift calculated using the field independent first order approximation [Curie law, Eq. (1.34)], instead of the exact field dependent value [Brillouin function, Eq. (1.37)] of the magnetic susceptibility. In fact, if the magnetic susceptibility is field dependent, as described by the Brillouin equation [Eq. (1.37)], because at high magnetic fields the electronic Zeeman energy is not much smaller than \( kT \) (Section 1.6) [16], pseudocontact shifts [described by Eq. (2.46)] become field dependent also in the absence of partial orientation [17], as well as contact shifts [Eq. (2.10)] [15].

The difference in population among the electron Zeeman levels can differ from what predicted from the Curie law even more than predicted by the Brillouin equation in the presence of ZFS effects. Thus, the consequent alteration of the pseudocontact shifts can be even larger than that due to Eq. (3.28), but at the same time it is a small fraction of the pseudocontact shift. Quantitative estimates have not been attempted. On the other hand, when a paramagnetic molecule is oriented mechanically by using orienting devices such as liquid crystals, a large and measurable change in hyperfine shift can be observed [18].

Another effect to be considered in the analysis of the chemical shifts of self-oriented molecules is the residual anisotropic chemical shift (RACS), due to the anisotropy in the orientational sampling of the chemical shifts, which results from the partial alignment of the molecule. It thus depends on the chemical shift anisotropy (CSA) tensor [19], defined from the difference between the chemical shielding tensor (Chapter 5) and its isotropic value (equal to the trace of the shielding tensor divided by 3). The residual anisotropic chemical shifts are described by the equation

\[
\delta^{\text{racs}} = \frac{B_0^2}{15\mu_b kT} \sum_{i,j} \delta^{\text{CSA}}_{ij} \cos^2 \theta_{ij} \Delta \chi_{ij}
\]

(3.30)
where the indices $i$ and $j$ run over the three axes $x$, $y$, and $z$, and $\theta_{ij}$ are the angles of the principal axes of the magnetic susceptibility anisotropy tensor, $\Delta \chi$, with respect to the principal axes of the CSA tensor, $\delta_{\text{CSA}}$. This effect can be evaluated from the knowledge of the CSA tensor, and thus from the molecular structure [20], and its evaluation can be important for a correct determination of the pseudocontact shifts. Residual anisotropic chemical shifts can be significant at high magnetic fields for nuclei with large CSA tensors, like amide $^{15}$N and carbonyl/aromatic $^{13}$C nuclei. They are usually negligible for protons.

### 3.5.2 PARTIAL ORIENTATION EFFECTS ON THE CONTACT SHIFTS

In the presence of partial orientation, either due to anisotropy of the susceptibility tensor or to external orienting agents, and of an orientation dependent $<S_z>$ [Eq. (2.6)], the contact shifts average to values that depend on the components of $S_{ii}$ along the main directions of the order tensor $S$ [analogously to Eq. (3.26) for pseudocontact shifts]:

$$
\delta_{\text{con}}^\approx = \left( \frac{1}{3} + \frac{1}{3} S_{xx} \right) \tilde{\delta}_{xx}^{\text{con}} + \left( \frac{1}{3} + \frac{1}{3} S_{yy} \right) \tilde{\delta}_{yy}^{\text{con}} + \left( \frac{1}{3} + \frac{1}{3} S_{zz} \right) \tilde{\delta}_{zz}^{\text{con}}.
$$

(3.31)

However, since the frame where the order tensor $S$ is diagonal can be different from the principal directions of the $g$ tensor, the axes of the $S$ frame will not coincide with the directions along which the contact shift values are calculated, $\delta_{xx}^{\text{con}}$, $\delta_{yy}^{\text{con}}$, and $\delta_{zz}^{\text{con}}$ [see Eq. (2.8)]. Therefore $\tilde{\delta}_{xx}^{\text{con}}$, $\tilde{\delta}_{yy}^{\text{con}}$, and $\tilde{\delta}_{zz}^{\text{con}}$ in Eq. (3.31) must be calculated by projecting the values of $\delta_{xx}^{\text{con}}$, $\delta_{yy}^{\text{con}}$, and $\delta_{zz}^{\text{con}}$ along the axes defining the frame where $S$ is diagonal

$$
\tilde{\delta}_{ii}^{\text{con}} = \delta_{xx}^{\text{con}} \sin^2 \alpha \cos^2 \beta + \delta_{yy}^{\text{con}} \sin^2 \alpha \sin^2 \beta + \delta_{zz}^{\text{con}} \cos^2 \alpha
$$

(3.32)

where $\alpha$ and $\beta$ are the spherical angles defining the position of the $i$ axis of the $S$ tensor in the frame of the $g$ tensor [21]. Partial orientation effects on contact shifts due to molecular self-orientation are usually not taken into account because considered a minor perturbation with respect to the precision of the experimental data.

### GENERAL REFERENCES


### REFERENCES

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