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Chapter 1 Multidimensional Methods and Paramagnetic NMR

Thomas Robinson, Kevin J. Sanders, Andrew J. Pell, and Guido Pintacuda

CRMN, Centre de RMN à Très Hauts Champs de Lyon (UMR 5280 CNRS / Ecole Normale Supérieure de Lyon / Université Claude Bernard Lyon 1), Université de Lyon, 69100, Villeurbanne, 5 rue de la Doua, France

*Corresponding Author: Guido Pintacuda; guido.pintacuda@ens-lyon.fr

1.1. Introduction

Paramagnetic centers are atoms or ions with unpaired electrons, including transition d or f metals but also organic radicals. They have a central role in many fields within life sciences, medicine, and industry. Unpaired electrons interact with the surrounding nuclear spins and change the appearance of their NMR spectrum in several ways, by altering chemical shifts, shift anisotropies and increasing relaxation rates. NMR of paramagnetic samples (paramagnetic NMR, pNMR) thus provides a direct probe of the electronic structures in many important compounds. It can disclose unique information in the study of the atomic-level properties of metal complexes, clusters, magnetic frameworks and metalloproteins, constituting essential steps for the design of new catalysts and new materials and for understanding biochemical processes inside cells.

The impact of NMR on paramagnetic molecules and materials, however, is hampered by the very same enhanced paramagnetic shifts, shift anisotropies and relaxation rates. These hinder the acquisition of the NMR experiments and the following spectral assignment and interpretation, and require the spectroscopist to modify the approaches that are optimal for diamagnetic substrates. The electronic properties of paramagnetic centers are widely different, and depending on the nature of the paramagnetic center, on the coordination environment, and on the nucleus investigated, NMR shifts and linewidths are affected to different extents, and different scenarios may be encountered. Figure 1.1 illustrates this point for some ions across the d and f transitions |1|, presenting the expected contributions to nuclear relaxation and chemical shifts for ¹H spins in close proximity of a metal center. Rapid electronic relaxation (with electronic spin-lattice relaxation times typically ranging between 10^{-13} and 10^{-8} s) enhance nuclear relaxation rates, which on the one hand contributes to broaden the lines up to hundred of thousands of Hz, and on the other hand reduces the spin-lattice relaxation times of the order of ms or lower. Large hyperfine coupling constants (up to several MHz for atoms in the bonding environment of a paramagnetic center) can result in contributions to NMR shifts of several hundreds of ppm. In materials, with a dense network of paramagnetic centers, these effects add up and result of shift and shift anisotropies of up to several thousands of ppm.

In the presence of large paramagnetic effects, 1D NMR experiments provide the most straightforward and sensitive fingerprint to probe the surrounding of a paramagnetic center. Assignment of these spectra can often be attempted exploiting the geometrical dependence of the paramagnetic effects, for exemple



Figure 1.1: Expected hyperfine shifts versus expected paramagnetic line broadening in ¹H resonances for a number of metal centers for directly metal bound (3.5 Å distance from the metal, subscript B) and non bound (6 Å distance from the metal, subscript NB) residues in tetrahedral ('tetra') and octahedral ('exa') coordination geometries. Reproduced with permission from ^[2]. Copyright (2014) Springer Nature.

by correlating spin-lattice relaxation times to the metal-nucleus distances from a structural model or (when available) a single crystal X-ray structure. As an alternative, resonances can be tentatively interpreted on the basis of the paramagnetic shifts predicted by rigorous quantum chemistry/density functional theory (DFT) calculations. Given the potential complexity of such spectra, the large linewidths, the overlap of the resonances, and the uncertainty of DFT methods in the reliable quantification of contact hyperfine interactions, however, 2D correlation spectra are clearly essential for a more extensive characterisation.

In this chapter we survey the experimental 2D NMR methods that have either been used on, or are applicable to paramagnetic systems. These comprise many NMR pulse sequences that have been designed for diamagnetic systems, including small- to medium-sized molecules in solution^[3], large biomolecules in solution^[4], solid materials^[5], and biosolids^[6]. We will discuss the modifications required in order to apply these methods to paramagnetic systems, and additionally present the tailored schemes, designed specifically to evaluate quantitatively paramagnetic effects. A more extensive discussion of these topics can be found in references^[1] and^[7].

1.2. NMR Methods for Paramagnetic Systems in Solution

1.2.1. Homonuclear correlations

Paramagnetic COSY. The COrrelation SpectroscopY (COSY) sequence is is the method of choice to identify coupled protons within each spin system ^[3]. In its most simple implementation, the sequence is composed of only two pulses, which is an advantage in that this minimises the potential for problems with non-ideal excitation to occur. Each diagonal-peak multiplet appears as an inphase array, while cross peaks are anti-phase in each dimension. This form of the spectrum has a disadvantage when the linewidth is comparable to or larger than the *J*-coupling, as the components of the cross-peak multiplet cancel to an extent, resulting in a low-intensity multiplet, whereas the diagonal peaks reinforce. Therefore it is often observed in rapidly-relaxing systems that COSY spectra exhibit very intense diagonal-peak multiplets that dominate the cross-peak multiplets. This problem is overcome by the Double-QuantumFiltered COSY (DQF-COSY) experiment (Figure 1.2 (a)), which yields antiphase square arrays for both the diagonal- and cross-peak multiplets, and hence a more equal balance of intensities. The double quantum filter also insures that only J- coupled spins contribute to the spectrum forbidding intense singlet resonances, though the overall sensitivity is reduced by a factor of 2 with respect to the simple COSY.

In both variants of the experiment, the signal intensity varies as $\sin(\pi J t_1)$ and so the typical evolution time (t_1) for a maximum cross-peek signal is roughly about 1/(2J). For ¹H–¹H correlation experiments, t_1 should thus extend to about 25 ms. Clearly this may present a problem for systems with large transverse PREs. In order to maximize the signal-to-noise ratio of the cross peaks, it is therefore important to tailor the time-domain weighting functions to the expected T_2 time constants. However for paramagnetic systems where $JT_2 \ll 1$ a weighting function is required with maxima at approximately $t_1 = t_2 = T_2$, and data points in the t_1 dimension should be acquired only out to a maximum time of $2T_2$.

When transverse PREs are relatively modest, as in the case of some 3d metal ions such as eg Ni²⁺, Co²⁺, or for Ln³⁺, the COSY experiment yields a substantial number of ¹H⁻¹H through-bond correlations despite larger dispersions of the signals. As an exemple, a ¹H⁻¹H COSY spectrum of (2-NCH₃-21-CH₃CTPP)-NiCl in CDCl₃ is shown in Figure 1.2 (d)^[8]. Interestingly, reduction of the recycle delays and acquisition times due to the fast relaxation times enables the acquisition of more data in a shorter amount of time using these optimised paramagnetic COSY parameters (typically 5-10 mins for 4-8 scans) compared to the standard COSY parameters (10-30 min for 1-2 scans)^[9].

Great care must be taken in interpreting the COSY spectra of param-



Figure 1.2: Pulse sequences for the two-dimensional DQF-COSY (a), TOCSY (b) and NOESY (c) experiments. Filled rectangles indicate pulses with a nominal flip angle of 90° and phase x, while the grey rectangle in the TOCSY experiment represents continuous irradiation during an isotropic mixing sequence. (d) Meso-phenyl region of the experimental two-dimensional ¹H–¹H DQF-COSY spectrum of (2-NCH₃-21-CH₃CTPP)-NiCl in CDCl₃ (the structure of the porphyrin ring 2-NCH₃-21-CH₃CTPPH is shown above the spectrum). (e-f) Experimental two-dimensional ¹H–¹H homonuclear correlation spectra of YbH(oep)(tpp) complex acquired in solution at 11.74 T. The spectra shown are (e) a TOCSY with an isotropic mixing time of 40 ms and (f) a NOESY spectrum with a mixing time of 40 ms. Adapted with permission from^[8] and from^[10]. Copyright (1996) and (2010) American Chemical Society.

agnetic molecules with slow rotational diffusion, where additional relaxationallowed cross-peaks may be observable complicating assignment. These additional cross-peaks, first described by Wimperis and Bodenhausen^[11] as well as Bertini^[12], are not the result of a through-bond coupling but rather of crosscorrelation from through-space (NOE) coupling between the nuclei as well as between the nuclei and the paramagnetic centre. The cross-correlation results in the two components of the doublet having different linewidths in the two dimensions, and so for zero *J*-coupling we do not obtain the perfect cancellation that we would see without cross-correlation. The result is that with cross-correlation, even in the limit of zero *J*-coupling, we can both generate an anti-phase coherence which is transferred to the second spin by the mixing period, and observe an anti-phase doublet in the cross-peak multiplet.

Paramagnetic TOCSY. The TOtal Correlation SpectroscopY (TOCSY) sequence provides an alternative, which compensates some of these weaknesses but is vulnerable to other ones. The form of the spectrum has a distinct advantage over COSY and DQF-COSY as all the multiplets are in phase. This means the cross-peak multiplets due to *J*-couplings that are comparable to the linewidth have a larger intensity, and so are easier to observe. In this sequence, however, mixing is accomplished by a continuous low-power RF irradiation with duration τ_m (Figure 1.2 (b)). This element may result in low sensitivity due to (i) the length of the mixing time giving large relaxation losses, and (ii) inefficient transfer due to the low RF field amplitude of the irradiation being insufficient to cover the spectral range of the paramagnetic shifts. The former problem is mitigated by compromising on the length of the mixing time, and ensuring that it is no longer than T_2 . A successful exemple of a ¹H–¹H TOCSY spectrum recorded on the Yb complex YbH(oep)(tpp) is shown in Figure 1.2 (e)^[10].

Paramagnetic NOESY. Either NOESY (Figure 1.2 (c)) or steady-state NOE experiments are recommended to complete the ¹H NMR spectrum assignment. These two experiments provide similar structural information but

NOESY has the advantage that the exchange and NOE cross-peaks can be observed in a single 2D spectrum compared with multiple 1D spectra for steadystate NOE experiments.

The standard NOESY pulse program needs to be modified for application to paramagnetic complexes. Mixing times need to be chosen as a compromise between the short relaxation times and comparatively long NOE cross-relaxation rates. Given the range of T_1 relaxation times within the complex, optimisation of the mixing time is an essential preliminary step. For protons with T_1 relaxation times significantly longer than the mixing time, the exchange integral approached a maximum as the mixing time increased. However, for protons with shorter T_1 relaxation times, the exchange integral reached a maximum before decreasing as relaxation began competing with exchange when the mixing time increased. Mixing times of 10-20 ms are usually found to be a good compromise for maximising the exchange cross-peak of all protons despite their differing T_1 relaxation times. A successful exemple of a ¹H–¹H NOESY spectrum recorded on the Yb complex YbH(oep)(tpp) is shown in Figure 1.2 (f) ^[10].

Exchange spectroscopy. NOE intensities are, in general, small for small molecules and reduced even further by the fast relaxation from coupling to the paramagnetic center. in case some excess ligand is also present in the complex solution, however, the same sequence can be exploited to observe exchange cross-peaks between the metal ligands in the complex and the excess ligands. If the exchange is sufficiently rapid, EXSY cross-peak intensities can be close to 100% even for short mixing times, allowing the assignment of protons in the complex using the free ligand assignments (Figure 1.3 (a)).

Two-dimensional ¹H EXSY has been used to study the slow-exchange dynamics of the two isomeric forms of molecules derived from the MRI contrast



Figure 1.3: (a) Experimental 2D ¹H EXSY spectrum of mer- $[Co(pq)_3](BF_4)_2$. Exchange cross-peaks are represented by the orange boxes and exchange cross-peaks between the complex and excess ligand are shown by the green boxes. (b) Experimental 2D ¹H EXSY spectrum of YbDOTA⁻ acquired with a mixing time of 5 ms. The geometries of the two isomers of YbDOTA⁻ are depicted above the spectrum. Adapted with permission from^[9] and from^[13]. Copyright (2019) and (2001) Wiley.

agent GdDOTA^[14] and YbDOTMA^[13] (Figure 1.3 (b)).

1.2.2. Heteronuclear correlations

Two experiments for one-bond correlations that have been successfully applied to paramagnetic molecules are the Heteronuclear Multiple-Quantum Correlation (HMQC) and the Heteronuclear Single-Quantum Correlation (HSQC) sequences. These experiments correlate the in-phase single-quantum coherence of the *I*-spin in t_2 against respectively multiple-quantum coherences of the *I* and *S*-spin of total coherence orders -2, 0, and +2 (HMQC, Figure 1.4 (a)) and single-quantum coherences of the *S*-spin in t_1 (HSQC, Figure 1.4 (b)). Heteronuclear coherences are generated and reconverted to *I*-spin coherences by a pair of heteronuclear INEPT sequences, with half-echo delays τ .



Figure 1.4: (a-b) Pulse sequences for the two-dimensional HMQC (a) and HSQC (b) experiments. The filled rectangles indicate pulses with a nominal flip angle of 90°, and unfilled rectangles indicate pulses with a nominal flip angle of 180°, and the grey rectangle represents broadband heteronuclear decoupling. The delays τ have an optimum value of $1/(2J_{IS})$ in the absence of relaxation. (c) Efficiency of the HMQC and HSQC transfers with J = 140 Hz at different ¹H T₂ values: i) no relaxation; ii) 10 ms, iii) 5 ms, iv) 2 ms, v) 1 ms. (d-e) Pulse sequences for the two-dimensional AP-HMQC (d) and AP-HSQC (e) experiments. (f) Efficiency of the AP-HMQC and AP-HSQC transfers with J = 140 Hz at the different ¹H T₂ values of panel (c).

When applied to paramagnetic systems, the most significant weaknesses in these sequences are the narrow bandwidth associated to the 180° pulses and the coherence losses due to paramagnetic relaxation during the echo delays τ .

The effects of the small bandwidth of conventional 180° pulses can be mitigated by the implementation of broadband inversion pulses (BIPs) of Smith et al^[15]. In the HMQC, the small number of pulses (four) reduces the effects of imperfections.

As for the duration of the INEPT delays, the peak intensity is proportional respectively to $\sin^2(\pi J_{IS}\tau)$ and $\sin^2(2\pi J_{IS}\tau)$, where J_{IS} is the one-bond heteronuclear *J*-coupling constant, and so the optimum value for τ is $1/(4J_{IS})$. For one-bond correlations between ¹H and ¹³C the coupling constant is typically 140 Hz, resulting in an optimum τ of 1.8 ms. However for short T_2 it is usually found that the optimum delay is shorter, as shown in Figure 1.4 (c) where e.g. a T_2 of 5 ms reduces the optimum value of τ to below 1.5 ms. The maximum peak intensity is reduced to $\simeq 30\%$ of the value expected with no relaxation.

In order to further mitigate sensitivity losses due to transverse relaxation in systems with short T_2 times, the inverse INEPT block, during which coherences are back-converted into observable, in phase H coherences immediately prior to acquisition can be omitted. This produces AP-HMQC and AP-HSQC sequences where an anti-phase splitting is detected in the direct ¹H dimension in the absence of ¹⁵N decoupling (Figure 1.4 (d-e)). Sensitivity is thus significantly improved as compared to the standard sequence. Figure 1.4 (f) shows eg that with the same T_2 of 5 ms, the optimum τ is again reduced to below 1.5 ms, but that the intensity of the peak is reduced only to $\simeq 60\%$. When signal linewidth is larger than doublet separation, the components of in phase doublets partly overlap and cancel signal. However, when the doublet is phased in dispersion mode, it gives rise to a "pseudosinglet" originated by the sum of the two dispersive components of the doublet. This contributes to the identification of broad, fast relaxing peaks and also to discriminate signals more severely affected by paramagnetic relaxation with respect to the others.

These detection schemes can be additionally combined via an inversionrecovery (IR) excitation block, which discriminates nuclear spins according to their T_1 relaxation times and enables a more convenient identification of residues at a short distance from a metal center. In the resulting IR-¹⁵N-HSQC-AP sequence (Figure 1.5), a suitable choice of the recovery delay causes a sign difference between fast-relaxing and slow-relaxing signals. Combined with a short duty cycle, this allows a significant suppression of the bulk envelope of the diamagnetic signals, easing the observation of paramagnetic ¹H resonances.



Figure 1.5: Overlay of a standard ¹⁵N-HSQC (thin lines) and IR-¹⁵N-HSQC-AP (thick lines) experiments acquired on the $[Fe_2S_2]^{2+}$ -CIAPIN1 domain. Peaks are labelled according to their assignment. Reproduced with permission from ^[2]. Copyright (2014) Springer Nature.

1.2.3. Long-range paramagnetic effects

Nuclear species at large distances from a paramagnetic metal center can be observed by employing the standard NMR experiments for diamagnetic proteins. Nonetheless, the presence of a fast-relaxing paramagnetic ion in the protein changes the appearance of the spectrum of the surrounding nuclei in several ways which are manifested as changes in chemical shifts, line widths, and onebond coupling constants (Figure 1.6 a-d):

• The pseudo-contact shift (PCS) changes the observed chemical shifts due

to interaction with the anisotropic susceptibility tensor of the paramagnetic center, and depends on the position of a given nucleus with respect to the magnetic susceptibility tensor of the unpaired electrons, via the distance between the nucleus and paramagnetic centre r, and the angles (θ, ϕ) that define the orientation of the electronic-nuclear vector with respect to the principal axis system (PAF) of the susceptibility tensor.

- The nuclear paramagnetic relaxation enhancement (PRE), due to a combination of the Solomon and the Curie contributions, depends on the distance r of the nucleus from the paramagnetic centre.
- The cross-correlated relaxation (CCR) between the Curie-spin and dipole-dipole relaxation both the distance r between the paramagnetic centre and the observed nucleus n, and the angle ϑ between the electronic-n vector and the bond vector defining an internuclear dipolar coupling.
- Finally, the residual dipolar coupling (RDC), due to alignment of the molecule with respect to the magnetic field, depends on the angles (Θ, Φ) that describe the orientation of an internuclear vector and the PAF of the magnetic susceptibility tensor.

Figure 1.6 (e) illustrates the information available from the different paramagnetic effects for an N–H amide bond of a paramagnetic protein, the 30 kDa complex between the N-terminal domain of the ϵ subunit and the θ subunit of Escherichia coli DNA polymerase III^[16]. All four paramagnetic parameters are readily measured by comparison of undecoupled ¹H–¹⁵N HSQC spectra recorded in the presence and absence of a paramagnetic metal ion. Line shape fitting of the antiphase doublets observed in the ¹H dimension yields the line width and frequency positions of each doublet component, and hence PCS, PRE, CCR, and RDC values. Given the explicit dependence on the threedimensional structure of the molecule (once the electronic chi tensor anisotropy and the correlation time τ_r are known), these effects can be used in combination with conventional restraints, such as those obtained from NOESY, to obtain the three-dimensional protein structure.



Figure 1.6: (a-d) Dependence of paramagnetic effects on local geometry: (a) pseudocontact shifts (PCS), (b) residual dipolar couplings (RDC), (c) cross-correlated relaxation (CCR) between the Curie spin of the paramagnetic protein and the dipole-dipole interaction between two nuclear spins, (d) paramagnetic relaxation enhancements (PRE). (e) ¹H⁻¹⁵N HSQC spectrum of the 30 kDa complex between the N-terminal domain of the ϵ subunit and the θ subunit of Escherichia coli DNA polymerase III in the absence and presence of a paramagnetic metal ion. The spectrum contains the anti-phase doublets of the two proteins, one binding paramagnetic Dy^{3+} (yellow and blue peaks) and the other with no metal ion (red and blue peaks). Straight dotted lines connect each paramagnetic resonance with the diamagnetic equivalent. Also shown is a cross section through a cross-peak, with two Lorentzian lineshapes fitted to the doublet components (dashed lines). The PCS is given by the overall change in chemical shift of the resonance, the transverse PRE is given by the overall line broadening, the CCR effects are measured from the difference in linewidth of the two components of the anti-phase doublet, and the RDC is calculated from the change in the splitting across the doublet. Reproduced with permission from^[16]. Copyright (2004) American Chemical Society.

1.2.4. Heteronuclear detection strategies

Conventional sequences exploit the larger gyromagnetic ratio of ¹H nuclei and use initial excitation and detection of ¹H to maximize the sensitivity. In paramagnetic systems, however, a larger gyromagnetic ratio also translates into larger transverse PREs, which in turn increases the line broadening observed in the ¹H dimension and accelerates coherence decay during transfer steps that involve ¹H. This leads to a reduction in sensitivity, countering the advantage associated to the initial excitation and detection of ${}^{1}H$. In these cases, a solution is offered by protonless NMR experiments, where better sensitivity is observed for resonances from the close proximities of a metal centre by performing both initial excitation and observation on ${}^{13}C$ or on ${}^{15}N$. For example, the use of ${}^{13}C$ COSY, ¹³C multiple-quantum experiments between CO and CA (COCAMQ), and ¹³C NOESY techniques was shown to be efficient for the detection of signals from nuclei as close as 4 Å to the Cu^{2+} ion in Cu^{2+}/Zn^{2+} superoxide dismutase (SOD), or as close as 8 Å to the lanthanide ion in Tb^{3+} -substituted human oncomodulin (OM) (in these molecules, ¹H-detection experiments are inefficient respectively from 10 and 16 Å from the metal ion). In the case of paramagnetic proteins, a large palette of two- and three-dimensional protonless NMR experiments has been designed for the sequential assignment of the backbone and sidechains (Figure 1.7). These include a combination of ${}^{13}C/{}^{15}N$ experiments such as CON, CANCO, CACO, CBCACO, and ¹³C TOCSY^[17,18].



Figure 1.7: Illustration of the sequential assignment procedure using ${}^{13}C/{}^{15}N$ experiments. The relevant one-bond *J*-coupling constants are given. Reproduced with permission from [18]. Copyright (2005) John Wiley and Sons.

1.3. NMR Methods for Paramagnetic Systems in Solids

1.3.1. Adiabatic Pulses

In solids, in addition to isotropic shifts, the high density of paramagnetic centers causes extremely large paramagnetic shift anisotropies, which can be on the order of 100-1000 ppm and are thus larger than the practicable RF-amplitudes. Under these conditions, square pulses do not provide the necessary bandwidth, and alternative pulse schemes have to be used to ensure broadband inversion or refocusing. Adiabatic pulses offer a solution to this problem, featuring an impressive ratio between the RF-power used and the achieved bandwidth, and providing a performance that is virtually independent from the resonance offsets.

Classical adiabatic pulses in solution are based on long low-power irradiations, while in paramagnetic solids under MAS efficient inversion is best achieved with the so-called Short High-power Adiabatic Pulse (SHAPs)^[19]. These elements are endowed with high power, which induces a larger effective field overcoming the modulation associated to the shift anisotropy, a wide and fast frequency sweep, which provides a uniform amplitude modulation for all the crystallites, and a short duration, which minimises signal losses due to the PRE.

Once optimised, SHAPs can be incorporated as inversion elements in more complex pulse sequences, or used for refocusing through a double spin echo, where a second SHAP compensates the frequency-dependent phase errors on the coherences induced by the first SHAP and leading to a dephasing of the signal in the powder^[20].

1.3.2. Homonuclear correlations

Spin diffusion. The most important mechanism for homonuclear correlation spectroscopy in solids is spin diffusion, in which we transfer longitudinal magnetization from one spin to another via a coherent homonuclear dipolar coupling interaction^[21]. This mechanism is particularly effective for ¹H nuclei, where it is referred to as proton spin diffusion (PSD), as the large gyromagnetic ratio gives large dipolar coupling constants, and therefore relatively rapid transfer. Spin diffusion exhibits good efficiency for systems showing moderate PRE, and at moderate spinning speed. Nevertheless the rate of transfer decreases inversely to the spinning speed, resulting in reduced transfer yields at fast MAS

and/or for systems with pronounced PRE.

Homonuclear recoupling. The rate of transfer can be increased by employing a homonuclear dipolar recoupling sequence during the mixing time. The most widely used technique for this in solid-state NMR is the Radio Frequency-driven Dipolar Recoupling (RFDR) experiment, which mixes nuclear coherences by way of a train of π pulses^[22]. Figure 1.8 (a-b) shows the corresponding pulse sequence, together with a RFDR spectrum correlating highly paramagnetic ¹Hs at the core of the iron-sulphur EhHiPIP protein^[23]. Despite the large hyperfine shifts (up to 100 ppm), the sequence provides a broadband, offset-insensitive route to efficient 2D correlations.

Due to the limited excitation bandwidth of π pulses in general, a long train of 50 or more pulses over 100s or 1000s of μ s may result however not only in spectral biasing due to accumulated pulse bandwidth issues, but potentially significant relaxation losses due to T₁ relaxation during this period. A method using $\pi/2$ pulses, which have much greater bandwidth than π pulses, over a shorter recoupling period is the single-quantum (SQ) – double-quantum (DQ) back-to-back experiment, with the pulse sequence given in Figure 1.8 (c). This experiment correlates the single-quantum resonance of two nuclei by way of the evolution of the a double quantum resonance of the pair; as such, the two single quantum resonances with frequencies ω_1 and ω_2 in the direct dimension will both have a frequency of $\omega_1 + \omega_2$ in the indirect dimension. This is illustrated on the 2D ³¹P BABA spectrum of MgP₄O₁₁ in Figure 1.8 (d) ^[25]. When applied to protons in paramagnetic organometallic complexes, the BABA experiment is a powerful method to resolve and assign ¹H peaks.



Figure 1.8: (a) Pulse sequence for the RFDR homonuclear correlation experiment. The phases ϕ of the 180° RFDR pulses vary according to the XY-8 supercycle $xyxyyxyx^{[24]}$. (b) Experimental two-dimensional ¹H–¹H dipolar correlation spectrum acquired with a microcrystalline, ¹³C- and ¹⁵N-labelled sample of the protein EhHiPIP I with RFDR mixing at 60 kHz MAS. Reproduced with permission from^[23]. Copyright (2017) American Chemical Society. (c) Pulse sequence for the BABA homonuclear correlation experiment. (d) Experimental 2D BABA spectrum of MgP_4O_{11} recorded using an MAS rotation rate of 14.2 kHz. It exhibits three correlated pairs of ³¹P nuclei, consistent with the structure composed of corrugated sheets of linked rings containing 4 or 16 PO₄ tetrahedra. Reproduced with permission from^[25]. Copyright (1996) Elsevier. (e) Pulse sequence for EXSY. (f) Experimental two-dimensional ⁶Li EXSY spectra acquired with monoclinic Li₃Fe₂(PO₄)₃ at 25 kHz MAS. The one-dimensional spectrum contains three distinct resonances for the three Li sites A, B, and C, and the EXSY spectrum, recorded with a long mixing time of 3 ms, exhibits cross peaks due to exchange between all three sites. Reproduced with permission from^[26]. Copyright (2010) American Chemical Society.

Exchange spectroscopy. The same two-dimensional EXSY sequence as for solution NMR can be used to obtain correlations between nuclear sites experiencing slow chemical exchange 1.8 (e). Also in this case, the mixing time is required not to exceed values of the order of T_1 . EXSY in paramagnetic solids has been mainly used to investigate the dynamics of lithium exchange in lithium-ion-conducting materials. Figure 1.8 (f) illustrates an example where 2D ⁶Li EXSY spectra were acquired on monoclinic Li₃Fe₂(PO₄)₃ and used to measure exchange between the three distinct Li sites in the material^[26].

1.3.3. Heteronuclear correlations

TEDOR. Because CP-based dipolar polarization transfers for paramagnetic materials are typically inefficient, it is necessary to achieve transfer of polarization from protons via short dipolar-recoupling sequences that use high-powered short RF pulses, such as the HETCOR variant of the TEDOR sequence, know also as Dipolar Insensitive Nucleus Enhanced by Polarization Transfer (DINEPT) sequence^[27,28]. This sequence uses rotor-synchronised π pulses to reintroduce, during a relatively short interval τ , the ¹H-¹³C dipolar couplings, otherwise averaged out by MAS, and is usually acquired as a ¹³C-detected experiment at intermediate MAS rates in the 30 kHz range. The 2D TEDOR sequence in Figure 1.9 has been proven to be particularly effective both on paramagnetic organometallic complexes and on metalloproteins. An example 2D TEDOR spectrum of the Fe(II) (S = 2) containing complex DIAD-Fe(II) dichloride (DIAD: 2,3-dimethyl-1,4-[(2',6')-diisopropylphenyl]-N,N'- diazediene) is given in Figure Figure 1.9 (c). Eight major resonances corresponding to eight of the nine C–H pairs in the molecule are observed, as well as a few

additional weaker resonances corresponding to more distant coupled C–H pairs. Heteronuclear correlations achieved by 2D TEDOR or similar techniques represent a major step towards assignment of resonances in small to medium-sized paramagnetic complexes, and indeed, with the aid of computational techniques may permit full assignment of the observed resonances.



Figure 1.9: (a, c) Pulse sequences for the TEDOR experiments with direct- (a) and indirect-detection (b). (c) ${}^{1}\text{H}{-}^{13}\text{C}$ TEDOR spectrum of the compound Fe(II)-DIAD acquired at 33 kHz MAS. (d) ${}^{1}\text{H}{-}^{13}\text{C}$ HSQC-TEDOR spectra of open-pore Ni-DUT acquired with short (66.6 μ s, left) and long (2.5 ms, right) τ_{TEDOR} recoupling at 60 kHz MAS. Long-range correlations are labelled in italics. (e) ${}^{1}\text{H}{-}^{13}\text{C}$ and ${}^{1}\text{H}{-}^{15}\text{N}$ HSQC-TEDOR correlation spectra of Co-SOD acquired at 100 kHz MAS. Reproduced with permission from refs^[28,29,30]. Copyright (2006) and (2020) American Chemical Society, (2021) Wiley.

HSQC-TEDOR. With the use of faster MAS it becomes possible to observe the ¹H spectrum directly, via a version of the TEDOR sequence with indirect detection, which benefits from the higher sensitivity of observing the nucleus with the larger γ_I (HSQC-TEDOR)^[31,32]. The uniformly short T₁ relaxation times observed in a paramagnetic solid sample, together with the absence of the requirement for high-power RF irradiations during the experiment, allow the use of very short recycle delays between acquisitions. Combined with the high sensitivity of ¹H detection, this produces a sensitivity boost, and for example in a 1.3 mm rotor at 60 kHz MAS, this scheme provides comparable sensitivity to the ¹³C-detected spectrum acquired in a 2.5 mm at 30 MAS, with a five-fold reduction in the sample volume.

The HSQC-TEDOR was recently employed for the characterisation of a Fe(II) organometallic complex^[32] and a metallo-organic framework containing Ni(II) paddle-wheel units^[29], where 2D correlation can be acquired within 30 minutes on 1–2 mg of samples at natural ¹³C abundance. Depending on the recoupling period employed, ¹H–¹³C correlations can be observed either over short distances corresponding to a single bond, or over longer distances, including correlations between ¹Hs and quaternary ¹³Cs (Figure 1.9 (d)). These HSQC-TEDOR spectra resolve and assign the individual frequencies of all the ¹H and ¹³ nuclei in the sample. The same approach was also used to elucidate the coordination sphere of a large paramagnetic Co(II) metalloenzyme, using correlations acquired on less than 0.5 mg of a large, uniformly ¹⁵, ¹³-labelled and fully protonated protein dimer (Figure 1.9 (e)) ^[30].

1.3.4. Long-range paramagnetic effects

As for solution NMR, the standard NMR experiments for diamagnetic proteins can be employed for observing nuclear species at large distances from a paramagnetic metal center, allowing the measurement of paramagnetic relaxation enhancements and of pseudocontact shifts. In deuterated protein at 60 kHz MAS, 2D and 3D ¹H-detected and CP-based correlations such as ¹H-¹⁵N CP-HSQC and (H)CANH correlations result in highly sensitive experiments capable to provide more than one hundred $PREs^{[33]}$ and $PCS^{[34]}$ between 10 and 24 Å from a paramagnetic metal ion in Cu(II)- and Co(II)-substituted superoxide dismutase. These data can be easily implemented as restraints in combination with internuclear distances and dihedral angles, improving the quality of an NMR-determined structure, in particular in the proximity of a metal ion.



Figure 1.10: ¹H-detected measurement of ¹⁵N PREs from the comparison of ¹H,¹⁵N CP-HSQC spectra of Cu²⁺,Zn²⁺-SOD and Cu⁺,Zn²⁺-SOD. (b) ¹H-detected measurement of ¹H and ¹⁵N PCS from the comparison of ¹H,¹⁵N CP-HSQC spectra of Co²⁺-SOD and Zn-SOD. (c-f) Bundles of SOD structures calculated (a) without paramagnetic restraints, (b) with ¹⁵N and ¹³C PREs, (c) with ¹H, ¹⁵N, and ¹³C PCSs, and (d) with both PREs and PCSs. The Cu and Co ions are represented by violet and pink spheres, and the mean NMR structure is depicted as an aquamarine ribbon. Reproduced with permission from ^[35]. Copyright (2013) American Chemical Society.

1.3.5. Separation of Shift and Shift Anisotropy Interactions

Due to large anisotropic effects induced by paramagnetic centers on the NMR resonances of nuclei in paramagnetic complexes, a significant number of sidebands or some or all of the resonances may be observed. This often results in spectra consisting of many isotropic resonances overlapping with spinning sidebands. Of course, this problem can be addressed simply by rotating at a faster rate, but in extreme cases even MAS rates up to 60 kHz cannot resolve this issue. 2D sideband separation experiments like the Magic Angle Turning (MAT) sequence^[36] or the Phase-Adjusted Spinning Sidebands (PASS) sequence^[37] address this issue by suppressing spinning sidebands in the direct (PASS) or indirect (MAT) dimensions, thus achieving a 2D spectrum correlating pure isotropic frequencies to mixed isotropic/anisotropic frequencies, the latter of which can be transformed to pure anisotropic frequencies with an appropriate shearing transformation. These experiments use a train of π refocusing pulses with unique timings in order to result in pure isotropic evolution, but the aforementioned bandwidth problems with π pulses can cause these experiments to not work properly for paramagnetic samples. Clément et al. addressed this problem in the MAT experiment by the inclusion of SHAP refocusing pulses, which was dubbed the adiabatic MAT (aMAT) sequence, and is given in Figure 1.11 (a)^[38]. The authors applied this methodology to untangle the ${}^{31}P$ resonances in the olivine-type mixed phase lithium-ion battery cathode materials with the stoichiometries $\text{LiFe}_x \text{Mn}_{1-x} \text{PO}_4$. The ³¹P MAS NMR spectra of these mixed-phases are composed of up to 32 unique isotropic resonances, each with potentially unique principle components of the anisotropy of their shift tensors.

To make matters worse, lines were severely affected by significant anisotropic bulk susceptibility broadening, resulting an a 1D MAS spectrum completely devoid of any appreciable resolution. The use of aMAT (shown in Figure 1.11 (b) on the LiFe_{0.5}Mn_{0.5}PO₄ composition) greatly enhanced the spectral resolution by suppression of spinning sidebands. Subsequent fitting of the isotropic projection of this spectrum permitted the identification of isotropic shifts for each of the 32 possible sites.



Figure 1.11: (a) aMAT pulse sequence and (b) 2D aMAT spectrum of LiFe0.5Mn0.5PO4 acquired with a MAS rotation rate of 60 kHz. Reproduced with permission from ^[38]. Copyright (2012) American Chemical Society.

1.3.6. Separation of Shift-anisotropy and Quadrupolar interactions

In this section we present experimental schemes that are designed to separate the contributions to the spectral lineshape from the paramagnetic SA and quadrupolar interactions. Though quadrupolar nuclei in paramagnetic systems have complicated spectra, the tensor parameters are useful indicators of the structural and electronic properties. There are two important types of quadrupolar nucleus (with integer- and half-integer-spins respectively) characterized by their different observable signal components, with half-integer spins exhibiting both central and satellite transitions signals, and integer spins only having satellite transitions. These two types of nuclei therefore need to be treated separately with specifically-tailored pulse sequences.

1.3.6.1. Integer-spin Quadrupolar Nuclei

Under static conditions we can in principle measure the effects on the spectrum of two different interactions: the isotropic shift and SA both evolve in the same way with frequencies that are proportional to the coherence order p, and the first-order quadrupolar interaction evolves with a frequency that is proportional to the satellite order d. The first pulse sequence that was developed to separate these two interactions is the shifting p-echo experiment of Antonijevic and Wimperis, and is shown in Figure 1.12 (a)^[39].

The *p*- and *d*-symmetry pathways are also shown. Therefore there are two echoes formed during the pulse sequence: the *p*-echo is formed at points where $t_1 = t_2$, and the *d*-echo is formed at $t_2 = 0$. The quadrupolar interaction echo ridge is therefore aligned along the t_1 -axis, whereas the shift/SA ridge echo is



Figure 1.12: Pulse sequences and symmetry-transfer pathways for correlating the paramagnetic shift anisotropy and quadrupolar lineshapes for spin I = 1 nuclei. Also shown are the transfer pathways for the coherence order p and satellite order d. The sequence in (a) is for the shifting p-echo experiment of Antonijevic and Wimperis in which the first-order quadrupolar interaction is refocussed at the end of $t_1^{[39]}$. The sequence for the shifting d-echo sequence of Walder et al. is shown in (b)^[40], where the SA is refocussed at the end of t_1 . The time points at which the shift/shift anisotropy, and the quadrupolar interactions are refocussed are indicated on the relevant symmetry pathways with arrows. Filled rectangles indicate pulses with a nominal flip angle of 90° and unfilled rectangles indicate pulses with a nominal flip angle of 180°. All pulses have phase x.

along $t_1 = t_2$. After an appropriate two-dimensional Fourier transform combined with a shear we obtain a spectrum in which the quadrupolar broadening is present only in one dimension whereas the shift/SA appears in the other.

There is room for improvement in this experiment as the coherence-order selection in each solid echo only retains half the total observable signal components, and so the sequence gives a maximum sensitivity that is one quarter of that theoretically attainable in the one-dimensional experiment. An improvement to the shifting *p*-echo experiment was proposed by Walder et al. who developed the shifting *d*-echo pulse sequence shown in Figure 1.12 (b)^[40]. Here the solid echo during the evolution period is replaced with a spin echo, with the result that the *p*- and *d*-symmetry pathways are altered so that it is the *p*-echo

that is now formed at $t_2 = 0$ and the *d*-echo that is formed at $t_1 = t_2$. As for the shifting p-echo experiment, complete separation of the two interactions is achieved using the same shearing and scaling transformations. The use of the spin echo is advantageous for two reasons: firstly the zero-frequency spike in the quadrupolar dimension is eliminated, and secondly the spin echo does not suffer from the reduction in signal intensity of the solid echo, with the result that the shifting d-echo experiment has double the sensitivity of the shifting pecho experiment. The disadvantage of the shifting d-echo sequence is the lower bandwidth of the 180° pulse compared to the 90° pulse this bandwidth issue has recently been tackled using adiabatic pulses. In order to improve the phase cancellation from the shift interaction and phase acquired due to the timedependent adiabatic pulse phase, a pair of adiabatic pulses have been added by Aleksis et al.^[41] before and after the $\frac{\pi}{2}$ pulse as illustrated in Figure 1.13a). The quadruple adiabatic shifting d-echo sequence appears to be superior since the spectrum of Figure 1.13b) shows no trace of any artefacts and yields a clear lineshape characteristic of the two interactions. Therefore this sequence is beneficial for complex materials with large shift anisotropies, and more than one local environment where the artefact would degrade the spectrum to the point of interfering with its assignment and interpretation.

Whilst the shifting p- and d-echo experiments have proven useful for characterizing ²H environments in paramagnetic systems, the experiments have the disadvantage of only being applicable to static samples, which result in spectra that suffer from low resolution and sensitivity. Magic-angle spinning alleviates these problems, but does require different pulse sequences in order to obtain a separation of shift/SA and quadrupolar effects. Such as separation and correlation has recently been achieved by Aleksis and Pell using the Phase-Adjusted Spinning Sidebands (PASS) experiment.^[42] The first order quadrupolar nuclei appears along an axis parallel to the direct dimension ω_2 while the shift anisotropy does so along the anti diagonal axis $\omega_2 = -\omega_1$. Hence the shift anisotropy only appears along the indirect dimension ω_1 while both quadrupolar interaction and shifts appear along the direct dimension ω_2 . A shearing transformation similar to that required for a shifting *d*-echo has to be applied to the spectrum in order to remove the shift anisotropy from the ω_2 dimension. It has been demonstrated that PASS shows high sensitivity and allows total separation of both interactions.

1.3.6.2. Half-integer-spin Quadrupolar Nuclei

If Central Transition (CT) is not broaden by the first order quandrupolar interaction, the satellite transitions (ST) are, and both CT and ST are are



Figure 1.13: Pulse sequences for and quadruple adiabatic shifting d- (a) echo sequences and the according experimental 2D spectra of ²H in CuCl₂ D₂O b). Here the filled rectangle represent $\frac{\pi}{2}$ pulses and rectangle with a diagonal represent adiabatic pulses. τ_1 , τ_2 and $\Delta \tau$ are free precession periods to facilitate correct echo formation. p_1 and d_1 are the symmetry pathways for the experiments. Adapted with permission from ^[41]. Copyright (2019) Elsevier.



Figure 1.14: (a) The PASS sequence, unless otherwise specified, the filled and empty rectangles represent RF pulses with flip angles $\frac{\pi}{2}$ and π , respectively. (b) Schematic representation of the time domain of the PASS experiment before and after shearing parallel to pseudo- t_1 dimension with a shear ratio of $\kappa = -1$ The blue, orange and green arrows represent time-independent (TI) p-echo ridge, the d-echo ridge and the time-dependent (TD) p-echo ridge. (c) Experimental ²H PASS spectra of two paramagnetic solids on the left CuCl₂,2D₂O t and on the right Ni(CD₃C00)₂,4H₂O. Both spectra have been sheared. The projection of the isotropic shift/quadrupolar dimension shows the spinning-sideband manifold, while the projection of the shift anisotropy dimension shows the profile of the spinning sideband intensities. Adapted with permission from ^[42]. Copyright (2021) American Institute of Physics.

broaden by the (C)SA and second order quadrupolar interaction therefore one must be especially cautious about their separation. Since the introduction of the multiple quantum magic angle spinning experiment (MQMAS) and the satellite transition magic angle (STMAS) experiment, the spectroscopist can rely on an efficient method to record resolved NMR spectra of half integer quadrupolar nuclei. However, in paramagnetic systems, half integer quadrupolar nuclei experience the inhomogeneous paramagnetic broadening from various sources. This includes contributions from the system itself, the most important of which is the anisotropic bulk magnetic susceptibility (ABMS), which arises for systems exhibiting an anisotropic magnetic susceptibility, and is due to the nature of the packing of the crystallites in the sample container, and contributions from the instrumental setup, such as temperature gradient across the sample as a result of frictional heating due to MAS, and which is particularly problematic for paramagnetic systems due to the strong Curie–Weiss temperature dependence of the shifts. These paramagnetic broadening effects broaden the individual sidebands due to MAS, and thus broaden the central transition resonance. When combined with the broadening due to the second-order quadrupolar interaction, this results in a central transition lineshape that is particularly difficult to interpret, thus requiring careful separation of the paramagnetic and second-order quadrupolar contributions.

Initially used separate the STs and enhance the CT signal intensity by a factor of $I+\frac{1}{2}$ the Rotor Assisted Population Transfer (RAPT) was used to estimate quadrupolar couplings on systems broaden by paramagnetic effects^[43] but still cannot separate shift/SA from quadrupolar coupling. The Correlation of Anisotropies Separated Through Echo Refocusing (COASTER)^[44] method has been successfully used, but requires sample spinning at the unconventional angle of 70.18° therefore this experiment remains difficult to implement on commercial ssNMR probes.

Carvalho et al recently proposed a new way of processing STMAS and MQMAS to separate in orthogonal dimensions the second-order fourth rank quadrupolar interaction tensor and the inhomogeneous paramagnetic broadening.^[45] This method is based on the Two dimensional one pulse (TOP) transformation. A first shearing is applied after which a new two-dimensional time domain system is constructed in which shift and ABMS broadening are separated along two separated axes. A second shear transformation is applied yielding a pure quadrupolar and a pure shift dimension. Figure 1.15 (a) shows the structure of an yttrium-aluminium garnets (YAG) (b) and (c) show TOP-STMAS spectra acquired on samples doped with Europium and Neodymium respectively.



Figure 1.15: (a) Structure of an Ln:Yttrium-aluminium garnet (YAG) and (b) a molecular cluster defined by the atoms present in the Ln-Y coordination shell. Three types of aluminium environments are represented : six coordinates (1) and four coordinates (2,3). (b-c) TOP-STMAS spectra acquired on samples doped with Neodymium and Europium respectively. Adapted with permission from ^[45]. Copyright (2020) John Wiley and sons.



Figure 1.16: (a) MAT spectrum of NaMnO₂ exhibiting six resonances with distinct observed shifts. Inset spectra correspond to extracted direct-dimension slices, and the observed shifts correspond to the centerbands marked by stars. RAPT pulses were applied before the MAT experiment to enhance the signal-to-noise ratio and suppress the signals arising from the satellite transitions. (b-c) Build up of the RAPT enhancement obtained for two of the ²³Na resonance of the MAT spectrum in (a) versus repetition frequency of RAPT pulses. Red circles represent data points while black lines are representation of the moving average. Adapted with permission from ^[46]. Copyright (2022) Elsevier.

The Use of RAPT-enhanced MAT experiment (or RAPT-MAT) allows to gain the needed resolution to plot the RAPT enhancement for a given resonance versus the repetition frequency of RAPT pulses.^[46] The maximum enhancement can be determined from such buildup and be used to extract the magnitude and range of the quadrupolar couplings. Figure 1.16 a) shows the resolved, RAPT-enhanced MAT spectra of NaMnO₂. The shift resolution allowed by this experiment is then used to obtain the buildup curves shown in Figure 1.16 (c).

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