Relaxation-Assisted Separation of Overlapping Patterns in Ultra-Wideline NMR Spectra

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Abstract

Efficient acquisition of high-quality ultra-wideline (UW) solid-state NMR powder patterns in short experimental time frames is challenging. UW NMR powder patterns often possess inherently low S/N and usually overlap for samples containing two or more magnetically distinct nuclides, which obscures spectral features and drastically lowers the spectral resolution. Currently, there is no reliable method for resolving overlapping powder patterns originating from unreceptive nuclei affected by large anisotropic NMR interactions. Herein, we discuss new methods for resolving individual UW NMR spectra associated with magnetically distinct nuclei by exploiting their different relaxation characteristics using 2D relaxation-assisted separation (RAS) experiments. These experiments use a non-negative Tikhonov fitting (NNTF) routine to process high-quality $T_1$ and $T_2^{\text{eff}}$ relaxation datasets in order to produce high-resolution, 2D spin-relaxation correlation spectra for both spin-1/2 and quadrupolar nuclei in organic and organometallic solids under static (i.e., stationary) conditions. It is found that (i) $T_2^{\text{eff}}$ RAS datasets can be acquired in a fraction of the time required for analogous $T_1$ RAS datasets, since a time-incremented 2D dataset is not required for the former; and (ii) Tikhonov regularization is superior to conventional non-negative least squares fitting, as it more reliably and robustly results in cleaner separation of patterns based on relaxation time constants.

1. Introduction

Many elements that are of great importance in chemistry, physics, biology, and materials science have isotopes that are unreceptive to the NMR experiment due to low gyromagnetic ratios ($\gamma$), low natural abundances, and/or unfavorable relaxation characteristics (i.e., long longitudinal and/or short transverse relaxation
time constants, denoted $T_1$ and $T_2$, respectively). For solid-state NMR (SSNMR), the problem of sensitivity is further exacerbated by the anisotropy (i.e., orientation-dependence) of the NMR interactions, which give rise to inhomogeneously broadened powder patterns that span wide spectral regions. So-called ultra-wideline NMR (UW NMR) powder patterns are extremely broad, possess low signal-to-noise ratios (S/N), and have poor resolution. Such spectra are commonly observed for both spin $I = 1/2$ nuclides affected by large chemical shielding anisotropies (CSAs) and quadrupolar nuclides ($I > 1/2$) influenced by both large quadrupolar coupling constants ($C_Q$) and/or large CSAs. Enhancement of signal and improvement of pattern resolution remain as prime challenges in UW NMR spectroscopy.$^{1,2}$

Several acquisition strategies have been developed to improve the low S/N ratios commonly observed in UW NMR spectra. Foremost among these strategies is the use of high magnetic field strengths; also common are specialized hardware and pulse sequences designed for the study of unreceptive nuclides. The former is ideal for half-integer quadrupolar nuclei, since the pattern breadths scale at the inverse of the applied magnetic strength, but is less effective for patterns arising from spin-half nuclei for which the CSA is dominant (breadths scale proportional to the field strength) or integer spin nuclei (breadths do not scale with field strength). The latter remain the most direct and cost effective way of enhancing the S/N. For instance, WURST (Wideband, Uniform-Rate, Smooth-Truncation) pulses$^{3,4}$ provide uniform excitation of UW NMR powder patterns, through the combined modulation of their amplitude and phase.$^{5,6}$ The so-called WURST-CPMG (WCPMG) pulse sequence (see Supporting Information, SI, Figure S1a), which uses a series of WURST refocusing pulses for $T_2$-dependent signal enhancement, has proven particularly effective for collecting high-quality UW NMR powder patterns for both spin-1/2 and
quadrupolar nuclei. Cross-polarization (CP) from abundant high-$\gamma$ to dilute low-$\gamma$

nuclei is also extensively employed for enhancing S/N, and is particularly useful for
studying unreceptive nuclides with long $T_1$ constants. Conventional CP employing
monochromatic, rectangular spin-locking pulses can have limited use in the
collection of UW NMR spectra due to its narrow excitation bandwidth. The
Broadband Adiabatic-Inversion Cross-Polarization pulse sequence (BRAIN-CP,
Figure S1b), which employs a frequency-swept WURST pulse on the low-$\gamma$ nucleus
during the spin locking period, provides the broad excitation bandwidth necessary for
rapidly collecting distortionless, high S/N UW NMR spectra. The BRAIN-
CP/WURST-CPMG pulse sequence (BCP for brevity) has been used to collect CSA-
broadened UW NMR spectra for spin-1/2 nuclides in inorganic materials, as well as
$^{14}$N NMR spectra of organic compounds, both under static conditions (i.e., stationary
samples).

More challenging remains the issue of improving site resolution in UW NMR
spectra. Spectral resolution in SSNMR experiments is usually improved by magic-
angle spinning (MAS), which spatially averages all anisotropic NMR interactions to
first order (e.g., the chemical shift and first-order quadrupolar interactions).

Unfortunately, for most UW NMR applications, MAS is insufficient for efficiently
averaging the anisotropic interactions, given the unrealizable spinning speeds that
are needed for dealing with powder patterns that are several hundred kHz to several
MHz in breadth. Moreover, for half-integer quadrupolar nuclides, it is not possible
to completely average the inhomogeneous broadening that results from the
quadrupolar interaction by spinning at any fixed rotor angle. In fact, it has been
demonstrated for MAS NMR spectra of both spin-1/2 and half-integer quadrupolar
nuclides with very broad anisotropic patterns that there are numerous challenges
associated with accurately extracting reliable tensor parameters, including (i) the difficulty of uniformly exciting the entire spinning sideband (SSB) manifold, (ii) the need for very stable MAS and an accurately set magic angle, (iii) low S/N due to the wide spread of spinning sideband frequencies, (iv) the need for multiple spinning speeds to accurately determine isotropic chemical shift values, and (v) residual inhomogeneous broadening that results from the second-order quadrupolar interaction, which is encountered even in the narrowest central-transition (CT) powder patterns of half-integer quadrupolar nuclei. Techniques like multiple-quantum MAS (MQMAS), satellite-transition MAS (STMAS) and hardware-related methods (e.g., DOR and DAS) are capable of averaging second-order quadrupolar anisotropies, but are only effective for quadrupolar nuclides with relatively small values of $C_Q$.

With these sensitivity and resolution challenges in mind, this work discusses a technique that can be used for resolving overlapping UW NMR powder patterns arising from magnetically distinct nuclides in static NMR spectra, while endowing them with enhanced S/N. As a starting point for separating overlapping powder patterns, we extend the idea of relaxation-assisted separation (RAS) initially proposed by Frydman et al., and exploit the different relaxation characteristics at magnetically inequivalent sites to resolve their powder patterns. Non-negative Tikhonov fitting (NNTF) routines are used to process both $T_1$ inversion recovery (IR) and $T_2$ Carr-Purcell Meiboom-Gill (CPMG) relaxation datasets. The first part of this paper introduces the key mathematical concepts used in the NNTF routine, and outlines the strategy for generating the ensuing two-dimensional (2D) RAS spectra. In the second part of this paper, the application of this approach is demonstrated for several experimental and simulated relaxation datasets that were collected for both
spin-1/2 and half-integer quadrupolar nuclides. In particular, it is demonstrated that significant experimental time savings are afforded by collecting $R_2$ RAS spectra (\textit{i.e.,} by using the NNTF routine to process one-dimensional $T_2$ CPMG datasets).

Additionally, it is shown that using a \textit{regularized} multi-exponential fitting procedure in the form of \textit{non-negative Tikhonov regularization} to generate $R_1$ and $R_2$ RAS spectra (\textit{i.e.,} by processing $T_1$ and $T_2$ datasets, respectively) allows for the separation of patterns arising from magnetically non-equivalent nuclei – even if they have similar relaxation constants.

\section{2. Theory and Numerical Methods}

All 2D relaxation and diffusion NMR data can be modeled according to a 2D Fredholm integral equation of the first kind:\cite{31}

\begin{equation}
    g(t, t) = K(s_1, s_2, t) f(s_1, s_2) dt + e(t, t)
\end{equation}

where $g(, t)$ is the 2D NMR signal acquired at times and $t$, $K(s_1, s_2, t)$ is the model function known as the \textit{kernel} that describes the 2D NMR signal, $f(s_1, s_2)$ is the \textit{density distribution function} representing the distribution of diffusion coefficients or relaxation time constants and $e(, t)$ represents the experimental noise.

Since the focus of this work involves collecting and processing $T_1$ and $T_2$ datasets for the purposes of separating overlapping powder patterns, the following discussion will be limited to Fredholm integral equations that describe these relaxation processes. Therefore, Eq. 1 can be rewritten to explicitly model $T_1$ or $T_2$ relaxation behavior:

\begin{equation}
    g(t, t) = K(R_j, n, t) f(R_j, n) dt + e(t, t)
\end{equation}
where $g(\tau, t)$ is the 2D $T_1$ or $T_2$ relaxation dataset, $K(R_j, \tau, t)$ is the kernel function describing the spins’ evolution frequencies and the relaxation rates $R_j$ (where $j = 1, 2$ denote $T_1^{-1}$ and $T_2^{-1}$, respectively), and $f(R_j, \tau)$ describes the distribution of NMR powder patterns separated on the basis of relaxation rate and frequency $\nu$.

Separating overlapping powder patterns originating from magnetically distinct sites according to differences in their $R_1$ and $R_2$ relaxation rates is achieved by extracting $f(R_j, \tau)$ from $g(\tau, t)$. This in turn requires: (i) Collecting $g(\tau, t)$ using either inversion/saturation recovery or CPMG in-plane refocusing pulse sequences, which encode the relaxation behavior in a pseudo-indirect dimension ($F_1$) and the spins’ evolution frequencies (chemical shifts, anisotropic patterns, etc.) in the direct dimension ($F_2$), as functions of $\tau$ and $t$, respectively. Corresponds either to the relaxation delay time that is incremented in an inversion/saturation recovery experiment or the times at which transverse magnetization forms coherent spin-echoes during a CPMG experiment. (ii) Fourier transforming $g(\tau, t)$ along $F_2$ for every value of $\tau$ to extract the frequency distributions for each inequivalent site, giving the new dataset, $P(\tau, \nu)$. (iii) Subjecting $P(\tau, \nu)$ to a multi-exponential fit for each frequency point to obtain the desired $f(R_j, \tau)$ dataset, which represents a preliminary 2D RAS spectrum. This is accomplished by defining an appropriate kernel function that specifically describes the relaxation process encoded in $F_1$; e.g.,

$$K(R_1, \tau, t) = \exp(i \tau) \left[ 1 - 2 \exp(-t R_1) \right]$$

and

$$K(R_2, \tau, t) = \exp(i \tau) \left[ \exp(-t R_2) \right]$$

for $T_1$ IR and $T_2$ CPMG datasets, respectively. Fitting the distributions contained within this kernel to the measured data for each value of $\nu$, can be formalized by the following Fredholm integral equation of the first kind:
This step is the most challenging in this kind of analysis, as it amounts to solving an ill-posed inverse problem. These problems – which are more formally described below – may fail to produce accurate and unique solutions in general, especially for datasets contaminated with noise (vide infra). (iv) Assuming that Eq. 3 has been solved, the last step in the RAS procedure involves post-processing the RAS spectrum to correct for numerical artifacts and/or the effects of anisotropic relaxation; details on how this is accomplished are also provided below.

2.1. Discrete Ill-Posed Inverse Problems

An inverse problem involves calculating a set of unknown input parameters, for a given set of known output data, according to a model function that describes some physical system or process.\textsuperscript{32,33} Inverse problems are classified as ill-posed if their solutions are either not unique or exhibit extreme sensitivity to noise (i.e., if a small perturbation to the data can cause a large fluctuation in the solution). To appreciate the ill-posed nature of RAS, Eq. 3 is rewritten in the discretized form that reflects the experimental manner in which these 2D NMR data are collected:

\[
P_i = K f_i + e_i
\]

where \( P_i \in \mathbb{R}^{m \times 1} \), \( K \in \mathbb{R}^{m \times n} \), \( f_i \in \mathbb{R}^{n \times 1} \) and \( e_i \in \mathbb{R}^{m \times 1} \) represent the discrete forms of the functions described in Eq. 3, and \( i \in \{1, \ldots, N\} \) with \( N \) representing the total number of frequency points acquired in \( F_2 \). Notice that \( f_i \) and \( P_i \) in Eq. 4 are expressed as 1D vectors, even though their functional analogues in Eq. 3 are two-dimensional. This discretized representation is valid because Eq. 4 is minimized for each column
belonging to $P(\cdot,\cdot)$ (i.e., for each frequency point, which is denoted with the index $i$).

The resulting solution vectors $f_i$ are then assembled to form the preliminary 2D RAS spectrum. Within this formalism, it is convenient to define a new vector, $\hat{P}_i$, representing the sum of the unadulterated noiseless data ($P_i$) and the experimental noise ($e_i$). Solutions to Eq. 4 can then be determined by a least-squares (LS) analysis

$$\min_{f_i} \| K f_i - \hat{P}_i \|^2$$

where $\| \cdot \|^2$ denotes the Euclidean norm, $l_2$. A singular value decomposition (SVD) of the kernel matrix, $K$, is a matrix factorization procedure that lends considerable insight into the nature of discrete ill-posed inverse problems. The SVD of the kernel matrix, $K$, appearing in Eqs. 4 and 5, is defined by the factorization:

$$K = U S V^T = \sum_{j=1}^{r} u_j v_j^T$$

Here, $U = (u_1, \ldots, u_m)$ and $V = (v_1, \ldots, v_n)$ are orthogonal matrices whose columns are the left and right singular vectors of $K$, $S = \text{diag}(s_1, \ldots, s_n)$ is a diagonal matrix whose non-negative entries are the singular values of $K$ arranged in decreasing magnitude as the index $j$ increases, and $r = \text{rank}(K)$. Two characteristic features of ill-posed inverse problems are: (i) the singular values of $K$ gradually decay to zero and (ii) the right singular vectors $v_j^T$ become more oscillatory as $j \to r$. Both of these features amplify the noise in the experimental data, thereby complicating the determination of an accurate solution. This can be visualized by
considering a generic solution to **Eq. 4**, which can be determined by using the Moore-Penrose pseudoinverse

\[ K^+ = \sum_{j=1}^{r} \frac{v_j u_j^T}{s_j} \]  

The resulting solution possessing the smallest \( l_2 \) norm can be represented as a sum of two components: one originating from the pure unadulterated data and the other originating from the noise. The latter can be represented as:

\[ K^+ e_i = \sum_{j=1}^{r} \frac{v_j u_j^T}{s_j} e_i \]  

As \( j \to r \), the magnitude of \( v_j \) increases, \( v_j \) becomes more oscillatory, and the contributions of the noise vector \( e_i \) become amplified, leading to solutions that are ultimately meaningless.

### 2.2. Stabilizing the Solutions of Discrete, Ill-posed Inverse Problems

Finding suitable solutions to inverse problems possessing ill-posed characteristics requires imposing additional constraints on the desired solution. Regularization refers to a variety of numerical methods that stabilize the solutions of ill-posed inverse problems, primarily by filtering out the high-frequency oscillations of the singular vectors associated with small singular values. This filtering process can take on many different forms of varying sophistication. The key to a successful numerical regularization scheme is to choose constraints that (i) effectively suppress the high-frequency components of the kernel matrix that amplify the noise, and (ii) return regularized solutions that are close approximations to the desired solution.

One of the most basic regularization schemes is the *non-negative least squares* (NNLS) algorithm, which was used to solve **Eq. 5** and generate \( R^1 \) RAS.
spectra in ref. 30 This algorithm imposes the regularized solution \( \hat{f}_{i}^{\text{reg}} \) to be non-negative according to:

\[
\hat{f}_{i}^{\text{reg}} = \min_{\hat{f}_{i} \geq 0} \left\| K \hat{f}_{i} - \hat{P} \right\|^2 \quad (9)
\]

This non-negativity constraint is valid in this case because the desired solution represents a distribution of non-negative relaxation rates/times. However, the NNLS algorithm fails to address the problematic small singular values; therefore, \( \hat{f}_{i}^{\text{reg}} \) remains sensitive to the noise.

**Tikhonov regularization**\(^{33,41}\) is perhaps the most popular method for stabilizing the solutions of such ill-posed inverse problems. This involves minimizing an ancillary constraint \( \left\| L (\hat{f}_{i} - \hat{f}_{i}^0) \right\|^2 \) with respect to the minimization of \( \left\| K \hat{f}_{i} - \hat{P} \right\|^2 \), which are commonly referred to as the solution semi-norm and residual norm, respectively, to give:

\[
\hat{f}_{i}^{\text{reg}} = \min_{\hat{f}_{i}} \left\{ \left\| K \hat{f}_{i} - \hat{P} \right\|^2 + \left\| L (\hat{f}_{i} - \hat{f}_{i}^0) \right\|^2 \right\} \quad (10)
\]

Here, \( L \) is either the identity matrix \( I_{n} \) or a discrete approximation to a derivative operator, \( \hat{f}_{i}^0 \) is an initial guess of the solution, and \( \lambda \) is a unitless positive variable known as the **regularization parameter**, which scales the magnitude of the stabilizing solution semi-norm with respect to the residual norm.

It is convenient to examine the filtering effects of Tikhonov regularization when **Eq. 10** is in standard form (i.e., when \( L = I_{n} \) and \( \hat{f}_{i}^0 = 0 \)). The regularized solution can then be written as:\(^{32,33,42}\)

\[
\hat{f}_{i}^{\text{reg}} = \frac{1}{2} \sum_{j=1}^{r} \lambda_j \hat{f}_{i}^j \hat{P}_j \quad (11)
\]
Tikhonov regularization adds $\frac{1}{2} j$ to each of the $\frac{1}{2} j$ so that as $j \rightarrow r$, $\frac{1}{2} \frac{j}{j} \rightarrow 0$,

thereby effectively dampening the contributions of both the small $j$ and oscillatory

$v_j$ on $\hat{f}_j^{\text{reg}}$. Moreover, the addition of the weighted side constraint, $\frac{1}{2} \left\| L(\hat{f}_j - \hat{f}_j^0) \right\|^2$, produces regularized solutions possessing smaller $l_2$ norms. Choosing an optimal value of $\lambda$ is crucial and can be achieved in a number of ways; in the present study, this was done by using the $L$-curve routine.$^{43}$

2.3. Non-Negative Tikhonov Fitting Routine:

Multi-exponential fits of $T_1$ and $T_2$ datasets were performed using a numerical algorithm combining Tikhonov regularization with non-negativity constraints. This non-negative Tikhonov fitting (NNTF) routine uses the built-in NNLS function within the MATLAB 8.1.0 environment to evaluate

$$\hat{f}_j^{\text{reg}} = \min_{f_j \geq 0} \left\| \begin{bmatrix} K & L & \hat{f}_j & \hat{p}_j & 0 \end{bmatrix} \right\|^2$$

(12)

where $L$ is the discrete second-order derivative operator that was calculated using the regularization toolbox for MATLAB$^{42}$ and $\hat{f}_j^0 = 0$. The processing procedure used to generate $R_1$ RAS spectra is similar to the method discussed in ref. 30; therefore, only the processing procedure used to generate $R_2$ RAS spectra is discussed herein (Figure 1). As mentioned above, WCPMG or BCP pulse sequences were used to collect a 1D CPMG echo train, which was rearranged into a 2D data matrix by sequentially placing each echo along the rows of the matrix with the points comprising each echo occupying the columns of the matrix (Figure 1a). These individual echoes were then apodized, zero-filled, Fourier transformed with respect
to \( t \), and magnitude processed (Figure 1b). The NNTF algorithm was then used to evaluate Eq. 12 for each frequency point using the optimal \( \lambda \) value\(^a\) determined from the L-curve routine\(^42\) and 1000 \( R_2 \) constants\(^b\) logarithmically spaced between 10 and 10000 s\(^{-1}\). The resulting solution vectors were then assembled to form the preliminary 2D RAS spectrum (Figure 1c). A post-NNTF processing routine was then employed to correct for numerical artifacts and anisotropic relaxation (see Fig. 1 caption for description). The signal intensity for each frequency bin falling within this specified region was then added to form the powder pattern for each magnetically distinct site with unique relaxation characteristics (Figure 1e, 1f). This pattern was then positioned at the average relaxation rate determined for the corresponding site in the final post-NNTF 2D RAS spectrum (Figure 1d). The post-processed spectrum is often displayed side-by-side with the RAS spectrum that has not undergone post-NNTF processing, since the latter provides information on the relaxation behavior \( (i.e., \) accurate measures of the relaxation constants and information on anisotropic relaxation), while the former provides an accurate measure of the NMR parameters for each of the overlapping spectra \( (\text{vide infra}) \).

3. Experimental Section

Samples. Samples of RbCH\(_3\)CO\(_2\), RbClO\(_4\), Ga(acac)\(_3\) (acac = acetylacetonate), (CH\(_3\)CH\(_2\)CH\(_2\)CH\(_2\))\(_2\)SnO (abbreviated as (n-Bu)\(_2\)SnO)), and SnPc (Pc = phthalocyanine) were purchased from Strem Chemicals, Inc. and used without

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\(^a\) The optimal \( \lambda \) value was calculated for each frequency point, and then the average of these values was used to evaluate Eq. 12.

\(^b\) In general, using more \( R_2 \) values in the NNTF routine gives a better multi-exponential fit at the cost of increased computer processing time. Using 1000 \( R_2 \) values in the NNTF routine maintained a balance between accuracy and overall computational time.
further purification. GaPcCl was purchased from Sigma Aldrich and used without further purification. The following w/w mixtures were prepared: 40:1 RbCH₃CO₂:RbClO₄, 10:1 GaPcCl:Ga(acac)₃, and 1:1 (n-Bu)₂SnO:SnPc. These sample ratios, while not necessarily representative of ratios of sites encountered in naturally-occurring or “real world” samples, were chosen in order to produce spectra that allow for clear visualization of the powder patterns from each distinct site. All samples – whether pure chemicals or co-mixed in appropriate w/w ratios – were ground into fine powders and packed in shortened 5 mm o.d. glass NMR tubes.

**Solid-State NMR Spectroscopy.** NMR experiments were performed under static conditions using a Varian InfinityPlus NMR console with an Oxford 9.4 T (ν₀¹H) = 400 MHz) wide-bore magnet operating at ν₀⁸⁷Rb = 130.79 MHz, ν₀⁷¹Ga = 121.794 MHz, and ν₀¹¹⁹Sn = 149.04 MHz. A Varian/Chemagnetics 5 mm double-resonance static wideline probe was used for all NMR experiments. Pulse width calibrations for all nuclides were performed on their respective solution-state standards. ⁸⁷Rb (I = 3/2) and ⁷¹Ga (I = 3/2) chemical shifts were referenced to 1.0 M solutions of RbNO₃ (aq) and Ga(NO₃)₃ (aq), respectively with δiso = 0.0 ppm for each nuclide.⁶,⁴⁴ ¹¹⁹Sn (I = 1/2) chemical shifts were referenced to neat Sn(CH₃)₄ (l) with δiso = 0.0 ppm.¹⁵

All direct excitation experiments used radio-frequency (rf) field strengths (i.e., ν₁ = ω₁/2π) between 10 and 30 kHz. Spin-locking fields ranging from 15 – 40 kHz were employed for cross-polarization experiments. WURST-80 pulses were used in all experiments, which were linearly swept over symmetric offsets with a total sweep range slightly larger than the total breadth of the powder pattern to be acquired. 15000 equally spaced points were used to define the amplitude and phase modulated waveforms for the WURST-A pulse and 900 equally spaced points were
used to define the amplitude and phase modulated waveforms for the WURST-B and
WURST-C pulses (see Figure S1 for the sequence’s scheme). Spectra of
compounds having protons were acquired using $^1$H continuous-wave (CW)
decoupling ranging between 10 and 50 kHz.

**Spectral processing and simulations.** All data were processed on a personal
computer using custom-written code for MATLAB; this code is available from the
authors upon request. Analytical simulations of NMR spectra were performed with
the WSOLIDS simulation package and SPINEVOLUTION, as noted. Further
experimental and processing details are provided in the SI and in the text.

4. Results and Discussion

There are four fundamental differences distinguishing the RAS method
described in this work and that of Frydman et al. In the current work: (i) Pulse
sequences employing broadband WURST pulses were used to collect all datasets.
This is essential for efficiently collecting distortion-free UW NMR powder patterns
that result from large anisotropic NMR interactions (i.e., CSA and quadrupolar) with
sufficient S/N. (ii) $T_1$ relaxation datasets were collected using broadband IR
experiments rather than SR experiments, because simulations indicate that the
NNTF processing of such $T_1$ datasets yield RAS spectra with higher resolution (as
also noted by Frydman et al.). (iii) All 2D RAS spectra were generated by
processing relaxation datasets with a MATLAB-based algorithm that combines NNLS
fitting with Tikhonov regularization (i.e., NNTF). Regularization is essential for
stabilizing the solutions of ill-posed inverse problems, and in this case, is crucial for
obtaining accurate multi-exponential fits of decaying signals. This is especially
important when processing datasets possessing limited S/N and/or multiple
overlapping patterns originating from magnetically distinct sites that have similar relaxation constants (vide infra). (iv) This NNTF algorithm was also applied to $T_2$ datasets (i.e., CPMG echo trains) so that overlapping powder patterns can be separated according to differences in effective $T_2$ constants (i.e., $T_2^{\text{eff}}$ denotes the effective $T_2$ constant that results under conditions of $^1$H decoupling, in which contributions to transverse relaxation from heteronuclear dipolar coupling are partially or wholly eliminated).

4.1 Theoretical Simulations of 2D RAS Datasets

There are several important factors that affect both the quality of the separations and the general applicability of the RAS technique to study challenging, "real-world" chemical systems. Some of these factors include the number of magnetically distinct sites (herein, only systems containing up to two magnetically distinct sites are discussed) and the difference in their relaxation constants, the S/N ratio of the relaxation datasets, the number of $F_1$ points (e.g., the number of CPMG echoes in a $T_2$ dataset), and the presence of spectral artifacts (e.g., DC offsets). To begin to address some of the issues that affect the success of a 2D RAS-based strategy for resolving inequivalent chemical sites, several $T_2$ relaxation datasets were simulated in which the aforementioned factors were independently manipulated or controlled. Subsequently, each dataset was processed with the NNTF routine in order to observe the influences of these factors on the 2D $R_2$ RAS spectra, and to determine how the acquisition or processing routines could be modified to produce spectra with optimal resolution and minimal distortion.

Figure 2 shows nine simulated $R_2$ RAS spectra, which were generated by applying the NNTF routine on simulated $^{35}$Cl CPMG datasets, each composed of
120 echoes (or equivalently each possessing 120 points in $F_1$) for two equally
populated chlorine sites with overlapping CT spectra. White noise was added to
each CPMG dataset prior to Tikhonov fitting to give desired S/N ratios of 100, 500,
and 1000 for the spectra shown in the first, second, and third column, respectively.
The $T_2^{(35\text{Cl})}$ constants for these two sites differ by a factor of 10, 4, and 2 for the
spectra shown in the top, middle, and bottom row, respectively. **Figure 2**
demonstrates how the NNTF algorithm separates the overlapping $^{35}\text{Cl}$ powder
patterns, for a given pair of $T_2^{(35\text{Cl})}$ constants as a function of the S/N ratio. The
best separation is achieved when the $T_2$ constants differ by a factor of 10 or more
($i.e., T_{2,A} = 1.0 \text{ ms and } T_{2,B} = 10.0 \text{ ms for site A and site B, Figure 2a-2c}$). The
separation between the patterns increases as the S/N ratio of the $R_2$ RAS spectrum
increases, from left to right across each row. When the $T_2$ constants differ by a
factor of 4 ($T_{2,A} = 2.5 \text{ ms and } T_{2,B} = 10.0 \text{ ms}$), which is the case presented in the
middle row, a S/N ratio of 100 is too low for the overlapped patterns to be clearly
separated (**Figure 2d**). Even so, the co-added projections of both patterns can still
be accurately extracted and fitted (**Figure S2**) by using the post-NNTF processing
procedure in this case. However, poor separation is achieved when the $T_2$ constants
differ by a factor of only 2 ($T_{2,A} = 5.0 \text{ ms and } T_{2,B} = 10.0 \text{ ms}$), as can be seen in the
bottom row for all three S/N ratios (**Figure 2g-2i**). Interestingly, with **a priori**
knowledge of the quadrupolar parameters for both sites, it is possible to resolve the
overlapping patterns using the post-NNTF processing procedure (**Figure S3**). It
should be noted that better separation might be possible when the $T_2$ constants for
the magnetically distinct nuclides differ by the same ratio, but have different
magnitudes ($i.e., 5 \text{ ms vs. } 10 \text{ ms and } 50 \text{ ms vs. } 100 \text{ ms}$). However, this is an issue
that will have to be resolved by conducting several new series of experiments, and is
not discussed further in this section. For these three simulated $R_2$ RAS spectra,
better separation is possible if more $R_2$ relaxation constants are used in the NNTF
algorithm (100 potential $R_2$ constants were used to process all nine spectra) and/or
when more echoes are collected (vide infra). In general, using more potential
relaxation constants in the multi-exponential fitting of relaxation data produces better
quality RAS spectra, with the only disadvantage being an increase in the
computational time. Furthermore, using a larger value of $\lambda$ than the one provided by
the L-curve routine, in combination with post-NNTF processing, could potentially
isolate each individual pattern (as was done for several of the experimental datasets
presented below). One caveat at this juncture is that there is not an accurate means
of evaluating the experimental errors in the $T_2$ values within the current numerical
regularization routine; rather, the 1D $T_2$ CPMG dataset can be fit using a multi-
exponential analysis and the resulting values can be compared against those
determined from the corresponding $R_2$ RAS spectrum. A full consideration of a
statistical analysis that separates experimental from processing uncertainties is
currently under consideration and beyond the scope of the current work.

Another parameter that influences the quality of $R_2$ RAS spectra is the
number of echoes collected in $F_1$ (i.e., the number of $F_1$ points). Figure 3 shows that
increasing the number of echoes leads to better separation of the powder patterns in
the $R_2$ RAS spectrum (due to increased resolution in the indirect dimension), which
may be especially useful for resolving inequivalent sites with very similar relaxation
constants (120, 240, and 480 echoes were simulated in Figure 3a, 3b, and 3c,
respectively). This is analogous to the collection of additional increments in standard
2D NMR experiments. The $T_2$ dataset simulated with 480 echoes gives an $R_2$ RAS
spectrum (Figure 3c) with the best separation between the overlapping patterns,
which makes it easier for the post-NNTF processing procedure to accurately extract
the projections for both site A and site B (Figure 3e and 3f, respectively).

Experimentally, however, it may not be feasible to collect so many echoes because
of either hardware limitations (e.g., a high duty cycle) and/or short $T_{2\text{eff}}$ constants. In
general, it is desirable to collect as many CPMG echoes as possible, which can be
achieved by simply increasing the number of CPMG loops in the pulse sequence.
One can also use techniques that increase the value of $T_{2\text{eff}}$, by attenuating
heteronuclear dipolar couplings, such as high-power $^1$H decoupling or isotopic
substitution of $^1$H with $^2$H. Another possibility is the use of variable-temperature
NMR to exploit temperature dependencies of the $T_{2\text{eff}}$ constants (where these
exist).47

4.2. $^{87}$Rb SSNMR of a 40:1 RbCH$_3$CO$_2$:RbClO$_4$ w/w mixture
$^{87}$Rb possesses several favorable NMR properties such as a high
gyromagnetic ratio ($\gamma = 8.78640 \times 10^7$ rad T$^{-1}$ s$^{-1}$, $v_0 = 130.79$ MHz at 9.4 T),
desirable relaxation characteristics (i.e., long $T_{2\text{eff}}$ and/or short $T_1$ relaxation time
constants), and a moderate nuclear quadrupole moment (NQM) of 133.5(5) millibarn.
$^{87}$Rb $T_1$ and $T_{2\text{eff}}$ relaxation datasets were collected for the model compounds
RbCH$_3$CO$_2$ and RbClO$_4$, as well as their mixture (vide infra). Each compound
possesses a single rubidium site (Figure S4a, S4b)44 with very different $^{87}$Rb
second-order CT powder patterns (quadrupolar NMR parameters have been
previously reported).44

The $T_1$($^{87}$Rb) and $T_{2\text{eff}}$($^{87}$Rb) relaxation time constants were measured for
each of the pure compounds. $T_1$ relaxation times were measured using a WURST-
CPMG IR sequence (Figure S1c), and $T_1$ constants were determined by sampling
five evenly spaced frequency points across the powder pattern; the five partially recovered powder patterns were then fit to standard formulas for each compound. This led to average $T_1(^{87}\text{Rb})$ values of ca. 109 ms and ca. 213 ms for RbCH$_3$CO$_2$ and RbClO$_4$ at room temperature, respectively (Table S3, S4). The $T_2^{\text{eff}}(^{87}\text{Rb})$ time constants were measured using the WCPMG echo trains (Figures 4a, 4b); exponential fitting of the resulting $T_2^{\text{eff}}$ decay curves led to average $T_2^{\text{eff}}(^{87}\text{Rb})$ of 1.16(7) ms and 14(1) ms for RbCH$_3$CO$_2$ and RbClO$_4$, respectively (Table S1). The details of collecting, processing, and analyzing the $R_1$ and $R_2^{\text{eff}}$ RAS spectra are explicitly described for these first two samples; similar methods were used for the remainder of the samples.

$T_2^{\text{eff}}(^{87}\text{Rb})$ echo trains were collected for RbCH$_3$CO$_2$ and RbClO$_4$ using the WCPMG sequence (Figures 4a and 4b, respectively) and processed as described in the SI. Figure 4d and Figure 4e show the WCPMG spectra of RbCH$_3$CO$_2$ and RbClO$_4$, respectively. Figure 4c shows the echo train of a 40:1 RbCH$_3$CO$_2$:RbClO$_4$ w/w mixture acquired with the WCPMG pulse sequence in ca. 40 minutes of acquisition time, and Figure 4f shows the resulting $^{87}\text{Rb}$ WCPMG 1D spectrum of the overlapping CT powder patterns. The 40:1 w/w ratio of this mixture was chosen due to the much stronger signal intensity of RbClO$_4$ in comparison to RbCH$_3$CO$_2$, resulting primarily from a $T_2^{\text{eff}}(^{87}\text{Rb})$ constant that is ten times longer and CT powder pattern that is six time narrower.

A $T_1$ dataset was collected for this mixture using the WCPMG IR pulse sequence (Figure 5a). The NNTF routine was then used to determine the distribution of $R_1$ relaxation rates (i.e., $R_1 = T_1^{-1}$) for each spectral frequency point. 1000 potential relaxation constants were used in all NNTFs (unless stated otherwise); the resulting 2D RAS spectrum (Figure 5b) separates the overlapping
resonances on the basis of frequency (direct dimension, $F_2$) and $R_1$ (indirect dimension, $F_1$).

It is clear from the contour plot in Figure 5c that the $T_1^{(87\text{Rb})}$ constants are too similar to produce a well-resolved $R_1$ RAS spectrum. The appearance of this spectrum is a consequence of the Tikhonov regularization used in the NNTF routine, which has the effect of broadening the powder patterns in the indirect dimension.

This loss in resolving power — which is akin to imposing a “line broadening” in the relaxation rate distribution — is primarily controlled by the magnitude of the regularization parameter $\lambda$ (see Figure S7). The optimal degree of regularization ($i.e.$, the value of $\lambda$) was determined by using the L-curve routine (see SI). While this allows one to discriminate overlapping patterns originating from inequivalent sites that possess similar relaxation time constants ($vide infra$), such regularization-imposed broadening often masks the differences arising from anisotropic relaxation.

Despite the very similar relaxation characteristics of the two sites, the 2D contour plot in Figure 5c displays regions in which the RbClO$_4$ or RbCH$_3$CO$_2$ powder patterns can be clearly identified (i) at points of maximum signal intensity (as indicated by the arrows) or (ii) best separation of the patterns (as indicated by the blue highlighted areas). At the points indicated by the arrows, $R_1$ ($T_1$) values of ca. 6.14 s$^{-1}$ (0.16 s) and ca. 17.84 s$^{-1}$ (0.056 s) are measured for RbClO$_4$ and RbCH$_3$CO$_2$, respectively. These values are slightly different than the ones that were measured by fitting the $T_1$ datasets for each of the individual compounds, primarily because of the large value of $\lambda$ employed in the NNTF, which causes a broadening of the powder patterns in $F_1$, thereby complicating an accurate measurement of the relaxation rates from the $R_1$ RAS spectrum. This dataset demonstrates, that in some cases, the areas of the RAS spectrum that give the best separation of the
overlapping powder patterns may be distinct from the areas that give increasingly accurate values of the relaxation constants for each of the sites.

The post-NNTF processing procedure discussed in the Theory Section was used to further refine the separation of these patterns. In this case, average $R_1$ values and co-added projections were calculated for each inequivalent chemical site by considering only the regions along $F_1$ that are highlighted in Figure 5c. The post-NNTF processed $R_1$ RAS spectrum (Figure 5d) clearly shows the separated $^{87}$Rb CT powder patterns, even though the $T_1(^{87}$Rb) constants for these compounds differ only by a factor of ca. 2 (Table S3, S4). These 1D projections of RbCH$_3$CO$_2$ and RbClO$_4$ can then be imported into an appropriate fitting program and their NMR parameters determined (Figures 5e, 5f); the resulting quadrupolar parameters are similar to those reported in the literature for both compounds.\textsuperscript{44}

An added advantage for using the NNTF routine over a basic NNLS algorithm to generate RAS spectra is the ability to apply the post-NNTF processing procedure over specific regions along the indirect dimension (\textit{i.e.}, in a “row-by-row” fashion along $F_1$), whereby the powder patterns may be partially or completely separated from one another. The post-NNTF processing procedure does not work properly for RAS spectra that were produced with an NNLS algorithm, since NNLS fitting is sensitive to the noise contained within relaxation datasets, which ultimately leads to distorted spectra for datasets having low S/N and/or for chemical sites having very similar relaxation constants (see Figure S8). Additionally, \textit{a priori} knowledge of the NMR parameters (\textit{e.g.}, the EFG and CS tensor parameters) for at least one of the overlapping patterns can greatly aid in defining the regions over which to apply the post-NNTF processing procedure.
The ability to resolve overlapped patterns according to differences in the $T_2^{\text{eff}}(^{87}\text{Rb})$ constants was also tested on this mixture by applying the NNTF routine to a 2D dataset generated by chronologically rearranging the 1D WCPMG train of echoes (Figure 4c). The reader is reminded that this 2D matrix is constructed from a 1D WCPMG $T_2$ dataset by sequentially placing each of the spin echoes along the rows of the matrix (e.g., a WCPMG echo train composed of 50 echoes with 100 points defined for each echo would form a 2D matrix of size 50×100). This 2D data matrix was Fourier transformed along the “direct” dimension (i.e., down each of the rows), magnitude processed, and then the NNTF routine was used to extract the distribution of $T_2^{\text{eff}}(^{87}\text{Rb})$ constants for each frequency point (i.e., down each of the columns). The ensuing $R_2^{\text{eff}}(1/T_2^{\text{eff}})$ RAS spectrum (Figure 6a) leads to two well-separated ridges – even if these show a substantial anisotropic $T_2$ dependence. The post-NNTF processing procedure was then used to calculate the co-added projections for each unique chemical site by adding up the total signal intensity pertaining to each of the well-separated patterns; when placed at their corresponding average $R_2^{\text{eff}}$ values, clear post-NNTF processed $R_2^{\text{eff}}$ separations are achieved (Figure 6b). Notice that for this mixture, the $R_2^{\text{eff}}$ RAS analysis is much better at separating the overlapped spectra than the $R_1$ RAS analysis, largely because the $T_2^{\text{eff}}(^{87}\text{Rb})$ constants for these two rubidium sites differ by a factor of ca. 10. Also, the S/N ratio in the $R_2^{\text{eff}}$ RAS spectrum (acquired with WCPMG) is higher than that observed in the $R_1$ RAS spectrum (acquired with WCPMG IR) for each increment in $F_1$. Additionally, more points could be collected in $F_1$ for the $R_2^{\text{eff}}$ dataset than that for $R_1$.

In general, the $R_2^{\text{eff}}$ RAS analysis is more robust than the $R_1$ RAS analysis, since the former simply requires a high-quality 1D CPMG dataset, which in turn
relies essentially on an optimized radio-frequency (rf) field and a long enough $T_2^{\text{eff}}$
constant to permit proper encoding of the transverse relaxation behavior. $R_1$ RAS, by contrast, is a 2D arrayed experiment requiring careful setting of the rf field and
sweep rate of the WURST-A pulse (i.e., to ensure the entire powder pattern is
uniformly inverted). Missets in these parameters can not only drastically increase
the overall experimental time, but can also affect the quality of the separations (vide infra). The projections of Rb$_2$CH$_3$CO$_2$ (Figure 6c) and RbClO$_4$ (Figure 6d) were fit
with NMR parameters similar to the ones reported in the literature. The approximate
values of the $R_1$($^{87}$Rb) and $R_2^{\text{eff}}$($^{87}$Rb) constants as determined from the RAS spectra
are shown in Table S2.

4.3. $^{71}$Ga SSNMR of a 10:1 GaPcCl:Ga(acac)$_3$ w/w mixture

$^{71}$Ga is a receptive half-integer ($I = 3/2$) quadrupolar nuclide, owing to its high
ratio ($\gamma = 8.18117 \times 10^7$ rad T$^{-1}$ s$^{-1}$, $\nu_0 = 121.794$ MHz at 9.4 T)
and high natural abundance (39.89%). The moderate NQM of $^{71}$Ga (107(1)
millibarn) most often results in broadened second-order CT powder patterns that can
span hundreds of kHz. These broad patterns often have inherently low S/N,
resulting in lengthy experimental times, and making it challenging to resolve multiple
overlapping patterns.

The $R_1$ and $R_2^{\text{eff}}$ RAS protocols were used to try to resolve the overlapping
$^{71}$Ga CT powder patterns resulting from a 10:1 gallium phthalocyanine chloride
(GaPcCl):gallium acetylacetonate (Ga(acac)$_3$) w/w mixture. The WCPMG spectrum
of this mixture (Figure 7c) shows the overlapped $^{71}$Ga powder patterns; the one with
a breadth of ca. 500 kHz corresponds to GaPcCl (Figure 7a) and the other (75 kHz
in breadth) corresponds to Ga(acac)$_3$ (Figure 7b). Applying the NNTF routine to the
$T_2^{\text{eff}}$ dataset of the 10:1 GaPcCl:Ga(acac)$_3$ mixture (Figure S5c), which was collected in ca. 47 minutes of acquisition time using WCPMG, yields a high-quality, post-NNTF processed $R_2^{\text{eff}}$ RAS spectrum (Figure 7d) in which the powder patterns corresponding to GaPcCl and Ga(acac)$_3$ are clearly separated. The 1D co-added projections for Ga(acac)$_3$ and GaPcCl are shown in Figure 7e and 7f, respectively. Both of these projections were fit with NMR parameters similar to the ones reported in the literature\textsuperscript{6}, which reinforces the fact that RAS is a suitable technique for not only identifying and separating overlapping powder patterns, but also useful for accurately obtaining the NMR tensor parameters that reveal detailed chemical information.

The NNTF routine was also applied to a $T_1$ dataset (Figure S6) for this mixture, which required ca. 13 hours of acquisition time using WCPMG IR. The resulting post-NNTF processed $R_1$ RAS spectrum is shown in Figure 7g.

Comparison of the spectra in Figures 7d and 7g clearly reveals higher S/N and better spectral resolution in the former, despite the fact that the $R_2^{\text{eff}}$ RAS spectrum was acquired ca. 16 times faster than its $R_1$ counterpart. Thus, while it is clearly advantageous to have the option to separate overlapping patterns based on either $R_1$ or $R_2$, it seems that the latter is preferable, since it only requires the acquisition of a single WCPMG spectrum.

4.4. $^{119}$Sn SSNMR of a 1:1 SnPc:(n-Bu)$_2$SnO w/w mixture

Tin possesses three NMR-active isotopes, with $^{119}$Sn being the preferred isotope for NMR experimentation due to its high receptivity resulting from a large gyromagnetic ratio ($\gamma = 10.03170 \times 10^7$ rad T$^{-1}$ s$^{-1}$, $\nu_0 = 149.04$ MHz at 9.4 T) and higher natural abundance (8.58%). Despite these relatively favorable NMR...
characteristics, $^{119}$Sn SSNMR spectra can be challenging to acquire since tin CSAs often broaden spectral breadths beyond both the excitation bandwidth of conventional monochromatic rf pulses, as well as the detection bandwidth of the probe. Furthermore, it is common for many tin-containing compounds to possess extremely long $T_1(^{119}$Sn) constants, necessitating recycle delays on the order of tens to hundreds of seconds in between scans, which prevent the retrieval of high-quality spectra in reasonable experimental timeframes. $^{7,49,50}$ $^{119}$Sn MAS experiments, which are used more often than static experiments, are subject to some of the experimental difficulties described in the Theory Section. $^1$H-$^{119}$Sn CP/MAS experiments are often employed to collect $^{119}$Sn powder patterns, exploiting the usually shorter recycle delays which depend on the $T_1(^1$H) constants. $^{51-53}$ However, since the excitation bandwidth over which CP is efficient is effectively determined by the length and power of the contact pulses, frequency-stepped acquisition must also be used to acquire these powder patterns, which in turn can lead to lengthy experimental times. $^{17,18,20,54}$ The recently developed broadband adiabatic inversion cross polarization (BRAIN-CP) pulse sequence effectively addresses the limited excitation bandwidths associated with so-called conventional CP experiments by using a frequency-swept WURST pulse as the X-channel spin-lock pulse (Figure S1b), which allows for the collection of high-quality UW NMR spectra under static conditions. $^{15}$

The BRAIN-CP/WCPMG (BCP for brevity) pulse sequence, which uses a train of WURST-CPMG pulses for refocusing (Figure S1b), was used to collect $^{119}$Sn spectra of (CH$_3$CH$_2$CH$_2$CH$_2$)$_2$SnO (dibutyltin(IV) oxide, abbreviated as (n-Bu)$_2$SnO) and tin phthalocyanine chloride (SnPc), which are shown in Figures 8a and 8b, respectively. The BCP pulse sequence uniformly excites the entire breadth of both
CSA-dominated powder patterns using relatively low-power spin-locking pulses (ca. 18 kHz and ca. 42 kHz on the $^{119}$Sn and $^1$H channels, respectively) and a contact time of 30.0 ms. The corresponding experimental times to collect both $^{119}$Sn spectra were ca. 10 minutes and ca. 30 minutes for (n-Bu)$_2$SnO oxide and SnPc, respectively, which is a significant time savings over direct excitation (DE) experiments (e.g., the $T_1$($^{119}$Sn) constant for (n-Bu)$_2$SnO is on the order of 100 s). The BCP spectrum of a 1:1 w/w mixture of these two tin compounds (Figure 8c) does not clearly reveal each of the unique $^{119}$Sn powder patterns that originate from each compound in the mixture. In this case, identifying and deconvoluting the contribution of each individual powder pattern may be difficult, making this mixture a good test case for 2D RAS. $R_1$ RAS analyses would be impractical for separating the individual $^{119}$Sn powder patterns, as the $\tau$ increments used in the collection of a $T_1$ relaxation dataset would have to be quite long in order to ensure a proper encoding of the $T_1$ relaxation behavior. A $R_2^{\text{eff}}$ RAS analysis was therefore attempted by applying the NNTF routine to the WCPMG $T_2^{\text{eff}}$ dataset (Figure S5f) acquired with the BCP pulse sequence using 100 CPMG loops, a recycle delay of 10.0 s, and 4096 scans (total acquisition time of ca. 11 hours). The resulting 2D $R_2^{\text{eff}}$ RAS spectrum of this 1:1 mixture (Figure 8d) easily resolves the $^{119}$Sn powder patterns. The $R_2^{\text{eff}}$($^{119}$Sn) constants determined from the $R_2^{\text{eff}}$ RAS spectrum are tabulated in Table S2. The projections of both powder patterns (Figure 8f and 8g, SnPc and (n-Bu)$_2$SnO, respectively) were fit with similar NMR parameters to the ones used to fit each of the individual patterns collected with the BCP pulse sequence. This example illustrates that the BCP pulse sequence can be used to collect high-quality $T_2^{\text{eff}}$ datasets that can then be processed using the NNTF algorithm to yield high-quality RAS spectra,
which is extremely useful when dealing with unreceptive nuclei (i.e., nuclei associated with broad CSA-dominated patterns, long $T_1$ constants and/or low values of $\gamma$).

5. Conclusions

2D $R_1$ and $R_2$ relaxation-assisted separation (RAS) analyses can be used to separate overlapping UW NMR powder patterns originating from magnetically distinct sites for both spin-1/2 and quadrupolar nuclei, provided that their relaxation characteristics are distinct. The WCPMG and BCP pulse sequences are robust and can be easily used to collect $T_1$ and $T_2$ datasets, which can then be imported into MATLAB and processed with the NNTF routine to give the corresponding RAS spectra. The NNTF routine is easily implemented within MATLAB and is straightforward to use. NNTF effectively addresses the problematic small singular values that are characteristic of ill-posed inverse problems. This is especially useful when processing $T_1$ and $T_2$ relaxation datasets having low S/N and when attempting to resolve overlapping patterns originating from sites having similar relaxation time constants. The combined use of NNTF with post-processing of $R_1$ or $R_2$ RAS spectra can greatly aid in achieving clear separation of powder patterns for instances where the separation is poor due to either (i) low S/N, (ii) similar relaxation constants among magnetically distinct nuclei, (iii) multiple inequivalent sites (each of which gives rise to a unique powder pattern, which may overlap with one another), and/or (iv) combinations of these factors. Moreover, a priori knowledge of the relaxation constants and/or the NMR parameters is beneficial when defining the range of relaxation rates in the NNTF routine and when using the post-NNTF processing procedure; however, this information is not required in order to collect and process
$R_1$ and $R_2$ RAS spectra. The experiments and simulations presented in this work demonstrate that in order to successfully resolve patterns originating from magnetically distinct sites in 2D RAS spectra, high-quality $T_1$ and $T_2$ relaxation datasets are essential. The WCPMG and BCP pulse sequences are indispensable to this end, since they can provide the necessary high-quality relaxation datasets in reasonable experimental timeframes. Moreover, the combined use of these pulse sequences with other sensitivity-enhancing techniques (e.g., the use of high magnetic fields, dynamic nuclear polarization, low-temperature NMR, etc.) is likely to open up larger swaths of the periodic table to routine analysis with $R_1$ and $R_2$ RAS methods. We hope that the ease with which $T_1$ and $T_2$ datasets can be collected and then processed with the NNTF routine will make RAS-based strategies effective methods for increasing spectral resolution. Our future work will include the continued development of the NNTF routine and related post-processing protocols, so that higher-quality RAS spectra can be produced. We will also investigate systems with two or more magnetically distinct sites in the unit cell, as well as those with very similar $T_{2\text{eff}}$ values.

6. Supporting Information

Also included in the supporting information: (i) information on the L-curve routine and how the optimal regularization parameter is chosen, (ii) details on spectral processing of 1D NMR spectra, (iii) figures of the 1D NMR spectra and associated analytical simulations for all compounds, as well as their 2D $T_1$ relaxation datasets, (iv) schemes of pulse sequences used to collect $T_1$ and $T_2$ datasets, (v) tables of the experimental NMR parameters, and (vi) figures of the separated and individual patterns obtained from the $^{71}$Ga RAS experiments and simulated $T_2$ RAS spectra.
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