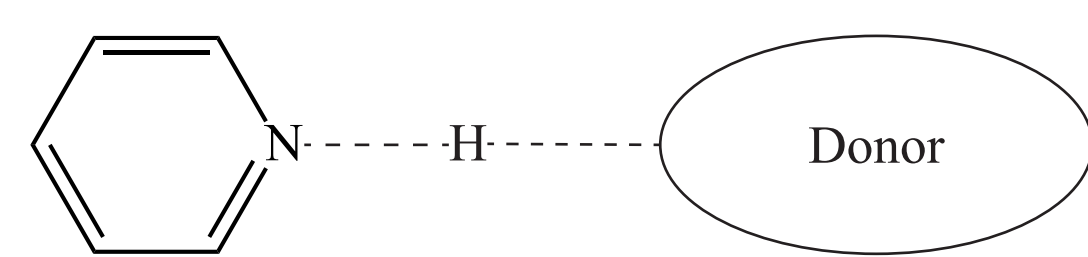


Introduction

Organic salts and co-crystals play a key role as essential matrices that encapsulate the structural configuration of active pharmaceutical ingredients (APIs). Acid-base salts and cocrystals are distinguished based on the position of a hydrogen atom between a hydrogen bond donor and acceptor. In salts, the hydrogen atom is transferred to the base (typically the API). Whereas cocrystals typically involve weak acids/bases, resulting in a retention of the hydrogen atom by the weak acid, or a partial transfer to the base. A precise determination of the hydrogen atom position will allow the accurate identification of the protonation state of such systems. However, it is challenging to precisely measure the hydrogen atom positions using standard X-ray diffraction techniques. Here, we focus on determining hydrogen atom positions in organic salts and co-crystals using nitrogen-14 solid-state NMR spectroscopy.



Scheme 1. Schematic showing pyridine (base) abstracting a hydrogen atom from a donor (acid).

Nitrogen-14 Solid State NMR

Nitrogen-14 is a spin (I) 1 quadrupolar nucleus with a high natural abundance of 99.6 %. However, nitrogen-15 is the preferred isotope for NMR even though it has a low natural abundance of 0.37 %, because it is a spin-1/2 isotope. Spin-1/2 nuclei show high resolution solid-state NMR spectra which allows multiple signals to be easily distinguished. Whereas solid-state NMR spectra of quadrupolar nuclei ($I > 1/2$) show spectra that are severely broadened by the nuclear quadrupolar interaction. However, ¹⁵N labeling is difficult and expensive, which motivates the development of ¹⁴N solid-state NMR spectroscopy.

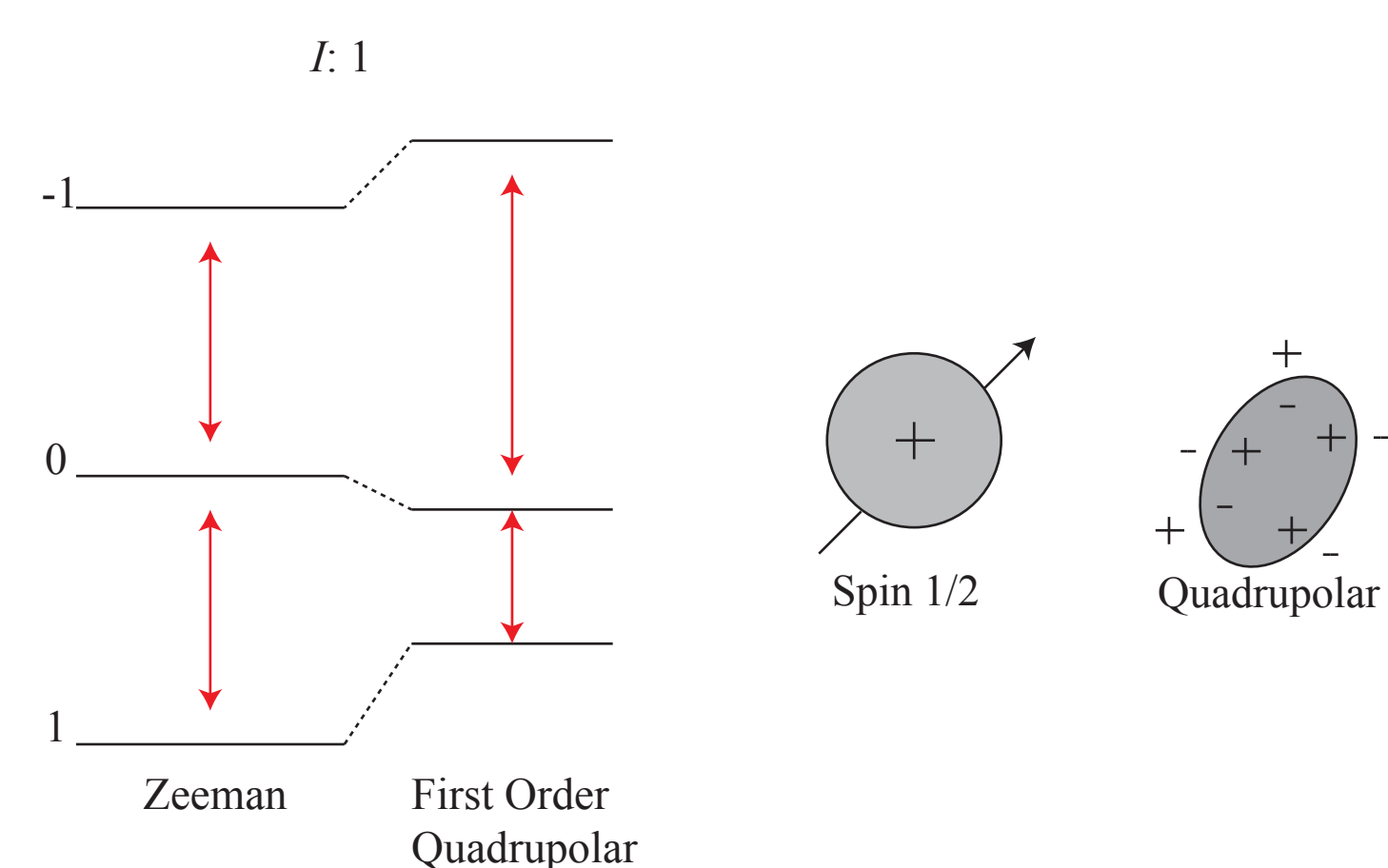


Figure 1. Figure showing the energy levels of a spin $I = 1$ quadrupolar nucleus under an applied magnetic field and the first-order quadrupolar effect (left). Model of spin-1/2 and quadrupolar nuclei (right).

Conventional methods include static ¹⁴N static solid-state NMR spectra which show spectral linewidth on the order of MHz due to the large first order quadrupolar interaction. However, magic angle spinning (MAS) can average the first order quadrupolar interaction leaving only a small second order quadrupolar effect on ¹⁴N solid-state NMR spectra.

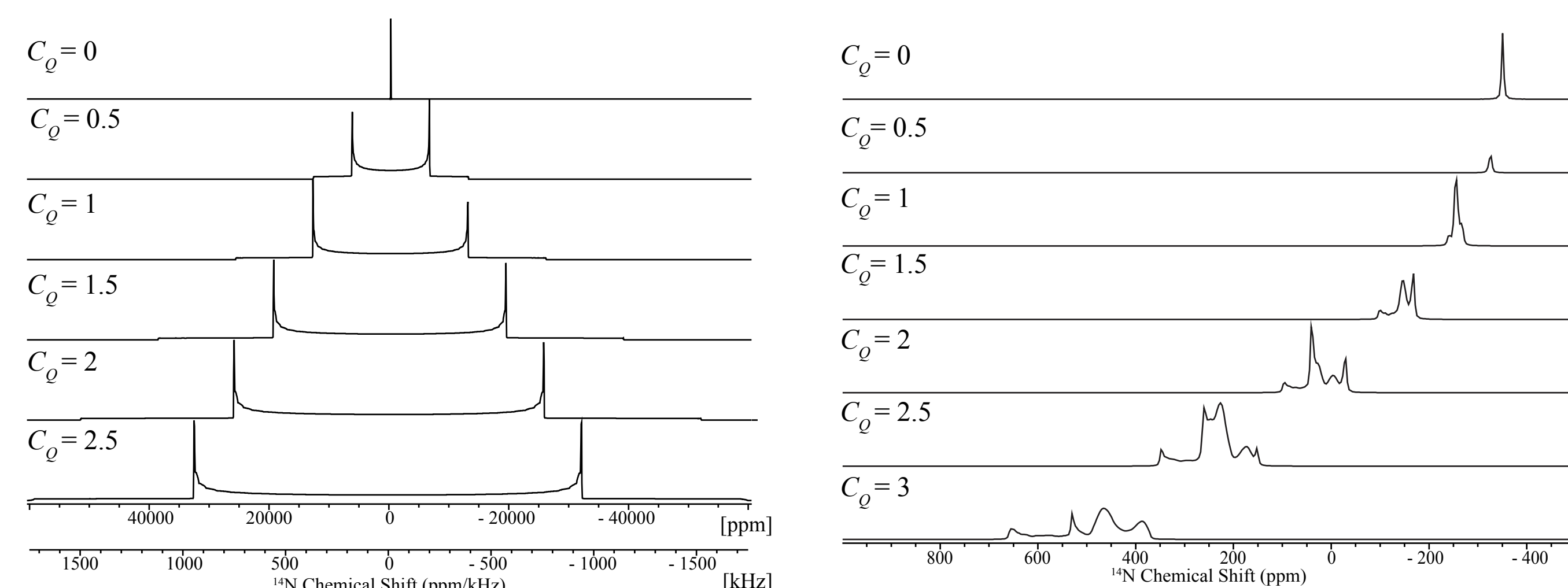


Figure 2. Plots showing the effect of increasing C_Q on static (left) and 50 kHz MAS frequency (right) ¹⁴N solid-state NMR simulated spectra. Isotropic shift was set to -350 ppm.

Fast Magic Angle Spinning

Fast MAS narrows ¹H linewidths in solid-state NMR, permitting proton detection of other low gyromagnetic ratio nuclei such as ¹⁴N. This technique allows ¹H-¹⁴N two-dimensional solid-state NMR spectra to be obtained which directly probes the N-H spatial proximity. Furthermore, ¹⁴N chemical shift and quadrupolar NMR parameters can be obtained by extracting slices from the 2D ¹H-¹⁴N solid-state NMR spectrum.

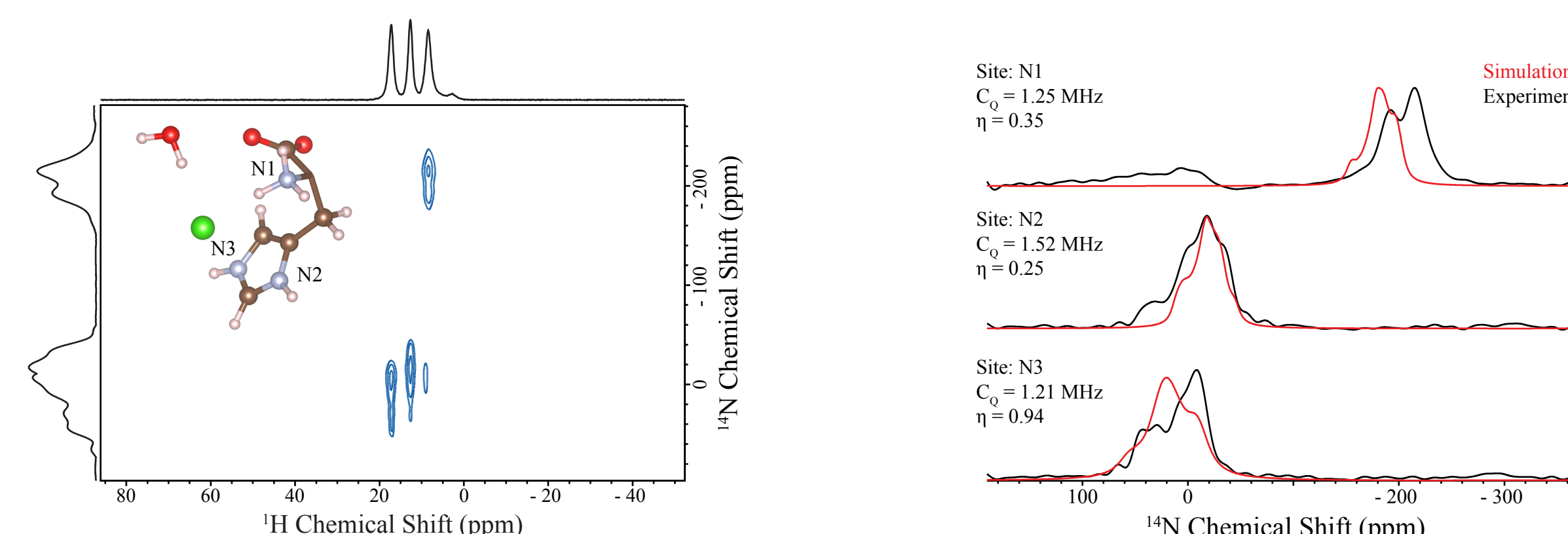


Figure 3. ¹H-¹⁴N 2D dipolar heteronuclear multiple quantum coherence (D-HMQC) solid-state NMR spectrum of Histidine HCl H₂O as a model (left). The ¹⁴N traces extracted from the 2D spectrum with the corresponding fits using the values from reference [2] are also shown (right).

Pyridinium Salts and Cocrystals

Pyridine is a widely used basic heterocyclic amine. Pyridine was chosen as the model system for our API due to the wide variety of salts and cocrystals that can potentially be synthesized using pyridinium bases.

Unlike X-ray crystallography, solid-state NMR can be used to precisely determine hydrogen atom positions. We first performed density functional theory (DFT) calculations on a series of pyridinium salts to optimize the hydrogen atom positions from the crystal structure, followed by calculations of NMR parameters. Figure 4 shows the corresponding ¹⁴N solid-state NMR spectra of these compounds.

Table 1. DFT-optimized structures of pyridine-based salts with the corresponding N-H distances and ¹⁴N NMR parameters.

Acid	N-H Distance (Å)	δ (ppm)	C_Q (MHz)	η
Fumaric Acid	1.088	-168	1.42	0.27
Maleic Acid	1.090	-170	1.39	0.35
HCl Acid	1.037	-181	0.67	0.06
Nitric Acid	1.074	-172	1.21	0.42
Tosylic Acid	1.067	-177	1.11	0.58

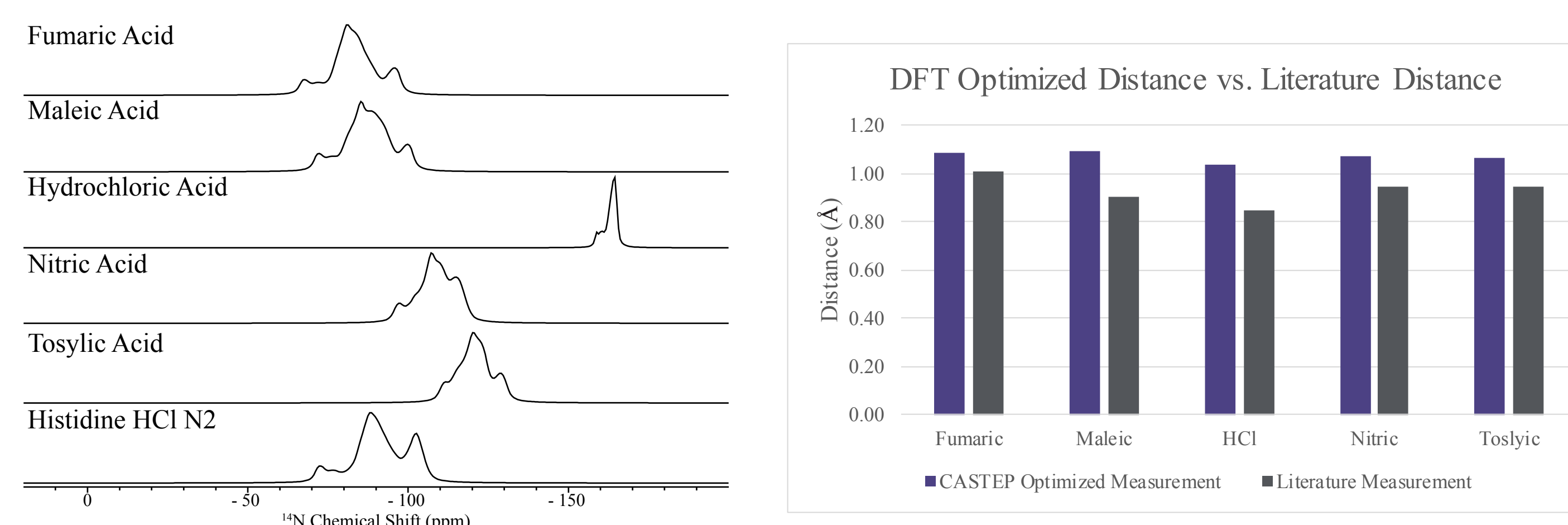


Figure 4. 50 kHz MAS (right) ¹⁴N solid-state NMR spectra of the various pyridinium salts (left). The position of the ¹⁴N signal is highly sensitive to the N-H distance. Plot comparing the DFT-optimized vs previously reported N-H distances (right).

¹H-¹⁴N Distance Measurements

To unambiguously measure the protonation state of a base, it is vital to precisely measure the distance between the nitrogen and hydrogen atoms. Solid-state NMR can be used to measure the dipolar couplings between ¹H and ¹⁴N, which will provide precise ¹H-¹⁴N distances. Rotational echo saturation pulse double resonance (RESPDOR) experiments can provide precise ¹H-¹⁴N distances. Figure 5 shows the potential for RESPDOR measurements to distinguish salts and cocrystals.

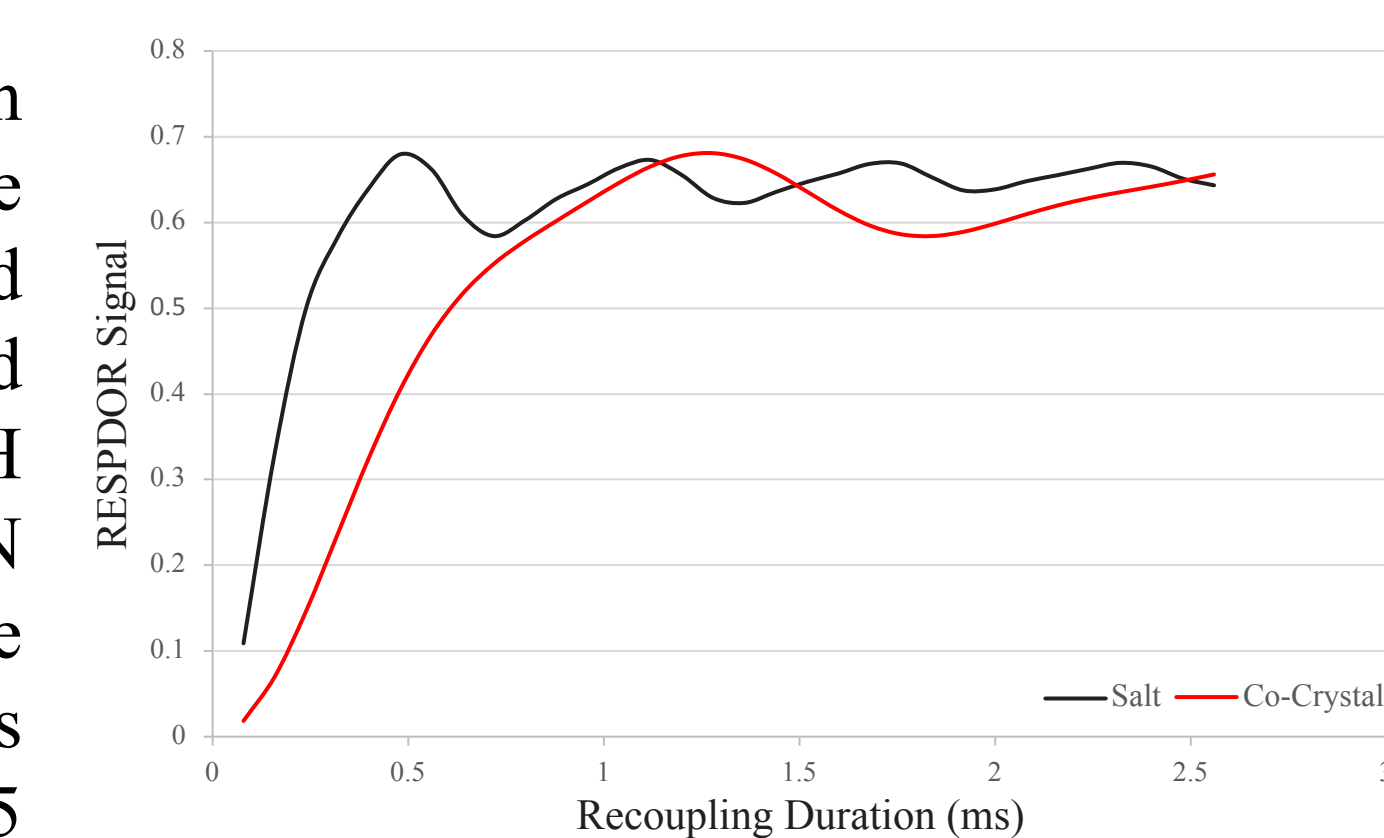


Figure 5. Calculated ¹H-¹⁴N RESPDOR curves for a model salt and cocrystal system containing ¹H-¹⁴N distances of 1.1 and 1.5 Å.

DFT calculations on the pyridinium compounds clearly show N-H distances of 1.04 - 1.09 Å, with hydrochloric acid, the strongest acid in the series, showing the shortest N-H bond distance of 1.037 Å. The other acids are slightly weaker and show slightly longer N-H distances. co-crystal. Figure 6 shows the calculated RESPDOR curves corresponding to the DFT-calculated N-H distances.

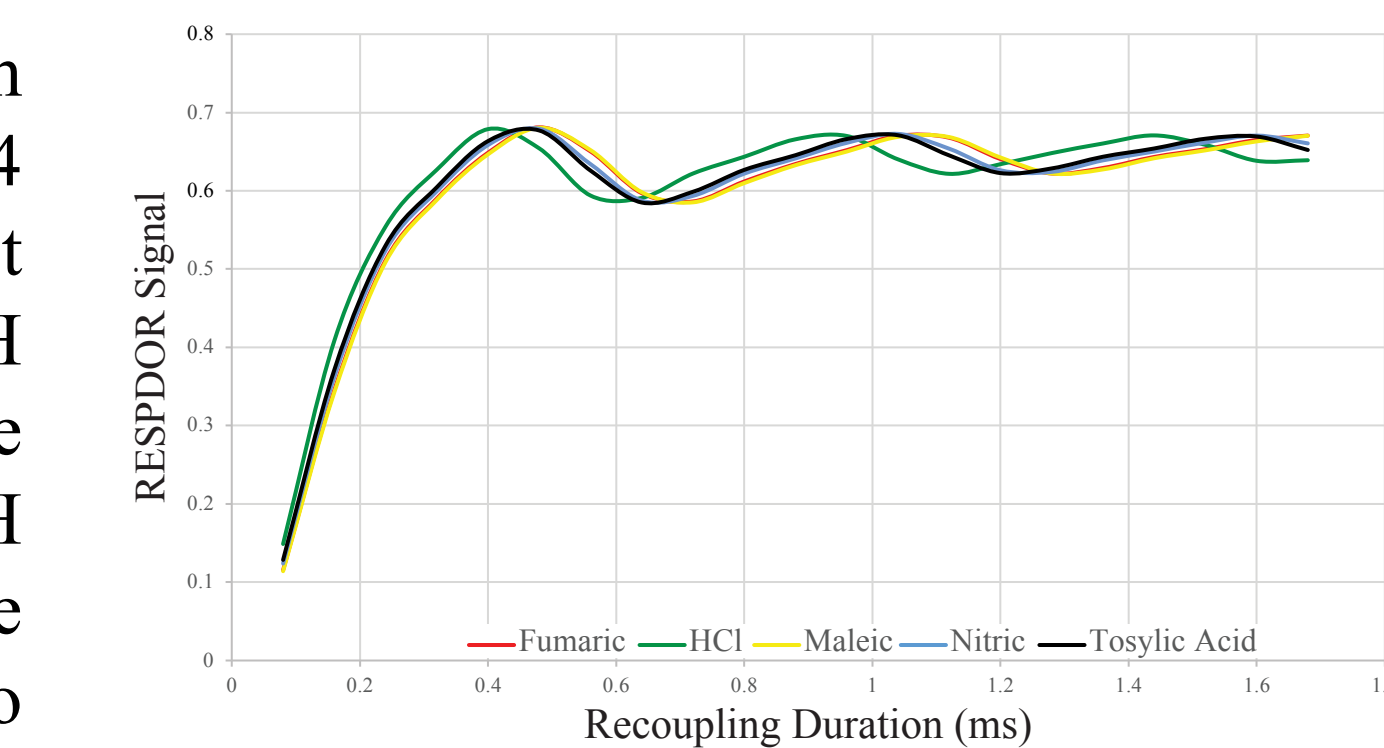


Figure 6. Calculated ¹H-¹⁴N RESPDOR curves for the series of pyridinium salts shown in Table 1.

Conclusions and Future Work

In conclusion, ¹H-¹⁴N solid-state NMR spectroscopy is a promising tool to probe N-H bond distances and local nitrogen and hydrogen atom environments in organic salts and co-crystals. Fast MAS, proton-detected 2D ¹H-¹⁴N correlations enable the rapid observation of ¹⁴N solid-state NMR spectra which are highly sensitive to the hydrogen atom positions. The position of the hydrogen atom can be precisely determined by measuring ¹H-¹⁴N distances using the RESPDOR technique. DFT calculations of NMR parameters allow the validation of the experimental measurements.

In the future we plan to expand our range of model systems to substituted pyridine bases, which will allow us to investigate salts and cocrystals with a larger spread of N-H bond distances. The demonstration of ¹H-¹⁴N 2D correlation and distance measurement techniques on a variety of compounds will establish this method as a powerful tool to probe salts and cocrystals. Ultimately these tools will benefit the drug discovery process.

Acknowledgements

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