Supporting Information for

Chemical exchange of labile protons by deuterium enables selective detection of pharmaceuticals in solid formulations

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1. Synthesis and experimental details

Partial deuteration of labile protons in molecular solids. Powders of L-histidine·HCl·H₂O [C₆H₃N₂O·HCl·H₂O], dopamine·HCl [(HO)₂C₆H₃(CH₂)₂NH₂·HCl] and microcrystalline cellulose (particle size, 20 µm) were purchased from Sigma Aldrich and used as received. The solubility of histidine is 50 mg/mL, and dopamine is 600 mg/mL in water, whereas microcrystalline cellulose is relatively insoluble in water and sparingly soluble in dilute acids and weak bases (e.g., NaOH solution). Approximately 10 mg of L-histidine·HCl·H₂O [C₆H₃N₂O·HCl·H₂O] and dopamine·HCl [(HO)₂C₆H₃(CH₂)₂NH₂·HCl] powders were dissolved in 500 microliters of D₂O, which ensures complete dissolution. The samples were suspended in a petri dish overnight at room temperature. Fine powders of partially deuterated samples were scratched from the petri dish to collect enough material for ssNMR experiments. For the high-temperature deuteration exchange experiment, powders of L-histidine·HCl·H₂O, and dopamine·HCl were suspended in 500 microliters of D₂O in a petri dish. For the blends with cellulose L-histidine·HCl·H₂O (20 wt.% and 30 wt.% of dopamine·HCl in microcrystalline cellulose was suspended in 300 microliters of D₂O. These suspensions were heated at 100 °C in order to facilitate the ¹H·²H exchange until evaporation of water and cooled down to room temperature. Fine dry powders are formed within a few minutes. These powders were scratched from the petri dish. The extent of deuteration is estimated by analysing 1D ¹H NMR spectra by comparing the relative integrals of the labile proton sites in pristine and deuterated samples.

Solid-state NMR spectroscopy. For the analysis of pristine compounds, all samples were used as received. Approximately 2.2 mg of each powder was packed into a 1.3 mm (outer diameter) rotor. All 1D ¹H MAS NMR experiments were carried out either on a 9.4 T, 18.8 T, or on a 28.2 T Bruker Avance NEO4 solid-state NMR spectrometer operating at a ¹H Larmor frequency of 400.1 MHz, 800.1 MHz or 1200.5 MHz. Each of them was equipped with a 1.3 mm double-resonance MAS probe head. All 2D ¹H–²H isotope correlation experiments were performed on a 18.8 T with 60 kHz MAS. The ¹H and ²H pulse lengths and dipolar recoupling periods (τrcpl) were empirically optimized using 1D versions of the D-HMQC pulse sequence. 1D ¹H MAS NMR spectra of neat compounds and blends were acquired with 32 coadded transients with a recycle delay of 10 s, leading to an experimental time of 6 mins each. All ²H MAS NMR spectra were acquired by coaddition of 1024 transients, leading to an experimental time of 16 mins each. ²H MAS NMR spectra can be acquired with 96 coadded transients in under a minute. All ¹H chemical shifts are calibrated with respect to neat TMS using adamantane as an external reference (¹H resonance, 1.85 ppm). All the ²H shifts are calibrated with respect to TMS using liquid D₂O as an external reference (²H resonance, 4.80 ppm).

Isotope correlation spectroscopy (iCOSY). All 2D NMR experiments were performed on a Bruker Avance Neo (18.8 T Larmor frequency of ¹H = 800.1 MHz, ²H = 122.8 MHz) spectrometer, using a 1.3 mm Bruker probe operating in double-resonance mode at a 60 kHz MAS frequency. The pulse sequence used to acquire the ²H–¹H HMQC spectra is reported in Figure S1. A pulse program suitable for Bruker spectrometers is provided in a previous study⁹ (see: supporting information of ref. 9, pages 10-16). SR4:⁵ recoupling elements were used to reintroduce the heteronuclear ²H–¹H dipolar couplings, using a duration τrcpl = 166 microseconds. The ¹H 90-degree pulse duration and the ²H pulse durations were 2.2 µs and 16.6 µs, respectively. For a L-histidine·HCl·H₂O, 80 t₁ FIDs were acquired using the States method to achieve sign discrimination in F₁ with a rotor synchronized increment of 16.6 µs, 32 transients were coadded with a recycle delay of 6 s, corresponding to a total experimental time of 4.2 h. For L-histidine·HCl·H₂O/microcrystalline cellulose blend (20/80 wt.%), 120 t₁ FIDs were acquired using the States method to achieve sign discrimination in F₁ with a rotor synchronized increment of 16.6 µs, 32 transients were coadded with a recycle delay of 6 s, corresponding to a total experimental time of ~7 h. For dopamine·HCl, 64 t₁ FIDs were acquired using the States method to
achieve sign discrimination in $F_1$ with a rotor synchronized increment of 16.6 µs, 8 transients were coadded with a recycle delay of 5 s, corresponding to a total experimental time of ~1.5 h. For the 30 wt.% dopamine.HCl blend with microcrystalline cellulose, 96 $t_1$ FIDs were acquired using the States method to achieve sign discrimination in $F_1$ with a rotor synchronized increment of 16.6 µs. 24 transients were coadded with a recycle delay of 5 s, corresponding to a total experimental time of ~4 h.

Figure S1. Schematic of HMQC pulse sequence (top) used in the present study to acquire one (1D) and two-dimensional (2D) iCOSY spectra. SR4: pulses are applied for the recoupling of the $^1$H-$^2$H dipolar interactions. The evolution during $t_1$, the recoupling pulses, and $^2$H pulses are rotor synchronized with respect to the sample spinning. The coherence pathways are provided at the bottom. See Ref. 9 (SI) for the pulse program.

**First-principles calculations using a periodic-DFT approach.** For calculations, input files were generated by taking molecular coordinates from previously published crystal structures deposited in the Cambridge database: L-histidine.HCl.H$_2$O (CCDC code: HISTCMO1) and dopamine.HCl (CCDC code: DOPAMN). All calculations were performed using the CASTEP 19.11 code. Geometry optimization was carried out using periodic density functional theory (DFT), and the NMR chemical shifts were computed using the gauge including projected augmented wave (GIPAW) method as described by Pickard and Mauri. The generalized density approximation DFT functional PBE (Perdew-Burke Ernzerhof) with the Tkatchenko-Scheffler (TS) dispersion correction scheme (DFT-D method) was applied with ultrasoft pseudopotentials. The Broyden–Fletcher–Goldfarb–Shanno (BFGS) optimization algorithm was used to geometry optimize unconstrained molecular systems. The Monkhorst–Pack grid of minimum sample spacing 0.07 × 2π Å$^{-1}$ was applied to sample the Brillouin zone (i.e., primitive cell in reciprocal space). The molecular geometry was converged with a maximum plane wave cut-off energy of 23.15 Hartrees. The atom positions in the unit cell were allowed to relax within the unit cell until the average forces, energies, and displacements remaining were below 3.6749 × 10$^{-7}$ Hartree/Å, 0.0011025 Hartrees, and 0.001 Å, respectively. For L-histidine.HCl.H$_2$O, and dopamine.HCl, all interatomic distances stated in this study correspond to the DFT optimized structures.
2. Estimation of the extent of deuteration (%) in molecular solids

The extent of deuteration, D(%), was estimated by comparing the $^1$H integral values of each labile proton site in pristine and deuterated samples using Equation 1, where $I_H$ (pristine) and $I_H$ (deuterated) are the peak integrals of labile protons in pristine and deuterated samples.

$$D(\%) = \frac{I_H(\text{deuterated})}{I_H(\text{pristine})} \times 100 \quad (1)$$

Figure S2 presents the 1D $^1$H spectrum before and after deuteration (at room temperature) of molecular solids utilized in this work, along with their 1D $^2$H spectrum post-deuteration. For overlapping sites, the proton integral values are measured by spectral deconvolution analysis. The $^1$H MAS spectra are presented by normalizing the peak intensities of CH$_2$ and CH (H2,3,4) sites for L-histidine.HCl.H$_2$O, and CH$_2$ sites (H2,2’, 3, 3’) for dopamine.HCl, in order to see the intensity losses for NH, NH$_3$, OH, and H$_2$O sites.

![Figure S2](image)

**Figure S2.** Solid-state 1D $^1$H MAS (top) spectra of pristine (black) and partially deuterated samples at different temperatures (green) together with the $^2$H MAS (bottom) spectra: (a) L-histidine.HCl.H$_2$O (b) dopamine.HCl (c) microcrystalline cellulose. Vertical yellow bands indicate the peaks of labile protons that undergo exchange with deuterium. All spectra were acquired at 18.8 T (Larmor frequency of $^1$H = 800.1 MHz) with 60 kHz MAS.
3. 1D $^1$H and $^2$H MAS NMR spectra of partially deuterated samples acquired at 9.4 T and 18.8 T

For partially deuterated L-histidine.HCl.H$_2$O, and dopamine.HCl, $^1$H and $^2$H MAS spectra acquired at different magnetic field strengths such as 9.4 T (400.1 MHz) and 18.8 T (800.1 MHz) are presented in Figure 3. All spectra were acquired at 60 kHz MAS to maintain a consistent spinning speed. Resolution and sensitivity enhancements in the $^1$H MAS spectra obtained at 18.8 T are evident from relatively better-resolved peaks, for example, in the 6-10 ppm range for L-histidine.HCl.H$_2$O as well in the 4-10 ppm range for dopamine.HCl. The resolution of the $^1$H MAS spectrum of L-histidine.HCl.H$_2$O is dramatically improved by going from 9.4 T to 18.8 T, due to the well-known effect that the quadrupolar interaction scales inversely with the magnetic field strength. Therefore, a high magnetic field improves the resolution in $^1$H as well as in $^2$H MAS NMR spectra, enabling better resolution to be achieved in the 2D $^1$H-$^2$H iCOSY spectra.

![Figure S3](image-url)

**Figure S3.** Solid-state 1D (a) $^1$H and (b) $^2$H MAS NMR spectra of L-histidine.HCl.H$_2$O, and (b) and (c) are the same spectra of dopamine.HCl, respectively. All spectra were acquired at 9.4 T (blue) and 18.8 T (red) with 60 kHz MAS with $^1$H Larmor frequencies of 400.1 MHz (9.4 T) and 800.1 MHz (18.8 T) to compare the sensitivity and resolution.
4. Analysis of $^2$H NMR spectra of L-histidine.HCl.H$_2$O

![Figure S4](image.png)

**Figure S4.** Solid-state 1D $^2$H MAS NMR spectra of L-histidine.HCl.H$_2$O (left) with full spinning sideband patterns acquired at 18.8 T (Larmor frequency of $^1$H = 800.1 MHz and $^2$H = 122.8 MHz) with variable spinning frequencies as indicated. Spinning sidebands are denoted by asterisks. The expanded regions of $^2$H MAS spectra (0-20 ppm) are shown in the right-hand side panel, whereby line narrowing of -ND, -ND$_3$, and -OD peaks are observed.
Figure S5. Lineshape analysis of a 1D $^2$H NMR spectrum of L-histidine.HCl.H$_2$O acquired at 18.8 T (Larmor frequency of $^1$H= 800.1 MHz and $^2$H = 122.8 MHz) with 60 kHz MAS.

Table S1. GIPAW DFT calculated $^2$H electric field gradient tensors and quadrupolar parameters of partially deuterated NH and NH$_3$ sites in L-histidine-HCl-H$_2$O along with experimental quadrupolar parameters obtained by lineshape fitting. $^a$

| $^2$H site | GIPAW-DFT calculated | Experimental |
|------------|----------------------|--------------|
|            | $V_{xx}$  | $V_{yy}$  | $V_{zz}$  | $C_Q$ (MHz) | $\eta_Q$ | $\delta_{iso}$ (ppm) | $C_Q$ (MHz) | $\eta_Q$ | $\delta_{iso}$ (ppm) |
| H5         | -0.063   | -0.094   | 0.157    | 0.11       | 0.20     | 17.9             | 0.14       | 0.11     | 16.8          |
| H7         | -0.120   | -0.149   | 0.269    | 0.11       | 0.59     | 13.0             | 0.20       | 0.13     | 12.4          |
| NH$_3$     | -0.117   | 0.315    | 0.246    | 0.16       | 0.05     | 8.1              | 0.05       | 0.13     | 8.3           |
| H$_2$O     | -0.150   | -0.200   | 0.350    | 0.22       | 0.13     | 4.9              | 0.14       | 0.12     | 5.3           |
| CH$_2$     | -0.130   | -0.530   | 0.270    | 0.17       | 0.06     | 2.4              | ---        | ---      | ---           |
| CH         | -0.120   | -0.140   | 0.260    | 0.18       | 0.06     | 2.0              | ---        | ---      | ---           |

$^a$ Errors on the $\delta_{iso}$, $C_Q$, and $\eta_Q$ are estimated to be ±0.1 ppm, ±0.04 MHz, and ±0.03, respectively.
5. 1D NMR of solid-state formulation of L-histidine.HCl.H₂O and microcrystalline cellulose

Figure S6. Solid-state 1D $^1$H (top) and $^2$H (bottom) spectra of pristine (black) and partially deuterated (green) solid-state formulation of L-histidine.HCl.H₂O (20wt.%), and microcrystalline cellulose (80 wt.%) acquired at 9.4 T and 60 kHz.
6. Analysis of $^2$H NMR spectra of dopamine.HCl

Figure S7. 1D $^2$H NMR spectra of dopamine-HCl (left) acquired at 18.8 T (Larmor frequency of $^1$H = 800.1 MHz and $^2$H = 122.8 MHz) at variable MAS as indicated alongside the spectrum. Spinning sidebands are denoted by asterisks. The expanded regions of $^2$H MAS spectra (0-20 ppm) are shown in the right-hand side panel, whereby line narrowing of ND$_3$ and OD peaks are observed, enabling the resolution of -OD sites at > 40 kHz MAS.
Figure S8. Lineshape analysis of 1D $^2$H NMR spectrum of dopamine.HCl acquired at 18.8 T (Larmor frequency of $^1$H= 800.1 MHz and $^2$H = 122.8 MHz) with 60 kHz MAS.

Table S2. GIPAW DFT calculated $^2$H electric field gradient tensors and quadrupolar parameters of partially deuterated NH$_3$ sites in dopamine·HCl along with experimental quadrupolar parameters obtained by lineshape fitting.$^a$

| $^2$H site | GIPAW-DFT calculated | Experimental |
|------------|-----------------|--------------|
|            | $V_{xx}$ | $V_{yy}$ | $V_{zz}$ | $C_Q$ (MHz) | $\eta_Q$ | $\delta_{iso}$ (ppm) | $C_Q$ (MHz) | $\eta_Q$ | $\delta_{iso}$ (ppm) |
| ND$_3$     | -0.131 | -0.144 | 0.275 | 0.18 | 0.05 | 7.3 | 0.04 | 0.05 | 7.5 |
| OD         | -0.152 | -0.195 | 0.347 | 0.23 | 0.12 | 7.5 | 0.21 | 0.17 | 7.9 |

$^a$ Errors on the $\delta_{iso}$, $C_Q$, and $\eta_Q$ are estimated to be ±0.1 ppm, ±0.04 MHz, and ±0.03, respectively.
7. 2D $^2$H-$^2$H NMR spectra of dopamine.HCl

Figure S9. Solid-state 2D $^1$H-$^2$H HMQC spectra of partially deuterated DOPA acquired with (b) $\tau_{RCPL} = 133.2$ µs and (c) $\tau_{RCPL} = 266.5$ µs, at 18.8 T with 60 kHz MAS.

8. Packing interactions in dopamine.HCl

Figure S10. Through-space packing interactions contributing to the correlations in the 2D $^1$H-$^2$H HMQC spectrum presented in Figure 4d.

Figure S10 presents the inter- and intramolecular $^1$H-$^2$H interactions and the distances between them, as indicated alongside the DFT geometry optimized crystal structure. The intermolecular proximity between the ND$_3$ group, the phenolic proton, and a hydroxyl group leads to the 2D peak at 7.8-8.2 ppm ($^1$H) and 7.0-9.0 ppm ($^2$H) as indicated by (I) and (II) in Figure 4d and Figure S10. The intramolecular proximity between the ND$_3$ group and neighbouring aliphatic -CH$_2$ groups (indicated by (III) and (VI)) leads to the signal at 7.0-9.0 ppm ($^2$H) and 3.5-5.5 ppm ($^1$H). The intra- and intermolecular proximity of ~2.4 Å and ~3.5 Å between phenolic protons and deuterated hydroxyl groups give rise to the signals indicated by (IV, V) in Figure 4d. The deuterated hydroxyl group is intermolecularly proximate to the -CH$_2$ protons, as indicated by (VII) in Figures 4d and S10.
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