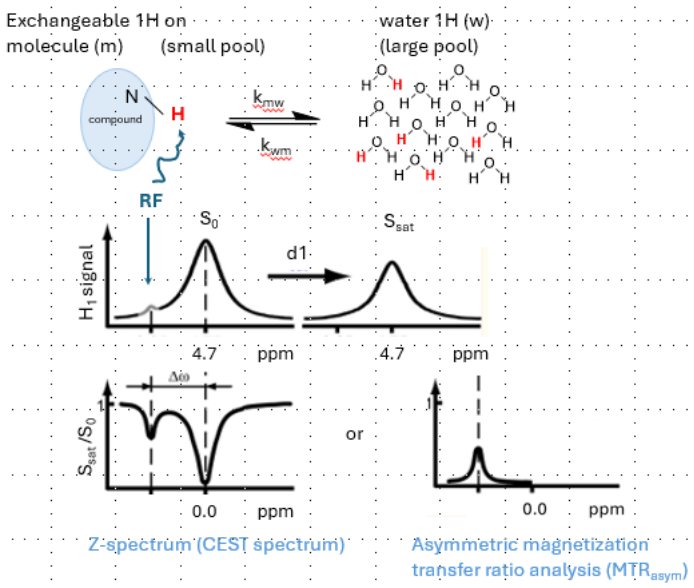
Chemical Exchange Saturation Transfer (CEST) NMR

20240705 (hh)

Summary

Chemical Exchange Saturation Transfer (CEST) is a technique in which exchangeable protons on molecules (m)/solutes are selectively saturated using RF irradiation. and transfer of that saturation onto bulk water is detected. The exchange of solute 1H with water protons will cause the water signal to become attenuated. Since the solute usually has a much lower concentration (uM to mM range) than the water (110 M), a single saturation transfer would not be detectable, but at sufficiently fast exchange rates (~ms) and long saturation times (s), longer irradiation will cause a buildup of the saturation effect which then becomes visible in the water signal. Exchangeable protons in solutes are present at low concentrations can therefore be visualized indirectly in a plot of normalized water saturation (S_{sat}/S_0) vs saturation frequency, called a CEST or Z-spectrum. A Z-spectrum usually shows a symmetric direct saturation (DS) around the water frequency which is set to 0. Beware: This is different from 0ppm in NMR spectroscopy!!!. CEST effect generally show up a asymmetric bands on one side of the spectrum only that can be found by so called magnetization transfer ratio (MTR) asymmetry analysis (MTR=1-(S_{sat}/S_0). A similar setup can be run for paramagnetic compounds and is then referred to as paramagnetic CEST (pCEST).



- **Sample Preparation:**

Prepare an aqueous solution of your compound. Salt should not affect CEST but literature suggests that using a desalting column is beneficial. Insert a capillary or 3mm NMR tube filled with D2O to be able to lock the sample and avoid water frequency shifts.
For paraCEST b(pCEST) measurements, it might be advantageous to remove uncomplexed metal ions if these affect T1/T2 of your sample.

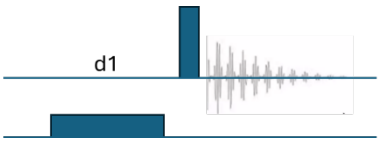
- **Setup and Acquisition**

1. Run a 1H NMR on your sample. If you have deuterated solvent in you sample, use a standard 1H, otherwise refer to the "No-D 1H and 13C NMR" guide. Check the shimming and note chemical shift of the water peak.

2. Set up a new experiment using parameter set **H1_CEST_2d.UW**. This will create a pseudo 2D experiment that can be conveniently worked up in MNova.

3. In ased set:

   a) **d1**: in this experiment d1 is used as the saturation time. The saturation pulse will be turned on during this time [s]. This parameter will need to be optimized. Values between 0.5s to 5s are common. Start with a short time.



b) **FQ2LIST** (frequency list on f2) should be set according to your experimental requirements. A number of lists are saved in the frequency list directory. If you would like to save your own list, please save under a new name using your initials so you can recognize it. Add one data point that is far removed from the region of interest. It will be used as an off resonance signal to determine the maximum signal.

   A typical list could include frequencies between -30ppm and 30 ppm in 1ppm steps. All frequencies need to be listed in Hz.

   Once you have set up a frequency list, note how many entries that list has. Change the display from ased to all **(A)** to see the 2D setup. Set **TD1** to the number of points in the frequency list used to create one experiment per frequency.

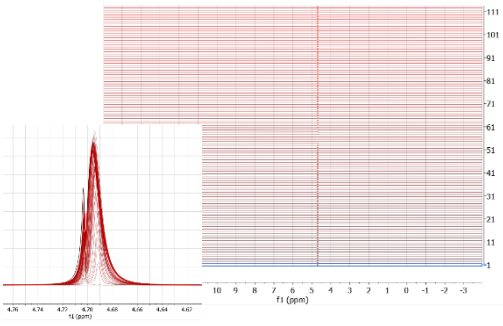
c) **O2p** is set to the center of H2O peak. This is where saturation will occur.

d) **CNST9**: Set the saturation B_1 field strength in Hz. This field is often reported in µT and cnst10 will display the calculated filed directly below. Typical values range from 1-30µT or 40 to 1200 Hz. This parameter will have to be optimized. 5µT or 200Hz might be a good starting point.

e) Do not run an rga – the water peak is very intense, rg 2 works well. Start the experiment using zg.

This is a pseudo 2D setup and will create a ser file for data storage.

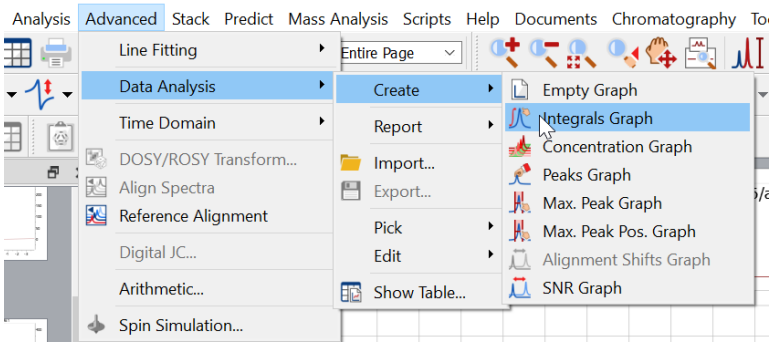
D. Data Workup



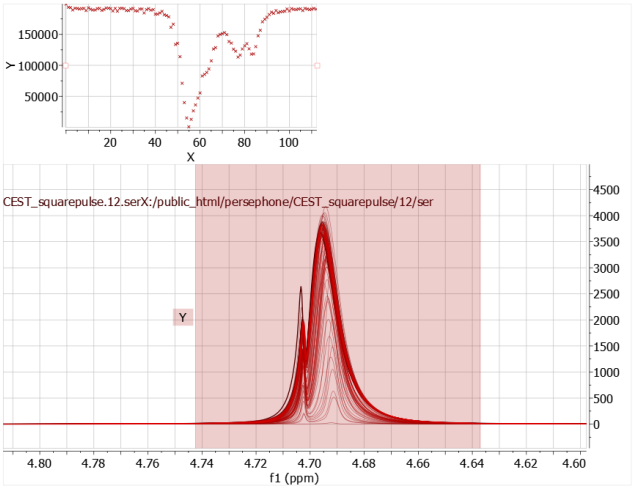
- Open the pseudo 2D dataset in MNova. You can pull in the ser file. It will create a stacked plot that consists of a single 1H spectrum for each point in your frequency table.
- Adjust the intensity until you can identify the water peak and the zoom into the area.
- Change the display settings  from “stack” to “overlay”.

- Under *Advanced* go to *Data Analysis* → *Create* → *Integrals graph.*

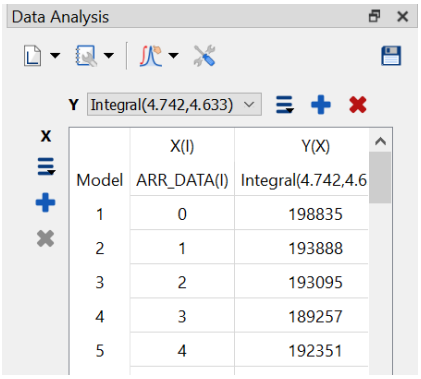
  The curser will turn into an integral and you can integrate all spectra in the overlay at the same time. Make sure to carefully include baseline on both sides of the peak.



- MNova will automatically create a graph using the integrations that will be displayed.



- It will also create a table with all the information. You can access the data under *View* → *Tables* → *Data Analysis* or by double clicking on the graph. Calculations can be run here or you can copy/paste the columns of interest into Excel.

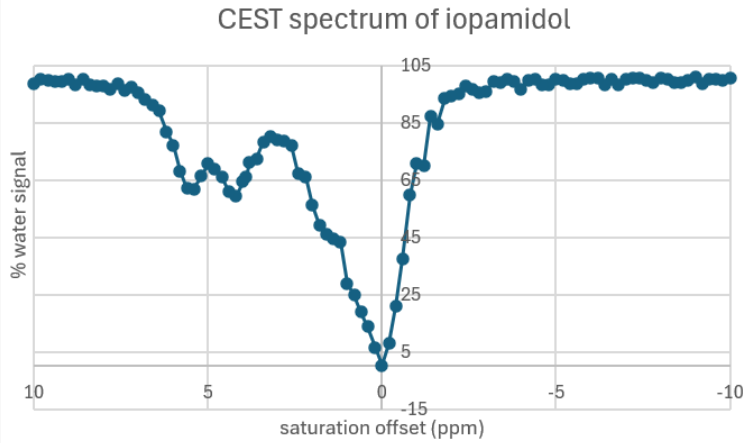


- Use frequency list values from the Bruker dataset (frqlist, open and copy values to excel) to calculate saturation offset in ppm by dividing by the strength of the magnet (e.g. 500). These values will be your x-axes. The water resonance is set to 0 here.

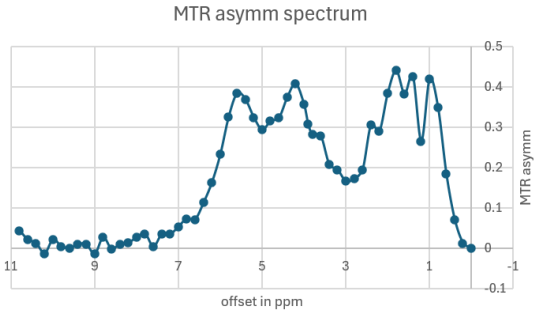
- For values on the y-axes, multiply the signal (S_{sat}) by 100 and divide by the value of the off-resonance signal (S_0). This is the % water signal.

- For the Z-spectrum plot % water signal vs saturation offset (ppm). Display x-values in reverse order.

CEST spectrum of iopamidol. Continuous wave saturation was applied to a 10 mM sample at 5 uT for 4s in 1ppm increments. The height of each water signal was normalized to the maximum water signal acquired during the experiment.



- MTR asymmetry can be calculated by subtracting data from the 2 sides from each other. A lot of assumptions are being made for this plot – so use with caution.



Literature:

1. G. Liu, Y. Li, M.D. Pagel. Design and Characterization of a New Irreversible Responsive PARACEST MRI Contrast Agent that Detects Nitric Oxide. *Mag.Res. Med*. **58** (2007) 1249-1256.
2. B. Yoo, M.D. Pagel. A PARACEST MRI Contrast Agent To Detect Enzyme Activity. *J. Am. Chem. Soc*. **128** (2006) 14032-14033.
3. Y. Li, V.R. Sheth, G. Liu, M.D. Pagel. A self-calibrating PARACEST MRI contrast agent that detects esterase enzyme activity. *Contrast Media Mol. Imaging* **6** (2011) 219–228.
4. P.C.M. van Zijl, N.N. Yadav. Chemical Exchange Saturation Transfer (CEST): what is in a name and what isn't? *Magn. Res. Med*. **65** (2011) 927-948.