# pH Measurement with a MRI-PARACEST Contrast Agent: Nd-DOTAM-Gly-Lys

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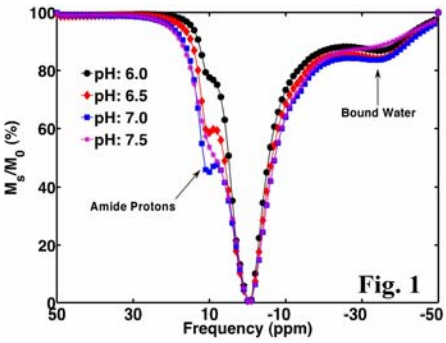
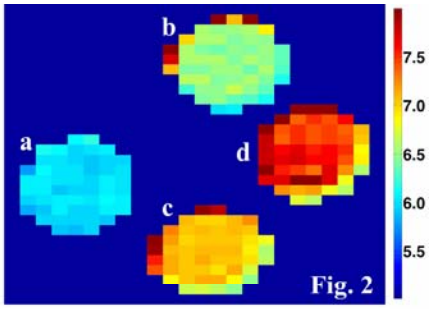
**Introduction:** Altered tissue pH is a common feature in pathological conditions where metabolic demand exceeds oxygen supply (i.e. stroke, tumors). Therefore in-vivo tissue pH may become a valuable biomarker of disease progression in conditions where oxygen is limited. Sherry *et al* and Aime *et al* recently described methods to measure and map pH by MRI using paramagnetic chemical exchange saturation transfer (PARACEST) agents [1,2,3,4]. They demonstrated that the saturation of the amide protons on the PARACEST agent and subsequent chemical exchange and saturation of bulk water (CEST effect) was pH dependent, and could be used to measure pH if the concentration of contrast agent was known. To eliminate the dependence on agent concentration, Aime *et al* developed a ratiometric method that combined information about the CEST effect from both the Lanthanide(III)-bound water and amide protons. However, because the bound water and amide protons had significantly different exchange rates with bulk water, the CEST effect from the bound water and amide protons was acquired in two separate experiments. In the current work, we report a novel compound (Nd-DOTAM-Gly-Lys) whose CEST effects from both bound water and amide protons can be observed under the same experimental conditions and therefore can be used as an effective PARACEST agent for pH mapping.

**Methods:** A new PARACEST agent containing neodymium (Nd) metal, cyclen ring, $CH_2CO$ linker, and glycine (Gly) - lysine (Lys) dipeptide sequence as functionalized side chains was synthesized (Nd-DOTAM-Gly-Lys). The relationship between pH (6.0-7.5) and the ratio of the CEST effect of bound water and amide protons was determined in a 10 mM solution of Nd-DOTAM-Gly-Lys on a 9.4T Varian MR Spectrometer at body temperature (37 $^{o}$C) by adding a frequency selective saturation pulse ($B_1$=22 μT and duration time = 10 s) prior to the standard FID pulse sequence (TR= 14 s). The ratio of the CEST effect from the amide protons and metal-bound water is given by $R = ((M_{a,off} - M_{a,on})/M_{a,off})/((M_{b,off} - M_{b,on})/M_{b,off})$, where $M_{a,on}$, $M_{a,off}$, $M_{b,on}$, and $M_{b,off}$ are the bulk water signal intensities with the saturation frequency centered on the amide protons (11 ppm) and off-resonance (-11 ppm), the bound water protons (-34 ppm) , and off-resonance (34 ppm), respectively. CEST images were acquired of a phantom consisting of four NMR tubes (pH 6.0, 6.5, 7.0, and 7.5) each containing 10 mM Nd-DOTAM-Gly-Lys at 37 $^{o}$C on a Varian 9.4T MRI using a fast spin echo pulse sequence (FOV: 20 × 20 $mm^2$, TR=7 s, 16 echoes, and TE=11 ms) preceded by a saturation pulse ($B_1$=20 μT, saturation range=-50~50 ppm in steps of 1 ppm, saturation time = 5 s). pH maps were created from the images acquired at ± 11 ppm (amide), and ± 34 ppm (bound water) using the relationship (R) given above.

**Results and Discussion:** The CEST spectra for Nd-DOTAM-Gly-Lys obtained as a function of pH are given in Fig. 1. The relationship between the ratio of the CEST effect from the amide protons and bound water, and pH was determined using the relationship given above, and was used to create pH maps. The CEST effect of the bound water was almost independent of pH, while the CEST effect of the amide protons was highly sensitive to pH. For example, the reduction in bulk water signal intensity due to amide proton saturation was 20%, 40%, and 55% for pH 6, 6.5, and 7.0, respectively. Inside brain tumors, the tissue is weakly acidic (5), Therefore the high CEST sensitivity of amide protons in this compound within the pH range 6 -7 makes this agent a possible candidate for the pH measurement of brain tumors. The relationship (pH = 0.5988R + 6.255) between pH and CEST ratio at 9.4T for 37 $^{o}$C was created using the average signal intensities at ± 11 ppm and ± 34 ppm in each tube, and was used to generate pH maps. The pH map is given in Fig. 2. Fig. 2a, 2b, 2c, and 2d correspond to pH 6.0, 6.5, 7.0, and 7.5 tubes, respectively. The standard deviation of pH distribution inside the pH maps is less than 0.2. The pH map reflects the real pH value of the solution inside the tube except some points at the rim. The large chemical shift of bound water of this compound also makes it a possible candidate for temperature measurement using the linear dependence of chemical shift on temperature. Further work is required to determine the relationship between the chemical shift of bound water and temperature, and the relationship between pH and the CEST effect ratio from amide protons and bound water under variable temperatures. Once established, this compound might be used to create pH map and temperature map simultaneously.




**Conclusion:** The relationship between pH and CEST ratio from both bound water and amide protons of a new compound (Nd-DOTAM-Gly-Lys, whose CEST effects from both bound water and amide protons might be obtained under same experimental conditions.) was determined, and a high-resolution pH map was generated for a phantom containing four solutions with different pH based on the relationship.

**Acknowledgements and References:** Funding provided by Robarts Research Institute and CIHR/UWO Strategic Training Initiative in Cancer Research and Technology Transfer. (1) Zhang SR *et al*, Angew Chem Int Ed 2002;41:1919-1921. (2) Ward KM *et al*, J Magn Res 2000;143:79-87. (3) Aime S *et al*, Magn Reson Med 2002;47:639-648. (4) Aime S *et al*, Andew Chem Int Ed 2002;41:4334-4336. (5) Gillies RJ *et al*, IEEE Eng Med Biol Mag 2004;23:57–64.