Using the linewidth of the amide proton CEST effect of MRI-PARACEST agents for pH mapping

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Introduction: Altered tissue pH is a common feature in pathological conditions such as stroke and tumors when metabolic demand exceeds oxygen supply. In vivo tissue pH may be a valuable biomarker of disease progression in conditions where oxygen is limited. The methods to measure and map pH using MRI PARACEST agents have been demonstrated based on the pH dependence of the CEST effect of amide protons (1,2). However, these methods require knowledge of agent concentration. Aime et al. have proposed the use of ratiometric methods (3) and Wegh et al. have proposed a method using only the amide proton site (4) to eliminate the dependence on agent concentration. However, these methods assumed a known temperature. We developed a method to measure pH using the amide proton site of a thulium (Tm^{3+}) complex with a DOTAM-Glycine (Gly)-Lysine (Lys) ligand: Tm^{3+}-DOTAM-Gly-Lys (5). The pH can be determined uniquely from the linewidth of the asymmetry curve of the CEST spectrum, which is independent of contrast agent concentration and temperature for a given saturation pulse.

Methods: CEST spectra were acquired as functions of temperature (34 to 40 ^{o}C), agent concentration (6, 10, and 15 mM) in aqueous solutions and (10, and 20 mM) in 3% bovine serum albumin, and pH (6, 6.5, 7, 7.5, and 8) on a 9.4 Tesla NMR spectrometer. The pulse sequence consisted of a continuous presaturation pulse followed by a hard 90-degree pulse. The presaturation time (10 s), the presaturation power (14 μT), and the repetition time (13 s) were fixed for all the experiments. CEST spectra were created to measure the linewidth of the CEST effect by recording the bulk water signal intensity as a function of the presaturation frequency from -60 ppm to 60 ppm with 1-ppm steps. The linewidth of the CEST effect is defined as the Full Width at Half Maximum (FWHM) of the asymmetry curve of the CEST spectrum (Fig. 1). The asymmetry curve was created by subtracting the negative frequencies from the positive frequencies with water referenced to 0 ppm. Spatial pH maps were produced for the 10 mM Tm^{3+}-DOTAM-Gly-Lys-containing phantoms with pHs 6.0, 6.5, 7.0, and 7.5. Images were acquired on a Varian 9.4T small animal MRI scanner at 38 ^{o}C using a two-dimensional fast low angle shot (FLASH) pulse sequence (field of view (FOV) = 25.6 × 25.6 mm^2, data matrix: 128 × 128, TR = 5.22 ms, echo time (TE) = 2.51 ms, and flip angle = 6^{o}) preceded by a continuous presaturation pulse (5 s). A series of images were acquired by varying the frequency of the presaturation pulse from -60 to 60 ppm with 1-ppm steps. pH maps were created using the linewidth of the CEST effect from each voxel.

Results and Discussion: The results demonstrated that temperature (34 to 40 ^{o}C), agent concentration, and the intrinsic magnetization transfer effect from macromolecules do not affect the linewidth of the CEST effect. The influence of the pH on the linewidth of the CEST effect is shown in Fig. 2a and 2b. It is clearly shown in Fig. 2 that the linewidth of the CEST effect increases when the pH changes from 6 to 8. The rate of increase is greater when pH is between 6.5 and 8 than that when the pH is between 6 and 6.5, which means that a more accurate measurement of pH can be obtained for a pH in the 6.5 – 8 range. The pH can be determined uniquely from the linewidth of the CEST effect. The pH map of the phantoms containing 10 mM Tm^{3+}-DOTAM-Gly-Lys solutions with pH 6.0, 6.5, 7.0, and 7.5 is shown in Fig. 3. The mean and standard deviation of the pH maps for pH 6.0, 6.5, 7.0, and 7.5 solutions are 6.47 ± 0.48, 6.60 ± 0.28, 7.04 ± 0.07, and 7.52 ± 0.06 pH units, respectively. As expected, much more accurate and precise pH maps were created for the solutions with higher pH. A standard deviation of less than 0.1 pH units was realized for solutions with pH ≥ 7.0.

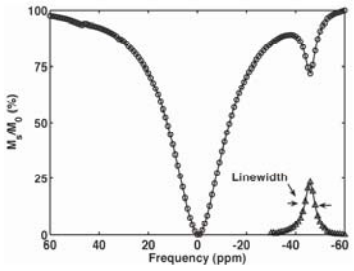

Figure. 1

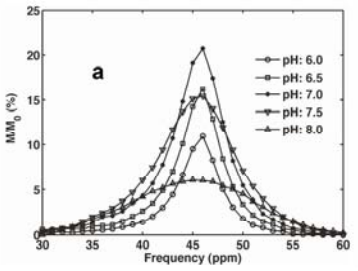

Figure. 2

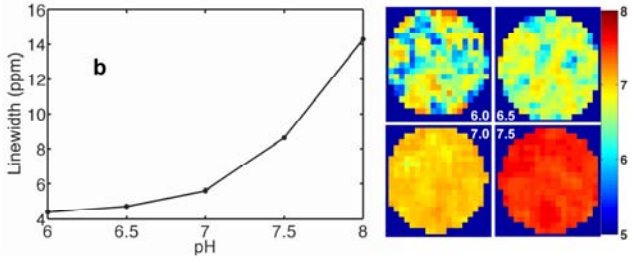

Figure. 3

Conclusion: pH mapping is feasible using the amide proton site of Tm^{3+}-DOTAM-Gly-Lys in a manner independent of temperature, agent concentration, and intrinsic magnetization transfer effect. The greatest sensitivity is in the physiologically relevant range (pH 6.5-8.0).

Acknowledgements and References: Funding provided by Ontario Institute of Cancer Research and CIHR/UWO Strategic Training Initiative in Cancer Research and Technology Transfer. (1). Zhang S, Wu K, Sherry AD. Angew Chem Int Ed Engl 1999;38:3192-3194. (2). Aime S, Delli Castelli D, Terreno E. Angew Chem Int Ed Engl 2002;41:4334-4336. (3). Terreno E, Castelli DD, Cravotto G, Milone L, Aime S. Invest Radiol 2004;39:235-243. (4). Wegh RT, Pikkemaat JA, Willard NP. WO Patent No. 2006-IB51237, 2006114739. 2006. (5). Wojciechowski F, Suchy M, Li AX, Azab HA, Bartha R, Hudson RHE. Bioconjugate Chem 2007;18:1625-1636.