Towards the Development of a Single PARACEST MRI contrast agent for tumor pH measurements

V. R. Sheth[1,2], G. Liu[3], Y. Li[1], and M. D. Pagel[1]

[1]Biomedical Engineering, University of Arizona, Tucson, AZ, United States, [2]Biomedical Engineering, Case Western Reserve University, Cleveland, OH, United States, [3]Radiology, Johns Hopkins University, Baltimore, MD, United States

Purpose: The in vivo measurement of tumor extracellular pH can be used to study the effects of pH-altering therapy and to predict the efficacy of pH-dependent therapy.[1] We have previously developed a single PARACEST MRI contrast agent, Yb-DO3AoAA, that can accurately measure pH without the need to account for the concentration of the agent.[2] In this study, we have investigated the effect of concentration and T1 relaxation time on the accuracy of the pH measurement. We have also established the link between the PARACEST effects of the agent and the pH measurement by applying an acid-base catalyzed exchange model to the analysis of the chemical exchange rates of the PARACEST agent.

Methods: 1,4,7,10-tetraazacyclododecane-1.4.7.10-tetraacetic acid o-aminoanilide (DO3A-oAA) was synthesized and characterized using standard chemical methods. Yb(III) DO3A-oAA complexes were prepared using standard methods. Solutions with different T1 times were prepared by addition of Gd-DTPA (Magnevist(R), Bayer HealthCare Pharmaceuticals). Solution pH was verified using a pH meter (ThermoElectron, Waltham, MA). T1 times were measured for solutions using spin-echo MR images acquired with a range of TR values. The PARACEST effect was measured on a 9.4T Bruker Biospec MRI using a RARE MRI pulse sequence with a RARE factor of 16 (TR/TE=6.0s/8.6s), prepended with a train of 300 Gaussian pulses applied at 21 uT for 5.49 sec to create steady-state selective saturation. The QUEST method was used to measure the exchange rates[3]. The estimated exchange rates of the amide and amine were fitted to the acid-base catalyzed exchange model (Eq. [1]) to reveal the underlying catalysis mechanisms.[4]

$$k_{obs} = k_0 + k_a \cdot [H^+] + k_b \cdot [OH^-] \quad [1]$$

Results:

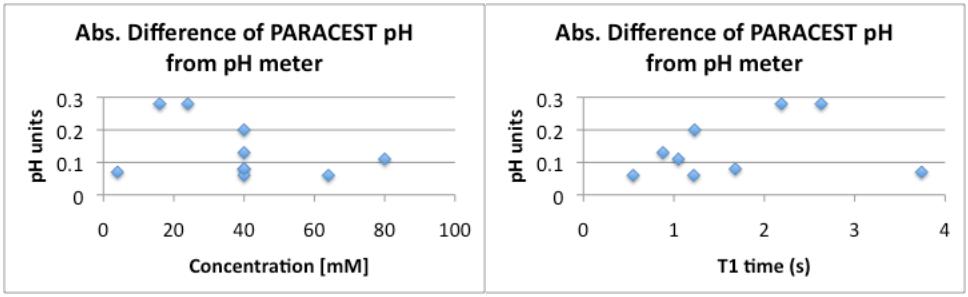

Figure 1 Agent: Yb-DO3A-oAA. **(a)** Concentrations above 5 mM show small absolute difference between PARACEST and a pH meter **(b)** T1 times greater than 500 ms show small absolute difference between PARACEST and a pH meter

Figure 1a shows that Yb-DO3A-oAA measures pH to within a range of 0.3 pH units over a concentration range of 5 mM to 80 mM. For T1 times great than 500 ms our agent is accurate to within 0.3 pH units, as shown in **Figure 1b.** The standard deviation for the difference between PARACEST and a pH meter is 0.1 pH units.

The amide proton showed typical base-catalyzed characteristics[5] with a base catalyzed exchange constant K_b of 6.71 x10^9 s^{-1} (K_0 = 517.85 s^{-1}, K_a = 8 s^{-1}), which was very close to the previously reported value of 5.57 x10^9 s^{-1}.[6] The pH dependency of the aryl amine was found to be more complicated. When pH <7.1, the agent showed base-catalyzed water exchange behavior with a base-catalyzed exchange constant K_b of 3.19 x10^{10} s^{-1} (K_0= 376.86 s^{-1}, K_a = 0 s^{-1}). In the pH range higher than 7.1, the experimental data demonstrated acid catalyzed exchange characteristics[4] with an acid-catalyzed exchange constant K_a of 6.06 x10^9 s^{-1} (K_0= 119.69 s^{-1}, K_b = 0.76 s^{-1}). The underlying mechanism for the aryl amine proton to swap from base-catalyzed to acid catalyzed exchange at pH 7.1 is likely due to a change in protonated state of the aryl amine group (i.e. pKa ~ 7.1).[7] The spontaneous exchange constants K_0 are also different above and below pH 7.1 due to the same reason.

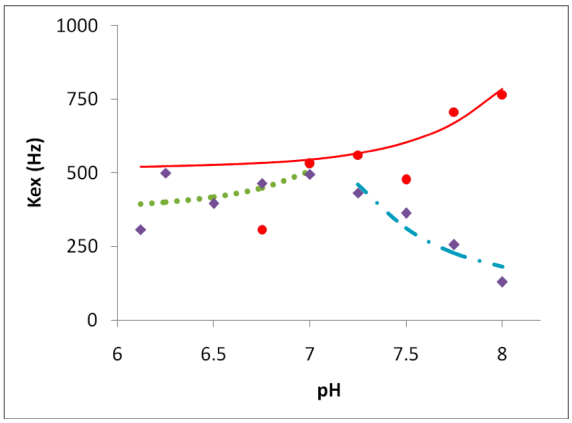

Figure 2: The exchange rate dependencies on pH of amide (red ●) and amine (blue ♦) labile protons. The red solid line was the modeled behavior for amide exchange process. The green dash line was the modeled behavior for the base-catalyzed exchange of amine protons in pH range of 6.1-7.0, and blue dash line was the modeled behavior for acid-catalyzed exchange at 7.2 -8.0.

Discussion & Conclusions: The Yb-DO3A-oAA PARACEST MRI contrast agent can accurately measure pH at concentrations as low as 5 mM and T1 times as low as 500 msec. The pH measurements with the PARACEST agent agree with pH measurements with a pH meter within a standard deviation of 0.1 pH unit, which is comparable to MR spectroscopic methods measuring pH.[8] The proton exchange at the amide site of the agent shows base-catalyzed properties, while the amine site changes from base catalyzed exchange to acid catalyzed exchange at pH 7.1, which explains the pH dependence of these PARACEST effects.

References

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