Four-Pool Modeling of Proton Exchange Processes in Biological Systems in the Presence of MRI-PARACEST Agents

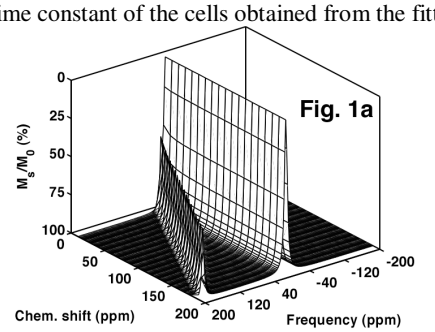
A. X. Li[1,2], R. H. Hudson[3], J. Barrett[4], and R. Bartha[1,2]

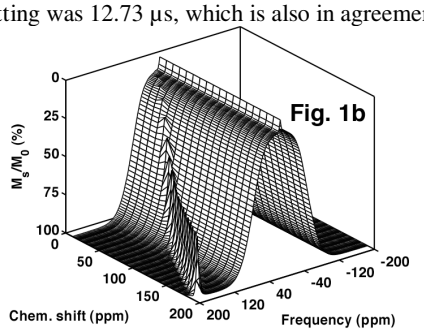
[1]Center for Functional and Metabolic Mapping, Robarts Research Institute, London, Ontario, Canada, [2]Department of Medical Biophysics, The University of Western Ontario, London, Ontario, Canada, [3]Department of Chemistry, The University of Western Ontario, London, Ontario, Canada, [4]Biotherapeutics Research Group, Robarts Research Institute, London, Ontario, Canada

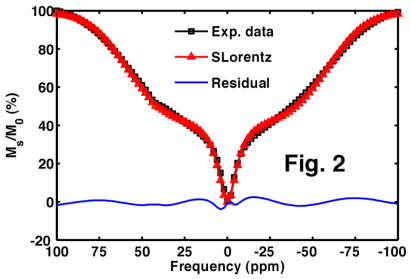
Introduction: A new class of paramagnetic (PARA) agent has recently been developed for magnetic resonance imaging (MRI) that generates contrast by chemical exchange saturation transfer (CEST) [1-6]. PARACEST agents can be used to measure pH [3,4], and temperature [1,6]. The proton exchange processes between the bound water and/or amide protons of PARACEST agents and bulk water in solution have been thoroughly modeled by Woessner et al. based on the modified Bloch equations with exchange terms using a two- or three-pool model [5]. However, the model proposed by Woessner et al. is not directly applicable *in-vivo* due to the inherent magnetization transfer (MT) effect observed from protons associated with macromolecules in biological systems. Bulk water in solution or tissue has a narrow spectral linewidth relative to the spectral linewidth of macromolecule bound water [7]. The magnetization transfer observed by selective saturation of the protons associated with the PARACEST agent leads to the partial saturation of the bulk water signal. Similarly, selective saturation of the broad macromolecule resonance also leads to partial saturation of bulk water. Saturation in the presence of both, leads to the greatest amount of saturation, but the result is not additive. In this work, a four-pool model is presented that incorporates the proton exchange processes between the bulk water (pool A) and the bound water of a PARACEST agent (pool B), the amide protons of a PARACEST agent (pool C), and the exchangeable protons associated with endogenous macromolecules (pool D). The chemical shift, relaxation times, proton exchange rate, and concentration of spins associated with each pool, as well as the saturation power and the duration of the saturation pulse is included in the model. The model was applied to estimate the bound water chemical shift and the transverse relaxation time of an agent-labeled (Eu^{3+}-DOTAM-Gly-Phe [8]) biological system consisting of Vero cells.

Methods: A four-pool model was developed to describe the proton exchange processes between pools A, B, C, and D. The magnetization in each pool can be described by the modified Bloch equations incorporating chemical exchange. The theoretical lineshape function of pool D is Lorentzian, but is commonly replaced with a superLorentzian lineshape function to model the MT effect of macromolecules. Vero cells were incubated with a 10 mM solution of Eu^{3+}-DOTAM-Gly-Phe for 12 hours. The cells were then washed to remove agent that did not penetrate the cell membrane. The CEST spectrum from agent-labeled cells was acquired on a 9.4 Tesla (400 MHz) Varian INOVA AS400 spectrometer at 37 ^{o}C using a standard pulse-acquire sequence preceded by a 14-μT continuous saturation pulse applied for 10 seconds (repetition time (TR) was 13 seconds). The saturation frequency offsets were varied from –100 to 100 ppm with 2-ppm step size. The bound water chemical shift and the transverse relaxation time of the agent-labeled Vero cells were estimated by fitting the experimental CEST spectrum to the four-pool Bloch equations.

Results and Discussion: As an example of the simulation, the effect of bound water chemical shift on CEST sensitivity is shown in Fig. 1. (T_{1A} = 2.5s, T_{2A} = 0.5s, T_{1B} = 0.1s, T_{2B} = 0.1s, T_{1C} = 0.1s, T_{2C} = 0.1s, T_{1D} = 0.1s, T_{2D} = 0.01ms, lifetimes of bound water, amide protons, and protons associated with macromolecules are 275 μs, 2 ms, and 20 ms, 10 mM contrast agent, 10% macromolecules, and a 10s saturation pulse with power 14-μT). The simulated CEST effect for a PARACEST agent in the absence of macromolecules (Fig. 1a) showed little variation (< 2%) as a function of the exchangeable proton chemical shift (10 - 180 ppm). However, in the presence of macromolecules (Fig. 1b) the net CEST effect due to the agent decreased substantially for chemical shifts within ± 100 ppm of bulk water because the CEST effect from the PARACEST agent was obscured by the broad MT effect from the macromolecule pool. Conversely, the MT effect only minimally obscures the CEST effect of the PARACEST agent when the exchangeable proton chemical shift is larger than the spectral width of macromolecule MT (e.g. beyond ± 100 ppm). Previous studies have documented the advantage of PARACEST agents with large exchangeable proton chemical shifts that more easily satisfy the slow exchange condition. Here we demonstrate that large exchangeable proton chemical shifts also increase *in-vivo* CEST efficiency by avoiding confounding MT from endogenous macromolecules. The CEST spectrum obtained from Vero cells labeled with the PARACEST agent is shown in Fig. 2 (squares). The broad background MT effect from macromolecules is clearly visible. The fitting result along with the residual is also given in Fig. 2. The estimated bound water chemical shift was 42.92 ppm, which is in agreement with the value obtained from PARACEST agent solutions (42.95 ppm). The T_2 time constant of the cells obtained from the fitting was 12.73 μs, which is also in agreement with previous reports (~10 μs).







Conclusion: A four-pool model based on the Bloch equations can be used to measure agent bound water chemical shift and magnitude in the presence of the broad MT effect from macromolecules. Such a model will increase the feasibility of *in-vivo* temperature and pH mapping using PARACEST agents.

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 SR *et al*, J Am Chem Soc 2005;127:17572-17573. [2] Zhang SR *et al*, J Solid State Chem 2003;171:38-43. [3] Ward KM *et al*, J Magn Res 2000;143:79-87. [4] Aime S *et al*, Magn Reson Med 2002;47:639-648. [5] Woessner DE *et al*, Magn Reson Med 2005;53:790-799. [6] Li AX *et al,* ISMRM 2007 Berlin, p3402. [7] Zhou J *et al*, Prog Nucl Magn Reson Spectros 2006;48:109-136. [8] Wojciechowski F *et al,* Bioconjugate Chem 2007;18:1625-1636.