EVALUATION OF THE CONCENTRATION OF LANTHANIDE IONS IN EX VIVO MOUSE TISSUE USING BMS NMR SPECTROSCOPY

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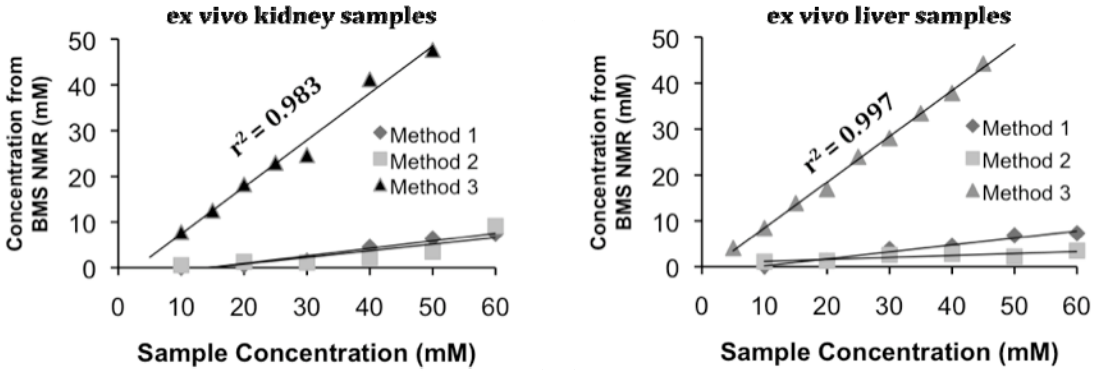
INTRODUCTION: The evaluation of lanthanide-based MRI contrast agents often requires the quantification of concentrations of the agents in ex vivo tissues to validate in vivo results. Inductively Coupled Plasma Mass Spectrometry is often used to quantify the concentrations of these agents, but this method is often expensive and not readily available (which slows turn-around time) [1]. Lanthanide ions cause a bulk magnetic susceptibility (BMS) that creates a concentration-dependent change in the NMR chemical shift that can be easily measured using a NMR spectrometer, which is less expensive and more readily available (which improves turn-around time) [2,3]. We investigated how BMS measurements can quantify the concentrations of lanthanide-based agents in tissues.

METHODS: BMS NMR measurements: Samples from chemical solutions or processed tissues were inserted in a 3 mm NMR tube within a 5 mm tube, and the solvent devoid of lanthanide ions was placed between the tubes. The NMR spectrum was recorded and the difference in chemical shifts was measured (Fig. 1). As little as 50 μL can be analyzed using this 'tube-in-tube' method. **Initial calibrations:** The BMS shift was calibrated with the concentration of Eu(III) and Tm(III) ions in water and in nitric acid, at different temperatures and magnetic field strengths. **Tissue preparations:** Kidney and liver tissues were harvested from SCID mice and treated with formaldehyde. Each tissue was spiked with an amount of $EuCl_3$ or $TmCl_3$ to create concentrations ranging between 5 and 50 mM. Although some samples were heated to 250-300°C, sonicated, and/or filtered, the best procedure for recovering lanthanide ions consisted of lyophilization and treatment with nitric acid. BMS NMR measurements were used to determine lanthanide ion concentrations based on the initial calibrations, and compared with the ion concentration in the initial tissue preparations. The yield, precision, and sensitivity of the concentration measurements were evaluated to determine the utility of this method.

RESULTS: Yield: Our optimized method for preparing tissues for the BMS NMR analysis generated an excellent yield of 92.1% and 93.9% for kidney and liver samples, respectively. Other preparation methods yielded recoveries that were an unacceptable 8-10%. Loss during lyophilization was identified as the primary source of sample loss during the optimized method, indicating that this method can be further optimized to improve yield. **Precision:** The standard deviation of precision for concentration measurements of a single sample was 1.9 mM and 0.74 mM for kidney and liver samples, respectively. The analysis of multiple samples can further improve the precision. **Sensitivity:** Based on the outstanding spectral resolution offered by NMR spectroscopy that leads to very accurate BMS NMR shifts, the minimum detection level for lanthanide chelates can be as low as 0.1 mM.

DISCUSSION: The use of nitric acid during sample preparation has been shown to release lanthanide ions from chelates. Therefore, our optimized preparation method is ideally suited for determining the concentrations of lanthanide-based MRI contrast agents in ex vivo tissues. The precision of the method for analyzing one sample is approximately 1 mM, although measuring multiple samples can improve this precision. The small sample volume of 50 μL facilitates the analysis of multiple sections of a single kidney, liver or other organs from a single mouse to improve the precision. The outstanding detection sensitivity approaching 0.1 mM is acceptable for the study of MRI contrast agents, which typically require a minimum concentration of 0.1 mM for detection with MRI.



REFERENCES: 1. Kantipuly CJ, Westland AD (1988). Talanta, 35, 1-18. 2. Corsi DM, Platas-Iglesias C, Bekkum Hv, Peters JA (2001). Magnetic Resonance in Chemistry, 39, 723-726. 3. Schneck J (1996). Medical Physics, 6, 815-850.