Developing DNP Mixtures with Long T_1s for In Vivo Metabolomics

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Introduction

Nuclear Magnetic Resonance (NMR) is an invaluable tool for determining chemical structure *in vivo, in vitro,* and *ex vivo.* Due to its inherent low sensitivity in detecting small molecules in animal studies, dissolution Dynamic Nuclear Polarization (DNP) may be implemented. DNP enables signal enhancement *in vivo* of up 4 degrees of magnitude.[1] This drastic improvement in signal to noise ratio (SNR) enables localized spectroscopy detection of small molecule metabolism in real time. Animal studies of hyperpolarized ^{13}C metabolite turnover can provide local biochemical information for pathology diagnostics as well as steady state enzyme kinetics.

Characterization of potential targets for polarization is based on spin lattice relaxation (T_1) rates that provide sufficient time to monitor signal enhancement once injected into an animal. Removal of trace metals and deuteration of solvents provide extended relaxation time and improve the mixtures application to *in vivo* models.

Experimental

Experiments were performed on the Avance III 500MHz spectrometer at the University of Florida Advanced Magnetic Resonance Imaging and Spectroscopy facility. Natural abundance ^{13}C T_1 characterizations were performed at physiological pH (7.4) at room temperature for Sodium Acetate, Sodium Oxalate, Sodium L-Lactate, Sodium Bicarbonate, Sodium Pyruvate, Folic Acid, and Sodium Butyrate. For each sample, simulated dissolution conditions were performed by addition of 4-oxo-TEMPO (5mM), Vitamin C (50mM), Ethanol (4%), (Deuterated PBS 1x), and EDTA (1mM).

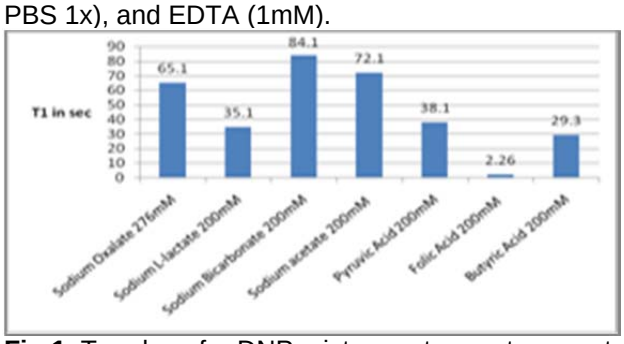

Fig 1. T_1 values for DNP mixtures at room temperature. Values are reported for the unprotonated carbon position.

Results and Discussion

Sample preparation for dissolution DNP is critical for high yield enhancement taking into account the safety of injection into a live animal. Longevity of signal enhancement after dissolution is controlled by both nucleus spin-lattice relaxation and RF tip angle applied during animal spectroscopy. [2] The goal of the study is to determine injection conditions for metabolic substrates that are physiologically relevant as well as practical to dissolution DNP.

Conclusions

Our analysis of several compounds show that six out of the seven have long enough T_1 times, under dissolution conditions, to be useful for application to dissolution DNP. Although further mixture concentration experiments will need to be performed, these results are positive indicators of dissolution parameters and metabolite selection.

Acknowledgements

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References

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[2] Bastiaansen, J.A.M., *et al*. Biochimica et Biophysica Acta **1830**, 4171-4178 (2013).