Brain NMR Metabolomics using Extracted Liquid Samples vs Focused Beam Microwave Fixed Solid Tissues

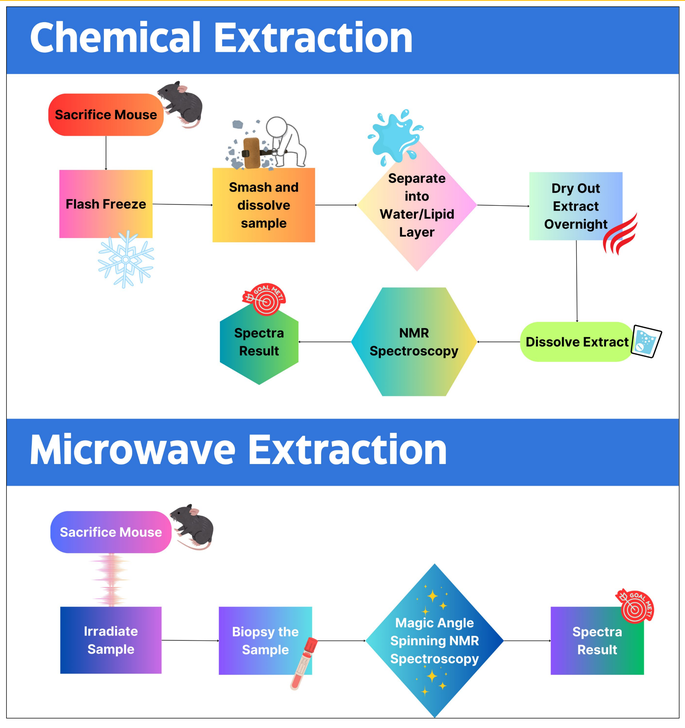
Cristian Zamora, *Zeinab Tashi, Patrick Jiang, Naren Raghu, Fernando Flores, Dr. Benjamin Bartelle*

School of Biological Health and Systems Engineering, Arizona State University, Tempe, AZ, USA

Introduction

The brain is known to have one of the highest metabolic rates out of the major organs and tissues in the human body (1). However, **current information about the brain is lacking** and treatments for neurodegenerative diseases are ineffective. The **brain metabolism has a direct relation to brain physiology**, neuronal function, and neurodegenerative diseases (2). This project **focuses on using Nuclear Magnetic Resonance (NMR) spectroscopy** to observe the metabolic profiles of rodent models. **NMR is an analytical technique** used to quantitatively measure the **structure of organic compounds** by utilizing the **magnetic properties** of the compound's nuclei. Currently, methods for analyzing the physiological brain and rodent models rely on NMR using liquid sample extraction (3,4). To improve upon this method, **Magic Angle Spinning (MAS) using solid samples** in NMR spectroscopy will be used to collect higher quality data by reducing the noise generated from liquid samples. Measurements of the solid sample fixed by the microwave will provide a snapshot of the brain. With the generated spectra being used to compare against liquid sample extraction to test effectiveness. Overall, by **removing liquid solvents and reducing the effect of postmortem conditions,** improvements of data quality are expected to be observed (5).

Flow Chart



Methods

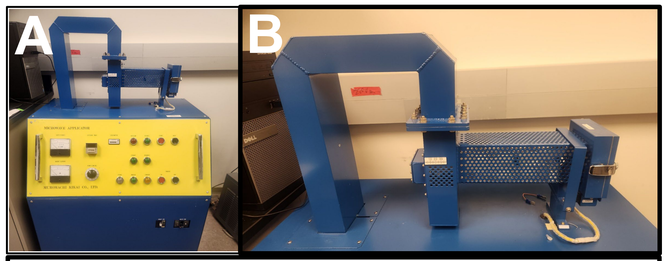


Figure 1: Focused Beam Microwave Irradiator
Analog controlled 6kW microwave with a liquid cooled magnetron tube (**A**) Microwave wave guide to irradiation chamber designed for anesthetized or immediate post-mortem mice (**B**)

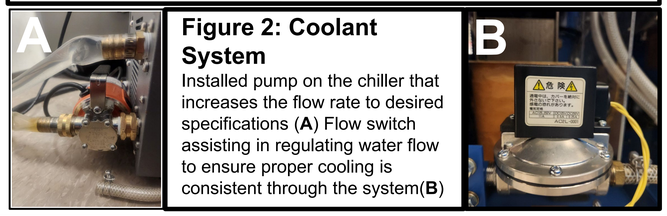


Figure 2: Coolant System
Installed pump on the chiller that increases the flow rate to desired specifications (**A**) Flow switch assisting in regulating water flow to ensure proper cooling is consistent through the system(**B**)

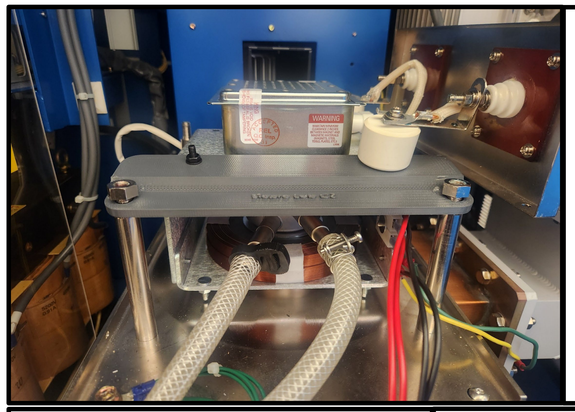


Figure 3: Magnetron

Yj1600 6kw Magnetron CW with a 3D printed custom housing made to fit it in place. Custom wiring, housing and coolant system fabricated. Allows for irradiation to safely be emitted to the sample

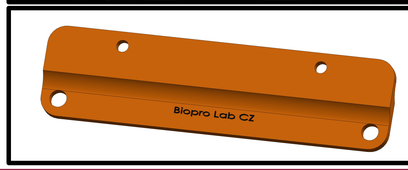


Figure 4: 3D Printed Part

3D printed part to custom fit new magnetron to housing, guides microwave emitter into waveguide

Results

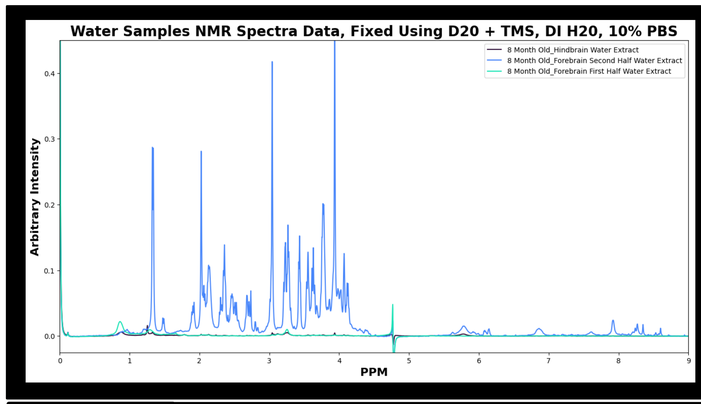
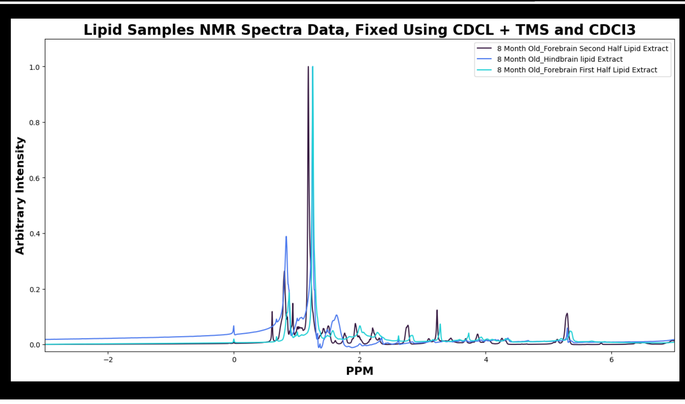


Figure 5: Water Soluble Extracts Measured by Liquid NMR Spectroscopy. NMR spectra of forebrain and hindbrain water soluble extracts from an 8-month-old mouse brain. Spectra are processed by normalizing peak intensity to a tetramethyl-silane (TMS) standard and scaled with TMS at 0 ppm.

Figure 6: Lipid Soluble Samples Measured by Liquid NMR Spectroscopy.

NMR spectra of forebrain and hindbrain water soluble extracts from an 8-month-old mouse brain. Spectra are processed and normalized against the TMS peak and shifted to 0 ppm.



Summary, Conclusions and Future Directions

The liquid sample extraction has provided data that can be compared to the Focused Beam Irradiation method. **Lipid samples had high-quality data** represented by Lorentz peaks, and the **water samples had very low-quality data** mainly consisting of noise. **In the future, focused beam microwave solid samples can be analyzed through MAS**, and higher quality data representing metabolites is expected to be found more efficiently with reduced noise, and less effects of postmortem conditions on the metabolites.

References

1) "PMC Home." *National Center for Biotechnology Information*, U.S. National Library of Medicine, pmc.ncbi.nlm.nih.gov/. Accessed 05 Nov. 2024.
2) 2. Nava, Arturo Sánchez López de. "Physiology, Metabolism." StatPearls [Internet]., U.S. National Library of Medicine, 12 Sept. 2022, www.ncbi.nlm.nih.gov/books/NBK546690/. Accessed 05 Nov. 2024.
3) 3. YouTube, www.youtube.com/watch?v=Sn3dNMv-67k. Accessed 05 Nov. 2024.
4) 4. Maher, Elizabeth A, et al. "Metabolism of [U-13 C]Glucose in Human Brain Tumors in Vivo." NMR in Biomedicine, U.S. National Library of Medicine, Nov. 2012, www.ncbi.nlm.nih.gov/pmc/articles/PMC3406255/. Accessed 05 Nov. 2024.
5) 5. Juras, Jelena A, et al. "In Situ Microwave Fixation Provides an Instantaneous Snapshot of the Brain Metabolome." Cell Reports Methods, U.S. National Library of Medicine, 18 Apr. 2023, pmc.ncbi.nlm.nih.gov/articles/PMC10163000/. Accessed 06 Nov. 2024.

Acknowledgements

I would like to give thanks to Dr. Benjamin Bartelle, Zeinab Tashi, Fernando Flores, Jennifer Brodsky, Patrick Jiang

Ira A. Fulton Schools of Engineering
Arizona State University