Analytical Methods for Metal Analysis in Rabbit Brain

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Abstract

The study and understanding of the brain has increased over the last 30 years with the introduction of new scientific techniques. In recent times, analytical chemistry has been used to determine the elemental composition of the brain and connect that to its function. Two methods of analytical chemistry that can be used are atomic absorption and laser-induced breakdown spectroscopy. LIBS allows for the identification of elements, specifically metals, in the brain while AA allows for the quantification of those elements. In this experiment, a rabbit brain sample was tested using both of the above methods in order to determine what metals were present in the brain. The results were then compared to other's experiments so that links between the metal accumulation and neurodegenerative diseases could be made. It was found that iron has a connection to brain function in Parkinson's disease. With further testing and new methods, an understanding to how the connection works can be investigated.

Introduction

The purpose of this experiment was to determine if metal concentrations in the brain were detectable using common analytical chemistry methods.

Specifically, laser induced breakdown spectroscopy, atomic absorption, and a scanning electron microscope were used to detect and quantify metals in a rabbit brain sample.

Laser-induced breakdown spectroscopy (LIBS) is an analytical chemistry method that allows for spatially resolved elemental analysis of solid samples using an infrared laser. The process involves hitting a solid sample with an infrared laser. The reaction with the sample and the laser emits a photon that is detectable with a camera, which measures the intensity of the photon and plots on a graph vs wavelength. Each wavelength corresponds to a given metal based on that metals excitability, and therefore any metal can be identified. The method is most often applied to common forensic evidence such as glass, paint, soil, etc. However, since it can be used on a variety of solid samples with metal concentrations within the instruments detection limits, it was hypothesized that LIBS could be used to test biological samples such as the brain. Upon research, little to no known data was found on LIBS being used to test for metals within biological samples. Therefore, trial runs had to be performed to determine an appropriate method for testing the rabbit brain sample with LIBS. First, the whole brain was tested with LIBS at wavelength ranges that corresponded to magnesium and sodium, two metals commonly known to be found within biological systems such as the brain. The trials showed peaks at the desired marks, confirming that LIBS was a useful method of testing, but the intensity changed depending on the location of the brain that was hit. The variations in intensity were attributed to the texture and thickness of the brain, correlating to how well the camera could detect the emitted photon. It was decided instead that a slice of the brain would produce better results as the texture and thickness would remain uniform at all wavelengths. Further testing was done on the brain slice at all wavelengths from 200 nm to 800 nm, with the intention of looking at the peaks produced to determine the metal composition.

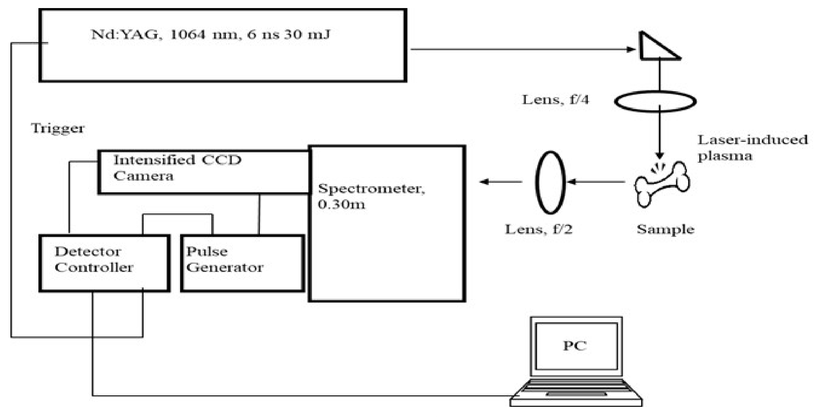

Figure 1. Laser-induced breakdown spectroscopy set-up

The determination of the metal composition of the brain allowed for the use of atomic absorption (AA) to quantify the concentrations of the metals. AA is a process that takes a liquid solution containing the desired metal and vaporizes it with a flame. As it is vaporized the metal emits photons that have an absorbance detected by that metals specific lamp in the instrument. Using this absorbance and a calibration curve made up of standards, the concentration of metals in the tested solution can be calculated. With the information from LIBS, standards of varying concentrations for six metals were made using metal salts. These standards were used to make calibration curves that produce equations to calculate metal concentration in the brain. For the brain solution, metal ions were extracted from the brain into an aqueous solution using concentrated acid and water. The solution was diluted for testing and absorbance values with each lamp were taken.

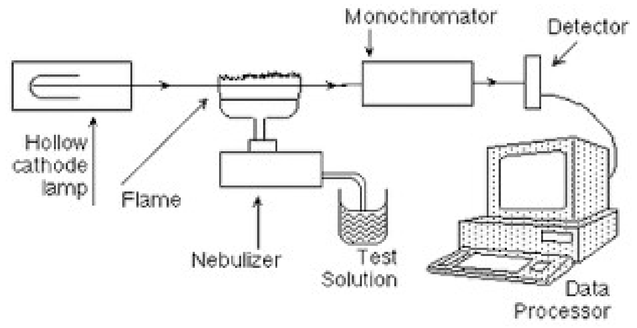

Figure 2. Atomic absorption flame set-up

Finally, images and elemental mapping of the brain were taken using the scanning electron microscope (SEM). The SEM is an instrument that takes high resolution microscopic images of small samples. One of its most useful functions is elemental mapping using x-ray detection, where the x-ray detection allows for elements in relatively high concentration to be mapped on an image of the sample. With the brain sample three metals were mapped as they had relatively high concentrations based on the other data collected.

Using this information, further testing is expected to be done. Specifically, iron will be focused on due to literature that suggests high accumulation relates negatively to brain function and neurodegenerative diseases. The process will involve continued use of the above methods, as well as the use of optical microscopy and H&E staining to get a closer look at the brain and its metal composition. The hope is that future studies will be able to use this information to determine the true effects of metal accumulation in the brain.

Experimental

The first step in this experiment was trace metal analysis with LIBS. To begin, a whole rabbit brain was placed on the stage and tested at two, 30 nm wavelength ranges, with center points of 280 nm (Mg) and 580 nm (Na). The tests involved one run within each range that had ten accumulations and a grating of 500 nm. Following data analysis of the initial sample, the brain was sliced, placed on a silicon slide, and frozen. Next, the brain slice was placed on the LIBS stage and tested at all wavelengths from 200 nm to 800 nm.

Three trials with ten accumulations at each 30 nm wavelength range were performed. The data was collected and peaks for sodium, magnesium, iron, potassium, copper, and zinc were found, among others.

Following LIBS, the rabbit brain was analyzed under the SEM. First, a small piece of brain was placed on a graphite sticker on the SEM plate and placed in the instrument and vacuum sealed. The brain was then observed under the microscope to see if any differing area were noticeable. Images of these areas were taken and saved and the measurement function on the instrument was used to show the scale of the brain features. Second, the elemental mapping for sodium, magnesium, and potassium, was performed for the sample. Three maps were made, each at a size 256x256 pixels with dwell times of -1 ms/pixel, -3 ms/pixel, and -10 ms/pixel.

To begin the AA experimentation, approximately 4 g of the remaining rabbit brain sample was placed in 1 mL of 70% concentrated nitric acid and 4 mL of water. This was left for 72 hours to allow for metal ion dissociation. The solution was gravity filtered to remove any remaining solid brain sample and the solution was diluted to 100 mL with water. Next, a series of standard solutions were made using metal salts and water. First, enough metal salt was weighed and dissolved in water with two drops of nitric acid to produce a 1000 ppm solution. This solution was then diluted by factors of 10 to produce 100 ppm, 10 ppm, 1 ppm, and 0.1 ppm solutions. Standards were made for sodium, magnesium, potassium, iron, copper, and zinc. Second, the standards were analyzed on the AA instrument using the flame method to produce a calibration curve. Each standard corresponded to its appropriate elemental lamp, and the trial began with a blank followed by the solutions from highest to lowest concentration. Only the 0.1 ppm, 1 ppm, 10 ppm, and 100 ppm solutions were analyzed on the instrument. Once absorbance values for standard solutions were collected, the brain metal ion solution was analyzed on the instrument. The brain solution was diluted by a factor of 10 for sodium, iron, potassium, and zinc due to the original values being outside the range of their respective calibration curves. The data was collected and calculations for metal concentrations in the brain were completed.

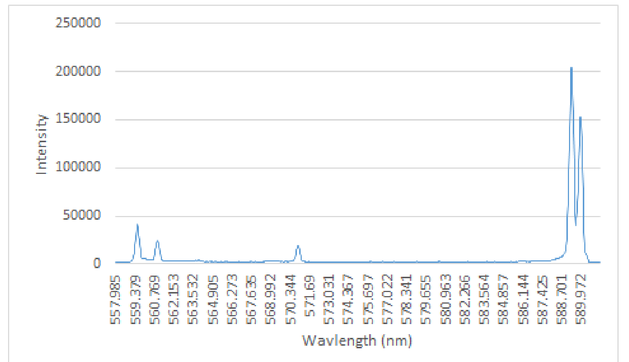

Figure 3. LIBS spectrum for Sodium in brain sample

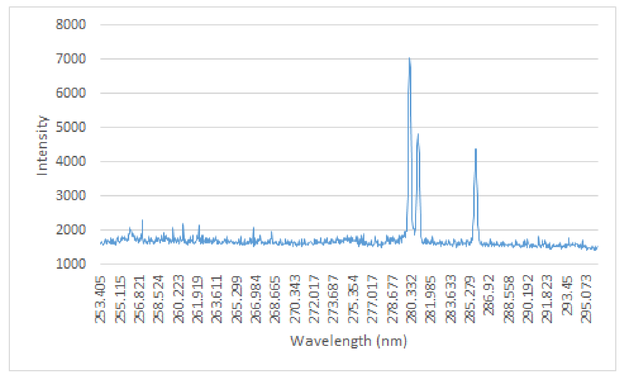

Figure 4. LIBS spectrum for Magnesium in brain sample

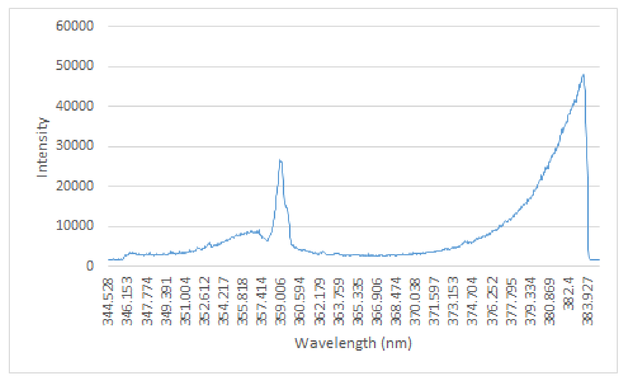

Figure 5. LIBS spectrum for Iron in brain sample

Metal	Na	Mg	Fe
Concentration (ppm)	20700	1450	272
Metal	Zn	K	Cu
Concentration (ppm)	53.5	26900	13.6

Figure 6. Calculated concentrations of each metal

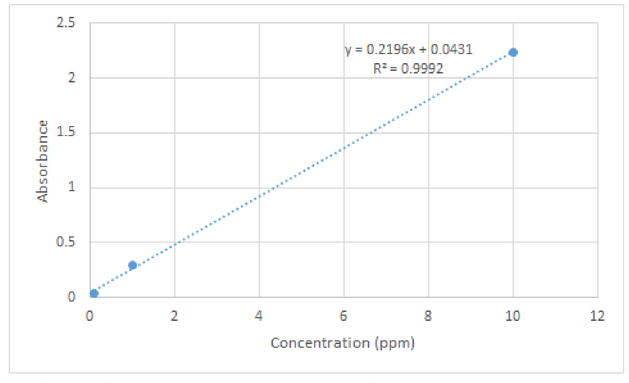

Figure 7. AA calibration curve for Sodium in brain sample

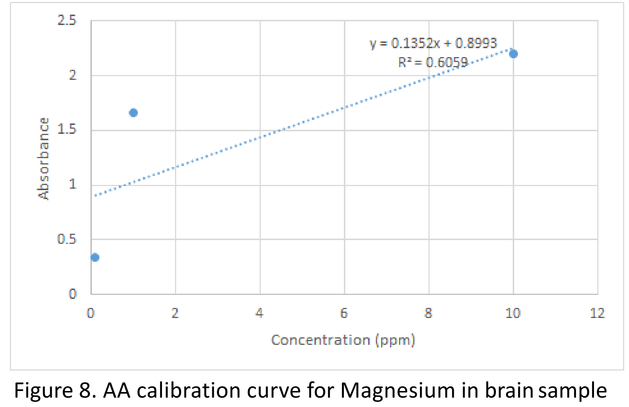

Figure 8. AA calibration curve for Magnesium in brain sample

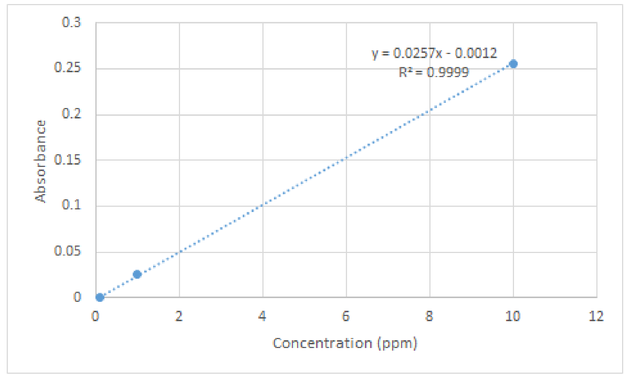

Figure 9. AA calibration curve for Iron in brain sample

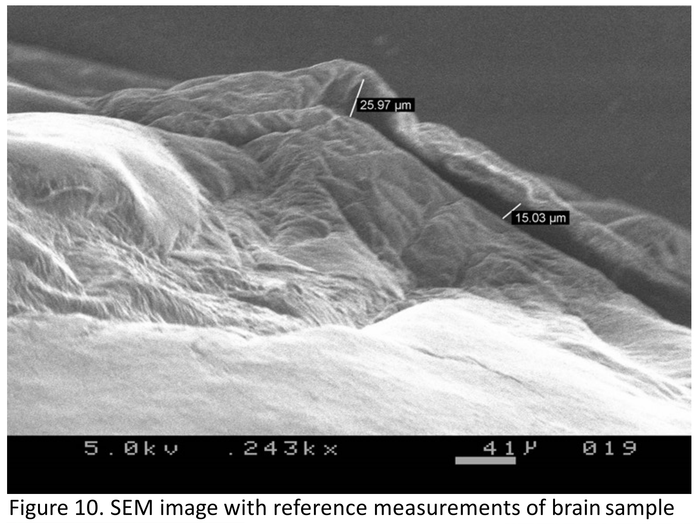

Figure 10. SEM image with reference measurements of brain sample

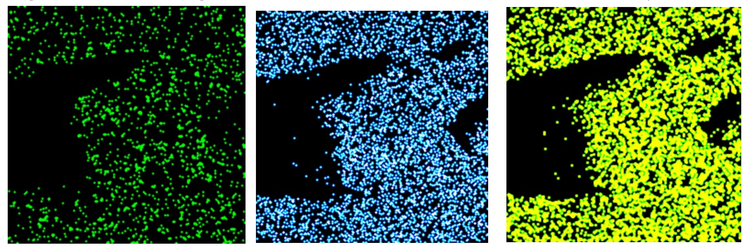

Figure 11. SEM mapping at 256x256 and -10 ms/pixel for potassium, magnesium, and sodium

Summary and Future Works

Analysis using LIBS showed various metals within the rabbit brain, including but not limited to sodium, magnesium, iron, potassium, copper, and zinc. Based on the AA analysis of these metals, concentrations of 20700 ppm sodium, 1450 ppm magnesium, 272 ppm iron, 26900 ppm potassium, 13.6 ppm copper, and 53.5 ppm zinc were found in the rabbit brain. It was also seen that sodium, magnesium, and potassium were in large enough concentrations to be elementally mapped using the SEM instrument. Upon further literary research, it was found that there is a possible correlation between iron accumulation in the brain and neurodegenerative diseases. One such disease is Parkinson's disease, which has shown a relationship between look to quantify the concentration of iron in a normal rabbit brain using LIBS, AA, SEM, H&E staining, and optical microscopy. Determining values for normal rabbit brains would allow for future studies to test for high accumulations in diseased brains using similar techniques. The hope is to determine if a true relationship exists, and if so attempt to find methods that would combat the accumulation and provide treatment to patients.

References

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