Volume 4, Number 1 | ISSN 2331-4621

THE FINGER LAKES JOURNAL OF SECONDARY SCIENCE

FLJSS

Edited by the New Visions Engineering Students at the Tompkins-Seneca-Tioga BOCES Career and Technical Education Center

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Editor's Note

Those who are committed readers of this Journal may have noticed that there has been a significant gap between the publication of this volume and the previous one. I regret this delay, but I've been given the extraordinary opportunity to start a new program, and this is its first year. I'm the teacher in the New Visions Engineering program through TST BOCES, located on the campus of Cornell University. It's an amazing opportunity for high-achieving high school seniors who would like to enter into the field of engineering. They get to visit all manner of interesting labs on campus, and take field trips to myriad places related to engineering. One of the most significant pieces of the program, however, is our articulation with a Cornell course on communication in science. In this course, my students get to work with undergraduate and graduate students as well as members of our local NPR station's science team on effective communication in the sciences.

It's been illuminating, to say the least. We've discovered that scientists are just not very good at communicating with each other, or with people outside their profession – or even just outside their particular field of study. One of the motivations for this Cornell course and our collaboration was an interesting set of statistics that was reported by the Pew Research Center in 2014*. They surveyed just over 2,000 members of the general population and about 3,700 members of the American Association for the Advancement of Science (AAAS), the largest general science society in the world. In the report that was generated based on this survey data, Pew found that 29% of the general population thought that, in comparison to the rest of the world, K-12 science education in America was "below average," while 46% of AAAS Scientists thought that it deserved that ranking. In the same report, 84% of scientists reported that the public's lack of scientific knowledge is a "major problem." Further, the *overwhelming* majority, 75%, of scientists indicate that a "major reason" for the lack of science knowledge is…"not enough K-12 STEM" Education. The final interesting bit is that only 40% of AAAS Scientists see "too few scientists who communicate their findings" as a "major reason" for this lack of public knowledge.

So this data, which, like all data, should be considered carefully for what it is and within a reasonable context, seems to indicate that most professional scientists think that it's mainly the job of K-12 educators to enlighten the population, and that any shortcoming in science knowledge isn't likely to be because they don't share their findings enough. What is hidden in here is perhaps the fact that professional scientists really *are* sharing a great deal of data…they're just doing it incredibly poorly.

At a recent American Society for Engineering Education conference, I attended sessions where many of the presenters had more words per slide than a dictionary did per page, and where not one of the people I approached at the poster session could give me a cogent description of their research in under two minutes (I timed them), much less one that a non-scientist could understand. So the public doesn't understand poorly-communicated science? Is that the public's fault? Is that the scientific community's fault? It's both.

The general population has a responsibility to take advantage of the education offered by the government, and the scientific community has a responsibility to keep the public informed, in a reasonable way, about the tax-funded goings-on in their labs. And as we always do in these notes, we come back to the fact that solid K-12 education is the one thing that can solve all of these problems. If everyone had a broader background in communication, which is what we're trying to achieve in my program, we could all base our decisions on freely-available, well-constructed and understandable science. It's fantastic to see the budding scientists who contribute to this journal work so hard at honing their communication skills; their effort will serve them and our country well.

D.M.S.
April, 2018
Ithaca, NY

* You can read the full Pew report here: https://goo.gl/fqQvWV

EFFECT OF ABOVE SEA LEVEL UNDERDEVELOPED COUNTRIES ON ENERGY GENERATION USING WIND TURBINES

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ABSTRACT

Twenty-first century alternative fuel sources such as biofuel and hydropower can be used to provide eco-friendly and non-polluting energy. Some countries use wind energy as their preferred type of energy due to its low cost and minimal interference with the environment around it. This experiment helped reveal whether wind energy could be utilized as an efficient energy source for above sea level underdeveloped countries as it had been seen that this was not the case for below sea level countries. If the average wind speeds of five underdeveloped countries were used to power a model wind turbine, then the energy produced would prove that wind energy is a viable energy source. The five countries chosen were Uganda, Guatemala, Sri Lanka, Peru, and Armenia as these countries are in a variety of locations and climates, and therefore could give a more accurate representation of how wind speeds are in general. Also, the average wind speeds of these countries were the same as that of Texas, 8 miles per hour (WeatherSpark, 9/6/16). Since Texas is currently the largest wind energy producer in the United States, this statistic helped to support the experiment. In order for the turbines to be viable, the turbines must have been able to produce enough energy for the population whilst not surpassing the income of those countries.

To test this hypothesis, model turbines were constructed using wood, and other easily accessible materials. The turbines were chosen to be horizontal axis turbines because they are the most prevalent turbines countries are using today. Using the data gathered over 60 seconds, the average kilowatt hours per month of one turbine was determined to be 187 – 264 kilowatt hours. The results of the experiment proved that 4 turbines would be successful in offering a viable energy source as the turbines used abundant materials like cheap metals and woods and had a low cost. Furthermore, the turbines were found to consume only about 10% of the countries' non-industrial and non-agricultural, so all three criteria for a viable energy source was fulfilled.

INTRODUCTION

The limited reservoir of fossil fuels such as oil, petroleum, and coal are being consistently depleted to provide electricity for developing countries. Alternative energy is important, as it utilizes renewable energy sources like wind and water to provide energy for a large spectrum of countries around the world. The research that was examined in this experiment was could wind energy could be utilized to provide an alternative and efficient energy source for above-sea level underdeveloped countries.

Underdeveloped countries are severely disadvantaged without efficient energy to compete and contribute to the growing global society. As of today, most human necessities rely on electricity and power to function, like refrigeration, lighting in a household, and the use of technologies to complete daily tasks like homework etc. Research by U.S Energy Information Administration (EIA.gov), supports this claim, by stating that "a person in the U.S uses about 10,932 kilowatt hours on average per year (as of 2014)". This exemplifies that without an efficient and effective power source, people residing in underdeveloped countries have little ways of contributing or competing in this society of developed countries. It also renders them much more liable to harmful pathogens as they lack the energy to power their numerous health facilities such as hospital equipment.

In a study of alternative energy sources, T. Johansson and his team explained the various ways energy could be produced without polluting the environment. Some of these ways included using water currents in the form of hydropower to power machines, as well as using bio-fuel as a cleaner alternative for heating a household. T. Johansson and his team compared the benefits of all these types of energy generation, and also showed examples of where they could be used such as in rural areas. Another study from T. Burton et. Al. examined a problem similar to T. Johansson, with a major difference being the focus on wind power. In their study, T. Burton et. Al. outlined the different environmental factors that could affect the amount of wind energy generated. One such factor was the elevation, as a turbine placed at a higher elevation, was seen to produce more energy in a certain amount of time when compared to a lower elevated one. Additionally, the amount of wind available as seen in wind speeds also affected the overall energy generated as the more wind the turbine captured, the more energy it was able to produce. Lastly, Jeffrey D. Mirocha et. Al.'s study talked about both types of wind turbines and how they are able to produce electricity from the wind around them. He talked about how the wind spins the blades and powers the generator which in turn produces usable electricity.

This research relates to larger issues because according the World Bank Organization (IAWP.org), as of 2013, there are 137 developing countries, meaning that 137 countries are not fully capable of accessing new technologies, innovations, or even bare necessities of survival like lighting and heat, because of their current deficient economical state.

The purpose of this project was to determine whether alternative energy sources could be utilized effectively (in regards to monetary cost, availability of resources, etc.) to provide energy to underdeveloped countries and their population. This was done in the hopes of raising those countries out of their current state and to enable them to contribute, compete, and also to survive in the growing and expanding society of today. This research hones in on simulation of alternative energy sources in above sea level underdeveloped countries. Wind energy was examined in the simulated conditions of wind speed and availability with a test group of five above sea level underdeveloped countries from around the globe. Those five countries were Uganda, Guatemala, Sri Lanka, Peru, and Armenia. (The average data covered all above sea level underdeveloped countries.) These selected countries then had their total populations and wind speeds averaged which was used in this experiment.

The hypothesis for this project was “If the average wind speeds of five underdeveloped countries were used to power a model wind turbine, then the energy produced would support that wind energy is a viable energy source”. This is significant because in 2013, according to Eia.gov, the state that produced the most energy using wind power was Texas. In that year, Texas produced “nearly 36 million megawatt hours of electricity ”, and Texas only has an average wind speed of 8 miles per hour. If Texas was able to produce such a large amount of power using wind turbines with wind speeds of only 8 miles per hour, wind energy should prove successful for the above sea level countries as well as their average wind speeds was also approximately 8 miles per hour. If this hypothesis was supported, it would be evident that above sea level underdeveloped countries are capable of utilizing a more efficient energy source than what they currently possess. This new energy source would not only reduce the pollution of the ozone layer because it is based upon renewable resources, but it would also allow these countries to have access to better resources which they could then use to benefit their countries as well as the state of underdeveloped countries as a whole. If this hypothesis was not confirmed, however, it would demonstrate that wind energy generation is not an effective way of providing alternate energy to underdeveloped countries as a whole, whether it is above or below sea level.

This research could lead to a significant improvement in the lives of people living in underdeveloped countries, because they would stop using harmful resources such as kerosene oil and diesel. These resources pollute the air and contribute to the growing problem of global-warming, which harms humans as well as the earth as a whole. They would probably restrain from using these resources, now that they have a cleaner alternative that still fulfills their energy needs. Lastly, this proposed research may persuade global authorities to start looking at other types of alternative energy and implement these types of energy generation in all countries where everyone could have access to electricity and an efficient power source that does not harm the environment, contrary to modern forms of electricity generation people are using today. If this is so, all countries would be able to contribute to the advancing world of technology in society.

MATERIALS

The materials used were one sheet of Aluminum sheet metal, one 8 amp DC motor , one thin metal rod (about 32 cm), one small metal strip, eight pairs of nuts and bolts, two semi-long wires (about 35 cm each), one roll of electrical tape, one 26 cm x 19 cm wooden platform, wood and metal working tools (Bench Grinder, Clamp and Drill, Power-Drill), one pair of strong scissors, one house fan, one metal strip, and one multimeter (Innova 3320).

METHODS

A horizontal-axis turbine was constructed using inexpensive materials which in this case were sheet metal and a block of wood. This type of turbine was constructed because according to Centurion Energy, and the U.S Energy Information Administration, the horizontal-axis variant of wind turbines is the most commonly used type of wind turbines throughout the world. This is because the horizontal axis turbine is more cost efficient and more of those turbines could be built with same budget, in comparison to the vertical axis alternative. It was important to use a horizontal-axis turbine, because underdeveloped countries do not have an abundance of money to begin with. With this in mind cost efficiency was a main focus when choosing which turbine to use within this experiment.

First, the blades were cut out of the Aluminum Sheet Metal for the wind turbine. Two 20 cm long and 11 cm wide blades were cut out of the sheet and the blades were then glued together. After this the small metal strip was centered and connected to the blades. Then, 3 holes were drilled in the metal strip (1 at either end and 1 in the middle) and screws and washers were placed into the two outermost holes. The small generator had a small metal cylinder protruding from the side which was placed in the last hole of the metal strip. Next, a pencil was used to mark off the thirds of both blades vertically. In this model, the turbine was intended to spin clockwise so the right third of both blades seen from the center hole of strip, was folded inwards at approximately 45 degrees. Likewise, the left thirds were folded outwards at about 45 degrees. The folds made the blades feel stiff rather than the prior flimsiness of the Aluminum sheet metal itself, while not hindering its ability to capture the wind. Lastly, since one washer came pre-attached to the fixture, that washer was just taken off and attached to the center hole as to secure the blades of the turbine to the generator itself.

After this, the tail of the wind turbine was assembled. This was done by using the second metal strip and hammering the end down using a hammer. The ends enclosed just enough for a folded piece of aluminum sheet metal to be placed inside. Screws were used to secure aluminum piece to extending strip. One hole was drilled into the strip, where the small cylinder of generator could be placed into. Then, electric tape was utilized to secure the metal strip to the generator after the cylinder was placed in hole. After that, the generator and tail device was placed with the blades and into the metal rod to merge all the pieces together. Following that, the platform was constructed to hold the wind turbine in place. Using the wood block and screws along with the drill gun, the metal rod was attached to the platform. The wood grinder was used beforehand to make the edges of the wood smooth and to make the surface even, to hold the turbine steady in place. Since the screw at the bottom of the platform makes the platform move around when under simulation, 4 more screws were drilled to the four bottom corners of the platform to keep it in place. After all this, the generator and attached parts were placed onto the metal rod and the whole device was assembled upright. Furthermore, two wires (ends stripped using wire clippers) were attached to the end of the generator and on the tail strip as to not interfere with the blades or rest of device.

To collect the data for the experiment henceforth, the small house fan was set to the same setting as the average wind speeds of the 5 countries. This average wind speed was found by averaging the wind speeds for each country using the Figure 3 below, and the populations of the countries was also averaged so they could be later used to determine how many turbines would be needed for the underdeveloped countries. The fan-wind turbine simulation was conducted for a span of 5 minutes where the high and low voltages were recorded in 1 minute intervals. Then, the average high and low voltages were determined and converted into watts. Watts is the most common measurement when electricity and power is involved and is the most used measurement when referring to the average power needed somewhere rather than the raw energy. That was then converted into kilowatt hours because that was what was this experiment was using for the approximate power used in the U.S. This data was then compared to the cost of the population to survive over approximately a year after which the appropriate conclusion to the question was determined (i.e. if it takes $100,000 to enable the population to survive for one year, the amount of energy needed must be compared with the experimental data, as well as the minimum cost of the turbines required to produce that approximate amount of electricity, etc.) The model turbine was based on the current model of horizontal-axis turbines which can be seen in Figure 1.

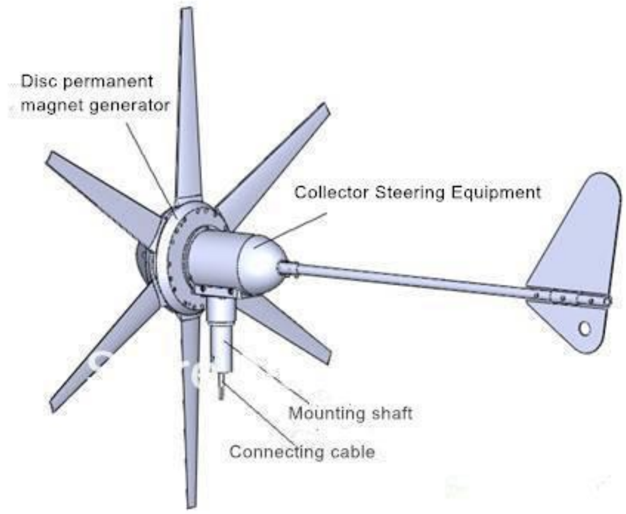

Figure 1. Design of Horizontal-Axis Turbine used in experiment.

There was no control group in this experiment as the turbines were calculating the amount of energy these average wind speeds were producing. Since the wind speeds of those countries were not used separately, having a control group would have been problematic and would not have served a valuable purpose in this study.

RESULTS

Country	Wind Speed (miles per hour)	Population (people)
Uganda	8 miles per hour	40,502,144
Guatemala	14 miles per hour	16,716,876
Sri Lanka	7 miles per hour	20,822,480
Peru	8 miles per hour	31,825,249
Armenia	3 miles per hour	3,020,000
Average	*8 miles per hour*	*22,577,350*

Figure 2. Population and Wind Speed per Country

Trial	Low Voltage	High Voltage
1	0.8 volts	1.1 volts
2	0.9 volts	1.3 volts
3	1.0 volts	1.4 volts
4	1.0 volts	1.3 volts
5	0.8 volts	1.2 volts
6	0.6 volts	1.3 volts

Figure 3: Low and High Voltages per Trial

Average Low Voltage	Average High Voltage	Average Range
0.85 volts	1.2 volts	0.85-1.2 volts

Figure 4: Average Voltages of 6 Trials

CONCLUSIONS

The purpose of this experiment was to see if wind energy generation using wind turbines could prove to be an efficient alternate energy source for above sea level underdeveloped countries. Some major findings were that one small turbine could produce an average of 187-264 kilowatt hours over the span of one month ([2,244-3168] /12). This meant that in order to fulfill EIA.gov's 911 kilowatt-hours standard for one person in America (10,932 / 12), 4 turbines would be needed per person. Initially, the two criteria that went into determining if the wind energy generation was "efficient," was that the wind turbine must be able to produce enough power to sustain the population of the country while at the same time costing less than the gross income. Additionally, the turbines had a very low cost, as these materials could be found throughout underdeveloped countries, which proved the initial hypothesis to be supported.

Although the hypothesis was supported by the fact that Texas produced enough alternative wind energy with an average wind speed of 8 miles per hour, the size and land the turbines were taking up were not accounted for. Each small turbine used in this experiment had a base area of 26 cm x 19 cm. This means that if the "4 per person" rule was put into effect, the total area of turbines required per person would be 1976 cm^2 or approximately 0.1976 m^2. Given that the majority of these above sea level underdeveloped countries have a lower GDP in relation to developed countries, they have a plethora of free land that they could take advantage of. For example, Uganda alone has approximately 930 square meters of free land for each of its 93,000 square miles of area (assuming that 1/100 of each square mile of land is not being taken up by industrial or agricultural structures). Even accounting for the total population of Uganda given this estimate of land, only about 8 million m^2 would be required for the turbines out of the 86,490,000 m^2 of free land available. Given this estimate, the third criterion of how much land the turbines would produce was not a problem as to account for the construction of the "4 per person" turbine rule as stated before, the turbines only required about 10% of the countries' non-occupied land. According to these figures, the notion of having 4 turbines per person in an above sea level underdeveloped country supply enough alternative energy to sustain the population is accurate.

Within this experiment, there was a small number of discrepancies and sources of error. One of these sources of error was that initially, the amount of land the turbines were taking up was not taken into account. Although this factor did not change the answer derived from the experiment, it would still have been beneficial to have considered this point in the beginning of the experiment rather than adding it whilst already conducting the experiment. Furthermore, it was determined that these above sea level underdeveloped countries would have these simple and low costing materials already available as pieces of metal are common as they are used in construction and also found in debris and other structures. Yet, if for some reason the underdeveloped countries did not possess these materials, which is improbable, the cost of one small turbine would be approximately 13 U.S. dollars. Given this number, the total cost on average of turbines needed for each country would estimate to about 1.17 billion U.S. dollars. Currently, Uganda alone is expending $750,000 on energy production every year which means in 2 years they would have repaid their investment in the turbines and they would begin to save money on energy every following year. In addition, to find the average wind speed of these countries, the internet and other acknowledged weather surveying websites were used. Although this was not such a substantial error, the data may have been more accurate if these countries were traveled to and the weather was surveyed there. However, this would have consumed a large amount of money which was not available to us. Lastly, in this experiment, a small model turbine was constructed and used rather than a more conventional wind turbine. This was because large conventional turbines usually cost thousands of U.S. dollars. Even though it would have been better to use conventional turbines to test this experimental question, using such large and costly conventional turbines would have been impossible within this experiment.

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THE EFFECT OF THE SIZE OF AN ELECTRODE ON THE ENERGY OUTPUT OF A FUEL CELL

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ABSTRACT

Today, there are many forms of alternative energy that are used in power plants. However, they are generally limited in many regions due to the high cost and poor efficiency of those systems. One of the most promising forms of alternate energy known of today is the fuel cell. Unlike most common alternative energy sources, fuel cells have a very high energy output efficiency. When energy (electricity) is produced, there is a rate of efficiency that shows how much of the energy source is being converted into electricity. Fuel cells have an efficiency rate of 60-80%, whereas solar panels have efficiency rates of 22.1%. The purpose of this experiment was to utilize the bacteria found in runoff from a stream to produce electricity in a microbial fuel cell (MFC). The hypothesis of this experiment was: If the size of the electrode of a fuel cell is doubled, then the fuel cell will have double the energy output. In order to conduct this experiment, two basic MFC's are created using two plastic containers with holes connected by a PVC coupling and tubing, which functions as a membrane for the fuel cell. Waste was placed in the anode, and oxygenated water was placed in the cathode. After recording data from 3 different trials for each fuel cell, the first cell peaked out at 1 volt and 0.05 milliamps, and the second cell peaks out at around 1.8 volts and 0.09 milliamperes. The first cell had enough voltage to power a small LED. The fuel cell with an electrode of double the surface area had an average of a 95% increase in voltage, and an average of a 92% increase in milliamperes. The hypothesis was unsupported, as the MFC with the larger electrode was not as efficient as the MFC with the smaller electrode. The results of this experiment indicate that there is potential for MFC's to produce renewable energy in a larger scale.

INTRODUCTION

A fuel cell is similar to a battery in that it contains many cells, but rather than storing energy like a battery does, a fuel cell produces electrical energy by utilizing chemical reactions. According to the U.S. Department of Energy, Fuel cells have an efficiency rate of 50-85%, and residential solar panels have efficiency rates of 22.1% at most. Efficiency rates refer to the amount of inputted energy that will be converted into usable electrical energy. Fuel cells are utilized in many different applications, especially in large cities, as a result of their high efficiency relative to solar panels. The resulting products of a hydrogen fuel cell includes heat, electricity, and water. These products are considered to be environmentally safe. Certain types of hydrogen fuel cells, such as MFCs, can reduce organic waste. This is important because of our current epidemic concerning non-renewable energy sources and the search for renewable energy. However, the development of fuel cells had some issues that need to be addressed.

The general area that was studied concerned the development and efficiency of different types of fuel cells that operate at lower temperatures. According to the Department of Energy, there are 7 well known types of fuel cells that are utilized in systems today, and only 1 of them operates at a 'low' temperature of around 80°C (176°F). This fuel cell is known as a PEM (Polymer electrolyte membrane) fuel cell, and generates electricity by combining oxygen and hydrogen to form water. The reaction of combining these two gases releases energy, therefore generating electricity.

Most factors of the efficiency of a fuel cell depend on the quality of the cell's structure. As stated by the Office of Energy Efficiency & Renewable Energy, PEM fuel cells are great for portable appliances due to their operating temperature, and their convenience of utilizing easy to access materials, such as hydrogen and oxygen. Normally, platinum is utilized as a catalyst to split hydrogen into hydrogen ions and electrons. The electrons then move across a wire to provide electricity, whilst the hydrogen ions move across a membrane that is only permeable to positively charged ions. On the other side of this membrane, negatively charged oxygen is readily available in the cathode, along with the electrons from the split hydrogen to recombine with the hydrogen ions to form water.

However, there were modifications of the PEM fuel cell that utilizes bacteria to generate electrons from organic material. Water is the home of many types of bacteria, some of them being anaerobic bacteria. Anaerobic bacteria are utilized in the MFC (microbial fuel cell) because they can undergo respiration without oxygen. This type of fuel cell uses the same type of concept in generating electricity, except it is very simple and is extremely accessible. The research question is concerning the efficiency of a MFC fuel cell, and specifically what would happen if the size of the electrodes used in the cell are changed.

If certain types of energy sources are more efficient, then this could result in a general increase of electricity output in all fuel cell builds. According to a study done by Bailey, global interests in renewable energies have placed increasing attentions on hydrogen-based fuel cells. It is also states that there is a critical need for an increase in development in clean energy technologies (Bailey et al. 1). Also in another study: "There has been a renewed interest due to global efforts to develop and use clean and efficient energy conversion technologies for sustainable development. Thus, this chapter presents an educative discussion on the current status of fuel cells and hydrogen technology." (Bello, 1). These articles discuss the main issues of fuel cells in society today, and it all comes down to the storage and usage of hydrogen. Hydrogen is generally stored in gas or liquid form, and both methods are very costly. According to the Department of Energy, targets for lower costs will not be achieved until the year 2020, "High density hydrogen storage is a challenge for stationary and portable applications and remains a significant challenge for transportation applications. […] By 2020, FCTO (Fuel Cell Technology Offices) aims to develop and verify onboard automotive hydrogen storage systems achieving targets that will allow hydrogen-fueled vehicle platforms to meet customer performance expectations for range, passenger and cargo space, refueling time, and overall vehicle performance." If the cost can be lowered to a more acceptable rate, fuel cells will be very appealing. Both of these articles emphasize the needs to improve existing technologies, and to put more focus into the development of storage methods. Bailey's article gives some solutions for the low number of workforce concerning fuel cells. Bello's article, however, brings more concern to the issues that fuel cells must overcome to be an exceptional alternative source of energy.

In articles by Chouler, an explanation on the functions and reasons for why MFC's were very appealing was given. "Microbial fuel cells are devices that directly convert the chemical energy in organic matter into electricity via metabolic processes of microorganisms [28]. […] In the absence of oxygen, the electrons are extracellularly transferred to the anode and flow through the external circuit towards the cathode thus producing electricity." (Chouler et al. 4). According to the studies, microorganisms are utilized to produce electrons which, in the end, produce energy. These microorganisms are also very easy to access, and are currently found in almost every source of water today. These conditions make this type of fuel cell very easy to access and helpful.

This could fix the issue of PEM fuel cells not being used, as it is important in finding a good alternative source of energy. These background studies were not quite as sufficient as they only provide the issues with fuel cells, and do not provide much of a solution for the issue. Since MFCs require electrodes for bacteria to grow on, the surface area of the electrode would affect the energy output of the fuel cell. Based on these findings, it was hypothesized that: If the surface area of an electrode in a MFC is increased, then the output of electricity would also increase.

If the hypothesis was confirmed, it would be supported that a larger surface area electrode was more efficient, and it would make sense to produce fuel cells with larger electrodes for the highest possible efficiency. But, if the hypothesis was incorrect, it would be supported that the surface area of the electrode in a fuel cell does not alter the energy output in any form. This would mean that there would be no reason to try to increase the surface area of the electrodes in a single fuel cell. Instead, it would be most efficient if a separate fuel cell was constructed.

Ultimately, more research is needed in this field because this type of fuel cell has undeniably vast energy appliances. Even if the actual output of the cell was low, there would be little risk in setting up this system. These fuel cells could be used in a stream, lake, or better yet, the sewage system. Since many types of bacteria dwell in the sewage from animals and humans, a MFC could be utilized inside of a water treatment facility. Since the bacterium in the MFC must perform respiration, they remove organic material similarly to a decomposer. Based off of the efficiency of MFC's, they will do exceedingly well in a city's' sewage system due to the bacteria's ability to decompose the waste entering the cell.

DESIGN OF EXPERIMENT

In order to collect and record the data, data tables were used, and charts were used to help compare the differences in performance when the fuel cell was utilizing the different sources of energy and supplies. A multimeter was used to obtain the data for these charts. Data was recorded at the same time of day to reduce miscalculations in the data. Electrodes was made in a rectangular shape in order to keep the surface area of the electrode as accurate as possible. The electrodes were flat as well, so there were only two sides where the bacteria were growing (back and front of the electrode). After recording data for 21 days, a conclusion was made concerning the efficiency and production of the MFC's. The purpose of this experiment was to test the efficiency of the fuel cells when the size of the electrode is changed. 2 fuel cells were constructed in this experiment, one with electrodes measuring 5 cm x 5 cm, and the other with electrodes measuring 10 cm x 5 cm.

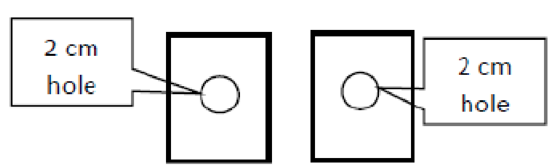
The equipment that most laboratories carry were not available, so only an electrical multimeter was used to record all data. Some variables that were kept constant included: room temperature, the fuel cell, the containers, and light conditions. All mud in this experiment was obtained from a nearby pond. If the stated hypothesis was supported, the electrical output of the MFC with the larger electrode would be the most productive and efficient.

MATERIALS

In order to complete the practical of this experiment, safety goggles, work gloves, and adult supervision at all times were required to be safe. The materials required to build the fuel cells include: 2 straight-sided plastic storage containers, about 11.4 cm. x 11.4 cm. x 17.8 cm, a drill press with 19 mm spade drill bit, a 2-millimeter (mm) drill bit, a digital multimeter, scissors, wire strippers, a 500 mL beaker, plastic wrap, aluminum foil, water, a pot and spoon, a glass rod, some electrical tape, 30 grams of agar, 15 grams of table salt, two 19 mm O-rings, two 12.7 mm PVC nuts, an aquarium pump and tubing, number 8 washers and nuts, two pieces of carbon cloth, 20 cm X 20 cm, nickel epoxy or other conductive epoxy, two pieces of 12-gauge copper wire, 45.7 cm in length, two 12.7 mm PVC couplings and a 12.7 mm PVC pipe. The pot, spoon, and glass rod were used to stir materials later in the procedure, and were not specific.

METHODS

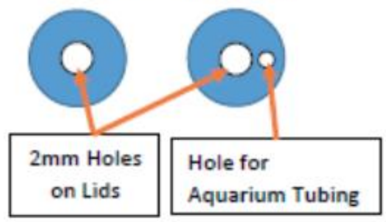
The permanent marker and ruler were used to make a mark (in the center) 4.45 cm from the bottom of both of the plastic containers. The containers were placed so that the marks are exactly opposite of and facing each other. Then, the 2 cm spade drill bit was used to drill a hole where the marks are located on the sides of both plastic containers. The holes were



Sides of the 2 Containers (Figure 1)

drilled slowly, as the drill might crack the container, and it would have been rendered useless. A 2-millimeter (mm) hole in diameter was drilled with a drill bit on both lids of the plastic containers. Plastic debris from the lids were brushed off.

Figure 2: Container Lids



Using the ruler, the outer diameter of the aquarium air pump tubing was measured, and a hole was drilled with the same measurement next to the first hole on one of the lids. The 19mm O-rings were placed on the sides with the threading of the 12.7 mm couplings. The threading side of the couplings were pushed through the holes on the side of both containers. From the inside of the containers, the 19mm washers were put on the threading of each coupling. The 12.7 mm PVC nuts were then screwed on each coupling. The PVC pipe was inserted into to the couplings on each container, and was checked to see if it was watertight. The PVC pipe was removed after confirmed that it was watertight. One end of the PVC pipe was covered securely with aluminum foil. The tube was placed, open end up, on a flat surface.

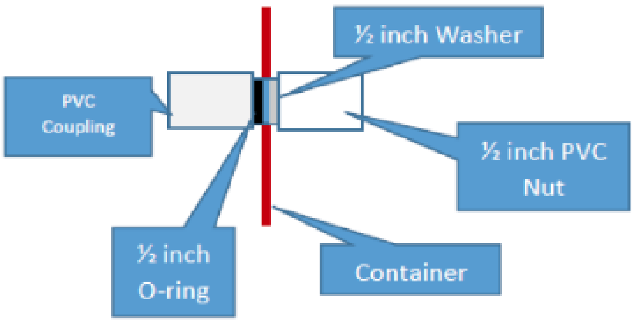


Figure 3: PVC Coupling Diagram and Set-Up

100 milliliters (mL) of water was measured and poured into a pot. The scale was used to measure out 10 g of agar. The measured agar was aside. 1 g of salt was measured. The pot of water was placed on the stove and brought to a boil. The agar was added to the boiling water and was stirred with the glass rod until it was dissolved. Once the agar was dissolved, the pot was taken off of the heat, and 1 g of salt was added into the mixture. The salt was stirred until it was dissolved. While the solution was still warm, the solution was poured into the PVC pipe. Once the pipe was filled, it was carefully moved to the refrigerator. The pipe was incubated at 2 degrees C for 8 hours, creating the salt bridge.

Next, the scissors were used cut the carbon cloth into 2 equal squares. 2 squares were 5 cm x 5 cm, and the 2 other squares were 10 cm x 5 cm. 2 pieces of copper wire were taken, and the wire stripper was used to strip off 15.24 cm of the insulation on one end of each wire (End A). 1 cm of insulation was stripped off from the other end of each wire (End B). Then, the conductive epoxy was prepared according to its directions. End A of one of the copper wires was epoxied to the carbon cloth, along its edges. This was repeated for the other copper wire. The epoxy was left to harden for 10 hours.

Once the epoxy was hardened, the connection between the carbon cloth and the copper wire was tested with the digital multimeter in resistance mode. One lead of the multimeter was placed on the carbon cloth and the other lead on End B of the wire. There was none or very low resistance (1–3 ohms). This was called an electrode.

The two containers were retrieved along with the salt bridges previously made. The pair of containers were connected together with the salt bridges. The salt bridges were inserted back into the two couplings on the containers. 2.8 liters of stream water was measured into a large bowl. 89.0 mL of salt was added into the bowl and was stirred with a spoon until the salt was dissolved. A container was filled with the salt solution. A screw was placed head up through the 2mm hole from the bottom of the lids of both containers. The screw was tightened slightly by putting a # 8 nut onto the top of the screw. An electrode was taken and End B of the copper wire was wrapped around the screw head, and was tightened onto the screw with the nut. This step was repeated for the second container lid. The lid with the two holes and the connected electrode was placed on one of the containers. The electrode was submerged. The lid of the container was tightened, and thus the contained was sealed.

Figure 4: Assembly Completed

The aquarium pump tubing was connected to the outlet of the aquarium pump, and used to oxygenate the water. The tubing was pushed through the free hole in the lid and the end of the tubing was submerged. Gloves were used to half-fill the mud into the next container. The mud was pushed down and tapped gently to ensure that no bubbles were present in the mud. Then, the second electrode was buried in the mud. Enough mud was placed into the container to cover the electrode. End B of the copper wire was wrapped around the screw head on the second container lid. The lid was placed onto the container to make sure that the electrode was hanging freely. The fuel cell was now completed, and output ready to be recorded.

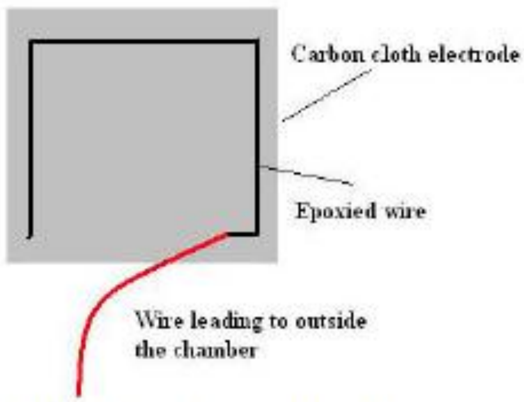


Figure 5: Completed Electrode

Figure 6: Completed Fuel Cell

ANALYSIS

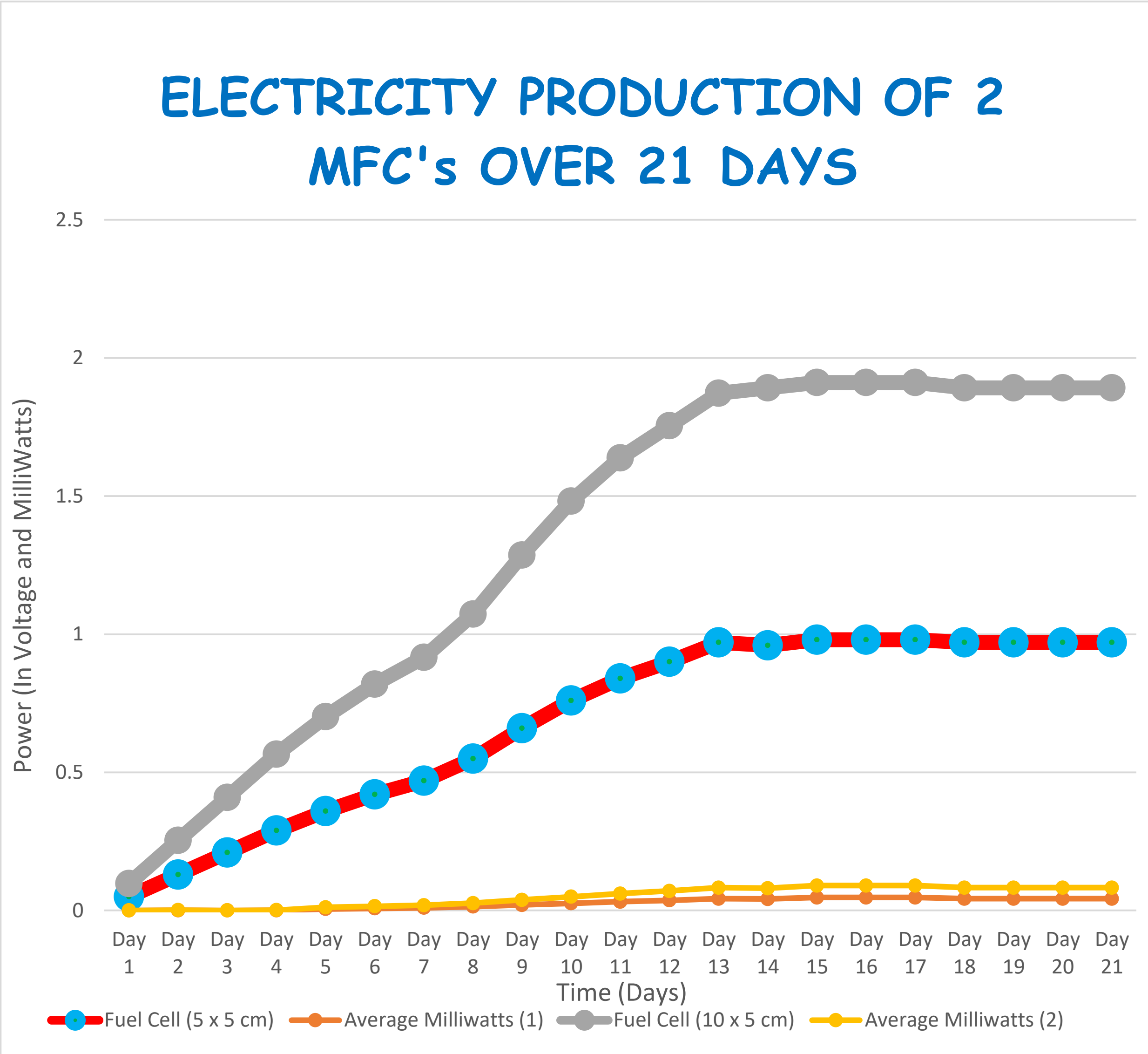


Figure 7: The chart displays the energy produced by the two fuel cells over 21 days. The recorded information is an average of the three trials.

When the 2 MFC's (microbial fuel cell) were constructed, they did not initially produce any voltage, and there were no indications of voltage increasing. After the first day of checking the voltage of the MFC's, there was a small amount of voltage being produced, 0.05 volts for the 1st fuel cell, and 0.09 for the second fuel cell. The next day, Day 2, voltage was measured at 0.13 volts, and 0.25 volts. The voltage of the fuel cells continued to rise about 0.07 for the first cell, and 0.13 volts for the second cell a day. The voltage of the cells stopped increasing at about Day 13, which gave a voltage of 0.97, and 1.872. The peak voltage and wattage for the cells remained stable, and remained stable until the end of the recording period, which was Day 21. The wattage of the trials was measured in milliwatts. Similarly, the wattage increased as the days continued but stabilized at Day 13. Sometimes the wattage increased to a peak of 0.05 milliwatts and 0.10 milliwatts, but it reduced to approximately 0.043 and 0.085 milliwatts during the next couple days.

CONCLUSIONS

The purpose of this experiment was to see if having a larger electrode in a MFC would be the most efficient way of generating electricity. The hypothesis of this experiment was: If the size of the electrode of a fuel cell is doubled, then the fuel cell will have double the energy output. When the experiment was completed, it was clear that the hypothesis was not completely accurate, and the cell with the larger electrode was in fact not as efficient as two separate fuel cells. Although the fuel cell with the larger electrodes did not produce exactly double the energy output, it would be more cost effective to build one fuel cell with a larger electrode than multiple fuel cells with smaller electrodes.

The MFC (microbial fuel cell), when first measured, produced no wattage. The MFC took two days to produce a current, and finally, after about 2 weeks, it reached its peak. The smaller and larger electrode MFC's produced close to 1 and 1.8 volts, and 0.05 and 0.9 milliwatts (respectively). A Cree Xlamp XM-L2 LED was used to apply load to the two fuel cells. When MFC with the smaller electrode was under heavy load for long periods of time, its voltage dropped significantly, averaging 0.7 volts. The MFC with the larger electrode, when under load, dropped to a voltage of 1.4 volts. The voltage then increased to its peak, and stabilized after having no load from the LED. Throughout all three trials, the data remained clear, and the peak power was always around the same. Testing time periods remained the same, which allowed the data to be uniform.

The voltage of the smaller electrode MFC briefly increased to 1.1 volts, then dropped to its average voltage of 1 volt. The larger electrode MFC briefly increased to 2.0 volts, then dropped down to its average of 1.8 volts. A 6 centimeter drop of the electrodes in the fuel cells increased the efficiency of the MFCs by about 20%, as opposed to a 3 centimeter drop of the electrodes in the fuel cell.

The main hindrance of these particular MFC's was their designs, as certain aspects of its components could be improved to make the fuel cell more efficient and productive. The size of the cathode and anode could be increased to a significantly larger scale, along with the sizes of the electrodes in them. The diameter of the salt bridge could be increased as well, so it can fit with the increased sizes of the other components. In total, these improvements might result in a more productive MFC.

In a larger scale area, like a wastewater facility, much larger fuel cells can be made. If these small fuel cells that were made in this project was able to light up a bulb with ease, a large scaled fuel cell with a non-stop supply of waste should have a significant impact on a small city. According to a study done in Penn State, they have managed to achieve 15.5 watts per cubic meter of electrode. When they calculated the output of a large scaled fuel cell at a wastewater plant that takes in waste from 100,000 people, the output would be 0.8 megawatts. This level of output could power over 500 homes. MFC's also help filter the organic matter in waste, so this is also beneficial in lowering the costs of cleaning wastewater. The U.S. spends about $25 billion a year in treating wastewater, but this can be reduced with the implementation of MFC's in these plants. MFCs can aid in the treatment of wastewater through the respiration of the anaerobic bacteria used in the fuel cells. The anaerobic bacteria decompose organic matter to produce enough energy to complete the reaction involved in the fuel cell.

FURTHER RESEARCH

The bacteria utilized in the MFC (microbial fuel cell) were capable of producing hydrogen and electrons in an anaerobic (no oxygen) environment. The electrons successfully moved across the wires, and the hydrogen ions passed through the salt bridge to the cathode. This results in a usable form of electricity, and a sizeable amount of it is produced. MFCs are recognized for having very long periods of time where they sustain their peak charge, which can be up to a few years. One improvement that could be made to the conducted experiment includes the utilization of higher quality materials for the fuel cell. If higher quality materials were utilized to assemble the fuel cell, there would be a higher level of efficiency due to less gases escaping from the cell. The containers used in this experiment were plastic containers, which may degrade at a more rapid pace than a metal container. Additionally, the cathode and electrode might be made of platinum, which is more effective at transferring electrical charges in fuel cells than copper. Other ways of improving the performed experiment may involve improved surface areas of the electrodes, and the determination of whether a fuel cell works in better conditions than others (variables that were constants in this experiment would be changed). Improving the surface area of the electrodes would be very beneficial, as the energy output of the fuel cell would be raised, while it keeps a compact size. Similarly, testing conditions and how they affect the energy output of a fuel cell would allow for an optimal condition where fuel cells would operate at their highest efficiency rates. All of these questions would be answered with further experimentation, but they require an experiment of their own.

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THE EFFECT OF PLACING A PIEZOELECTRIC ELEMENT IN THE SOLE OF A SHOE ON GATHERING PIEZOELECTRIC ENERGY

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ABSTRACT

Piezoelectricity is an electric charge that accumulates in certain solids in response to mechanical stress, or pressure. An experiment conducted by Moure, A., M.a. Izquierdo Rodríguez, et Al. in 2016, analyzed how the unused vibrational potential of roadways could provide energy. By implementing piezoelectric cymbals, which are similar to the piezoelectric disks used in this experiment but larger and more durable, they covered a section of roadways in Madrid. The results of the experiment conducted under these scientists prove the possibility of collecting energy through piezoelectric means. Though the aforementioned experiment tested for the feasibility of piezoelectricity as an alternate energy source, the results of this experiment demonstrate that there are many adjustments to be made before implementing its use.

It was hypothesized that a piezoelectric energy harvester in the sole of a shoe is able to gather enough electricity to power a small household appliance if the generated electricity reaches the threshold determined by the device. This experiment used 3 piezoelectric disks,that were placed under a right shoe's sole. The disks were spaced apart to cover the surface area of the heel of the foot as this area of the foot provided the largest amount of pressure. After numerous trials, the average energy per step was approximately 0.379v per step. The raw energy generated provides enough power to light a LED, but compiling and storing enough energy for a full battery charge would need much more energy. Effectively, these results proved that hundreds of thousands of steps would be needed to fully charge a smartphone.

INTRODUCTION

In today's society, one of the main focuses in energy conservation and the future involves alternative energy. Energy generation, efficiency, and conservation are key aspects when trying to utilize a form of alternate energy. Furthermore, alternative energy sources stimulate a brighter and cleaner future. Piezoelectricity, originally discovered in the late 1800's, is a form of energy that is created from applying pressure to a special crystal. The arrangements of atoms within this crystal are asymmetrical and remain generally neutral. As the crystal is squeezed, atoms are pushed farther apart and closer together creating electrical impulses from the interactions of positive and negative energies. Piezoelectric generators take advantage of these crystals and produce electricity from daily activities such as walking and moving.

If piezoelectric disks can effectively generate enough energy, it can be considered a legitimate source of alternate energy. Due to piezoelectric sources having a unique role in ways that could be implemented in very small spaces, such technologies are useful for those who are interested in obtaining additional energy without additional effort. Piezoelectricity is an electric charge that accumulates within certain solids in response to mechanical stress, or pressure. Piezoelectricity can be employed in many situations, and one of the most effective ways to implement this technology would be in the sole of a type of footwear. The generated electricity from walking or producing movement while the footwear is worn is accumulated and stored into a battery. Eventually, the stored energy would become a viable way of charging another device.

Two studies that were conducted under the same realm of piezoelectric energy have each used different ways in which energy was harvested. The authors of, "A Novel Piezoelectric Energy Harvester Using the Macro Fiber Composite Cantilever with a Bicylinder in Water" Rujun Song, Tao Xie et Al. in 2015 used a piezoelectric energy harvester equipped with two piezoelectric beams and two cylinders. This experiment was primarily conducted through producing energy from water pressure. This demonstrated that the faster the water was flowing, the more energy was being produced. The maximum output for this study as the combined sum from both beams (21.86 µW) was obtained when the water velocity was measured to be around 0.31 meters per second. The connection between this and the actual experiment described the relationship between water velocity and energy output, as compared to foot pressure and energy output. If the same relationship of pressure and energy exists in both, then the larger the input (pressure) the higher the output will be.

Another study conducted by Y. G. Leng et Al. in 2015 ignored the traditional nonlinear piezoelectric cantilever energy harvester and involved the assembly of an elastic-support model for enhanced harvesting from random vibrations. The study proved that "elastic-support systems are capable of adapting to random excitations […] through which maximum power output […] of the system can be accomplished." The design of the energy harvester in the sole of the shoe should be able to be the most efficient as found in the case previously mentioned. Though there have been many studies around the field of piezoelectricity, there is a lack of any real life adaptations or applications of piezoelectric generators, despite the positive results in these studies.

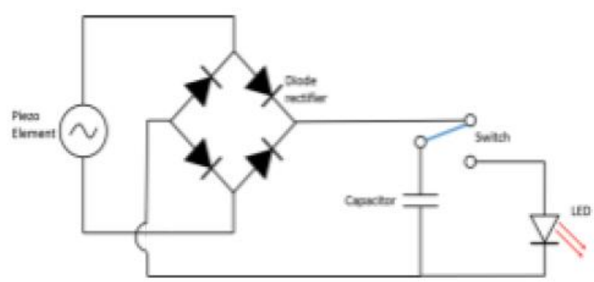


Figure 1: An example of a wiring diagram of a piezoelectric generator. The diode rectifier, is the 4 way bridge rectifier.

Previous studies discuss the scientific and mathematical reasoning behind gathering these forces and converting them to energy. When applied to a scenario that appears consistent throughout the course of one day, the value of piezoelectric generators became much more valuable. There are many other

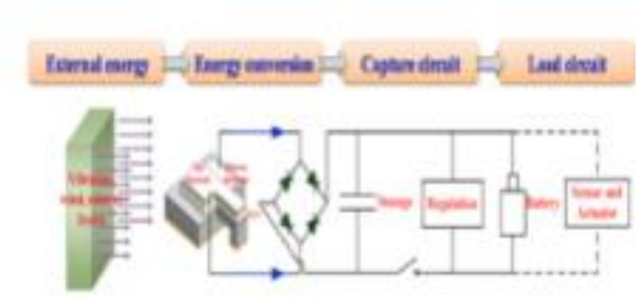


Figure 2: The process in which mechanical energy is being converted into piezoelectric energy is shown above.

uses that piezoelectricity can have on someone’s lifestyle but not necessarily through a shoe or in this type of application of the energy. Fire alarms, speakers, and watches can take advantage of energy to become louder, and last longer, respectively. Using piezoelectricity would be a feasible alternative energy source that can be used on a daily basis. Production of piezoelectricity does not require more energy, instead of having to perform extra actions to acquire energy, movement that is already being conducted produces unused potential energy. The piezoelectric generator used the stress produced from these actions to generate electricity. When the piezoelectric energy harvester in the sole of a shoe gathered enough electricity to meet the threshold determined by the device, then the energy was used to power the device.

MATERIALS

In order to conduct the experiment, there were several materials that needed to be purchased. The first item was a comfortable shoe that had a detachable sole so that the piezoelectric material could be placed underneath specifically a Nike Revolution 2 Running Shoe. The second item was a pedometer to track the number of steps; the third item was the “Basic kWh Meter 14mm 120 volt, 2-wire, External CT Meter. 50 Amp. Pulse output EKM-15E (One kit)” This specific kit measures the amount of energy something has and in this case the capacitor held the energy which was attached to the piezoelectric material. The spare battery charging kit was another item that was used to transfer the electricity from the capacitor to the battery. A bridge rectifier was used to convert the AC energy into DC voltage so it could be used by small household electronics that run on DC energy. A cell phone and a matching charger was needed as well to see whether the energy gathered would be able to charge a device. A soldering kit was used so that the components could be connected and the circuit could be completed.

EXPERIMENTAL DESIGN

In order to test and collect data for this experiment, a multi-meter was necessary to find the exact measurements of electrical outputs. A human subject was needed to walk in the shoe that the equipment was attached to and walk for a set amount of steps (100, 200, and 300). Using a certain number of steps was better than a set distance because everyone’s step per meter is different and this would have provided mixed results. After results were gathered, an approximate conversion unit that accounts for the amount of energy generated per step was used to find the average of the three trials. The energy from the capacitor was converted through the formula: $\frac{1}{2} * v^2 * C$. Finally, the gathered energy was used in attempt to charge a small device, in this case a Samsung Galaxy smartphone.

Figure 3: A variation where the piezoelectric element is inside the sole instead of directly above it. This leads to less mechanical stress in contrast.

The methods within other studies used generators that were made and tested in labs where everything was precise and controlled. The methodology used in this experiment was chosen because it represented a relatable situation to an everyday occurrence that happens when the product is being generally used. It is also optimal because the hypothesis focuses on pressure exerted from someone's feet and this method would most accurately represent an average person's feet movement.

One of the most prominent factors that had to be controlled was the terrain and how compacted it was. There would be a difference of results from that of a pebbled driveway compared to a paved street. The difference could have accounted for a margin of error however to prevent this, only one type of terrain was used in this experiment. Weather conditions had to be taken into consideration as well to avoid source of error thus, in general, the experiment was conducted in one type of weather. The final condition would be the surface area of the shoe; sticking to one specific shoe size and measuring the sole before conducting the experiment would confront this issue.

Figure 4: The Battery Kit that is being used in the project, comes with 4 batteries and a 4-port charger

Although there are many outcomes to this experiment that can be considered a "success", to actually support the hypothesis, not only would energy have to be generated and stored, it must be a large enough output that it would be able to power a small device efficiently and give off enough energy to do so for a set amount of time. If and only if these conditions are met, then the hypothesis would be confirmed as a success. An example of a disconfirming result may be that energy was generated, but was not powerful enough to power or charge the set electrical device.

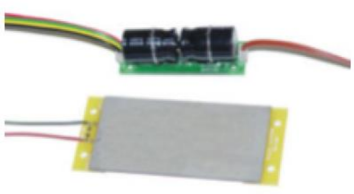


Figure 5: Mock Piezoelectric Energy Harvester. The sheet is an example of piezoelectric element, there are two capacitors attached as well.

METHODS

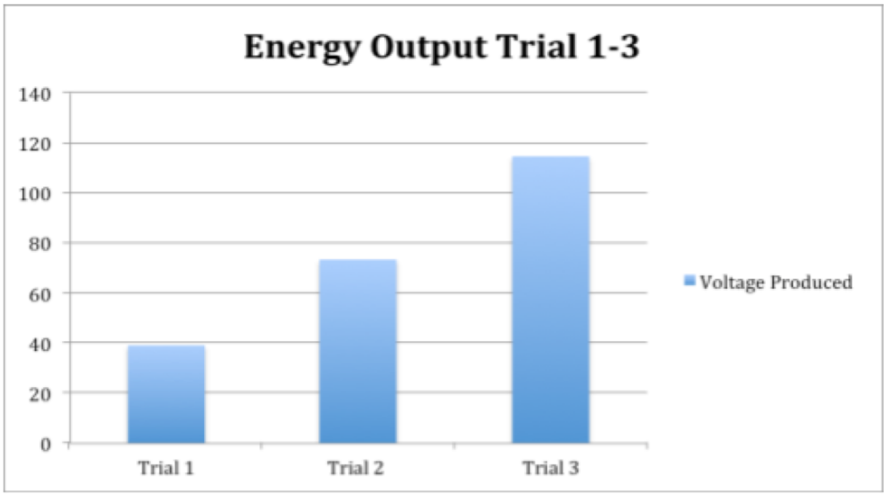
Initially the sole of the shoe was adjusted to accommodate the disks and make sure everything was spaced appropriately. The disks were glued to the sole of the shoe and connected by soldering wires onto them. Afterwards the output of the disks was connected to a 4-way bridge rectifier where the energy was converted, then connected to the capacitor, and finally to the battery. Once the connection had been established, the multi-meter was used to check and make sure that energy was being produced, and held by the capacitor. Three different trials 100, 200, and 300 steps were conducted. Each trial was done twice to ensure that the results were accurate. The distances were specifically chosen because a small amount of steps would produce energy that wouldn't be sufficient enough to even power a light bulb. The output meter was used to check how much energy was going through the wiring. To conclude, the battery was taken from the set up and connected to a charger then the charger was connected to the device and the results were recorded after observing the charging time and rate.

Figure 6: Shoes, soldering equipment, pedometer, etc. All materials to complete experiment.

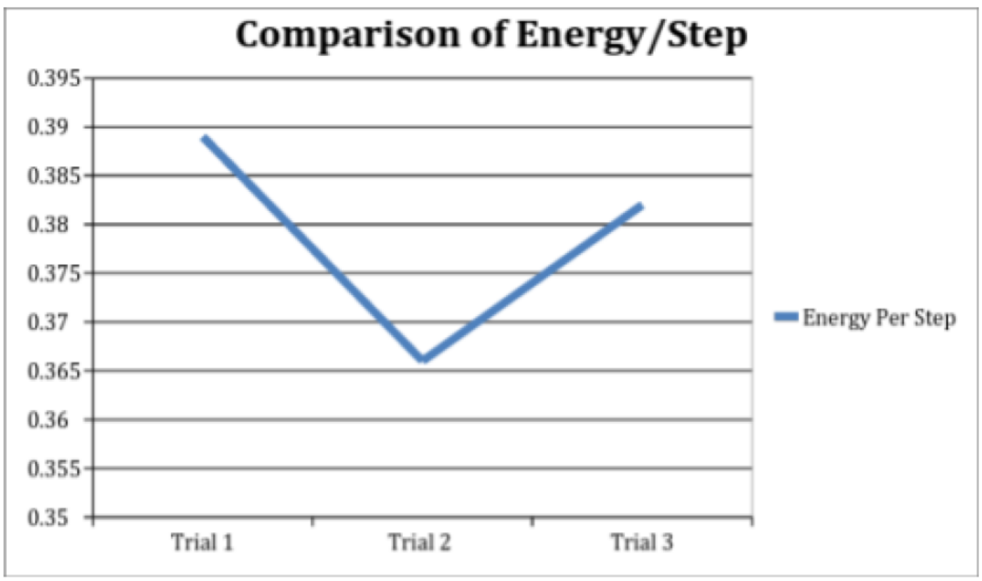
RESULTS

Trials	Steps	Energy Gathered	Energy Per Step	Feasible Use?	Energy Comparison
1	100 Steps	38.9v	.389v/step	No, very little energy output.	This wouldn't provide optimal energy
2	200 Steps	73.2v	.366v/step	No, very little energy output.	This wouldn't provide optimal energy
3	300 Steps	114.6v	.382v/step	LED lighting is an option.	Provides enough energy for LED.

Table 1: This table represents the data that is gathered. It shows the trials and the amount of energy that is being produced for every set amount steps.



Graph 1: The above graph shows a gradual increase in voltage produced from walking the set amount of steps. The voltage increases at a constant rate. The formula for calculating energy from capacitor is: ½*v2*C



Graph 2: This graph shows the change in energy per step as the amount of steps increase, the changes seem drastic due to the Y-value increase.

ANALYSIS

The data gathered through the research was analyzed through a set of charts and graphs. Graph one shows the direct relationship between voltage produced and the amount of steps taken; its results were recorded in table one. At one hundred steps 38.9 volts were produced, at two hundred steps 73.2 volts were produced, and finally at three hundred steps 111.4 volts were produced. The increases between these intervals does not follow an absolute linear path. This further exemplifies the trend that more steps are equivalent to more energy. It can be observed that as the amount of steps increases, the amount of energy increases as well. Graph two, describes energy created directly from footsteps. Initially the amount of energy per steps is high, it decreases during trial two, then however equalizes when it reaches trial three. The first two trials show there are very limited practical uses for such amount of energy, the last trial provides enough energy to power an LED for a limited amount of time. Overall the energy produced from the piezoelectric generator is insufficient.

CONCLUSIONS

The purpose of the experiment was to determine if piezoelectric energy would be an alternative energy source that can be used on a daily basis. The energy produced from the in-sole generator was economically friendly and clean, however the only limitation was the amount of energy that was produced. Unlike previous research around the subject of piezoelectricity, this experiment was based on real-life scenarios. Having a human subject test the effectiveness of the generator gave accurate results as to what could be expected if another person were to use the generator as well. The importance of this information is valuable when determining whether or not the shoe is a practical source of gathering energy.

It was found that although energy was being produced from this circuit, it was not enough to charge a smartphone completely. However, that didn’t limit its potential use. The circuit could have been hooked up to an LED and been used as a flashlight in situations when light was needed and there wasn't a power source nearby. As for the hypothesis, it was not supported. It was found that energy that was being produced did not meet the minimum threshold established by the device and the device did not charge. There is a far way to go before piezoelectricity can become a reliable source of energy. Past research on piezoelectricity found it to be potentially useful, as did this experiment.

The main source of error throughout the conducted experiment resulted from immediately checking the capacitor and amount of energy it contained. Capacitors are generally imprecise and unreliable in circuits. One characteristic they have is to conduct various voltages, which was the reason they were utilized in this experiment. Another source of error would be inconsistent strides. The difference in steps and pressure will provide a varying degree of difference in energy output. The final source of error would be walking pace, the faster something would hit the ground the greater the impact would be. Walking faster would mean more pressure was exerted, and if the walking rate were not consistent then the amount of energy output would be off as well. An example of such an occurrence can be seen in trial two, in which graph 2 displays energy per step. The energy per step is dependent upon how fast or how hard the shoe was hitting the surface to generate electricity. Differing results in each trial indicated the inconsistent walking patterns.

FURTHER RESEARCH

For further experiments, more piezoelectric disks could be applied and arranged in more optimal positions to receive the most amount of tension, which would thus produce more energy. Placing the disks in different places and testing these comparatively could produce an entirely new experiment. Exploring the effects on placement of piezoelectric disks can prove useful when determining the best location for the most amount of energy produced while having the least amount of output.

Another research topic would be to explore the effects of stacking piezoelectric disks on their energy output, and determining how the output is changed by the amount of them stacked on top of each other. If piezoelectric disks are stacked, there would be more room for electric generation. Walking speed is a separate variable that can be explored. The relationship between how fast the user is walking and the energy output is an important area of interest that can be further researched to find a possible correlation. Lastly the effects that the size and shape of the piezoelectric disks can have on the on the results of the experiment can be studied. All aforementioned suggestions to the performed experiment would require completely new research to be properly validated.

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Isotope Labelled Quantification Method for Desmosine by MS/MS

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ABSTRACT

Desmosine and Isodesmosine are two unique amino acids involved in elastin cross-linking that differentiate elastin from other proteins. As the tissue loses its elastin, the degraded elastin travels into the body fluid: serum, blood, plasma or urine. The proportion of desmosine in elastin can provide the amount of elastin present in the given biological sample. The quantitative information is imperative in assessing the biological functions of proteins and their modifications. To aspire to that, it is important to develop a calibration curve in order to obtain quantitative information on the unknown concentration of a sample of desmosine. In this experiment, we have developed a novel method to quantify desmosine using synthetic isotopically labeled desmosine (des-D4) as a standard. Mass spectrometry, an analytical tool was used which can easily differentiate labelled desmosine from unlabelled desmosine. We were able to create calibration curve which includes three linear graphs, one reproduced data points and sensitivity of the instrument.

INTRODUCTION

Elastin is a fibrous glycoprotein that plays a crucial role in various connective tissues that depend on elasticity. It provides these tissues with the ability to stretch and recoil. However, in the event of damage to the elastin, which may be due to long exposure to sunlight and aging, the tissue starts to lose elastin and the degraded elastin travels into the body fluid: serum, blood, plasma or urine. Desmosine, as well as Isodesmosine, are what gives elastin its structure and are only found after elastin breaks down. Therefore, they act as important biomarkers to the degradation of elastin.

Desmosine and Isodesmosine are both pyridinium amino acids that act as crosslinks in elastin by binding to the elastin's four lysine side chains. Emphysema, Williams Syndrome, Costello Syndrome, and Chronic Obstructive Pulmonary Disease (COPD) are all diseases that are caused by a disruption in elastin. Validated analytical methods that allow the accurate and precise quantification of Desmosine and Isodesmosine in human biological samples like tissue, plasma or urine are mandatory prior to the analysis of clinical samples in order to guarantee the reliability of the obtained results by assessing the levels of desmosine in the given sample.

Stable isotope labeling is a powerful quantitative method for accurately determining the concentration of the desired compound of interest; methods are continually being developed. Stable isotope labeling provides chemically isotopically equivalent but different internal standards for each compound of interest for direct comparison of mass spectral signal intensities that represent relative abundance.[1] In this experiment, we have developed a fully validated Mass Spectrometry/Mass Spectrometry (MS/MS) method that allows the analysis of free Desmosine in order to evaluate the potential use of biological samples that contain Desmosine as a biomarker. We have used isotopic labelled deuterated desmosine as a standard to create a calibration curve for desmosine.

Similar research, creating calibration curves, has been conducted such as "Quantitative high-performance liquid chromatography-tandem mass spectrometry method for the analysis of free desmosines in plasma and urine." [2] In the study, researchers worked to create a calibration curve for plasma and urine samples with labelled deuterium isodesmosine as the standard. They used ultra-filtration with two-step solid phase extraction to reduce the ion suppression effect in addition to using analytical column filled with porous graphitic carbon material to retain the polar desmosine analytes. A successful calibration curve was created through the use of these methods and mass spectrometry.

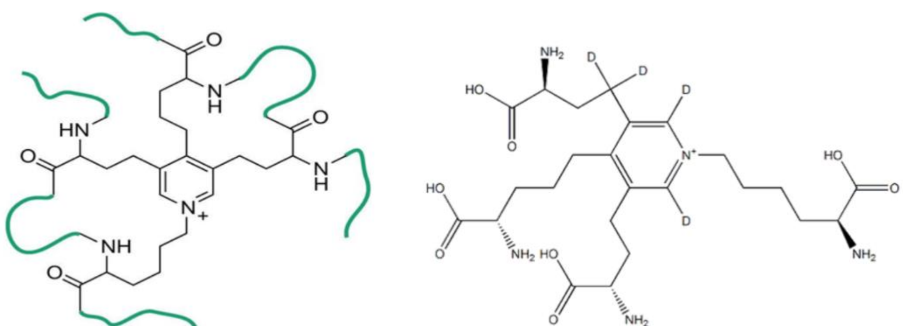

Figure 1. The figures show the structure of desmosine and deuterated desmosine (D4).
Left: Unlabeled desmosine (Image from www.nature.com) Right: Labeled desmosine (Image from www.chemicalbook.com)

INSTRUMENTATION

Matrix-Assisted Laser Desorption/Ionization (MALDI LTQ-XL) is a soft ionization technique used in mass spectrometry. MALDI LTQ-XL was chosen to be the main instrument used in the lab due to its speed, relative accuracy, and convenience. It can easily differentiate the similar chemical compounds, such as unlabeled and labelled desmosine, on the basis of their mass.

Figure 2. The MALDI LTQ-XL machine.
(Image from www.selectscience.net)

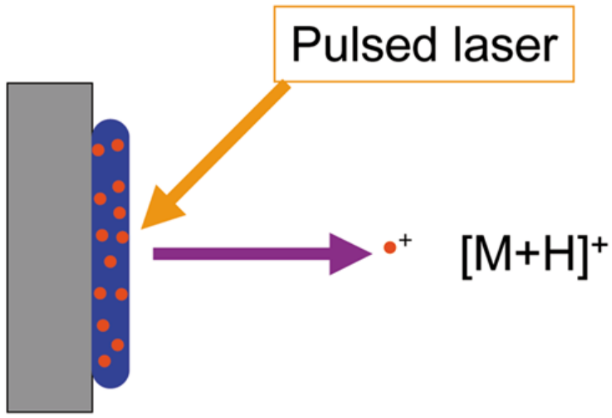

Figure 3: Ionization of a sample (Image from www.wikibooks.org)

The purpose of using α-Cyano-4-hydroxycinnamic acid (CHCA) matrix with the sample before running in MALDI was to allow the sample to crystallize faster after it has been pipetted on the designated Linear Trap Quadrupole (LTQ) plate and to prevent the laser from directly hitting and decomposing the sample. When running LTQ, the metal plate with the spotted samples was placed into the MALDI source, and was then sent to the linear ion trap[3], which traps the ions using radiofrequency (RF) electric field.[4] If further study into a specific fragment was necessary, MS/MS or MS2 could be performed by sending the separated ions to the Higher-Energy Collisional Dissociation (HCD) collision cell, where they collided with gases, such as argon, creating further fragmentations.[5]

HYPOTHESIS

It is initially hypothesized that when the ratio of the different concentrations of desmosine to a constant concentration of labelled desmosine (D4) is plotted, it will result in a linear graph, otherwise known as a calibration curve, with the variables: concentration ratios (des/D4) and average area ratios (des/D4).

MATERIALS

Chemicals

Desmosine of 1mM concentration with a mass of 526 amu and a purity of 99.5% ± 0.2% was purchased from Elastin Products Company, Inc (Owensville, Missouri, USA); and Deuterated-desmosine of 0.1mM concentration with a mass of 530 amu and a purity of 99.5% ± 0.2% was purchased from TRC Canada. Another item that was used in this experiment was CHCA (Alpha Cyano 4 Hydroxy cinnamic Acid) matrix.

Others

The mass spectrometer, MALDI LTQ, was primarily used in obtaining data with additional equipments: LTQ plate, 2 µL micropipette, 10 µL micropipette, 20 µL micropipette, and 100 µL micropipette.

METHODS

Reagent Preparation:

The CHCA matrix was prepared by centrifuging matrix powder with 70% of AcN (Acetonitrile) and 0.1% TFA (Trifluoroacetic acid) for 4 minutes after a minute vortex, using only the supernatant during the experiment. HPLC (high performance/ pressure liquid chromatography) water was used to make dilutions while preparing the samples.

Sample preparation:

The unlabelled desmosine has a starting concentration of 1.0 mM and the labelled desmosine, D4, has a starting concentration of 0.1 mM. The concentration of D4 remained constant throughout the whole experiment. The unlabelled desmosine sample is prepared differently for each respective trial. Preparation of 5 different concentrations of unlabelled desmosine were made for trial 1. The concentrations that were prepared were: 0.025 mM, 0.050 mM, 0.075 mM, 0.100 mM, and 0.250 mM. These concentrations were prepared using the formula: $M_1V_1=M_2V_2$, where the volume of the resulting dilution can be found with three other known variables. For example, the volume of the 0.250 mM concentration can be determined as such:

$$M_1V_1=M_2V_2$$
$$(1.0\text{ mM})(5\ \mu\text{L})= (0.250\text{ mM})\ V_2$$
$$V_2= 20\ \mu\text{L}$$

Each respective concentration for trial 1 was prepared using the formula to determine the final volume. 2 µL of each final unlabelled desmosine of different concentrations were extracted and placed in each distinct test tube. 2 µL of 0.1 mM labelled desmosine was then placed in each test tube. The concentration of both trial 2 and 3 were prepared in the same manner, using the formula: $M_1V_1=M_2V_2$ to determine the unknown final volume. For trial 2 and 3 these concentrations were prepared: 0.025 mM, 0.100 mM, 0.125 mM, and 0.25 mM. 1 µL of the resulting mixtures of unlabelled and labelled desmosine were then separately extracted and diluted with 9 µL of CHCA matrix (to help prevent the sample from being hit too directly by the laser beam as well as to cleanse the sample of any salts or minerals), then were pipetted on the MALDI-MS plate. (4 replicates for each sample were spotted).

Table 1: Concentrations of Unlabeled Desmosine in the matrix mixture (mM)

Trial 1	Trial 2	Trial 3
0.00125	0.00125	0.00125
0.0025	0.0050	0.0050
0.00375	0.00625	0.00625
0.0050	0.0125	0.0125
0.0125	-----	-----

Table 1: The concentration of 0.1 mM D4-desmosine in the matrix with desmosine would be 0.5 mM and then in the matrix would be 0.05 mM.

Detection Limit (sensitivity of the instrument)

The detection limit was determined by taking 0.25 mM of desmosine and diluting it by a factor of five. This was decided to be an appropriate dilution factor due to the high starting concentration of the sample as well as the fact that a dilution factor of 2 would produce too many data points. The sample was diluted as many times as possible until traces of the sample were too low to be detected by the MALDI instrument.

Reproducibility

The experiment was repeated by a experienced faculty in the lab to ensure that results were reproducible.

RESULTS

Figure 4: MS/MS spectra of the mixture of desmosine/D4 with the matrix

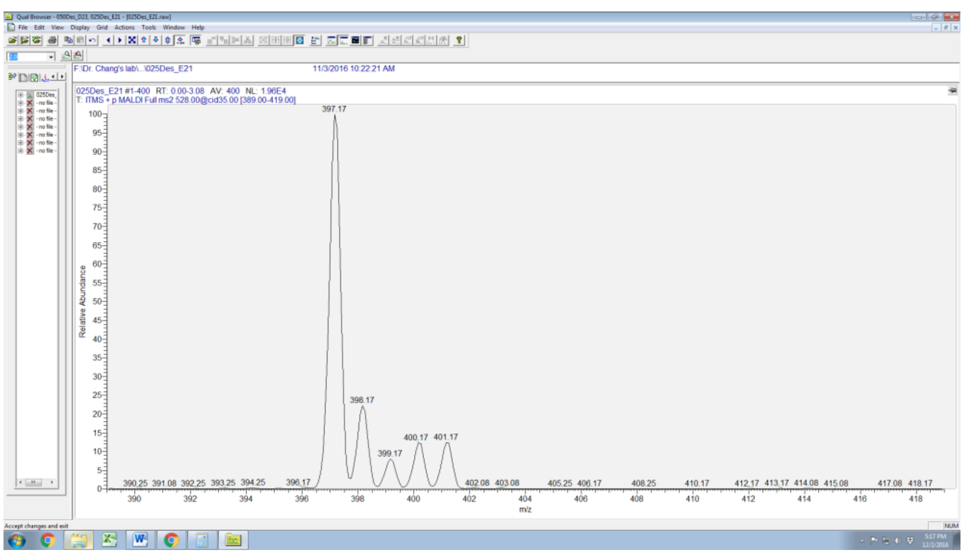

Figure 4(a): Trial 1, 0.0125 mM (desmosine) : 0.05 mM (D4)

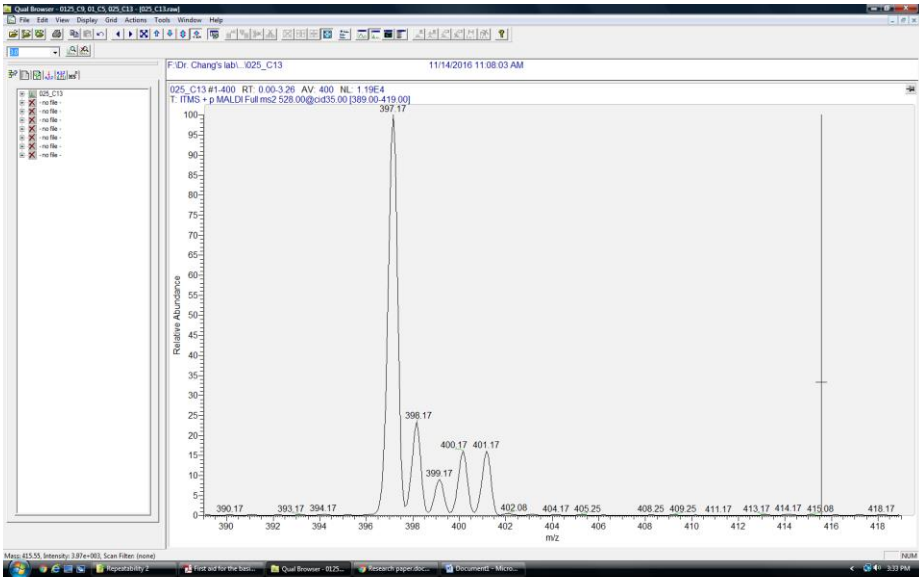

Figure 4(b): Trial 2, 0.0125 mM (desmosine) : 0.05 mM (D4)

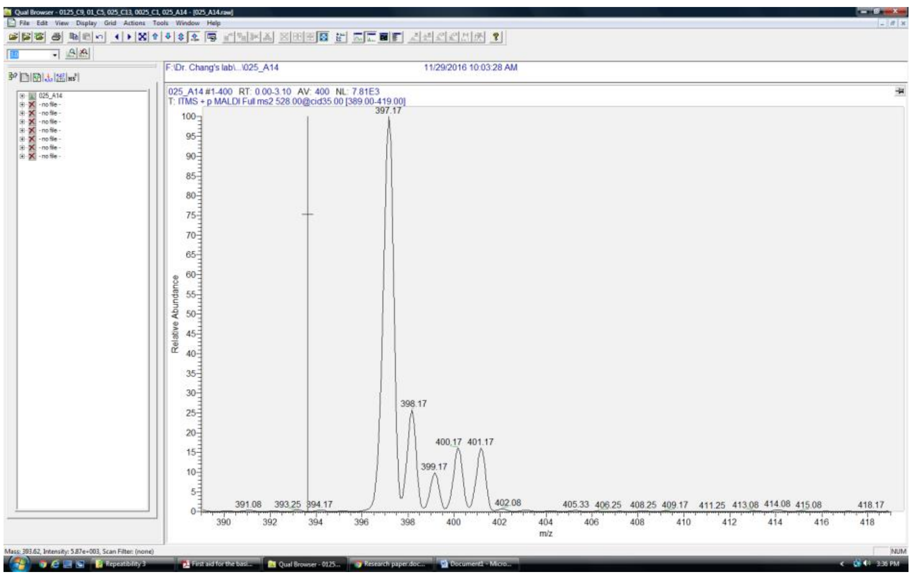

Figure 4(c): Trial 3, 0.0125 mM (desmosine) : 0.05 mM (D4)

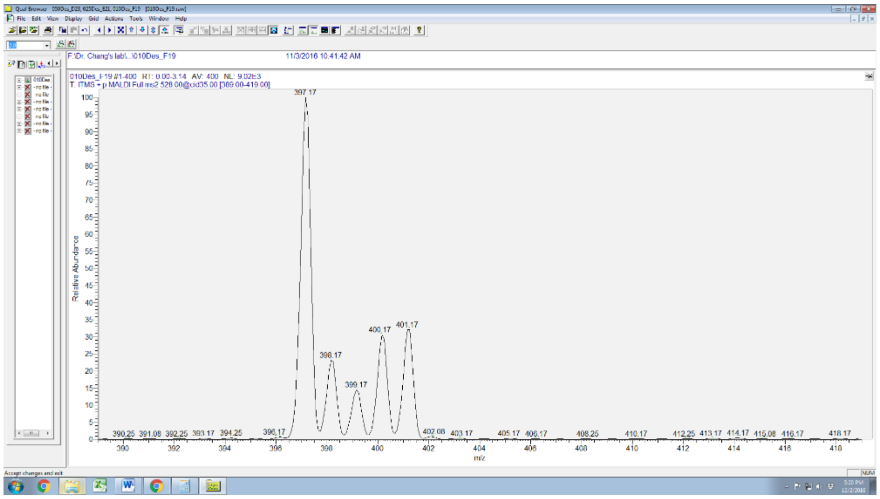

Figure 4(d): Trial 1, 0.0050 mM (desmosine) : 0.05 mM (D4)

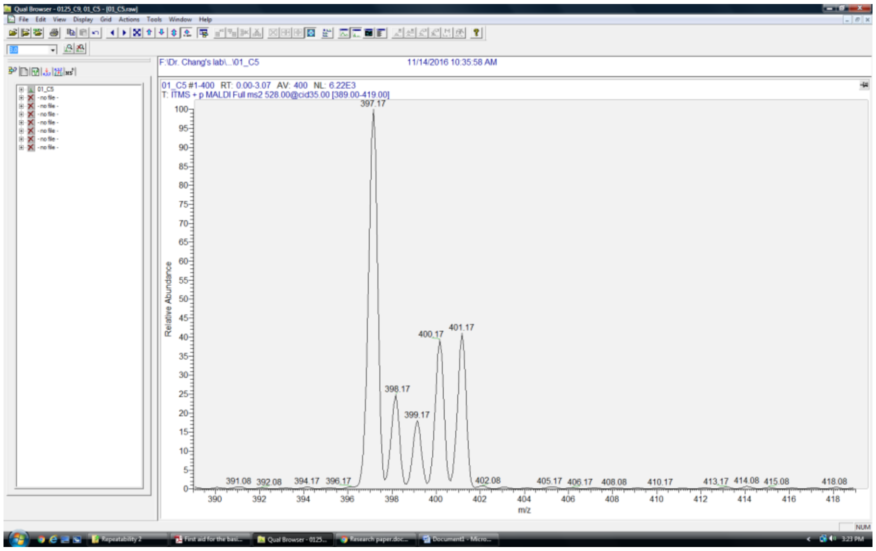

Figure 4(e): Trial 2, 0.0050 mM (desmosine) : 0.05 mM (D4)

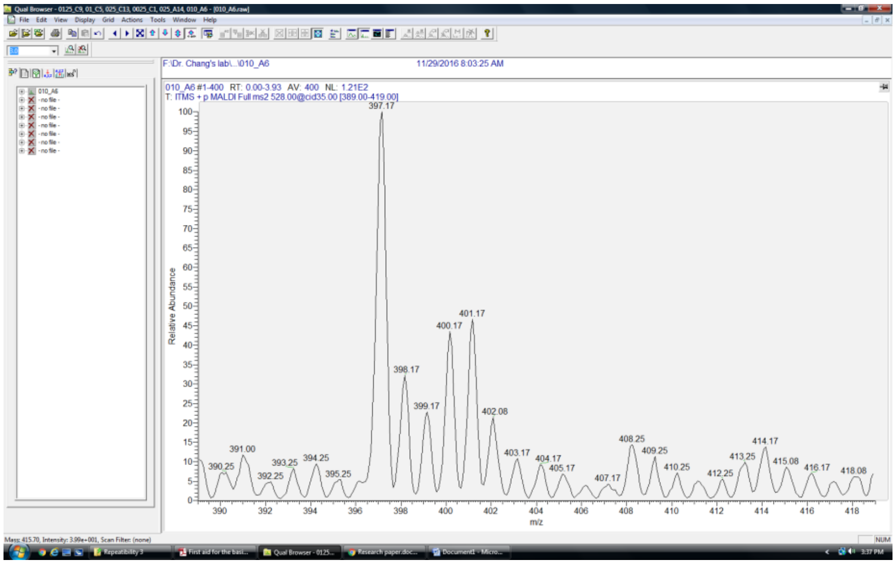

Figure 4(f): Trial 3, 0.0050 mM (desmosine) : 0.05 mM (D4)

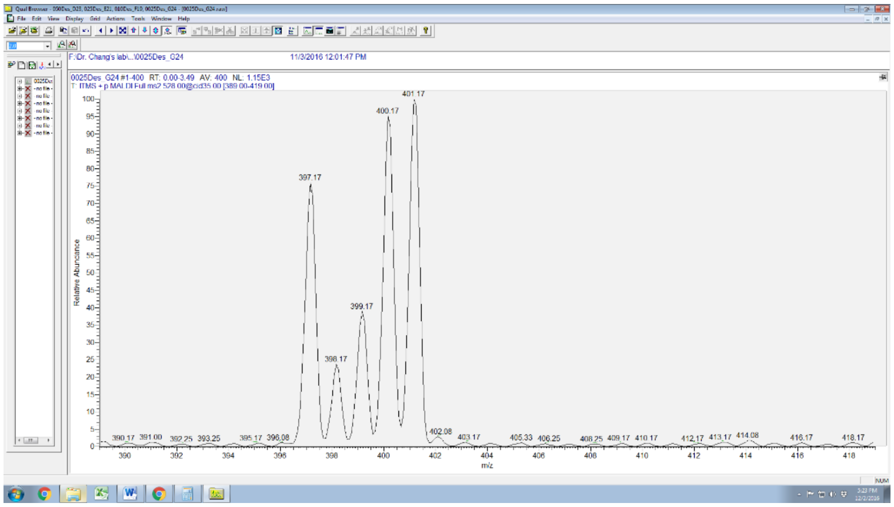

Figure 4(g): Trial 1, 0.00125 mM (desmosine) : 0.05 mM (D4)

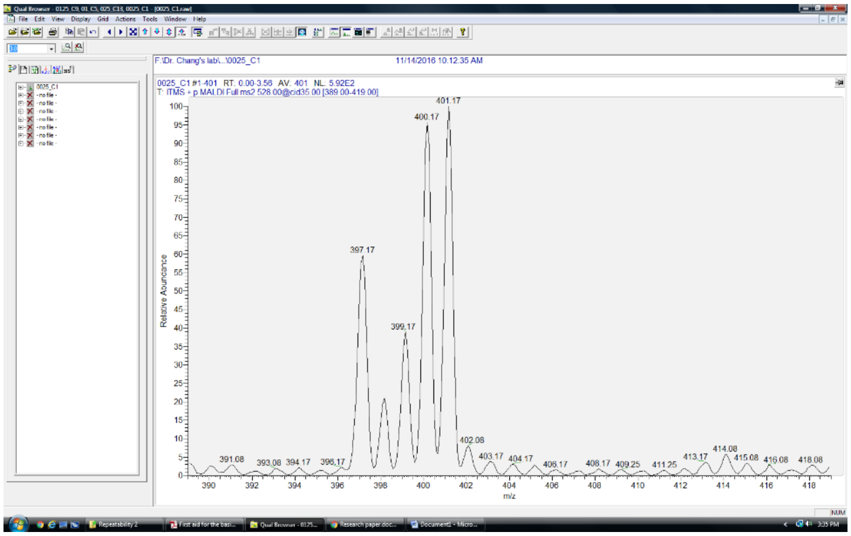

Figure 4(h): Trial 2, 0.00125 mM (desmosine) : 0.05 mM (D4)

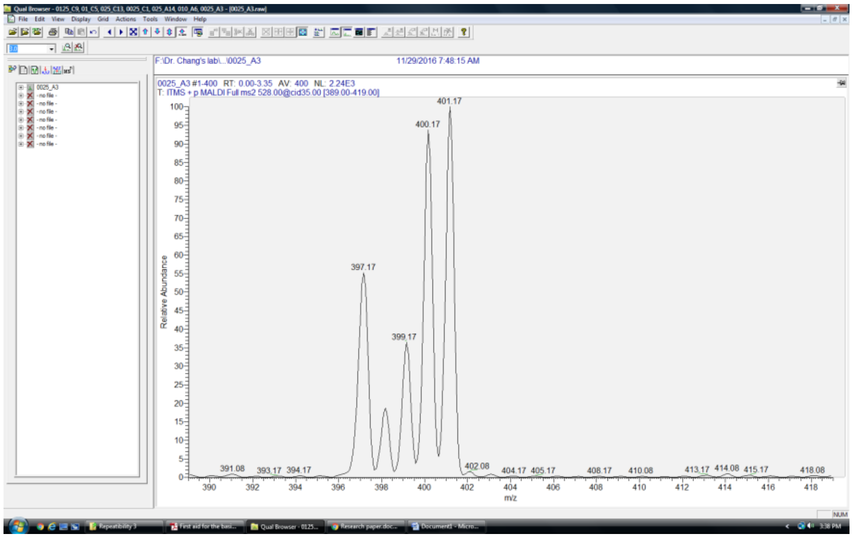

Figure 4(i): Trial 3, 0.00125 mM (desmosine) : 0.05 mM (D4)

Table 2: Concentration of desmosine and D4-desmosine samples after different dilutions, their concentration ratios and average area ratios with respective error.

Trial 1

Before mixture (mM)		After mixture (mM)		After matrix (mM)		Concentration ratio	Average area ratio	
conc des	conc D4	conc des	conc D4	conc des	conc D4	des/D4	des/D4	error
0.025	0.100	0.0125	0.05	0.0012 5	0.005	0.25	0.690	0.005
0.050	0.100	0.0250	0.05	0.0025 0	0.005	0.50	1.397	0.031
0.075	0.100	0.0375	0.05	0.0037 5	0.005	0.75	2.068	0.094
0.100	0.100	0.0500	0.05	0.0050 0	0.005	1.00	2.750	0.048
0.250	0.100	0.1250	0.05	0.0125 0	0.005	2.50	7.027	0.075

Table 2(a): Trial 1

Trial 2

Before mixture (mM)		After mixture (mM)		After matrix (mM)		Concentration ratio	Average area ratio	
conc des	conc D4	conc des	conc D4	conc des	conc D4	des/D4	des/D4	error
0.025	0.1	0.0125	0.05	0.0012 5	0.005	0.25	0.603	0.005
0.100	0.1	0.05	0.05	0.005	0.005	1	2.306	0.019
0.125	0.1	0.0625	0.05	0.0062 5	0.005	1.25	2.718	0.024
0.250	0.1	0.125	0.05	0.0125	0.005	2.5	5.791	0.083

Table 2(b): Trial 2

Trial 3

Before mixture (mM)		After mixture (mM)		After matrix (mM)		Concentration ratio	Average area ratio	
conc des	conc D4	conc des	conc D4	conc des	conc D4	des/D4	des/D4	error
0.025	0.1	0.0125	0.05	0.0012 5	0.005	0.250	0.576	0.006
0.100	0.1	0.05	0.05	0.005	0.005	1.000	2.103	0.041
0.125	0.1	0.0625	0.05	0.0062 5	0.005	1.250	2.959	0.020
0.250	0.1	0.125	0.05	0.0125	0.005	2.500	6.178	0.116

Table2(c): Trial 3

Figure 5: Linearity graphs from the three trials

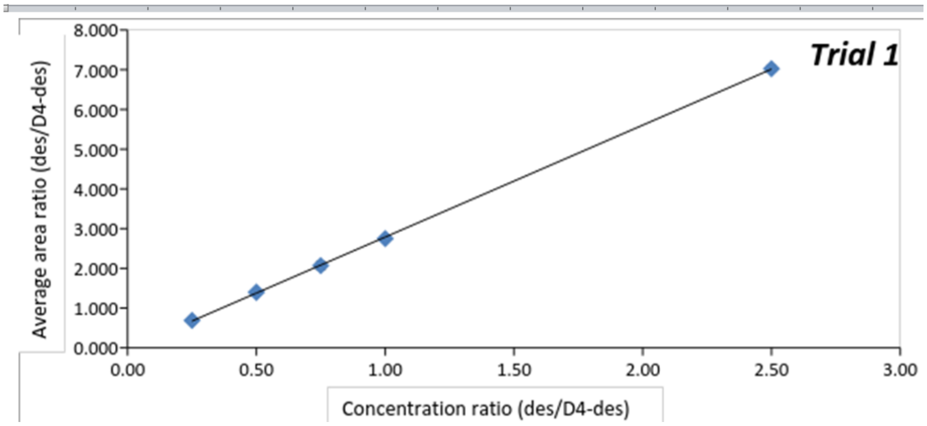

Figure 5(a): Trial 1

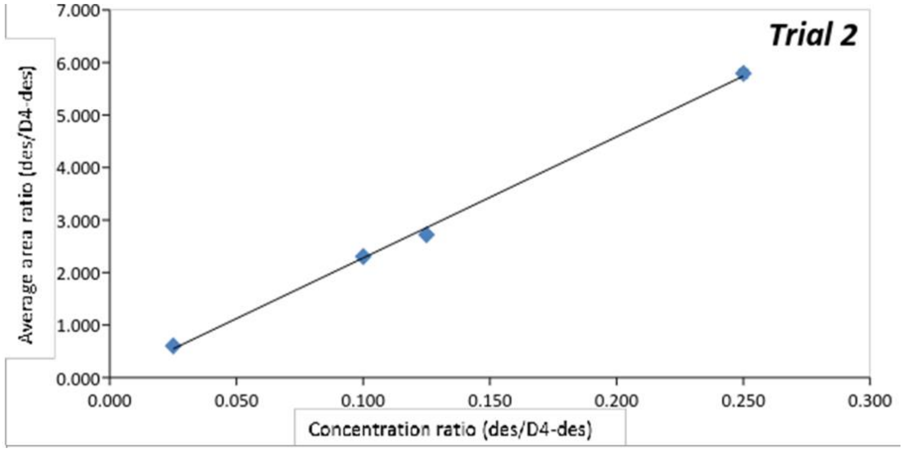

Figure 5(b): Trial 2

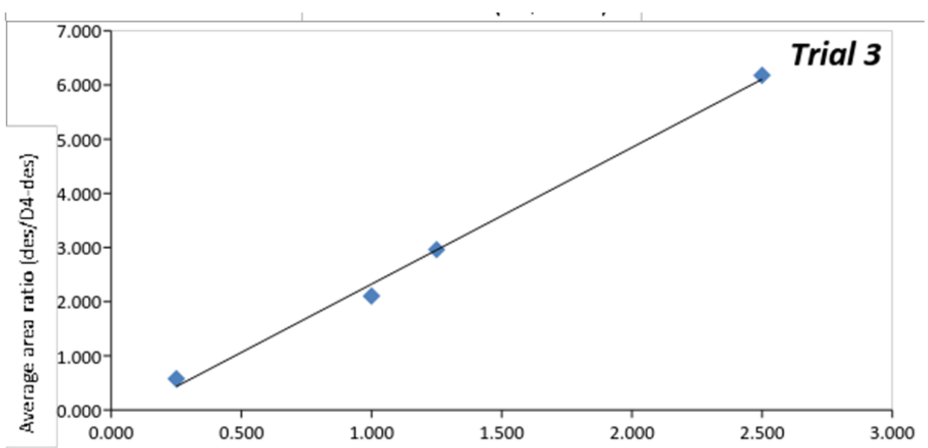

Figure 5(c): Trial 3

Concentration ratio	Average area ratio	
des/D4	des/D4	error
0.25	0.623	0.001
1.00	2.386	0.012
2.50	6.332	0.017

Table 3: The averages of the area ratio from above three trials

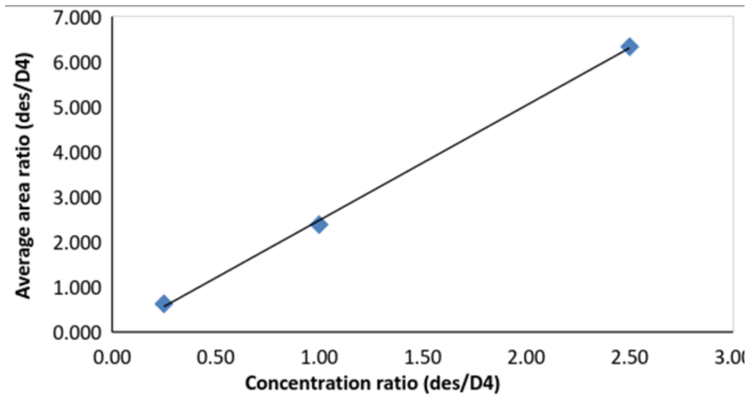

Figure 6: The graph shows precision in the three trials

Figure 7: Mixture samples of des/D4-des diluted by factor of 5 with the starting concentration of 0.125 mM/0.05 mM

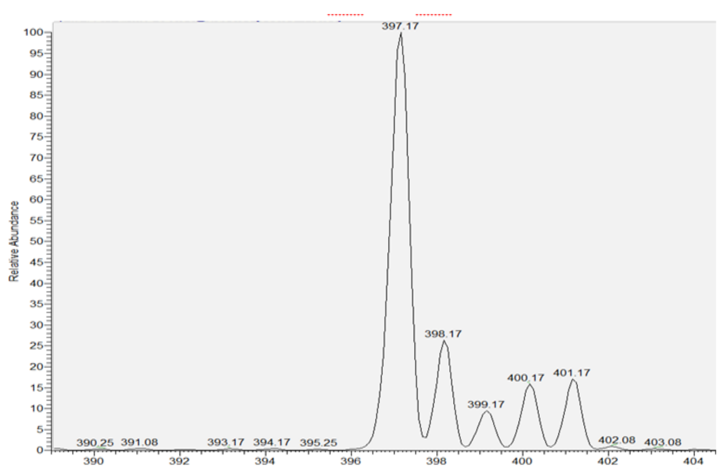

Figure 7(a): Concentration (mM) of 0.125/0.05, des/D4-des; this considered as 1 equivalent

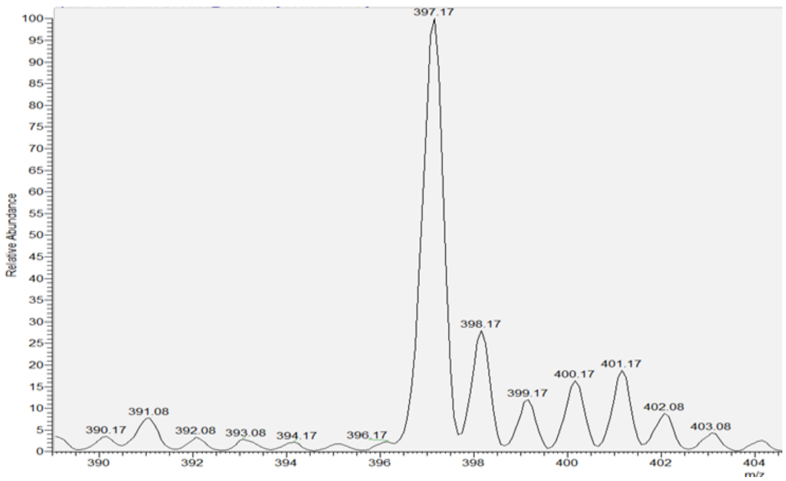

Figure 7(b): Concentration (mM) after first dilution of 0.125/0.05, des/D4-des; this considered as 0.02 equivalents

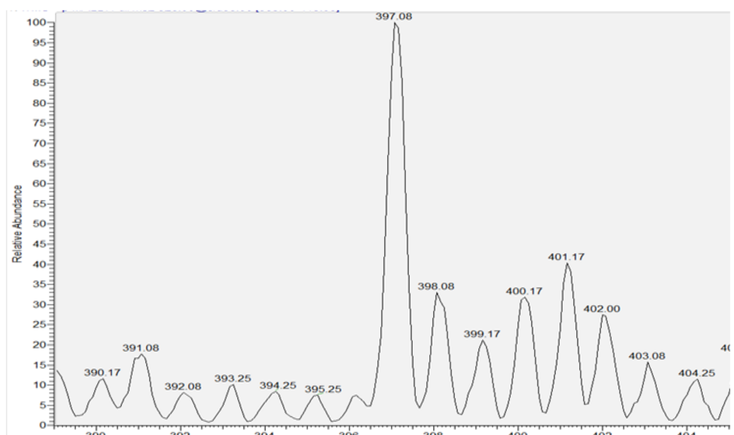

Figure 7(c): Concentration (mM) after second dilution of 0.125/0.05, des/D4-des; this considered as 0.004 equivalents

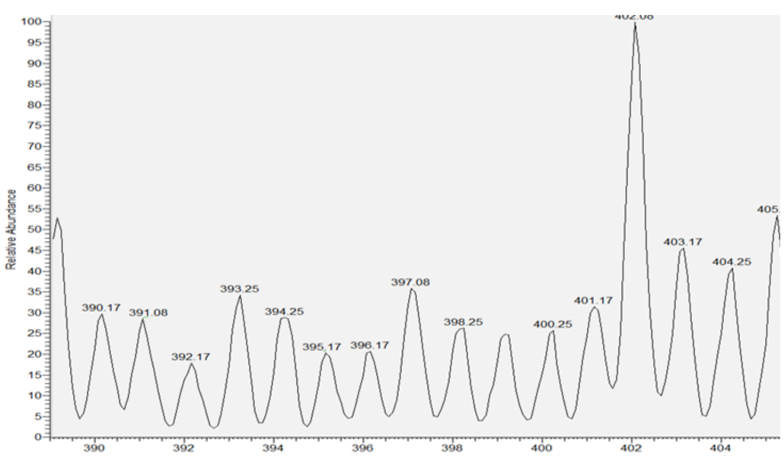

Figure 7(d): Concentration (mM) after third dilution of 0.125/0.05, des/D4-des; this considered as 0.0008 equivalents

Equivalent of des/D4-des	Fraction ratio [des/(des+D4-des)]
1	0.8606++
0.2	0.844
0.04	0.723
0.008	0.508

Table 4: 0.25mM and 0.1mM concentration of desmosine and D4-desmosine were mixed and three serial dilutions were made by a factor of five.

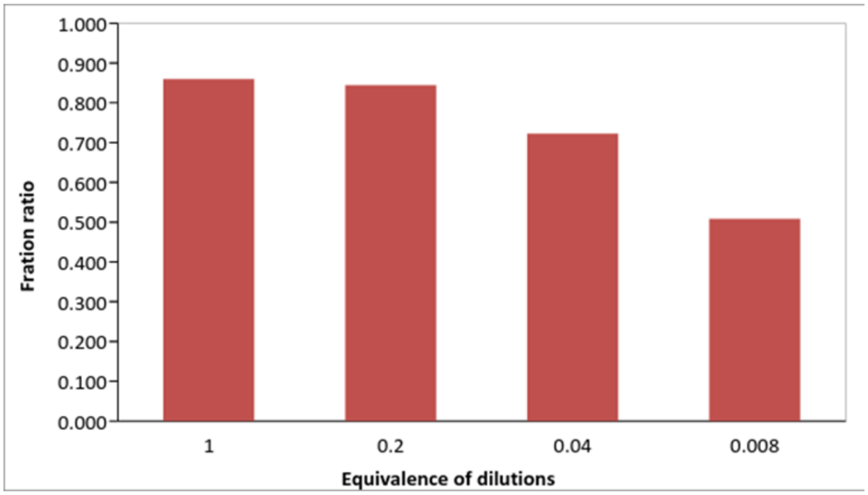

Figure 8: The bar graph above shows the dynamic range of the mixture of des:D4-des that can be detected in MALDI-MS/MS

Before mixture (mM)		After mixture (mM)		After matrix (mM)		Concentration ratio	Average area ratio	
conc des	conc D4	conc des	conc D4	conc des	conc D4	des/D4	des/D4	error
0.025	0.1	0.0125	0.05	0.0012 5	0.005	0.25	0.854	0.003
0.1	0.1	0.05	0.05	0.0050 0	0.005	1	3.811	0.038
0.125	0.1	0.0625	0.05	0.0062 5	0.005	1.25	4.050	0.017
0.25	0.1	0.125	0.05	0.0125 0	0.005	2.5	7.872	0.024

Table 5: The experiment was repeated by a PhD student in the lab to check the reproducibility of the results

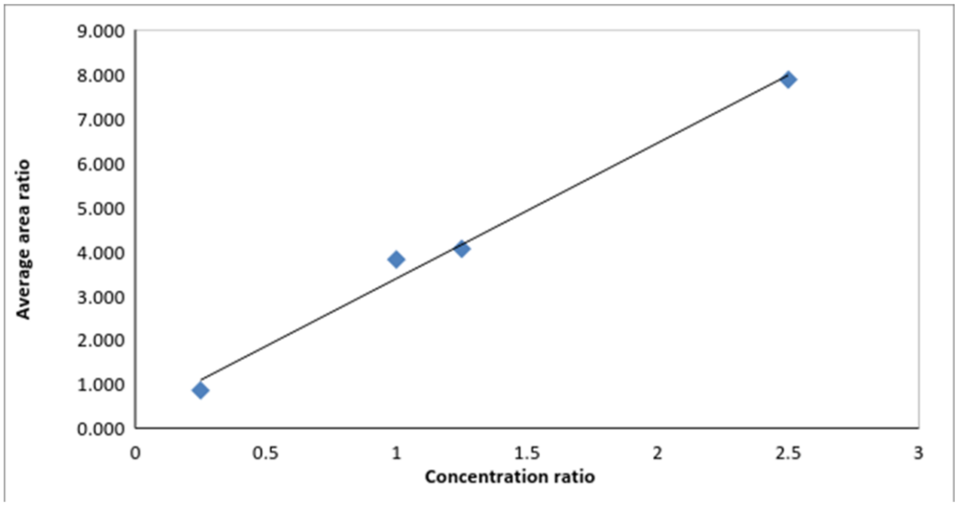

Figure 9: Calibration curve after the reproducibility of the results

DISCUSSION AND CONCLUSIONS

This paper describes a novel quantitative strategy combined with isotope labelled MALDI-MS/MS to quantify desmosine from the lab samples. We have successfully produced a linear calibration curve using a mixture of isotopically labelled and unlabelled desmosine standards. Reproducibility, precision and dynamic range has been determined.

Linearity

Since labelled and unlabelled desmosine can easily be detected in Mass Spectrometry, we were able to conduct this experiment very easily and efficiently. Desmosine was prepared for different concentrations to have approximately four to five data points for all three trials and reproduced trial. The most ideal results were displayed in the 0.025 mM, 0.100 mM, and 0.250 mM unlabelled desmosine concentrations as it produced the ideal peaks of 397 and 401. However, the concentration of D4-desmosine was kept constant throughout the experiment. The reproduced trial, with one constant concentration (des:des/D4): 0.125 mM/0.05 mM, is displayed in figure 7. Table 1 provides the concentration of mixture Des:D4-des with matrix in mM concentration. After preparing the sample mixture (des:D4) with matrix, each concentration mixture was pipetted four times (so that there are multiple trials) on the MALDI plate in order to obtain the average of the area under the two specific peaks. Peak at 397 amu and at 401 amu corresponds to Desmosine and D4-desmosine respectively. In each mass spectrum, we look at the relative abundance keeping either of the two peaks 100% relative to the other. In figure 4, as the concentration of desmosine gets lower its peak height also gets smaller which makes the peak from D4-desmosine to look higher in relative to desmosine. This fact of proportionality between concentration and peak height of a compound in MS makes it possible to conduct this experiment and produce a calibration curve, shown in figure 9. All three trials are provided in table 2 with its linear graphs in figure 5 respectively. The graphs were plotted with concentration ratio (des/D4) on x-axis and average area ratio (des/D4) on y-axis. The R^2 values on the graph provide a statistical measure of how close the data points to the fitted regression line. For all three trials the R^2 values are very close to 1 that shows the data points are close to the fitted regression. Table 3 and Figure 6 provide how precise the three trials are. In this case R^2 is 0.9993, which is almost one, shows that the trials were precisely done.

Dynamic range

The sensitivity of the instrument was detected by conducting a serial dilution by a factor of five, which is decidedly chosen, for the 0.125/0.5 des/D4-des concentration ratio, which is considered as 1. Table 4 shows all the fraction ratios that correspond to the respective concentration ratio. The instrument could not detect the concentration ratio after the second dilution as can be seen in Figures 7 and 8. In Figure 7a, 7b and 7c the peaks at 397 amu and 401 amu look consistent which means that the instrument was able to detect the concentration because the fraction ratios for all three are very close to each other. However, in Figure 7d the peaks at 397 amu and 401 amu are absent, also the fraction ratio is very low this concludes that the instrument can not detect the third diluted concentration. The concentration after third dilution comes out to be 500 nM for desmosine and 800 nM for D4-desmosine. In general, the dynamic range for the MS instrument in Dr. Emmanuel Chang's lab a York College-CUNY is 1000 nM -100 nM and our result was between this range.

Reproducibility

In order to see if the experiment would provide the same results if conducted by a third party, we asked a PhD student in our lab to repeat the same protocol. Table 5 and Figure 9 provide the data points and linear graph of the reproduced trial. The result are little off from the above three trials. This could be because the new stock of D4 with same concentration was prepared at this time while older stocks of same-concentrated D4 for the above three trials were prepared with a long passing time ranging in days.

Future plans

The project is ongoing; the method's reproducibility and accuracy need to be continuously performed to validate the calibration curve. Further study can be done on the effectiveness of this method on human biological samples. The information gathered in future research can be helpful for medical research, in which this method can hopefully be used to help patients determine the extent of their diseases regarding the breakdown of elastin.

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ACKNOWLEDGEMENTS AND CONTACT INFORMATION

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We would like to thank Dr. Emmanuel Chang for his supervision, Manjeet Kaur for her guidance, and Praikkumar Rathod for his encouragement. We would also like to thank York College for providing us with the necessary facilities and equipment to conduct our studies.