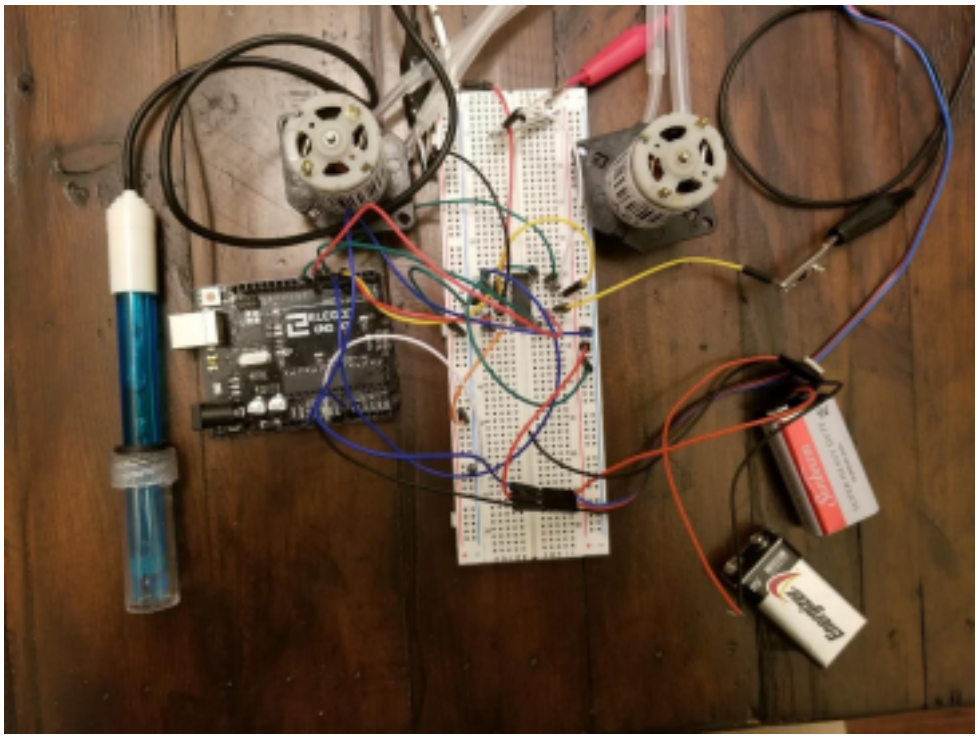

A DIGITAL DUAL-HORMONE ARTIFICIAL PANCREAS

A Year Two Study

Waleed Randhawa

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Research Paper

Every year, diabetes kills 1.5 million globally. Diabetes directly affects 422 million people in the world. Diabetes is the result of a malfunctioning pancreas. The experiment will focus on treating Type 1 diabetes. Children, as young as two years old, can fall victim to Type 1 diabetes. Type 1 diabetes begins as an infection; the body can mistake the insulin-producing cells, better known as beta islet cells, for the infectious cells and demolishes the wrong cells. As a result, the body has no more cells that can produce insulin. Occasionally, the body will also demolish the alpha islet cells which are known to produce glucagon. Glucagon are known to increase blood sugar levels, whereas, insulin decreases blood sugar levels. The absence of insulin, glucagon, or both can cause severe conditions known as hyperglycemia and hypoglycemia.

On the other hand, Type 2 diabetes is most likely to be caused by genetics or a person's lifestyle and food choice. If doctors were able to develop an artificial pancreas, many people in the world would be cured. Currently, people with Type I and Type II diabetes periodically check their glucose levels using disposable test strips and glucose meters. Patients inject an insulin formulation or a glucagon formulation into the bloodstream to control blood glucose levels. Doctors require patients with diabetes to inject insulin after each meal.

Current treatments have multiple disadvantages. The quantity of injected insulin or glucagon may be reduced when traveling through the body's subcutaneous tissue. The quantity of insulin or glucagon may never reach the bloodstream and may degrade or reduce inside the body's tissues, especially the body's subcutaneous tissue. If patients fail to monitor their glucose levels or take the improper dose of insulin or glucagon even at the correct time, their blood sugar levels will drastically increase or decrease. Furthermore, people suffering from diabetes are not sure of the exact quantity of insulin that needs to be injected. If a patient's blood glucose levels immediately increase or decrease, the patient will be unaware of the dangers that may occur.

The experimenter, acknowledging the magnitude of the problem, will develop a system that can replace a diseased pancreas in the near future. The artificial pancreas will model the exact functions of the endocrine portion of the pancreas. This system will combat the disease through continuous monitoring of blood glucose level and inject the appropriate amount of insulin and glucagon accordingly. Closed-loop delivery systems allow little, if any, patient interference. Once the technology of dual-hormone delivery systems is perfected, Type 1 and

Type 2 diabetes patients will be able to use the systems to control their glucose levels. Patients

all over the world will be able to control glucose levels with ease; patients will no longer worry about spontaneous cases of hypoglycemia, or hyperglycemia.

Previous Experiments

An experiment, conducted by Roman Hovorka, Janet M. Allen and many other scientists, aimed to discover if closed-loop insulin delivery systems could control overnight glucose levels in 17 patients aged from 5 to 18. The participants had been affected by Type 1 diabetes for 5-7 years. The experiment comprised of three different studies, focusing on patients who consumed self-selected meals, patients who consumed a large meal with a high content of carbohydrates and patients who consumed a light meal with additional exercise in the afternoon. All patients were monitored from 8pm to 8am with closed loop delivery systems to detect nocturnal hypoglycemia.

The time in the target range for plasma glucose was higher during closed loop delivery when compared to standard insulin delivery. In addition, closed loop delivery doubled the target range time after midnight, making all hypoglycemic events asymptomatic. The experiment found that closed-loop delivery with evening exercise allowed the patient's glucose levels to be inside the target zone for the longest period of time. Moreover, closed-loop insulin delivery systems consistently outperformed continuous infusion at low and high glucose concentration levels. Despite all the encouraging results, closed-loop glucose control involved sensing errors which are required to be improved before a safe launch into the public health industry.[1]

This experiment helped to understand the comparison between the closed-loop insulin delivery system and standard insulin infusion. It proved that the closed-loop insulin delivery system is highly effective and can be customized to an individual's lifestyle (meal size, meal type, and duration of evening exercises). Moreover, closed loop (automatic) insulin delivery significantly reduced the risks of nocturnal hypoglycemia. However, improvements in automation and sensing can further refine the closed loop system as a viable solution for many Type One diabetes patients.

[1]Stockman, J.a. "Manual Closed-loop Insulin Delivery in Children and Adolescents with Type 1 Diabetes: A Phase 2 Randomised Crossover Trial." *Yearbook of Pediatrics*2011 (2011): 127-28. Web. <http://www.thelancet.com/pdfs/journals/lancet/PIIS0140-6736(09)61998-X.pdf>29 June 2017.

Stuart A Weinzimer, Garry M Steil, Karena L Swa, Jim Dziura, Natalie Kurtz and William Tamborlane conducted an experimental study to compare fully automated closed-loop

insulin delivery system and semi-automated hybrid control. Seventeen adolescents between the age of 15 to 18 underwent 34 hours of closed-loop control, 8 with full closed-loop control and 9 with hybrid closed-loop control. All the subjects were diagnosed with type I diabetes. In the FCL (full closed loop) group, all the insulin was given under the control of computer-based algorithm. Whereas in HCL (hybrid closed loop), a bolus of rapidly acting insulin is manually given about fifteen minutes before the meal.

In this experiment, the closed loop delivery system consists of continuous glucose monitors, insulin pumps, and a control algorithm. The glucose sensor detects the glucose level in the patient's body and inputs the values into the control algorithm. The algorithm provides the insulin pump with the amount of insulin needed to restore normal glucose levels. The insulin pump's only job is to supply the right amount of insulin to the patient's body. During the course of the experiment, the glucose sensors accurately tracked patients' glucose levels. According to the continuous glucose sensor, 85% of all patients' glucose levels were between 70 and 180 mg/dl when using closed loop delivery systems, outperforming open-loop system which only maintained target blood glucose level in 58% of the patients. In addition, HCL displayed better daytime, nighttime, and peak postprandial glucose level when compared to FCL. [2]

This experiment was helpful because it exhibited that hybrid closed-loop control system was more effective than full closed-loop. A subcutaneous insulin sensor can enhance the regulation of blood glucose levels during the daytime, after midnight and postprandial. However, the accuracy of the subcutaneous sensor alarm is critical for clinical approval. This study clearly showed that even with today's technology human intervention is essential to accurately monitor and regulate blood glucose levels.

Pratik Choudhary, David Kerr, and many other scientists conducted an experiment in the United Kingdom to evaluate low glucose suspend (LGS) feature of Veo insulin pump in response to hypoglycemia. Thirty-one patients with a mean age of 41.9 years were studied over a period of

[2] Weinzimer, Stuart A., Garry M. Steil, Karena L. Swan, Jim Dziura, Natalie Kurtz, and William V. Tamborlane. "Fully Automated Closed-Loop Insulin Delivery Versus Semiautomated Hybrid Control in Pediatric Patients with Type 1 Diabetes Using an Artificial Pancreas." Diabetes Care. American Diabetes Association, 01 May 2008. Web. <http://care.diabetesjournals.org/content/diacare/31/5/934.full.pdf>30 June 2017

three weeks. Patients were divided into four different groups by the average duration of their hypoglycemia. Low glucose suspends systems (LGS) were programmed to sound alarms when the glucose levels reach a certain threshold set by the patient. It was hypothesized that patients

with most hypoglycemia at baseline will benefit the most.

During the study, the Low Glucose Suspend (LGS) was used for the maximum of two hours after hypoglycemia was detected. 75% of these occasions occurred overnight. Low Glucose suspend largely decreases the risk and duration of nocturnal hypoglycemia. 93% of the patients claimed they felt a difference and rarely experienced hypoglycemia. 166 LGS episodes were detected and 66% of the daytime episode were treated within 10 minutes. During night time, the mean response time to an alarm is more than 60 minutes which further reinforce the need for automated delivery systems. The experiment proved that the insulin pump with LGS reduces the nocturnal hypoglycemia among the high-risk patients. [3]

This experiment was valuable because it studied older adults with Type One diabetes suffering from hypoglycemia. LGS features ensure improved patient care through the reduction of risk involved in both low or high glucose levels. The experiment stressed the need of insulin pump therapy during nighttime when patients are not as responsive. Furthermore, an insulin pump must be capable to provide additional glucose when needed. The experiment validates the effectiveness of the first fully automated insulin delivery system in response to glucose levels.

Antonios E. Pantaleon, Mikhail Loutsenko, Garry M. Steil and Kerstin Rebrin conducted an experiment with the approval of National Institute of Health Research to evaluate the effect of closed-loop insulin delivery system after meals. Eight dogs with diabetes were tested. Prior to the study, the dogs were treated with the subcutaneous open-loop insulin infusion method for one week to determine the daily dose of insulin. All dogs were healthy and lived in kennels. The insulin delivery method applied the proportional-integral-derivative model, delivering insulin in proportion to the glucose above target. Closed-loop control began 8:00 a.m.; blood samples were taken every 20 minutes until noon when the dogs were provided with a standard meal. For the next 3 hours, the scientists withdrew blood samples every 10 to 20 minutes. Experiments on each dog are separated by an interval of five days.

[3] Choudhary, Pratik, John Shin, Yongyin Wang, Mark L. Evans, Peter J. Hammond, David Kerr, James A.M. Shaw, John C. Pickup, and Stephanie A. Amiel. "Insulin Pump Therapy with Automated Insulin Suspension in Response to Hypoglycemia." Diabetes Care. American Diabetes Association, 01 Sept. 2011. Web. <http://care.diabetesjournals.org/content/diacare/34/9/2023.full.pdf>30 June 2017.

The scientists discovered that after meals were consumed, glucose levels significantly increased. However, the closed-loop system provided a rapid reaction to the rising postprandial glucose hike, which led to the glucose levels dropping before returning to normal. Insulin delivery remained elevated for up to 6 hours after the noon meal was digested. Each dog

received 1.5-time high insulin dose as compared to the total daily dose to compensate for the delay in subcutaneous insulin absorption. Although glucose levels returned to normal after meals, high insulin delivery rates still were maintained to ensure normal blood glucose levels. Closed loop systems retained a high delivery rate despite normoglycemia. [4]

This experiment was valuable because it tested an automated closed-loop system on eight dogs suffering from diabetes. The study found that additional insulin is required well past the meal time when glucose levels were normalized, especially to accommodate subcutaneous absorption delay. Moreover, the experiment also suggested that humans and dogs both have a similar peak insulin absorption time, between 40 to 44 minutes. This study also suggested that closed-loop insulin delivery systems are effective on dogs, therefore, further experimentation on canines can benefits humans the near future.

Benyamin Grosman, Jacob Ilany, Anirban Roy conducted an experiment in Israel to test the safety and effectiveness of the Medtronic Hybrid Closed-loop system. Nine patients with Type 1 diabetes were remotely monitored for 571 hours inside a protected home with closed circuit television. The closed-loop control was linked to an Android phone via Bluetooth. The Android controller allowed doctors to view patient glucose levels and insulin delivery. The patients were permitted to consume homemade meals or dining out but the system required meal announcements with the estimated carbohydrate content. Postprandial glycemic was compared between the suggested parameters of dietitian- made meals and subject controlled meals.

During the supervised outpatient monitoring of Hybrid Closed-loop delivery, participants' glucose levels were within 70-180 mg/dl target zone 80% of the time. The mean glucose sensor reading was close to the target zone for the longest amount of time with personalized parameters as compared to default parameters. Over 24 hours, when the default parameters were being evaluated, 68 percent of the time patients' glucose levels were in the target zone. With

[4]Panteleon, Antonios E., Mikhail Loutseiko, Garry M. Steil, and Kerstin Rebrin. "Evaluation of the Effect of Gain on the Meal Response of an Automated Closed-Loop Insulin Delivery System." Diabetes. American Diabetes Association, 01 July 2006. Web. <http://diabetes.diabetesjournals.org/content/diabetes/55/7/1995.full.pdf>30 June 2017.

personalized parameters, 87 percent of the time patients' glucose levels were inside the target zone. On average, 53-72 percent of meal carbohydrate estimation error was recorded when reported by the patients. However, the estimation error did not significantly impact the postprandial glucose level due to the advance adaptability of the Medtronic insulin delivery system. [5]

This experiment was useful because it studied Type 1 diabetes patients who were living in their home. It showed that closed-loop systems can overcome many barriers using sensor readings, blood glucose meter readings, and meal announcements. Blood glucose levels significantly rose after meal consumption. However, in the study, the closed-loop system overcame the difficulty by requesting a meal time announcement. In addition, the study discovered that target blood glucose level can be maintained despite slight under or overestimation of meal carbohydrate content. This experiment concluded if a closed loop or hybrid loop system are ever released for public use, it must include mealtime announcements and a patient's personalized parameter input features**.**

An experimental study was conducted by Richard Bergenstal and his fellow colleagues at the International Diabetes Center, Minnesota, to evaluate the safety of the hybrid closed-loop Insulin Delivery Systems with type I diabetes. One hundred and twenty-four patients, with age 14 to 75 years, with type I diabetes for at least two years, participated in this experiment for a period of 5 months. All the participants had been using insulin pumps for at least 6 months and had glycated hemoglobin less than 10%. The experiment aimed to investigate the continuous glucose monitors, insulin pump and the control algorithm. Every midnight, the algorithm autonomously adjusts the parameters for each individual patient to regulate blood glucose level.

No major episodes of hypoglycemia occurred over the course of the study. However, there were 28 events that were related to the closed-loop system malfunctioning compared to 117 events which were not because of the insulin delivery system. About 66 to 72 percent of the time patients' blood glucose levels were in the target zone when using a closed loop system. Judging

[5] Grosman, B., J. Ilany, A. Roy, N. Kurtz, D. Wu, N. Parikh, G. Voskanyan, N. Konvalina, C. Mylonas, R. Gottlieb, F. Kaufman, and O. Cohen. "Hybrid Closed-Loop Insulin Delivery in Type 1 Diabetes During Supervised Outpatient Conditions." Journal of Diabetes Science and Technology. U.S. National Library of Medicine, 03 May 2016. Web. <https://www.ncbi.nlm.nih.gov/pubmed/26880389>01 July 2017.

from the experiment, the researchers at the International Diabetes Center concluded that the system needs many improvements before releasing to the public. [6]

This experiment was unique because it was performed on a board age group ranging from 14 to 75 years. It proved a longer testing period was required to validate the effectiveness of a closed loop delivery system. Although the system reduced the hypoglycemic events from 117 times to

28 times, still the difference of glycated hemoglobin could have caused multiple malfunctions in the system. This study proved that a larger group of patients must be divided into smaller groups according to age and lifestyle to provide a better insight into the effectiveness of the automatic closed-loop system.

An experiment conducted by Ahmad Haidar, Laurent Legault and Ammar Al-Khateeb [7] aimed to compare standard insulin pump therapy with dual-hormone closed loop delivery. Both systems were tested on 15 adults with type one diabetes. Patients were required to visit the facility twice during the test. During the visit, which lasts 14 hours, patients exercised for 30 minutes in the evening. Following exercise, the patients consumed a meal, carbohydrate was regulated to ensure that glucose levels did not spike. Before bedtime, patients were given a small snack. Patients slept at the facility overnight during both visits. Through this experiment, scientists aimed to discover if the addition of glucagon delivery will make any Artificial Pancreas more effective. Based on previous studies, the scientists hypothesized that the dual hormone closed loop system, when compared to a single hormone insulin delivery system, would increase the amount of time the patient's blood glucose levels were in the desired range, by more than 22 percent.

As predicted by scientists, the dual-hormone closed loop system outperformed the single hormone insulin delivery system. The study confirmed the dual-hormone closed loop delivery is capable of preventing hypoglycemia. As compared to standard insulin delivery, only one patient experienced hypoglycemia during treatment with the dual-hormone closed-loop delivery system.

Although the inclusion of glucagon delivery may lead to hyperglycemia, scientists found a

[6] Digitale, Erin. "For Slumbering Diabetics, a Way to Detect Low Blood Sugar and Stop Insulin Delivery." News Center. N.p., 07 May 1970. Web. <https://med.stanford.edu/news/all-news/2014/05/for-slumbering-diabetics-a-way to-detect-low-blood-sugar-and-stop-insulin-delivery.html>30 June 2017.

[7] Haidar, Ahmad, Legault, Laurent, Dallaire, Maryse, Alkhateeb, Ammar, Coriat, Adèle, Messier, Virginie, Cheng, Peiyao, Millette, Maude, Boulet, Benoit, Lhoret, Rémi R. "Glucose-responsive insulin and glucagon delivery (dual hormone artificial pancreas) in adults with type 1 diabetes: a randomized crossover controlled trial." CMAJ, 05 March 2013. Web. <http://www.cmaj.ca/content/cmaj/185/4/297.full.pdf>01 July 2018.

decrease in risk of hyperglycemia during the trial. Additionally, dual-hormone closed loop delivery was found to be more effective during the patient's 30-minute exercise routine. All in all, dual-hormone closed loop delivery may be the solution to treating hypoglycemia during the day and night.

This experiment has shown the need for a glucagon component in every artificial Pancreas.

Dual-hormone delivery clearly outperforms current single hormone insulin delivery systems, around the clock. With the inclusion of glucagon delivery, the patient's blood glucose levels will normal during exercise, after meals and throughout the night. An Artificial Pancreas that aims to operate in clinics successfully, without human intervention, needs glucagon delivery.

Ahmad Haidar and T.M Peters[8]conducted a study to discover the benefits of a dual-hormone artificial pancreas. Six previous studies were analyzed to compare dual-hormone delivery systems compared with the standard insulin delivery system. All studies have confirmed that single hormone (only insulin) and dual-hormone (insulin and glucagon) systems outperform subcutaneous insulin infusion pumps. In the experiments that were reviewed, dual-hormone systems delivered small doses of glucagon along with large doses of insulin. Although the dual hormone systems are more expensive than single-hormone systems, the inclusion of glucagon delivery allows more rapid insulin delivery while ensuring glucose levels do not drop dangerously and preventing hypoglycemia.

The dual-hormone artificial pancreas closely replicates the actual functions of a human pancreas. In an experiment among 28 patients with Type 1 diabetes that lasted over 48 hours, only three episodes of hypoglycemia occurred when treatment with dual-hormone artificial pancreas was taking place. Often, during exercise patients with type 1 diabetes suffer severe cases of hypoglycemia due to the decline in glucose levels. Currently, to normalize blood sugar levels during exercise, patients must consume carbohydrates. Unfortunately, this method leads to hyperglycemia. Single-hormone systems suspend insulin delivery during cases of hypoglycemia, however, this method is not efficient and may lead to severe health issues, such as nausea and fainting. In the experiment, not only did the dual-hormone system lower the cases of hypoglycemia during exercise, but it was also more effective than single hormone systems when

[8]Peters, T M, and A Haidar. "Dual-Hormone Artificial Pancreas: Benefits and Limitations Compared with Single Hormone Systems." Current Neurology and Neuroscience Reports., U.S. National Library of Medicine, Apr. 2018. Web. <www.ncbi.nlm.nih.gov/pubmed/29337384.> 26 July 2018.

reducing the risk of nocturnal hypoglycemia. Dual-hormone artificial pancreas can lower blood glucose levels using insulin delivery and eliminate the risk of hypoglycemia using glucagon delivery.

As evaluated by this research paper, dual-hormone systems significantly reduce the risk of hypoglycemia. Dual-hormone systems help patients throughout the night and during exercise. The inclusion of glucagon delivery reduces the risk of dangerous conditions related to Type 1

diabetes, such as severe hypoglycemia and hyperglycemia. Dual-hormone systems are more effective than single-hormone systems when normalizing blood glucose levels during hypoglycemia. Glucagon delivery helps make life more stable, convenient, and enjoyable for patients with type 1 diabetes.

Hypothesis

Previous experiments testing closed loop delivery have revealed that automated insulin delivery systems help control hyperglycemia around the clock. The most effective system was the dual-hormone Artificial Pancreas, which uses insulin delivery along with glucagon delivery. When under single hormone (only insulin) treatment patient glucose levels were in the target zone 65 percent of the time. However, under dual-hormone treatment, 85 percent of the time patients were in the target zone.

In previous experiments, the glucose sensor was consistently accurate; the glucose sensors rarely detected the wrong glucose levels except postprandial when glucose levels rose exponentially. In Stuart Weinzimer and his associates' experiment, delivery rates after meals could not match the rising glucose levels. However, when using Hybrid Closed loop, doctors were able to pump additional insulin after meals resulting in HCL outperforming Full Closed Loop. However, Ahmad Haidar's study proved dual-hormone systems could deal with spikes in blood glucose levels post-prandial. Dual-hormone systems also helped lower the risk of hypoglycemia during exercise. The inclusion of glucagon cells allows for rapid insulin delivery, which lowers the risk of hypoglycemia and hyperglycemia around the clock.

The current experiment is important because it stresses the need to develop a dual hormone Artificial Pancreas. Most existing insulin delivery systems require some form of human

intervention. In many instances, mostly following the consumption of meals containing large amounts of carbohydrates, the dual-hormone systems required human intervention, Although current systems provide better blood glucose level control, the goal of this experiment is to further develop a cost-effective integrated prototype of a fully automated dual-hormone delivery system that effectively operates without any human intervention. Using a microcontroller, Arduino, programmed with an integrated algorithm that is capable of fixing glitches that occur during testing, the system will model the endocrine function of an actual pancreas. Developing a

successful system is extremely critical for preventing and curing cases of hypoglycemia and hyperglycemia, thus helping all patients with Type I diabetes.

In this engineered Artificial Pancreas, vinegar and a basic solution will be used to represent insulin and glucagon, respectively. Both systems will attempt to neutralize a basic or acidic solution. With time, as the solution is neutralized the flow decreases until it completely stops. However, if the systems would somehow malfunction and continue pumping vinegar even after neutralization, a basic solution representing glucagon will be needed to normalize blood sugar levels. Even if there is such a glitch, the system will still operate without any human intervention.

Based on previous research, it is hypothesized that the dual-hormone Artificial Pancreas will accurately neutralize, the pH, of the solution more than 95 percent of the time; the pH will be in the target zone more than 95 percent of the time. It is further hypothesized, due to the inclusion of glucagon delivery, that the final pH of the solution will drop beneath the target zone (pH of 7.25-7.5) three times or less out of 120 tests. Both the digital single and dual-hormone Artificial Pancreas will neutralize the more effectively and efficiently, due to better digital control provided through Arduino combined with the digital pH sensor.

Materials and Procedures

The experiment being conducted aims to engineer a dual-hormone Artificial Pancreas using an Arduino Uno R3 that can control blood glucose levels of patients with Type 1 diabetes. When using the dual-hormone system, patients will no longer be monitoring their own glucose levels; rather, an automated continuous glucose monitor will always be sensing blood glucose levels glucose levels. The dual-hormone system will consist of a solderless breadboard, a jump

wire kit, two 9-volt batteries, a digital pH module, an Arduino Uno R3, and two peristaltic liquid pumps. The rest of the materials are liquids such as vinegar, water, and baking soda. Adult supervision may be required when dealing with liquids such as baking soda and vinegar.

In the first part of the experiment, a customized algorithm needs to be created and uploaded into the solution. Experience with circuit boards along with Arduino coding (essentially C++ coding language) is essential to creating an accurate algorithm that ensures the solution is neutralized. Knowledge of diabetes is needed in order to evaluate the overall performance of a closed loop

system. The process of the closed-loop system is similar to the systems that are being tested on humans. However, instead of insulin being pumped into the bloodstream vinegar is pumped into the basic solution and vice versa.

In order to produce accurate readings, the digital pH sensor must be calibrated. First, the probe of the sensor needs to be placed in a neutralized liquid (pH 7), an acidic liquid (pH 4) and a basic liquid (pH 8). Once the sensor is calibrated it should send the pH information to Arduino control system. The control algorithm, uploaded to the Arduino, will determine the amount of baking soda or vinegar needed to neutralize the solution.

The automated delivery system will be tested with substances such as vinegar, water, and baking soda. A basic solution, consisting of a quantity of baking soda diluted in water, represents high glucose levels and the acidic solution, vinegar, represent low glucose levels and insulin. When the sensor detects high glucose levels the pump will send insulin (vinegar) to neutralize the basic solution, likewise, when the pH sensor detects an acidic solution, glucagon (another basic solution) will be sent to neutralize the solution. If the sensor detects large amounts of acidity or basicity in the solution, the algorithm will be programmed to increase the speed of the peristaltic pump, which will help neutralize the solution efficiently. A neutralized solution represents normal or healthy glucose levels. The motor should automatically stop pumping acidic or basic substances when the substance becomes neutralized. In order to test the effectiveness of the pump and the sensor, the pH of the liquid can be tested after the experiment is conducted using litmus paper, bromothymol blue, and the digital pH sensor's readings.

Core Science

The pancreas is located in the upper left section of the abdomen. There are two separate parts of the pancreas, the endocrine and exocrine function. The exocrine tissue is 95 percent of the pancreas and produces enzymes to help the body digest meals. Whereas, the endocrine system consists of islet cells and glucagon. Islet cells produce and release insulin into the bloodstream to control rising glucose levels. Insulin lower glucose levels and glucagon raise glucose levels during hypoglycemia. Without insulin, the body would suffer from dangerously high glucose levels and would be combatting a surplus of carbohydrates, fats, and proteins.

Whereas, hypoglycemia (low blood glucose levels) occurs when the body's islet cells are unable to produce glucagon. This experiment aims to create a dual-hormone system that models the endocrine function of the pancreas.

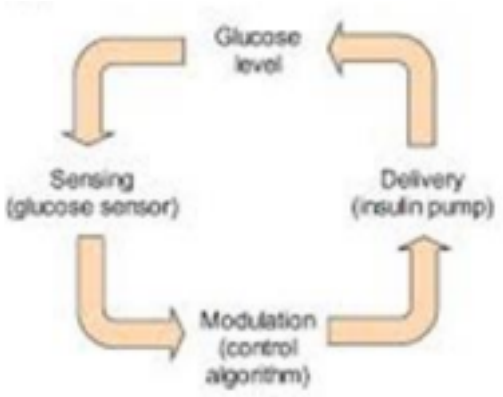

Figure 1 Closed-loop insulin delivery system

As shown in Figure 1 closed loop systems are automatic insulin delivery systems. Patient glucose levels are detected by a continuous glucose monitor (CGM). The sensor sends the glucose levels to the control algorithm located in the main controller. Based on the sensor's output the algorithm calculates the amount of insulin needed to balance the patient's glucose levels. After calculating the amount of insulin required, it signals the pump to deliver that amount.

The delivery is done by an insulin pump and the glucose levels steadily rise or drop according to the amount of insulin that was provided. For example, if the sensor detected the patient's glucose levels rising, it would inform the control algorithm. The algorithm would determine that the insulin pump needs to pump additional insulin to the bloodstream. The

process continues until the patient's glucose levels reach healthy levels. The sensor informs the algorithm about the glucose levels. The algorithm would then instruct the insulin pump to stop delivering the insulin. However, suspending insulin may not normalize blood glucose levels efficiently. Therefore, glucagon is needed to prevent severe hypoglycemia. If the glucose levels would drop beneath healthy levels, the algorithm would determine the amount of glucagon needed to normalize glucose levels. The pump would then deliver the amount of glucagon needed. The glucagon component of the system was added to ensure the solution was neutralized even when the system overshoots, and the solution becomes acidic.

This experiment is aiming to create a digital dual-hormone system and compare the

results with a digital single hormone system as well as a previously constructed single hormone closed loop system. Blood, glucagon, and insulin are inaccessible for the project; the experimenter will use simple liquids instead. A basic solution replaces high blood glucose levels and a neutralized solution represents closed to normal glucose levels. Closed loop systems pump insulin into the bloodstream when the sensor detects increasing glucose levels and glucagon when low blood glucose levels are detected. Whereas, in the experiment, the insulin pump will provide vinegar to neutralize the basicity in a substance and the glucagon pump will neutralize the solution using baking soda. Once the solution is neutralized the sensor will inform the Arduino that glucose levels are normal and the pump will turn off.

When measuring the acidity or basicity of the solution the pH scale is utilized. Acidic substances have pH levels below 7 and give hydrogen ions away. An example of a base is vinegar or lemon juice. Water and most neutral substances have pH levels of 7. Basic substances such as baking soda and bleach have pH levels above 7. They are known to accept hydrogen ions. In the experiment, when pH levels of the solution are acidic or below 7 the pump does not release any vinegar into the solution. However, when the pH levels are above 7 the conductivity sensor will instruct the pump to release vinegar to neutralize the solution. The chemical reaction for the neutralization is given by the following equation:

$$NaHCO3+CH3COOH\rightarrow CO2+H2O+CH3COONa$$

Where,

- $NaHCO3$ is baking soda

- $CH3COOH$ vinegar
- $CO2$ is the carbon dioxide gas generated by the chemical reaction
- $H2O$ is the water is the byproduct of the chemical reaction
- $CH3COONa$ is sodium acetate

Equal amounts of vinegar and baking soda result in a neutralized solution representing normal blood glucose levels. Basic solutions can conduct electricity because the substance has a lower electric resistance. Neutral solutions have high electric resistance making it difficult for electric currents to flow through the liquid. In the experiment, the sensor has the ability to detect if a solution is basic, neutral or acidic. The conductivity sensor contains two metal wires. When the solution is conductive, the electric current is allowed to flow through the conductivity sensor.

However, if the substance is not conductive the pump will not turn on. The conductivity sensor's results will be compared to with a newly included digital pH sensor to determine the most effective configuration.

In this year's experiment, a digital pH sensor will be used. The digital pH sensor is more accurate; it will reduce the error in the neutralization process. The digital pH sensor consists of an electrode and a microchip. The pH electrode consists of a glass ball that has direct contact with the solution. On the inside of the electrode is a metal wire that conducts electricity. The wire is connected to the microchip of the sensor and submerged in an electrolyte solution. The bottom portion of the glass probe is coated in a hydrated gel layer. All parts of the probe work together to produce a pH reading of the solution. The electrolyte will begin leaking into the solution. A battery connected to the wire will charge the hydrated gel layer of the glass ball. The hydrogen ion atoms in the solution will then be attracted to the gel layer. The charge created by the hydrogen ions in the solution will be sent to the pH module's microchip through the metal wire. The microchip can then find the pH of the solution using the Nernst equation.

The equation is as follows:

$$E = E^{\circ} - \frac{RT}{nF} \ln Q_c$$

Ultimately, the number of hydrogen ion atoms inside the glass probe compared to the hydrogen ion atoms outside of the gel layer will determine the pH of the solution. If more

hydrogen ion atoms are attached to the outside of the gel layer than inside the probe, the solution will be acidic (pH level below 7). On the other hand, if more hydrogen ions are inside the glass probe than attached to the gel layer, the solution will be basic (pH above 7). However, if the number of ions in both locations are the same, the solution will be neutral (pH 7). After finding the pH of the solution, the pH microchip will send the information to the Arduino R3. Arduino's algorithm, combined with the digital motor control (breadboard) will then neutralize the solution.

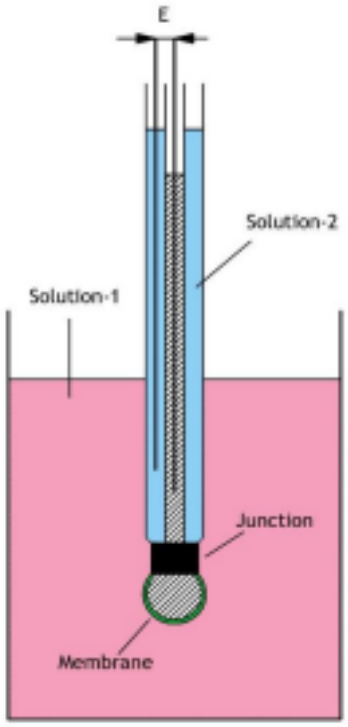

Figure 2 A diagram showing a pH probe immersed in a solution. The glass membrane is surrounded by a green layer which is the gel layer. The number of hydrogen ions attached to the outside of the gel layer compared to the number of hydrogen ions inside the glass membrane will determine the pH of the solution.

In the experiment, a closed-loop system will be developed using Arduino and two 12-volt motors, each representing an insulin and glucagon pump. Although Arduino will be programmed using Arduino coding language, (similar to C++) electronics will still need to be used to connect the motor to Arduino and digital motor control..

In the year two experiment, to further reduce the error in the current prototype, Arduino, a microcontroller, will be programmed to automatically turn the insulin and glucagon pumps on

and off according to the current state of the solution. Arduino's program will replace the potentiometers, MOSFET, and resistors from last year's experiment. The program will determine the amount of vinegar or baking soda needed to neutralize the solution as well as the speed of the peristaltic pump, which will be proportional to the pH.

Arduino's program will regulate the speed of the motor and determine when the motor should turn on according to the pH of the solution. Furthermore, Arduino (using the program) will also figure out which motor will effectively neutralize the solution based upon the acidity or

basicity of the solution. The insulin pump will only pump vinegar when the solution is basic. Whereas, the glucagon pump will pump baking soda when the solution is acidic. Arduino will be responsible for choosing the right motor depending on the current state of the solution. In order for the solution to be neutralized, the Arduino must be programmed to turn on the glucagon and insulin pump based on the digital pH sensor readings.

First, the pH sensor will detect the potential of hydrogen in the solution. Then, the microchip in the sensor would send the pH of the solution to Arduino. If the pH is greater than 7, the Arduino program would turn on the insulin pump. However, if the pH is less than 7, then the glucagon pump would begin to pump baking soda to neutralize the solution. Once the solution is neutralized or has a pH of 7, the Arduino will inform the glucagon and insulin pump to turn off.

The speed of the pumps will be proportional to the pH of the solution. Arduino will control the speed of the pumps using Pulse Width Modulation (PWM). PWM is a type of digital signal that is used to create a square wave, a signal that is not switched completely on or off. The time for which the signal stays on is known as the pulse width. If the pulse width can be modified fast enough, it will seem as if the signal is at a constant rate. PWM can also be used to change the speed of an object, by altering the amount of time the object stays on and off. For an example, if the on time is greater than the off time the speed of the object will be comparatively fast. However, if the off time is longer than the on time than the speed of the motor will be slower. In order to use PWM in Arduino, a call to analog Write () can be used. This value inside the brackets can range from 0 to 255, 0 being that the signal is always off and 255 meaning the signal is on a 100 percent of the time.

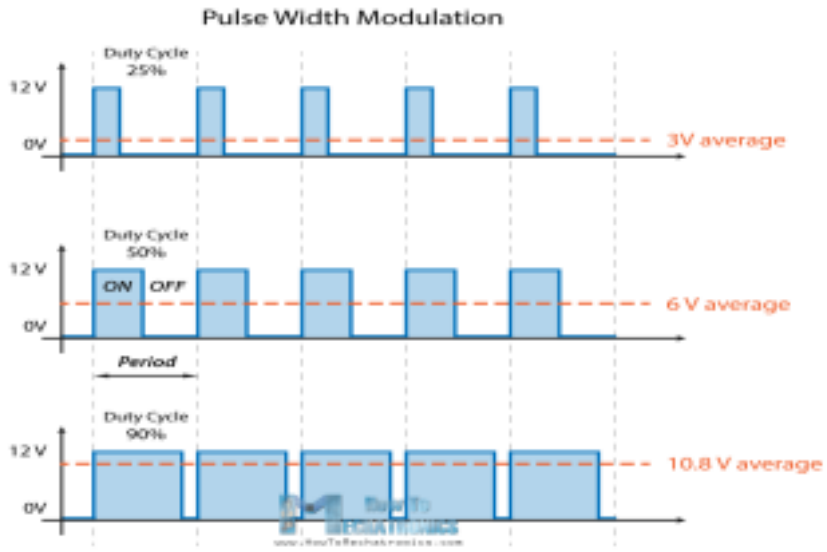

Figure 3 A diagram showing how the pulse widths change with each duty cycle. The longer the duty cycle the longer the pulse widths are. The average value of the voltage that is traveling to the device depends on the amount of time the signal is on compared to the amount of time the signal is off.

Using PWM will enable Arduino to regulate the speed of the motor depending on the acidity or basicity of the solution.

Although batteries will be used to power the motor, Arduino will control the motors using an L293D chip. This chip drives both motors using Arduino's logic. The L293D chip can drive two motors simultaneously in any direction. In this experiment, the chip will drive the insulin and glucagon pump. As found in the previous experiments, delivery glucagon along with insulin will allow for rapid insulin delivery, ensuring no cases of hypoglycemia (ph. below 7) and even hyperglycemia (ph. above 7) occur.

The L293D chip consists of sixteen pins (eight on each side), which help drive both motors at the same time. The chip consists of two H-bridge circuits, which can drive the motors separately. A single H-bridge circuit has the capacity to apply a voltage in any direction which is desired. Most importantly, the chip contains a PWM input driver, which allows Arduino to control the speed of the liquid pump. Combining PWM with H-bridge control will allow Arduino to have full control of the motors.

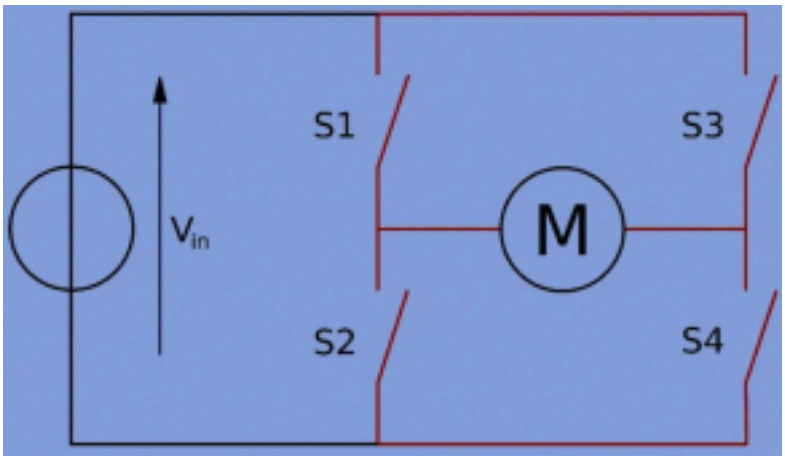

Figure 4 This diagram shows that an h-bridge circuit has 4 switches, S1, S2, S3, and S4. When S1 and S4 are closed, the voltage is applied across the motor. Whereas, when the S2 and S3 are closed the voltage is inverted.

Research Summary

The research and experimentation aim to find a new way to control high and low blood glucose levels. A dual-hormone Artificial Pancreas is being constructed. Dual-hormone closed loop systems consist of a continuous glucose monitor, a control algorithm and an insulin pump. However, blood, insulin, and glucagon are inaccessible. Therefore, a dual-hormone system in the experiment is a digital and simplified prototype, mainly composed of a digital pH sensor, a programmed Arduino R3, and two liquid peristaltic pumps. Vinegar represents insulin and the initial solution mixed with baking soda represents high blood glucose levels. When the pump releases vinegar into the solution, a chemical reaction will occur and the solution will become neutralized. On the other hand, if the solution were to become acidic, the glucagon pump will pump a basic solution into the main solution to neutralize an acidic solution, which represents low blood glucose levels. A neutralized solution represents normal blood glucose levels.

Multiple previous experiments faced conflicts such as sensing error and lag. However, closed loop systems constantly outperform other insulin treatments. Hybrid closed-loop control was the most effective when combined with meal announcements. Doctors were allowed to pump additional insulin in order to match the higher postprandial glucose levels. Automatic insulin delivery is most effective at night when the patients and doctors are asleep. Additionally, the addition of glucagon delivery was beneficial during nighttime and exercise. During treatment

with dual-hormone systems glucagon patients almost never experienced hypoglycemic symptoms. The addition of glucagon allowed for rapid insulin delivery lowering the risk of

hyperglycemia.

Based on previous research, it is hypothesized that the dual-hormone Artificial Pancreas will accurately neutralize, the pH, of the solution more than 95 percent of the time; the pH will be in the target zone more than 95 percent of the time. It is further hypothesized, due to the inclusion of glucagon delivery, that the final pH of the solution will drop beneath the target zone (pH of 7.25-7.5), three times or less out of 120 tests. The digital system will also neutralize the solution more effectively and efficiently due to better digital control provided through Arduino combined with the digital pH sensor.

The dual-hormone Artificial Pancreas is the best option for patients with type 1 diabetes. According to multiple previous experiments, the dual-hormone system maintains healthy glucose levels around the clock. Patients using the dual-hormone system will not be in grave danger if the sensor glitches, and the motor overshoots. With the inclusion of glucagon delivery, the dual hormone system is capable of treating both hypoglycemia and hyperglycemia. Patient's will no longer need to manually inject insulin or consume carbohydrates. The commercial introduction, of the digital dual-hormone Artificial Pancreas, will provide insulin and glucagon as effectively as a healthy functioning human pancreas.

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Statement of the Problem

Researchers and scientist have been collaborating to construct an Artificial Pancreas capable of normalizing abnormal blood glucose levels 24 hours, a day, seven days a week. Previous experiments testing closed-loop insulin delivery systems failed to show significant progress in the functionality of the Artificial Pancreas device. The greatest possible threat to the

integrity of the Artificial Pancreas are malfunctions with the sensing of the system. The Artificial pancreas should pump insulin when glucose levels become higher than normal and pump glucagon when the glucose levels are lower than normal (this function is only found in dual hormone Artificial Pancreas). However, after meals or food consumption blood glucose levels spike and the closed-loop system cannot keep up. Occasionally, the sensor lags when measuring the patient's glucose levels and thus the control system does not have the accurate glucose level readings. This may lead to a surplus of insulin or glucagon, which leads to hyperglycemia, or hypoglycemia, respectively. The experiment hopes to discover a cost-effective solution to control blood glucose levels for Type 1 diabetic patients, without human intervention, using insulin and glucagon.

Purpose

Many scientists have been working on the invention of single and dual-hormone artificial pancreases, to help the millions of diabetes patients in the world. The purpose of the experiment is to construct a cost-effective digital dual-hormone closed-loop insulin delivery system that is capable of normalizing high and low blood glucose levels, more efficiently than a single hormone system. Previous studies have shown dual-hormone systems (consists of glucagon and insulin pump) outperforms single-hormone systems (only uses an insulin pump) when normalizing high and low blood glucose levels. If the results in the experiment were to prove that dual-hormone systems normalize blood glucose levels effectively, these systems could eventually be used clinically. This brand of technology would appeal to diabetic patients around the world. The dual-hormone Artificial Pancreas would substantially lower the risk of hyperglycemia and hypoglycemia.

Hypothesis

Prior research has exposed the faults, ineffectiveness and inefficiency of current methods to treat type 1 diabetes. However, multiple experiments have shown successful test results when using a closed loop Artificial Pancreas. According to Benyamin Grossman, patient's blood glucose levels were inside the designated target zone, 70-180 mg/dl more than 80 percent of the time. However, the system occasionally glitched causing too little or too much insulin to enter

the patients' bloodstream. Single hormone systems do not have the capacity to treat low blood glucose levels, as the systems can only pump insulin). Once the motor overshot, and the pH levels were above the target zone, human intervention was required. Therefore, in order to establish a true Artificial Pancreas, glucagon delivery is needed.

An experiment, conducted by Ahmad Haidar, proved that a dual-hormone Artificial Pancreas is more effective than a single-hormone Artificial Pancreas when normalizing blood glucose levels. Due to the inclusion of glucagon, which raises low blood glucose levels, the dual hormone system significantly lowered the risk of hypoglycemia during exercise and nighttime. During treatment with dual-hormone systems, only one patient experienced symptoms of hypoglycemia. This result confirms that a dual-hormone artificial pancreas is more effective than a single hormone artificial pancreas.

In this experiment, a baking soda solution with many different concentrations will be created to represent high blood glucose levels. 3 grams, 2.5 grams, 4 grams and 7.5 grams of baking soda will be mixed into 100 mL of water. The digital dual-hormone Artificial Pancreas and the digital single hormone Artificial Pancreas will be tested with each of these concentrations. Both digital systems should be able to neutralize the solution using baking soda or vinegar without any human interference.

Larger concentrations of baking soda such as 7.5 grams will take more time to neutralize than lower concentrations in the solution. The system motor will pump insulin or glucagon based on the pH of the solution. If the pH is greater than the target zone (pH 7.25-7.5), the insulin will be used. If the solution's pH level is lower than the target zone a basic solution (glucagon) will be used. If the pH of the solution is extremely far from the target zone, the motor will pump vinegar or the baking soda faster than the usual rate. However, if the pH is closer to 7, the motor will pump the substance slower than usual. This factor is implemented in the Arduino code, to avoid the motor overshooting which leads to inaccurate results. Previous research has proven that

the lower the concentration of the baking soda (2.5 grams, 3 grams and 4 grams), the more accurately the Artificial Pancreas will neutralize the solution. Furthermore, previous experiments have shown that dual-hormone systems are more effective than single-hormone systems.

Based on previous research, it is hypothesized that the dual-hormone Artificial Pancreas will accurately neutralize, the pH, of the solution more than 95 percent of the time; the pH will be in the target zone more than 95 percent of the time. It is further hypothesized, due

to the inclusion of glucagon delivery, that the pH of the solution will drop beneath the target zone (pH of 7.25-7.5), three-time or less out of 120 tests. The digital system will also neutralize the solution faster more effectively and efficiently due to better digital control provided through Arduino combined with the digital pH sensor.

Variables and Controls

1. <u>Independent Variable: Concentration of Baking Soda in 100 mL of water</u> • The variable will be changed manually by adding less baking soda when making the solution to run the experimentation.

- The variable changes the time it will take for the solution to be neutralized by the pump.
- The concentration of baking soda will also change the amount of vinegar

that is needed to be poured in the container.

2. **Dependent Variable: Time it takes for the solution to become neutralized** • This variable will be measured by starting a timer from the moment the motor starts until the motors stop vinegar to neutralize the baking soda solution.
   • When the motors stop the solution should be neutralized.
3. **The Control Group:**
   • None
4. **The Controls**

The following should remain unchanged between each run of the experiment (year 1):

- the pH sensor
- the configuration of the circuit
- the Arduino code (unless proven to be faulty)
- the same acidic solution (vinegar)
- The insulin and glucagon pump

Materials

Breadboard

1. L293D chip
2. Jumper wires
3. 2 9-volt batteries
4. 2 double-sided Alligator clips
5. 2 12-volt peristaltic pumps (insulin and glucagon pump)

Main control system

1. Arduino Uno R3

    a. programmed with code that controls the motor

2. pH module

    a. microchip (processes information)

    b. pH probe (submerged in liquid during testing)

Solution

1. Graduated Cylinder with 250 mL capacity
2. Digital water scale
3. 250 pH strips
4. 1-2 liter of baking soda
5. 1-2 liter of vinegar
6. 3 mixing water/ plastic Tupperware

Procedure

Part I: Creating the Circuit

1. Place the L293D in the middle of the breadboard

2. Connect the top insulin pump (12-volt pump) using 2 alligators clips, then connect jumper wires from the alligator clips to the L293D chip

3. Connect the glucagon pump (12-volt pump) using 2 alligator clips, connect jumper wires to the alligator clips, then attach the jumper wires to the opposite side of the L293D chip (opposite side of the insulin pump).

a. the schematics of both connections will allow Arduino to drive both motors **b.** the glucagon pump will only be used with the dual-hormone system, this step is only required for the

dual-hormone system

4. Use the jumper wires to attach the L293D chip to the Arduino so that Arduino's logic can be used to drive the motors (i.e. motors can operate according to the code) **5.** Two 9-volt batteries will be connected to the L293D so that the chip is capable of running both motors at full power **6.** The pH module will be directly connected to the analog pins of Arduino **Part II: Calibrating**

the Sensor

1. The sensor needs to be placed in a liquid

2. The sensor needs to be placed in an acidic solution, basic solution, and neutral solution

3. The desired pH value, to be associated with the different types of solutions, should be assigned using the calibration code

Part III: Creating the Liquid Solution

1. Add any quantity of baking soda, however, the quantity has to be less than 8 grams **2.** Mix the baking soda with 100 mL and pour the solution into the container **3.** Pour 200 or more mL of distilled white vinegar into another container **4.** Add one teaspoon of bromothymol blue into each container

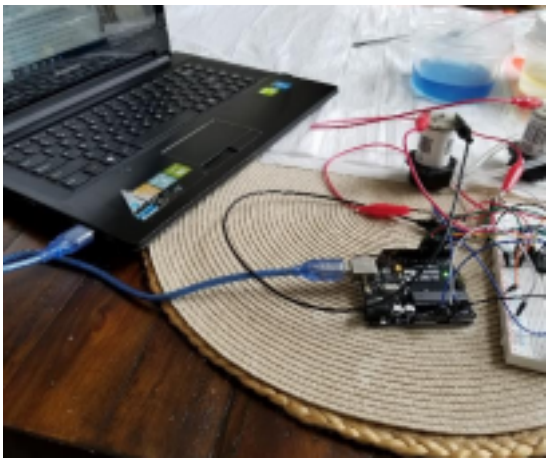
Part IV: Setting up the Experiment

1. Make sure all the pipes are placed in the right container

2. Ensure the pH sensor is placed in the correct container and completely submerged in the solution

3. All the wires should be secured tightly on the right connection on the breadboard to ensure the circuit functions properly

4. Upload the program from the computer to Arduino R3



Part V: Recording Observations and Finding

1. Record the initial pH of the solution

2. Record the pH of the solution after the experiment

3. Record the amount of time the system took to neutralize the solution; the time should begin when the motor starts pumping; the time ends when the motor stops pumping any

substance.

Year Two Results

Digital Single Hormone Results

Testing Day 1-4

Concentration Time(s) Average Time pH level Average pH
2.5 20 17 7.4 7.36871
2.5 10 7.3
2.5 12 7.6
2.5 14 7.32
2.5 9 7.35
2.5 17 7.42
2.5 19 7.65
2.5 15 7.53
2.5 12 7.56
2.5 13 7.45
2.5 13 7.42
2.5 25 6.76
2.5 19 7.3

2.5 16 7.4
2.5 19 7.32
2.5 15 7.48
2.5 11 7.32
2.5 13 7.29
2.5 17 7.26
2.5 12 7.32
2.5 14 7.35
2.5 15 7.33
2.5 20 7.2
2.5 17 7.5
2.5 22 7.69
2.5 19 7.05
2.5 22 7.64
2.5 21 7.17
2.5 25 7.54
2.5 27 7.49
2.5 24 7.02

Testing Day 5-9

Concentration Time(seconds) Average Time pH Average pH
3 18 22.66667 7.54 7.453667
3 21 7.51
3 23 7.56
3 12 7.61
3 22 7.53
3 19 7.49
3 29 7.34

3 30 7.09
3 21 7.48
3 34 7.12
3 18 7.64
3 20 7.51
3 19 7.45
3 22 7.48
3 21 7.55
3 20 7.52
3 25 7.5
3 26 7.48
3 19 7.49
3 28 7.58
3 33 7.05
3 18 7.48
3 15 7.68
3 22 7.48
3 29 7.21
3 18 7.53
3 14 7.73
3 44 6.9
3 21 7.55
3 19 7.53

Testing Day 10-14

Concentration	Time (seconds)	Average Time	pH	Average pH
4	32	29.633	7.52	7.422333
	26		7.56	

4 22 7.49
4 19 7.51
4 40 7.58
4 28 7.67
4 31 7.28
4 33 7.26
4 29 7.52
4 26 7.51
4 30 7.5
4 29 7.58
4 31 7.47
4 45 6.8
4 29 7.38
4 27 7.78
4 42 7.03
4 28 7.01
4 29 7.45
4 36 7.78
4 23 7.32
4 28 7.26
4 22 7.51
4 28 7.5
4 27 7.54
4 24 7.51
4 33 7.19
4 35 7.15
4 29 7.53
4 28 7.48

Testing Day 15-17

Concentration Time(seconds) Average Time pHAverage pH
7.5 99 54.88235294 7.54 7.435
7.5 63 7.4
7.5 56 7.5
7.5 52 7.5
7.5 50 7.62
7.5 63 7.28
7.5 72 7.64
7.5 45 7.48
7.5 30 7.6
7.5 40 7.8
7.5 35 7.54
7.5 74 7.32
7.5 85 7.49
7.5 59 7.56
7.5 52 7.78
7.5 55 7.53
7.5 72 7.45
7.5 48 7.59
7.5 37 7.37
7.5 36 7.25
7.5 45 7.64
7.5 62 7.54
7.5 72 7.4
7.5 32 7.68

	7.5 57 7.17
	7.5 43 7.35
	7.5 42 7.45
	7.5 65 7.56
	7.5 32 7.39
	7.5 35 7.05
	7.5 35 7.38
	7.5 47 7.21
	7.5 86 6.93
	7.5 90 6.8

Dual-hormone System Results

Day 20-22

Experiment Iteration	Concentration (g) pH Average pH
1	7.5 7.41 7.35
2	7.5 7.42
3	7.5 7.41
4	7.5 7.39
5	7.5 7.4
6	7.5 7.39
7	7.5 7.45
8	7.5 7.36
9	7.5 7.28
10	7.5 7.32
11	7.5 7.48
12	7.5 7.36
13	7.5 7.38

14	7.5 7.28
15	7.5 7.4
16	7.5 7.29
17	7.5 7.28
18	7.5 7.36
19	7.5 7.37
20	7.5 7.34
21	7.5 7.29
22	7.5 7.28
23	7.5 7.32
24	7.5 7.33
25	7.5 7.32
26	7.5 7.31
27	7.5 7.34
28	7.5 7.36
29	7.5 7.31
30	7.5 7.29

Day 23-25

Experiment Iteration	Concentration (g) pH Average pH
1	4 7.31 7.364
2	4 7.32
3	4 7.29
4	4 7.33
5	4 7.36
6	4 7.4
7	4 7.29

8	4 7.3
9	4 7.4
10	4 7.42
11	4 7.48
12	4 7.4
13	4 7.49
14	4 7.42
15	4 7.36
16	4 7.31
17	4 7.28
18	4 7.32
19	4 7.33
20	4 7.39
21	4 7.36
22	4 7.34
23	4 7.41
24	4 7.32
25	4 7.34
26	4 7.32
27	4 7.39
28	4 7.46
29	4 7.38
30	4 7.4

Day 26-29

Experiment Iteration	Concentration (g) pH Average pH
1	3 7.32 7.37233

2	3 7.35
3	3 7.33
4	3 7.32
5	3 7.39
6	3 7.35
7	3 7.49
8	3 7.45
9	3 7.46
10	3 7.29
11	3 7.42
12	3 7.41
13	3 7.37
14	3 7.32
15	3 7.31
16	3 7.33
17	3 7.38
18	3 7.34
19	3 7.32
20	3 7.31
21	3 7.41
22	3 7.34
23	3 7.42
24	3 7.4
25	3 7.43
26	3 7.34
27	3 7.37
28	3 7.46

29	3 7.26
30	3 7.48

Day 30-32

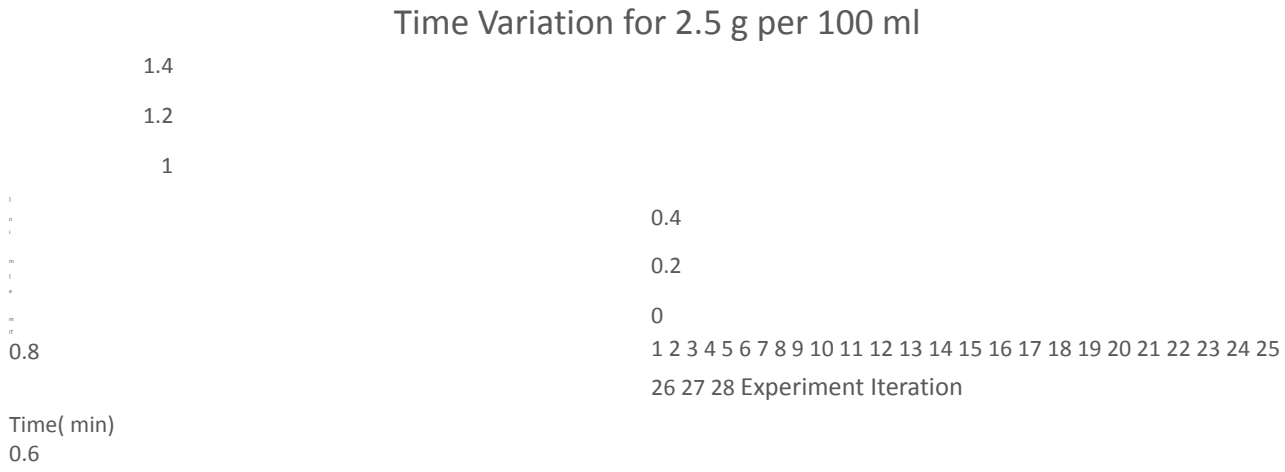
Experiment Iteration	Concentration (g) pH Average pH
1	2.5 7.36 7.347666667
2	2.5 7.49
3	2.5 7.32
4	2.5 7.31
5	2.5 7.28
6	2.5 7.26
7	2.5 7.31
8	2.5 7.29
9	2.5 7.36
10	2.5 6.95
11	2.5 7.74
12	2.5 7.42
13	2.5 7.31
14	2.5 7.29
15	2.5 7.33
16	2.5 7.41
17	2.5 7.09
18	2.5 7.3
19	2.5 7.38
20	2.5 7.29
21	2.5 7.26
22	2.5 7.38

23	2.5 7.17
24	2.5 8.02
25	2.5 7.36
26	2.5 7.42
27	2.5 7.39
28	2.5 7.34
29	2.5 7.28
30	2.5 7.32

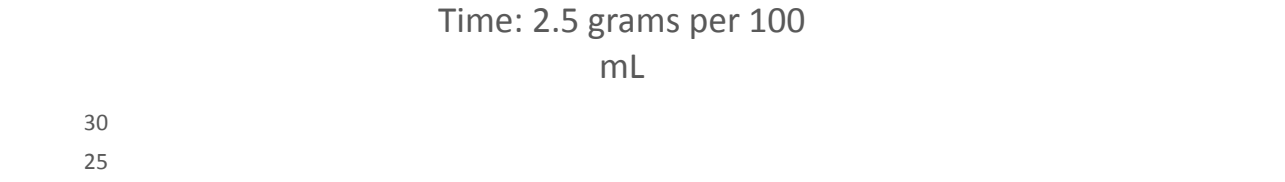
Measuring Efficiency- time taken to neutralize (Graphs) Year One vs. Year Two-Single Hormone Systems

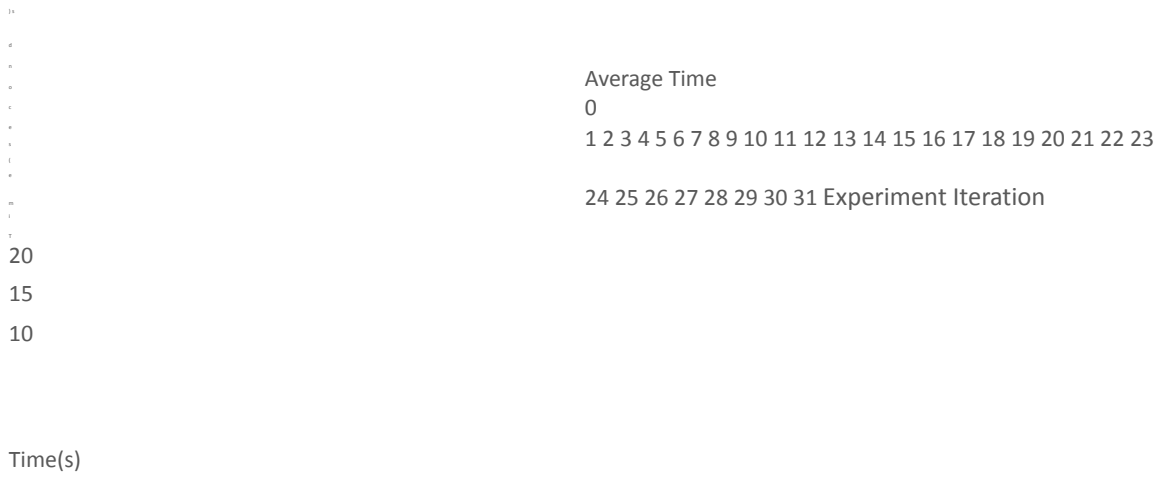
Concentration: 2.5 grams per 100 mL

Year One



Year Two-Single Hormone



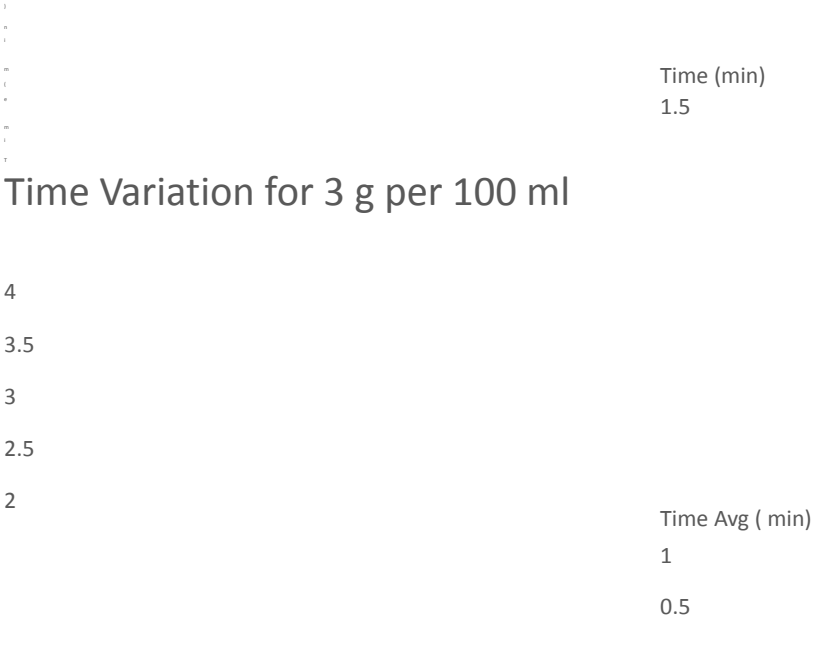


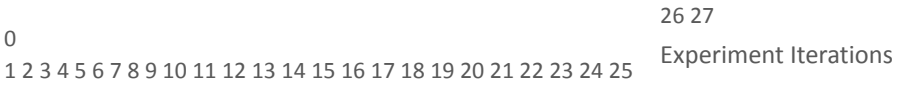
The graphs show the time the system takes to neutralize a 2.5-gram solution. The 2.5-gram solution was the lowest concentrated solution. The year one closed loop

Concentration: 3 grams per 100 mL

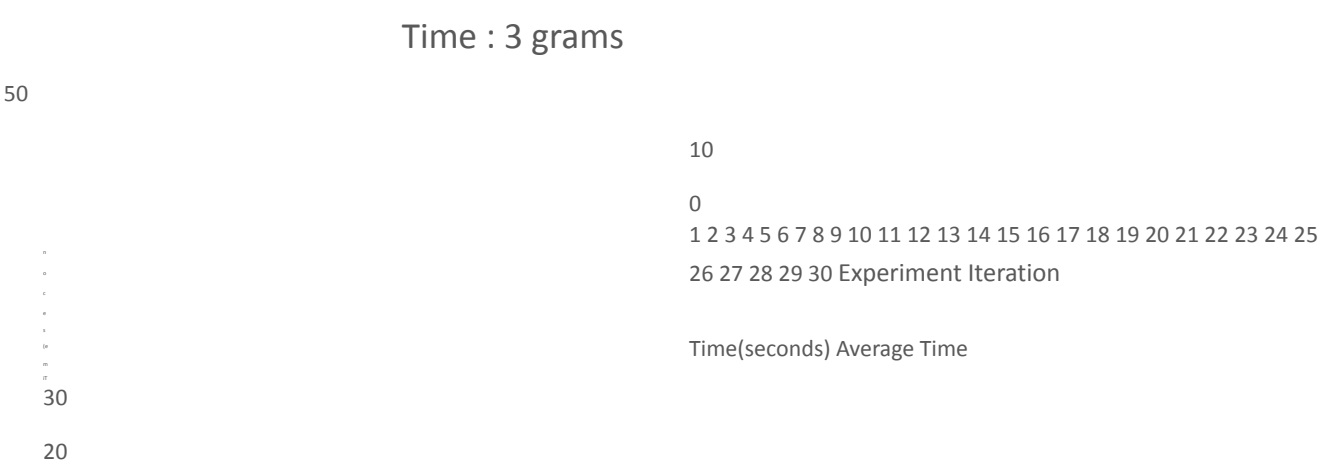
system takes over a minute to neutralize the solution. The year two digital system was far more efficient, neutralizing the solution in about 17 seconds.

Year One





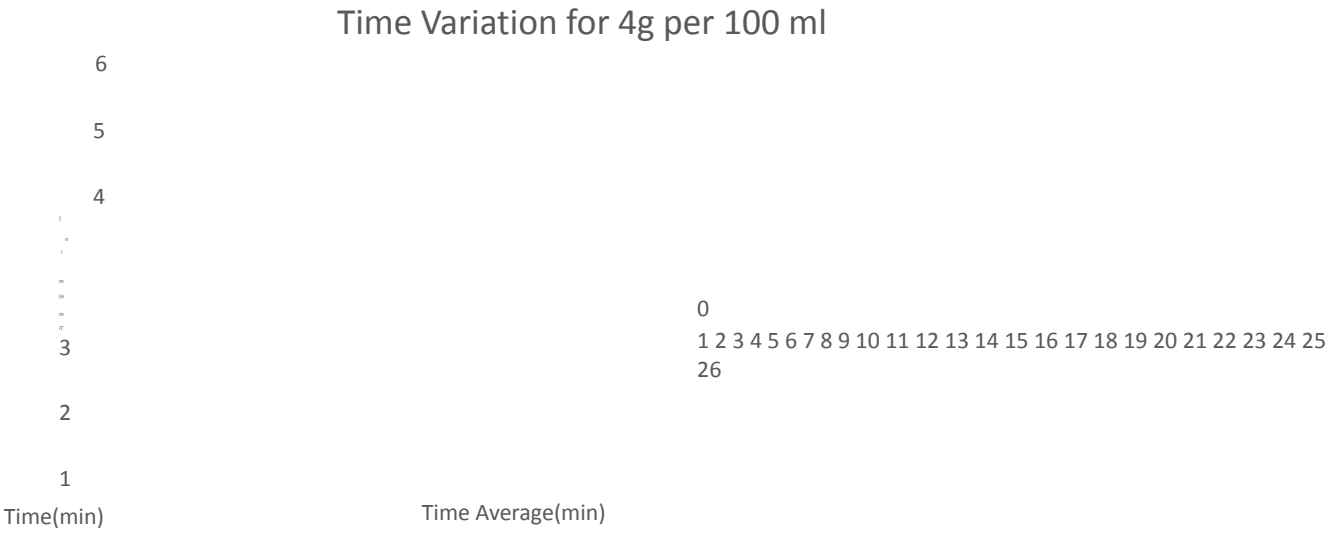
Year Two- Single Hormone



The graphs depict the time taken to neutralize the 3-gram solution. The year two digital system neutralizes the system in just over twenty seconds. The year one closed loop system neutralizes the solution in over two minutes. The year two digital system is extremely efficient.

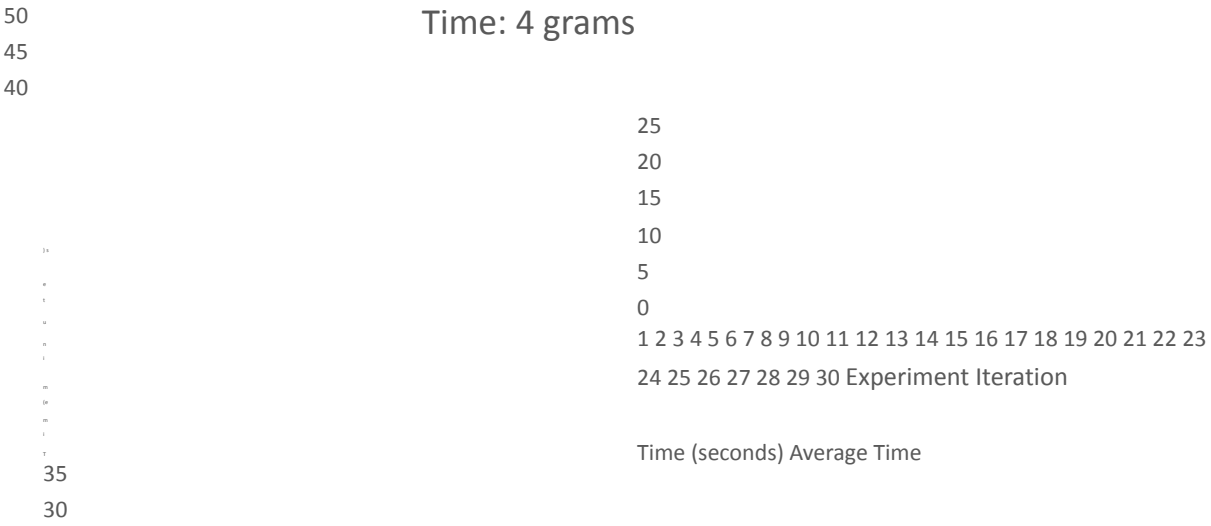
Concentration: 4 grams per 100 mL

Year One



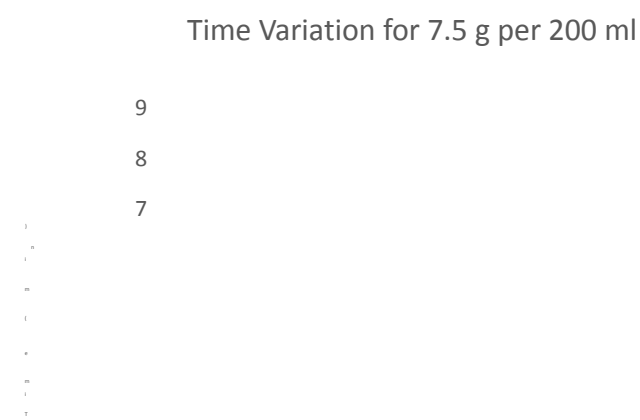
Experiment Iteration

Year Two- Single Hormone



The graphs show the efficiency of both systems when neutralizing a 4 gram-solution. The year two digital system outperforms the year one closed loop system. The average time for the year two digital system is just over thirty seconds. On the other hand, the average time taken for the year one system is over three minutes.

Concentration: 7.5 grams per 100 mL Year One



20

0

Time(min)

Average Time(min)

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17

18 19 20 21 22 23 24 25 26 27 28 29 30 31

32 33 Experiment Iterations

6

5

4

3

2

1

0

Year Two Single Hormone Time(seconds) Average Time

Efficiency of Single Hormone System: 7.5 grams

120

100

80

60

40

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17

18 19 20 21 22 23 24 25 26 27 28 29 30 31

32 33 34 Experiment Iteration

The graphs show the time in which the 7.5-gram solution was neutralized. The graphs depict the efficiency of both systems. Evidently, the year two single hormone system neutralizes the solution more efficiently. The year two system takes an average of under one minute to neutralize the solution. Meanwhile, for the same solution, the year one closed loop system takes

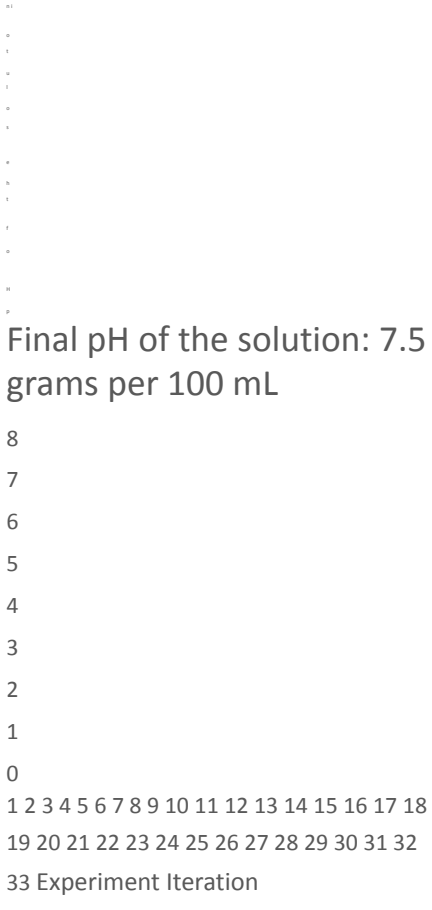
over 7 minutes.

Measuring Accuracy- pH of the solution after neutralization process (Graphs)
Year One vs. Year Two Single Hormone system vs. Dual-hormone System

Target pH range: 7.25-7.5

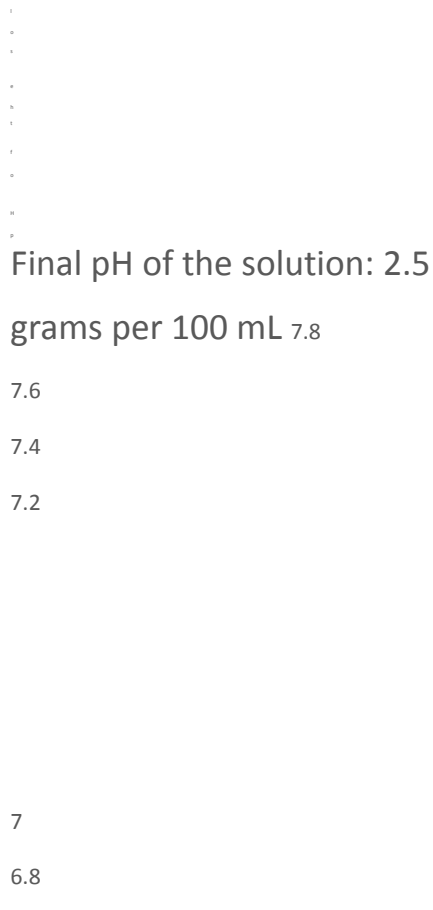
Concentration: 2.5 grams

Year One



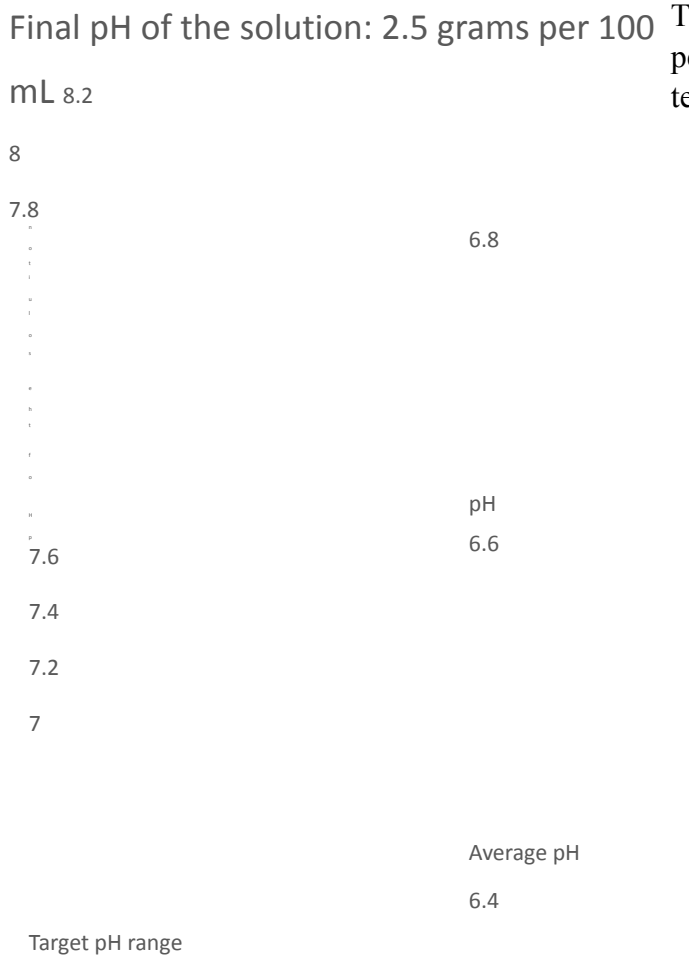
Year Two-Single Hormone

This graph represents the final pH of the solution, when testing the previous year's system with a 7.5 grams solution. The system was not very accurate. The pH dropped to as low as 4. The average pH fails to be within the target pH.



This graph represents the final pH of the solution when testing this year's single hormone system with a 2.5-gram solution. The Year Two single hormone system outperforms the previous year's system. The outliers produced by the system were caused by glitches in the sensor

Year Two-Dual-hormone

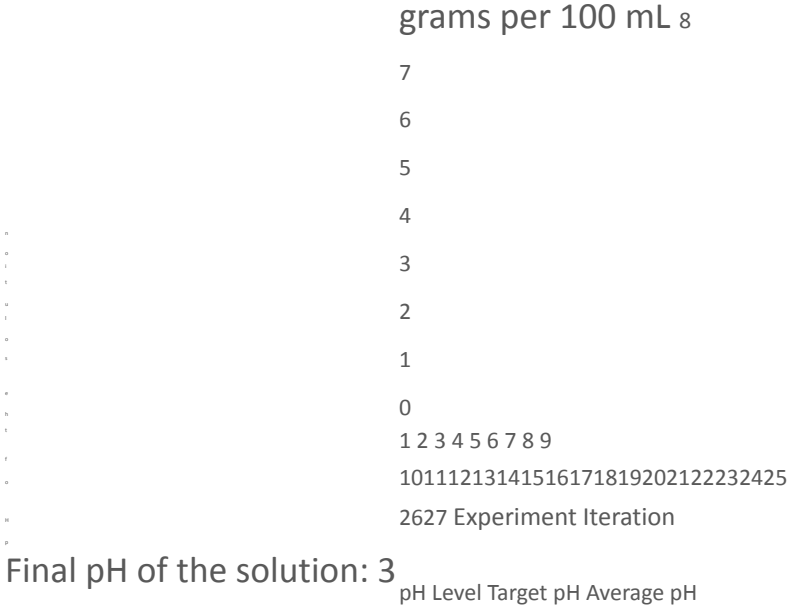


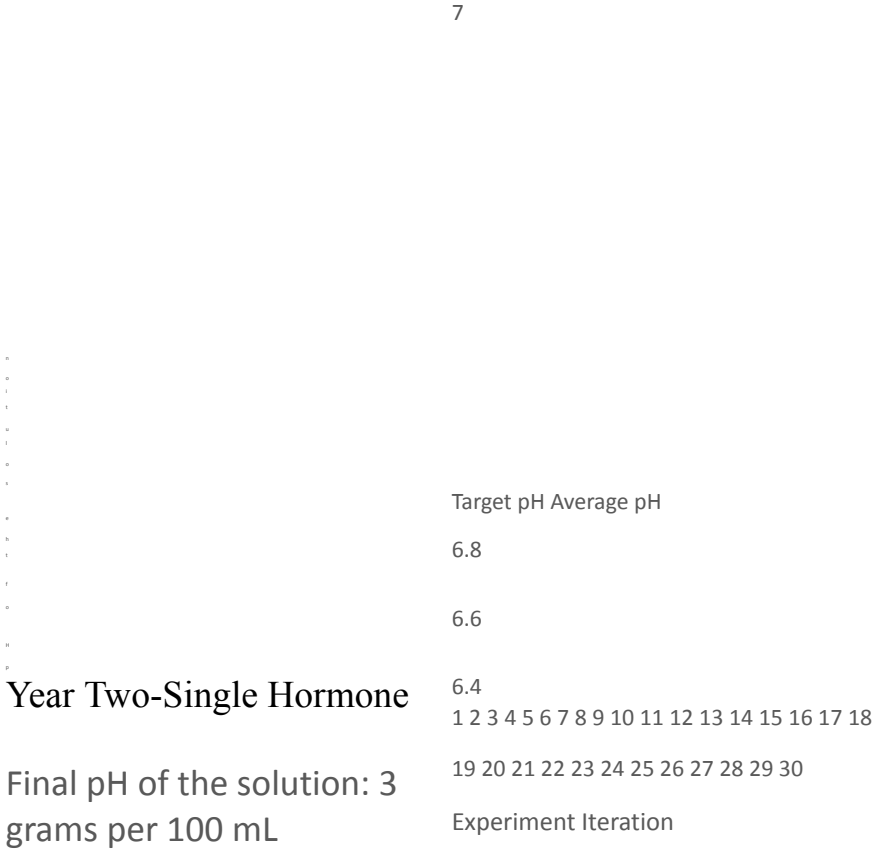
This graph shows the outstanding performance of the dual-hormone system when tested with a 2.5- gram solution. The dual-hormone system outperformed the single hormone and the previous year's system. The dual-hormone Artificial Pancreas' average was within the target range. However, outliers were caused by the sensor's inability to sense low levels of basicity.

Concentration: 3 grams

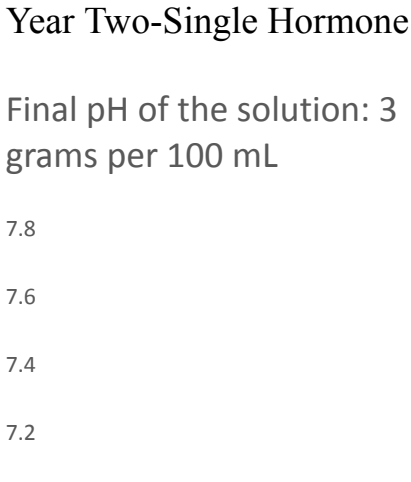
Year One

This graph shows the ineffectiveness of the previous year's system when tested with a 3-gram solution. The spikes, in the graph, highlight the inconsistent neutralization process. The average pH is acidic (pH is below 7) meaning the system was ineffective.





Year Two-Single Hormone

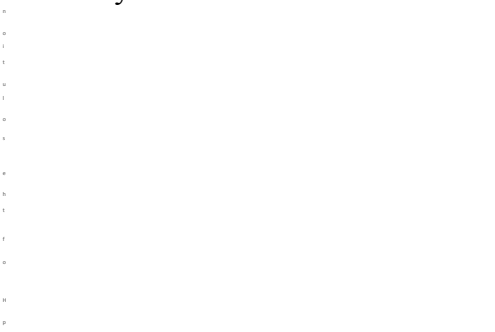


Year Two-Dual-hormone

This graph shows the performance of the single hormone system when tested with a 3-gram solution. The single

hormone system once again outperformed the previous year's system. However, due to the absence of glucagon, often, the solution became acidic. The average pH was within the target zone, displaying the overall effectiveness of the system.

This graph displays the overall performance of the dual-hormone system when tested with a 3-gram solution. When neutralizing a 3-gram solution, the dual hormone system was the most effective system. Due to the inclusion of glucagon delivery, the pH level never dropped beneath or above the target zone. The dual hormone system is extremely accurate.



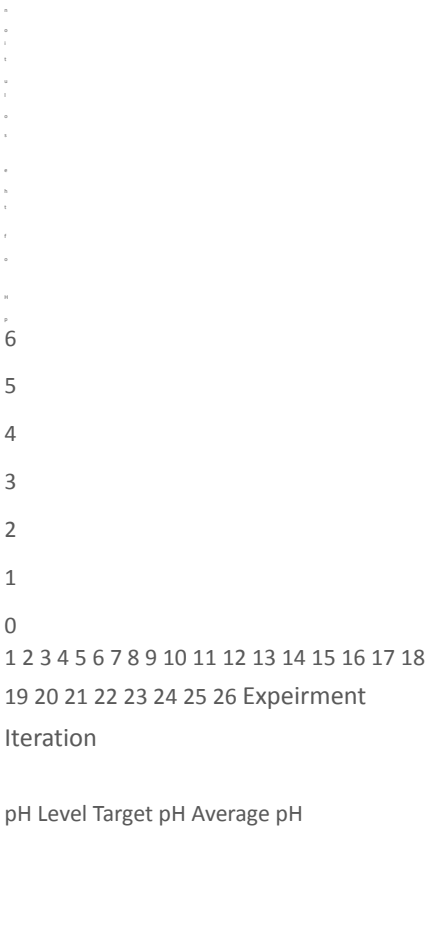
Final pH of the solution: 3 grams per 100 mL

Concentration: 4 grams

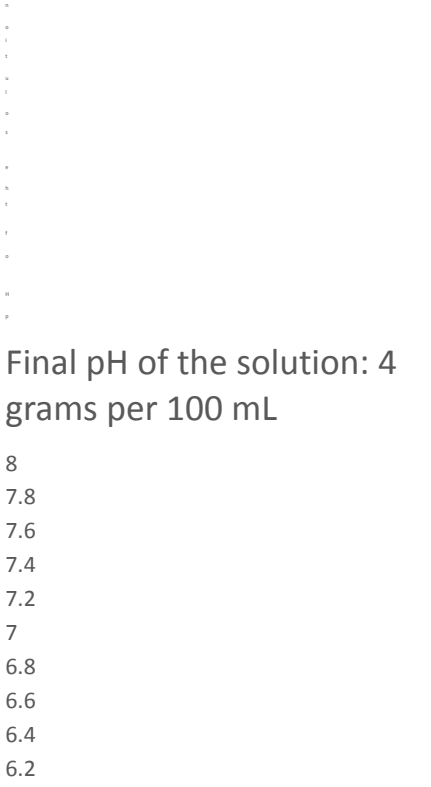
Year One

Final pH of the solution: 4 grams per 100 mL

This graph shows the results produced from the year one system when neutralizing a 4-



However, there were many outliers, where the pH of the solution was not within the target

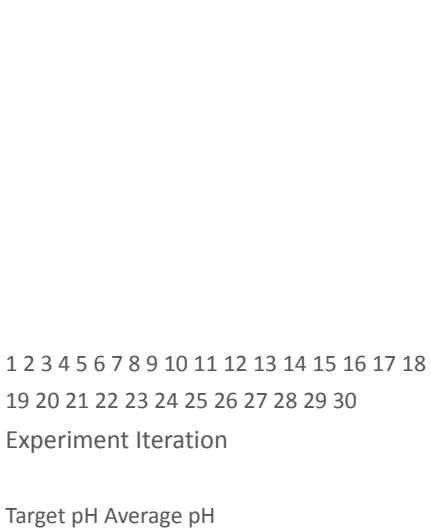


48

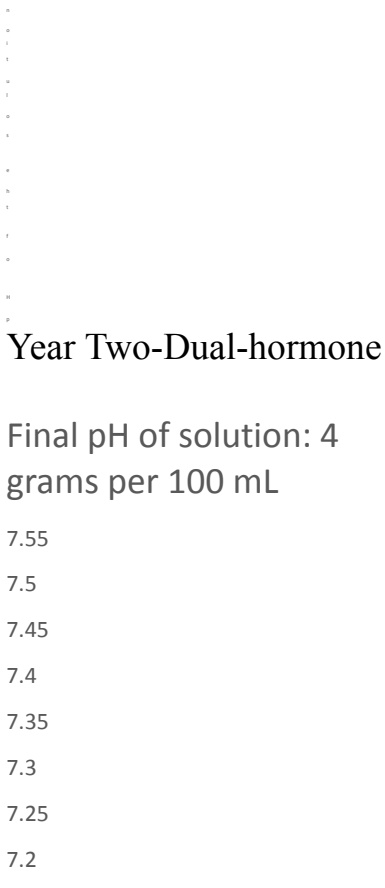
Year Two-Single Hormone

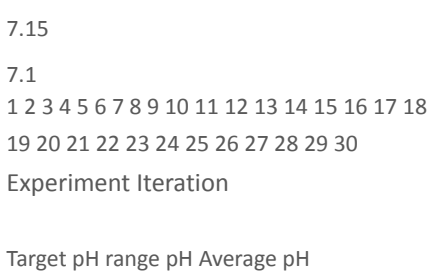
gram solution. The year one system was not very effective. The motor overshot leading to many outliers, particularly obvious from experiment 21 to 26. However, the average pH was close to the target pH range.

This graph shows the final pH of the solution when testing the Year Two single hormone system with a 4-gram solution. The single hormone system performed well. The average pH was also within the target zone.



Year Two-Dual-hormone



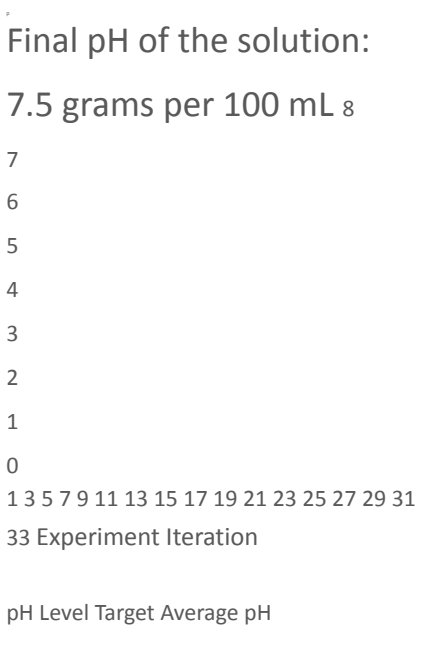


This graph shows the final pH of the solution when testing the dual hormone system with the 4-gram solution. There were no outliers. The pH of every single test (and average pH) were inside the target zone. The dual hormone system outperformed the other two systems.

Concentration: 7.5 grams

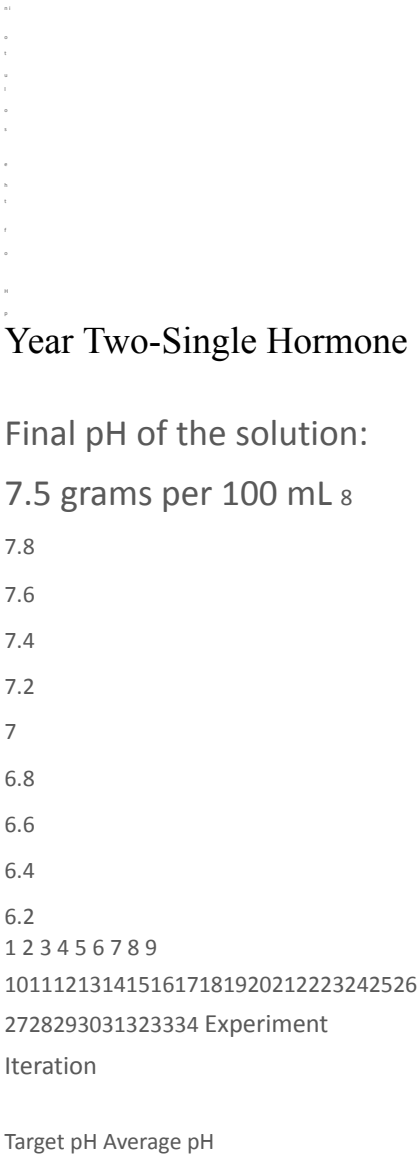
Year One

This graph displays the final pH of the solution when the year one system was tested with a 7.5- gram solution. From test 23 to 33, the pH of the solution is well below the target zone. The year one closed loop system did not effectively neutralize the solution.



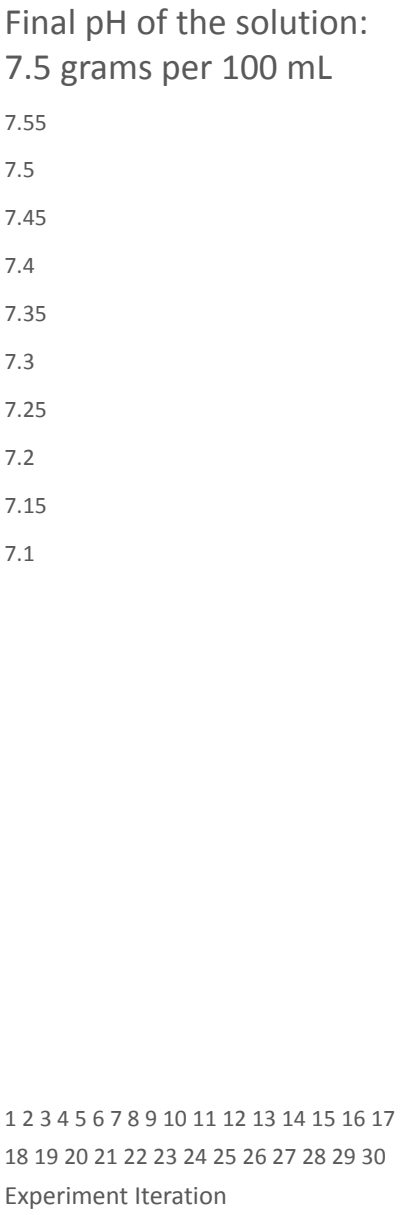
the pH was always inside the target zone. Due to the inclusion of glucagon delivery, the dual-hormone system outperformed the other devices when testing a 7.5-gram solution.

Year Two-Single Hormone



This graph shows the final pH of the solution when testing with the single hormone system. There are many outliers. Often, the motor overshot and sometimes never even turned on. The single hormone system was not very effective with a 7.5-gram solution.

Year Two-Dual-hormone



This graph is comprised of the data collected from the dual hormone's test results with a 7.5- gram solution. In the results, there were not outliers;

Target pH range pH Average pH

Observations

In the Year Two experiment, two different types of integrated, digital systems were engineered. The single hormone system, consisting of only one motor (the insulin pump) was tested with four different concentrations of baking soda per 100 mL of water, 2.5 grams, 3 grams, 4 grams, and 7.5 grams. The dual-hormone system, consisting of two motors (the insulin and glucagon) was also tested with the same concentrated solutions.

In order to increase the efficiency and effectiveness of the system, a completely new and improved system had to be developed. Contrary to the previous year's system, the new and improved Artificial Pancreas were digital, consisting of an Arduino R3 micro-controller. The new digital systems consisted of a more accurate digital pH sensor which replaced the crude conductivity sensor. The main control system was programmed to neutralize the solution, through a code that was developed and uploaded to Arduino.

However, Arduino was unable to directly drive the motors. Arduino's general-purpose input-output (GPIO) pins cannot drive the motor directly as the amperage is limited to only 40 mA. The current required to run the pump motor is much higher than that. Furthermore, turning the motor on and off causes current spikes that can damage the Arduino. After research, the L293D chip was found. The L293D chip can simultaneously drive two motors. The chip provides current driving circuitry for the motor and also protects the Arduino from the current spikes. With the ability to drive one or two motors, the chip would drive the motor's (the insulin

and glucagon pump) based on Arduino's logic. The motors were also connected to 18 volts of supply (two 9-volt batteries) ensuring they would pump the liquid fast, to create a more efficient system.

A target pH zone was created after multiple tests, to create more meaningful results. It was observed that the pH, of the final solution, never corresponded to the target pH, meaning all results were inaccurate. Acknowledging the lack of pinpoint accuracy in the single hormone system, the target range was increased from only pH 7.5 to pH 7.25 to pH 7.5.

In order to neutralize the solution, and have the pH of the solution within the target zone (pH 7.25-7.5), Arduino needed to be accurately programmed. Initially, the code for the single hormone system stated that if the pH was higher than the target zone, which is pH 7.25 to pH 7.5, the motor will pump vinegar at full speed, until the solution's pH is within the target zone. This created problems. It was observed that the sensor lagged, sending delayed information to

Arduino. Due to the lag, the motor overshot, creating an acidic solution. The code was modified. If the solution's pH was further away from the target zone, the motor would run faster. As the solution's pH approached the target zone, the motor would run slower. When the pH of the solution was inside the target zone, the motor would shut off.

Learning from the flaw in the single hormone system's code, the code for the dual hormone system was altered. The insulin (vinegar) pump would be programmed identically to the single hormone system's insulin pump. However, with the inclusion of the glucagon (represented by a basic solution with the ability to raise the pH of the solution) pump, if the pH dropped beneath the target zone, the glucagon pump would need to turn on. The glucagon pump's speed was also scaled accordingly to the pH. If the solution became extremely acidic, the glucagon pump would run faster. When the pH of the solution approached the target zone, the glucagon pump would run slower. When the pH of the solution was in the target zone, the glucagon and insulin pump would shut off.

The single hormone system was tested with four different concentrations. After each result, the pH of the solution, as well as the time, of neutralization were recorded. The time would later be compared to the previous year's module the final pH of the solution would be compared to the dual-hormone system as well as the previous year's module.

During experimentation with the single and dual-hormone system, there were two containers, container A, consisting of baking soda which represents a patient's bloodstream, and container B, consisting of vinegar, which represents insulin. Tests with the dual-hormone system

included container C, consisting of a basic solution, which represents glucagon. For the single hormone system, the time, the pH, and the concentration of the initial solution were recorded. For the dual-hormone system, the pH of the neutralized solution was recorded. The data showed impressive efficiency and effectiveness of the new single and dual-hormone system compared to the previous year's system.

The time, of neutralization, was recorded by a stopwatch. It was observed that as the concentration of the baking soda decreased, the time for neutralization also decreased. As in the previous year's experiment the time, for neutralization, is proportional to the concentration of baking soda in the solution. Compared to the previous year's system, the single hormone system was extremely efficient, neutralizing 7.5 grams of baking soda solution in under one minute. The

previous year's system neutralized a 7.5-gram solution in over 7 minutes. The digital single hormone system was extremely efficient, validating the hypothesis.

Throughout the experiment, the pH of the solution was measured with the digital pH monitor. While measuring the pH of the solution, for the single hormone system, many outliers were observed. The single hormone system only had one motor. If the system overshot, and the solution became acidic, there would be no solution. This shortcoming became a huge flaw. When testing with small concentrations of baking soda, such as 2, 3, and 4 grams the motor would take seconds to neutralize the solution. Often, due to sensor lag, the motor continued to run even after the solution was neutralized, creating an acidic solution.

The single hormone system could neutralize an acidic solution, due to the absence of glucagon delivery, therefore creating inaccurate results. Surprisingly, even with this major flaw, the average pH, of all the tests, for all four concentrations, remained inside the target zone. For 2.5 grams the average pH was 7.37. For 3 grams, the average pH was 7.45. For 4 grams the average pH was 7.42 For 7.5 grams the average pH was 7.44. It was hypothesized that the digital single hormone system was to be more effective and efficient than last year's closed-loop system. It was observed that digital single hormone system was more effective and efficient than last year's closed-loop system, validating the hypothesis.

The dual-hormone system was also tested with the same four concentrations of baking soda. The dual-hormone system was created to improve the accuracy of the system. It consisted of a glucagon pump which can neutralize an acidic solution, removing inaccurate results. When testing with the dual-hormone system, only the final pH of the solution was recorded. It was

hypothesized, based on previous research, that less than 3 results would occur where the pH was underneath the target zone. Throughout testing, the dual-hormone system accurately neutralized all types of solutions. When the motor overshot, and the pH would drop below the target zone, the glucagon pump always turned on. The pH was raised until inside the target zone. Once the pH was inside the target zone, both motors shut off.

The dual-hormone system was most accurate when testing with the 7.5-gram, 4-gram, and 3-gram baking soda- solution. There were no outliers, and the system was extremely consistent. However, the system especially struggled when tested with the 2.5-gram solution. Often, the glucagon failed to turn on, even when the solution became acidic. The pH of the solution dropped beneath the target zone three times, the pH, of the acidic solutions were 7.17,

7.09 and 6.95. Furthermore, due to the low concentration of baking soda, the pH sensor glitched. The pH sensor's glitches also caused the glucagon pump to overshoot, leading to a basic solution. Overall, the dual-hormone system outperformed the single hormone system.

Conclusions

In this year's experiment, a digital dual and digital single hormone Artificial Pancreas were constructed to effectively neutralize a baking soda solution, which represents a patient's bloodstream. **Based on previous research, it was hypothesized that the Dual-hormone**

Artificial Pancreas will accurately neutralize, the pH, of the solution more than 95 percent of the time; the pH will be in the target zone more than 95 percent of the time. It was further hypothesized, due to the inclusion of glucagon delivery, that the final pH of the solution will drop beneath the target zone (pH of 7.25-7.5) three times or less out of 120 tests. The digital system will also neutralize more effectively and efficiently due to better digital control provided through Arduino and the digital pH sensor. The dual-hormone system and the single hormone system were both tested with four different concentrations of baking soda.

The hypothesis was validated by experimentation. Out of 120 tests, only three times the pH of the solution was not below the target zone's lower threshold (pH 7.25). Meanwhile, the average pH for all concentrations, for both the single and dual-hormone systems, were inside the target zone. Compared to the previous year's results, both digital systems outperformed the analog closed loop system. The digital systems neutralized solutions more effectively and efficiently.

The dual-hormone system was able to neutralize the solution 95 percent of the time, due to the inclusion of glucagon delivery. If the motor overshot, and the solution became acidic, the glucagon would turn on and neutralize the solution again. The single hormone system produced less accurate readings because it did not have glucagon delivery. Glucagon delivery was successful and had the ability to raise the pH level. The glucagon pump delivered a basic solution. Adding basicity to an acidic solution leads to neutralization. Therefore, turning on the glucagon pump, when the solution is acidic, will neutralize the solution. The effectiveness of the glucagon pump is shown by the results.

The use of the digital pH sensor removed errors previously caused by the conductivity sensor used in the last year's experiment. When testing with the digital pH sensor, the readings were accurate and stable, leading to better results. Unlike the previous year's conductivity sensor, the digital sensor was not corroded by acid. The digital pH sensor accurately performed well in all concentrations except for 2.5 grams. For this concentration, the pH sensor lagged and

was unable to sense basicity in this concentration. When the motor pump vinegar into the solution, the sensor was unable to detect change. The motor continued to pump vinegar at a fast rate, leading to an acidic solution. On three occasions, when the motor overshot, and the solution became acidic, the digital pH sensor was unable to detect acidity in the solution. This also led to inaccurate results.

The increased input voltage helped the digital systems outperform the previous year's experiment. Instead of 12 volts, 2 9-volt batteries, or 18 volts were applied to the motor(s). Applying more volts increased the speed of the motor(s), leading to a more efficient system. The solution became neutralized faster.

The creation and implementation of Arduino's code made the digital systems more accurate. Instead of relying on resistors, a MOSFET, potentiometers, and other analog electronic devices, this year's experiment used computer programming to ensure the solution's pH remained inside the target zone (pH 7.25-7.5) at all times. To prevent the motor from overshooting, the speed of the motor was adjusted according to the current state of the pH. If the pH was relatively distant from the target zone, the motor was driven relatively faster. If the pH was closer to the target zone, the speed of the motor was relatively slower. With the assistance of the digital pH sensor, Arduino allowed the pH of the solution to control the motors. Once the pH was inside the designated target zone, the motors stopped. If the motors overshot, the code instructed Arduino to turn on the glucagon pump which would neutralize the solution. Due to the program created for Arduino, the digital systems were more accurate.

If this concept could work would real-life subjects, the system could become commercial and help many people. Patients suffering from extreme hyperglycemia or hypoglycemia would require rapid insulin or glucagon delivery. Once the patient's blood glucose levels were normalized the glucagon and insulin pumps would shut off. This system could help treat Type 1 Diabetes, globally.

Recommendations

All of the previous year's recommendations were made. A more accurate digital pH sensor was used. The system was converted from analog to digital using Arduino. A more integrated motor control system was used to ensure the increased efficiency of the peristaltic pumps

Throughout experimentation, the dual-hormone system was effective and efficient.

However, when testing with the 2.5-gram solution, multiple errors occurred. The sensor caused these errors. Unable to sense the low levels of basicity, the sensor sent incorrect information to the main control system (Arduino) causing the motor to overshoot. Even after the pH of the solution was within the target zone, the motor continued to run, causing the solution to become extremely acidic. Arduino's algorithm did not account for extremely acidic or basic solutions. The insulin pump continued to run until the container overflowed. The sensor's lag created inaccurate results. To improve results, a better, larger sensor should be used. A larger sensor would detect the average pH across the entire container. A better sensor would help eliminate lag and it would help create a more accurate Artificial Pancreas.

The dual-hormone system, consisting of an insulin and glucagon pump, was only tested with an initial basic solution. The system's full capacity was not tested. The dual-hormone system could have been tested with an initial acidic solution, to prove its functionality with low and high blood glucose levels.

The Artificial Pancreas should be more mobile. If the system were powered through a mobile phone, it would be extremely convenient. A notification pushed out through the mobile phone could alert the patient if blood glucose levels became abnormal. The device would then take the necessary steps needed to normalize the blood glucose levels. The Artificial Pancreas could travel with patients, constantly monitoring their blood glucose levels and ensuring their blood glucose levels remained within a healthy range at all times.

Bigger containers should be used when experimenting. Bigger containers would better resemble the inconsistency of blood glucose levels in the human bloodstream. After testing with bigger containers, the system could be further refined to be tested with actual patients. The combination of a more integrated sensor, a more convenient and mobile Artificial Pancreas, and the use of bigger containers would create a more effective Artificial Pancreas that could eventually be used clinically and commercially across the world.

Appendix

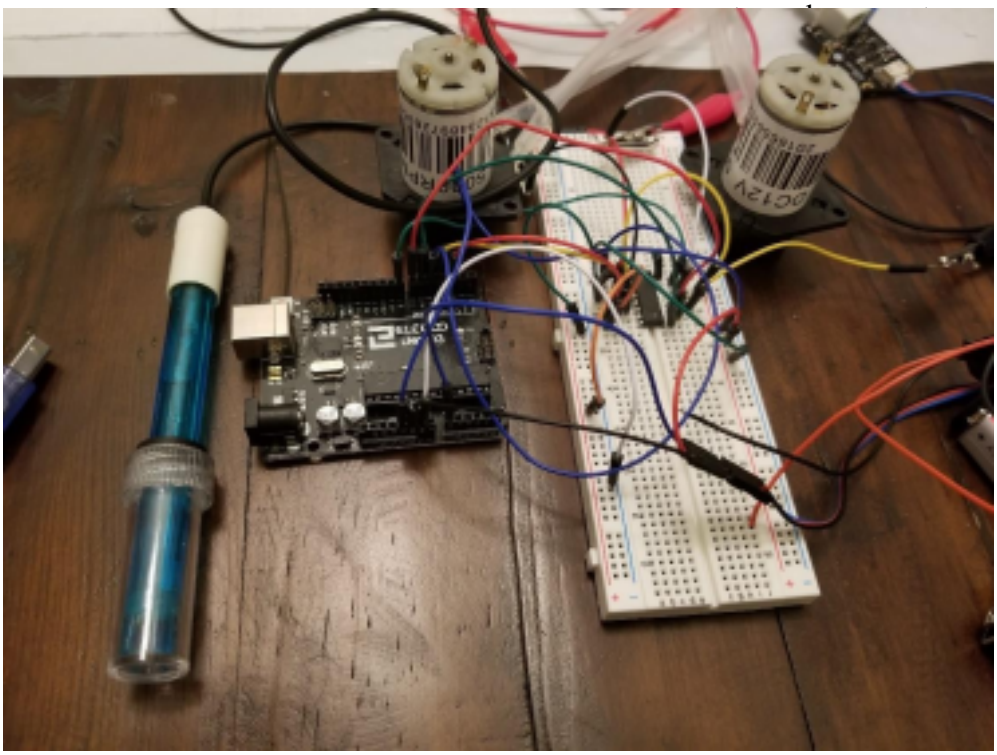
This picture shows the glucagon pump along with the basic solution. The glucagon pump was used to raise the pH of the solution, if the solution became acidic. The glucagon pump was

only used when testing with the dual-hormone system.

This is a picture of the insulin pump along with vinegar. When the solution was basic, the insulin pump turned on and vinegar was used to lower the pH of the solution. The insulin pump was used when testing with the dual and single hormone system.

This picture shows the digital pH sensor inside of the baking soda solution. The digital pH sends the pH of the solution to Arduino. Throughout experimentation, the digital pH sensor was

This picture shows the entire dual-hormone system. It shows the breadboard, the insulin and glucagon pump, the pH sensor, and most importantly the Arduino chipboard. These components collaborate with Arduino's code to create an effective dual-hormone Artificial Pancreas.



Digital Single Hormone Artificial Pancreas Code

```
#include <TimerOne.h>

int M1_PWM=5; //define pin on Arduino board

const float min_ph=7.3; //constants will remain constant
throughout  experimentation

const float max_ph=9.9;

const float target_ph=7.5;

int M1_D1=4; //establishing the direction of the

motor int M1_D0=3;

const int ph_sensor_pin=0;

float PWMMOTORvalue; //speed of insulin pump
```

```
float ph_value;
float voltage;
void setup () { //initialize pin modes, inputs and outputs
pinMode(M1_PWM, OUTPUT);
 pinMode(M1_D1, OUTPUT);
 pinMode(M1_D0, OUTPUT);
 digitalWrite(M1_D0, 0);
  digitalWrite(M1_D1, 1);
 Serial.begin(9600);
 Timer1.initialize(1000000/5);
 Timer1.attachInterrupt(callback);
}

void callback () {
 voltage=analogRead(ph_sensor_pin)*5.0/1024;//scaling the pH value
accroding to the voltage
 ph_value=voltage*3.5;//finding the pH value
 Serial.println(ph_value); // for troubleshooting
 if (ph_value>=target_ph)// if the solution is basic
```



```
 {
 PWMMOTORvalue=150+(ph_value-target_ph)*(255-150)/(max_ph
min_ph);//if the solution is extremely basic the motor's speed
will  increase
 analogWrite(M1_PWM, PWMMOTORvalue);//if the solution's pH becomes
closer to the value of the target pH the motor's speed will decrease
 Serial.println(PWMMOTORvalue);//print PWM value, for
troubleshooting (above 150, which is the minimum threshold that
the  motors runs, below 255, which is the max value for PWM)
 }
 else // if the solution is not acidic Arduino will skip the
directions above
 {
 analogWrite(M1_PWM, 0);// when the pH of the solution reaches the
target pH, the motor will shut off
```

```
 }
}
 void loop() {
}
```


Digital Dual-Hormone Artificial Pancreas Code

```
#include <TimerOne.h>
int M1_PWM=5;
int M2_PWM=6;
const float min_ph=7.3;
const float max_ph=9.3;
const float target_ph=7.5;//upper threshold
const float min_phgluc=3;
const float max_phgluc=7.25; //lower threshold, establishing
target  zone
const float target_phgluc=7.25;
int M1_D1=4;
int M1_D0=3;
```

```
int M2_D1=7;
int M2_D0=8;
const int ph_sensor_pin=0;
float PWMMOTORvalue;
float ph_value;
float glucagonpump;
float insulinpump;
float voltage;
void setup () {
 pinMode(M1_PWM, OUTPUT);
 pinMode(M1_D1, OUTPUT);
 pinMode(M1_D0, OUTPUT);

 pinMode(M2_PWM, OUTPUT);
 pinMode(M2_D1, OUTPUT);
 pinMode(M2_D0, OUTPUT);
```



```
 digitalWrite(M1_D0, 0);
 digitalWrite(M1_D1, 1);

 digitalWrite(M2_D0, 1);
 digitalWrite(M2_D1, 0);
 Serial.begin(9600);
 Timer1.initialize(1000000/5);
 Timer1.attachInterrupt(callback);
 }

void callback () {
 voltage=analogRead(ph_sensor_pin)*5.0/1024;//scaling the pH value
accroding to the voltage
 ph_value=voltage*3.5;//finding the pH value
```

```
 Serial.println(ph_value);

 //analogWrite(M2_PWM, 200)

 if (ph_value>=target_ph)// if the solution is basic, the following
steps will be followed, this part is identical to the single
hormone's  system, the insulin pump's logic

 {

 PWMMOTORvalue=150+(ph_value-target_ph)*(255-150)/(max_ph
min_ph);//if the solution is extremely basic the motor's speed
will  increase

  analogWrite(M1_PWM,  PWMMOTORvalue);//if  the  solution's  pH  becomes
closer  to  the  value  of  the  target  pH  the  motor's  speed  will
decrease,  prevents the motor overshooting

 Serial.println("insulin"); //inform what pump is on

 Serial.println(PWMMOTORvalue);//print PWM value, for
troubleshooting

 }

 else if(ph_value<=target_phgluc) //if the pH value is less than the
lower threshold
```



```
 {

 PWMMOTORvalue=150+(target_phgluc-ph_value) *(255-150)/(max_phgluc
min_phgluc);//scaling the glucagon pump

 analogWrite(M2_PWM, PWMMOTORvalue); //the PWM value, or the speed
of the glucagon pump will depend on the pH of the solution

 Serial.println ("glucagon"); //inform what pump is running

Serial.println (PWMMOTORvalue); //troubleshooting

 }

 else //if the pH is not below or above the target zone, the pH is
inside the target zone

 {


 analogWrite(M2_PWM, 0); //the glucagon pump is shut

off  analogWrite(M1_PWM,0); //the insulin pump is shut

off

 Serial.println("neutral"); //included so the timer can stop when
this message appears, the pH of the solution is within the target
```

```
zone
 Serial.println(PWMMOTORvalue); //should appear as less than 150,
meaning the motors are switched off,



}
void loop()
}
```