SenseNC

VAES
North Carolina State University

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Summary

We are SenseNC, the team representing North Carolina State University and the USA. We are an interdisciplinary team of engineers working together to design, model, and market a biosensor to detect valproic acid.

The biosensor we have modeled possesses a multilayered enzyme electrode for detection of free valproic acid in human serum. The serum sample containing bound and unbound VPA will travel through an outer membrane layer to the first enzyme layer. After reacting, the resulting product will travel through another membrane to the second enzyme layer and then oxidized into hydrogen peroxide. Current will be measured from the hydrogen peroxide product. The current measured will be plotted against initial concentrations of VPA to determine the initial free VPA concentration in any serum sample. An impedance measurement will also be taken to determine the concentration of bound VPA. A lookup table, containing experimentally measured values, is then consulted to estimate the concentration of the bound VPA. The accuracy of our impedance measurements is +/- 0.2% within the frequency range of 10 Hz to 20k Hz. While our low power potentiostat circuit can expect a typical current measurement of 80 pA, which is much lower than our expected current measurements.

Biosensor System and Assay

Molecular Recognition and Assay Reagents

The molecular recognition is accomplished by diluting a serum sample such that all bound VPA in the sample becomes free. After dilution, the solution will be placed on an electrode with a surface area of 1cm^2 where two sequential, enzymatic reactions will take place: 1) acyl coenzyme synthase (ACAS) and 2) acyl coenzyme A oxidase (ACAO). Each enzyme reaction takes place within a membrane isolated layer. The outer membrane separates the added sample from the first immobilized enzyme, ACAS. Here the VPA will undergo a reaction with ACAS to be converted into Acyl-CoA [3].

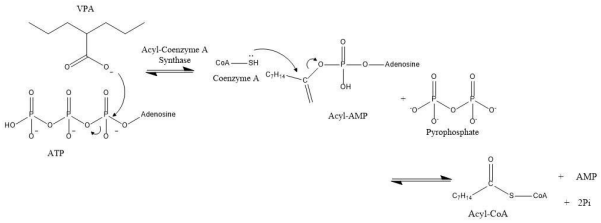


Figure 1: *Reaction Scheme for Valproic Acid Reacting with Acyl-Coenzyme A Synthase*

The reaction products will then travel through a semipermeable membrane to the ACAO layer where the second enzyme reaction takes place. Acyl-CoA reacts with ACAO to produce Enoyl-CoA and Hydrogen Peroxide [3].

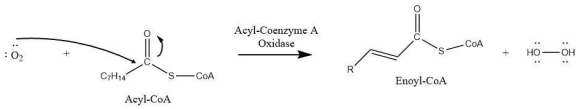


Figure 2: *Reaction Scheme for Acyl-CoA reaction with Acyl-Coenzyme A Oxidase*

The hydrogen peroxide produced from this reaction is a crucial component of the biosensor recognition, as the current generated is proportional to the amount of total VPA utilized in the enzyme reaction.

The current is measured from the electrons in the hydrogen peroxide. The Cottrell equation was used to calculate the current generated with respect to time as the reactions proceed. The produced current can then be transduced to determine the initial VPA concentrations.

$$i = \frac{nFAc_j^0\sqrt{D_j}}{\sqrt{\pi t}}$$

Figure 3: *Cottrell Equation used for Plotting Current vs. Time*

Physical Transduction

Due to interactions between VPA and blood plasma proteins, a large portion of total VPA can be in a bound state [4]. In order to accurately measure valproic acid, the amount of bound VPA must be taken into account when measuring total VPA. The concentration of bound VPA is measured using impedance spectroscopy and total VPA is found using a 3 electrode potentiostat. From these, the amount of total VPA is back calculated. This general process is shown in *Figure 4*.

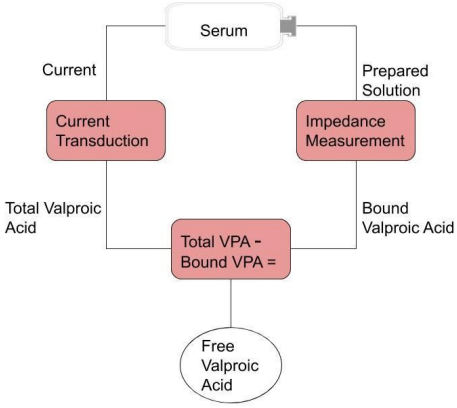


Figure 4: *Using the transduction and measurement, the two results can be subtracted to find the free valproic acid.*

A small current is produced from the detachment of free electrons in hydrogen peroxide produced by the second enzymatic reaction as described in the previous section.

The magnitude of produced current is directly proportional to concentration of free valproic acid. Working, reference, and counter electrodes are present in solution during this reaction. The working electrode is potential-controlled and where current measurement occurs, the voltage output of this electrode is measured in reference to the reference electrode which has constant potential and no current flow. The counter electrode passes current, completing the circuit and allowing measurement to take place.

An AC voltage of varying frequencies is then swept across the remaining serum to determine impedance. The impedance we measure will then be correlated to a concentration of bound VPA, from a lookup table in our software. The lookup table will contain concentrations of bound VPA with a specific impedance value. To determine the impedance of the serum, a voltage with known peak-to-peak value is applied across a known resistance value which produces a current through the resistor. The current through the resistor is measured by the AD5941, the same voltage is applied to the sample and the current produced is measured. The two currents and known resistance value is used to determine the impedance of the serum as described in *Figure 5.*

$$|Zunkown| = \frac{IrcalMag}{IzunknownMag} \; x \; Rcal$$

Figure 5: *AD5941 impedance calculation.*

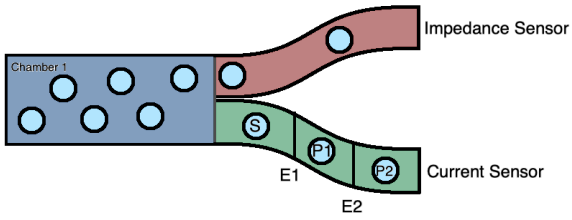
Finally, to calculate the free Valproic acid we subtract the concentration of bound from total Valproic Acid.

Cartridge technology

A blood sample from a finger prick will be the main input. Next the sample will be introduced to the reaction chamber one. In this chamber VPA is diluted and then the sample is split into two separate paths.

The first path is where our impedance sensor is used, allowing us to find the concentration of bound serum albumin. The second path contacts our electrode where enzymatic reactions occur.

Figure 11: *Fluidic Cartridge.*



Reader Instrument and User Interaction

The user will interact with an LCD screen on a handheld-size device that will be controlled with a thumbwheel and two buttons. The buttons, thumbwheel, and LCD screen will be built onto the MSP430 microcontroller running C code that is optimized for low power consumption. Additionally, an IOT module connected to the MSP430 can send the user data through WiFi across a preselected baud rate.

The user interface on the device will have 4 menu options, as shown in *Figure 6*: Display for the amount of Valproic Acid in the sample, 2) Debugging tool for our team, 3) Network screen for local wifi, 4) Display detailed measurements of the latest models.

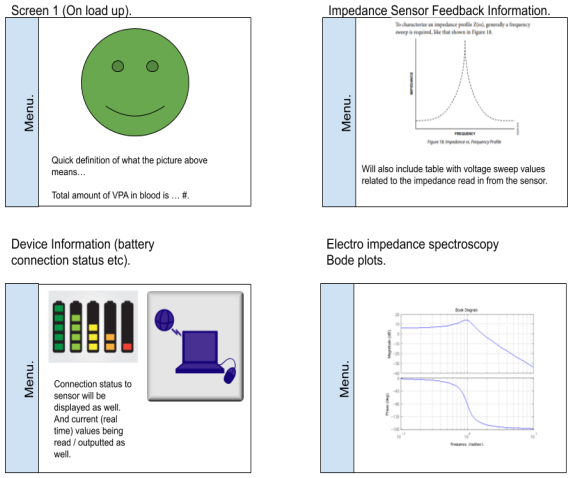


Figure 6: *User Interface.*

Technological Feasibility

Molecular Recognition

First, we modeled the two enzyme reactions mentioned previously (Acyl Coenzyme Synthetase and Acyl Coenzyme Oxidase reactions) using Michaelis-Menton enzyme kinetics. These two reactions were commonly referred to as Enzyme Reaction 1 (E1) and Enzyme Reaction 2 (E2). The constant values (max velocity (Vmax), turnover number (Kcat) and Michaelis constant (Km)) vary with substrate and were obtained from literature [1,2,5,6]. For E1, the constants used were for nButyric Acid in place of Valproic acid and for E2 the constants used were for butyryl CoA as an estimate for Valproyl CoA. These assumptions were made to base the calculations of the reaction rates for our designed biosensor. In a lab setting, we would have been able to calculate the constants for VPA and Valproyl CoA experimentally and use that data to base the rest of our calculations.

Table 1 shows the table of constants used to calculate the rates of E1 and E2, specifically for a starting concentration of 300µg VPA/mL . Modeling allowed us to tune the initial enzyme concentration to achieve desired operating parameters - the initial enzyme concentration for E1 and E2 are 100x and 50x less, respectively, than the maximum substrate concentration.

Table 1: *Michaelis-Menten Constants for a Starting Substrate Concentration of 300µg/mL*

	E1 - nButyric acid	E2 - Butyryl COA
Km	0.32 mM	131.9 uM
Kcat [1/s]	15.04	0.225
Km [mol/cm^3]	3.20E-08	1.319E-07
Vmax [mol/s*cm^3]	3.12832E-07	9.36E-09

Using the calculated reaction rates we were able to determine the amount of product produced from the E1 and E2 reactions. The biosensor is designed so that the E1 reaction will run to completion (60 seconds) and subsequently the E2 reaction will run until there is approximately 10% consumption of the starting substrate (20 seconds). After the E2 reaction has run for 20 seconds, the reaction will be stopped and the sensor will be turned on to measure current produced. The Cottrell equation was used, with the assumption that E1 and E2 reactions are diffusion limited, to calculate the current generated from the products of the E2 reaction versus time after the sensor has been

turned on. *Figure 7* shows the cottrell plot for seven different starting concentrations of VPA.

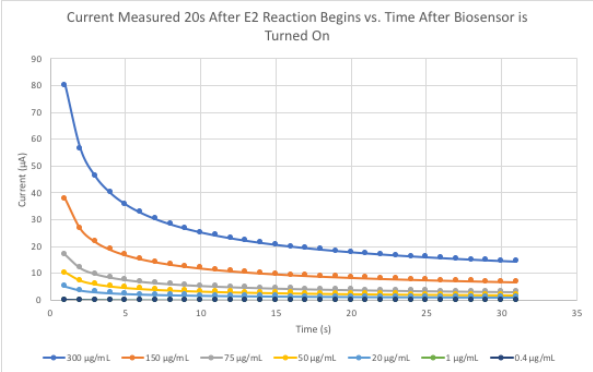


Figure 7: *Current Measured from E2 Products Versus the Time After the Biosensor is Turned On*

As seen in *Figure 7*, large, decaying slopes are present initially with higher starting concentrations of VPA due to an increased enzyme reaction rate. Because of this, our sensor design includes a 20 second stabilization period in order to measure currents not drastically changing and better fit our calibration plot as shown in *Figure 8.*

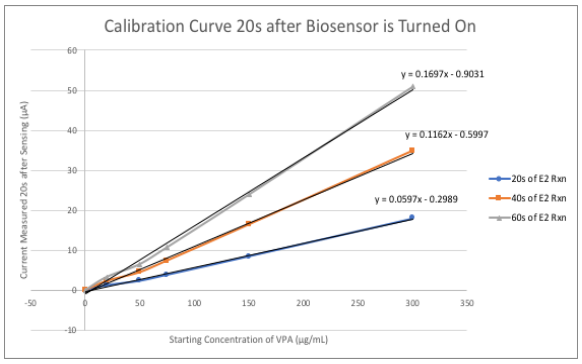


Figure 8: *Calibration Curves when E2 is Ran for 20, 40, and 60s*

Figure 8 shows the calibration curves we will use to back calculate concentration from measured current in our sensor.

Physical Transduction

Producing an exponentially decaying current vs. time graph *(Figure 9*) was achieved by creating a 0.1V potential difference between two electrodes. The potential difference allowed electrons to move from a higher voltage to a lower voltage. The current range we expected to measure was 0.1-10 µAmps. Once a current is measured we used the Cottrell equation to determine the concentration of total valproic acid in the serum.

This process requires the device to be a steady state before we can accurately measure the current. If there is any feedback, we can read negative values and jeopardize the accuracy of the system. If we apply the same potential difference over a long period of time, we will begin to read negative feedback.

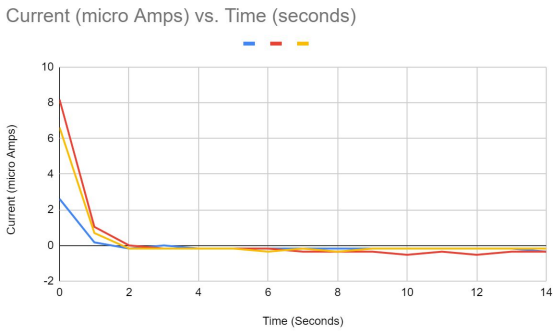


Figure 9: *Exponentially decreasing Current vs. Time graph.*

To measure the impedance of our serum we applied a voltage with a small range of frequencies across the system. The range of frequencies we used correlated to the greatest change in impedance values we measured from the AD5941. This drastic change in impedance value allowed us to get the most response from the serum. Once we measure the impedance, with correlating frequencies, we will use a lookup table to estimate the concentration of bound VPA.

Our main concern with this system design is that we were unable to develop the lookup table mentioned above. Another disadvantage is the need to estimate a concentration of bound VPA from previous samples, tested in a lab environment.

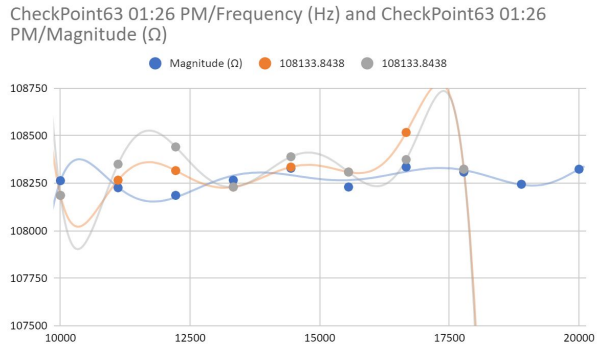


Figure 10: *Impedance vs. Frequency graph.*

Fluidic Cartridge

The fluidic cartridge contains several chambers, which will add the length/ width of the device. The separation of the two different samples will also add to the complexity of the microfluidics of the device.

Based on limited testing it is unknown to how our device will perform with the multi chamber system. In the future we will have the ability to create a physical system and test the usability/accuracy.

Reader Instrument

Developing a hand held model will allow for easier storage/use. Our user interface is simple and has an intuitive design. Interaction with the device is limited to a thumbwheel and buttons.

A downfall to this design is the screen size and use of simple methods of receiving input from the user. It would be more optimal to interact with a touch screen and have a larger screen to read from. But, the small screen design allows for a slimmer fit.

Originality

Team Section

The novelty of the VAES is the use of the double enzyme reaction to quantify the amount of free VPA in human serum. We had discovered both the acyl-CoA synthase and the acyl-CoA oxidase reactions after performing extensive research on biosensors that detect the presence of fatty acids. We thought that, with VPA being a short chain fatty acid, we could apply it to the same reactions being used in those biosensors with adjustments. With the information gathered from the team's research, we recognized the ability to use the combination of the two enzymes in our biosensor. The VAES sensor represents the interaction and reaction between the blood serum and these acyl-CoA enzymes together to produce interpretable data about the targeted protein, VPA. The synchronous timing between these two reactions in our biosensor allows for the further innovative development of the VAES. When testing our ideas by performing calculations of simulated experiments, our team was able to see the dependability of the two enzyme reactions on one another. On the transduction and usability for the consumer side of the sensor we are using a single device to measure both the impedance and current of our serum. The ability to rely on a single analog front end will allow us to create a slimmer device for easier storage. Our ability to send users information via bluetooth is also a radical inclusion into our system. This way the user is able to bring their results with them on their cellular device.

Team Supervisor Section

Valproic acid is a very difficult analyte to sense in a portable or point-of-care system. First, the parameter to quantify is “free” VPA, which would require complex separation systems. To overcome this challenge, the team developed a differential sensing technique. ***To quantify free VPA, the total VPA concentration and the amount of binding proteins (e.g. serum albumin) will be measured, and from these values the “free” valproic acid can be determined.*** To acquire a sample with free VPA, the sample can be diluted beyond the equilibrium binding constant of VPA and serum albumin. This was a uniquely novel exploration and application of chemical engineering knowledge to the problem. This technique relies on extensive modelling of the equilibrium binding of valproic acid and serum albumin at the various concentrations of both to determine optimal dilution. The students conducted the initial calculations of such models from empirical results found in the literature. Future efforts would collect and input this data into the models.

To quantify serum albumin in the patient sample a simple impedimetric sensor was designed based on the literature. However, to measure total VPA, the SenseNC Team aimed to build a novel multi-stage electrochemical sensing platform. While learning about different types of electrochemical sensors, the SenseNC Team was very interested in attempting to mimic very popular enzymatic/amperometric sensing devices. Unfortunately, there were no readily available dehydrogenases or oxidases for valproic acids, but multiple were found for other short chain fatty acids. Accordingly the team investigated the potential to use a sequential or ***multi-step sensing process to make valproic acid into a compound that could be detected via enzymatic/amperometric sensing.*** This resulted in a design to use multiple enzymes to modify valproic acid with acetyl-CoA, which can then be catalyzed and measured amperometrically. The students designed and simulated the entire chemical and electrical system. As the team supervisor, I aided in the instruction of the fundamental principles of thermodynamic equilibrium for affinity binding, electrochemical principles like the Cottrell Equation and Nersnt Equation, so the students could carry out their simulation of the sensors.

Translation Potential

Business Model Canvas

See **Appendix Figure 1** for the business model canvas.

Stakeholder Desirability

Valproic acid is an anticonvulsant medication usually administered orally via 250 mg tablets or syrup with a concentration of 250 mg per mL [7]. The therapeutic range for total VPA ranges from 50 to 120 micrograms per mL [4]. It is generally prescribed at low doses that increase until the most effective dose is found. This need to gradually increase dosages is a result of the high protein-binding tendency of VPA to serum albumin. The percent of protein binding ranges from 90 - 95% [4]. With such high protein binding, therapeutic drug monitoring becomes necessary as slight shifts in serum albumin concentration levels can cause changes in the amount of VPA in the bloodstream [4]. High VPA concentration can lead to adverse effects that, depending on the severity, can become life threatening.

There have been several studies carried out showing that basing drug dosages on the amount of free drug is more effective than using the total amount [4]. By utilizing the VAES we have developed, physicians can ensure that their patients are receiving the most effective daily doses of valproic acid. This is especially true with patients that have conditions that greatly affect their serum albumin levels. By being able to monitor the free VPA levels in these patients, physicians can reduce the potential risk of VPA poisoning due to protein concentration shifts. In addition, the sensor can also reduce the costs associated with the treatment of epilepsy or bipolar disorder via valproic acid. It is a more cost effective as well as less-time consuming method of determining VPA concentration in serum. Generally, patients have to get their blood collected and sent out for laboratory testing. According to Labcorp, the expected turnaround time is 1 - 3 days for the current immunoassay test utilized to determine VPA concentrations [8]. With the VAES, the turnaround time would be significantly shortened to just three minutes, making the process of monitoring VPA levels more efficient. By decreasing the amount of time required for the therapeutic drug monitoring of these patients, the cost of VPA treatment can be reduced as well.

A need for VAES is demonstrated in the markets for medical devices as well as therapeutic drug monitoring. The United States has the largest medical device market in the world valuing at $160.8 billion dollars in 2019 [9,10]. In 2017, the United States' market comprised 40% of the global medical device market [9]. Its growth is driven by innovation as the medical industry is constantly looking for new and improved ways to treat their patients. With this being the case, the introduction of a new sensor would most likely result in a favorable outcome. In addition, antiepileptic drugs possess the greatest share in the market for therapeutic drug monitoring due to their complicated pharmacokinetics [11]. Since VPA is an antiepileptic drug, it is also favored by the TDM market as well.

Business Feasibility

Our key resources for this project have been the NC state lab spaces and materials as well as guidance from medical professionals and patients. We have had meetings with both Micronit and a local physician in the Raleigh area. Micronit proved to be an invaluable partener to the electrical team, as they helped in the development of the biosensor's microcontroller. Meeting with a local physician helped to understand the role valproic acid plays in healthcare and the potential impact our device could have. In these meetings, and through the research using NC State's labs and

materials, we gathered the necessary information to produce a business plan for our device.

To produce the VAES we also require expertise on FDA regulation, marketing, and mass production. In the commercialization of this product, we will market to physicians as well as insurance providers in hopes that they will prescribe the product to their patients. We will maintain the collaboration with these key partners through customer service programs and frequent reviews.

Financial Viability

The product will be sold to patients through provider prescription. There are over 5 million patients in the United States alone that are taking valproic acid, and they make up our target market [5]. In time, we hope to expand this market to include European patients as well. Since we are selling the product through medical providers, it is important to include that the American Academy of Neurology has approximately 36,000 members, all of which are neurologists or neuroscience professionals. These are the specific providers that we will market the VAES to.

The revenue streams for the device include the device itself, replacement parts, and possible subscriptions for regular replacements. The associated development costs include those required for sensor assembly, FDA approval, marketing/advertising, and continued research. In the future, mass production will also become an important factor in our cost analysis as well. The electrical components for our sensor have cost a total of $421.55. Much of the costs for the chemistry team's research have been covered by the university. In regard to the FDA, the medical annual device fee for market entry in 2020 is $5,326 [12]. Other costs to consider include the costs for employees and possible taxes related to development or sales. Based on these costs, we estimate that the cost for this biosensor will start at around $875.00 and decrease as mass production lowers the cost per sensor. This unit price will cover the necessary costs for materials and leaves enough profit margin to contribute to the other production and distribution costs. See **Appendix Table 1** for the cost chart for each biosensor.

Team and Support

Team Members

McKenna Downey - B.S. Chemical Engineering - Team Captain - Chemistry and Business Team - McKenna worked to delegate tasks and responsibilities to the team as a whole as well as within the chemistry and business team in order to keep our team on track and working together. She also worked with the chemistry team in the lab and remotely to perform research, develop ideas and produce calculations for the enzyme reactions in the biosensor.

Sucheta Malladi - B.S. Electrical Engineering - Sucheta led the team on designs for the user interface and current/impedance sensor. Her experience in signal processing has allowed us to precisely coordinate greatest areas of change in our impedance sensor, allowing us to receive more accurate measurements.

Aryana Ortiz - B.S. Chemical Engineering - Chemistry and Business Team - Aryana performed research for the chemistry team to determine the best method of sensing for VPA. She reviewed literature to help find the necessary constants for the enzyme reactions and assisted in the development of the excel model.

Meekhel Patel - B.S. Computer Engineering - Electrical Team - Meekhel was part of a team that created the current and impedance sensor designs. He ran impedance tests across a variety of capacitor and resistance values, to find the ideal frequency sweep range for calculating VPA concentrations.

Shannon Pinnell - B.S. Electrical Engineering - Electrical Team - Shannon was part of a team that created current and impedance sensor designs. She ran PSPICE simulations to confirm results from other tests and created guidance documents for many of the concepts used in the sensor electrically.

Molly Powell - B.S. Biomedical Engineering - Chemistry and Business Team - Molly performed research for the chemistry team in understanding possible processes for VPA detection, much of which involved literature review. She also worked with the business team on developing the business model. Molly also worked to find funding sources and handled the financing needs for the group.

Sydney Stine - B.S. Chemical Engineering - Team Captain - Chemistry and Business Team - Sydney helped delegate responsibilities out to the whole team to make sure the project was completed in a timely manner, as well as kept the team organized and up to date on deliverables and assignments. She also helped perform calculations in Excel to model the enzyme reactions and current produced.

Joshua Wilson - B.S. Electrical Engineering - Electrical Team - Joshua was a part of a team that created current & impedance sensor designs. He also helped to develop code that was able to operate the sensor's backend protocols.

People Who Have Given Support

Dr. Micheal Daniele - Dr. Michael Daniele - Team Advisor - In addition to providing advice and expert analysis, Dr. Daniele navigated all financial issues associated with the project. He made it all possible and we are grateful for his support.

Katie Kilgour - Ph.D. Chemical Engineering - Provided guidance and knowledge with assay development and system design.

Brendan Turner - Ph.D. Biomedical Engineering - Gave advice and direction with initial assay development and modeling of the assay.

Sponsors

NCSU Office of Undergraduate Research - Provided funding and admin support across the project.

Final Remarks

Nothing worth doing is ever easy. After accounting for personnel, technical and organizational mishaps, SenseNC 2020 has been no exception. This year, SensUs was especially unique due to the unforeseen circumstances from COVID-19. On top of the struggles we were already facing, we were challenged with finishing the project remotely without access to our labs and other resources on our campus. However, if one were to ask if those struggles were worth the end result, every single member of SenseNC would answer “absolutely”. The lessons learned, friendships formed, and challenges overcome far outweigh the rough patches experienced during development of the VAES. Our mission is not only to build a biosensor each year, but to also cultivate newer members so that they can carry the team forward in future years and achieve greater success. All of the difficulties faced will serve as lessons for those continuing members and strengthen SenseNC as it continues to grow and thrive.

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Appendix

Figure 1. Business Model Canvas

Business Model

SenseNC

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Problem
- The concentration of valproic acid in a patient's blood can vary and be unpredictable at times because of its sensitivity to serum albumin
- It is difficult to find/maintain an efficient and healthy therapeutic window for valproic acid
- in 2007 VPA toxicity was responsible for 1,835 cases requiring treatment at US healthcare centers

Solution
- Give doctors a way to measure the VPA levels in their patient's blood to determine the best amount of VPA to prescribe

Value Proposition
- A faster and simpler method to measure the free valproic acid concentration in the blood
- Portable and user friendly
- To be used for therapeutic drug monitoring for doctors

Customer Segment
- Medical offices
- Market to more than just epilepsy since the drug also treats bipolar disorder and some other ailments/diseases

Key Resources
- NC State lab space
- Materials
- Research
- Information from medical professionals and patients
- Certification by FDA

Unfair Advantage
- May take time for the sensor to collect stats on each individual's binding rates of VPA in their blood and become effective at telling the patient how much VPA they can take at any given time
- there are not a lot of these VPA sensors for point of care. Therefore we have an unfair advantage in the market because our competition is small.

Key Partners
- Micronit
- Doctors
- Health Insurance Providers

Revenue Streams
- The device
- Replacement parts
- Possible subscription for replacements
- Revenue likely to come from insurance companies due to US health care's reimbursement model

Customer Relationships
- Medical professionals using the product for therapeutic drug monitoring and VPA prescription
- Insurance companies

Key Activities
- Research, fund, design, and build the device
- Get FDA approval
- Market/Advertise
- Build connections with insurance and healthcare providers
- Sell and distribute the product

Distribution Channels
- Could be distributed by healthcare providers

Cost Structure
- Product prescribed by doctor, patients split costs with insurance
- Possibly given as a prescription

Customer Segments
- Targeting patients in the United State because of size and accessibility of market
- Approximately 3.4 million people in the United States have epilepsy (1.2% of the population)
- Approximately 6 million people in Europe have epilepsy
- The American Academy of Neurology has approximately 36,000 members, all of which are neurologists or neuroscience professionals

Unique Value Proposition
- More reliable dosing of VPA and confidence in knowing how much of the drug is circulating through your bloodstream and active at a given time
- Sensor will give the dose of VPA needed based on it's collected data (from the individual's serum) on binding rates of VPA to albumin

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Table 1. Cost Calculations for Biosensor Device Based on Materials, Labor, and Profit Margin

Components	**Unit Price ($)**
Electrode Chemical Solution	$30.00
MSP430FR2355 LaunchPad™ development kit (MSP-EXP430FR2355)	$15.59
Rechargeable Li-ion Cell Button Coins Battery (LIR2032 40mAh 3.6V)	$1.38
Micro USB to double crocodile clip cable	$1.46
Wire (55A0111-14-DS-ND)	$1.12
AD5941 Dev Kit (505-EVAL-AD5941ELCZ-ND)	$281.25
Power Supply Housing	$5.99
Materials Total	$336.79
5 Engineers @$30/hr	$6000/week
100 devices/week	$60
Total with Labor	$396.79
FDA Medical Device Fee	$5236/year
100 devices/week	$1.01
Development, Approval, Marketing Costs	$250.00
Total Cost Per Device	$647.80
Expected Revenue Per Device	$863.73