KERJ Biologics Blood Potassium Measurement Device

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Executive Summary

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When treating patients in field EMS environments, it is difficult to reliably treat patients due to a lack of diagnostic testing. Conditions such as a hyperkalemic cardiac arrhythmia are easy to confuse with other cardiac conditions without a potassium blood level reading. Paramedics currently have the medications to treat hyperkalemia, but are unable to reliably recognize patients with elevated potassium and provide the correct treatment for the condition.

Our group aimed to create a portable test for blood potassium levels that can be used by field providers to identify hyperkalemia. By providing quantitative potassium measurements, providers can easily confirm when a patient has elevated potassium levels in their blood. Giving EMS providers in field environments diagnostic testing for hyperkalemia will allow for an elimination of the fatal effects of misdiagnosis and incorrect treatment, and allow providers to be confident in the correct treatment of hyperkalemia patients.

In order to create a portable blood test, we have developed a potassium specific solid state ion selective electrode. By utilizing a potassium ionophore, we have constructed electrodes that clearly show potassium selectivity and have begun to collect data that suggests we are able to generate electrical potentials in the physiological range of potassium concentration. At this point, our work has been hampered due to the variability between electrodes and nonsensical data that is sometimes generated by using a reaction meant for a single use multiple times in attempt to generate calibration data.

In parallel with the development of the electrodes, we have developed the other components of our device; test strips to contain the single use electrode blood tests, and a handheld device to analyze test strip results and provide a potassium measurement to the user. At this time, we are able to reliably make multilayered test strips and have been using the test strip interface with the electrodes as a way to test the function of our electrodes with less error and difficulty. Our interface device currently has a button controlled screen interface that has display to a user a concentration in potassium in mmol/L. The development progress we have had with the test strips and interface device means that once we have perfected the electrode design we will be able to rapidly move towards having a user ready device.

At this time, we have made substantial progress in developing our three major subsystems; the electrode, test strips, and meter interface. Future work should include optimizing the layers of the ion selective electrode so they are consistent and provide the same potential across multiple individual membranes. This is crucial for developing a quality control standard for single use disposable test strips which cannot theoretically be tested more than once. Then full testing procedures can be done with other ions, whole blood samples, and in different temperatures. Once these testing procedures have been completed to generate calibration data and prove the effectiveness of our electrode, we will be able to move forward to developing a final device that is ready to be marketed and sold to EMS providers.

Table of Contents:

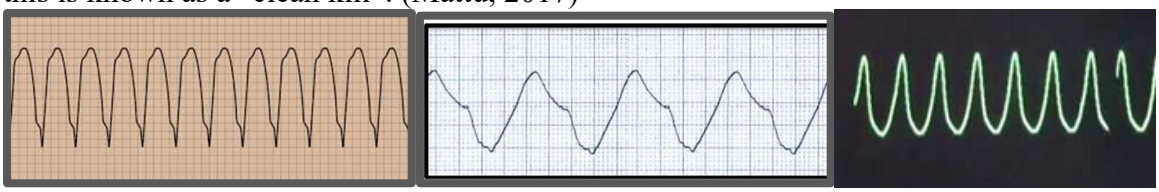
Introduction:

Emergency medical personnel are often faced with difficult patient care decisions due to a lack of diagnostic tools. Currently EMTs and paramedics have to largely rely on physical symptoms and vital signs to diagnose and treat a patient's condition. While these methods are largely successful, there are many varying conditions which display the same physical symptoms and, without the availability of or time for laboratory testing, misdiagnosis can easily occur. This report details the progress of our group in our attempt to create a portable blood potassium measurement device for use by paramedics in ambulances to diagnose hyperkalemia. We discuss the design solution we have come up with, preliminary testing data, the current state of device subsystems, and guidance for future development of this technology.

Background:

Patients with hyperkalemia (high potassium) can have a wide array of dangerous heart problems which are difficult to identify using current EMS methods. High potassium interrupts normal electrical conduction within the heart and leads to irregular heart rhythms called arrhythmias. If left untreated, many cardiac arrhythmias will lead to cardiac arrest and death. One of the common arrhythmias that can cause cardiac arrest is called wide complex tachycardia. In normal cases, this arrhythmia is treated with amiodarone or lidocaine; anti-arrhythmic medications that return the heart to a normal rhythm. (NYS Dept of Health, 2017)

When a wide complex arrhythmia is caused by hyperkalemia or a typical conduction problem, identical visual cues are revealed on the cardiac monitors used by paramedics. The only difference between the two conditions is a slightly altered heart rate, which varies from patient to patient and can be easily missed by paramedics. If a potassium caused wide complex arrhythmia is misdiagnosed as wide complex ventricular tachycardia, a patient will be given amiodarone or lidocaine as treatment. Due to the way that these medications interact with and further block potassium ion channels, a paramedic administering these standard cardiac drugs will kill a patient. A patient given anti-arrhymics with hyperkalemia will have their heart almost immediately stop and will die as a result of the intervention given to them. This type of unnecessary death is a risk that comes from treating patients without the diagnostic information available to hospital providers. When a provider kills a patient as a result of their interventions this is known as a "clean kill". (Mattu, 2017)



Wide Complex Tachycardia | Wide Complex Hyperkalemia | Cardiac Monitor Display

Figure 1: Comparison of Wide Complex Tachycardia and Hyperkalemia Rhythm. Wide complex tachycardia and hyperkalemia differ on a rhythm strip due to the time of each wave; however on a monitor the arrhythmias are almost identical in appearance.

Paramedics have the medications to treat hyperkalemia already as part of their available treatment options, but are not able to reliably diagnose the condition in the field. If a provider is able to identify hyperkalemia, they can easily treat a patient with sodium bicarbonate and calcium chloride to immediately lower potassium levels back to a normal range. If no treatment

is given for an arrhythmia, a patient may progress into cardiac arrest and die, and if arrhythmias are misdiagnosed a patient will be given the incorrect treatment and almost certainly die. (NYS Dept of Health, 2017) (Mattu, 2017)

Currently, the only diagnostic test used on ambulances is of blood sugar measurement through a glucometer. The success of a glucometer comes from its use as a point of care tool; a device that can be brought to patients and provide information pertinent to patient care quickly. A glucometer is useful as the symptoms of both abnormally high and low blood sugar are almost identical within diabetic patients. Despite this, the treatments for each condition vary tremendously, and, if a patient is given medication to decrease low blood sugar or to increase high blood sugar, then the patient may develop permanent neurological damage, possibly leading to death. The introduction of glucometers to EMS medical professionals has allowed diabetic emergencies to be treated with improved patient outcomes. The low cost and high positive benefit of glucose testing devices has allowed this technology to thrive in EMS. We aim to replicate the success and benefit of glucose testing with potassium measurement for EMS providers. (Pollak, 2011)

The only other point of care device available to measure potassium is the ISTAT system by Abbott Labs. This device is prohibitively expensive at $6500 for a refurbished model and in the experience of our customer; the ISTAT is extremely fragile and temperature sensitive, and therefore not suitable for use in field environments (Block Scientific, 2019). In order to make a device feasible for adoption by field providers, our device needs to be a low cost and durable system similar to glucometers.

In order to address the lack of a portable and rapid blood test of potassium that could prevent accidental deaths and save lives in EMS settings, we aim to develop a glucometer style point of care potassium test. This device measures the concentration of potassium within a blood sample in order to provide paramedics with the quantitative data needed to make accurate treatment decisions. Similarly to the glucometers in common use, our device will increase the amount of information available to providers to insure an improved decision-making process and more saved lives in pre-hospital settings.

Problem Statement:

Emergency medical personnel are faced with difficult situations in the field regarding the triage of conditions and patient care decisions due to a lack of diagnostic tools. A portable point of care blood testing device which measures potassium concentration would provide valuable information regarding the severity of a patient's condition, and lead to better decisions regarding field care and transport destinations when presented with patients with renal failure or cardiac arrhythmias. Providing EMS paramedics with field blood testing capabilities for potassium provides additional information that can be used to save patient with hyperkalemic arrhythmias from dying due to incorrect treatment and save lives by identifying patients with high potassium. We aim to develop a portable device that can test the concentration of potassium present in a whole blood sample for use in field triage decisions.

Description of Stakeholders and Scenario:

Emergency medical personnel have to interact with a wide variety of patients in extreme situations with little quantitative information. In a variety of situations, it is beneficial for a provider to have a measurement of blood potassium before initiating care. Some of the medical situations that require a blood potassium level include patient with kidney failure, severe crush injuries, and cardiac arrhythmias with an unknown cause.

When a patient has a cardiac arrhythmia there may be a variety of different causes. If the cause of an arrhythmia is unknown, the arrhythmia may progress further to cardiac arrest, and eventually death. Cardiac muscle is especially sensitive to changes in blood potassium due to the unique channels in cardiac tissue. When blood potassium is lower or higher than the normal expected ranges, heart muscle may start to beat irregularly or stop beating altogether (Pollak, 2011). When a patient has an abnormal ECG or is in cardiac arrest, blood potassium is one of many potential causes. If hyperkalemia is detected as the cause of a cardiac arrhythmia, a patient can be treated with sodium bicarbonate and calcium chloride (NYS Dept. of Health, 2016). Currently, hyperkalemia is detected by attempting to notice characteristics in an ECG instead of relying on quantitative data (NYS Dept. of Health, 2016). If hyperkalemia is misdiagnosed as wide complex tachycardia or another arrhythmia and a patient is given the antiarrhythmic medications such as lidocaine or amiodarone; the patient will be killed by the provider intervention (Mattu, 2017). Both providers and patients have high stakes when treatment is given for any condition. If a provider is right then a patient gets better and the provider can continue to practice medicine. When a provider is incorrect and gives a patient treatment what worsens their condition or causes death, then the patient loses their life and a provider can face lawsuits and the end of their career. Both sides of treatment have a lot to lose if a diagnosis goes wrong, so a measure of blood potassium would dramatically lower the stakes when a patient needs to be diagnosed with hyperkalemia.

Patients with renal failure are expected to receive dialysis on a regular basis in order to maintain normal blood concentration of many components, including potassium. If a patient is a new developer of renal failure or has not received dialysis regularly as they are supposed to, they may have an increased level of blood potassium (Pollak, 2011). As described above, an increase on blood potassium may cause these patients to be at risk of cardiac arrhythmia and arrest.

A rare but extremely dangerous situation that may occur in prehospital medical care is severe crush injuries. These are often a result of disasters such as building collapse and car crashes when someone's limbs are caught under something heavy and cuts off circulation to that extremity. If trapped for extended periods of time, potassium will build up in the limb (Pollak, 2011)(NYS Dept. of Health, 2016). The buildup of potassium occurs as a result of destroyed muscle cells releasing their internal potassium into the blood (Healthline, 2017). When the limb is freed as a patient is extracted from the situation, the potassium in that limb will re-enter blood circulation and can cause cardiac arrest.

The device would commonly be used in field emergency scenarios, therefore measurements could be taken anywhere where patients are found. The wide array of environments could include freezing cold winter nights or hot summer days. In addition to treating patients where they are found, this device would be used when transporting patients to hospitals. This could include either ambulances or helicopters depending on patient severity and the distance to facilities; and each presents an experience where devices must be able to withstand forces such as shaking and dropping.

A measure of blood potassium is useful in providing the best patient care possible in each of the scenarios mentioned above. Each of these patients has a condition severe enough to warrant placement of an IV. As part of the IV placement process, it is extremely easy to gather a sample of blood either by dripping directly from the line or extracting with a syringe. This blood sample would be introduced to a disposable element and inserted into our device for processing. By the time the provider finishes flushing, securing, and starting fluids into the patient's IV, our device would provide a clear numerical readout of the patient's blood potassium level. Based on the expected design of our device, it is likely that the provider would only need a drop of blood from the IV line in order to obtain a reading, similarly to how a drop is taken from the line for glucose readings with a glucometer. Once armed with the quantitative data given from our device, a provider will be better able to move forward with their differential diagnosis and catch hyperkalemia before the condition causes death either from natural progression or incorrect provider intervention.

In order for our device to be useful as a field diagnostics tool, it needs to meet quality and performance specifications. To provide information, the device needs to provide a quantitative measure of potassium concentration in the physiological range. In order to be used in a field environment the device needs to be durable and able to withstand typical rough use. Finally in order to be utilized by EMS agencies the device needs to be low cost, similar to a glucometer.

Design Overview:

To remedy the aforementioned problem, we have designed a handheld meter which utilizes single use, disposable test strips to measure potassium. Though a prototype version of the device is shown in Figure 2, a concept rendering of what the device could look like in the final stage is shown below as well. Like glucose testing strips, the reagents necessary for measuring potassium are embedded in a polyester test strip assembly. Specialized polymer layers in a solid contact ion selective electrode (SC ISE) are used to convert the concentration of potassium into a voltage which can be delivered to the meter and measured. During use, the user will insert the strip into the meter, obtain a small drop of blood from a newly inserted IV line, and the meter will read blood potassium. This is an identical workflow to how blood glucose is already taken in emergency medicine. We have designed the device to be least intrusive to established practices, and the use of test strips provides a testing system which EMS providers are already trained in. A systems level design diagram is provided in Figures 3 and 4, which highlights the 3 subsystems of our device; the meter, the strip, and the ion selective electrode.

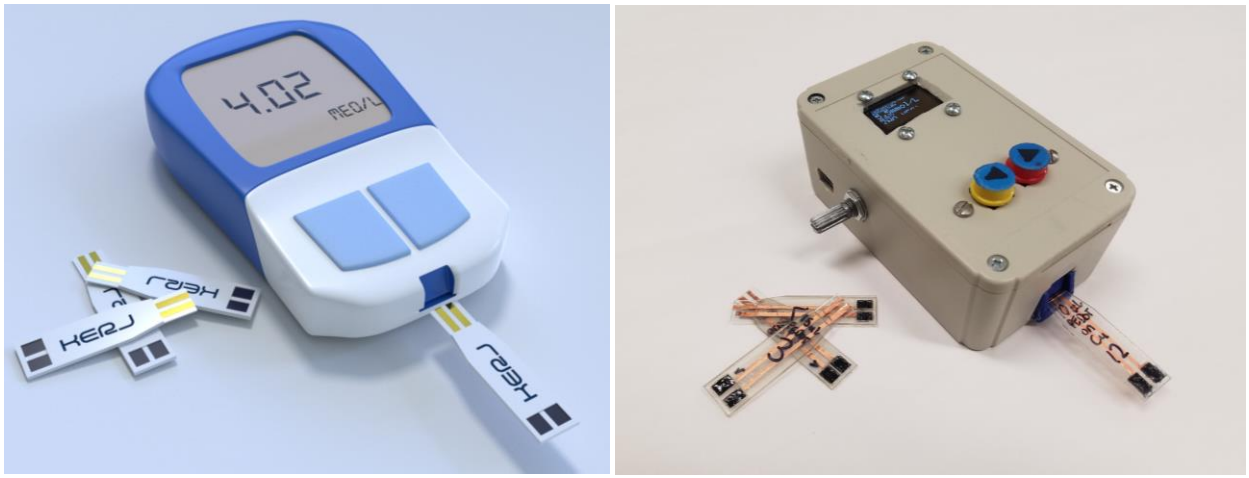


Figure 2: (right) Concept rendering of what a finished product could look like. (left) The prototyped version of our potassium meter.

Systems Level Design:
It is helpful to define some terms related to the various subsystems of our device in order to better understand the systems and their interactions.

Disposable Test Strip: Made from polyester film, this small, rectangular component contains the features and materials necessary to generate a potential based on the potassium concentration of a sample. The sample is applied to the wells in one end and the other end is inserted into the meter. The generated potential then travels down a conductive trace to the handheld meter.

Handheld Meter: A handheld device to which the strip will be inserted and which will interpret a small voltage, process the signal, and display the result.

Strip Inserter: A small plastic component with a slot and pin connectors which the strip is inserted into. Detailed drawing in the Appendix, Figure 17

Solid Contact Ion Selective Electrode (SC ISE): A multilayered polymer membrane which is embedded in the test strip, is selective for potassium, and uses redox reactions to generate a potential which travels to the reading end of the strip.

Valinomycin: A naturally found antibiotic which acts as a selective potassium shuttle.

PVC Membrane: A viscous plastic in which the selective components are mixed to provide ion selectivity.

PEDOT-PSS: poly(3,4-ethylenedioxythiophene) polystyrene sulfonate: an ionic polymer which assists in ion to electron transduction through redox reactions

Reference Electrode (RE): Similar construction to the ISE, however, it is not selective for potassium and provides a reference voltage.

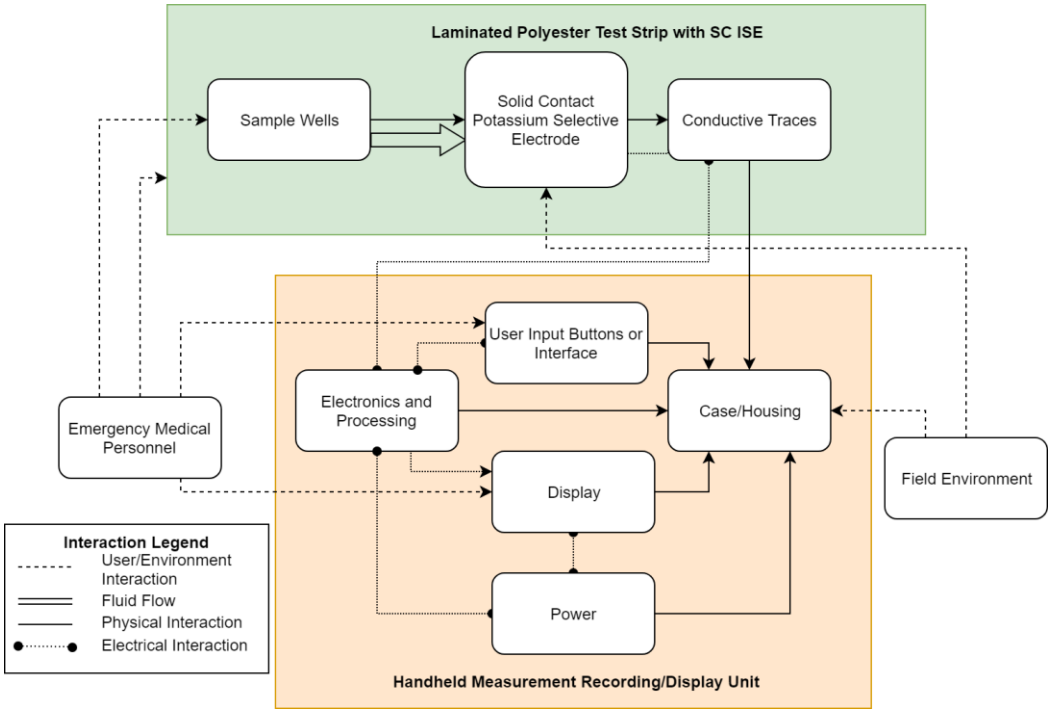


Figure 3: System level breakdown showing the two subsystems in orange and green. Interactions are shown on the basis of physical, fluid, environment, or electrical contact.

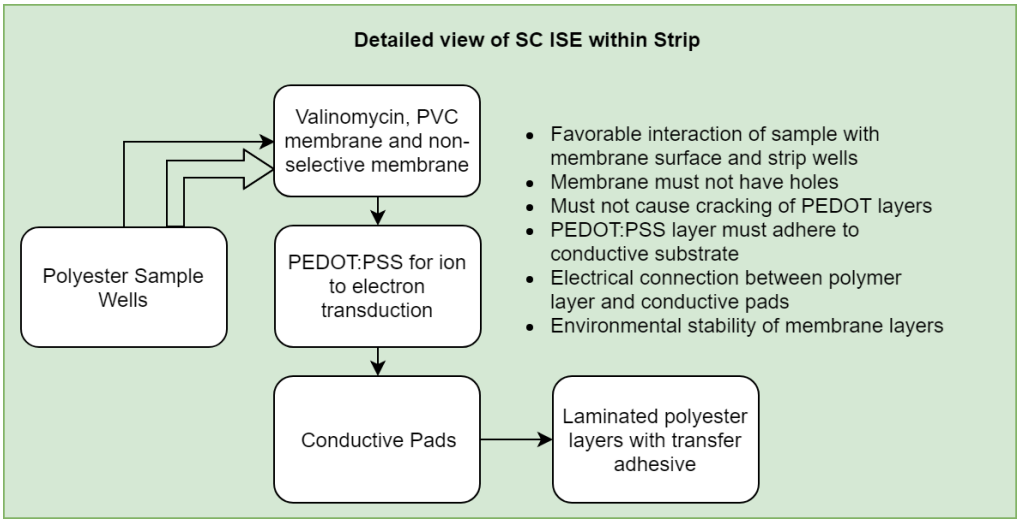


Figure 4: Breakdown of the SC ISE/RE and its interactions with the rest of the strip assembly

Design Specifications:

Table 1: Design Specifications

Potassium Selectivity	Responds to potassium and not other ions such as sodium or calcium
Physiological Potassium Measurement	Potassium detection range between 1mmol/L and 10mmol/L
Potassium Concentration Differentiation	Concentration levels are differentiation with a resolution of 0.1mmol/L between 2.5mmol/L and 6.5mmol/L, the physiologic range for pota(Healthline, 2017)
Whole Blood Measurement	Device is able to measure potassium levels in whole blood samples
Rapid Response Time	Potassium reading occurs within 5 minutes; ideally less than 1 minute
Single Use	Tests are reliable for a single sample use
Wide Operating Temperature	Detect potassium between -30F and 100F
Small Form Factor	Device is palm sized; 3in x 3in x 1in max
Battery Operation	Powered by 2 or less commonly available batteries
Durable	Withstands drops onto hard surfaces from 6ft without damage
Low cost	Device costs less than $50. Individual tests cost less than $10

Detailed Design:

Potassium Selective Membrane

Ion selective electrodes are widely used to measure concentrations of various ions. For this reason, it was decided that we would attempt to minimize this technology for our application. We employed valinomycin, a potassium selective ionophore, in order for our membrane to select specifically for potassium. When a sample contacts the membrane, valinomycin will bind potassium ions in the sample and shuttle these ions across the polymer membrane. The potassium ions are then released into the solid contact layer of the ISE and bind to PSS. The dissociation of PSS from the PEDOT within this layer results in the donation of an electron to the conducting substrate layer. We should then be able to compare this potential with the stable potential of an impermeable membrane (reference electrode), resulting in a potential difference due to potassium binding. Ideally, this will generate a measurable potential which scales with potassium concentration.

The actual ion selective aspect of our ISE is composed of three crucial layers. The "ion-selective membrane" layer is composed primarily of PVC polymer (Fig. 5). Each batch of this layer was composed of 0.125g PVC, 410uL DOS, 2mL THF, 0.8mg $KBPh_4$, 20uL Acetone, and 5mg valinomycin. However, in our reference electrodes, this formulation excluded valinomycin. This mixture was mixed thoroughly by vortex, resulting in a clear, viscous liquid. The "SC with redox capacitance" layer acted as a solid contact and was composed of PEDOT, a conducting polymer, and NaPSS, a dopant to increase the redox capacity of the layer. Each batch of this layer was composed of 10mL of 3wt% PEDOT dissolved in DI water and 4mL of 0.1mM NaPSS in DI water. After vortexing, this mixture became a very viscous, black fluid. Our final layer, the "electron conducting substrate" was copper. In large scale experiments, 300um copper plates were used. Upon beginning smaller scale experiments, strips of adhesive copper tape were used instead.

Several coating methods were used during the membrane fabrication process. We first attempted to coat the large copper plates by hand-painting the PEDOT onto the plates and allowing it to dry. We then used the same method in adding the PVC polymer layer. We were aware that this method would not give us homogenous layers, but this was intended to be a proof of concept experiment to test for potassium selectivity. One of the primary aims of this project was to minimize the time necessary for a potassium test. Thus, the thickness of the PVC layer would eventually need to be minimized to reduce the time necessary for potassium shuttling.

An issue we ran into during the coating process was cracking of the PEDOT layer after the addition of the PVC. We determined that this was due to the rigidity of the PEDOT layer once dried and the tendency of the PVC layer to contract during the drying process. To address this issue, we decided to use spin coating as a new coating technique. This process was used for both the PEDOT and PVC layers and we no longer observed any PEDOT cracking. However, we did observe some small breaks in the PVC layer after spin coating. There were small aggregates in the PEDOT layer which we concluded were harmless cross-linked polymers. However, these aggregates did result in occasional breaks in the PVC layer. Because of this and the cracking issues, we decided to take a new approach in the ISE layer fabrication process.

SiN nanoporous chips presented a possible solution to our PEDOT cracking and PVC layer problems. These chips contain a shallow trench on one side between the chip's surface and the nanoporous membrane. We decided to try and fill these trenches with our ISE layers in reverse order (PVC then PEDOT), eliminating the possibility for PEDOT cracking. In addition, the nanoporous membrane would act as a filter for blood cells, allowing only plasma to contact our membrane, thus minimizing the possibility of cell lysis at the membrane surface. This method proved to solve our layering problems. However, further experimentation is necessary for testing this method for potassium selectivity and concentration scaling. In addition, engineers may want to consider a polymer additive to the PEDOT layer in order to reduce its hydrophilicity, allowing for improved interaction with the very hydrophobic PVC layer.

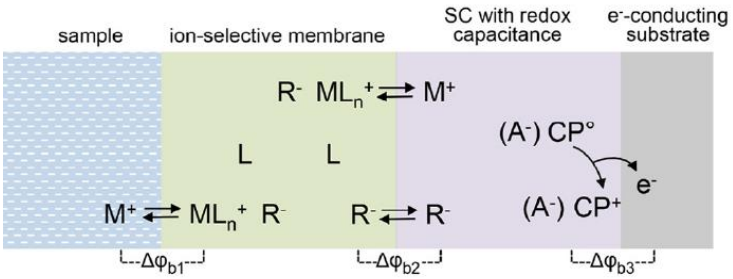


Figure 5: Basic theory behind solid state ion-selective electrodes - redox capacitance model. M is potassium, R- is an anion (TPB), A- is PSS, and CP is PEDOT in our system (Hu et al, 2016).

Test Strip Design and Manufacturing

The choice of designing a test strip apparatus as one of the main device phenotypes was for several reasons. Originally, we were pursuing constructing a conventional selective electrode, which involves a selective membrane, inner filling solution, and silver silver chloride wire. After a lot of deliberation we decided to switch to a more difficult solid state electrode design which better fit the needs of someone working in a field environment. A strip based system had become a suitable option to hold the solid contact layers. EMS providers are already comfortable with using disposable test strips, as taking a glucose reading is standard practice. Additionally, testing large scale prototypes was very difficult. The testing solutions had to be precisely placed on both a selective and reference electrode all while bridging the gap between them to complete a circuit. So the test strips actually serve as an easy testing platform which allows you to easily apply a sample to small windows of exposed electrode.

We researched existing test strip solutions for glucose meters, and found several helpful sources describing their manufacturing. Liberty Medical provides a video on how they manufacture glucose test strips. Polyester (PET) is commonly used as a substrate, upon which conductive traces, reagents, adhesive, and insulating layers are applied (Liberty Medical, 2019). Conductive traces are often screen printed or laser etched into the base layer. Our strip design is based loosely around the materials and manufacturing techniques for existing test strips, with scalability in mind.

The test strips we have prototyped are constructed from laser cut 300um PET film. A diagram of the layers is shown in figure 6. They consist of four layers which have been customized to hold the ion selective electrode polymers. The first is a base layer which is cut to fit the strip inserter on the meter. Upon the first layer there are conductive traces and pads we

have prototyped using copper tape and then silver ink. To construct the next several layers, an acrylic transfer adhesive (468MP) often used in industry is pre applied to large sheets of the polyester we are using. Rectangular holes are cut in the second layer of polyester-adhesive to act as wells for the ISE components. A final layer consists of a large rectangular well to hold the liquid sample for reading. When constructing the test strips, the liner of the adhesive is peeled off to reveal precisely cut adhesive strip layers which create a seal when two layers are pressed together. The ISE components are deposited into the wells either layer by layer or as a complete sub assembly. We have prototyped with both methods, which still need to be optimized. The final layer is peeled and pressed onto the assembly to complete the test strip.

The use of an Epilog Zing laser cutter was crucial in the production of test strips. After concluding that hand cutting was not precise enough for our purposes, we transitioned to using a laser cutter. It allowed us to cut custom strip shapes with precise dimensions based on CAD models shown in the Appendix, Figure 16. DXF files were exported from Fusion360 to Inkscape, where they were printed to the laser cutter software. The polyester layers were cut at a high speed and low power to avoid thermoplastic deformation. This manufacturing process is scalable, as shown in figure 7, and it allowed us to test with a reproducible platform.

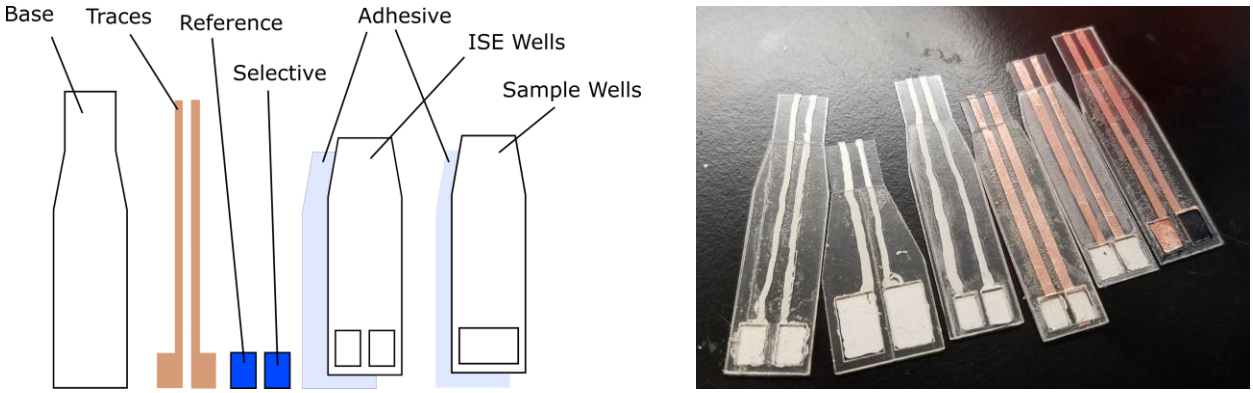


Figure 6: (left) An exploded view showing the different layers of the test strip. (right) An image of test strip prototypes with copper tape and silver ink as conductive traces. The wells are visible surrounding conductive pads, and as the image shows, the strips are fully customizable to many sizes.

Figure 7: A laser cut sheet of polyester with adhesive pre-applied. Consistent strips can be made using this method, and they can be easily customized to fit certain dimensions.

Electronics Design

Amplification Circuit

Amplification of input signals was done with an AD623 instrumentation amplifier. The amplifier was fed with +-6V and the negative input was grounded. The gain of the amplifier is determined as G=1+(100K/Rg) where Rg is a resistor value. In our testing we predominantly used a gain of 100 (Rg=1k).

Our tests showed a high amount of 60Hz noise, overwhelming our signals. In order to reduce the noise present an RC low pass filter with a cutoff frequency of 0.6Hz was added to the input of the amplification for testing.

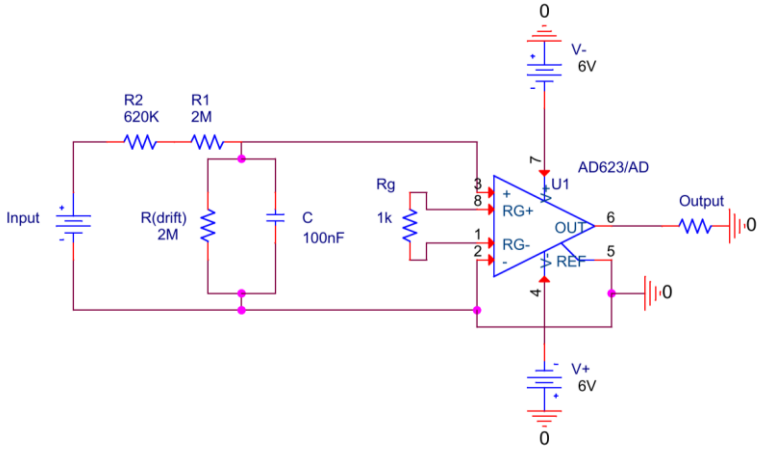


Figure 8: Amplification Circuit Design. A circuit with an instrumentation amplifier and 0.6Hz RC filter was designed for testing of electrodes.

Testing Equipment Setup

Testing equipment included the previously described amplification circuit, a DC power supply, and an oscilloscope. The power supply was used to power the amplifier with +-6V. The power supply ground was connected to the end of the RC filter. The Electrode leads were connected to the beginning and end of the RC filter. The reference electrode was connected to the end of the filter, and thus the reference electrode was grounded. The oscilloscope was connected to measure from the amplifier output to ground.

User Interface

The user interface device consists of an LCD screen, 2 off-mom push button switches, a DHT11 digital temperature sensor, and will ultimately include a strip reader and the amplification circuit. The interface is run by and arduino nano microcontroller and currently requires 2 9V batteries as a power supply.

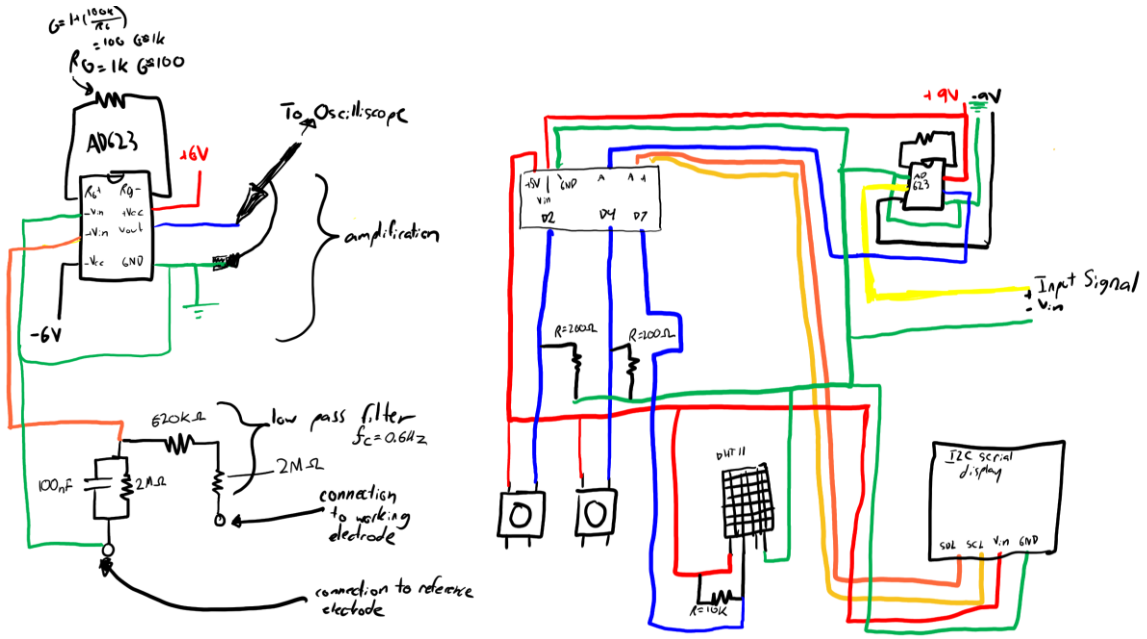


Figure 9: Design Schematic Sketches. Wiring schematics for the amplifier system and full device interface.

The user interface begins by prompting the user to insert a sample and press any button. The microcontroller then collects temperature data and input data from the amplifier. Currently, the device displays a potassium value between 2.5mmol/L and 6.5mmol/L, "LOW", or "HIGH" based on the input from a potentiometer. Once the test strips and electrodes are finalized the device will use calibration data to calculate the potassium in a sample based on the amplified input signal.

Throughout our design process there have been 3 prototype interfaces. The first was done on a breadboard, the second was a full design placed on a protoboard, and the third is a simplified interface with only one battery and no amplifier that was placed in an enclosure for concept demonstration.

Evaluation of Design:

The components of our device which needed to be evaluated include response time, size, estimated cost, reproducibility, selectivity, and overall EMF response. On first observation our device is of an appropriate size, and can easily be inserted into a glucometer-like device. The price is slightly higher than ideal with a $10.00 price tag per test strip. However, when compared to other commonly-used materials within the ambulance, this was not determined to be an unsatisfactory result.

Three different ISE prototypes were examined for selectivity, reproducibility and EMF response. These include a thick-film drop casted copper sheet, a spin-coated copper sheet, and a test strip model. The thick-film copper sheet was initially tested at high concentrations of sodium and potassium without use of a reference electrode. This electrode was composed of our ISE layers superimposed upon a copper plate through a drop casting-technique. During testing, wires were attached directly to the copper plate and placed in the solution. Due to the thick layers, three to five minutes were needed for the results to stabilize. Five minutes was determined by the customer to be too long for efficient results in the field. Therefore, it was

necessary that the layer thickness be reduced. The results of this testing protocol can be seen below in figure 10.

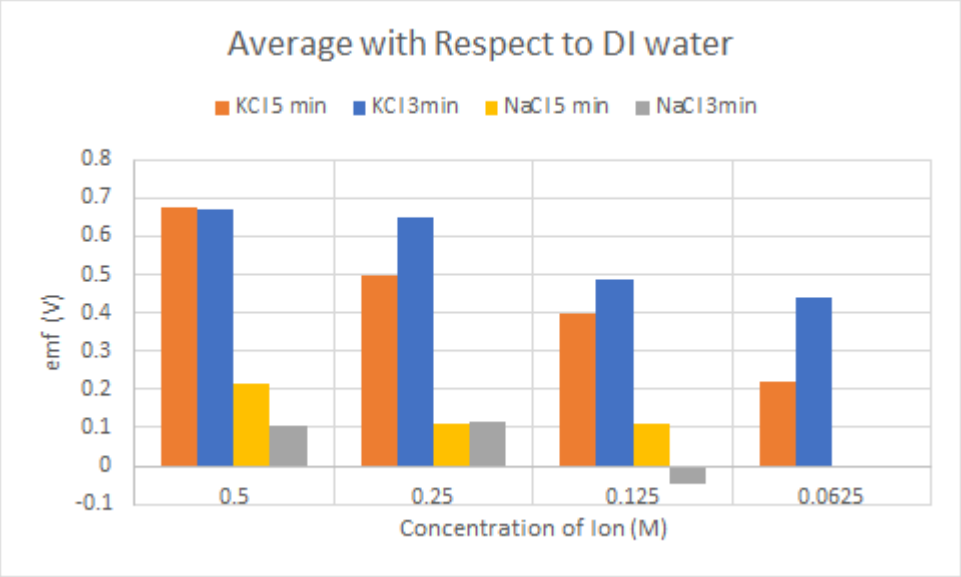


Figure 10: Thick film ISE Test Summary The above table demonstrates the emf response for potassium and sodium solutions at varying concentrations and moments in time

The average Additional results determined that, at these concentrations, the electrode provided voltage relative to potassium concentration and potassium selectivity over sodium. This selectivity appeared to increase with decreasing concentration, suggesting significantly higher selectivity at physiological concentrations. This established proof-of-concept for our ISE at high concentrations, .0625-.5M. Despite these encouraging results, the ISE did not demonstrate a significant EMF response at physiological concentrations. As an attempt to remedy this, the ISE formula was altered to included a greater concentration of PSS-.

Following these copper sheets, we decided to place our ISE layers directly onto the testing strips. This was beneficial in that it allowed us to easily apply a reference electrode to our measuring system. An additional change was the large increase in PSS- concentration following thick film tests. Testing procedures included the addition of potassium and sodium solutions, within physiological pH, directly onto both the ISE and reference electrode. The EMF response measurement was taken through connection to both electrodes. There were several drawbacks that became apparent during this testing produce. Although the device appeared to be selective for potassium there was no distinguishable correlation between ion concentration and EMF response. Instead of the anticipated response, voltage appeared to increase in magnitude over repeated tests regardless of ion concentration. A sample of this occurrence is provided below.

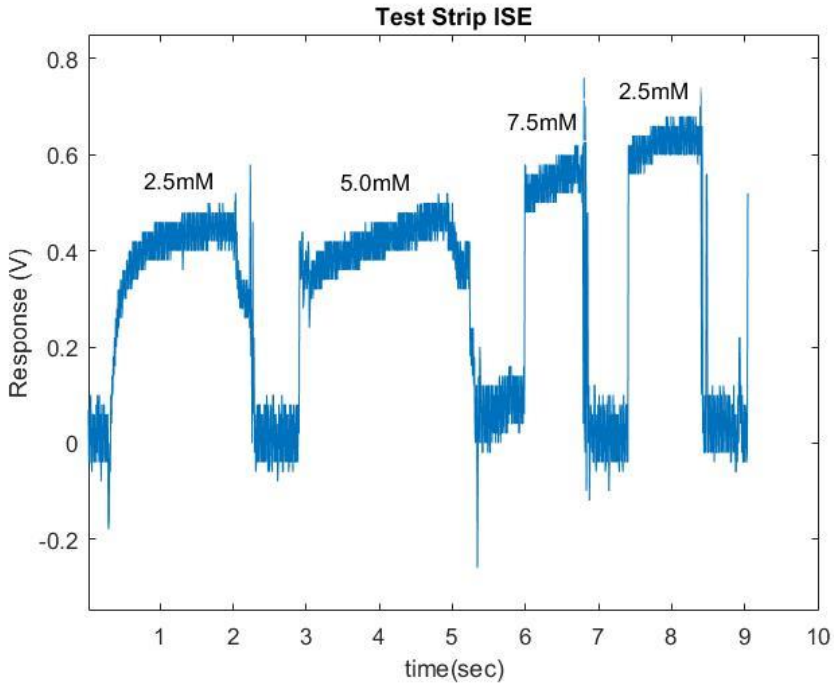


Figure 11: Results of thick film test-strip ISE. The results do not demonstrate the earlier encountered relationship between emf response to potassium concentration.

Despite repeated testing, the voltage appears to follow a trend of gradual increase. This unusual result was determined to be an effect of ISE de-lamination and degradation along with a possible diminishment of PSS- within the PVC layer. As the ISE layers delaminate, the electrode becomes less selective and a greater number of ions are capable of interacting with the copper plate to generate an increasing voltage. Additionally, as the PSS- becomes diminished, the regions of intact ISE loose their impact on the overall response. Despite these unfortunate results, the EMF response was potassium sensitive and was generated within milliseconds of applying the solution. This was a significantly more ideal time frame. In order to prevent delamination and to generate a more standard, reproducible test a spin-coated ISE was developed.

The spin-coated ISE produced significantly smaller layers, but did not appear to generate more homogenous test-strips due to the webbed nature of their overall appearance. During testing, wires connected both the reference and the selective ISE. The ion solution was then sandwiched between the reference and the selective electrode. These results were highly promising. An example of the response is shown in the figure below.

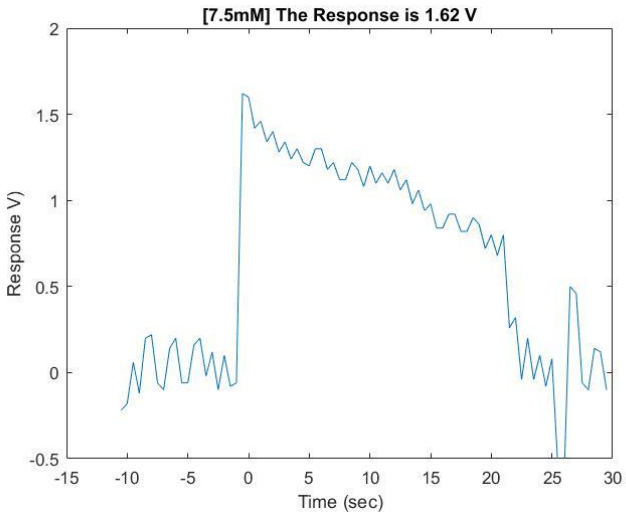


Figure 12: Sample Signal: The standard response of our spin-coated ISE upon encountering an ionic solution. The initial response is large and then decays rapidly over time.

It should be initially recognized that the response at any concentration generates a large peak which then gradually decays as time progresses. This demonstrated a lack of stability on the part of our electrode, possibly indicating a limited amount of PSS- within the conductive polymer layer. Additionally, we were concerned about which portion of the response would generate the most accurate concentration curve. When examining the emf response as a function of concentration it was determined that this initial time point generated the response closest to ideal.

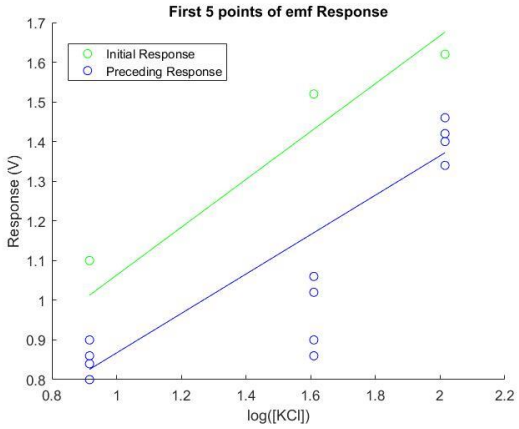


Figure 12: Spin-Coated Electrode Calibration Curve: Green represents the initial response, and generates a calibration curve of ~.06mV. Blue represents the preceding four points and generates a calibration curve of .05mV.

Figure 13 demonstrates the initial response of the spin-coated plate with green representing the first recorded value of the response and blue representing the subsequent four values. The ideal ISE generates a linear calibration curve of response as a function of the natural log of the ion concentration with a slope of .059mV. The initial response, shown in green, was the closest data series to this value and possessed a best fit curve with a slope of .060mV. This lead us to believe that the initial response was the most accurate for use in a calibration curve. However,

work should be undergone to reduce the decay of the response. It is hypothesized that an increase in PSS- concentration may lead to signal stabilization.

Additional tests were undergone to determine the selectivity of the ISE with regards to potassium over sodium. These were performed at significantly greater concentrations than physiological ranges. The results are depicted in the figure below.

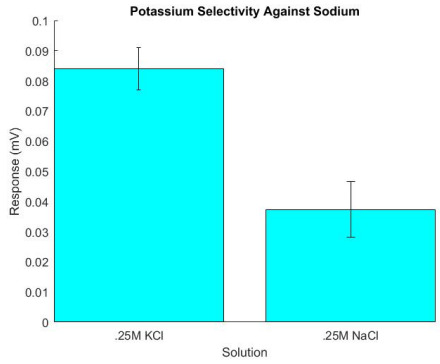


Figure 13: Spin-Coated ISE Selectivity: As shown above, the ISE is selective for potassium over sodium at high concentrations

Although these concentrations are high, they do demonstrate that the ISE is selective for potassium over sodium. Extrapolating from the previously performed thick-film testing, it is estimated that the ISE will demonstrate even greater selectivity for potassium over sodium within solutions of lower concentrations.

The customer appeared to be satisfied with the response time, and is receptive to future attempts to produce this device. Further evaluation should be undergone following improved reproducibility to fully characterize this device. Such tests should examine the impacts of additional ions, temperature, and pH on the EMF response. These will be essential in generating predictions of failure. Additionally, durability, shelf life, and accuracy should be examined. Accuracy and reproducibility are particularly important in generating a safe, effective device. Without these tests this device can not be safely implemented into the field of health care.

Considerations for Further Development:

Manufacturing

Manufacturing of our device is something we have kept in mind during the prototyping phase in order to make our design scalable moving forward. Though we have a proof of concept for our device, changes in the manufacturing of the ISE will certainly change the magnitude, selectivity, and longevity of the device. We must consider three aspects of manufacturing; the case and electronics, the test strip, and the ISE/RE assembly.

For the housing, injection molding is the most common method of manufacturing small, two piece snap or screw fit plastic cases. This manufacturing process requires the creation of a metal mold which fits into a machine that does the injection. The startup process is rather expensive, with many mold quotes coming in at around $13,000-15,000 dollars (Rex Plastics, 2019). However, production is very cheap once the mold has been created. The constraints of injection molding are important to keep in mind, so features like ribbing and curved corners must be included in a final CAD model. The internal electronics of the case will need to be changed from an Arduino to a custom microcontroller and amplifier, and examples of these specifically

design for POC testing are made by Texas Instruments. Finally, the strip inserter will need to be purchased from international retailers or made ourselves. As described before, it accepts a test strip and is often directly mounted to a PCB.

The ISE manufacturing and strip manufacturing are not independent from one another. The ISE consists of two liquid polymeric solutions which are deposited onto a conductive substrate sequentially, with drying steps between. This was the most challenging aspect of the project. There are a lot of variables to deal with, such as solution concentration, layer thickness, and layer adhesion. A common problem we had to deal with was the PEDOT layer cracking and adhesion to the underlying substrate. Moving forward, there are several manufacturing methods which might make the ISE more reproducible. Drop casting, screen printing, spin coating, and inkjet printing are all manufacturing methods currently used in industry. We have experimented with drop casting and spin coating, with mixed results. Inkjet printing is being used more in industry for printing conductive polymers like PEDOT:PSS for photovoltaic applications (Singh, Katiyar and Garg, 2015). Once the layers are optimized and we know the appropriate thickness, the manufacturing method will be chosen or modified. Future considerations also involve whether our ISE/RE will manufactured separately and then embedded in the strip, or if the layers can be deposited directly into the plastic layers of a test strip during production. One opportunity is to use microporous silica nitride chips with trenches to stabilize membrane layers. While we did not focus as a team on this, the McGrath lab is very interested in utilizing the chips as a platform for delicate polymer layers. An image is shown below in Figure 15 which is an example of what this system would look like.

The plastic test strips we have designed serve as a convenient testing platform and a physical object which EMS providers are familiar with. Moving forward for mass production, die cutting might be used instead of laser cutting. In this process, a metal stamp/die is used to punch out precise shapes from plastic film, which can enter the process in roll form. The starting form has a high cost due to the creation of the custom stamp, but afterwards the process is relatively cheap, at around 80 dollars/hour according to Thrust Industries (Thrust Industries, 2019). Screen printing or stencil printing is a method commonly used to print conductive traces, and is something that can be scaled up. A stencil is applied to a base material, and conductive ink is spread over or stamped on the stencil (Metrohm.com, 2019). We imagine this process could be applied to a whole flat sheet of base material before die cutting. A video by Liberty Medical shows their method of manufacturing test strips, and they use a gold coated polyester film which is laser etched into the different traces, which would be another option (Liberty Medical, 2010). Adhesive can be applied to subsequent layers and then they can be die cut as well. Roll to roll manufacturing can be used to bind the layers together. Figure 14 is a hypothetical manufacturing procedure to construct our strip subsystem with inkjet printed electrodes. In the future, additional modifications to the test strip and ISE design might allow for use with a fingerstick. This will involve capillary channels designed into the plastic layers, and might require hydrophilic coatings.

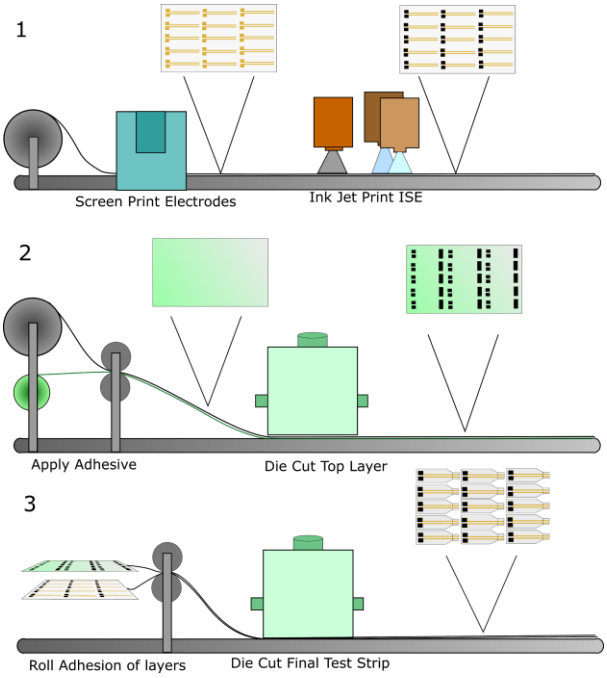


Figure 14: Hypothetical manufacturing process for test strips utilizing inkjet printing of ISE. Utilizes die cutting in different phases to create test strips with ISEs embedded.

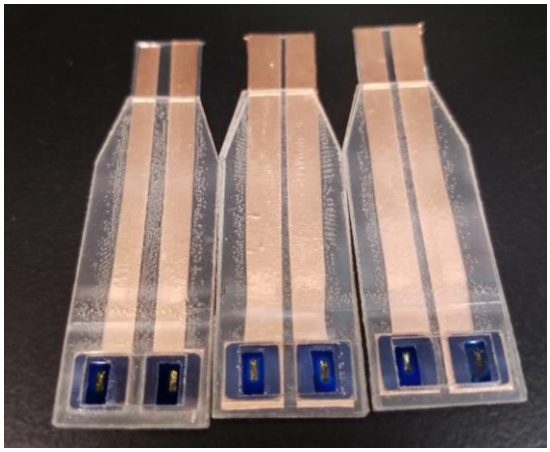


Figure 15: Prototype test strips which have SiN nanoporous chips embedded in them. ISE applied to small window in the center of each chip.

Regulatory

In considering the necessary regulations on devices such as ours, we needed to look at substantially equivalent devices, as there are currently no widely used potassium point of care devices on today's market. These substantially equivalent devices were determined to be glucometers and Abbott's i-STAT system. Both devices are classified as Class II medical devices by the FDA, so it is safe to conclude that ours would likely hold the same classification and therefore, it would be subject to the 510(k) Premarket Notification process (Class 3 Device Recall, 2010;*510(k) Summary, 2006)*. By FDA regulations, there are slightly different constraints between home-use and point-of-care glucose meters (FDA *DiaTribe, 2016)*. For home-use devices, 95% of measurements performed by the meter must deliver glucose values within 15% of the true value. In this case, the "true value" is defined as the glucose value as determined by a comprehensive lab test. In addition, 99% of the measurements performed by these devices must deliver values within 20% of the true value. Point-of-care devices, or devices intended to be used in a professional healthcare setting, are understandably held to a higher standard of accuracy. For these devices, 95% of tests must deliver values within 12% of the true value and 98% of tests must deliver values within 15% of the true value. However, these constraints, put forth in 2016, are simply "guidelines" set in place by the FDA and not necessarily requirements. Nonetheless, devices that perform within this desired range are much more likely to receive FDA approval than devices that do not perform at this level.

There are also a number of testing protocols that devices such as our have been subject to in the past (*510(k) Summary, 2006)*. The four primary testing protocols include the "Within-run Precision Evaluation", the "Intermediate Precision Evaluation", the "Linearity Evaluation

Study", and the "User Evaluation." These protocols are designed to evaluate measurement consistency across meters and test strip lots, precision across handling by multiple users, the accuracy of results across the claimed measurement range, and the functionality of the device while in the hands of potential users respectively. Each of these evaluations should be carefully considered when designing a final device for FDA approval.

Implementation

After obtaining FDA approval for our device, we will need to go through a substantial process to implement our device on ambulances. Focusing on New York State as the primary launch market, we would need a protocol change in order to get our device in the hands of paramedics. Our customer, Dr. Cushman, is one of the most influential medical directors in the state; and he brought this project to us because he wants to implement a protocol chance that would require paramedics to have the capability to measure blood potassium. For this protocol change to take effect, there are many levels of EMS bureaucracy that need to be appeased.

The first step to implementation, after obtaining field data to prove a device's effectiveness, is to have a vote by the NYS REMAC to add a new protocol to the state guidelines. The REMAC is made of medical directors and other prominent members of the state EMS community. This committee created and updates yearly the collaborative EMS protocols that most of the state abides by. Since there are many varying opinions on the committee, if a group does not agree with a protocol there is a significant possibility that a protocol addition may be voted down and have to wait a year to be revisited again. Once the REMAC votes to add a protocol, there are still many more people down the chain that can stop implementation. The medical director of a region may choose not to implement a protocol in their jurisdiction if they do not believe in the update or do not feel like the protocol is relevant for the patient and geographical characteristics of their region. Medical directors of urban areas with hospitals close to most patients may not choose to implement certain procedures due to short transport times and directors in rural areas may choose to give their EMS providers as many protocol tools as possible to keep patients alive until they get to a hospital. Once regional medical directors approve a protocol, the director of an agency decides if the agency is approved to implement the new protocol. Directors who don't have trust in the competency of an agency to utilize a new protocol or feel that the agency does not have the call volume or variety to justify implementation may stop an agency from using new protocols. If an agency's medical director allows implementation of a protocol, the agency operations and training department may have the option to decide in allowing their employees to practice the new protocol. If a protocol is not mandatory to implement and expansive to utilize, an agency may choose not to use the new protocols given to them.

Most of this EMS chain of approval can be moved through without problems when a device has low risk and high benefits. If a procedure is easy to add into care standards with minimum training, has little or no negative effects on patients, and has the potential to improve patient outcomes then it is likely it will be widely implemented. Measuring blood potassium with a form factor that resembles a glucometer would need little additional training, cannot negatively affect a patient unless in the case of an extreme false value, and can save patients from dying of cardiac arrest or incorrect treatment. In this case, it is likely that all levels of the EMS chain would be supportive of protocol change to mandate potassium measurement in all cases where hyperkalemia is a potential differential diagnosis. This support is completely dependent on data that proves our device is fast and reliable in providing information, that our device is affordable

for agencies to implement, and that our device is able to handle the extreme use scenarios of field medicine.

Market Benefits

The primary purpose of this device is the improvement of care and the increased survival rate of potassium-induced cardiac arrhythmias. However, there are some economical advantages to such a device as well. Point of care devices have to ability to lead to more precise triage and patient care decisions (Soremekun, 2013; Lavery, 2000). Although this is primarily important for providing the best care possible, it has the added benefit of decreasing overcrowding within emergency departments, a condition linked with increased mortality and decreased quality of care (Derlet, 2000). Additionally, it has been linked to lost revenue on the part of the hospital and the ambulance companies (Bayley 2005; McConnell, 2013). For this reason, a potassium point of care device has the added benefit of recounting some of this lost revenue for the health care companies involved.

Project Management:

The team set a standard three meetings a week: Monday from 10:00-1:00, Wednesday from 10:00-1:00 and Friday from 12:00-1:00. Meetings with our supervisor typically lasted between 12:30-1:00 on Fridays while meeting with our TA occurred on Wednesday, during our assigned meeting slot. These weekly meeting were shown to be of use, and allowed for frequent updates, intermediary deadlines, and a set time for project advancement. They facilitated discussion among all group members and greatly aided in our progress this semester. Each member of the group was deemed responsible for specific areas of the project. Through such division we were capable of working on several aspects of the project at once to increase progress speed. The total combined hours of the team members is estimated to be around 720 this semester.

The majority of our ISE chemical components were taken from the storage supplies of the McGrath Laboratory. Therefore the majority of our budget was spent on the electrical components of our meter and the physical components of our test strip. The total budget expended on electronics was around $90, all of which was distributed between AD623 Op-Amps, DHT11 Temperature Sensors, LCD Displays, and an Arduino. These electrical components were spread across three prototypes, and a testing apparatus. When manufacturing the test strips the plastic components totaled $50 while the adhesives summed to around $40. Not all of the adhesive or plastics were applied to the final device, but were important steps in the prototyping process. No additional cost was spent on specialized equipment. However, a blade based cutting machine as well as an Epilog Zing laser cutter was borrowed from the McGrath lab for use in the cutting of test strip material. A small spin coating machine was also used for polymer layer experiments.

Table 2: Prototyping Budget

Item	Project System	Supplier	Cost Total
Arduino UNO	Electronic	Borrowed	-
Aruino Nano	Electronic	OOYKE	$13.86
AD623	Electronic	Analog Devices	$31.55
Resistors	Electronic	Borrowed	$5.00
16x2 LCD w/ I2C Breakout	Electronic	LGDehome	$17.99
DHT11 Sensor	Electronic	OctagonStar	$9.96
Tactile Switches	Electronic	Adafruit	$2.50
Wires	Electronic	Borrowed	-
Breadboards	Electronic	Borrowed	-
PCB	Electronic	DIY or Manufacturer	$50.00
SD Card Reader	Electronic	Sparkfun	$9.00
FPC Connectors	Electronic	Digikey Various	$2.00
9V Batteries	Electronic	Borrowed	$5.00
3 Polyester Sheets (24"x48")	Test Strip	ePlastics	$52.75
Copper Foil	Test Strip	Borrowed	-
3D Printed Connector Holder	Case	Rettner	-
Valinomycin (125mg)	Membrane	Purchesed through McGrath Lab	$372.00
THF (1L)	Membrane	Purchesed through McGrath Lab	$60.00
PVC (10g)	Membrane	Purchesed through McGrath Lab	$74.00
KBPh4 (5g)	Membrane	Purchesed through McGrath Lab	$47.00
PEDOT (5g)	Membrane	Purchesed through McGrath Lab	$232.00

Timeline of Completed Events

- Pre-semester~ Selection of Potassium and research into basic ISE function
- January 2019~ Finalization of Initial Interface Design
- Jan 29 ~ Selection of solid ISE over Liquid ISE + clarification of membrane properties
- Jan 31~ Discussion with Dr. Pete Shrager about electrode possibilities
- February 1 ~ Realization that clean room is not necessary, and fabrication can be performed within the McGrath lab
- February 4 ~ Finalization of test strip design with two reference electrodes contained in a microporous chip
- February 6 ~ Purchase of test-strip plastic, request for free sample of pressure sensitive adhesive, and purchase of test strip-device connectors
- February 14 ~ Meeting with Greg Madejski to discuss the manufacturing of the polymer-strip interface
- February 15~ Finished research on Reference electrode layers, finalizes ISE membrane layers, and order final electrode components
- February 18~ Test strip Polymer Materials arrived
- February 22 ~ completion of amplification circuit
- February 25~ Reduction of Initial prototype into an arduino nano
- March 4 ~ Completion of open-faced test strip design
- March 8 ~ Completion of research regarding characterization of ISE's and anticipated response
- March 11~ SD Card Adaptor strip inserter prototype completed

- March 13~ Arduino prototype is deemed incompatible with Orion Probe, and examination of said probe is terminated
- March 14~ Completion of electrical circuit testing
- March 15~ PEDOT/PSS- (the remaining ISE component arrives)
- March 27~ Initial Testing with thick-film, drop-cast ISE layers without use of a reference electrode
- March 29~ Housing for the connection of the strip to the SD card reader is completed
- April 5~ Testing of ISE with increased PSS- concentration and a reference electrode upon the test strip apparatus
- April 11~ Additional testing of test strip apparatus upon alteration of solvent to acetone in an attempt to prevent cracking
- April 18~ Manufacture and subsequent Testing of Spin-coated ISE layers
- April 23~ Application for the Mark Ain Business competition
- April 30~ Mark Ain Business Competition Semi-final Application
- May 2 ~ Housing Interface Prototype Completion
- May 3~ Mark Ain Business Competition Semi-final Presentation

Lessons Learned:

A problem that engineers will have moving forward is the reproducibility of our test strips. Unfortunately, as we have learned from testing, with the ISE solid contact redox capacitance model that we are using, test strips are only reliable for a single test and the thicknesses and volumes of materials used are very important. Thus, it is very difficult to develop a calibration curve when we can not guarantee that all of our strips are identical. This is likely an issue to be solved in the future by an industrial engineer and has been done in glucose test strips, we are just unable to solve this issue with our resources.

A problem that we frequently encountered with our testing was the presence of 60Hz noise when measuring small signals. Even with a 0.6Hz filter, we were still having a large amount of noise attenuated by our amplifier. When designing a device that measures small input signals, it is important to minimize noise using high attenuation filters in order to get any meaningful results.

We also learned that when designing and developing this device, it was very beneficial to divide the design into subsystems. If too many people were working on one aspect of the device at the same time, there is simply not enough work for all team members to do. We chose to assign one team member to each subsystem of our project and this resulted in a very productive design process.

Weekly team meetings were very productive and useful during the design process. These meetings were often used strictly for design and experimentation while memos and the bulk of our major reports were completed outside of the design lab. Meetings promoted highly productive conversation between our team members and also allowed us to set definitive deadlines for assignments and experiments.

Future Recommendations:

First and foremost, additional prototyping with improved manufacturing methods would be essential to any continuation of this project. In order to generate any additional progress in calibrating or testing the ion-selective electrode, each ISE layer or every ISE needs to be

standardized in thickness and homogeneity. This is best performed through a highly regulated and precise manufacturing procedure. Once the ISE components have become reproducible, the ISE can be further characterized through additional testing for selectivity, pH interference, temperature interference, and shelf-life. Additional alterations to the ISE formula may be needed such as a higher PSS- concentration or a thicker PEOT layer to optimize sensitivity or emf response. One method which may assist in stabilizing the layers is the implementation of a microporous chip into the ISE construction. This may prevent delamination or degradation of the ISE layers while applying some reproducibility to the membrane thickness.

For practical implementation of our device, many parts of our electrical design will need to be optimized. The first and most obvious step is to design or use another smaller microcontroller better suited to our device needs. The arduino platform is useful for its easy use and programming, but the microcontroller requires a power source of at least 5V, is a relatively large device for the palm sized form factor we are aiming for, and has much more utility than we will need for this purpose. The next component that needs to be replaced is the instrumentation amplifier used. The AD623 is useful because it is rail-to-rail and can scale a signal up to 5V so we can utilize the full range of arduino input capabilities, but it requires + and - Vcc to operate rail to rail; using a single supply does not allow amplification of small signals properly. An amplifier that can operate with either a single supply or 2 small coin batteries would be ideal for our desired form factor. The screen we are currently using is relatively small (about 1in diameter) because of the limitations of our experience. A larger LCD screen could be easily implemented by an electrical engineer with device design experience in order to make the display easier to read.

Once designs have been finalized for the test strips and electronics that compose the physical measurement device, a casing should be designed to contain all the elements of the system. Our aim is for a case to resemble a glucometer. A custom circuit board and microcontroller should easily be able to fit into a palm sized case with small replaceable coin batteries as a power source. A case should be designed to be durable like glucometers. The device should be able to withstand multiple drops into hard surfaces such as concrete, operate in cold winter and hot summer conditions, withstand body fluids such as blood contacting the device, and be sterilized with bleach wipes without deterioration.

After the test strips and electrodes have been improved to yield consistent interpretable results and the device design has been finalized, pilot testing will need to be done in order to collect data for FDA approval and EMS implementation. Once our device has been FDA approved, we can begin the process of large scale manufacturing and sales.

One interesting point brought up during the Mark Ain business competition was the possibility to license intellectual property for the production of potassium test strips. Companies like Accu-Chek and Liberty Medical already make glucometer housings and glucose test strips. Instead of reinventing the wheel and making another meter, it may be possible to utilize existing meters. Including a special coding strip for the meter which tells the measurement system to return a value for potassium concentration would be a very interesting concept. Then, only one handheld device would be needed and multiple strips for different analytes can be used.

Conclusion:

At the beginning of the semester, KERJ Biologics was tasked with developing a device which could sense multiple whole blood analytes in the field environment. EMS providers in the field have very little quantitative diagnostic information upon which treatment decisions are

based, so such a device would be very helpful to heave. After reviewing biomarkers like glucose, lactate, potassium, and a novel brain injury protein, we decided to pursue the construction of a potassium sensing device. Imbalances in potassium cause dangerous cardiac conditions if not treated timely, so we aimed to develop a quick, portable potassium testing device for whole blood taken from an IV. Initially, we pursued a conventional ion selective electrode design, which uses a half cell selective for potassium ions to generate a voltage. After deciding this design did not fit the needs of a field environment, we pursued a solid contact ion selective electrode design. Our prototyped device contains three systems. One is a meter, similar in form factor to a glucometer, into which a test strip is inserted and a potassium concentration is measured. It contains an amplification circuit, microcontroller, and display. The second part of our design is a disposable plastic test strip which contains the ISE. The ISE is embedded within the strip and consists of a series of polymer layers which converts a potassium concentration into a voltage. A significant amount of time was spent working on a proof of concept, as this required optimization of our layer manufacturing technique. Drop casting and spin coating were used to produce prototypes resulting in the development of a calibration curve. Additional experiments show that the ISE is selective for potassium. This being said, much more optimization in the manufacturing and testing process needs to occur before a finalized device is produced. There are a significant amount of variables and chemistry techniques involved, and testing needs to be done in a variety of conditions with whole blood samples. However, our prototype demonstrates serious proof of concept and the scalable strip based form factor presents an interesting platform to move forward from. It seems possible to bring cheap, effective, point of care diagnostics to the field in the near future.

Acknowledgements:

We would like to acknowledge Mike Klaczko from the McGrath lab for his assistance in the fabrication of our potassium selective membranes. We would also like to acknowledge Kilean Lucas for help cutting strip materials and Greg Madejski for his knowledge on microfabrication processes.

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Appendix: Detailed Figures

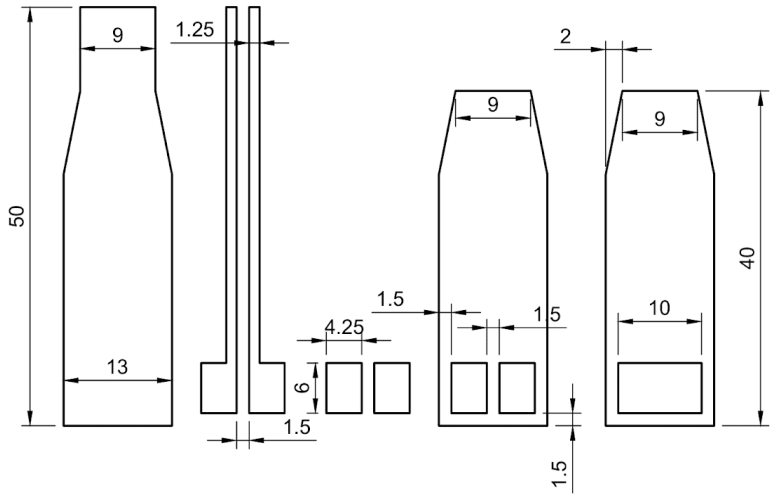

Figure 16: Detailed design drawings of test strips. Dimensions are shown in millimeters. The first and last two layers are those cut out using an Epilog Zing laser cutter. The last two cross sections also have adhesive pre-applied for precise cutting.

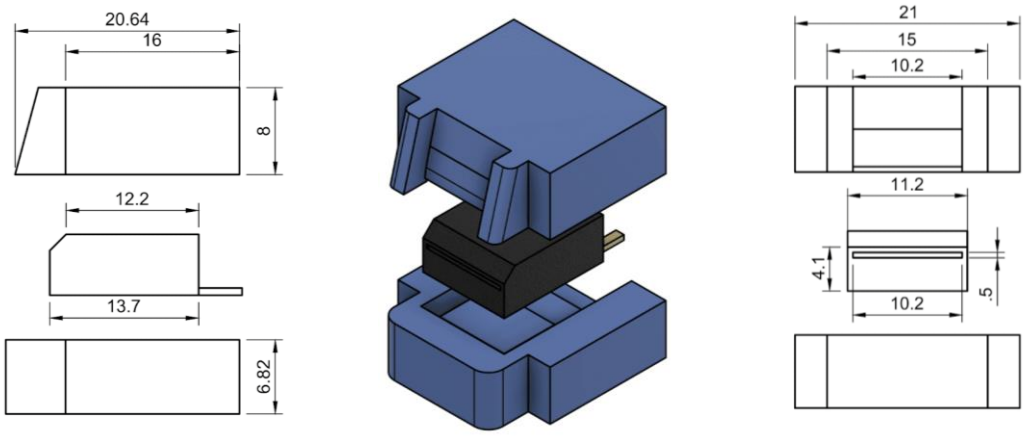

Figure 17: Strip inserter CAD model, dimensions in millimeters. We took a strip inserter from an existing glucometer (black object) and 3D printed a housing for it to make inserting the strip easy.