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# Phosphate Contaminant Detection in Water Through a Paper-based Microfluidic Device

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**SANTA CLARA UNIVERSITY**

Department of Bioengineering

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UNDER MY SUPERVISION BY

Brandon Miura, Alex Wagner, Philip Wu

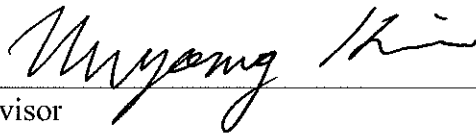
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Phosphate Contaminant Detection in Water Through a  
Paper-based Microfluidic Device

BE ACCEPTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS  
FOR THE DEGREE OF

**BACHELOR OF SCIENCE**  
IN  
**BIOENGINEERING**

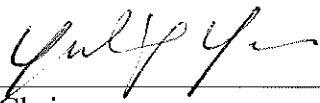
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# **Phosphate Contaminant Detection in Water Through a Paper-based Microfluidic Device**

By

**Brandon Miura, Alex Wagner, Philip Wu**

All students contributed equally to this work

## **SENIOR DESIGN PROJECT REPORT**

Submitted to  
the Department of Bioengineering  
of  
SANTA CLARA UNIVERSITY

in Partial Fulfillment of the Requirements  
for the degree of  
Bachelor of Science in Bioengineering

Santa Clara, California

2016 - 2017

## **Abstract**

This report describes a project aimed at developing a low-cost, portable, on-site, user-friendly system for detecting different concentrations of phosphate in drinking water. Phosphate is a natural chemical, but toxic in large concentrations; detection is therefore important to avoid drinking contaminated water. Despite this fact, no cheap, and/or non-toxic system for phosphate detection is yet on the market.

The detection system utilizes a paper-based microfluidic device to automate the electrochemical detection process, which normally requires expert use of lab equipment. When combined with a portable potentiostat that works with a mobile app, the device will allow untrained users to determine if any source of drinking water contains unsafe levels of phosphate without equipment or training, and to communicate that information to a central database for further analysis. Those of any background, particularly in developing countries, will be able to maintain health and raise awareness about clean water.

Microfluidic devices are useful tools for the detection of water contaminants, but there is a gap in technology for the detection of phosphate. Our phosphate detection system is a paper-based microfluidic device with an already-developed voltammetry device that automates the detection process so that any user can safely find phosphate in water. The system will provide a binary analysis about whether the water is safe to consume or not. Completion of the project provides a valuable tool to both average customers in developing countries and scientific researchers in determining the safety of drinking water.

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Thank you to the Miller Center for Social Entrepreneurship for supporting this project through the Roelandts Family Grant. Additionally, we thank the School of Engineering at Santa Clara University for supporting us financially. Our achievements would not be possible without these generous contributions.

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# Chapter 1: Introduction

## 1.1 Background and Motivation

### *The Dangers of Phosphate Contamination*

Phosphate is an inorganic chemical compound found in agricultural fertilizers. If unregulated, the usage of phosphate eventually leads to phosphate contamination of drinking water [1]. In the United States, the Environmental Protection Agency (EPA) defines the limit for phosphate level in streams and rivers to 100 parts per billion (ppb) [2]. However, water sources in India contain phosphate levels as high as 8,400 ppb [3]. While less immediately hazardous than some better-studied contaminants, too much phosphate ingestion can negatively impact bone and kidney health in the long term [4].

### *Need for low-cost and accurate alternative*

Detecting these dangerous levels of phosphate in water is important, but it is not easy. Since phosphate contamination is most prevalent in developing countries, there needs to be a low-cost and accurate testing method. However, existing methods for finding phosphate rely on expensive equipment and trained lab technicians [5]. These resources are not readily available in rural and low-income areas where large populations may be at risk of ingesting this contaminant. Since water is necessary, our portable detection device will greatly improve the health of these communities because it can test if their drinking water is safe to consume.

Our research expands the water testing technology that would help developing nations improve the quality of water.

## 1.2 Literature Review

### *Research in Microfluidic Devices*

Microfluidic devices are often designed to be implemented in developing areas because of their small-scale size, low cost, and wide range of uses. There have been many microfluidic devices designed specifically for the detection of particular contaminants. While not all microfluidic devices designed for contaminant detection are for water, there is a significant amount of research in microfluidics for water contamination because many developing countries have been identified with having unsafe drinking water. These research findings are discussed below [6, 7, 8].

### *Bioluminescent-cell-based device for copper, zinc, and potassium dichromate detection*

The processes by which microfluidic devices have been designed varies by the target contaminant. For example, a bioluminescent-cell-based microfluidic device was designed to detect copper, zinc, and potassium dichromate in water supplies by using a specific type of cell as the sensor [6]. This approach used a plastic microfluidic chip that had living cells as the main tool for detecting the contaminants. Unfortunately, this microfluidic device is very difficult to design and manufacture because it utilizes living cells. Additionally, the materials necessary to manufacture the device are relatively expensive because of the plastic used and the culture of the cells [6].

### *Paper-based device using a gold nanosensor for arsenic detection*

Another microfluidic device designed for water contamination detection is a paper-based microfluidic device, which uses a gold nanosensor to detect arsenic in water [7]. The device has proven effective in areas suffering from low arsenic contamination. It utilized paper as the main structure of the microfluidic chip, and could be easily manufactured. However, the gold nanosensor leads to a high cost per test, which is approximately \$6.80 for each test for a gold nanosensor from Dropsens (Asturias, Spain).

### *Compatibility of PDMS for project*

A third design was created to test whether poly-dimethylsiloxane (PDMS) can be compatible with microfluidic devices [8]. This was tested by observing the effects of various chemical solutions on the shape and integrity of PDMS. It was found that PDMS, a cheap plastic, was a good material for microfluidic devices since it can resist deformation from chemicals. Although this was a significant discovery for microfluidic devices, the study did not create an application with microfluidic devices.

Even though the first two examples could detect contaminants in drinking water, their high cost and difficult fabrication make them harder to implement. If they could be made at a lower cost, they would be better suited to aid poor families and communities in developing countries. The third example does not directly aid these families and communities either, as it lacks an application geared toward them.

Building off these and other microfluidic device discoveries, our microfluidic device will be usable by anyone and will be able to detect phosphate. Phosphate has only recently become a target for detection.

## **1.3 Project Goal**

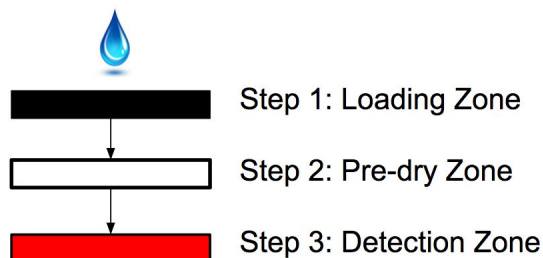
### *Purpose of Project*

The purpose of this project was to build an affordable, portable, accurate, and user-friendly device to detect phosphate concentrations in drinking water. This device can test water samples and contributes to the advancement of providing safe drinking water to people around the world.

The main goal of the device is automate the detection process so that the user will not need to handle any of the hazardous chemicals, but only needs to add a water sample and wait for the result.

### *General overview of process*

The device automates the flow of a water sample through a paper-based device in order to perform the testing of phosphate as shown below.



*Figure 1: Overview of water sample flowing through device*

The water sample (Figure 1: step 1) travels through a paper-device that has been pre-dried with the detection chemical sodium molybdate (Figure 1: step 2). The water sample then travels to a screen-printed sensor. Then the device applies cyclic voltammetry to a three-electrode system (Figure 1: step 3) to determine the phosphate concentration.

The device combines with a miniature potentiostat and a mobile application developed by the Department of Electrical Engineering and the Department of Computer Engineering at Santa Clara University. All three components are portable, affordable, and accurate.

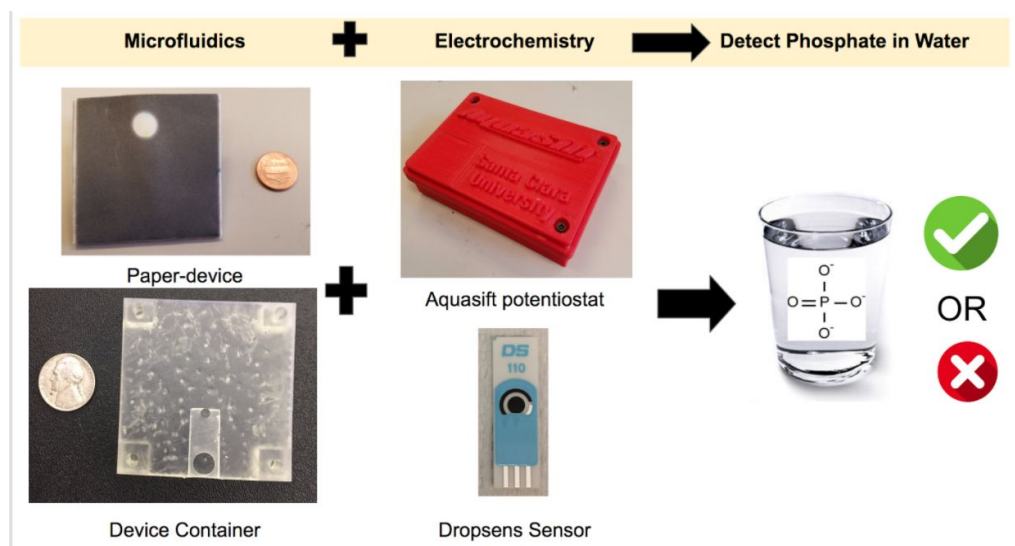


Figure 2: Project goal with components

As Figure 2 illustrates, the device combines microfluidics and electrochemistry to detect the phosphate in water, but does so using a paper-based container and low-cost Dropsens sensor (Asturias, Spain) to keep cost low and quality high. This combination of technology yields an advanced technical solution at a very low price.

## 1.4 Previous and Concurrent Work

This project was a continuation of the Phosphate Detection Project [9] funded by the Roelandts Grant in 2015-2016 school year. This project was now funded by a new Roelandts Grant in 2016-2017 school year. The previous senior design teams optimized and created a proof of concept device to detect phosphate in water sources [9].

This project expanded and carried out the proof of concept with a physical device. In the future, this device could be built upon in order to test for multiple contaminants.

## Chapter 2: Systems Level Overview

### 2.1 System Overview

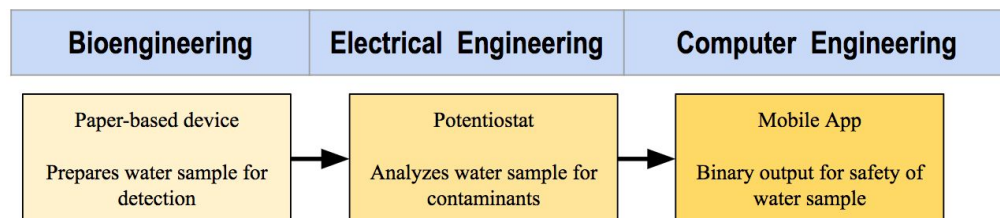
#### *Overview of Subsystems*

There are several interdisciplinary components of the project:

- The paper-based microfluidic device (Bioengineering)
- The Aquasift potentiostat (Electrical Engineering)
- The mobile tracking application (Computer Engineering)

The user-friendly device was built to help public health organizations determine what water sources are safe to drink. This device provides critical information about water quality for those without easy access to this type of technology. If enough communities use this technology, the number of known contaminated water sources will be increased substantially.

The paper-based microfluidic device is comprised of three subsystems: the paper-based device, the device container, and the electrochemical parameters. These subsystems are meant to automate the detection process so that the user does not need to make any measurements or handle any of the chemicals. Essentially, this system will prepare the water sample for the electrochemical parameters system that measures the water sample for unknown phosphate concentrations.



*Figure 3: Block diagram of how subsystems interact with each other.*



## 2.2 Customer Needs Analysis

### *ASSURED Features*

For the device to be implemented effectively, the customer's needs must be considered in the design process. We followed the ASSURED requirements, established by the World Health Organization (WHO) for designing Point-of-Care Diagnostics [10], to ensure that customer needs are satisfied in the developing countries that lack safe drinking water.

The following table lists the ASSURED features:

*Table 1: Project Goals with the ASSURED Requirements*

Requirements	Goal
Affordable	Cost < \$ 10
Sensitive	Detect 0 ppb to 150 ppb (Drinking limit 100 ppb)
Specific	No false positives
User friendly	Minimal training required; easy to use
Rapid and robust	< 30 minutes response time
Equipment minimum/free	Minimal handling of chemicals
Delivered to end users	Portable, handheld

### *How planned device met ASSURED features*

The system was designed to be low cost and to accurately detect phosphate levels between 0 ppb and 150 ppb. This range goes beyond the safety limit of phosphate concentration of 100 ppb set by the WHO [2], in order to reliably detect phosphate levels in drinking water that are health hazards. Additionally, the paper-based device includes all necessary chemicals and prepares the user's water sample required for testing so that there is no training necessary. The system is portable and safe for the customers.

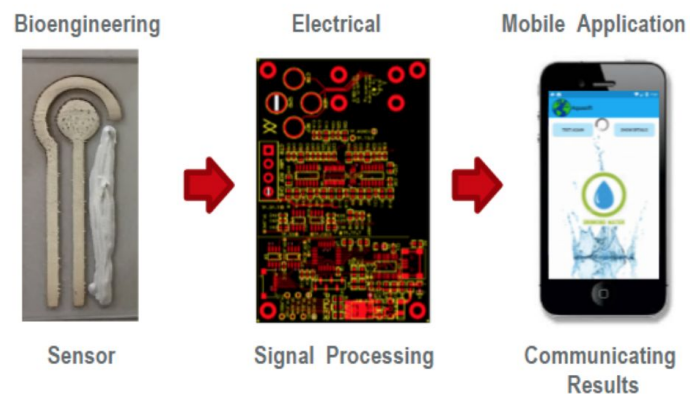
## Chapter 3: Functional Analysis

### 3.1 Functional Decomposition

#### *Overview of steps for system detection*

The main function of our project is to detect phosphate contaminants in water sources and deliver this data to a mobile application to indicate whether a water source is contaminated with phosphate. The product consists of three sections: a bioengineered paper-based microfluidic device, an electrical signal processing unit, and a mobile application. These sections work together to accurately prepare a water sample with the appropriate detection chemicals so the potentiostat measures the water sample.

The following figure summarizes how the three systems interact:



*Figure 4: System Levels Overview*

- The paper-based device interacts with a water sample and prepares it for testing.
- The water sample then interacts with a sensor and sweeps through a range of voltages generated by the potentiostat in the electrochemical analyzer.
  - This generates a corresponding current measured by the sensor and processed in the electrochemical analyzer.

- This data is translated into information communicated to a mobile application on a local phone, which indicates to the user if the water sample is safe to consume.
- The mobile application documents the locations of both contaminated and safe water sources.

## 3.2 Performance Requirements

### *Projected Goals and Objectives for Device*

Our project had several main objectives that we hoped to achieve to overcome the limitations of the more commercially available microfluidic devices. These are outlined in the table below:

*Table 2: Projected Goals and Objectives*

Goal	Objectives	Results	Comment
<i>Safe-to-Use</i>	<ul style="list-style-type: none"> <li>● No need for handling of chemicals</li> <li>● Detection chemicals pre-dried on paper to minimize chemical handling</li> </ul>	Successful	Overall safe to use – user does not need to handle any chemicals.
<i>Accurate</i>	<ul style="list-style-type: none"> <li>● Detect a lower limit of 50 ppb of phosphate</li> <li>● Replicate lab-setting tests within a 5% range</li> </ul>	Successful	Detects down to 50 ppb of phosphate.
<i>Paper-based</i>	<ul style="list-style-type: none"> <li>● Paper-based components to make it low cost</li> </ul>	Successful	Transports water through the paper device.
<i>Portable</i>	<ul style="list-style-type: none"> <li>● User can carry device around in their hand</li> </ul>	Successful	Device is smaller than a tablet.
<i>Disposable</i>	<ul style="list-style-type: none"> <li>● Device can be safely disposed of after usage</li> </ul>	Further research	Sensors need to be replaced for each test.
<i>Affordable</i>	<ul style="list-style-type: none"> <li>● Device will cost less than \$5.00/test</li> </ul>	Successful	Further research could reduce this cost more.

### *Expected outcomes:*

We expected that the final device would be: accurate, portable, safe-to-use, and paper-based, and affordable. An easily disposable device was a desired goal; however, given that each detection test required a new sensor, this goal was less achievable since discarding the sensor is not environmentally friendly. We also hope that further research

and improvements can be made to the system in order to drive down the overall cost of the detection system.

Our project provided a valuable tool to both average customers and scientific researchers in determining the safety of drinking water.

## Chapter 4: Team and Project Management

### 4.1 Experimental Challenges

In order to run an accurate test, our device was expected to work similarly to running the experiment in a lab. There were a few challenges that we had during experimentation, which are listed below.

#### *Challenge 1: Optimizing pre-dried chemical concentrations*

This challenge involved optimizing the pre-dried chemical conditions to control the concentrations of each chemical. This was our most difficult challenge. Additionally, it was very difficult to figure out ways to begin testing, and was expected to become more difficult if our experimental ideas were insufficient. Based on previous lab tests, it was known that the solution necessary for detecting phosphate must have a certain concentration of sulfuric acid and sodium molybdate. In order to obtain accurate readings from our system, we had to find the optimal amount of each chemical to dry onto our paper-based microfluidic device in order to get the correct final solution for the sensor.

Our team planned to run preliminary tests with Whatman paper with chemicals pre-dried on it to gather information about how much of each chemical we were obtaining when water was added to the paper. From this data, we expected to find relationships between the volume of the chemicals dried with the amount of chemicals obtained as well as between the concentration and its effects on the accuracy of the concentration detection.

#### *Challenge 2: Wax ink melting duration*

Our next challenge came after the printing of the paper-based microfluidic device. Since the design was printed onto paper using wax ink, the channels were only defined on one side of the paper. Because of this, water could flow below the ink walls. To fix this the

device was placed on a hot plate and heated in order to melt the wax through the thickness of the paper. It was found that leaving the device on the hot plate for too long would melt the wax into the channels and the channel design would be ruined. Therefore, it was a challenge to find the optimal melting temperature and duration in order to only melt the wax through the thickness of the paper and not across the channel design.

Our team ran separate tests for heating by adjusting the temperature or the time. These tests were expected to show what conditions were best for melting the wax ink through the full thickness of the paper.

### *Challenge 3: Accuracy and Sensitivity*

Our last main challenge was maintaining accuracy and sensitivity. To detect phosphate between the concentrations of 0 ppb to 150 ppb, we had to ensure that our testing process was working accurately even as the concentrations increased. Additionally, the results from the AquaSift device were expected to differ by a constant value between the microfluidic device and normal lab equipment.

In order to combat this challenge, our team experimented with discrepancies between tests for a large set of experiments. This allowed us to analyze the data and make sure that we were able to maintain accuracy over a wide range of phosphate concentrations. There was not a significant change in relationship between increasing concentration and the resulting increase in detected phosphate by the AquaSift device.

Table 3 below is a summary of the risks and mitigation strategies involved in the project.

*Table 3: Risks, Consequences, and Plan of Action*

*The table lists risks from least impactful (top) to most impactful (bottom).*

<b>Risk</b>	<b>Consequence</b>	<b>Plan of Action</b>
<ul style="list-style-type: none"><li>• Hazardous chemicals</li></ul>	<ul style="list-style-type: none"><li>• Potential toxic threat to eyes and skin</li></ul>	<ul style="list-style-type: none"><li>• Wear proper lab safety gear (gloves, lab coat, eye glasses).</li><li>• Follow all safety guidelines when working with chemicals</li></ul>
<ul style="list-style-type: none"><li>• Inconsistent data from different paper-based microfluidic designs</li></ul>	<ul style="list-style-type: none"><li>• Return to brainstorming phase, re-optimize testing procedures</li></ul>	<ul style="list-style-type: none"><li>• Use good laboratory practices to have consistent testing procedures</li></ul>
<ul style="list-style-type: none"><li>• Microfluidic design cannot be used due to chemical drying conditions or size that can be printed</li></ul>	<ul style="list-style-type: none"><li>• Return to brainstorming phase for the microfluidic design.</li><li>• Create new design and redo chemical pre-drying optimization</li></ul>	<ul style="list-style-type: none"><li>• Careful pre-planning of device design and preliminary pre-drying testing to get an estimate set of conditions necessary for optimal testing solution</li></ul>

## 4.2 Management

### *Overview of Team Roles*

Our team was committed to making tangible progress on a weekly basis. The three main participants, Brandon Miura, Alex Wagner, and Philip Wu, met once per week with our advisor, Dr. Ashley Kim. We shared and discussed the latest results and ideas for refining the device's design, as well as established weekly goals. Additionally, the three main participants met in the lab at least once per week to perform the necessary tests for the project.

We took charge of a particular component of the design process:

- Alex focused on designing new iterations of the wax and paper components.
- Brandon handled the designing and 3D printing of plastic components of the device.
- Philip focused on testing these designs to determine if each component worked as intended. He also focused on the pre-drying aspect of the device.

The team stayed in close collaboration through weekly meetings, continual communication, and regular consultation with research advisor Dr. Kim. This ensured that the features of the plastic components complemented the paper components, and any team member could contribute to any part of the design process if needed.

### 4.3 Timeline

This project started October 2016. The Gantt Chart below shows the project timeline.

Table 4: Gantt Chart of Project Timeline

Task	Owner	Month									
		Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	
Submit grant proposals	Alex, Brandon, Philip										
Preliminary design presentation	Alex, Brandon, Philip										
Research microfluidic device fabrication	Alex, Brandon, Philip										
Brainstorm and test initial designs	Alex, Brandon, Philip										
Conceptual design review	Alex, Brandon, Philip										
Optimize wax printing protocol	Alex, Brandon, Philip										
Optimize pre-drying conditions	Philip										
Optimize device-design (paper & plastic)	Alex, Brandon										
Brainstorm and test device features	Alex, Brandon, Philip										
Conclude testing	Alex, Brandon, Philip										
Complete final thesis	Alex, Brandon, Philip										
Senior design conference	Alex, Brandon, Philip										

The most crucial components and goals, such as the optimization of the pre-drying conditions and the device design, took the most time to complete. Additional testing could be done following the completion of this project in order to optimize the device even further.

### 4.4 Project Budget

The following budget accounts for the cost of producing and testing many iterations of the design and therefore includes large quantities of lab materials.



Table 5: Project Budget

Item	Use	Unit Cost (USD)	Quantity	Total Cost (USD)	Source
DropSens DRP-110 Sensors	For testing (75 in one order)	310	5	1550	MetrOhm
Whatman paper sheets	For paper-device (100 sheets in one order)	56	2	112	GE Healthcare Life sciences
Sodium molybdate	Buffer solution for dissolving phosphate	44.6	1	44.6	Sigma-Aldrich
Sulfuric acid	To adjust Ph for test conditions	23.6	1	23.6	Sigma-Aldrich
Phosphate Standard (100 mL)	For calibration and tests	44.2	1	44.2	Sigma-Aldrich
Xerox ColorQube 8580 black Solid Ink (1 cartridge)	For printing	154.99	1	154.99	Xerox
Pipette tips, disposable lab tools, etc. (Variable)	For testing	43.8	Variable	43.8	Sigma-Aldrich
			Total:	1973.19	
Shipping, handling, & tax	For price fluctuation, shipping & handling, and tax			42.32	
			Supplies total:	<b>2015.51</b>	
			Amount Requested:	<b>2294.00</b>	

Although the Project Budget seems significantly higher than the intended cost of the device, all of these expenses led to discovering an ideal device design. Projected costs for the production and testing of at least fifty design iterations are included. The final device, including highly reusable components, costs under \$10; accounting for reuse, the total cost per test is under \$5.00, as seen in Table 6 on page 30.

## Chapter 5: Subsystem: Paper-device

### 5.1 Whatman Paper Choice

#### *Whatman Paper chosen for Device*

Paper-based microfluidic devices use capillary action, which allows liquids to travel through paper naturally, in order to control the flow through the device. Typically, these paper-based microfluidic devices are cheap to manufacture but only work with liquids. These qualities are optimal for our device because we aim to manufacture it at a low cost and only plan to use it for water.

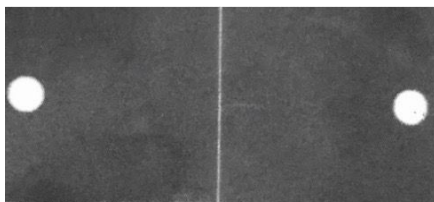
The material choice for this paper-based component was Whatman paper from GE Healthcare Life Sciences (Buckinghamshire, United Kingdom) because of its optimal flow rate property and its low cost (\$56.00 for 100 sheets or \$0.58 for each sheet). On each sheet, we can print 8 paper devices. Since Whatman grade 1 Chromatography paper has a flow rate of 130 ml/min and a nominal thickness of 180  $\mu\text{m}$ , a water sample can travel through the paper relatively quickly. Our design for the paper device optimizes the flow of water through the device.

#### *Choice of Double Layer for Paper*

A double layer device was chosen because it allows water to flow through the device and increases the surface area that interacts with the water. Merely increasing the concentration of the pre-dried sodium molybdate had little effect on the testing. In Figure 5 below, the white area is the paper area while the dark area indicates where the wax melted on the paper device. Since some of the water is absorbed by the paper or “lost” within the device, the diameter of the circle paper was chosen to best ensure that enough water sample reaches the sensor at the end of the device. The dimensions of the paper itself were designed to be small to make the device portable.

### *Printing the paper-based device*

We printed this design using the Xerox ColorQube 8580 printer and wax ink. We then placed the paper device on a hot plate to ensure that the wax could melt through the paper. The black area on the paper device indicates the melted ink. In the figure below, we display the double-sided paper-device that we pre-dried on for experimentation.



*Figure 5: Paper-based device*

## **5.2 Pre-drying sodium molybdate**

### *Choice of pre-drying*

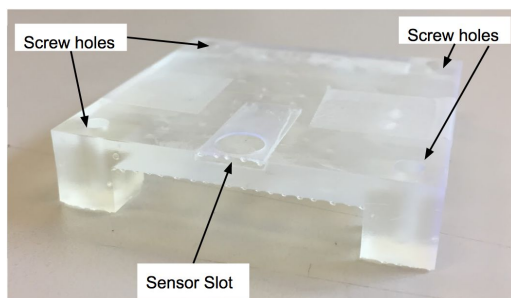
Since safety was a crucial goal of our project, we decided to pursue a pre-drying method in order to store the chemicals within the device itself. In pre-drying, a paper is soaked with a certain volume of liquid, then allowed to dry over a beaker overnight. This provides a sufficient amount of time for the liquid to evaporate, leaving a solid on the paper. This paper can later be rehydrated, pulling off solid particles from the paper. This entire process ensured that the chemicals were incorporated in the device, instead of being added manually by the user, which could present a hazardous exposure to chemical contamination.

### *Pre-drying with sodium molybdate*

One of our detection chemicals is sodium molybdate, which is very important in carrying out the reaction necessary to detect phosphate (see Section 7.2 for more information). In pre-drying for our project, we pipetted a drop, 75 $\mu$ L in volume, with a 50 mM concentration of sodium molybdate onto the white circle of our paper device. We would rehydrate this area with our water sample later.

## Chapter 6: Subsystem: The Device Container

Because we did not want the user to interact with water sample or detection chemicals, the paper needed to be stored within a container. The device container ensures that the user does not touch the water sample, helps store the paper-device, and automates the testing process.



*Figure 6: View of Device Container*

### 6.1 Substrate Choice

#### *Design of Device Container*

For the device to hold the water, the material needed to be sturdy but also chemically inert so that it would not react with any of the water sample or detection chemicals. We chose resin for its durability, light weight, and chemical inertness.

We designed the container in this fashion in order to be of equal height to the potentiostat, and to be of equal width and length to the paper device as mentioned above. In order to ensure that the user would not contact the water sample while it sat on the sensor, we designed a slot for the sensor.

### 6.2 3-D Printing Process

The device container was designed using SolidWorks CAD software. Based on measurements of the potentiostat and paper device, the STL file of the container was created and taken to Santa Clara University's Maker Lab to be 3-D printed. The 3-D

printer used was a resin-cured printer made by FormLabs. This printer was used due to its high-resolution options which were necessary in order to have an accurate slot for the sensor. Lower quality prints resulted in erroneous sensor slots; the sensor either did not fit or had too much space around it, which led to leaking of the water sample.



*Figure 7: Resin-cured printer by FormLabs*

Once the container was printed, it was cleaned using isopropyl alcohol ( $> 90\%$ ) and compressed air in order to clear off any uncured resin. It was also sanded down to clear off the supports generated while printing. The container was set aside in the sunlight in order to let the resin finish curing, which occurs when resin is exposed to UV light.

### **6.3 Container for Sulfuric Acid**

Since acid is toxic and hazardous to handle, we needed to ensure that the user will not touch the acid. However, since high concentrations acid cannot be stored on a piece of Whatman paper, we decided to store the acid with a holder printed with resin. We used a resin-printed device as a storage unit for the acid because of its chemical inertness.



*Figure 8: Top part of the device container*

## Chapter 7: Electrochemical Parameters

Many of the electrochemical parameters were researched and determined by the previous senior design group [9]. This system summarizes many of their findings that were relevant to this project.

### 7.1 Electrode Choice

A sensor is composed of a three-metal-electrode system that is used in voltammetry testing. The three electrodes are called counter, working, and reference, referring to their function. The figure below illustrates the detection process.

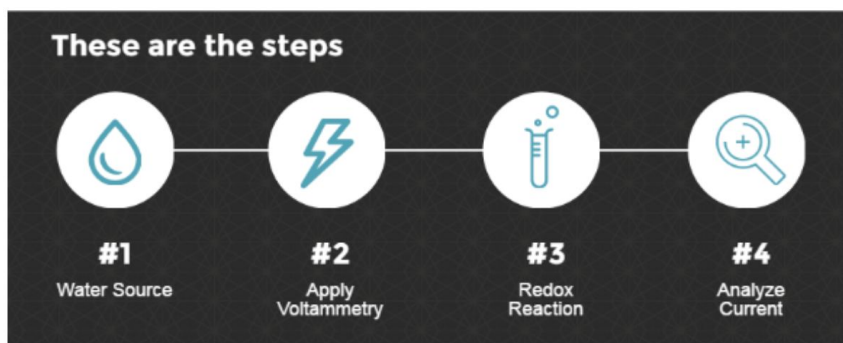


Figure 9: A simple overview of the electrochemical principles

Figure 10 explains the specific voltammetry process that occurs during testing for a three-metal-electrode system or sensor.

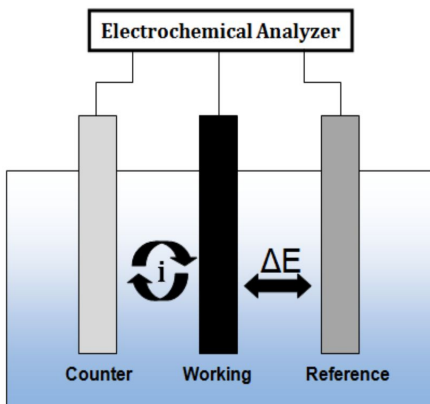


Figure 10: Three-Electrode Design

Within the system, the working electrode sweeps through a range of potentials with respect to the reference electrode (Figure 10,  $\Delta E$ ). When a reaction occurs at different potentials in a solution (Figure 10, I), electrons will flow, and the counter electrode allows the current, or flow of electrons, to be measured. For our project, the electrochemical analyzer was a separate unit, called the potentiostat or the Aquasift.

The chosen sensor (which contained the three-electrode system) for this project was the DRP-110 sensor from Dropsens (Asturias, Spain) which has a silver reference electrode, carbon working electrode, and carbon counter electrode. These three chemicals were chosen for their chemical-inertness, and conductive nature to respond to electrical signals. They were also selected for their affordability.

## 7.2 Redox Reactions

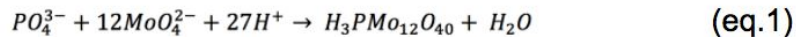
### *Oxidation-Reduction (Redox Reaction)*

For the sensor to accurately measure phosphate concentrations, an oxidation-reduction, or redox, reaction must occur. In a redox reaction, electrons flow from one species to another; the oxidized species loses electrons while the reduced species gains those electrons.

### *Reaction Tracked*

Since phosphate is a non-electroactive species, it needs to react with molybdate in an acidic environment to form a molybdophosphate complex [11]. The complex reacts to cyclic voltammetry by allowing different current levels to flow through the system at different potential values. The current variation results in a graph characterized by two peaks in the IV curve. Each peak corresponds to a step in the reduction of molybdenum (Mo), from Mo (VI) to Mo (IV) and from Mo (IV) to Mo (II), which corresponds to peak 1 and peak 2 respectively [12]. These two reduction peaks were analyzed in our results (Section 8.5).

This reaction allows the phosphate levels to be indirectly measured.



*Figure 11: Reaction between Phosphate and Sodium Molybdate*

## 7.3 Buffer Solution

In order to determine if a solution does not contain any phosphate, a buffer solution needed to be selected to serve as a sample that did not have any contaminants. Since the complex mentioned above needs an acidic environment, the buffer solution has sulfuric acid ( $H_2SO_4$ ) to drive the pH down to 1.011. Additionally, the solution has sodium molybdate ( $Na_2MoO_4$ ) as the main component because it makes phosphate electro-active as discussed above in section 7.2 [12].

## 7.4 Voltammetry

### *Cyclic Voltammetry used for testing*

Another important parameter of the electrochemistry pertains to voltammetry, which includes the type of voltammetry, the voltammetric window used, and the scan rate. In order to see the current peak from the reduction of molybdenum, cyclic voltammetry is employed from a potential of 0.3V to -0.3V and back to 0.6V.

### *Voltammetric window and scan rate for detection peaks*

These two potentials frame the voltammetric window, which ensure that the peak heights are identified in the reduction of molybdenum. These peak heights correlate to the level of phosphate in the water sample.

The rate at which the voltages are applied to the system is also important. This is known as the scan rate of voltammetry. Higher voltage scan rates result in higher current peaks, in which larger concentration differentiation at low concentration levels occurs. This method provides more accurate and sensitive results for phosphate concentrations.



However, applying voltages too quickly can cause current saturation. The scan rate used for this test was 900 mV/s based on summer research conducted by Philip Wu.

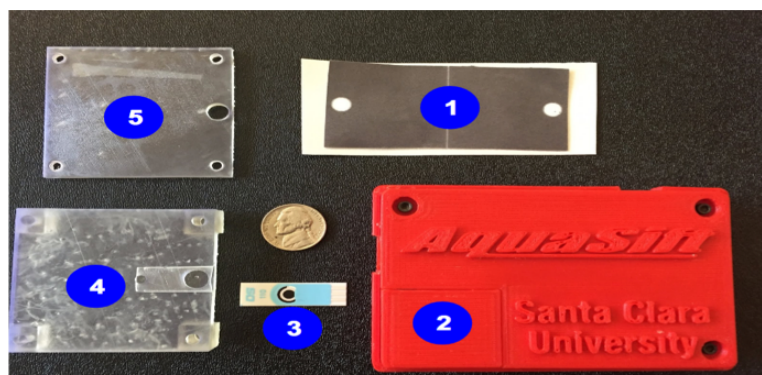
## **7.5 Wait Time**

The amount of time that the phosphate is allowed to mix with the molybdenum in the acidic buffer is also important for the electrochemical detection of phosphate. This ensures that the reaction occurs between the chemicals. After waiting thirty minutes to let the reaction occur, a voltammetry test can be run. This wait time is important to ensure that every phosphate compound is used in the reaction described in section 7.2.

## Chapter 8: Test and Results

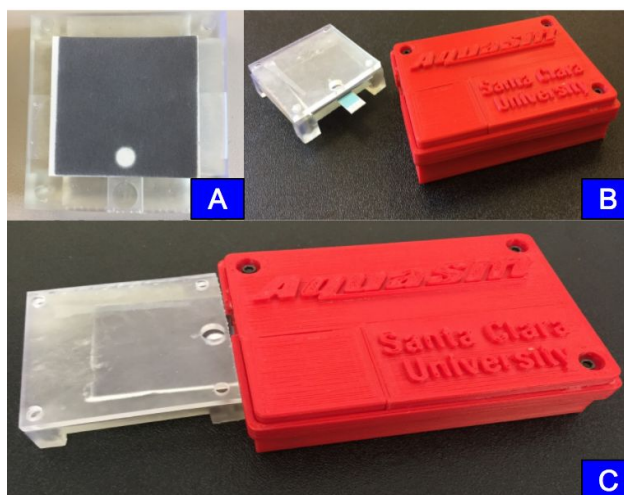
### 8.1 Assembly

In order to have a functioning system, all three subcomponents need to be coupled with a portable potentiostat. The following figure gives a simplified and actual assembly of the system.



*Figure 12: Individual components of the device:*

1. Paper device, printed with wax ink.    2. Aquasift voltammetry device.    3. Dropsens sensor.  
4. Device Container (bottom)    5. Device container (Top)



*Figure 13: Assembly of Device.*

- 13a - Paper device placed on top of device container; 13b - Device container and paper device assembled;  
13c - Paper-based device along with Aquasift potentiostat

## 8.2 Equipment

### *Solutions*

To conduct the tests for the detection of phosphate levels in drinking water, three solutions were used: phosphate standard, sodium molybdate, and sulfuric acid.

- Phosphate Standard (Phosphate Standard for IC- 38364) was purchased from Sigma Aldrich (St. Louis, MO) at a concentration of 1000 mg/L and was diluted with deionized water to the desired concentrations.
- Sodium molybdate (23465) was purchased from Sigma Aldrich (St. Louis, MO) in an anhydrous powder form as a source for molybdate.
  - It was dissolved in deionized water to the desired concentration of 50 mM.
- Sulfuric acid was purchased from Sigma Aldrich (St. Louis, MO) at a pH of 1, and diluted to a concentration of 0.5M.

### *Potentiostat and Sensor*

- The potentiostat device that both ran voltages through the system and measured the current flowing through the sensor was built by the Department of Electrical Engineering.
- The screen-printed carbon electrode sensor (DRP-110) used to apply voltage to the water samples and to measure the current was purchased from Dropsens (Asturias, Spain).

## 8.3 Experimental Methods

### *Amount of water sample for testing*

The detection of phosphate was carried out using a specific set of protocols.

Concentrations of phosphate (some with buffer or 0 ppb, others with different concentrations of phosphate from 50 ppb to 150 ppb) of 200  $\mu$ L volume were added to the top compartment of the device that holds the acid container.

The selected volume of water sample was chosen so that a desired final volume of 100  $\mu\text{L}$  arrived at the detection zone when the water sample flowed through the device. The rest of the procedure was automated. Water sample traveled through the device and pre-dried area as shown below:

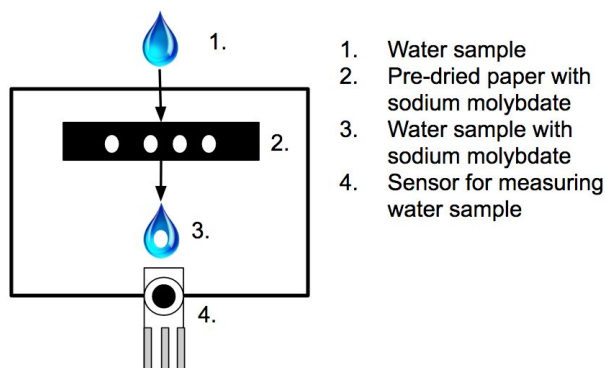


Figure 14: Schematic of pre-drying and water channel flow

#### *Electrochemical parameters for testing*

After waiting for thirty minute incubation period, the water sample should be in contact with the circular detection zone of the sensor. The potentiostat, connected directly to the sensor, applies a potential of 0.3 V to -0.3 V with a negative initial scan polarity at a scan rate of 900 mV/s. The resulting reduction peaks are measured, analyzed, and correlated to a specific level of phosphate as a current value as shown below in an ideal current vs. concentration curve.

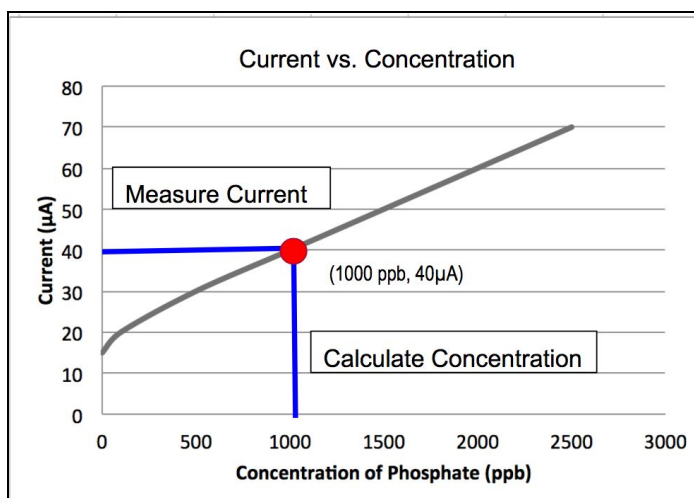


Figure 15: Ideal current vs. concentration graph

## 8.4 Control test: Non-pre-dried testing

In order to ensure that our device matches accurately with an in-lab setting, a series of non-pre-dried tests were run. These experiments were meant to be a control test as a comparison for our device. Phosphate concentrations were prepared in a 10 mL beaker supplied by the Santa Clara University Bioengineering labs. The water sample was pipetted onto the Dropsens sensor attached to the Aquasift as shown in the figure below:



*Figure 16: Non-pre-dried control test*

The results of the non-pre-dried test are discussed in the following section (Section 8.4).

## 8.5 Comparison of Results

### *Device testing*

In order to assess if the manufactured device is able to detect phosphate, we added varying concentrations of phosphate solutions to the device, and generated a voltage and current curve from the testing. The average current signal of each concentration level was taken, and converted into a graph of concentration compared to current. This linear regression line enabled us to calibrate a standard curve and determine the phosphate level of unknown concentrations. The figure below shows the comparison between the non-pre-dried and pre-dried testing:

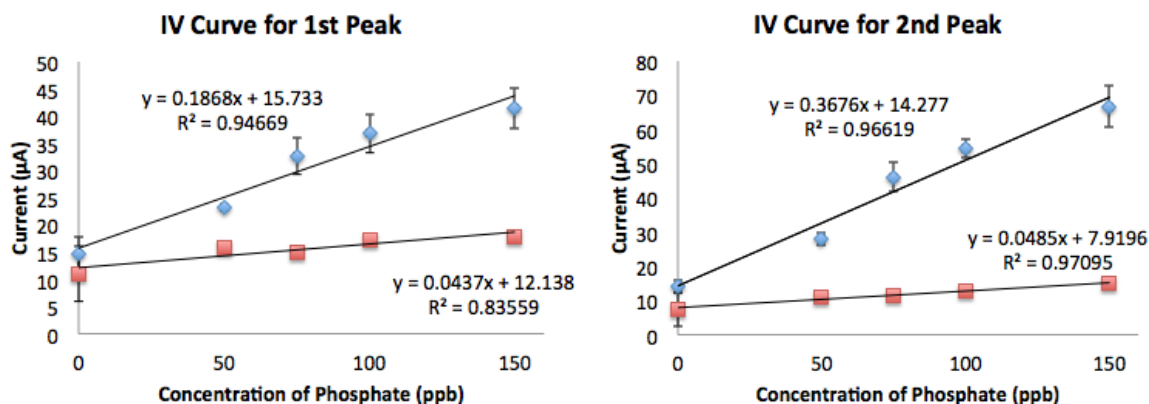


Figure 17: Comparison of current vs. concentration for pre-dried test (red) and non-pre-dried test (blue) for 1st and 2nd reduction peaks.

### Pre-dried vs. Non-pre-dried test

Directly comparing the slope of the pre-dried test to the slope of the non-pre-dried test, both have linear regions from 0 ppb to 150 ppb, and match fairly closely to each other. The same trend of increasing concentrations and increased current is seen for both the pre-dried and non-pre-dried tests.

The pre-dried device had higher values, as indicated by the steeper slope of 0.1868 for the 1st peak and 0.3676 for the 2nd. This higher current value may have resulted from how the phosphate sample was prepared within the device. However, this higher value means that the device is more sensitive and a good predictor of phosphate in a water sample. Both tests resulted in a  $R^2$  close to 1, indicating a high degree of fit to the line.

### Measuring an unknown phosphate concentration

With an unknown phosphate concentration sample, we can read a current signal numerical value from the potentiostat. This value correlates to a concentration level using the equation of this line. This is how the unknown phosphate concentration is determined. The process is illustrated in Figure 18 below.

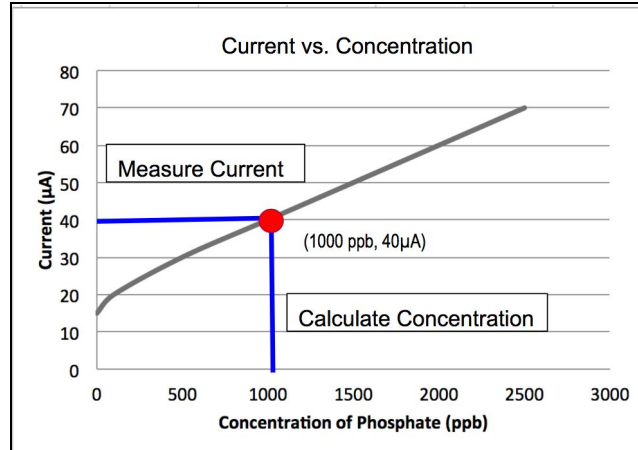


Figure 18: Calculating an unknown concentration of phosphate

### *Unexpected Results*

During our experiments, we were able to determine some parameters and unexpected outcomes. In some of our experiments, we observed a blue liquid on the sensor. This indicated that another form of reaction was occurring. This blue color detection method is often used for colorimetry [13].

It was not erroneous, but not necessary to indicate the reaction is completed. Residual ions or leftover phosphate from previous tests also had a minimal effect on our data points.

## Chapter 9: Cost Analysis

### 9.1 Cost of Device for Developing Countries

The cost of each voltammetry test must be less than \$5.00 to be considered affordable.

The cost of our device is outlined below in the following table.

*Table 6: Breakdown cost for one device*

Item/Test	Cost
Aquasift Device (Multi-usage)	\$70.00
Device Container (Multi-usage)	\$7.00
Sensor	\$4.53
Whatman Paper	\$0.07
Wax Ink	\$0.01
Total cost	<b>\$81.61</b>
Cost per measurement (reused for 1000 tests)	<b>\$4.69</b>

*The total cost accounts for the price for buying each unit. The cost per measurement accounts for the fact that the Aquasift Device and the Device container are multi-usage items and can be used for over a hundred or theoretically even a thousand tests.*

### 9.2 Commercialization Potential

This platform will significantly reduce the cost of phosphate detection since typical lab testing exceeds \$400 in California [5]. This product can be sold at its manufacturing cost to developing communities or non-governmental organizations that will help distribute the device. This product has the potential to be integrated into a larger platform that can test for other contaminants such as arsenic, nitrate, and cadmium that utilize voltammetry. Although no conclusive research has been conducted, there only needs to be minor adjustment of voltammetry tests and the pre-dried paper to implement a multi-detection device. Future groups will complete the required research and design a business plan for this integrated platform.



## **Chapter 10: Professional Standards and Constraints:**

### **Clinical Impact**

#### **10.1 Science, Technology & Society**

The goal of this project was to develop a portable, affordable device that can be combined with a potentiostat, a sensor from DropSens, and a mobile application to allow for testing of drinking water samples. Testing the water sources within communities that are economically disadvantaged, the public community can be informed about how safe their drinking water is.

However, there are potential consequences of implementing this design: if there are no safe water sources nearby, the community may be forced to travel longer distances to find healthier water to consume.

Despite these possible negative results of this proposal, the benefits of clean water for these communities would significantly outweigh the adverse health effects of unclean water and greatly benefit the developing communities.

#### **10.2 Economic Impact**

Affordability was our main economic consideration for the development and design of the device. A resin container with a paper-based device inside was the best and most economical solution. Hence, Whatman Chromatography paper was the appropriate choice, based on its functionality and affordability, especially when compared to PDMS, which is more commonly used for microfluidic devices but more costly than paper. Furthermore, the design of our channels was kept simple to facilitate manufacturing and keep production costs at a minimum. The overall cost of the device is affordable by any community that might benefit from it.

### **10.3 Health & Safety**

Health and safety of the user are of the utmost priority. The pre-drying technique and the device container for the paper-device prevents the user from touching the toxic detection chemicals. The user will likely have no previous experience with hazardous chemicals, and will need to be informed about how to handle the device.

### **10.4 Usability**

Usability is a key feature of the phosphate detection because the users could have varying backgrounds in education and skill level. We considered our users to be largely non-English speaking with minimal background in operating devices. To have an extensive impact, we designed the device to be user friendly and intuitive in function. The user simply needs to add a water sample in the device to start the testing process and press on button to start the testing. The casing of the device will clearly show directions, mainly using images, to demonstrate the process.

### **10.5 Sustainability and Environmental Impact**

Current colorimetric methods used for phosphate detection involve using large volumes of toxic chemicals. Hazardous spills and contamination could result if the device is not handled properly. To prevent any spilling, all the chemicals used in detection are stored within the device. This aspect of the product makes it more environmentally and user friendly than current testing methods.

However, the Dropsens sensors are single-use only and must be disposed of after one test. This is a concern for sustainability and the environment as the product will be generating waste. However, the sensors are designed with non-toxic materials such as glass, carbon, and silver. The users will need to be educated on proper disposal of the sensors after testing.

## **10.6 Civic Engagement**

Since this product is intended for developing countries, it must pass the regulatory water standards in developing countries. In the United States, there are many municipal governments that monitor public water sources. These include the U.S. Environmental Protection Agency and the Center for Disease Control and Prevention at the national level. Locally, Santa Clara Valley Water District [14] refers residents to Alpha Analytical Laboratories, Inc. to get private drinking water analyses. Public water source records of contaminant levels at water treatment plants are available online.

# Chapter 11: Ethical Analysis

## 11.1 Ethical Justification

*700 million people with no access to clean water*

More than 700 million people do not have access to clean water [2], and are forced to drink unsafe water that results in adverse health effects and even death. By providing a rapid, accurate, and easy-to-use method to continuously monitor water quality, these areas will be able to identify contaminants in the water and take the necessary steps to address the issue.

Certain engineering virtues must be used for this device because our users should have the best product possible with the most accurate results from the testing. Our approach was the most beneficial for a broader community, and could help minimize harm due to unclean water.

## 11.2 Engineering Virtues

There are certain technical and professional ethical factors that are imperative and required for our project. These are critical for professional engineers; we have considered three areas for this project:

### **1. Right to clean water**

The United Nations has stated that “clean drinking water and sanitation are essential to the realization of all human rights” [15]. If the detection of a hazardous contaminant such as phosphate is ignored, it is not possible to ensure that a person’s drinking water is completely safe, denying them an essential right.

## **2. Equal access to technology**

Access to technology that promotes health and human rights should be as widely available as possible so that it may benefit as many people as possible. And yet, current phosphate detection methods are too costly for everyone to access them. More remote, less privileged people deserve the same ability to assess water safety those with lab equipment or abundant resources have.

That is why a key component of this project is the low-cost production of our device. Since unsafe drinking water is most prevalent in the most disadvantaged communities, affordability remains a necessity. The use of paper and resin will ensure that those who need this technology most will be able to afford it. This project aims to be usable by anyone, especially those with contaminated water sources.

## **3. Environmental concerns**

Rising population leads to rising agricultural and mining activity, which in turn leads to more phosphate in water. It is not highly surprising that a previously ignored water contaminant such as phosphate would reach dangerous concentrations now, when the health of the environment is being negatively impacted in many other ways by human activity or natural causes. It is an increasingly important responsibility to combat the decline of environmental health. Devices that raise awareness of where pollution is greatest, and that have eco-friendly or biodegradable components of their own, are important steps toward this goal.

## **11.3 Stakeholders**

The development of this device impacts many groups: not only the communities that use the device, but also the developers and manufacturers.

### *Users*

The users will directly benefit from the device because they will use it determine if their water is safe for drinking purposes.

### *Manufacturers*

The manufacturers will benefit financially by mass producing the devices. However, if they should build faulty devices or use incorrect materials for the device, the manufacturers will experience negative effects such as a hurt reputation, and will discourage people from using this product despite its beneficial potential.

### *Developers*

The last group of stakeholders is the group of developers themselves. Since they created the device, they must carefully conduct various tests to ensure functionality, accuracy, and ease of use for the device. These tests verify the proof of concept proposed by the previous senior design project, and ensure that it functions reliably and accurately. If these tests are not properly conducted, the user of the device runs the risk of drinking unclean and toxic water. This outcome affects the reputation of the developers and could prevent them from pursuing future endeavors to improve this water detection technology.

## **11.4 Ethical Challenges and Tradeoffs**

### *Challenges for Project*

Throughout this project, we considered the challenges that could arise. In order to minimize risk to the developing communities, we designed our device to be as intuitive as possible. This would be addressed by providing manuals on the specific instruction of how to use the device and a clear outline of the hazards that must be mitigated. These instructions will include simple graphic instructions for users with limited technical knowledge.

### *Fulfillment of ASSURED requirements*

The product satisfies the ASSURED requirements mentioned earlier in order to be an ethical device for the users. To fulfill those requirements for our device, we had to make several tradeoffs. For example, we utilized paper and the pre-drying method so the detection chemicals could be easily stored within the device and prevent the user from spilling or touching the hazardous chemicals. These were some of the tradeoffs used to help enhance the performance and maintain environmental standards.

Since the public is most likely to be affected by the risk of using this product, they need to understand the risks and provide their informed consent to use the product.

## **11.5 Conclusion of Ethical Challenges**

After careful analysis of the ethical benefits and risks of our device, we determined that it is safe to use in the field through our accurate testing of the device. The product will benefit many communities while the benefits of the device outweigh the small potential for risks. These benefits include: affordable cost, more accurate testing methods, rapid testing time, improvement for health, and improved sanitation methods for developing regions.

## Chapter 12: Project Summary

### 12.1 Conclusions & Future Work

#### *Project summary*

Microfluidic devices are useful tools for the detection of water contaminants, but there is a gap in technology for the detection of phosphate. In order to address this gap, our team designed a phosphate detection system that is inexpensive, portable, on-site, and user-friendly. The combination of a mass-producible paper-based microfluidic device with a potentiostat automates the detection process so that any user can safely find phosphate in water. The project provides a valuable tool to both average customers and scientific researchers in determining the safety of drinking water.

This project demonstrated how this portable, paper-based device could accurately measure phosphate concentration values from 0 ppb to 150 ppb. We designed it to cost less than \$5.00. However, we hope to drive down the cost even further in the future.

#### *Future considerations for device improvements*

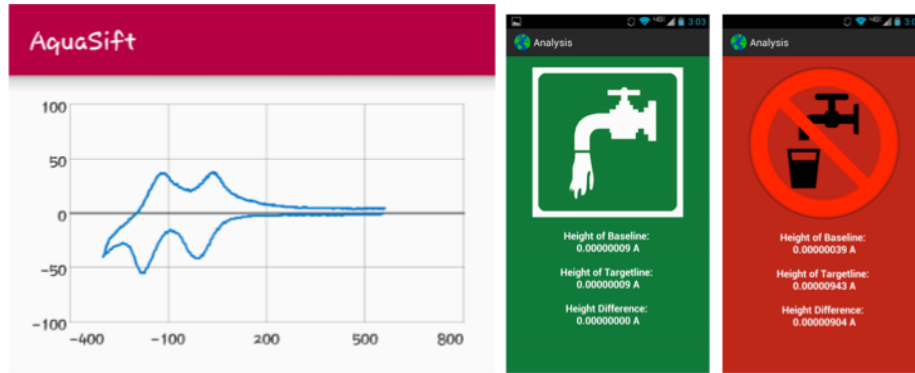
We are already considering further improvements, beyond this project's current scope, that could be made to the phosphate detection system in the future as part of other projects. For the paper-based microfluidic device, additional channels and chemicals can be included for the detection of contaminants besides phosphate, such as arsenic. This will allow our device to detect multiple contaminants in water so that customers do not need to purchase more than one detection system.

Further, for the mobile app that connects with the AquaSift device, an online map could be generated using the results that people get around the globe. This map could be used to show the areas that have the most phosphate contamination and be beneficial for further



phosphate detection research. The mobile app will output a binary output for the safety level of the drinking water.

The following figure displays a proposed app to be used with device and the Aquasift unit.



*Figure 19: Mobile application that will be used with device and Aquasift unit*

As we have shown, the development of a low-cost phosphate detecting device was successful, and demonstrates the capability for extending this technology to other water contaminants, giving developing countries the same control over the purity of their water supplies as enjoyed by advanced economies.

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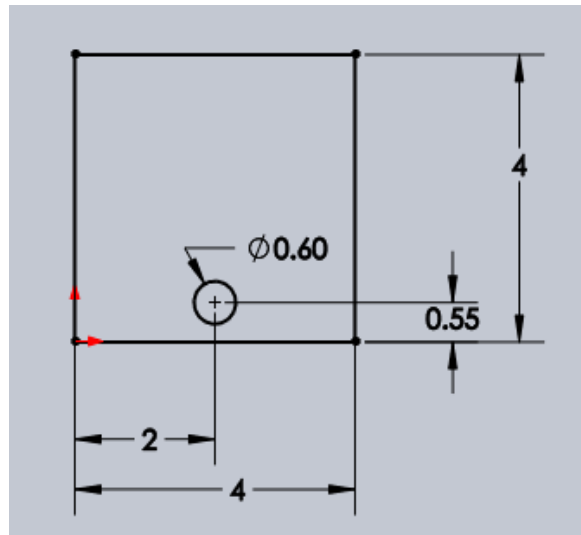
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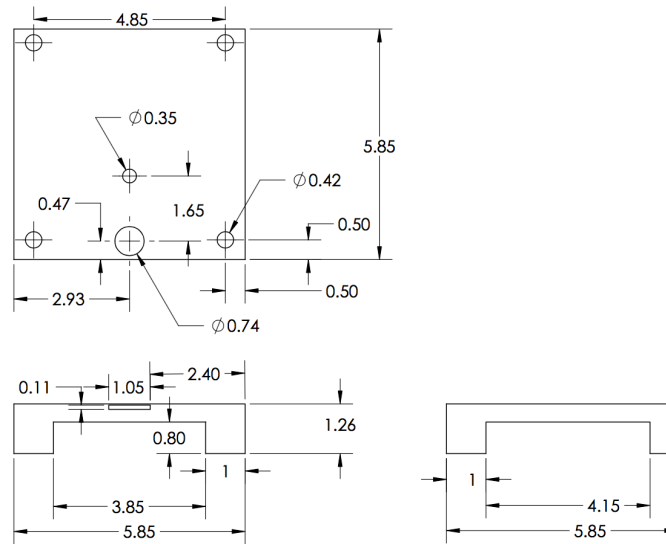
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## Appendix A-1: Solidworks Paper device design

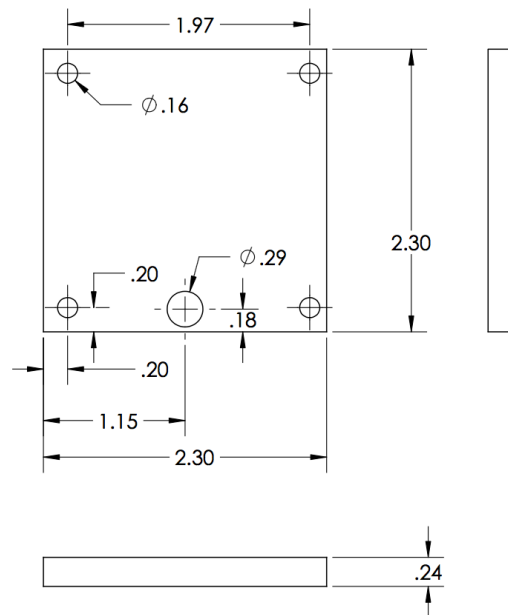


*Solidworks dimensions of paper-based device (Whatman paper)*

## Appendix A-2: Solidworks Device Container Design



*Solidworks dimensioning of Device Container with sensor slot (bottom)*



*Solidworks dimensioning of Device container (top)*

## Appendix A-3: Budget

### *Project Budget*

Item	Use	Unit Cost (USD)	Quantity	Total Cost (USD)	Source
DropSens DRP-110 Sensors	For testing (75 in one order)	310	5	1550	MetrOhm
Whatman paper sheets	For paper-device (100 sheets for one order)	56	2	112	GE Healthcare Life sciences
Sodium molybdate	Buffer solution for dissolving phosphate	44.6	1	44.6	Sigma-Aldrich
Sulfuric acid	To adjust Ph for test conditions	23.6	1	23.6	Sigma-Aldrich
Phosphate Standard (100 mL)	For calibration and tests	44.2	1	44.2	Sigma-Aldrich
Xerox ColorQube 8580 black Solid Ink (1 cartridge)	For printing	154.99	1	154.99	Xerox
Pipette tips, disposable lab tools, etc. (Variable)	For testing	43.8	Variable	43.8	Sigma-Aldrich
			Total:	1973.19	
Shipping, handling, & tax	For price fluctuation, shipping & handling, and tax			42.32	
			Supplies total:	<b>2015.51</b>	
			Amount Requested:	<b>2294.00</b>	