

PORTABLE GLUTEN BIOSENSOR

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Approved by:

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Dear Dr. Riley,

This letter is to inform you of the completion of the Project Report detailing the engineering design of a biosensor for the detection of gluten, which is enclosed within. This design project was commissioned by you for the ABE 498a course at the University of Arizona Fall 2008. The design problem was to develop a biosensor that could be used in a restaurant setting and detect levels of gluten in food that would be harmful for individuals with celiac disease. This project report details the design process used for the development of the sensor, as well as how the completed design meets the original objectives and specifications.

To develop a biosensor for the detection of gluten, the sensor was broken down into three essential processes: processing of a food sample, addition of reagents to the substrate, and analysis of the sample. Each of these components were investigated and researched, and multiple solutions were developed. An optimal solution was determined for each process, which were then integrated into a cohesive final device for commercial use.

For the final result, a crescent blade is chosen for processing. Syringe pumps are used to move and dilute the sample and present the detection chemical (TMB). A reciprocating pump will be used to flush and clean the entire device during and between uses. The optimal design for the analysis portion is a method similar to the Enzyme-Linked ImmunoFlow Assay (ELIFA) technique. A color change caused by the ELIFA technique is quantified and then converted into a signal that can be read by the user through a system of LEDs.

In conclusion, our resulting final design meets most needs specified in the original design problem, as well as the design specifications. Our completed design is portable and able to detect gluten in foods within the ranges that would be harmful to people with celiac disease. It is easy to use, requiring no technical background, as well as being reusable. Certain components would have to be replaced and refilled, however for repeated use. The current cost of construction, \$598.66, is higher than the initial constraint of \$250, but it is estimated that a mass produced product will cost lower than the price of the prototype. The cost for the replaceable items was \$4.45 for each use. Overall, it is recommended that the prototype be completed and tested because estimations were used in the design which actually need to be determined experimentally.

Sincerely,

Kevin Miller

James Nimlos

Jeremy Williams

TITLE: Portable Gluten Biosensor

ABSTRACT: A small portable biosensor device of (40cm × 20cm × 15cm) was designed for the use of detecting gluten in food above the potentially dangerous level of 20ppm. A crescent mixer was used for processing and milling in the device in order to grind the sample into a homogenous liquid state. A 40% ethanol (v/v) mixture is then used to extract the soluble gliadin portion for analysis. The gluten solution would be transported through a flow-through ELISA system, where gliadin particles will become immobilized. The chromogen 3,3',5,5' – tetramethylbenzidine (TMB) will then be run through this system, producing a color change that can be read optically. The optical sensor detected gluten up to 20ppm which is put into a series of op-amps and translated to a binary response with a system of two LEDs. The cost for the prototype device is estimated to be \$598.66, although this would be reduced in mass production of the device.

KEYWORDS: Gluten, Biosensor, Portable, ELISA, Celiac Disease

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STATEMENT OF ROLES AND RESPONSIBILITIES: The overall design and prototype could be broken down into three different sections: blending/mixing of the food sample, a fluidics system to transport material between the sections and measure out precise volumes, and the detection of the gluten content and output circuit components. Kevin Miller was responsible for the design of the detection method and output circuit, as well as the construction of these parts in the prototype device. He was also responsible for testing these components of the device. Jeremy Williams responsibilities included design and construction of the fluidics portion of the device. James Nimlos was responsible for construction and design of blender portion of the device. Each member was also responsible for presenting their portion of the device in various presentations as well as writing their corresponding portion of the paper.

Contents

Introduction.....	1
Statement of the Problem.....	1
Background.....	1
Overview of Project Report.....	1
Project Goals.....	2
Original Design Specifications.....	2
Approach.....	2
Particle Reduction and Homogenization.....	6
Pump/Pipeline Design.....	8
Analysis Component Design.....	14
Estimates and Calculations.....	18
Experimental Calculation of Mixing.....	18
Experimental Calculation Pipe Flow.....	18
Calculations of Dual Syringe Pump.....	19
Pump Calculations.....	19
Sample Standard Curve.....	20
Testing of Light-Source LED & Photodiode.....	21
Testing of ELISA Flow-Through Method and Development of Preliminary Standard Curve.....	23
Determination of Path Length.....	24
Absorbance Analysis and Determination of Output Voltage.....	25
Calibration of Output Signal.....	25
Material List.....	27
Economic Analysis.....	28
Results.....	28
Technical Description.....	28
Analysis Segment.....	29
Final Design Specifications.....	31
Testing and Calibration.....	31
External Constraints.....	31
Safety Considerations.....	32
Operation.....	32
Conclusions.....	32
Recommendations.....	33
References.....	34
Appendix A: Moody Chart.....	36
Appendix B: Equations.....	37
Appendix C: Cole Parmer Valve Catalog.....	39
Appendix D: Motor.....	40
Appendix E: TaosInc TSL13S-LF LTV Chip.....	41
Appendix F: EZ Gluten- Gluten Detection Kit.....	45

List of Figures

Figure 1: Design Approach for Project (JCW)	3
Figure 2: Design Schematic Flow Chart	4
Figure 3: Design Schematic	5
Figure 4: Prototyping Design	8
Figure 5: Regimes for transport of solids through pipe (re-drawn from Liu, 2003)	9
Figure 7: Dual Syringe Pump	11
Figure 8: Syringe Pump Design	11
Figure 9: Cad Picture of Syringe Pump	12
Figure 10: Geared Ratio of Syringe Pump	12
Figure 11: Reciprocating Pump from Edmund Scientific	13
Figure 12: VDC Precision Micropump	13
Figure 13: ELISA Method	15
Figure 14: Processing circuit of output voltage	16
Figure 15: Flow Cell equipped with screen printed carbon electrode	17
Figure 16: Standard Curve: Graphical Representation of Data from Immunotech	20
Figure 17: LED Data	21
Figure 18: LED Data 2	22
Figure 19: Preliminary Standard Curve (Test 2)	24
Figure 20: Inverting Op-Amp Setup	26
Figure 21: (1) Conventional ELISA. (2) Flow Through ELISA. (3) ELIFA	29

List of Tables

Table 1: Standard data for gliadin standards (Immunotech, 2008)	20
Table 2: Flow Through ELISA Test Results	23
Table 3: Design Cost Analysis	27

List of Equations

Equation 1: Classification of slurry flows	37
Equation 2: Shear velocity for slurry flows	37
Equation 3: Friction factor	37
Equation 4: Pressure Gradient of psuedohomogeneous flows	37
Equation 5: Slurry/mixture Viscosity	37
Equation 6: Mixture Density	37
Equation 7: Stokes Setting Equation	37

Introduction

Statement of the Problem

Celiac disease (CD) is an autoimmune disorder that causes an adverse reaction to gluten in the body. A reliable and portable device or product for the detection of gluten in foods would greatly benefit individuals suffering from CD and help them to safely maintain a relatively gluten-free diet. According to multiple studies, a gluten-free diet is characterized by one that lacks the consumption of wheat, rye, and barley products (Liu, 2003; CSA, 2008). This does not necessarily exclude other similar food products, e.g. oats, as there is possible gluten contamination or similar gluten-like proteins (Perry, 1973; CSA, 2008). The danger is the ubiquitous use of these three primary sources in various common food categories consisting of breads, pastas, and doughs. Gluten proteins are found in many other food products as binding agents such as frozen dinners, sauces, salad dressings, and soups. The gluten is used to hold flavors or create viscosity in these food products. The product will be used to timely detect gluten in common foods or public settings to prevent accidental gluten over-consumption. Currently, there is no product available to accomplish this without significant amount of time and laboratory techniques. The device will be consistent with recent studies to identify foods which contain more than the safe maximum dosage, 20 ppm of gluten. This represents 20 parts gluten to a million parts of any type of food. This applies to all amounts of food eaten because higher amounts of gluten content cause problems with digestion in people suffering from celiac disease.

Background

Gluten is a major component of wheat products, consisting of the proteins gliadin and glutenin, which are conjoined with starch. Celiac disease is an autoimmune disorder that causes sensitivity to gluten and an inability to properly digest it. As no cure or treatment for celiac disease is available, afflicted individuals must avoid ingesting gluten by limiting their diet to gluten-free foods or food products low in gluten. Varying degrees of gluten sensitivity are found in individuals with celiac disease, but the WHO and the UN have classified gluten-free foods as products containing gluten concentrations no more than 20 parts per million (ppm) (CSA, 2008). This is generally accepted as the lower threshold of concentrations safe for individuals with celiac disease.

Because people with celiac disease must avoid gluten-containing products, there is a need to test foods for their gluten concentration. Even foods marketed as gluten-free often have higher than acceptable gluten concentrations, as the FDA has not yet set a standard in the United States. Additionally, because of the ubiquity of gluten, contamination is common in gluten-free foods, and foods that people would expect to not have gluten, such as ketchup, often contain gluten as a thickening factor. The need to test for gluten in foods is especially relevant in restaurants, where nutritional information is not readily available and where gluten contamination is common. Gluten testing kits are available, but contain multiple components and are not convenient to a restaurant setting.

Overview of Project Report

This report details the process used for the design of the portable gluten biosensor, as well as the final optimal solution found. The original design problem and background are first detailed, followed by an explanation of the approach for a solution. A technical description of the final design and final design specifications are provided then an overview of the operation, external constraints, and an

economic and material analysis of the final product. Finally, a critique of the final design is provided based on how well the product fits the need and the original design specifications, along with a recommendation on how to proceed further.

Project Goals

The purpose of this project was to develop a design for an effective solution for helping individuals with celiac disease determine if certain foods are safe for them to eat, i.e. have acceptably low levels of gluten concentration. This problem was solved by the development of a potentially commercializable sensor for the detection of gluten in foods, primarily for use in a restaurant setting.

Original Design Specifications

The device must be able to detect gluten in food products at concentrations as low as at least 20 parts per million, and must provide a qualitative response to the user on the level of gluten present. It must also be easy to use and require no background knowledge of science. The ability to process a variety of food products, at least pastas, breads, and gravies, and test them for the presence of gluten is also required. Additionally, the device should be compact and portable, ideally the size of a cell phone. It should cost no more than \$250, and be able to run off of a rechargeable battery or easily replaceable batteries.

Approach

We first approached this design project through identification of the design problem and the subsequent design specifications. The WHO (World Health Organization) and UN (United Nations) have determined that for foods to be classified to be “gluten-free” and therefore safe for consumption for individuals with celiac disease, these foods must contain a gluten concentration of no more than 20 ppm. For this reason, we determined that our sensor must be able to detect concentrations at least this low. Additionally, since this sensor would be used primarily in a restaurant setting, it was decided that the sensor must be portable, ideally the size of a cell phone, and require no external power source. Commercially available gluten detection kits were found consisting of several components and processes. Based on these currently available products, we decided that our sensor should combine and automate the processes of these kits and be able to handle the processing of the food sample, addition of reagents, analysis of sample, and provide an output signal to the user. The sensor was divided up into these three processes: food processing and mixing, mass transfer, and analysis. Each team member was responsible for heading the design of different major portions of the design: James Nimlos the food processing portion, Jeremy Williams the mass transfer solution, and Kevin Miller the analysis section.

The entire device is connected by pipes sealed with valves and has a simple flow from beginning to end. The user will first be adding water and placing the small amount of sample food into the first homogenizing chamber where a crescent bladed blender is used, where a 40% ethanol mixture (v/v) is used for extraction. The gliadin portion of gluten becomes soluble in the alcohol fraction. After the sample is thoroughly mixed, a system of syringe pumps will extract the correct amount of the gliadin containing alcohol, as well as the correct amount of buffer containing antibody/enzyme. The two solutions will then be mixed in the pipe system, and then flowed through the flow-through ELISA assay and into the waste. A different syringe pump will take an amount of TMB and flow it through the flow-through compartment and into the cuvette for analysis. In the last step, the detection solution is analyzed for color change using an LED and light detection device and the signal is transferred to an OpAmp that will be calibrated to reach the zener voltage of a zener diode for a sample of 20 ppm gluten, providing a voltage regulator that differentially allows a green LED to be

either on or off and be seen by the user. After deciding on these specifications, we examined different alternative solutions for each process

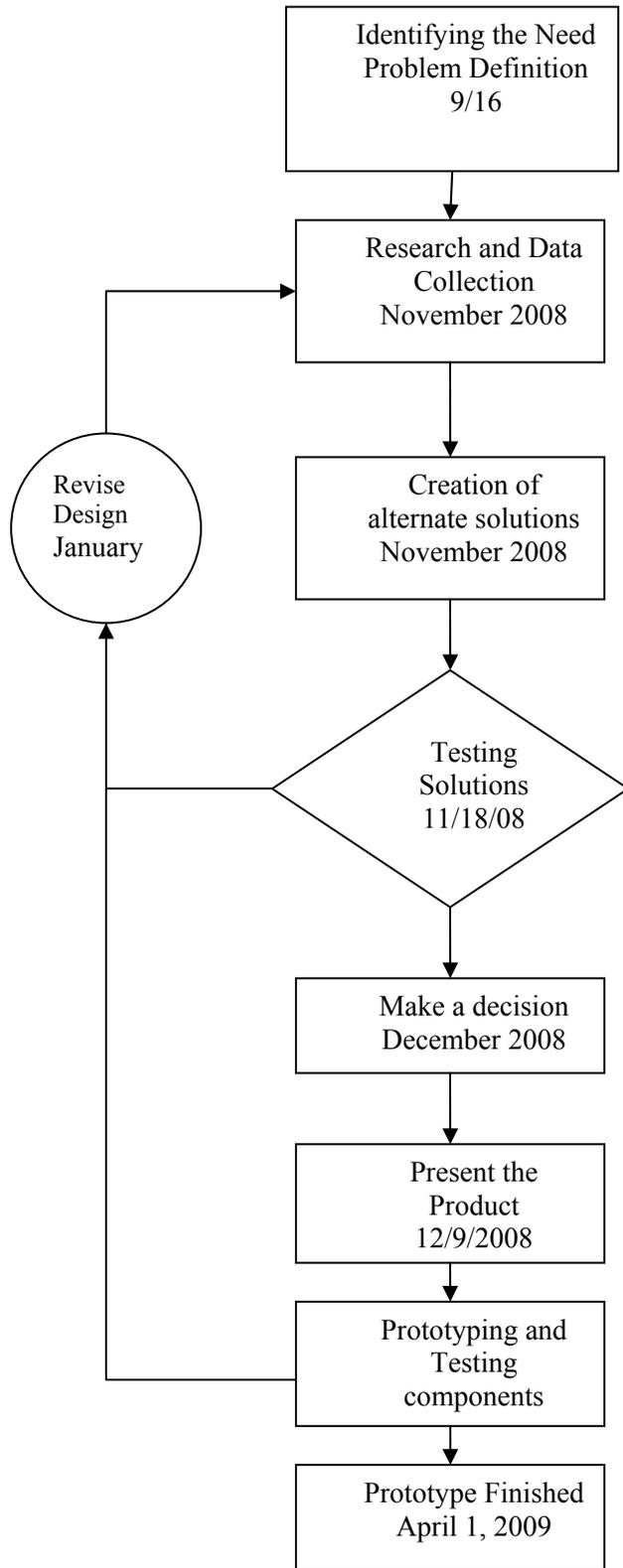


Figure 1: Design Approach for Project (JCW)

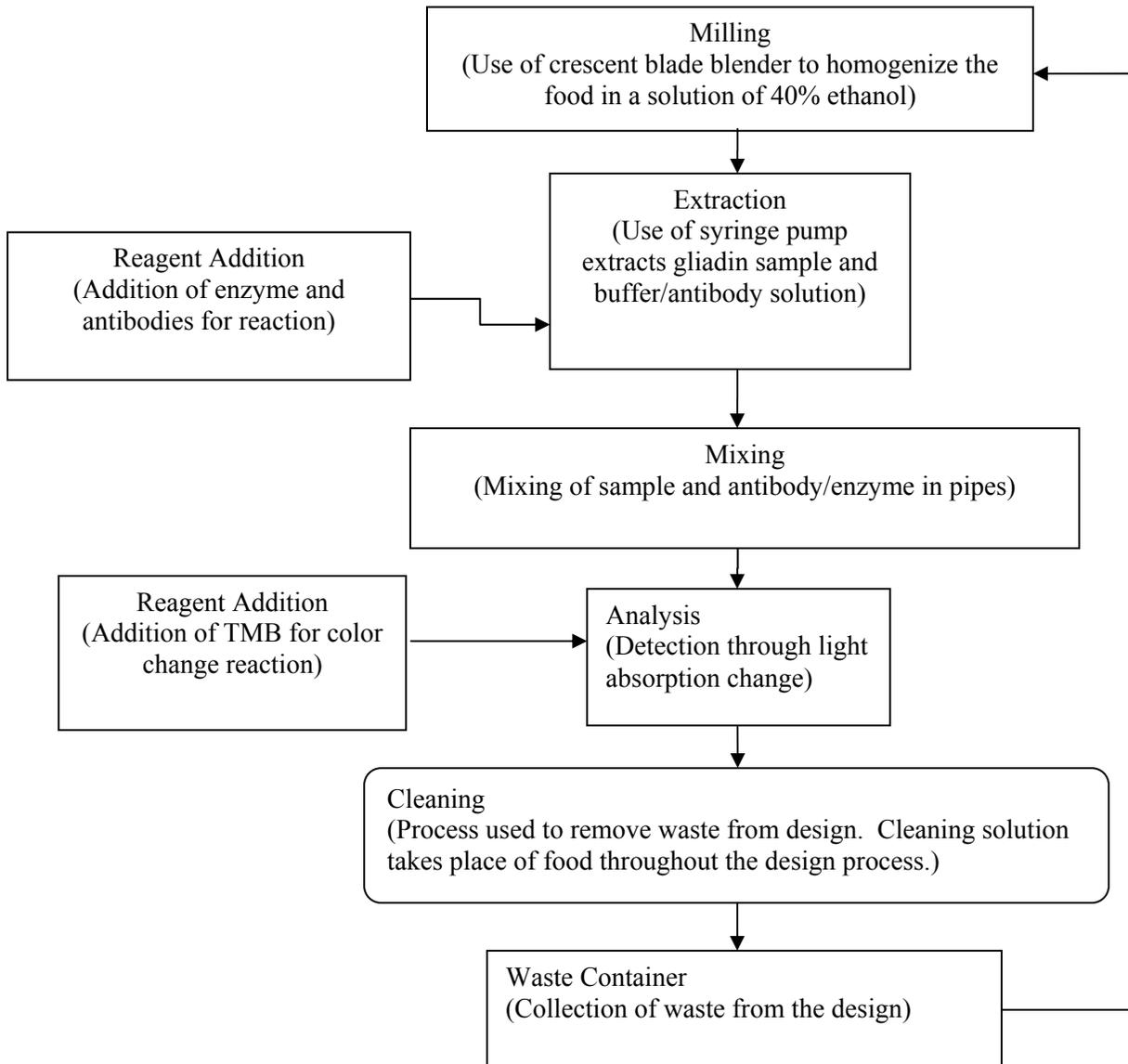


Figure 2: Design Schematic Flow Chart

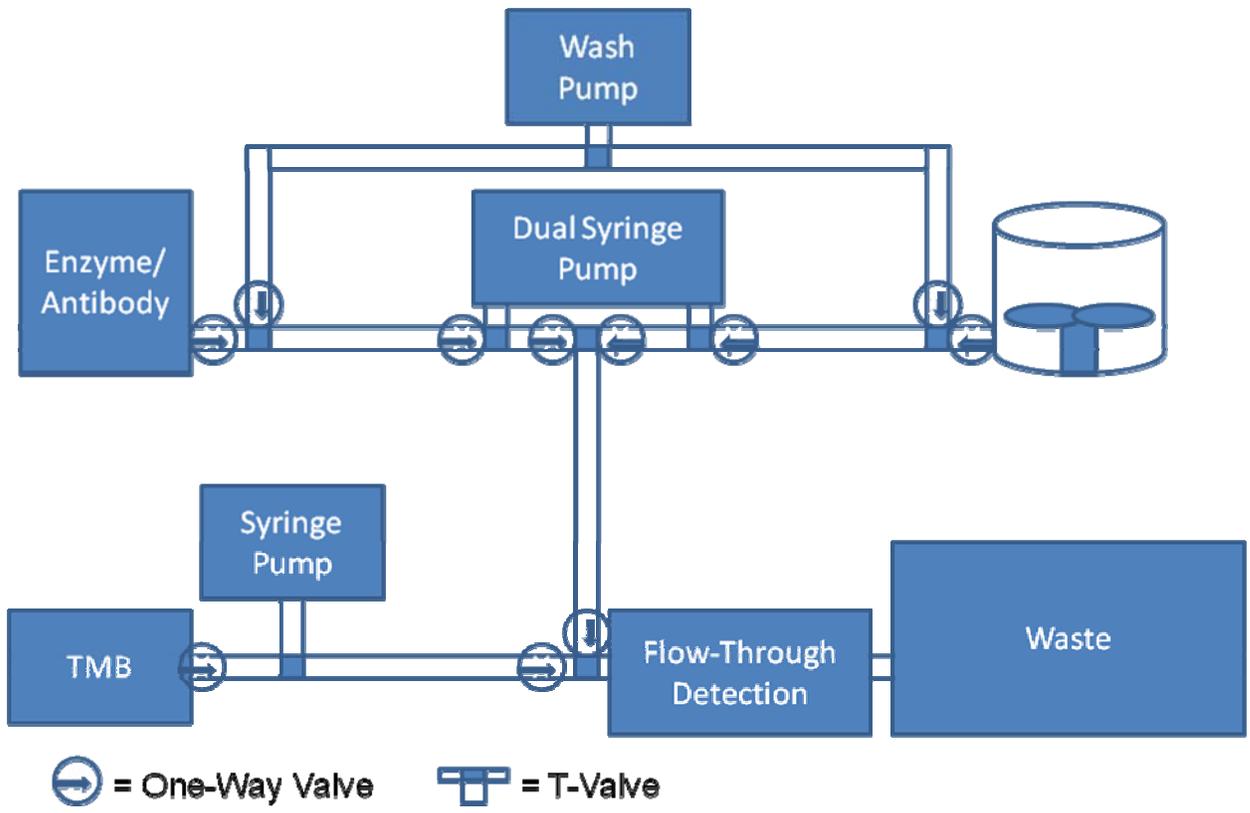


Figure 3: Design Schematic

Particle Reduction and Homogenization

The first process completed within the device is blending and homogenizing the food sample in order to extract the gluten. Originally, there were three options for design: one, two, or three mixing chambers. The specifications included creating the correct dilution for analysis, simplicity, and mixing the enzyme-antibody complex with the extraction fluid. The three tank approach for serial dilutions was rejected for cost reasons. A single tank design is the most simple and cost effective though with two tanks there is greater dilution and mixing. At first, the use of only one tank for both mixing and extracting was determined ineffective because, through calculations for the analysis section, the overall dilution needed to be approximately 1:1000 (Estimates and Calculations): one tank would be too bulky and multiple dilutions would be more efficient. However, with the new solution for analysis being the ELIFA method and the decision to use a syringe pump scheme, there is more control over different components of the process making the dilution calculated extraneous to the design of the blender. Instead, the focus will be on the effectiveness of the proportions and design for extraction to the fluidic system.

To extract the gluten from the food, the samples must be broken down to release all available gluten. This involves particle reduction in order to allow for the protein to be suspended in the fluid. The best approach is to use a design similar to commercial blenders; however there is no public research on the design or specifics of blender technology. Therefore general information was obtained through observation of current, non-patented products and equation were sought to mimic or over-compensate for the calculations necessary in the design process.

Normal mixer designs consist of the interior volume of the tank and the impellor type which then define the specifications. The interior volume of the tank needed to be able to create a concentration that is workable for the analysis and as small as possible. The impellor needed to be effective at particle reduction and mixing. With these specifications in mind, the design was imagined.

It was concluded that the device should be very simple for users to add the appropriate amount of food sample. It needed to be easy to take pieces from an entire hypothetical helping of food being sure to not leave out or exaggerate portions of the food, e.g. a pickle on a hamburger. The tank volume was centered on the acceptable sample size of approximately 1 cm^3 as the amount to be added to the detection device for reading which coincides with that used in the *Official Methods of Analysis* published by AOAC International (2006). A diameter (D_t) of 5.0 cm resides within the approximate specifications of a portable detector, and using specifications from the book by Doran titled *Bioprocess Engineering Principles*, the height of the tank (H_t) is best at 6.25 cm (1998).

Many different options were researched in order to find an impellor suiting the characteristics necessary. A Helical Ribbon mixer is often used for high viscosity applications because it is resilient to strain and powerful. The outer diameter of the ribbon (D_r) in a 3cm diameter tank was calculated to be 2.97cm and the width of the ribbon (W) was 0.3 cm. The height of the ribbon (H_r) was 3 cm also based on the specifications laid out by Doran (1998).

The estimated power requirements were determined using the laminar equation presented by Doran, and the physical properties of molasses as a food sample (1998, Bortnikov 1974). The requirements were 0.06 W and fit in the category of any electric motor on the market (Equation 10). Therefore, the motor found was the least expensive, which is GM6 Miniature Gear-motor: Offset Shaft found at hobbyengineering.com (Appendix D). The motor is priced for individual sale at \$7.99 each with advertised large quantity discounts at \$5.59 each, making this section very inexpensive.

The rotation rate specified by the company is 145 RPM at 5V and using formulas provided by Doran and Delaplace, the estimated mixing time is 30 seconds (1998, 2000, Equation 11)

The process of mixing the food and enzymes will not experience high viscosities; therefore the Rushton turbine was selected for applicability because it is extremely efficient (Doran 1998). For convenience, the mixing tank was determined to be the same size; however baffles were added to the specifications denoted by Doran to improve vertical mixing (1998). In order to create the desired dilution for analysis, 0.6 mL of the first solution was decided to be added to the second tank, along with water and the enzyme mixture.

Using the specifications from Doran, the dimensions of the turbine were calculated. The height from the bottom of the chamber (H_i) was 1cm along with the diameter of the turbine (D_i). The paddles were given the dimensions of paddle length (L_i) = 2.5cm and paddle width (W_i) = 2.0cm. The baffles within the chamber were calculated to 0.3cm for optimal vertical mixing.

In the second tank, the power requirements were much less at 8.57×10^{-7} W and the same motor was chosen (Equation 10, Appendix D). The necessary mixing time was calculated and rounded to 23 seconds to ensure proper mixing (Equation 12).

By observation however, most blenders and grinders on the market use two or four blade-like wings extending from the center point of rotation therefore the solutions presented for typical mixing were discarded and a new design was required. In two blade designs, they are crescent shaped with the inner radius sharpened and facing the direction of rotation. The four blade designs have two blades directly across which are relatively flat and another two angled downwards. Both were considered to use and it was concluded to use the crescent blade design because it is most often used for slicing as in our application. The impeller's radius can be estimated to be a significant portion of the interior radius of the chamber. Using measurements from food processors, there was approximately a 12:13 ratio between the Diameter of the impeller (D_i) and D_t . The radii will be calculated using geometric principles before creation however other considerations need be addressed simultaneously.

Component Testing

A design has been created to begin testing and optimizing the different components to be used in the homogenization portion of the prototype. Seals must be created between the motor and the tank however it was determined that the bearing set onto the shaft should be water resilient because it is full of oil based products and not open to the environment for that reason. The container does however need to be water proof for application so o-rings were employed along with a simple restraint. A picture and section view can be seen below (Figure 5). The piece consists of a fluid container, two compression plates to create seals, and bolts to hold it together.

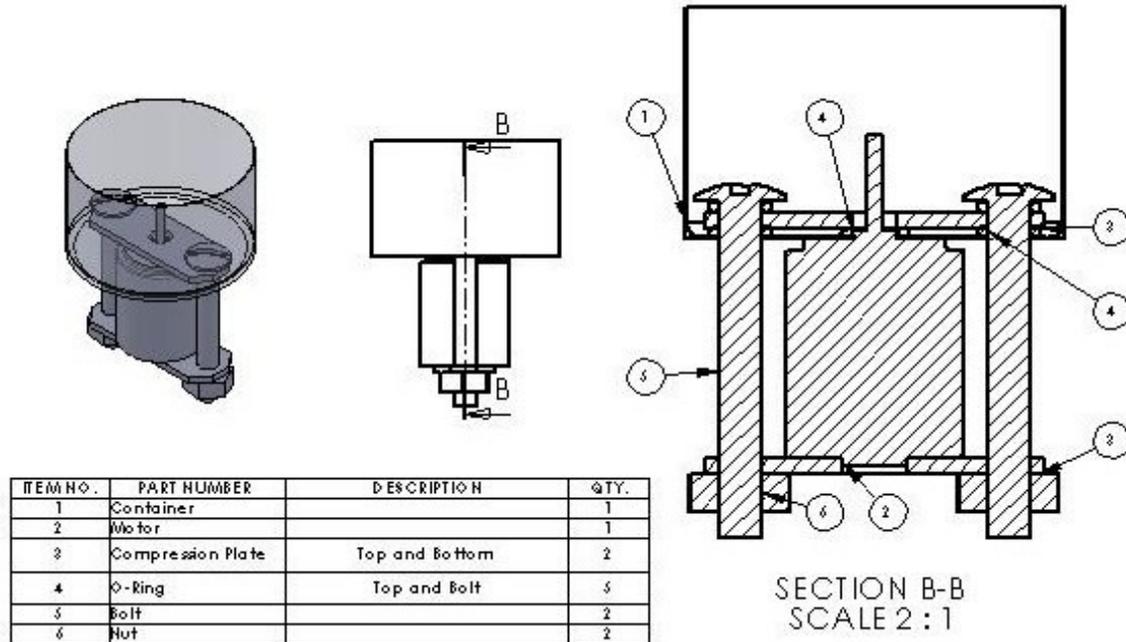


Figure 4: Prototyping Design

This design allows for motors to be easily strapped into the container and then impellers are attached to the rotation shaft of the motor. This is being constructed in order to test the efficiency of different experimental blade shapes and sizes to find the most efficient.

Pump/Pipeline Design

The basic necessities of the pump were dependent on a few factors. These factors included:

- The flow in the pipes must be homogeneous due to the detection method requirements
- There has to be enough pressure to push a slurry solution through the pipes

There are four regimes for transport of solids through a pipe according to *Pipeline Engineering* (Liu, 2003). These regimes include Stationary-Bed Flow, Moving-Bed Flow, Heterogeneous Flow, and finally Homogeneous Flow. Stationary-Bed Flow is described as when particles settle on the bed of the pipe and do not move. This happens when the solid being transported is very coarse or has a low velocity in the pipes. Moving-Bed Flow is when the particles settle on the bottom of the pipe and move through sliding, rolling or saltation. This is due to low velocity of flow or a large particle size. Heterogeneous is when particles are fully suspended but not uniformly distributed (non-homogeneous). This is due to a low velocity or large particle size. Finally Homogeneous Flow occurs when particles are fully suspended and are uniformly distributed. Figure 2 shows the particles size vs. mean velocity for the four regimes.

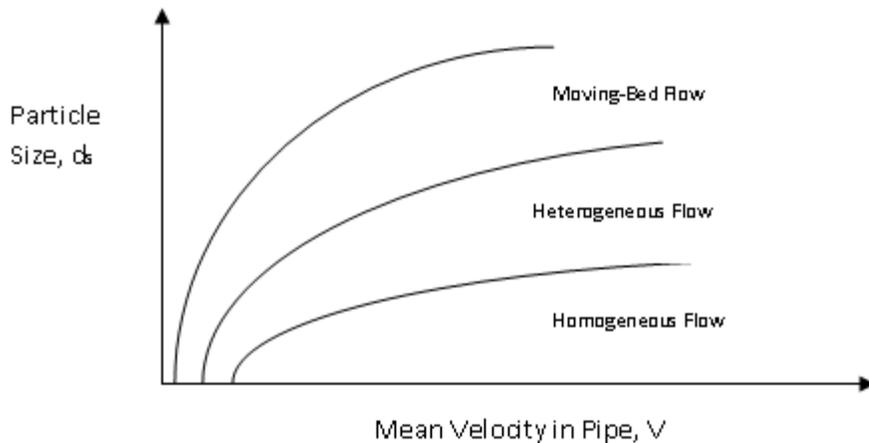


Figure 5: Regimes for transport of solids through pipe (re-drawn from Liu, 2003)

The flow in the pipes had to be homogeneous in order to keep the concentrations the same. As slurry solutions flow through pipes, the solid must travel at the same average speed of the fluid and be completely and evenly dispersed. From this, a homogenous slurry solution was thus the only acceptable flow. This required a small particle size during the processing of the food and a large velocity of flow in the pipes. In order to accomplish this, the assumption was made that there either had to be larger pipes, or a stronger pump to increase velocity.

Pump cost was looked into for the use in the design. The price range for micro pumps ranged from \$350 and upwards. Although the \$350 price may have been acceptable for the design, the pump could not have performed for the whole fluidic system. This conclusion was made due to the complexity of the fluidic design. As can be seen in Figure 3. *Schematic Design*, there are multiple pipes over short distances that are connected to the different chambers. There was not an ideal way use one pump for all of the fluid movement without greatly increasing the pipe length, material costs, and overall design complexity. Due to this, it was decided to not use pumps and to use the force from gravity for the acceleration of the fluid. With this idea, the previous question of pump strength or pipe size was answered. The pipe size was the only variable now in the flow design for the system.

The size of the pipes was determined to be $\frac{1}{4}$ " due to the low costs of the pipes as well as the accessibility of the size. The tubing that was used was purchased from Cole Parmer, a chemical equipment company. The smallest tube size available was $\frac{1}{4}$ " in diameter. This was selected as it was the cheapest and smallest that could be found. The design does not require specific material for the pipes which allowed any selection to be made. The pipes also needed to be tubing in order for them to be pinched closed by the valves as well as being movable and not rigid.

With this decision made, the fluidic system was designed to release flow through hand valves at the dispense of the user. The volume would flow into the middle space of the pipe with the left valve open and then out after the right valve was opened. This approach still allowed the design to be cheap but did not allow it to be user friendly.

The method of using gravity to move the fluid throughout the pipes was examined and found that the solution did not stay homogenous throughout the pipe flow. A simple test was run involving crushed bread as well as noodles (about thumb size) crushed and placed in about 25mL of tap water. The solution was then mixed and run through $\frac{1}{4}$ " "spaghetti" irrigation tubing. The food particles

clumped together and did not move through the pipe and instead would become lodged in the pipes. From this it was determined that the second solution of using gravity for movement of fluidics would not work.

A third solution involving pumps that do not require power was examined. Push pumps have been looked into and designs created in order for the user to simply push down on the pump with his finger and release a specific amount of solution. A simple design can be seen in Figure 6 below. Another method involving the use of the turbine in the mixing stage of the design to push the solution into the detection chamber has been created. The power requirement would already be fulfilled from the use of the turbine to mix and would not create another source of power drain in the fluidic portion. This solution has not been fully developed at this point of the design.

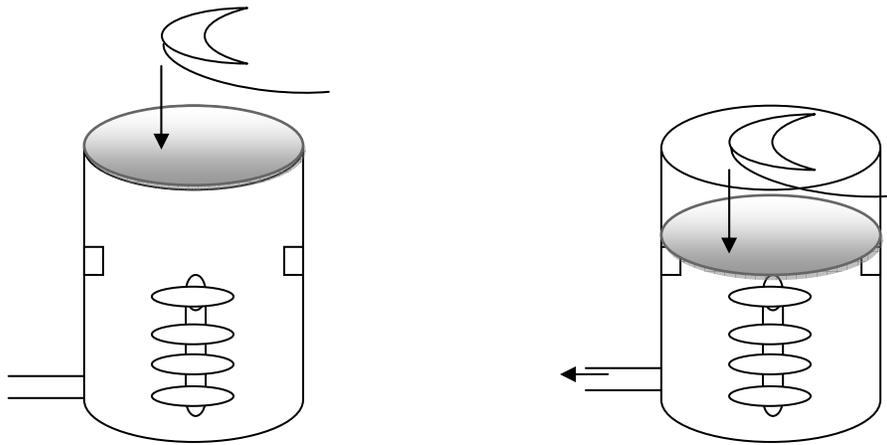


Figure 6: Push Pump Design

An examination of the primary and alternative solutions was made as follows. The first solution examined included the use of small centrifugal pumps to transfer the fluid throughout the system. This solution involved expensive costs including the pump of \$350 and extra tubing. This solution would allow the device to be more user-friendly but at a large cost increase. The second solution examined included the use of gravity to transfer the fluid throughout the system. This solution would be very inexpensive by only requiring the cost of the pipes (\$0.75). This would increase the requirement of the user to interact with the device but would allow the device to be very inexpensive. Due to testing, this solution was discarded as the slurry solution did not stay homogenous in the pipes. The final and primary solution examined included the use of push pumps to propel the fluid forward. This solution was assumed to be inexpensive when compared to solution 1 and more expensive in comparison to solution 2. It was more user friendly than solution 2 but less than solution 1.

A decision was made to test the viscosity of the food solution in reference to water. This was done by a basic viscosity test using a graduated cylinder filled with first water and dropping a steel ball a specific length and timing the fall. The was then done again with food particles dissolved into water and the viscosity values were calculated according to the results. These values and calculations can be seen in the Appendix F and in the calculations section respectively.

Tests showed that the viscosity of food solution was close to that of water, however could not be considered water. Due to this calculation it was decided to include a filter into pumping system in order to exclude the food particles but allow the dissolved gluten in alcohol to be transported through.

This concept increased the frictional losses of the fluid system but allowed for an easy method of transportation.

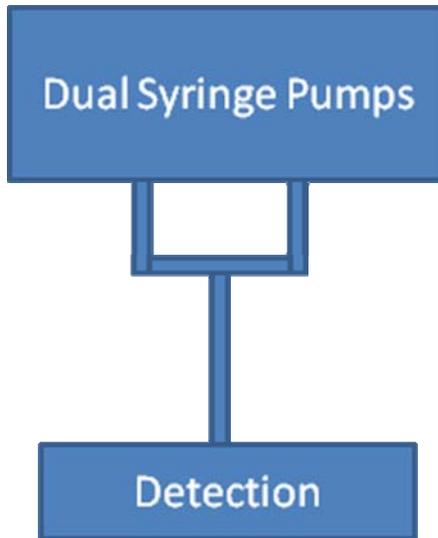


Figure 7: Dual Syringe Pump

The fluid system requires the fluid quantities of the gluten solution and enzyme/antibody complex solution to be mixed proportionally at a 1/50 ratio due to the results from the AOAC methods of extraction method 991.19. Due to this fact the method of fluid transport was once again brought up to question as the previous solutions did not accommodate this. A syringe pump system was devised that would allow the fluid to be pumped simultaneously and at different velocities. A schematic of this can be seen in figure 7. The syringe pumps will suck fluid from two separate locations simultaneously and then begin to pump them out down the tubes to combine at the t-valve and finally go through the detection method. The design for the syringe pump can be seen below in Figure 8.

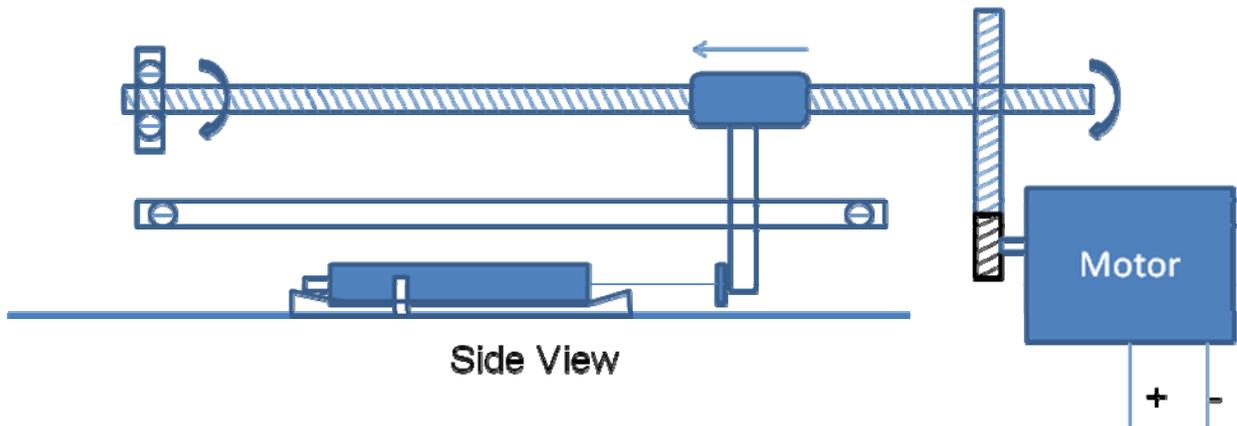


Figure 8: Syringe Pump Design

This syringe pump was designed to use a DC motor connected to a set of gears to turn a worm screw. This worm screw was then used to move a propeller which would be used to push or pull the plunger

on the syringe at the needed speed. The plunger speed would finally create pressure inside the plunger and create a fluid velocity that was needed for the system.

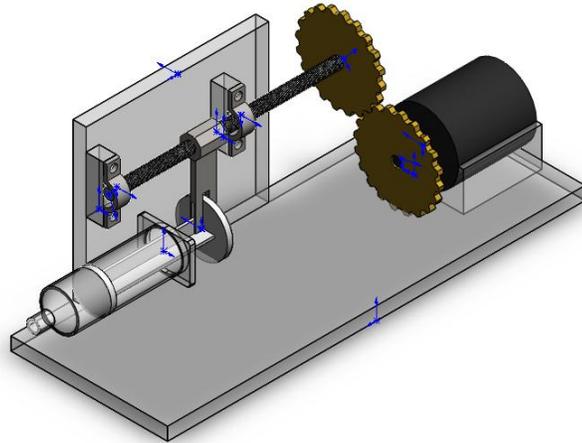


Figure 9: Cad Picture of Syringe Pump

The syringes used were 5mL and 1ml in order to hold the 1/50 radioed volumes of 4.9mL and 0.1mL. The syringes were secured into the base of the device using clamps and held in place by lips on the surface as can be seen. The nut ring-propeller connected to the worm screw was designed to have the same pitch screw on the inside and to move in the forward or backward direction due to the force of the connected propeller to the wall. The propeller portion was connected to both the front and back of the plunger in order to pull it and push it. The gears on the motor and worm screw were plastic for economic purposes and had a pitch of 1/14”.

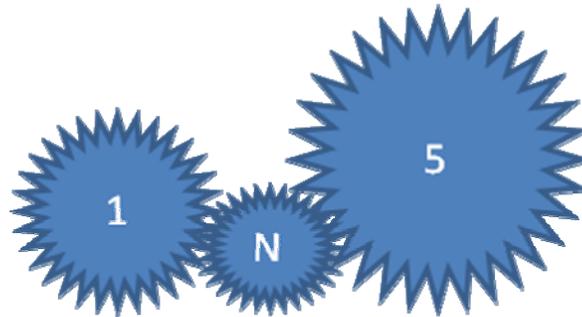


Figure 10: Geared Ratio of Syringe Pump

The gears on the end of the worm screw will rotate at a specific speed in reference to the velocities needed on the plungers. This can be seen in the calculations section of the report. The ratio that was used came out to be a 1:5 ratio in consideration of the 1/:50 dilutions of gluten solution to enzyme/antibody complex solution.

The fluid design had a need of using a buffer wash to first clean the antibodies in the detection system of excess gluten, and second to completely remove any wasted fluid or particles from the system. This caused the need for a pump that would have the power and constant flow rate to complete the task. A reciprocating pump was chosen from Edmund Scientific for the design.



Figure 11: Reciprocating Pump from Edmund Scientific

The pump was capable of pumping 1 pint/min at 12" high which was compared to the head loss of the fluid system. The pump power capabilities were far greater than the frictional losses showing that it was able to optimally perform for the design. These calculations can be seen in the calculations section. The values of frictional loss did not include the loss from a filter which would be considered to be great. This was decided to be done in future testing.

The Reciprocating pump used alternating current as a power source in the design. Due to the fact that the rest of the design used DC battery power the choice of using the reciprocating pump was abandoned. The alternative design used to replace this was to add a switchable t-valve at the mouth of the blender and enzyme antibody containers. This allowed for the dual syringe pump to be used for the pumping of the PBS wash as well.

The addition of the TMB to the detection chamber needed to be very specific volumes and velocities that could be controlled. Due to this a 12 VDC Precision Micropump was purchased. The pump has a bent axis design allows for infinite adjustment from 0 to 100 micro-Liters which was precisely the volumes of TMB that needed to be added. The pump was powered by a 1.8 degree-per-step stepper motor with five wire opto-sensor. The control board has potentiometer speed control, forward/reverse switch and manual mode for single step operation.

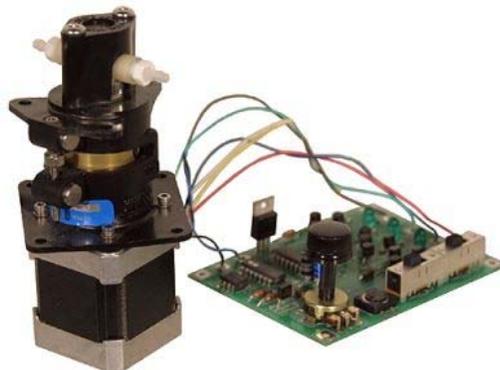


Figure 12: VDC Precision Micropump

Analysis Component Design

For the design of the analysis component of the sensor, different detection methods used by biosensors were investigated, such as the glucose biosensor used by diabetics, as well as currently available commercial gluten detection kits. The criteria selected for a successful analysis portion of the sensor were that it would be able to accurately and effectively detect gluten in a variety of food samples with different mechanical and chemical properties (at least breads, pastas, and gravies), as well as be able to detect at least as low as 20 parts per million (ppm), the upper threshold concentration established by the WHO and UN as “gluten-free” and safe for consumption for individuals with celiac disease (CSA, 2008).

Several alternative solutions were developed for the analysis section. A system similar to glucose biosensors was considered, where immobilized enzymes would react with the sample, causing reduction that could be converted into an electrical signal. This idea was rejected due to the lack of easily available enzyme systems that would react specifically with gluten. A system based on the sandwich ELISA (Enzyme-Linked ImmunoSorbent Assay) technique was developed instead, similarly to how the gluten detection kits worked. A currently available commercial product from ELISA Technologies, Inc. demonstrates this concept. In this detection kit, a food sample is ground to a fine consistency, then added to a gluten extraction medium, then a sample of the extract is placed into a test tube, and finally test strips are placed into the extract, which induces a color change that can be read by the user (ELISA Technologies, Inc., 2008, Appendix F).

The sandwich ELISA technique involves immobilizing antibodies on a surface, in this case the analysis chamber, to which the gluten analyte would later attach. A secondary antibody which is complexed with an enzyme, horseradish peroxidase (HRP), is then added to the analysis chamber which then attaches to the now immobilized gluten. A chromogen is then added which react with the enzyme, producing a color change in the medium. This color change can then be read by measuring the absorbance at a specific wavelength (635 nm).

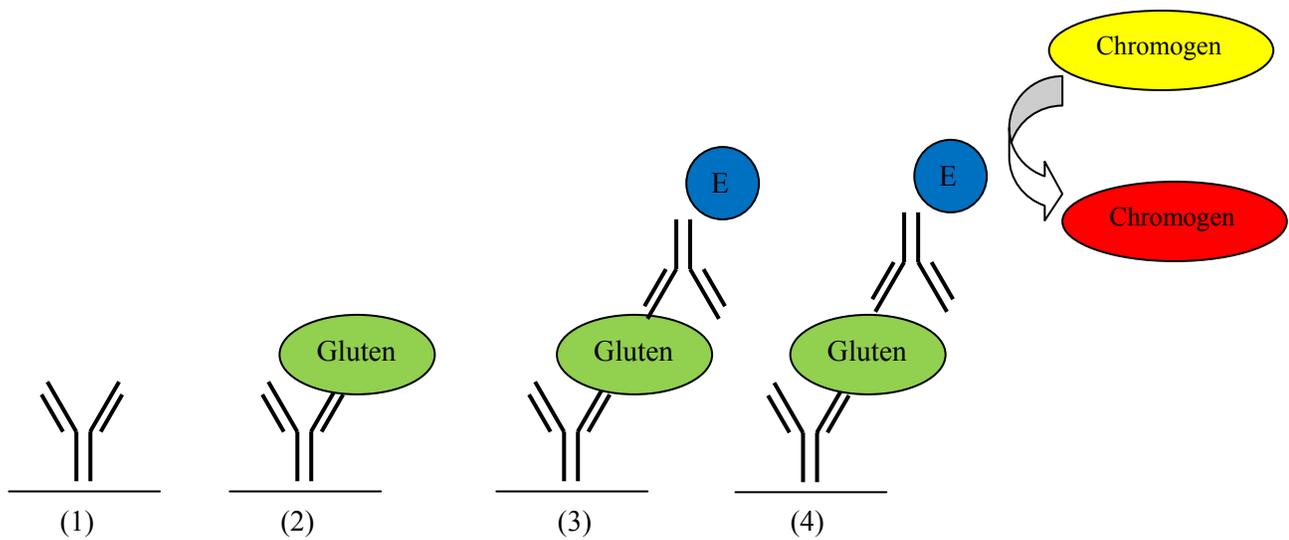


Figure 13: ELISA Method

Absorbance is the relationship of how much light is absorbed by a medium, and is given by Equation 13. Light source at a set intensity, an LED in this case, is shown through the analysis chamber, and the intensity of the light leaving the chamber is then read using a photodiode chip which converts this light intensity into a voltage. Several options were considered on how to convert this output voltage into a signal that is read by the user. A voltmeter was first considered, but was rejected on the grounds that it would increase the overall cost of the unit (\$30-\$40 dollars), and potentially provide too much information to the user, when all that is necessary is a binary signal to the user. A cheaper alternative using two differently colored LEDs (green and red), a system of several op-amps, and a Zener diode was developed.

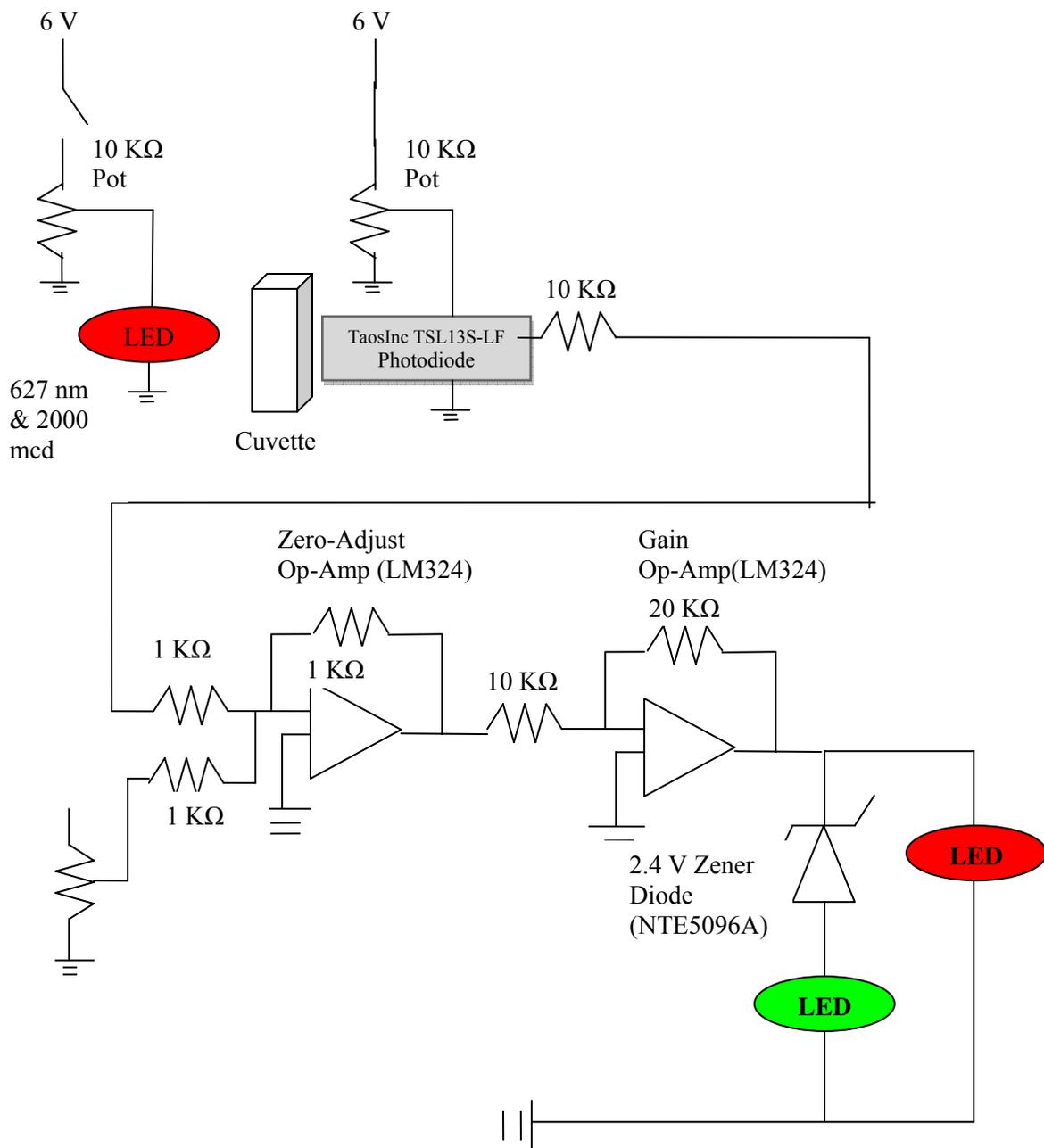


Figure 14: Processing circuit of output voltage

This circuit would function by amplifying the outputted voltage from the photodiode chip through the op-amp. The photodiode chip functions by using a PN junction. As photons from the light source strike the photodiode, electrons are excited and electron-hole pairs are created. The presence of these free electrons then move toward the cathode of the system in order to maintain equilibrium in the diode, creating a current as they travel. In this system, the user would turn a switch, turning on the light source which travels through the cuvette holding the sample for analysis. The photodiode then creates a voltage relative to the intensity of the luminous intensity of the light, which first travels into

a zero-adjust op-amp configuration. This is used to cancel out any ambient light of the system. The second op-amp has a set gain, which amplifies the voltage so that, for a sample of 20 ppm, the output voltage signal after going through the op-amp would be equal to the zener voltage of the zener diode (see Estimates and Calculations for calibration). Then, the circuit would split, with a zener diode with a specific zener voltage acting as a voltage regulator followed by a green LED in one path, and a red LED in the other. Due to the zener diode, samples less than 20 ppm will produce a voltage greater than the zener voltage, thus following the path with the green LED and turning it on, while samples above 20 ppm will produce a voltage below the zener voltage will not turn on the green LED and flow only through the path turning on the red LED. Thus, the green LED acts as a safety indicator, with it being lit indicating a safe food sample, while the red LED serves as a power indicator, showing that the circuit is functioning.

Two optical chemicals were considered for this technique: 3,5,3',5' – tetramethylbenzidine (TMB) and 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS). ABTS was rejected, however, as it requires storage in cooler temperatures, while TMB can be stored at room temperature for up to one year (SDT, 2008). Additionally, some TMB products were found to be water-based and therefore easily disposable (Clinical Science Products, Inc., 2008). With the final approach selected, a prototype design was created.

An alternative system using an amperometric detection method rather than an optical detection system was also considered and evaluated. This method would still utilize the ELISA method described earlier. As TMB and peroxide react with the horseradish peroxide, the TMB becomes oxidized and can be read amperometrically, or by monitoring the change in current generated by the oxidized TMB. An automated amperometric detection system involving TMB and HRP was described by Fanjul-Bolado et. al (2005). The authors use a flow injection analysis (FIA) system to automate the process, where the oxidized TMB is reacted on a screen-printed carbon electrode. In this system, the ELISA reaction would be performed in a separate chamber, after which the oxidized TMB would be flowed through the flow cell containing the electrode, where the change in current could be read via a voltmeter. This method of detection was disregarded in favor of the optical detection method because, as the authors note, it is less accurate and more time intensive than optical methods. As it is important that the final device be both very accurate to ensure safety and as fast as possible to provide prompt analysis, the optical method was deemed preferable.

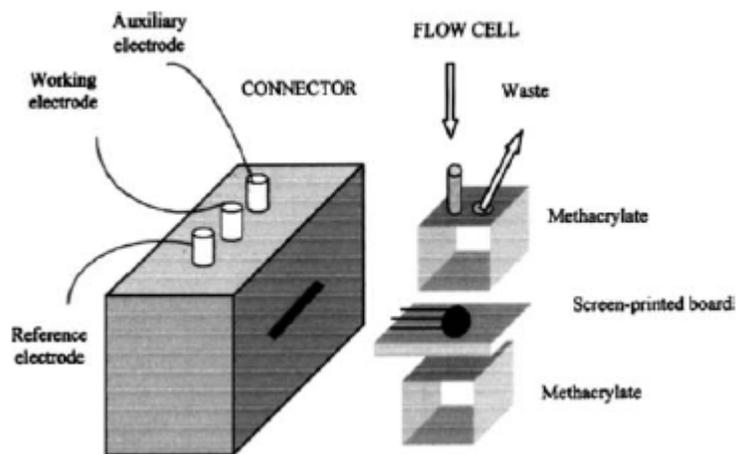


Figure 15: Flow Cell equipped with screen printed carbon electrode

Estimates and Calculations

Experimental Calculation of Mixing

In order to calculate the volumes of the tanks a simple equation was used by subtracting the volume of the significant portion of the mixing column from the total interior volume.

The volume of the tanks were found to be 24.15cm^3 and 25.97cm^3 for the 1st and 2nd tanks respectively. These allowed for the dilutions to be calculated in each tank. The first, having an addition of approximately 1cm^3 was simply 1:24.15 and in order to find the necessary addition to the second chamber a ratio equation was used.

The addition volume was calculated to 0.627mL to create the correct dilution for analysis.

To find the impeller power requirements, the physical properties of molasses were used as the sample with an original size of 1cm^3 added. First the Reynolds number was found to be lower than 100 and then the correct equation for laminar flow was used. In the following equation, N_i is the rotations per second, D_i is the diameter of the outside of the ribbon, ρ is the density of the fluid, and μ is the absolute viscosity. In the next equation, P represents power, k_1 is the non-turbulent power constant (equal to 1000 for helical ribbons), and other variables are the same.

The power required was found to be 0.06 W or 8.04×10^{-5} Hp. This was assumed acceptable for the Rushton turbine also considering the additional decrease in density and viscosity.

The mixing time for the helical ribbon used one equation from Delaplane and the other from Doran (2000, 1998) respectively.

The mixing time was calculated using the rotation rate of the motors (1.75s^{-1}) and found to be 28.8 seconds or approximately 30 seconds. The calculation for mixing in a non-turbulent Rushton turbine is extremely complicated, therefore it was assumed to be $\text{Re} > 10^5$ and the following equation was used.

The variable t_m was found to be 22.85 seconds or also approximately 30 seconds for assurance of full mixing.

Experimental Calculation Pipe Flow

In order to determine the velocity needed in the pipe flow the following analysis was completed. C_T represents the volume concentration of the solid at the top of the pipe and C_A represents the volume concentration of the solid at the pipe axis. A ratio of C_T/C_A was assumed to be 0.8 showing that the pipe flow was pseudohomogeneous. The settling velocity V_S was estimated by assuming that it was the same as that of a sphere of the same material density, having a diameter d equal to the particle size, d_s (estimated at 1mm), of the particles. The settling velocity was assumed to be 0.116 m/s as an average from Jimenez, 2003 table. The table shows the settling velocity and particle size of several referenced experiments all depending on shape factor and other parameters. The assumption was made considering that the particles of food would have a shape factor close to 1.0 and thus the closest data of 0.9 was used. Equation 1 was then used to calculate u_* using a κ of 0.35. The value of 0.35 was assumed due to the assumption in *Pipeline Engineering* (Liu, 2003) made stating that most slurry

solutions' κ are just under 0.4. Finally, equation 2 in appendix B was used to calculate the required velocity needed in the pipes. The value of the friction factor was determined using equation 3 with the set pipe diameter.

There were calculations throughout the pipe system in order to deliver specific amounts of fluid to the next reservoir. Such calculations included designing valves to be 2.18 cm apart in order to deliver exactly 0.7 mL of fluid from reservoir 1 to reservoir 2. This was done using basic geometries of a pipe or cylinder.

The pressure gradient was calculated from equation 4 in appendix B. Pseudohomogeneous slurries can be treated in the same manner as homogeneous flow (Newtonian and non-Newtonian fluids) except during the time the pump or pressure is shut down. The ratio between shear and the velocity gradient were assumed to be constant for this system due to the small pipe size. Because of this, the flow can be treated as Newtonian and thus use equation 4. When using equation 4, the viscosity and density were supposed to be that of the mixture rather than that of water to calculate the Reynold's number for determining f_m . Equations 5 and 6 to calculate these parameters are presented in appendix B and follow from those of Liu (2003).

The calculation for the velocity of flow in the pipe system was done as follows. For each of the fluid pipe flows the force of movement was created from gravity acting on the fluid in the pipes as well as a reservoir above. The first flow in the system is that of water into the milling tank 1. The fluid has a reservoir of 2cm above the pipe and a density of .998g/cm³. The Bernoulli equation is then used to calculate the velocities and pressures at different positions in the pipe assuming that initial and final pressures are 0. The velocity of the slurry flow from the first milling tank into the second tank came out to about 100 cm/s. These calculations are now irrelevant due to the use of pumps.

Calculations of Dual Syringe Pump

There were a few calculations done for the syringe pump. First the velocity of the plunger of the syringes based on the velocity needed in the pipes was calculated. There was a need of a 1cm/s flow in the pipes across the detection system. This was done by multiplying the velocity times the ration of the needed fluid volume by the ratio of the pipe to syringe areas. This translation came out to velocities of 0.193cm/s and 0.0356cm/s for the first and second syringe respectively. These velocities were then related to the rotational speed of the worm screws. The worm screws had a pitch of 1/14" which means a distance of 1/14" per rotation. This in cm came out to 0.1814 cm per rotation. These were then related to the velocities of the plungers to determine the rotation speeds. These came out to 1.06 rotations/s and 0.2 rotations/s.

The gears ratio was determined based on the rotational speeds of the worm gears. This allowed for the determination of the size of the gears in comparison to each other. The rotational speed of 1.06 rotations per second was divided by that of 0.20 rotations per second to get the ratio of 5.3. This ratio showed that the gears had to be different by a factor of 5.

Pump Calculations

The pump used to move the wash buffer was shown to supply enough power as shown below. First the major and minor head losses were determined for the pumping system. The major head loss represents the frictional losses due to the length of the piping and by surface material. The minor represent the frictional losses due to changes in pipe direction, height, and fluid flow. The values

came out to 0.0143cm for minor and 9.7×10^{-7} cm for major. The equations for minor and major headloss can be seen in the equation appendix.

Sample Standard Curve

To determine a standard curve for the absorbance of a gluten ELISA system, a standard curve would need to be developed experimentally. A sample standard curve for the gluten ELISA kit Immunotech Corporation using TMB was found. This system used the gliadin component of the gluten as the active component:

Calibrators	Gliadin (ng/mL)	Abs.	Abs.-Abs.CAL ⁰
0	0	0.404	-
1	20	0.942	0.538
2	40	1.249	0.845
3	80	1.638	1.234
4	160	2.018	1.614
5	320	2.370	1.966

Table 1: Standard data for gliadin standards (Immunotech, 2008)

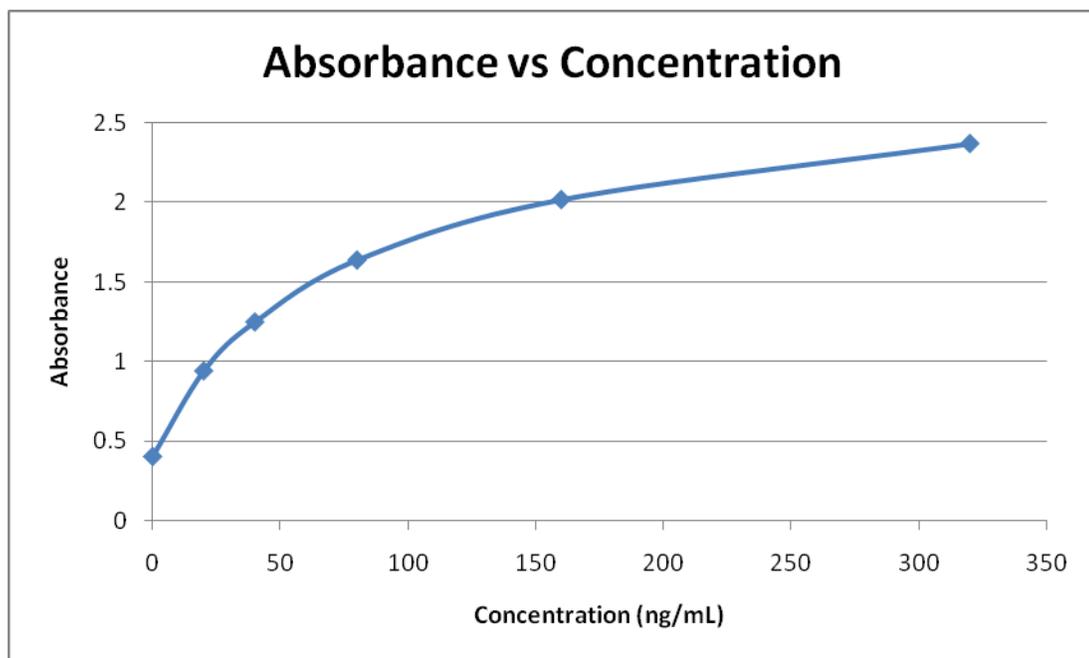


Figure 16: Standard Curve: Graphical Representation of Data from Immunotech

A standard curve based on the specific characteristics of the final biosensor would have to be developed experimentally. These specific characteristics that the standard curve is dependent upon include the dimensions of the analysis chamber, the volume of sample and TMB used, the amount of immobilized antibody, and the amount of mixing. To develop this standard curve, samples of known gliadin concentration are used to relate absorbance to concentration. The absorbance of samples with

unknown concentrations can be compared to this curve, and from this their concentration can be determined.

Testing of Light-Source LED & Photodiode

To mimic the color change TMB, food coloring was diluted to several concentrations and the response of the photodiode, in output voltage, for these concentrations was recorded. Initially, a 12000 mcd LED light source was used, which saturated the photodiode at almost all luminous intensities tested, producing an output of around 4.5 V. A 2000 mcd light source was then tested, and yielded the following results:

Concentration (v/v)	Output Voltage (V)
0	2.56
0.00005	2.114
0.00025	1.812
0.0005	1.592
0.005	0.2522

Output Voltage v Concentration

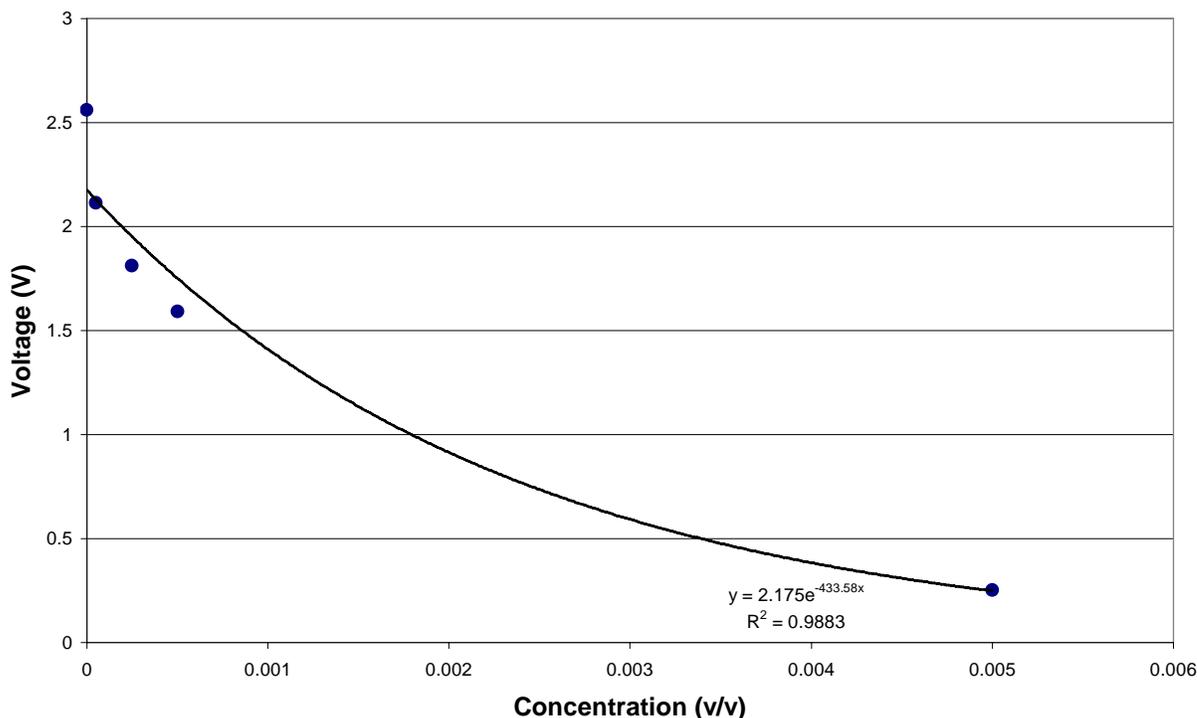


Figure 17: LED Data

The data shows an exponential regression as the concentration of food dye is increased. The response of the photodiode as the luminous intensity is increased was also measured. In this test, the amount of voltage going into the light-source LED was set, thereby setting the corresponding intensity of the light. The output voltage produced by the photodiode was then measured.

VLED	Voutput
0.003	0.0084
0.1254	0.0094
0.1999	0.0091
0.3784	0.0096
0.619	0.0099
1.527	0.616
1.628	1.154
1.634	1.539
1.647	1.872
1.655	1.854
1.666	2.619
1.667	2.753
1.68	3.112
1.695	3.554
1.732	4.92
1.812	4.97
1.952	4.97
2.142	4.98

V_{LED} v V_{Out}

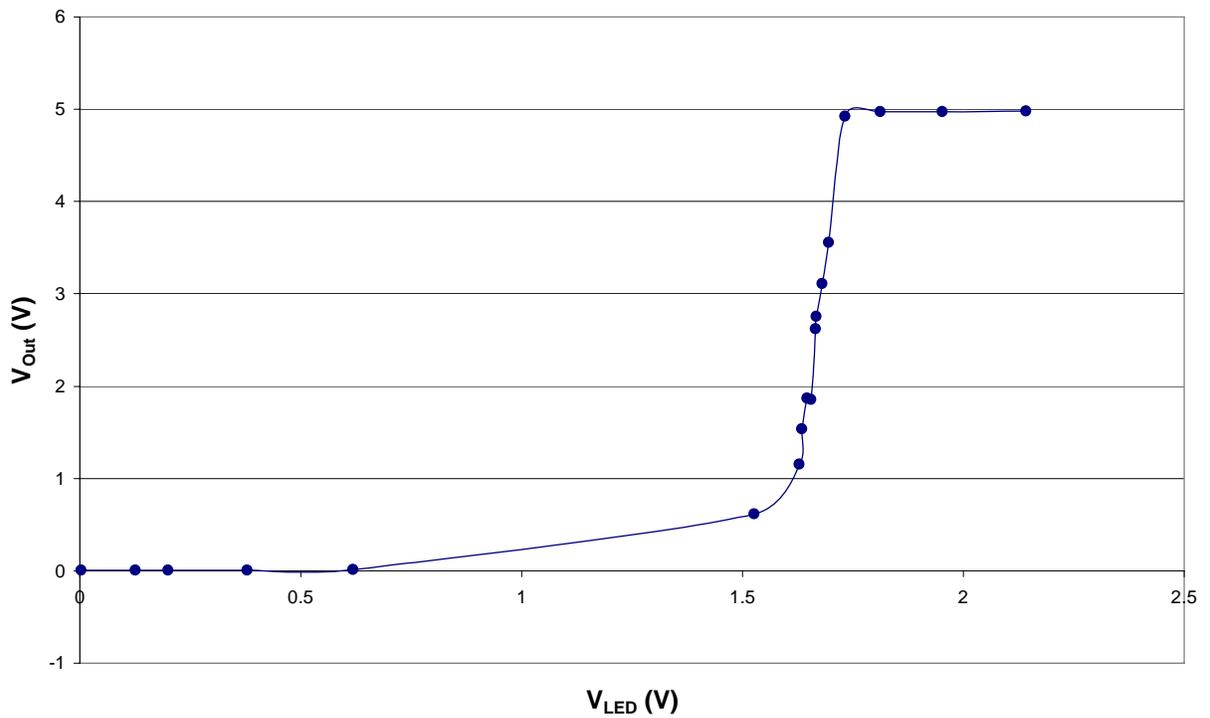


Figure 18: LED Data 2

These tests produced mostly expected results, with the response of the LED remaining mostly constant for low light intensities, then entering a more linear region, and finally becoming saturated. The device should be calibrated so that the photodiode is getting an input within the linear range, as this will produce the greatest response for slight changes in light intensity. As the data collected is somewhat contradictory for this linear range, further data should be collected to better analyze the linear range of the photodiode.

Testing of ELISA Flow-Through Method and Development of Preliminary Standard Curve

Testing of the flow-through ELISA method was performed, and showed a proof of concept for the method. The testing was performed using a standard sandwich ELISA protocol (Abcam Inc., 2009), but instead of reacting the reagents in a standard ELISA well plate, the reaction was performed in a 1 inch segment of PVC tubing, similar to the replacable components of the final device. Additionally, the food sample and secondary antibodies were added together, instead of sequentially as in the protocol, as this is the method that would be performed in the final device. The results of the tests can be seen in the table below, with the voltage being the outputted voltage from the photodiode.

	Concentration (ppm)	Photodiode Output (V)	Normalized Data
Test 1 (4 min TMB incubation)	200	0.726	0.398901
	20	0.714	0.392308
	Blank	1.82	1
Test 2 (4 min TMB incubation)	200	0.166	0.043387
	20	0.304	0.079456
	10	2.182	0.570308
	Blank	3.826	1
Test 3 (2 min TMB incubation)	200	2.009	0.421174004
	20	0.139	0.029140461
	10	3.59	0.752620545
	Blank	4.77	1
	Water	4.95	
	Unreacted TMB	4.94	
	Flow w/o TMB	4.95	

Table 2: Flow Through ELISA Test Results

Output Voltage v Concentration

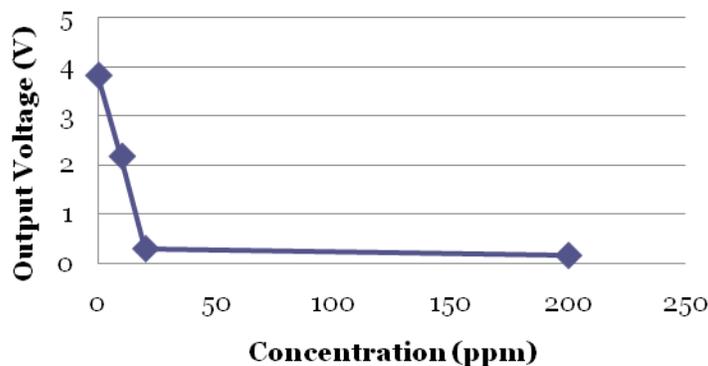


Figure 19: Preliminary Standard Curve (Test 2)

These results show that the system would adequately be able to distinguish between sample greater than or less than 20 ppm. In both test 1 and 2, the voltage output for 20 ppm and 200 ppm is very close, and then suddenly rises for concentrations less than 20 ppm. Although the output for the 20 ppm sample is much lower than expected, it is still lower than samples less than 20 ppm, again suggesting that the system can distinguish this 20 ppm threshold. Although the time for incubation ELISA was reduced from the protocol (2 hours), the samples were still incubated for a much longer time than would be appropriate for our final device (45 min). This was done to maximize the response from the samples. Further testing should be done to determine the minimum incubation time required to receive a response, and if any other methods could be done to reduce this time (i.e. inducing turbulent flow for mixing or addition of a heating element). Additionally, more data points for other concentrations should be tested to produce a more complete standard curve.

Determination of Path Length

To determine the path length required for the analysis segment, the path length used by ELISA wells was used as a reference. These wells have a diameter of around 6.5 mm, and a final volume of 200 μL of TMB (Nunc, 2008). Using the equation $V = \pi r^2 h$, the height of the TMB liquid in the well, and therefore the path length used by the plate reader, was determined to be $h=1.51$ mm. A plate reader was found using a light intensity of 13,126.49 mcd (Bees Medical Unlimited, 2008). The LED light source used has an intensity of 1,200 mcd (Superbright LEDs, 2006), the path length required for the sensor was found assuming proportionality between the systems' path length and initial light intensity:

$$b_1 / I_1 = b_2 / I_2$$

Using this equation, it was determined that the path length should be 0.138 mm ($1.51/13126.49 = b_2/1200$) if proportionality between the systems was required. However, since this path length is impractical for our design, as most cuvettes are manufactured with a path length of 1 cm or 0.5 cm, so cuvette of 1 cm was used instead.

Absorbance Analysis and Determination of Output Voltage

For the analysis segment, the Beer-Lambert Law was used for calculations (see equation 13 and 14).

A sample standard curve from Immunotech for TMB at 635 nm was used as a reference to base dimensions, sample size, and incubation time. If proportionality between the Immunotech system and our biosensor, the amount of gluten volume and TMB to be added to the system will both be 200 μL , and have a cross sectional area of 33.183 mm^2 . The path length would be 1.38 mm. The incubation time, if static, would be 30 minutes, but through proper mixing, this time could be decreased.

However, a path length of 1.38 mm would be too small to effectively implement. The analysis chamber would have to have the antibodies immobilized periodically, which would be challenging using such a small chamber. It was determined that a cuvette of standard size (1cm x 1cm x 4.5cm) would be more effective for ease of use and replacement. To determine the volume of sample and TMB used based on this cuvette size, it was determined that the price of TMB to be used would be the limiting factor. Ideally, the entire cuvette would be filled with the sample and then the TMB to decrease the necessary reaction time, this would require a volume of 3.5 mL. The cost for using this much TMB per use would be \$7.67. It was decided that a volume of 1mL for TMB would be an acceptable compromise, as this would provide an adequate amount for measurement in the system, and would be around \$2.20 per use. Cartridges of TMB for 25 uses could be sold for around \$55.00.

To determine the output voltage threshold corresponding to safe gluten levels, Equation 13 was used. For example, using the absorbance curve from the Immunotech ELISA system, the safe gluten concentration threshold of 20 ppm (corresponding to a final concentration of 20 ng/ml for the analysis section), will provide an absorbance of 0.942, and an initial irradiance of 31 $\mu\text{W}/\text{cm}^2$ (irradiance used to calibrate chip), the output irradiance was determined to be 3.54 $\mu\text{W}/\text{cm}^2$. Based on the specification sheet for the photodiode (See Appendix E), the output voltage should be around 2.35 V.

Calibration of Output Signal

For the calibration of the output signal from the photodiode, the system must be calibrated such that:

$$\text{For a 20 ppm sample: } V_o \times \beta = V_z$$

V_o = Photodiode output voltage, β = Gain from op-amp, V_z = Zener voltage of zener diode

For example, using the sample output voltage calculated from the Immunotech ELISA system of 2.35 V, and the NTE5096A Zener Diode zener voltage of 4.7 V, the gain of the op-amp must be calibrated to 2. The gain of the op-amp is dependent on the resistors used, using the equation:

$$\beta = -R_2/R_1$$

For inverting set-up:

$$R_2$$

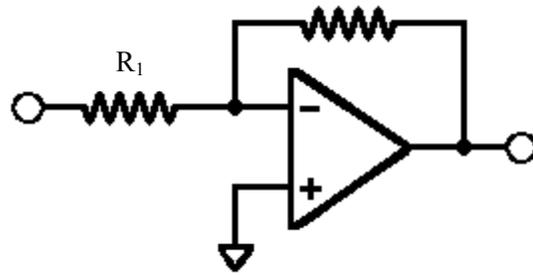


Figure 20: Inverting Op-Amp Setup

Material List

Item	Prototype Cost
Electronic Components	\$7.50
Detection Reagents & Antibodies	\$500.00
Fluidics – Valves	\$10.61
Fluidics - Syringes	\$1.55
Motors	\$4.05
Gears	\$4.95
Pump & Tubing	\$140.00
Various Screws & Bolts	\$10.00
Various Plastic Parts	\$20.00
Total	\$698.66

Table 3: Design Cost Analysis

Economic Analysis

The current cost of production for our prototype has been approximately \$598.66. If our device were to be mass produced, this cost would likely be much lower, as many components, such as the antibodies, were ordered in excess of what the average user would need. Also for a mass produced device, plastic containing pieces would be necessary, and it has been determined that the plastic molded pieces will total less than \$10.00 if mass produced. We expect the value of a mass produced product to be around \$250.00. The cost for the replaceable items was \$1.00 for each use, based on prices for TMB and antibodies. At retail the object would need to sell at a much higher price, but would still be less expensive than many commercially available medical products.

Results

Technical Description

Homogenization Segment

For the blending of the food sample to create the first homogenous mixture, a helical ribbon was used in a 24.15cm³ tank to create a 1:24.15 dilution of the initial sample. The Merkle-Korff Industries, Inc. Item # 8501 DC Gearmotor would rotate the mixer at 1.75 s⁻¹ and to a fully mixed state after approximately 30 s. A portion of the first solution would be added to the second mixing chamber was baffled and housed a Rushton turbine. The same motor was used for the second volume and has a similar mixing time to the first. The motor uses 0.14Amps and has sufficient torque specifications for both applications.

Pipe Segment

For the fluidic system of the gluten detector device, the first solution used included the force of gravity for pressure on the fluid systems with valves for control. The device was set up to be able to be turned on a specific side in order to create flow for the tubing and valves. The method for this can be seen in the operations of this report. The fluidic system can be seen in the Solidworks drawing located in the appendix. The system consisted of 5 pinch valves, 9cm of tubing, and a 3-way valve. These items can be seen in appendix C. The valves were used in order to control the flow of water as well as deliver specific amounts of volume to the device. These volumes include 0.7 mL for the rushton turbine and 200 uL for the detection device. These volumes are delivered using the double valve method seen in figure 4. The valves were located a specific distance away from each other in order to deliver the needed volume. The separation distance is calculated by taking the wanted volume and dividing by the area of the pipes. This method was used to transfer the slurry solution from the milling container to the mixing container and the solutions from the mixing container to the detection device.

With the determination that the fluid does not stay homogenous in gravitational fluid flow, another solution was approached. The use of push pump was examined and selected as the primary solution. For the fluidic system of the gluten detector device, the main principle used syringe pumps using a DC motor and gears as the driving force. There was also a reciprocating pump used to pump the wash throughout the whole system in order to clean and refresh the antibodies, however, after further research this was scrapped and an addition of valves were used with the syringe pump. The system consisted of 11 one way valves and 8 t-valves. These items can be seen in appendix C. The valves were used in order to control the flow of water as well as allow multiple pathways for the fluid. A 12VDC fluidic motor was used to pump the TMB across the detection area.

Analysis Segment

For the design of the analysis component of the sensor, the method of detection was based off of the ELISA (Enzyme-Linked ImmunoSorbent Assay) technique. This process involves measuring the absorbance of a color change in the medium the gluten sample is contained. Although it was initially proposed that disposable and replaceable cuvettes units would be sold to the user with antibodies already immobilized on the surface, this idea was considered inefficient as it would require multiple washings to remove excess gluten and antibody particles, and the reaction would have to be stopped and read by the user at a very specific time. An alternative system was developed where the ELISA reaction is separated from the cuvette where the TMB chromogen is analyzed. Two solutions were designed, and currently require testing to determine the optimal choice. These solutions used a flow-through system, where solutions are flowed through some device for a set time rather than statically placed in a container for a set time as in a conventional ELISA. This flow-through concept was chosen because it eliminates the need for washings and the need to stop the reaction. The proposed solutions that need testing are a disposable, replaceable glass tubing with antibodies immobilized on the inner surface, and a replaceable nitrocellulose filter with immobilized antibodies that the solutions would flow through. This latter option is also known as ELIFA (Enzyme-Linked ImmunoFlow Assay) and there are currently commercially available products utilizing the concept (ThermoFisher Scientific, Inc. 2008).

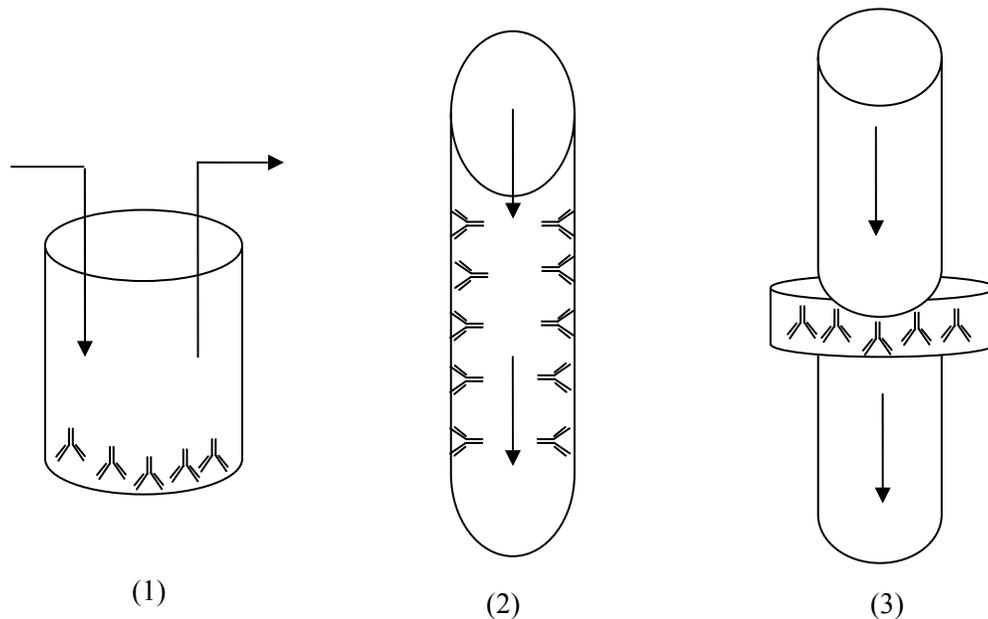


Figure 21: (1) Conventional ELISA. (2) Flow Through ELISA. (3) ELIFA

The gliadin will become complexed with the antibody-enzyme (horseradish peroxidase) complex during the addition of the enzyme-antibodies in the mixing stage. The 1 mL volume of sample/enzyme antibodies will be measured out by the user flipping valves in sequence. This volume will then flow through the ELISA system, where antibodies would be immobilized on the interior of the glass tubing. The ELIFA system involving a membrane which the antibodies would be immobilized on was deemed inefficient, as food particles would likely be caught on the membrane as

well. After flowing through this system, the effluent should flow into the waste container. The user would then measure out the 1 mL of 3,5,3',5' - Tetramethylbenzidine (TMB) using the same valve system used to measure out the sample, and then would flow through the system containing the immobilized enzymes. The user would have to turn a valve before adding the TMB, allowing it to flow into the cuvette for analysis rather than into the waste. After waiting a set amount of time (which is dependent on the required incubation time of the ELISA system and has yet to be determined), the user would then turn on the absorbance reading LED. The absorbance of the medium can then be read at 627 nm by shining light at a set intensity and wavelength of 627 nm through the medium and measuring the light intensity exiting the medium. The absorbance is given by Equation 8.

The TSL13S TaosInc Light-to-Voltage sensor converts the intensity of the light into a voltage, which can then be processed into an output signal which is read by the user. The relationship between the absorbance and the concentration of the analyte can often be determined through Beer's Law (Equation 9). The signal processing is performed by a series of op-amps and zener diode, and outputted to the user through the use of a red LED and a green LED, which are on the exterior of the device and can be seen by the user. The device will be calibrated such that, at sample concentrations less than 20 ppm, the output voltage after amplification by the op-amp will be greater than the zener voltage of the zener diode. This will cause the green LED to light up, while a sample of concentration greater than 20 ppm will have an output voltage less than the zener voltage and cause both green LED to turn off. Thus, the output on whether the food is safe to eat is a binary output where the user looks to see which LEDs are on. This output circuit has been tested and verified using known voltages generated from a power source, and the LED/photodiode has also been tested. The LED/photodiode system was tested using the RL5-R120008 LED from SuperBright LEDs. This LED saturated the photodiode in tests involving nothing between the LED and photodiode, as well as most tests with various solutions of blue food dye in water (to simulate reacted TMB) in a cuvette between them. Only in very dark blue solutions was the LED not saturated. It was determined that the LED, which had a luminous intensity of 12000 mcd, was providing a more than 600,000 times the test value for the photodiode (test value of 0.018 mcd) when no sample is present. New LEDs of lower luminous intensity, 1200 mcd, were ordered and installed to correct this problem.

For the purposes of our sensor, the Beer-Lambert Law equation cannot be used because the concentration is not the concentration of gliadin in the sample, but rather the concentration of reacted TMB in a period of time. For this reason, a standard curve must be determined experimentally using standards with known concentrations of gliadin. If a time was found where the ratio of reacted TMB was equal to the concentration of gliadin in the sample, Beer's Law could be used, using a TMB molar absorptivity of $1.36 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ at 648 nm (Everhart, 1995). Sample standard curves were found for gliadin from the Immunotech corporation (Immunotech, 2008).

This sample standard curve is developed using standard ELISA well sizes (6.5mm diameter), as well as a sample size of 200 μL (Nunc, 2008). Absorbances used for the detection would ideally come from the mostly linear portion of the standard curve (i.e. 0.1 – 1), corresponding to a range of 0 – 30 ng/mL using this given standard curve. Since we would be analyzing food samples with a gluten concentration of between 0 – 30 ppm (0 – 0.03 mg/mL), our samples would have to be diluted by a factor of 1:1000 to give concentrations similar to the ones used in this standard curve (i.e. 0.03 mg/mL = 30000 ng/mL, 30000 ng/mL /1000 = 30 ng/mL). Finally, for this standard curve the TMB was incubated for 30 min. under static conditions. For our proposed flow-through assay method, the incubation time should be lower based on commercially available ELIFA systems (ThermoFisher Scientific, Inc., 2008), but the exact time needed to be determined experimentally.

Our test experimentations of the flow-through ELISA method have shown that our system would be able to distinguish between samples greater than or less than 20 ppm and quantify the amount of

gluten in the sample. Additionally, we have developed a preliminary standard curve relating gluten concentration to outputted voltage from the photodiode using test samples of 200, 20, 10 and 0 ppm (Fig. 15). However, the time done in these experiments was too long to be practical (45 min), and further testing should be done to determine the minimum amount of time required to receive a response. Additionally, further testing should be done to produce a more complete standard curve through the testing of additional concentrations.

Final Design Specifications

The device has been designed to be able to detect gluten concentrations below 20 ppm and distinguish between samples greater than or less than 20 ppm. The size was about 40 cm x 30cm x 20cm. The time for a test was estimated to be around 45 minutes. The device cost came out to \$250.00 for the mass production cost, with a \$1.00 cost per use, and \$698.66 for the initial prototype cost. It was capable of testing all types of food. The device was not user friendly as it required many steps that could have been difficult for the average user to understand and complete.

Testing and Calibration

Analysis Segment

For the testing of the analysis segment, the minimum amount of time for the flow-through ELISA should be determined. Additionally, the preliminary standard curve developed further with the addition of more concentration data points to better relate concentration of gluten to output voltage.

Process 1 – Sample Homogenization

In order to calibrate the effectiveness of the mixing time, a sample of bulky food with known gluten content will be added and tested for accurate readings. If the reading fails then the mixing time will be re-determined through trial and error.

Process 2 – Enzyme Addition

The second processing tank can be calibrated using a simple saline addition method. A sensor will be placed in a separated Tank 2 for testing. Then water will be added along with a saline solution similar to the concentration of the enzyme solution used. The saline sensor is monitored and readings are taken until complete homogenization is reached. This is compared to the calculated mixing time and either confirmed or re-determined through trial and error.

External Constraints

The device would provide an ethical benefit socially, allowing the user to better monitor their diet and increasing their health. However, because the sensor uses non-reusable reagents, there is waste created by the product, and refills of these reagents would have to be provided, increasing the economic and environmental costs. Although the sensor itself should remain functional and reusable for long periods of time, fluid and reagent refills hamper its sustainability. The device also used designs similar to some currently existing products, such as the syringe pump and blenders, which could cause some intellectual property disputes, although it is unlikely. Finally, there is some safety risk associated with false negatives that could cause the user to ingest unsafe foods. The device should be made sure to be as reliable as possible for this reason.

Safety Considerations

There are some safety risks associated with the current design: the sensor contains moving sharp blades, so there is an injury risk, as well as a shock risk from the electrical components. However, since these parts are contained within the interior of the product and are small, the risk factor for serious injury is minimal. It is possible that, due to the high light intensity of the LED used for detection, that if the user stares directly into this LED, could cause ocular damage. However, this LED is enclosed within the system, and the user would have to open the device up and turn on the LED to be exposed to the high intensity light. The LEDs used for the output signal are much lower intensity and should pose no such similar risk. The major safety factor present is if the device malfunctions, the user could potentially ingest an unsafe food sample, causing intestinal damage. Although a single malfunction would not pose a lethal risk and instead only a risk of indigestion or other minor health problems, prolonged malfunction could result in more serious health risks. While our device is reliable and would not malfunction regularly, the potential danger is present.

Operation

1. Find a sample of food to be tested.
2. Add approximately 1 cm³ or 1 g of food into the grinding container.
3. Close the lid and activate grinder.
4. Push button to activate syringe pumps.
5. Push button to activate flow of wash solution.
6. Push button to activate metered pump for TMB flow.
7. Flip switch to activate output circuit.
8. Check LEDs for output: Green LED on means food contains below 20 ppm gluten, Green LED off means food contains more than 20 ppm gluten.
9. Push button to activate flow of wash solution.

Conclusions

The purpose of the design was to develop an effective solution for detecting small amounts (below 20 ppm) of gluten in all food types. The goal was for the device to be small and portable (handheld) and to be autonomous. A cheap and small sized product with complete autonomy would have great success in the market. Our proposed solution would meet the necessary demands of this device.

Our current design and prototyping has been completed. The testing has been completed for each of the components of the device, and found that each of them performed satisfactorily for the needs of the system. Testing of the flow-through ELISA method has revealed that the system should be able to give an accurate output to the user indicating the gluten content. The blender component has been seen to function properly and able to grind food samples and extract the gluten content. The dual syringe pump and step motor pump have also been tested and found to be able to pump and measure the proper proportions of fluids. The connecting tubing and power systems still need to be developed and perfected, however.

Overall, it is anticipated that our final prototype will meet the initial constraints set by the client. The device will be able to detect concentrations of gluten around the crucial 20 ppm, and be able to give an output to the user informing them if the product is safe to eat. The current cost of construction,

\$598.66, is higher than the initial constraint of \$250, but it is estimated that a mass produced product will cost lower than the price of the prototype, with a cost of \$250 and a \$1.00 cost per use. Although the device is not yet within the time practical time limits for a commercially viable device, with a time for the flow-through ELISA of around 45 minutes. However, we figure this could be reduced in further prototypes, by inducing mixing or heating elements and be brought down to an appropriate level. One constraint that is likely not to be met by our prototype is size, which is anticipated to be (40cm x 30cm x 20cm), which is not small enough to be portable. However, it is likely possible to miniaturize our various systems in the prototype to produce a portable device in the future. Overall, the designed device and anticipated prototype solves the problem that the client wanted solved.

Recommendations

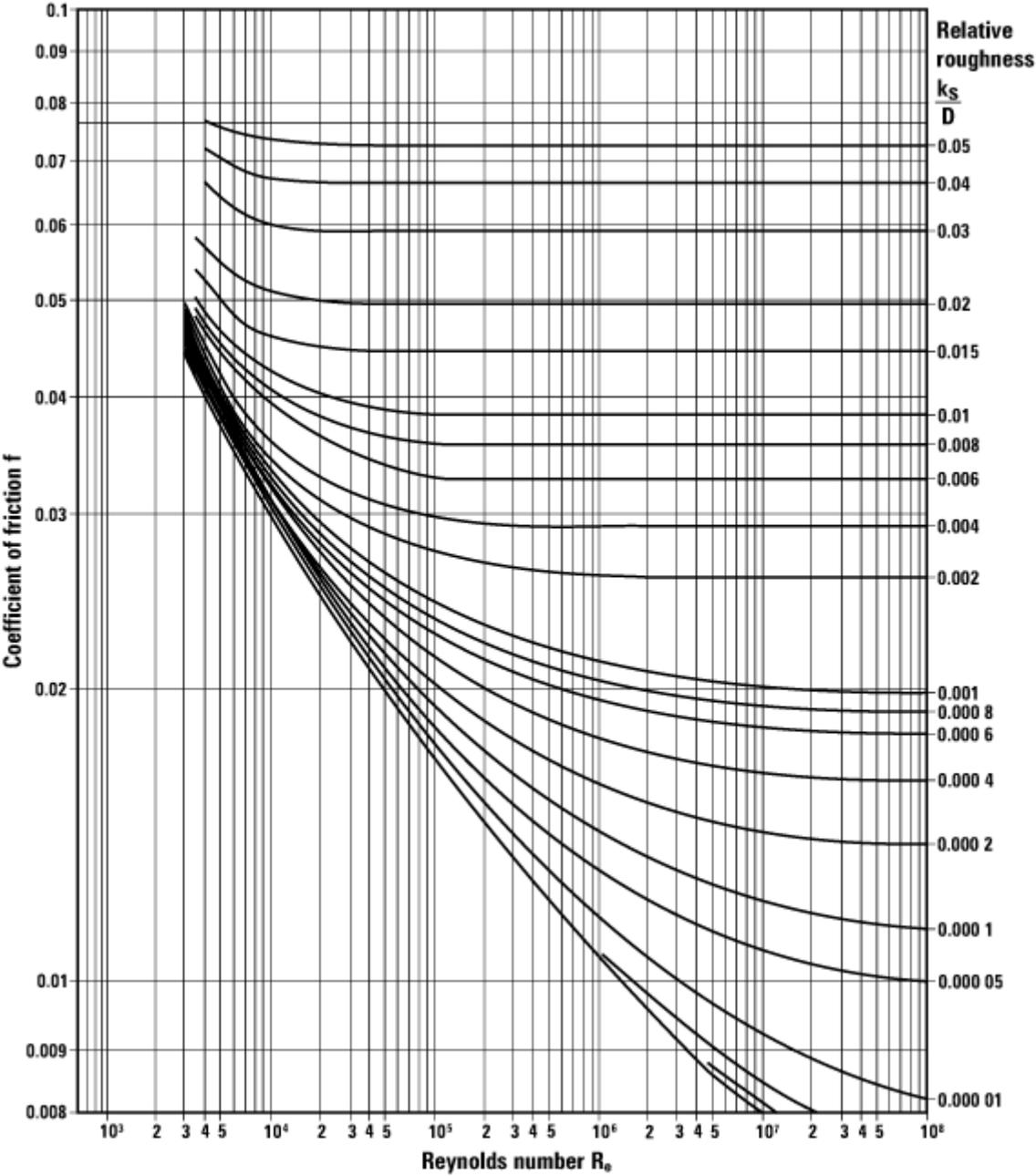
The project is recommended to continue patenting, prototyping and testing. The absolute time requirements of the flow-through ELISA should be determined, as well as a more complete standard curve should be developed. The tubing, valve, and power systems still need to be perfected and tested, although preliminary systems are in place. Scale down of the systems should be begun to miniaturize the device and bring it down into the original design specifications and become truly portable. Finally, a patent should be pursued for the device in order to protect the intellectual property and work done.

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Appendix A: Moody Chart



Appendix B: Equations

Pump/Pipeline

Equation 1: Classification of slurry flows

$$\log \frac{C_T}{C_A} = -1.8 \frac{V_s}{\kappa u_*}$$

Equation 2: Shear velocity for slurry flows

$$u_* = \sqrt{\frac{\tau_0}{\rho}} = V \sqrt{\frac{f}{8}}$$

Equation 3: Friction factor

$$f = \frac{64}{\text{Re}}$$

Equation 4: Pressure Gradient of pseudohomogeneous flows

$$i_m = \frac{\Delta p_m}{L} = f_m \frac{1}{D} \frac{\rho_m V^2}{2}$$

Equation 5: Slurry/mixture Viscosity

$$\mu_m = \mu \left[1 + 2.5C_v + 10.05C_v^2 + 0.00273 \text{EXP}(16.6C_v) \right]$$

Equation 6: Mixture Density

$$\rho_m = \frac{\rho_s}{S + C_w - SC_w}$$

Equation 7: Stokes Settling Equation

$$V_s = \frac{1}{18} \frac{g(\rho_p - \rho_f)D^2}{\nu}$$

Equation 8: Volume

$$V_t - V_c = V_{actual}$$

Equation 9: Volume Addition to Second Chamber

$$V \left(\frac{1}{24.15} \right) = 25.97 \left(\frac{1}{1000} \right)$$

Equation 10: Power Requirements for Helical Ribbon

$$\text{Re} = \frac{N_i \cdot D_i^2 \cdot \rho}{\mu}$$
$$P = k_1 \cdot \mu \cdot N_i^2 \cdot D_i^3$$

Equation 11: Time Requirements for Helical Ribbon

$$N_i \cdot t_m = 12.6$$
$$t_m = 4t_c$$

Equation 12: Time Requirements for Rushton Turbine

$$N_i \cdot t_m = \frac{1.54 \cdot V_{actual}}{D_i^3}$$

Equation 13: Absorbance

$$A = -\log_{10}(I_1 / I_0)$$

A = Absorbance, I_1 = Exiting light intensity, I_0 = Emitted light intensity

Equation 14: Beer-Lambert Law

$$A = \epsilon lc$$

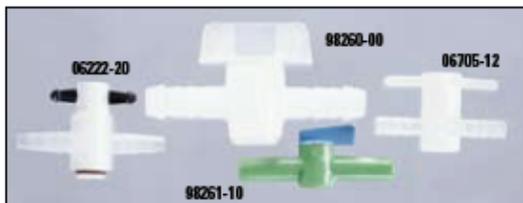
A = Absorbance, ϵ = Molar Absorptivity, l = path length, c = concentration

Appendix C: Cole Parmer Valve Catalog



Two- and Three-way Stopcocks with Hose Barb Connections

PP stopcocks have plugs made of PTFE and are autoclavable. Maximum temperature is 275°F (135°C); maximum pressure is 2 psi. PVDF stopcocks are rated for 464°F (240°C); maximum pressure is 5 psi. LDPE stopcocks are rated for 167°F (75°C); maximum pressure is 7 psi. HDPE stopcocks are rated for 203°F (95°C); maximum pressure is 7 psi.



Cat. no.	Tube ID	Orifice size	Material	Price/ea ¹
Two-way stopcocks				
K-06222-20	1/4" to 3/8"	2 mm	PP	
K-06222-40	1/2" to 3/4"	4 mm		
K-98260-00	3/4"	1/4"	LDPE	
K-98260-10	1/2"	3/8"		
K-98261-10	10 mm	6.8 mm	HDPE	
K-98261-20	14 mm	10.7 mm		
K-98261-30	16 mm	12.7 mm		
Three-way stopcocks; follow flow pattern 2				
K-06225-20	1/4" to 3/8"	2 mm	PP	
K-06225-40	1/2" to 3/4"	4 mm		
K-06705-10	6 mm	4 mm	PVDF	
K-06705-12	8 mm	6 mm		
K-06705-14	10 mm	8 mm		

¹Quantity discounts available

GO to page(s) 2167-2217
For our full selection of flexible and rigid tubing in formulations for all of your applications.

Keck® Ramp Clamps®

Unique wheel-in-groove clamp design permits one-handed flow control. Simply remove wheel to insert tubing, then roll wheel back into clamp to your desired flow rate. Made of pure polybutylene terephthalate (PBT) thermoplastic polyester; safe to 392°F (200°C). All four sizes accept tubing with a maximum wall thickness of 3/32" (2.4 mm). Autoclavable.



See page 317 for more Keck® adapters, clamps, and clips.

Catalog number	Max tubing OD	Price/pkg of 12
EG-06835-06	3/16"	4.5 mm
EG-06835-08	1/4"	6 mm
EG-06835-07	3/8"	10 mm
EG-06835-10	1/2"	14 mm

EG-06841-00 **Clamp assortment kit.** Contains 8 each of 06835-05, -03, -07, and -10 above; 32 pieces total

Stopcocks with Flow Indication

PVC bodies, EPDM seals and seats. Flow indicator on handle. Maximum pressure is 150 psi at the maximum temperature of 120°F (49°C); maximum vacuum is 29.9" Hg.



Catalog number	Connection	Orifice size	Price
K-06225-50	NPT(F) x NPT(F)	1/4"	
K-06225-52	NPT(M) x NPT(M)	1/4"	
K-06225-54	NPT(M) x hose	1/4"	
K-06225-56	Hose x hose	1/4" to 1/2"	
K-06225-58	NPT(M) x NPT(F)	1/4"	

K-06225-60 **Universal stopcock kit.** Comes complete with two 1/4" NPT(M) adapters, two hose barb connectors accepting 1/4" to 3/8" ID tubing, and a wrench

Stopcocks for Glass and Rigid Tubing

Stopcocks have wetted parts constructed of PFA. An O-ring made of Viton® protects the glass tube when you tighten the compression nut. Stopcocks also accept rigid tubing such as PTFE.

Available in two-way and three-way styles. The three-way stopcocks follow flow pattern 2 (see left).



Two-way stopcock



Three-way stopcock

Tube OD	Two-way stopcocks		Three-way stopcocks	
	Cat. no.	Price	Cat. no.	Price
6 mm	K-30503-01		K-30503-10	
8 mm	K-30503-02		K-30503-12	
10 mm	K-30503-04		K-30503-14	

Stopcocks with Compression Fittings

Stopcock bodies are made of PFA. Plug is made of PTFE. Maximum pressure is 50 psi.

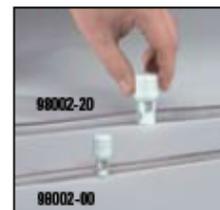


06373-85

Catalog number	Tube OD	Orifice size	C _v	Price
K-06373-85	1/4"	1/4"	0.38	
K-06373-86	1/2"	1/2"	0.44	

Manual Pinch Valves

Fluid contacts only the tubing—for use where preventing contamination is critical. Obtain precise and repeatable flow rates using easy-to-read metered scale on valve body. Valves are made of Delrin®. Tubing is not included—order flexible tubing with durometer (Shore A) of 60 or less from our "Tubing" section on pages 2167-2217.



98002-20

98002-00

Catalog number	Tube OD	Price
K-98002-00	3/32" to 1/4"	
K-98002-10	3/8" to 3/4"	
K-98002-20	3/4" to 1/2"	

Appendix D: Motor

Miniature Gear Motor: Offset Shaft

<http://www.hobbyengineering.com/H1211.html>

HOBBYENGINEERING Department

A supply store for people who want to build robots, electronic gadgets, kinetic art or anything else that moves, beeps or flashes.

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- ◆ ICs

Miniature Gear Motor: Offset Shaft



The GM6 is a baby version of our popular GM2 gearmotor, again designed by Mark Tilden to have tighter tolerances and better performance than a regular toy-style motor. These smaller, quicker 120:1 gear reduction ratio'd motors are sure to stir ideas!

This gearmotor starts rotation at 0.58 volts, turning 7RPM drawing only 46.5mA. At 5 volts, this unit really humms, spinning at 145RPM and drawing 88mA no-load (610mA stall producing 20in*oz torque). We've successfully used these units very well on solarengine circuits.

Like it's big brother, this gearmotor has a built-in safety clutch which engages at approximately 25in*oz (at 6.5V / 650mA). This motor also has a unique 2mm (0.8") crest diameter chrome-finish splined shaft can be pushed through the output gear to either side of the motor, and is also solderable! (If you do want to solder to it, yank the shaft right out, solder, then press it back in).

These motors have an overall size of 37mm x 21mm x 27mm (1.45" x 0.83" x 1.06"), and weigh in at only 20 grams (0.7oz). A tidy package, with pretty good power! Unlike the GM2 or GM3, these motors do NOT come with any sort of wheel or mount.

Quick Facts	
Manufacturer's Part Number:	GM6
Buy Now:	Add to Cart

Stock Number	Buy	Product Title	Price		Status
			Qty	Price	
H01211-01F	Add to Cart	Miniature Gear Motor: Offset Shaft	1	\$7.99	In Stock
			2 to 9	\$7.19	
			10 to 24	\$6.79	
			25 to 49	\$6.39	
			50 or more	\$5.99	

Accessories

Item	Title
1897	1.25" Rubber Wheel

Other products in the Geared DC Motors (Assembled) category

Item	Title
------	-------

Appendix E: TaosInc TSL13S-LF LTV Chip

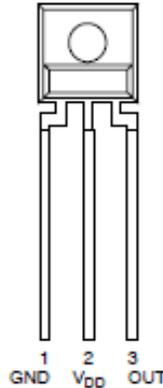


TSL12S, TSL13S, TSL14S LIGHT-TO-VOLTAGE CONVERTERS

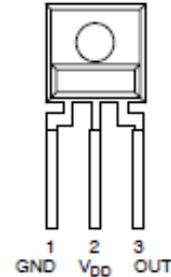
TAOS001E - SEPTEMBER 2007

- Converts Light Intensity to Output Voltage
- Monolithic Silicon IC Containing Photodiode, Transconductance Amplifier, and Feedback Components
- Single-Supply Operation . . . 2.7 V to 5.5 V
- High Irradiance Responsivity . . . Typical 246 mV/(μ W/cm²) at $\lambda_p = 640$ nm (TSL12S)
- Low Supply Current . . . 1.1 mA Typical
- Sidelooker 3-Lead Plastic Package
- RoHS Compliant (-LF Package Only)

PACKAGE S
SIDELOOKER
(FRONT VIEW)



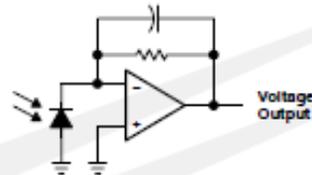
PACKAGE SM
SURFACE MOUNT
SIDELOOKER
(FRONT VIEW)



Description

The TSL12S, TSL13S, and TSL14S are cost-optimized, highly integrated light-to-voltage optical sensors, each combining a photodiode and a transimpedance amplifier (feedback resistor = 60 M Ω , 20 M Ω , and 5 M Ω , respectively) on a single monolithic integrated circuit. The photodiode active area is 0.5 mm \times 0.5 mm and the sensors respond to light in the range of 320 nm to 1050 nm. Output voltage is linear with light intensity (irradiance) incident on the sensor over a wide dynamic range. These devices are supplied in a 3-lead clear plastic sidelooker package (S). When supplied in the lead (Pb) free package, the device is RoHS compliant.

Functional Block Diagram



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Texas Advanced Optoelectronic Solutions Inc.

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1

TSL12S, TSL13S, TSL14S LIGHT-TO-VOLTAGE CONVERTERS

TAOS051E - SEPTEMBER 2007

Available Options

DEVICE	T _A	PACKAGE - LEADS	PACKAGE DESIGNATOR	ORDERING NUMBER
TSL12S	0°C to 70°C	3-lead Sidelooker	S	TSL12S
TSL12S	0°C to 70°C	3-lead Sidelooker — Lead (Pb) Free	S	TSL12S-LF
TSL12S	0°C to 70°C	3-lead Surface-Mount Sidelooker — Lead (Pb) Free	SM	TSL12SM-LF
TSL13S	0°C to 70°C	3-lead Sidelooker	S	TSL13S
TSL13S	0°C to 70°C	3-lead Sidelooker — Lead (Pb) Free	S	TSL13S-LF
TSL13S	0°C to 70°C	3-lead Surface-Mount Sidelooker — Lead (Pb) Free	SM	TSL13SM-LF
TSL14S	0°C to 70°C	3-lead Sidelooker	S	TSL14S
TSL14S	0°C to 70°C	3-lead Sidelooker — Lead (Pb) Free	S	TSL14S-LF
TSL14S	0°C to 70°C	3-lead Surface-Mount Sidelooker — Lead (Pb) Free	SM	TSL14SM-LF

Terminal Functions

TERMINAL NAME	NO.	TYPE	DESCRIPTION
GND	1		Power supply ground (substrate). All voltages are referenced to GND.
OUT	3	O	Output voltage.
V _{DD}	2		Supply voltage.

Absolute Maximum Ratings over operating free-air temperature range (unless otherwise noted)[†]

Supply voltage, V _{DD} (see Note 1)	6 V
Output current, I _O	±10 mA
Duration of short-circuit current at (or below) 25°C (see Note 2)	5 s
Operating free-air temperature range, T _A	-25°C to 85°C
Storage temperature range, T _{stg}	-25°C to 85°C
Lead temperature 1.6 mm (1/16 inch) from case for 10 seconds (S Package)	260°C
Reflow solder, in accordance with J-STD-020C or J-STD-020D (SM Package)	260°C

[†] Stresses beyond those listed under "absolute maximum ratings" may cause permanent damage to the device. These are stress ratings only, and functional operation of the device at these or any other conditions beyond those indicated under "recommended operating conditions" is not implied. Exposure to absolute-maximum-rated conditions for extended periods may affect device reliability.

NOTES: 1. All voltages are with respect to GND.
2. Output may be shorted to supply.

Recommended Operating Conditions

	MIN	NOM	MAX	UNIT
Supply voltage, V _{DD}	2.7		5.5	V
Operating free-air temperature, T _A	0		70	°C

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2

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TAOS001E - SEPTEMBER 2007

Electrical Characteristics at $V_{DD} = 5\text{ V}$, $T_A = 25^\circ\text{C}$, $\lambda_p = 640\text{ nm}$, $R_L = 10\text{ k}\Omega$ (unless otherwise noted) (see Notes 3, 4, 5)

PARAMETER	TEST CONDITIONS	TSL12S			TSL13S			TSL14S			UNIT	
		MIN	TYP	MAX	MIN	TYP	MAX	MIN	TYP	MAX		
V_{OM}	Maximum output voltage	4.6	4.9		4.6	4.9		4.6	4.9		V	
V_O	Output voltage	$E_s = 8\ \mu\text{W}/\text{cm}^2$	1.5	2	2.5						V	
		$E_s = 31\ \mu\text{W}/\text{cm}^2$				1.5	2	2.5				
		$E_s = 120\ \mu\text{W}/\text{cm}^2$							1.5	2		2.5
		$E_s = 16\ \mu\text{W}/\text{cm}^2$		4								
		$E_s = 62\ \mu\text{W}/\text{cm}^2$					4					
		$E_s = 240\ \mu\text{W}/\text{cm}^2$								4		
R_s	Irradiance responsivity	Note 6	248		64		16			mV/ ($\mu\text{W}/\text{cm}^2$)		
V_{OS}	Extrapolated offset voltage	Note 6	-0.02	0.03	0.08	-0.02	0.03	0.08	-0.02	0.03	0.08	V
V_D	Dark voltage	$E_s = 0$	0	0.08		0	0.08		0	0.08		V
I_D	Supply current	$E_s = 0$		1.1	1.7						mA	
		$E_s = 8\ \mu\text{W}/\text{cm}^2$				1.1	1.7					
		$E_s = 120\ \mu\text{W}/\text{cm}^2$						1.1	1.7			

- NOTES: 3. Measurements are made with $R_L = 10\text{ k}\Omega$ between output and ground.
 4. Optical measurements are made using small-angle incident radiation from an LED optical source.
 5. The 640 nm input irradiance E_s is supplied by an AlInGaP LED with peak wavelength $\lambda_p = 640\text{ nm}$.
 6. Irradiance responsivity is characterized over the range $V_O = 0.2$ to 4 V. The best-fit straight line of Output Voltage V_O versus irradiance E_s over this range may have a positive or negative extrapolated V_O value for $E_s = 0$. For low irradiance values, the output voltage V_O versus irradiance E_s characteristic is non linear with a deviation toward $V_O = 0$, $E_s = 0$ origin from the best-fit straight line referenced above.

Dynamic Characteristics at $V_{DD} = 5\text{ V}$, $T_A = 25^\circ\text{C}$, $\lambda_p = 640\text{ nm}$, $R_L = 10\text{ k}\Omega$ (unless otherwise noted) (see Figure 1)

PARAMETER	TEST CONDITIONS	TSL12S			TSL13S			TSL14S			UNIT
		MIN	TYP	MAX	MIN	TYP	MAX	MIN	TYP	MAX	
t_{dr}	Output pulse delay time for rising edge (0% to 10%)	Min $V_O = 0\text{ V}$; Peak $V_O = 2\text{ V}$	13		1.7		0.9			μs	
	Min $V_O = 0.5\text{ V}$; Peak $V_O = 2\text{ V}$	2.3		1.2		0.6					
t_r	Output pulse rise time (10% to 90%)	Min $V_O = 0\text{ V}$; Peak $V_O = 2\text{ V}$	20		7.2		2.6			μs	
	Min $V_O = 0.5\text{ V}$; Peak $V_O = 2\text{ V}$	10		6.5		2.9					
t_{df}	Output pulse delay time for falling edge (100% to 90%)	Min $V_O = 0\text{ V}$; Peak $V_O = 2\text{ V}$	2.3		1.2		0.8			μs	
	Min $V_O = 0.5\text{ V}$; Peak $V_O = 2\text{ V}$	2.2		1.1		0.7					
t_f	Output pulse fall time (90% to 10%)	Min $V_O = 0\text{ V}$; Peak $V_O = 2\text{ V}$	10		6.8		2.9			μs	
	Min $V_O = 0.5\text{ V}$; Peak $V_O = 2\text{ V}$	9		6.4		2.8					

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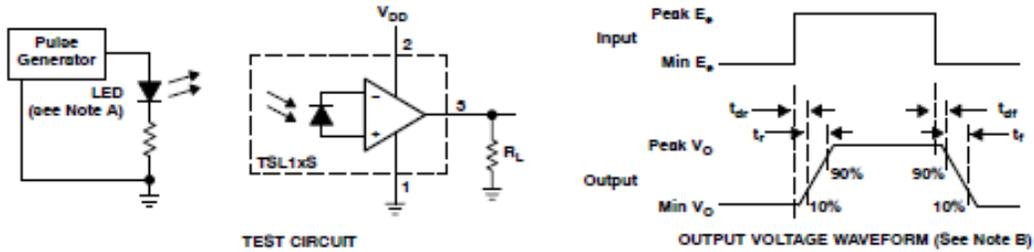
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3

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TAOS051E – SEPTEMBER 2007

PARAMETER MEASUREMENT INFORMATION



- NOTES: A. The input irradiance is supplied by a pulsed AlInGaP light-emitting diode with the following characteristics: $\lambda_p = 640$ nm, $t_p < 1 \mu s$, $t_r < 1 \mu s$.
 B. The output waveform is monitored on an oscilloscope with the following characteristics: $t_s < 100$ ns, $Z_i \geq 1$ M Ω , $C_i \leq 20$ pF.

Figure 1. Switching Times

TYPICAL CHARACTERISTICS

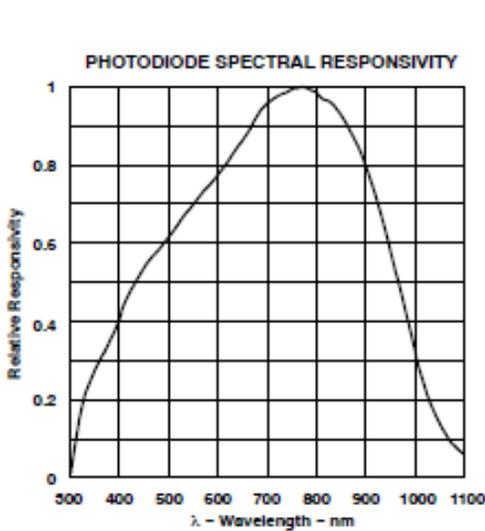


Figure 2

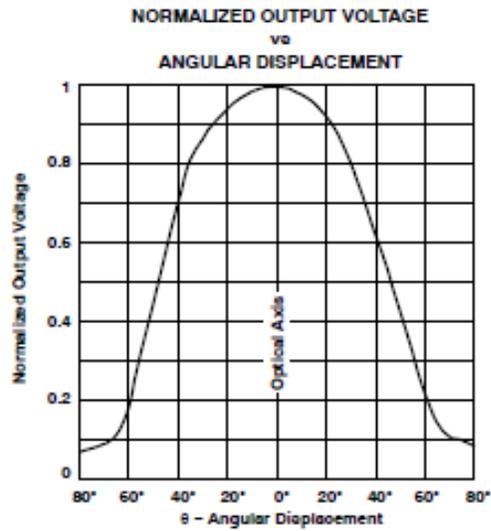


Figure 3

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Appendix F: EZ Gluten- Gluten Detection Kit



- ⊕ Easy to Use Home Test
- ⊕ Simple Small Portable
- ⊕ Test Individual Ingredients in Foods
- ⊕ Test Finished and Cooked Products
- ⊕ Quickly Detects the Presence of Gluten in Foods
- ⊕ Detects Levels of Gluten as Low as 10 ppm

In the EZ Gluten™ Test, A food sample is ground to a fine consistency,



then added to the gluten extraction solution and mixed.



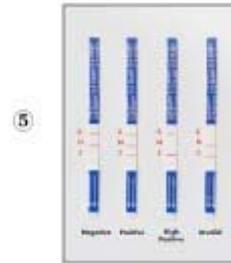
A few drops of the sample extract are placed into a test tube.



The EZ Gluten™ test strip is placed into the test tube to absorb the sample extract.



After 10 minutes, the test strip can be read for the presence of gluten.



Call us Toll-Free
1-866-222-4729
For Pricing and Shipping



ELISA Technologies, Inc.

EZ Gluten™ - 2 pk Kit \$ 25.00

EZ Gluten™ - 5 pk Kit \$ 60.00

EZ Gluten™ - 10 pk Kit \$110.00

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