Warren J. Baker Endowment for Excellence in Project-Based Learning
Robert D. Koob Endowment for Student Success

Excellence in Project-Based Learning

Proposal Cover Page

Title of Project:

Application of Polymer Bound Boronic Acid for Glucose Detection in Printed Microfluidics Devices

Student Applicant(s)	Signature	Cal Poly Email	Department
Spencer Schultz		spschult@calpoly.edu	Chemistry and Biochemistry

Faculty Advisor: Andres Martinez **Department:** Chemistry and Biochemistry

Faculty Advisor email: awmartin@calpoly.edu **Telephone:** 805-756-2744

Anticipated Start Date: January 5, 2015

Anticipated End Date: December 4, 2015

Total Funds Requested ($): $2500

Signature of Faculty Advisor: _____ **Date:** _____

PROPOSAL NARRATIVE

I. Abstract

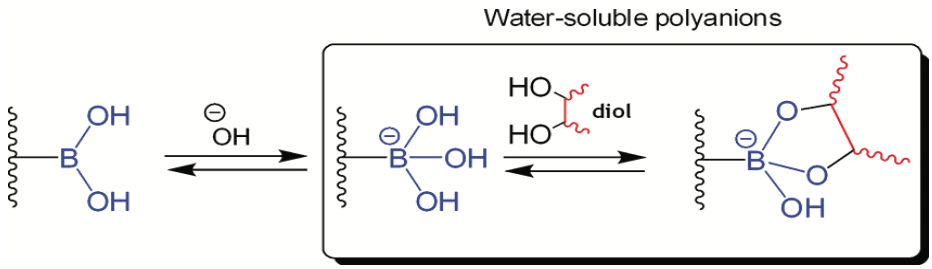
The goal of this research project is to develop a simple and quantitative chronometric glucose test on printed microfluidic devices using polymer bound boronic acid (PBBA), which selectively binds to glucose, instead of enzymatic reactions. PBBA promises to enable cheaper medical diagnostics without requiring the expensive and often complex instrumentation that is normally utilized in medical diagnostics. PBBA should also increase the shelf life of glucose tests as the material is not as susceptible to degradation as the enzymatic reagents that are currently in use. The final device will be optimized to measure glucose concentrations in the range of 0.5 to 10 mM using 20 to 40 µL of a liquid sample in a time frame under 30 minutes. The results gained from this project will serve as a foundation for further research into the use of non-enzymatic polymer-based assays in printed microfluidic devices.

II. Introduction

Point-of-care of care diagnostic assays offer great promise for expanding access to healthcare to the most remote settings around the world. Printed microfluidic devices are one type of point-of-care device that have shown promise as a general platform for low-cost, portable and simple to use diagnostic tests. Many of the tests conducted on printed microfluidic devices rely on enzymatic reactions for specificity or signal amplification. The

problem with enzymatic tests is that the enzymes are not particularly stable and tend to denature when stored on the devices within days or weeks. In order to overcome this limitation, this proposal aims to explore the use of polymers for detecting analytes on printed microfluidic devices. Polymer bound boronic acids (PBBA) is an interesting material to start this work because it undergoes a change in its solubility when exposed to glucose. This response to glucose will be harnessed to produce a simple glucose sensor.

When PBBA is under neutral conditions crosslinkage occurs between the boronic acid groups, which form a continuous hydrophobic network that is insoluble in water. Under basic conditions, the boron can act as an electrophile and pull a hydroxyl ion from the solution. This transforms the boronic acid groups into water-soluble polyanions as denoted in Scheme 1.



Scheme 1: Ionization and Diol Complexation Equilibria of Bornoic Acids in Aqueous Solutions

A diol such as glucose can bind to the boronic acid through a dehydration reaction under neutral conditions. This reaction prevents crosslinking from occurring and will make the PBBA change from being insoluble to being soluble in water. This property makes the material a good candidate for use as an assay in microfluidics devices as the material could act as a temporary barrier that only allows for solution passage in the presence of glucose.

III. Objective(s)

The objective of this research project is to develop a simple and quantitative chronometric glucose test on printed microfluidic devices using polymer bound boronic acid. In

order to achieve this objective, methods for fabricating the devices and methods for applying the polymer to the devices will be developed. The polymer and the devices will be optimized for the specific application in order to detect clinical ranges of glucose.

IV. Methodology

The printed microfluidic devices will be designed using Auto-Cad. A simple device with a sample inlet, a reagent storage zone and test zone will be designed (Figure 1). The pattern for the devices will then be printed onto Whatman No. 1 chromatography paper using a Xerox solid ink printer. Finally, the devices will be heated in a convection oven at 195^{o}C for 1 minute and then allowed to cool to room temperature slowly under ambient conditions.

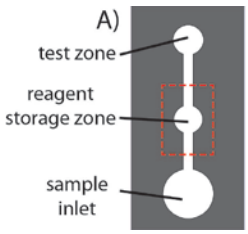


Figure 2: Design of the printed microfluidic device.

The PBBA will be deposited onto the reagent storage zone of the devices by first dissolving the polymer in a basic solution and applying a small volume of the resulting solution on the device. An acidic solution will then be applied to the polymer to neutralize the base and cause the polymer to become insoluble, thus the polymer will become entrapped within the matrix of the chromatography paper. The pH for the basic solution and the acidic solution will be optimized in order to allow for effective deposition of the polymer.

Glucose solutions with concentrations ranging from 0.5 to 20 mM will be prepared in a phosphate buffer in order to simulate physiological conditions. These solutions will be tested by adding the solution to the sample inlet of the device and monitoring the time it takes for the solution to wick into the test zone. Solutions with higher concentrations of glucose are expected to be able to dissolve the polymer faster and wick across the channel in a shorter

amount of time. A calibration curve of time to wick across the channel versus concentration of glucose in the sample will be prepared and the results will guide further optimization of the device.

The amount of polymer deposited, the shape of the reagent storage zone and the polymer itself will be optimized in order to obtain enough sensitivity from the test to distinguish between glucose concentrations with differences of no more than 1 mM and with an overall limit of detection of at least 0.5 mM. The polymer will be optimized by synthesizing various PBBAs with variations in the polymer size, the boronic acid density, and the monomeric density within the copolymer itself. The modifications of the polymer that will be attempted will depend upon the results from the initial tests with the glucose solutions. For synthesizing new polymers, Spencer will travel to the University of Florida to work in the laboratory of Professor Brent Sumerlin.

Once the single channel system is optimized, a final device for detecting glucose will be developed for detecting glucose concentrations in the range of 0.5 to 10 mM using 20 to 40 µL of sample within a 30 minute time period from sample introduction to final result. The final device will comprise multiple channels that will allow for negative control tests, positive control tests, and a sample tests to be conducted simultaneously. The negative and positive controls will aid in calibrating the results of the device to account for variations in environmental conditions such as humidity and temperature and also to ensure that the results are valid. We will also conduct side-by-side experiments using the polymer-based devices and conventional enzymatic-based devices to compare the accuracy, precision and shelf life of the two types of devices.

V. Timeline

Fabrication of devices: January 2015-February 2015

Optimization of methods for depositing the polymer: January 2015-April 2015

Testing and optimization of the device for detection of glucose: February 2015-September 2015

Synthesis of polymers at the University of Florida: April 2015

Preparation of the final glucose test and comparison to current enzyme-based tests: September 2015-December 2015

VI. Final Products and Dissemination

The research for this proposal will be used as the basis for a Masters thesis by the student investigator in order to fulfill the thesis requirement for obtaining a Masters of Science in Polymers and Coating Science. Results will also be submitted for publication, used as a basis for a patent application, and presented at the American Chemical Society's national meeting. Furthermore, this research will be used as a foundation for further study of the application of non-enzymatic assay in printed microfluidics devices.

VII. Budget Justification

The $1500 allocated in the budget for Out-of-State Travel is for travel to the University of Florida in Gainsville, Florida. At this present time, the facilities and expertise for making the polymer bound boronic acid material are not present at Cal Poly, so I will travel to Florida to be trained to make the material. The $100 allocated for Postage and Shipping will be for shipping material produced during this visit from Gainsville, Florida to Cal Poly. The $900 allocated for Non-computer Supplies and Materials will be used to purchase lab supplies necessary to make the devices and determine a feasible application method. These materials include: Whatman #1 Chromatography Paper, Xerox Solid Ink, Scotch Thermal Lamination Pouches, Pipet Tips (1000, 200, and 10 µL), 1.5 mL Micro-centrifuge Tubes, 15 mL Centrifuge Tubes, Glucose, Glucose Oxidase, Horseradish Peroxidase, Erioglaucine (blue dye), ABTS, and Phosphate Buffer.

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PROPOSAL BUDGET

Student Applicant(s): Spencer Schultz	
Faculty Advisor:Andres Martinez	
Project Title: Application of Polymer Bound Boronic Acid for Glucose Detection in Printed Microfluidics Devices	**Requested Baker Endowment Funding**
Travel *subtotal*	**$1500**
Travel: In-state	**$**
Travel: Out-of-state	**$1500**
Travel: International	**$**
Operating Expenses *subtotal*	**$1000**
Non-computer Supplies & Materials	**$900**
Computer Supplies & Materials	**$**
Software/Software Licenses	**$**
Printing/Duplication	**$**
Postage/Shipping	**$100**
Registration	**$**
Membership Dues & Subscriptions	**$**
Multimedia Services	**$**
Advertising	**$**
Journal Publication Costs	**$**
Contractual Services *subtotal*	**$**
Contracted Services	**$**
Equipment Rental/Lease Agreements	**$**
Service/Maintenance Agreements	**$**
TOTAL	**$2500**

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California Polytechnic State University
San Luis Obispo, CA 93407

October 23, 2014

RE: Letter of support for Baker and Koob Endowment proposal

Title of Project: Application of Polymer Bound Boronic Acid for Glucose Detection in Printed Microfluidics Devices

Student Applicant: Spencer Schultz (Polymers and Coatings program, Chemistry Department)

Dear Baker and Koob Endowment Proposal Selection Committee,

Spence Schultz is proposing a project that will lead to a new option for monitoring glucose concentrations in a portable, point-of-care format. Glucose tests are important for monitoring blood glucose levels and urine glucose levels. All currently available glucose tests rely on enzymatic reactions for specificity and signal amplification. While these tests work well, they tend to have a short shelf life because the enzymes denature over time. Spencer will explore the use of polymer bound boronic acid instead of enzymes for detecting glucose, which could lead to sensitive, inexpensive and long-lasting glucose sensors. The project is highly interdisciplinary as it will incorporate elements of polymer chemistry, analytical chemistry, device fabrication, and will ultimately have applications in clinical settings. Spencer will be supported by three faculty with expertise in all the areas required for successful completion of this project: myself, with expertise in analytical chemistry and device fabrication; Professor Phillip Costanzo, with expertise in polymer synthesis; and Professor Brent Sumerlin at the University of Florida, who is also an expert in polymer synthesis and is the leading expert in the synthesis and applications of boronic acid polymers. I envision that one or two undergraduate students will also become involved in the project, under Spencer's supervision, during the summer of 2015.

Spencer is a master's student in the chemistry department's polymers and coatings program. He has the right background and skillset to take on this project and this project will be part of his master's thesis work. Spencer is highly motivated and has already demonstrated that he can quickly learn the techniques that he will need to complete the project. Spencer also has excellent verbal and written communication skills. The funds from the proposal will provide

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Spencer with the opportunity to travel to the University of Florida and work in Professor Summerlin's laboratory where he will learn the techniques for synthesizing the boronic acid polymers. This, in turn, should help foster a long-lasting collaboration between our department's polymers and coatings program and the polymers program and the University of Florida.

The bulk of the research will be carried out in a dedicated research space in building 180, room 365. The laboratory is equipped with all the equipment required to fabricate the devices, conduct the assays and optimize the results. I will also provide spencer with all the basic supplies he will need that are not detailed in the budget, things like gloves and glassware. While Spencer is new to the area of device fabrication, he will have my full support as he pursues the project, and I will be able to teach him any techniques they he is not already familiar with. We will also hold weekly meetings to discuss his results and future work.

Please do not hesitate to contact me if you have any additional questions about Spencer's proposal. Best regards,

Andres W. Martinez
Assistant Professor
Department of Chemistry & Biochemistry
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