UMSAEP UM-UWC Interim Report

Visit to Columbia, Missouri, 11 April – 27 June 2019

Multi-component electrochemical sensors based on cucurbit [8] uril and their boronic acid substituted bis-pyridinium salt receptors.

Submitted by
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UM-Columbia Host: Prof Timothy Glass
Head of Department: Chemistry
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1. Overview

The research exchange visit to the University of Missouri (Columbia) represented the first steps towards a mutually beneficial research collaboration between the electrochemistry driven research focus of SensorLab (UWC) and the Organic Chemistry synthetic expertise offered by the Chemistry department (Columbia, Missouri). SensorLab researchers introduced the electrochemical approach to analytical reporting extensively studied in their laboratories (UWC) as a suitable protocol for sensitivie reporting of glucose. The Glass organic synthesis group provided the host-ligand system comprised of cucurbit[8]uril and various boronic acid ligands as the active components of a solution system for titration with glycolipids and fluorescence reporting of the binding efficiency. In this collaborative project we evaluated the feasibility of a parallel electro-analytical protocol proposed by Baker for evaluation of the binding efficiency of the Glass analytical protocol for glucose detection.

2. Proposed Objectives (*abbreviated*)

We proposed the design of a multi-component electrochemical sensor system which binds to glucose which would have advantages such as lower cost, potential for repeat usage and improved durability compared to enzyme-based systems currently in use. The sensor system was based on two boronic-acid containing receptors which are bound together non-covalently by a cucurbituril macrocycle first reported by the Glass group (see Figure 1).

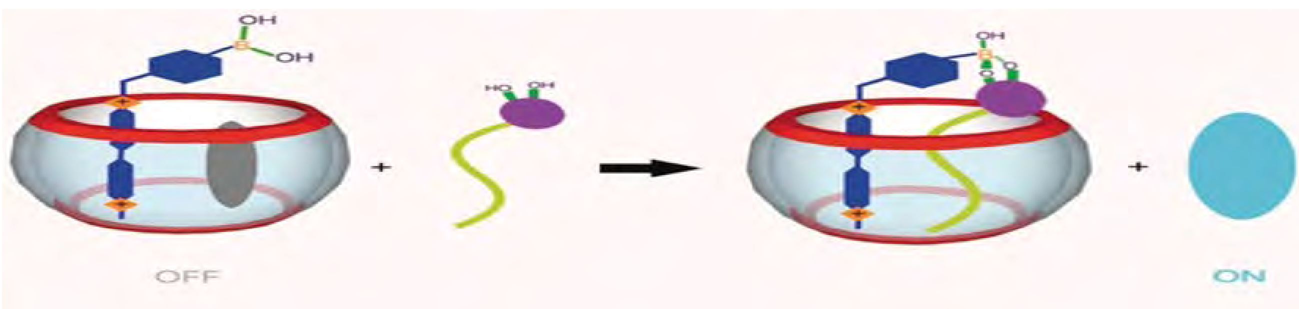


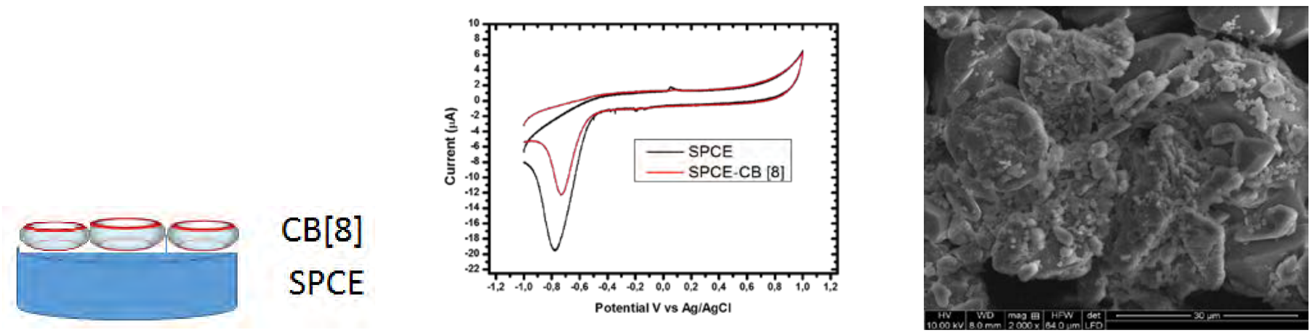
Figure 1: Flourescence sensing protocol for glucose detection (Ming Xu et al, 2018, Angewandte Chemie)

The receptors have a viologen (dication) base because cucurbiturils (CBs) are well known to bind to cation guests. Binding to glucose causes the receptors to rearrange in the macrocycle. Boronic acids are well known to become negatively charged (boronate), therefore we expect significant changes in the electrochemistry of the sensor upon binding glucose. Following a parallel approach to the fluorescence sensing an electrochemical layer by layer approach was designed using $K_3Fe_3(CN)_6$ as the internal reporting redox molecule (similar to the dye in Glass concept). The redox compound is fully dissociated in aqueous systems and the oxidation and reduction of the Fe^{2+}/Fe^{3+} system forms a highly efficient redox indicator for binding as the charge within the CB host changes.

So, we would like to see if a change in redox potential correlates with the addition of glucose. If significant changes are observed, it may be interesting to coat simple electrodes with our sensor to see if a model 'test strip' can be developed. It would also be important to check for a response upon

Objective 1: CB as host agent

Siyabulela Hamnca worked closely with Ming Xy to understand the behavior of the CB, boronic acid ligands and the fluorescence reporting of glucose sensing. He then evaluated different wasy of fuixing the CB to an electrochemical transducer interface which we selected to be the commercially available scrren printed electrodes (Dropsens) which is graphenated electrode with high surface area. CB prepared as a drop casting solution was coated onto the commercial electrode and allowed to dry overnight. Electrochemistry and Scanning electron microscopy was used to confirm the immobilization of the CB and evaluate the redox behavior of the CB modified electrode.



(a) (b) (c)

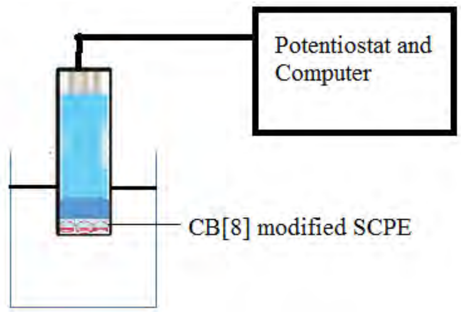
Figure 2: (a) Schematic of sensor assembly, (b) Cyclic voltammetry and (c) Scanning electron microscopy of the SPCE/CB sensor

The modification of the SPCE was deemed efficient for preparation of a rapid screening tool to evaluate the insertion and binding of the boronic acid ligands. The SPCE/CB ligands were stable and ready to use in a reasonably short time, with very good reproducibility (RSD < 15%)

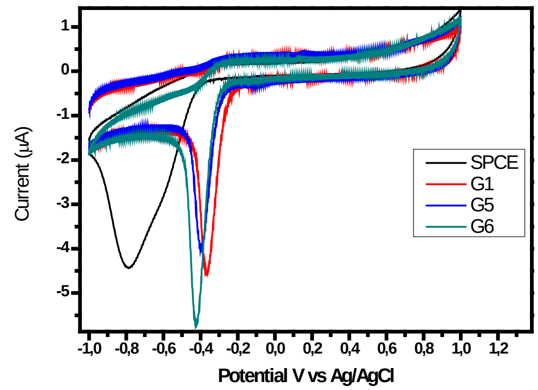
Objective 2: Evaluation of the selected boronic acid ligands

A series of boronic acid ligands were synthesized , purified and characterized by the Glass group with a view to correlate stereochemical effects of ligand synthesis with the efficiency of bidning glucose. The Glass group proposed three most likely ligands to evaluate in the electrochemical approach based on the preliminary titration results monitored by fluorescence in their work.

Therefore the ligands (labeled G1, G5 and G6 for ease of reference) were subsequently studied by cyclic voltammetry to evaluate their individual redox behavior. The CB modified electrode was placed into an electrochemical cell, containing 0.1 M KCl as the electrolyte. The background signal was recorded before the addition of any ligand or redox probe. The oxidation and reduction current corresponding to the each boronic acid ligand was then recorded independent of each other. The data was reported as an overlay graph to evaluate similarities and differences (figure 2)



(a)



(b)

Figure 2: (a) Experimental set up for the evaluation of ligands and (b) Cyclic voltammetry response of G1, G5 and G6 ligands

The ligands shows characteristic peaks for boron oxidation and reduction according to the chemical reactions proposed:

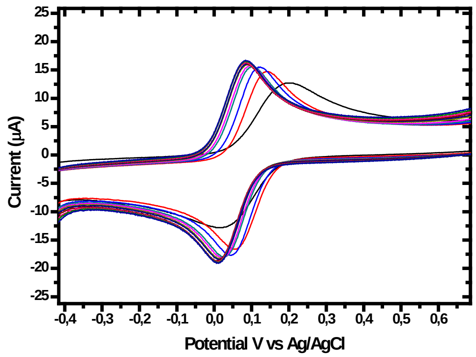
$$RH_2BO_2 + 2H^+ + 2e^- \rightarrow R\text{-}B + 2H_2O$$

$$Br + 6\ OH^- + 6\ e^- \rightarrow BrO_3^- + 3H_2O$$

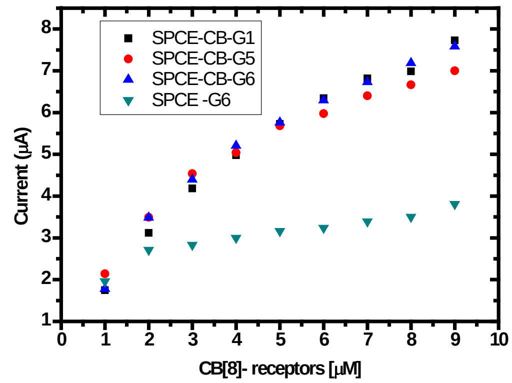
The CB-ligand complexes did not produce distinctive potential shifts which could be related to binding, but the change is redox currne t may be used in the construction of an amperometric sensor for the evaluation of the remaining ligands in the series

Objective 3: Evaluation of ligand binding to immobilized CB

The experimental setup (Figure 2a) was applied to the evaluation of the ligand binding to immobilized CB at the SPCE interface. The redox marker Fe^{2+}/Fe^{3+} was added as a fixed concentration in all experiments (internal standard) to monitor changes in current as the ligand binds to the SPCE/CB electrode. Ligand additions were continued until the Fe^{2+}/Fe^{3+}signal showed no further decrease, to indicate completeness of binding reaction. A control test was also done to evaluate the effect of ligand binding to the SPCE electrode without the CB immobilized (control). We observed that the G1, G5 and G6 ligands showed strong and comparable binding affinity to the CB which was observed to clearly separated from control experiment (Figure 3)



(a)



(b)

Figure 3: (a) Cyclic voltammetry redox probe current response to ligand binding and (b) associated binding curve for each ligand (n=3)

The binding parameters were calculated and the efficiency reported (Bmax/Kd) as 2.1 for G1, 2.75 for G5 and 2.32 for G6. This means that the 3 selected ligands bound strongly to the immobilized CB even though some of the host portals may be blocked to the surface adsorption.

Based on the above very promising results a self assembled approach was proposed for the design of a ligand functionalized CB[8] sensor for the detection of glucose. The initial results obtained with the CB[8]-ligand sensor was not very satisfactory due to an exaggerated glucose concentration range which was not compatible with the sensor. However we believe that the dilution of the gluscose analytical concentration may result in a fast and efficient sugar sensing system, which may be applied to glucose of other sugars. The optimization and application of the newly developed sensor will form part of ongoing research and collaboration between the Baker- Glass research groups

3. Status of Proposed Objectives

4. Additional Noteworthy Activities

4.1 Scientific:

4.1.1 *Baker and Glass collaboration*

Prof Baker and Prof Glass were very pleased with the promising outcomes of the first visit and have agreed that this area of collaboration should be pursued on an ongoing basis. The first was (2019) was focused on (a) introduction to the host and ligand mechanisms of the cucurbit[8]uril boronic acid fluorescence based reporting of glycolipid detection (b) introduction to electrochemistry concepts and how these could be used to develop a small scale portable sensor system for glycolipid quantification. The successful outcome of the 2019 visit was based on the demonstration of step by step sensor system assembly and characterization, which is a completely novel proposal for in situ sensing of glucose. The sensor design and characterization data has been drafted as a research paper for joint author publication in (Baker, Glass) in Electroanalysis (2019).

4.1. 2 *Baker Sheila Grant collaboration*

The proposal expanded on the in situ deposition of electrospun polyamic nanofibers (i) molecularly imprinted or (ii) surface functionalized with the an antigen of choice. The immunosensor thus produced may then be applied to a sample solution containing the TB antibodies and through the efficient transduction provided by the polymer interface the signal may be modelled as an impedimetric or amperrometric output . This is a common electroanalytical strategy adopted by Baker in her research with great success. However the orientation of the antigen on the transducer impacts directly on the availability of the antigen for binding to the antibody in solution and the efficiency of this type of sensor may vary greatly of the orientation of the antigen is not carefully controlled. It is in this respect that we believe the careful control of nanofiber synthesis (and even alignment) during electrospinning will add value to the integrity of the electyrodynamic interface during analytical application. These immunosensors may be designed with a variety of diseases in mind but for this project we had identified particular TB Ag/Ab detection strategy for SensorLab, which would be preceded by bovine TB Ag/Ab affinity sensors, developed with the Grant groups interests in mind. We believe that the work proposed will have **great potential for publication** and conference presentations, cementing the UM-UWC linkage programme in a truly collaborative partnership focusing on scientific development, publication and human capacity development. However these were realistically identified as long term goals that would require a repeat visit (at least one more time) to finalise the output of the first visit in a scientifically validated manner. Unfortunately there were no UM students available to take over the research investigations from Siyabulela (due to the time of year) and we would like to motivate for a return visit by Baker student to continue working with Prof Dave and Sheila Grant on the Ag/Ab functionalized electrospun polyamic acid immunosensors.

4,3 Integration and Logistics:

Prof Baker and Mr Hamnca were accommodated at Tara apartment unit 806 located at 1133 Ashland Rd within 10 minutes walking distance of the main campus. The commitment of the staff at Mizzou involved in the linkage programme as well as the greater ideological objectives of the linkage programme between UWC and UM was truly underscored by the care and friendly reception by colleagues at the International office (UWC) and Prof Rod Uphoff's team (UM). The Mizzou campus is user friendly, very efficient and very homely with a vibrant cultural atmosphere that makes it easy for visitors to get comfortable in a short space of time and really settle into enjoy Mizzou life. Ground transportation to the Columbia campus as well as in and around the town was efficient and easily accessible. Siyabulela had the opportunity to play football (soccer) in the Stankowski Field, next to the MizzouRec center and joined a soccer team that participated in Sunday soccer league. During his stay at the University of Missouri, he was invited to lunch with the University of Missouri president, Prof Mun Y. Choi, along with visiting students and staff from the University of the Western Cape.

Acknowledgements:

We would like to take the opportunity to thank all the staff at the International relations office (UWC) as well as Prof Uphoff, Ms Ashley Rhode and all the colleagues at Mizzou for a warm and welcoming reception and very stimulating and productive research engagement. We want to specifically acknowledge the co-funding received from the International relations (UWC) c/o Prof Umesh Bawa. We also want to appreciate the constructive engagement with all Mizzou staff in Chemistry, but particularly the commitment of Prof Sheila Baker, Prof Tim Glass, PhD student Ming Xu and the research group of Prof Glass.

Prof PGL BAKER
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