Abstract

Mobile Phone-Based Electrochemiluminescent Detection of Biogenic Amines

Nic Heckenlaible

Due to their ability to cause food poisoning, the presence of biogenic amines in food represents a significant potential health risk for individuals in all parts of the world. Currently, few rapid and low-cost methods exist for the analytical detection of biogenic amines outside of the well-equipped laboratory. Electrochemiluminescent (ECL) detection offers a potential solution for this problem. In ECL, an analyte and a luminescent reagent undergo an oxidation-reduction reaction to produce visible light in an amount proportional to the concentration of the species being studied. Whereas previous research with ECL required a lab-based camera system for optical detection, work in recent years has proven the feasibility of using a mobile phone camera for detection. With some improvements, a phone-based ECL detection method could be applied to the detection of amines (in this case histamine, putrescine, and cadaverine) outside the constraints of a traditional lab setting.

The proposed research will investigate and improve a phone-based detection system for ECL in biogenic amines. A light-tight housing will be designed, 3-D printed, and implemented into the phone-based detection system. Precision studies will determine the housing's ability to eliminate visual background noise. The optimal red-ox potentials will be determined for each of the amines as a measurement of maximum light intensity produced. For each amine, trials will be run across a span of concentrations to examine the relationship between concentration and intensity and determine the limit of detection. Trials will be conducted using both a Samsung Galaxy S7 Edge smartphone and a Charge Coupled Device camera from Thor Labs to compare each method's quality of detection. Lastly, similar concentration studies will be run on samples of milk spiked to known concentrations of histamine to compare the precision of each detection method on real-world samples.

Because biogenic amines lack a rapid detection method outside a conventional lab, a fully portable phone-based field kit could provide lifesaving prevention of food-borne illness in developing countries. The progress from this research would be instrumental in satisfying the need for a low-cost and universally accessible detection method for biogenic amines.

Proposal Narrative

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1. Background and Significance

Biogenic amines are an important and widely studied class of molecules. They are linked to important physiological processes, most commonly including food-related illnesses such as histamine poisoning. Biogenic amines such as putrescine and cadaverine (Figure 1) have been found to limit the body's ability to neutralize excess amounts of histamine, further potentiating illness. Because of the possible risks, it is important to be able to analytically test food samples for the presence of biogenic amines. As these molecules are primarily produced by the decarboxylation of amino acids in bacteria, they can be used as a measure for microbiological activity in food and by extension food quality. Unfortunately, there are few rapid methods for the detection of biogenic amines outside of the well-equipped laboratory.[1]

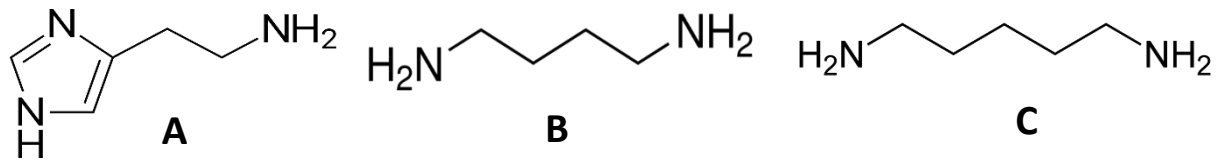

Figure 1. Chemical Structures for (A) histamine (B) putrescine (C) cadaverine

Electrogenerated Chemiluminescence (ECL) detection[2] offers a potential solution for this problem. ECL is an analytical detection method in which an analyte and a luminescent reagent, tris(2,2'-biryridly)ruthenium(II) (known commonly as $Ru(bpy)^{2+}$), undergo an oxidation-reduction reaction at a controlled potential and emit visible light that can be quantified. Because the analyte is the limiting reagent, the intensity of light observed serves as an analytical measurement of the analyte concentration. To illustrate this, the commonly studied mechanism of the reaction between the amine 2-(dibutylamino)-ethanol (DBAE) and $Ru(bpy)^{2+}$ is shown in Figure 2.[3] As the reaction occurs, both reagents are oxidized at the surface of the electrode. Once

oxidized, the analyte (DBAE) forms a free radical (R·) and donates an electron to $Ru(bpy)_3^{3+}$, causing it to enter an electrically excited state from which it subsequently relaxes. The process of $*Ru(bpy)_3^{2+}$ relaxing to its ground state releases a photon which is detected with an optical sensor. Given this mechanism, a solution containing an excess of $Ru(bpy)_3^{2+}$ and a biogenic amine of unknown concentration (replacing DBAE in the mechanism) can be measured with ECL detection to provide a near instantaneous result. Previous work in our lab has found that the optimal oxidation potential for $Ru(bpy)_3^{2+}$ and DBAE solutions is 1.1V.[7]

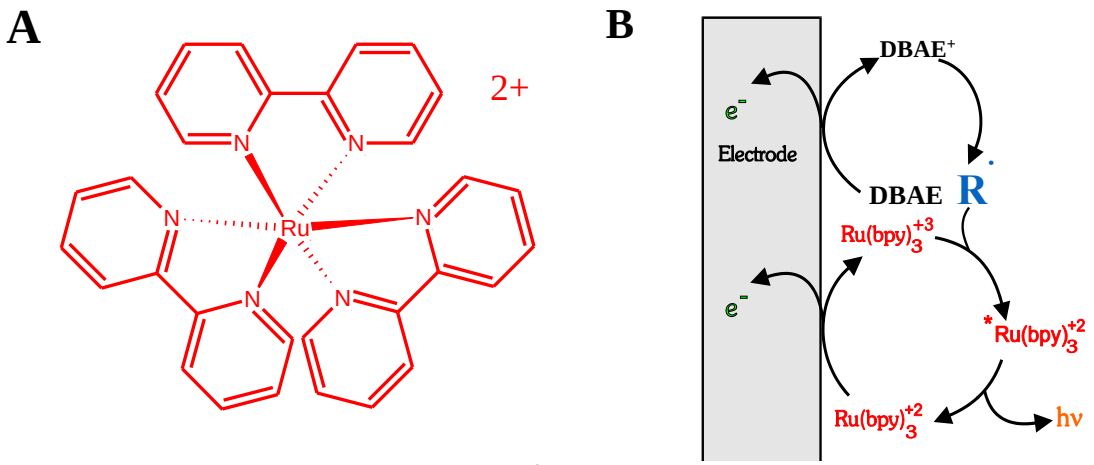

Figure 2. (A) Chemical structure of $Ru(bpy)^{2+}$ (B) Oxidative-Reductive mechanism of $Ru(bpy)^{2+}$ and an amine such as DBAE. Both species are oxidized on the electrode surface, then the $DBAE^+$ species rearranges to a reducing agent which donates an electron to $Ru(bpy)_3^{3+}$. $*Ru(bpy)_3^{2+}$ relaxes from its excited state, releasing a detectable and quantifiable photon.

Conventionally, optical sensors like photomultiplier tubes (PMTs) have been used, but other groups such as Delaney et al.[5] have demonstrated the efficacy of using a mobile phone for imaging certain ECL processes. Using a mobile phone as an optical detector is advantageous because a mobile phone is portable, smaller, and more available than a conventional laboratory camera setup. Mobile phone detectors have been demonstrated as colorimetric detectors for telemedicine applications and in resource-limited settings[6]. Delaney et al.'s work first showed that a phone based detection system for ECL is possible, but there are improvements to be made to fully realize a rapid, accessible, and cost-effective method. The goal for our work is to

improve the quality and feasibility of a phone-based detection system through several means, and apply the technology to the detection of biogenic amines. Our current method for image collection involves taking a long-exposure image over the course of the first 8 seconds of potential application. Once images have been captured, image analysis software (ImageJ) is used to analyze the average red pixel intensity on the appearance of the surface of the electrode. Previous research in our lab has found a linear trend between red intensity and analyte concentration. Preliminary data from a concentration study of the amine DBAE and $Ru(bpy)_3^{2+}$ (shown in Figure 3) demonstrates the trend observed between DBAE concentration and observed red pixel intensity. Further work is being done in the spring 2018 semester to further characterize and improve the linear relationship observed.

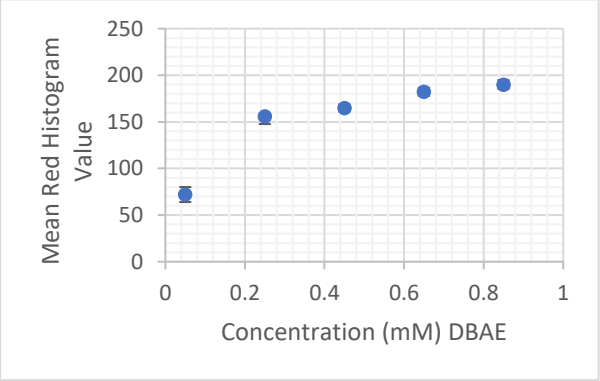

Figure 3. ECL signal as a function of amine concentration. Solution was DBAE in 0.45M $Ru(bpy)^{2+}$ at 1.1V in 0.1M PBS. ECL imaged using a Samsung Galaxy S7 Edge.

One key step forward towards a cheaper detection method is the use of Screen Printed Carbon electrodes (SPC electrodes). SPC electrodes are small scale electrodes that can be fabricated in-house and serve as a miniaturized, disposable substitute for conventional laboratory electrodes. SPC electrodes are manufactured by screen printing an inexpensive and easily made carbon ink onto plastic sheets. Similar screen printing methods are often used to fabricate commercial glucose sensors, a testament to the feasibility of mass-produced disposable electrodes. A small drop of silver ink is painted onto each electrode to serve as a reference

electrode and a reservoir made from precisely cut packing tape is attached to retain the solution. SPC electrodes can be made rapidly, in large quantities, and for very low cost per electrode (<2¢ per unit). Previous work in our lab has shown SPC electrodes to be consistent and dependable candidates for disposable electrodes.[7]

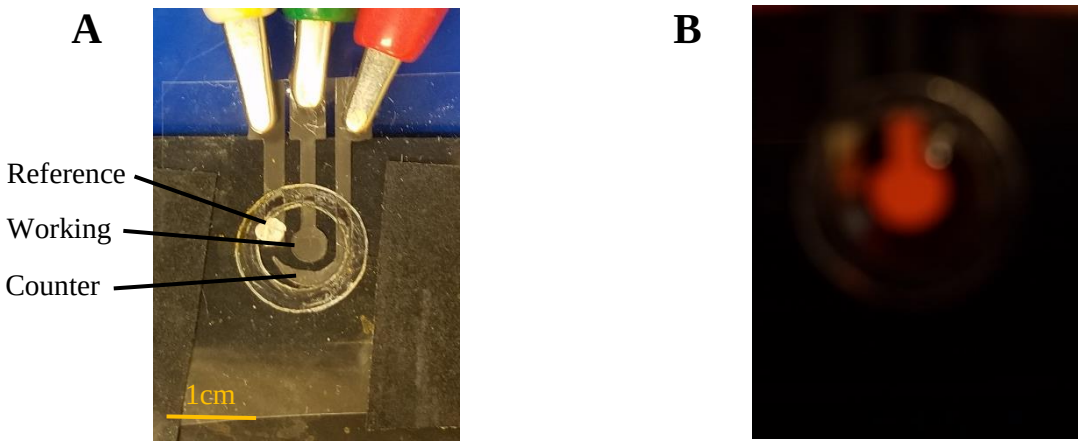

Figure 4. (A) Photograph of SPC electrode in working position with solution absent (B) Sample image from ECL detection trial of DBAE and $Ru(bpy)^{2+}$

The combination of these concepts is the groundwork for the goal of creating a fully portable and handheld rapid detection method for potentially harmful biogenic amines. A phone-based detection kit consisting of a 3-D printed housing case, a screen printed carbon electrode, and custom software could fill the need for a low-cost detection system and help to minimize the risk of food-borne illness in developing countries or places without easy lab access. The work proposed in this project is progress towards that goal.

2. Experimental Plan

2.1 Light-Tight Apparatus Design and Implementation

While previous research on phone-based detection of ECL has been done in open-air low-light conditions in the lab, a finalized phone-based detection system will require a portable light-tight housing case to be able to negate environmental noise and give a more precise limit of detection (LOD).

The first segment of work in this project will involve designing, prototyping, and testing a 3-D printed housing to hold the electrode and mobile phone in a consistent and light-tight fashion. The nature of 3-D printing enables iterations of designs to be rapidly fabricated, tested, and reworked. Designs will be created using SketchUp software and printed using a Formlabs Form 2 printer already operating within Dr. E. Gross's lab.

To examine the projected improvement in the consistency of ECL detection, precision studies will be run with and without the use of the light-tight case. Previous research has determined that a solution comprised of 5 mM $Ru(bpy)^{2+}$ and 0.45 mM DBAE in a pH 7.4 0.1M phosphate buffer at 1.1V produces ECL intensity sufficient for consistent analysis.[6] Standard deviation in the red light count of 10 trials before and after implementation will serve as measure of consistency and repeatability of ECL detection.

2.2 Oxidation Potential Optimization for Histamine, Putrescine, and Cadaverine

Following the implementation of a light-tight case, work will need to be done to confirm the optimal potential for ECL signal in each of the 3 amines. Cyclic voltammetry has previously been performed to determine that the optimal oxidation potential of $Ru(bpy)^{2+}$ and DBAE solutions (1.1V), along with $Ru(bpy)_3^{2+}$ and several other biogenic amines (1.1-1.3V for putrescine, spermidine, and spermine). However, the non-negligible variation observed in the optimal oxidation potentials of other biogenic amines warrants separate investigation into the optimal potentials for histamine, putrescine, and cadaverine.[4] A range of potentials from 0.9V to 1.6V will be applied to each solution and ECL intensity will be recorded. The potential that results in peak ECL intensity in each amine will be used moving forward. The range of optimal potentials also serves as a measure of the robustness for the SPC electrode system.

2.3 Examination of Histamine, Putrescine, and Cadaverine Using Phone-based Detection System

Next, the limit of detection and linear range of detection will be determined for each amine. The limit of detection is used as an evaluative method to compare detection methods. Solutions in a range of 5 concentrations for each amine will be prepared and trials will be run (n=5) at each concentration. Background trials will also be run on solution containing only $Ru(bpy)_3^{2+}$ (n=10). With the background subtracted, the relationship between concentration and ECL intensity will be analyzed to determine the limit of detection and linear range of the signal.

2.4 Examination of Histamine, Putrescine, and Cadaverine Using Conventional Lab Camera

To compare the quality of results from a phone-based detection system with a lab-based detection system, the same 5 concentrations of each amine will be tested and examined with the lab camera, a Charge Coupled Device (CCD) camera from Thor Labs. Similar background trials will be run (n=10). The limit of detection will be calculated for the lab-based system and compared to the phone-based system.

2.5 Examination of 'Real-World' Sample

Finally, as an introductory foray into non-ideal solutions, samples of milk will be spiked to known concentrations of histamine and $Ru(bpy)^{2+}$ to examine the phone-based system's ability for detection in 'real-world' situations. Previous literature has stated amounts of 50mg of histamine per kg of food as being sufficient to cause food poisoning in adult humans.[1] Given this fact, concentrations in a range from 0 to 0.80mM will be sufficient to cover the threshold of safety (~.45mM). In the same way as above, trials will be run (n=5) at each concentration to establish the relationship between histamine in a food sample and ECL intensity. Limit of detection and linear range will be calculated for the phone-based detection method. The analysis will be mirrored for the lab-based detection method. Identical concentrations of milk sample will be tested and LOD and linear range established for the lab camera system.

3. Project Schedule

Week:	*Objectives:*
1	A precision study will be run on an unmodified phone-based detection system. Self-tutorial of 3-D design will begin as the light-tight case is designed and prototyped.
2	Design for light-tight case will be finalized and a final copy printed. A second precision study will be run after the implementation of the case. The data will be analyzed.
3	Solutions will be prepared in 5 concentrations for each amine. Oxidation potential optimization trials will be run for each of the 3 amines.
4	The images and data from optimization trials will be analyzed. The concentration study for biogenic amines will be performed using the phone-based system.
5	The images and data from the previous concentration study will be analyzed. The concentration study for biogenic amines using the lab-based system will begin.
6	The lab-based system concentration study will be concluded and images and data will be analyzed. Real-world milk samples will be prepared.
7	The concentration study to characterize response in milk samples using phone-based system will be conducted.
8	Images and data for phone-based system concentration study will be analyzed. The concentration study to characterize response in milk samples using lab-based system will begin.
9	The concentration study using lab-based system will be concluded. Images and data will be analyzed.
10	Final data compilation and analysis will be performed. 2-3 days extra time alotted in case of unexpected delays in previous work.

4. Conclusion

In this project, the efficacy of using a mobile phone-based detection method for ECL analysis of biogenic amines will be evaluated. The presence of biogenic amines in food represents a significant potential risk to the health of individuals in all parts of the world, thus a low-cost and fast-acting method of detection would be beneficial to both developing areas and areas without instant lab access alike. Furthermore, mobile ECL detection promises to be a rapid analytical detection method for numerous analytes beyond biogenic amines. An exploration into the practice of phone-based detection will lay the groundwork for a potentially life-saving innovation in rapid detection methods.

6. References

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Budget

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Funds are requested for the following consumables and chemicals:

Item	Description	Expense
3-D Printer Resin	▪ *Opaque resin for case printing*	$200
Chemicals	▪ *Histamine* ▪ *Cadaverine* ▪ *Putrescine* ▪ *Silver Ink* ▪ *Milk Samples*	$300
	Provided by Stipend	+$500
	Remainder	$0

*All of the instrumentation and electrode fabrication materials required for this project are already present in Dr. E. Gross's lab.