DEVELOPMENT AND OPTIMIZATION OF AMMONIUM-SENSITIVE CHEMFETS FOR AQUEOUS SENSING

by

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A THESIS

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Primary Thesis Advisor

Ion sensing is an increasingly important field of research for regulating and detecting high concentrations of ions harmful to the environment. One such ion is the ammonium ion which, because of eutrophication, destroy local ecosystems. ChemFETs have been a promising design for sensing agriculturally relevant ions such as the ammonium ion. The component that allows the sensor to detect these relevant ions is the ion selective membrane which is made up of three components: the receptor, the polymer, and the ionic additive. This study seeks to find trends in how the sensor performs when increasing the amount of receptor molecules in the ion selective membrane while simultaneously decreasing the amount of ionic additive. Furthermore, this study's conclusions can be used to base future ammonium ChemFET designs for future optimization.

Acknowledgements

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Section 1: Introduction

Eutrophication and the need for nitrogen sensing

Nitrogen is a crucial nutrient required for plant growth [1]. Due to a limiting amount of nitrogen found in the soil, farmers usually provide their crops nitrogen in the form of nitrogen-based fertilizers. The main ingredient in these fertilizers is ammonium nitrate (NH_4NO_3) which is soluble in water. However, using these fertilizers often risks overfertilizing the crops with nitrogen resulting an excess amount of nutrients. When it rains, the excess nutrients "runs-off" in the rainwater and collects in rivers, streams, lakes, or any neighboring bodies of water. This excess amount of nitrogen in our water systems promotes the growth of algae blooms, a process known as **eutrophication** [2].[1] Algae absorb the oxygen from the water resulting in the loss of fish and local wildlife by starving fish of oxygen. Additionally, these algae blooms create a sheet over the surface of the water which reduces the sunlight beneath the surface of the water which can cause harm to underwater flora that requires light to survive. These blooms can also be poisonous which could harm any wildlife that uses these water systems for drinking water [3]. Additional consequences of eutrophication are the $2.2 billion annual costs in the United States alone: including the cost to prevent the loss in aquatic biodiversity, increased cost to buy bottled water due to pollution, recreational activity losses, decreased property value, and cleanup costs [4].

The result of overfertilization has led to a current need for a real-time chemical sensor to detect nitrogen **concentrations** in soil and water, dubbed "total-N" content

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[1] Bolded terms defined in glossary

[5]. Chemically sensitive field effect transistors (ChemFETs) development has been well studied as promising **ion**-sensing chemical sensors. These devices are beneficial to other chemical sensors due to their large potential for miniaturization, fast response time, low cost, low power consumption, recyclability, and their compatibility with electronics [6][7][8].

ChemFETs

The main FET schematic is made up of three **terminals**: the source, drain, and gate with a channel connecting the source and drain [8]. The gate and source are separated by two additional layers: a water solution containing a target charged **analyte**, and a **semi-permeable membrane** that has been applied to the FETs surface called the ion-selective material [8]. A schematic of this is shown in Figure 1.

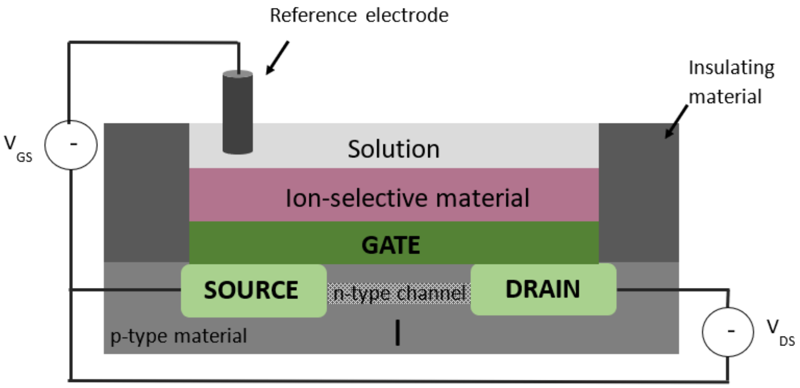


Figure 1: General schematic of a ChemFET for ion sensing.

The ion-selective material is made up of three primary components: a polymer that acts as the membrane and adheres to the surface of the FET, a **receptor** molecule that bind to the target analyte, and an ionic additive which acts as a **counter-ion** to guide the target analyte to the receptor. The interaction between the receptor and the charged analyte results in the formation of a concentration gradient visualized in Figure 2. This gradient creates a measurable response between the source and drain terminals of the FET [9].

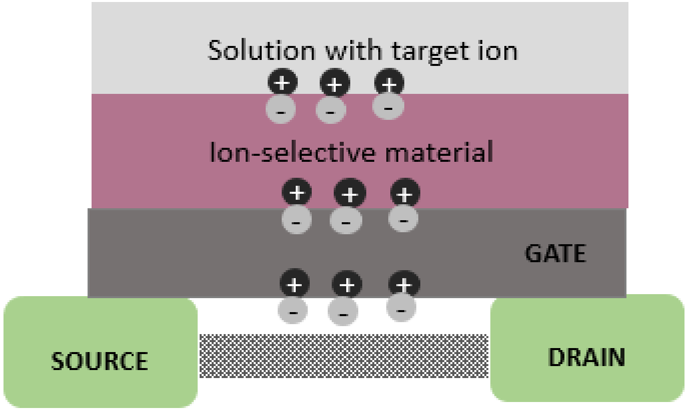


Figure 2: Interaction between charged analyte and FET

The charged ion of interest binds to the receptor molecules found in the ion-selective material. This attracts the opposite charge to the surface of the ion-selective material and pushes the same charge to the other side of the ion-selective material. This pattern repeats and allows charge to pass through the source and drain to complete the circuit.

Developing and optimizing an ammonium sensor

There has been exhaustive research within the last 30 years into the development and application of ChemFETs for total N sensing. Prior studies have shown promising designs for detecting nitrate (NO_3^-) [10] and for ammonium (NH_4^+) [11] which are the ions that make nitrogen-based fertilizers. However, there is a lack of knowledge on how different formulations of the ion-selective material affect the sensors performance. This study will explore three different formulations for an ammonium sensor each outlined in Table I with the overarching goal in finding trends in how different amounts of receptor and ionic additive in the ion selective material's composition affects the ChemFET's performance in detecting ammonium. These formulations best model previously studied ammonium-selective ChemFETs in the literature and represent a low, medium, and large amount of the receptor molecule present in the ion-selective material.

Table I: Composition of three ChemFETs for ammonium sensing

Components:	Receptor (w%)	Ionic additive (w%)	Polymer (w%)
Formulation I	0.45	4.54	95.0
Formulation II	2.5	2.5	95.0
Formulation III	4.0	1.0	95.0

Receptor, ionic additive, and polymer amounts are listed by their weight percent, i.e., how much they make up the ion-selective material by weight. The polymer is kept constant at 95% by weight while the receptor and ionic additive change with a 1:10 ratio by weight in formulation I, a 1:1 ratio in formulation II, and a 4:1 ratio in formulation III.

Section 2: Materials and Methods

Materials

Constructing the ChemFET

Unmodified FETs with a silicon nitride (Si_3Ni_4) gate, which corresponds to the gate, drain, source, and p-type material in Figure 1, were purchased from Winsense^{TM}. The FETs were wire bonded to small circuit boards having the source and drain electrically accessible through small **vias**. Wires were soldered to these vias to allow connection to the source and drain and any exposed connections were coated with epoxy as an insulating material (Figure 1). After waiting a day for the epoxy to dry, polymer membranes were **drop-cast** onto the FET surface corresponding to one of the three formulations described in Table I. During all drop cast processes, thirteen 1.6 µL drops of the drop-cast solution were applied to the FET surface via a micro pipette spaced 15 minutes apart to allow for the **solvent** to evaporate. Following the drop-cast, the sensors were placed in an oven at 80°C overnight to ensure all remaining solvent has evaporated. After the drop casting process, the ion selective material remains, covering the surface of the FET (Figure 1).

Drop-cast solutions

The drop-cast solutions are made up of three primary compounds: nonactin, the receptor molecule that binds ammonium, polyvinyl chloride (PVC) to act as the polymer, and potassium tetrakis (4-chlorophenyl) borate to act as the ionic additive. The structure of these molecules can be found in Figure 3 below:

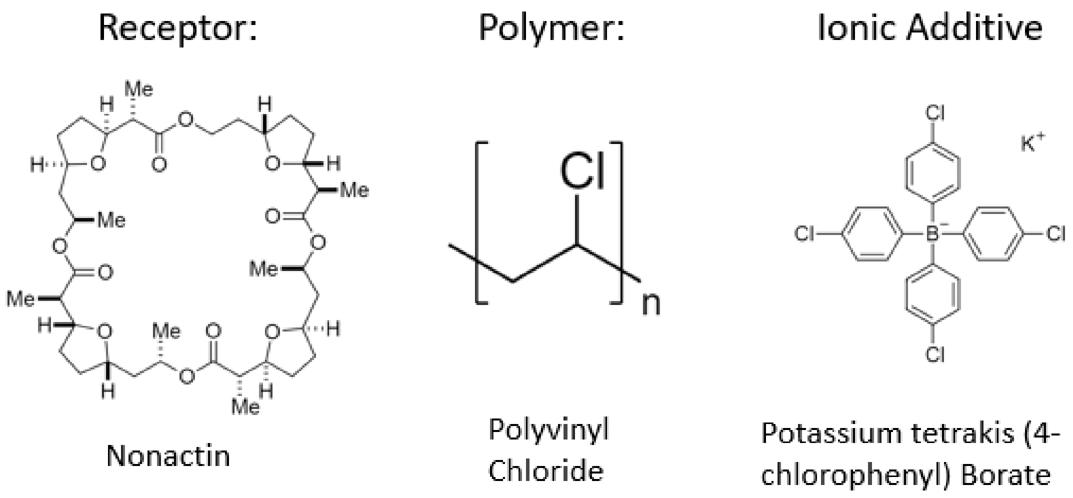


Figure 3: Structure of the three components that make up the Ammonium ChemFET's ion selective material.

Methods

Contact Tests

A contact test involves measuring a ChemFETs sensitivity to ions present in a solution of water. Each test involved a set of identical four ChemFETs and a **reference electrode** submerged in the same solution which were connected electronically to a computer for analysis. Before each test, ChemFETs were preconditioned by soaking the FETs in solutions containing a high concentration of target analyte solution (typically 1 M) for at least 30 minutes. Following preconditioning, the sensors were rinsed in deionized water (DI water) before the first measurement. Tests were made up of 3 runs in which the FET was submerged in a solution containing a known amount of ammonium ion. The first and last runs were performed stepwise from low to high concentrations while the second run from high to low. For each measurement, the response of the sensors was collected for 5 minutes with the final response being recorded. After a measurement, all sensors and the reference electrode were rinsed with DI water and dried with a Kimwipe prior to the next measurement. The average signal obtained in each run was plotted over the logarithm of the concentration of the ammonium ion (a_n). Plotting this data produced a graph that looked similar to Figure 4. The linear portion of the graph corresponds to the sensor's sensitivity to ammonium. Sensitivity can be thought of as how responsive the sensor is to a change in the ammonium concentration. This linear portion of the graph is described by the Nernstian Equation (Equation 1). The Nernstian equation also provides an upper limit of how large the sensitivity can be at -59.2 millivolts per **decade** (mV/dec) while the intersection between the linear and flat line portion is the detection limit of the sensor. The detection limit corresponds to the minimum concentration of ammonium that the sensor can detect. Past the detection limit the sensor can no longer discernibly detect any change in the concentration of ammonium.

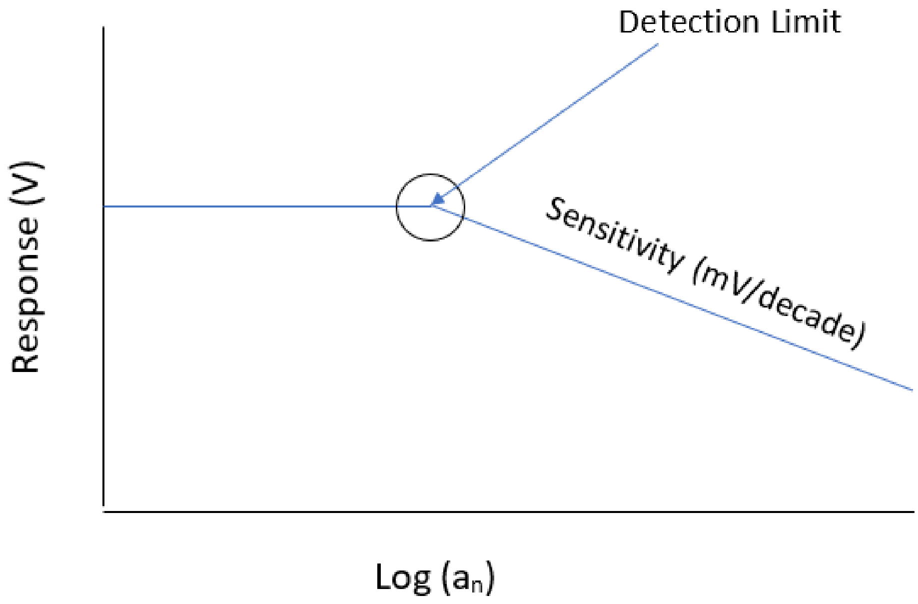


Figure 4: Ideal results from a contact test

The sensitivity (slope) of the graph is defined by the Nernsian Equation (Equation 1) resulting in the slope having a maximum limit of 59.2 mV/decade. A perfect response would involve having slopes as close to this figure as possible.

$$E_{cell} = E^0_{cell} - \frac{59.2\ mv}{n}\log(a_n) \qquad (1)$$

Fixed Interference tests and Selectivity Coefficients

Unlike the contact tests, there is no waterbody that will only have the ammonium ion and nothing else present. There are a host of other ions in our lakes, ponds and streams and these ions might be **interferents** to the ammonium sensor. Prior studies have shown that nonactin, the receptor molecule for the ammonium sensor, can recognize and bind to other ions such as the potassium ion (K^+) [12]. Thus, it is important to determine how well the sensor can detect ammonium while potassium or other interfering ions are present in solution. Fixed interference experiments are a way to quantitatively determine how interfering ions affect the sensor's performance. To do fixed interference experiments, triplicate runs were conducted in the same manner as the contact tests, however a fixed amount of interferent was added to these solutions. For example, for determining potassium's interference, each solution included 0.001 M K^+ in addition to the ammonium concentration. The resulting graph should look very similar to the graph shown in Figure 4. By dividing the detection limit by the concentration of the fixed interferent the selectivity coefficient can be determined. The selectivity describes the sensor's responsiveness to the interfering ion. For example, if the selectivity coefficient = 0.01, the sensor would be 100 times more responsive to ammonium over the interferent.

Section 3: Results and Discussion

Testing the FETs in water and PIPES buffer solutions

At first, all three formulations were tested in water solutions containing ammonium nitrate to characterize the performance of each formulation. These results are summarized in Figure 5 below. The results show high sensitivities ranging from -45 to -52 mV/dec and all show low detection limits (on the order of 10^{-4} M). However, unexpectedly at the 'low' concentration portion of the graph, the response does not show a flat line and instead the graphs have a small but noticeable slope averaging at ~7 mV/dec.

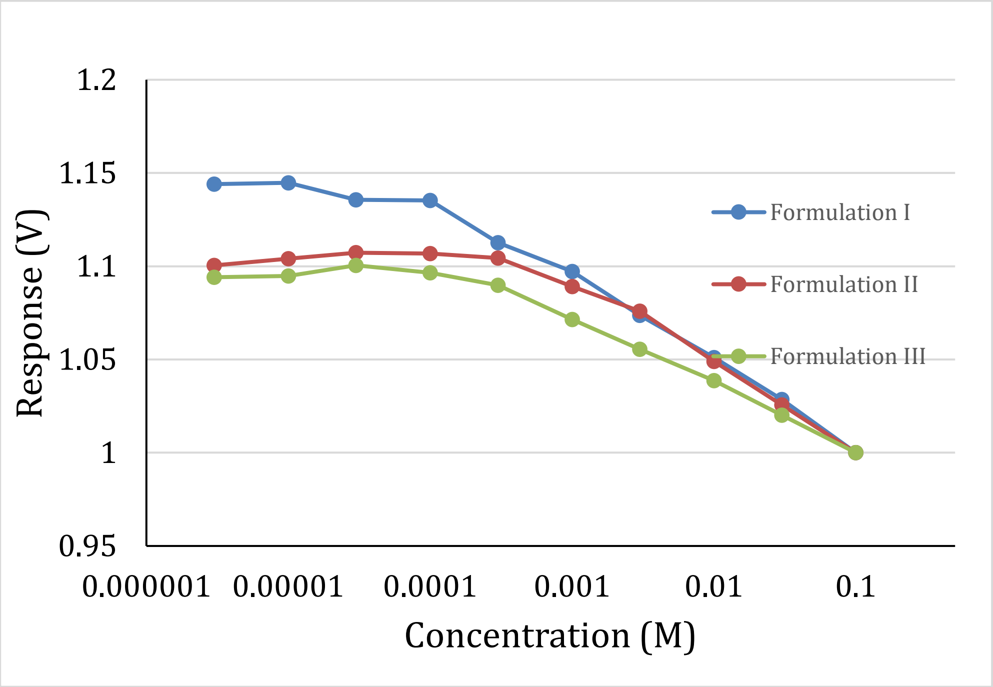


Figure 5: Average response of each formulation in DI water.

The slopes of formulations I, II, and II are -49.14 mV/dec, -52.42 mV/dec, and -45.21 mV/dec respectively. Additionally, the detection limits for each formulation are also 0.064 mM, 0.069 mM, and 0.021 mM respectively.

This slight slope at low ammonium concentrations is not expected as the sensor should show no change in response resulting in a flat line. Since this is not observed it results in a poor determination of the detection limit. The detection limit is determined by finding the intersection between the flat and linear portions of the graph. If the flat portion has a curve this results in an incorrect determination of the detection limit. Likely, this slight response is due to the experiment being conducted in DI water. In usual tap water there are a host of dissolved ions present in addition to the water molecules themselves. DI water removes these excess ions which is useful for conducting experiments as it removes a lot of the impurities that would otherwise be present which could interfere with gathering reliable data. Consequently, due to a lack of anything for the sensor to detect, the sensor likely is not responding to anything besides random static noise at low ammonium concentrations.

To resolve this error in the results, the sensors were rerun in various PIPES (Piperazine-N,N'bis(2-ethanesulfonic acid)) buffer solutions. The goal is for the buffer to provide something for the sensor to detect at low ammonium concentrations in a controlled manner that will result in the sensor showing no change in response at the low concentration levels. PIPES was chosen over other alternative buffers primarily due to it being a stable buffer for testing at neutral pH and the molecule itself should not interfere with the FET response as an interferent [13]. To prepare the buffer PIPES is dissolved in water and a strong base is added until the desired pH is reached. However, it is possible that the base added could introduce an interferent for ammonium sensing so three different bases were attempted: potassium hydroxide (KOH), lithium hydroxide (LiOH), and sodium hydroxide (NaOH).

For potassium and lithium PIPES buffers, the sensors showed poor Nernstian response, averaging at -10 mV/dec and -13 mV/dec respectively (supporting Figures 1-4). Likely, these are caused by too much potassium and lithium being present in the solutions and thus the sensor is only responding to a constant amount of interferent in the solutions, and not the change in ammonium concentration. In sodium buffer however, the sensors showed a better response as seen in Figure 6.

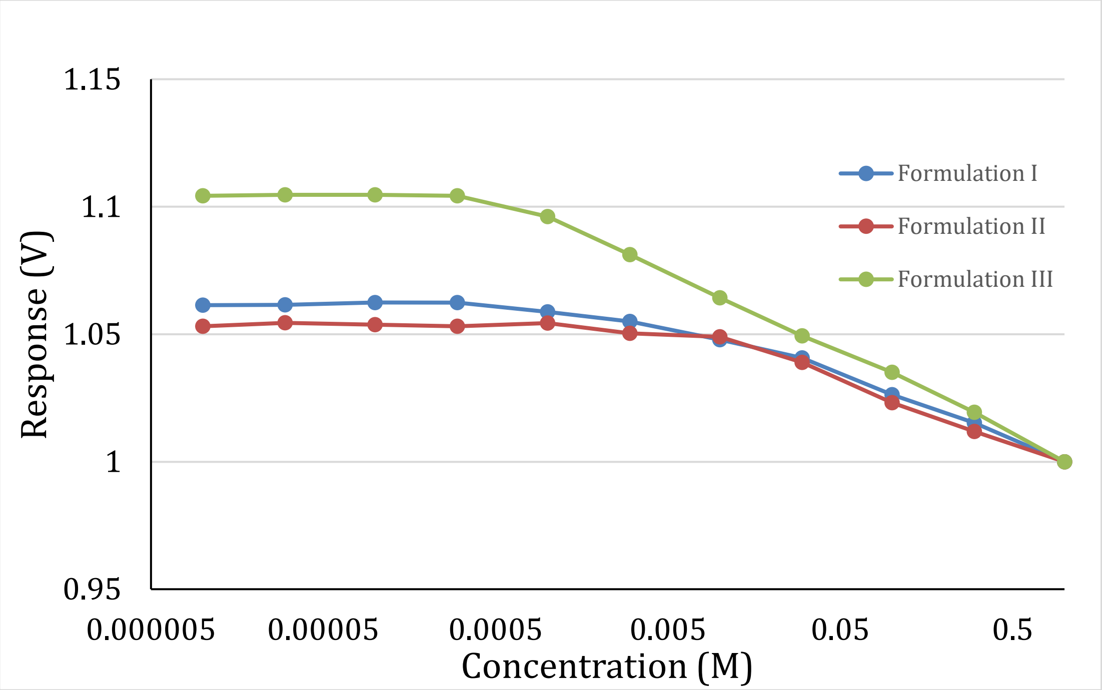


Figure 6: Average responses of each formulation in 50 mM Na^+ PIPES buffer solution

The slopes of Formulation I, II, and II are -27.86 mV/dec, -26.74 mV/dec, and -39.92 mV/dec respectively. The detection limits are 0.753 mM, 0.72 mM, and 0.13 mM respectively. Sodium concentration was determined by an approximation to be 0.087 M resulting in selectivity coefficients of 8.66E-02, 8.28E-02, and 1.49E-02 respectively.

The initial goal of flattening the slope at the low concentration end was achieved in sodium PIPES buffer with each sensor formulation showing a response of less than 1 mV/dec across the last 4 points. The determination of the detection limits is thus more accurate. Interestingly the slopes of Formulations I and II saw a significant decrease between the test in sodium PIPES buffer and DI water. Formulation III on the other hand did not have as significant of a decrease in its response. Additionally, the detection limits all showed an increase by an order of magnitude when compared to the DI water experiment. Likely, these findings are the result of sodium being added to the solution. The exact sodium concentration in solution can be estimated using the Henderson-Hasselbalch equation (Equation 2)

$$\mathrm{pH} = \mathrm{pKa} + \mathrm{Log}\left(\frac{[\mathrm{B}]}{[\mathrm{A}]}\right) \tag{2}$$

Where [B] is the concentration of base present in the solution, which can be estimated as the concentration of NaOH present, and [A] is the concentration of acid present, in this case the PIPES molecule. Using Equation 2 and solving for [B] results in 0.087 M. This figure is an estimation of the amount of sodium present in the solution.

Fixed Interference Tests

As evident from the buffer tests, it is important to understand how much other ions interfere with the sensor's performance. To do this, a constant amount of an interfering ion was added to each solution. The following results can be used to find the selectivity coefficient, a measurement of responsiveness described in Section 2. The data for sodium was determined using the results from the sodium buffer runs. A summary of the results for these runs is shown in Table II (also see supporting figures 5-6).

Table II: Selectivity Coefficients of Fixed Interference Experiments

Components:	K^+	Li^+	Na^+
Formulation I	0.355	1.61×10^{-2}	8.66×10^{-2}
Formulation II	0.807	8.90×10^{-3}	8.28×10^{-2}
Formulation III	0.352	3.70×10^{-3}	1.49×10^{-2}

$[K^+] = 0.001$ M, $[Li^+] = 0.1$ M, $[Na^+] = 0.087$ M

From Table II it becomes evident that the sensors respond to the different interferents to varying degrees. It responds to potassium the most, followed by sodium, followed by lithium. This makes sense when considering the size of each ion and how the size compares to ammonium. Potassium has an **ionic radius** of 1.38Å which is most similar to ammonium's ionic radius of 1.48Å. Sodium and lithium ions have an ionic radius of 1.02Å and 0.76Å respectively [14]. This size trend following the responsiveness trend makes sense when considering the receptor, nonactin, as a glove with a pocket about the size of the ammonium ion. Other ions can fit in the pocket, but they do not fit it perfectly. Ions that better match the size of the ammonium ion fit this pocket better, which explains why the sensor responds to potassium the most.

Comparing the Three Formulations & Future Considerations

In all experiments, formulation III appeared to be the most consistent and reliable. While it did have the lowest overall response in DI water at -45.21 mV/dec the sensitivity is still comparable to the other two having responses around -50 mV/dec. It should be noted that formulation III had the lowest detection limit at 2.10×10^{-4} M in water, where formulation I and II had a detection limit of 6.40×10^{-4} and 6.90×10^{-4}

respectively. A lower detection limit is crucial for a sensor as this is the lowest concentration the sensor can reasonably detect.

In the sodium buffer and fixed interference experiments formulation III also had the lowest selectivity coefficient as shown in Table II. Like the detection limit, this is important as the selectivity coefficient determines how responsiveness the sensor is to other ions in solution. This might mean that having 4% nonactin (receptor) in the ion selective material is ideal for ammonium sensing. Future work should involve testing more interferents such as calcium and magnesium ions as these two ions are also commonly found in ground water [15]. Additional future work could also involve increasing the amount of nonactin to more than 4%. However, it should be noted that this could also result in a decrease in the overall sensitivity of the sensor. While formulation III did have comparable sensitivity to the other two formulations, it was consistently lower by around 2-5 mV per decade in all experiments except the sodium buffer experiment. Further optimization therefor should focus efforts on slightly increasing or decreasing the nonactin present in the sensor using formulation III as a starting point.

Glossary

Analyte: A substance that is the subject of analysis.

Concentration: Measurement of the amount of a particular substance present in a solution. Usually given in units of molarity (M) which is the amount of substance (in **moles**) per unit volume of solution (in liters).

Counter-ion: an ion having an opposite charge to an associated substance.

Decade (dec): Unit for measuring ratios on a logarithmic scale, one decade corresponding to a ratio of 10 between two numbers (e.g., 1 and 10 and 10 and 100 are each 1 decade apart).

Drop-cast: Formation of a thin solid by dropping a liquid solution onto a flat surface and evaporating the solvent.

Eutrophication: Excessive richness of nutrients (e.g., nitrogen) in lakes or other water. body which promotes excessive plant growth and death of fish and other wildlife from lack of oxygen.

Interferents: A substance other than the analyte that can be measured or prevents the measurement of the analyte.

Ion: A positively or negatively charged atom or molecule.

Ionic radius: The distance between the nucleus and the outermost electron of an ion

Mole: A measurement of the total number of atoms or molecules in a substance. One mole is equal to 6.022×10^{23} atoms or molecules.

Receptor: A molecule that binds to and recognizes a particular analyte.

Reference electrode: An electrical conductor that carries current into different media with a known electrical potential (i.e., voltage).

Semi-permeable membrane: A thin layer or boundary that allows certain molecules or ions to pass through it.

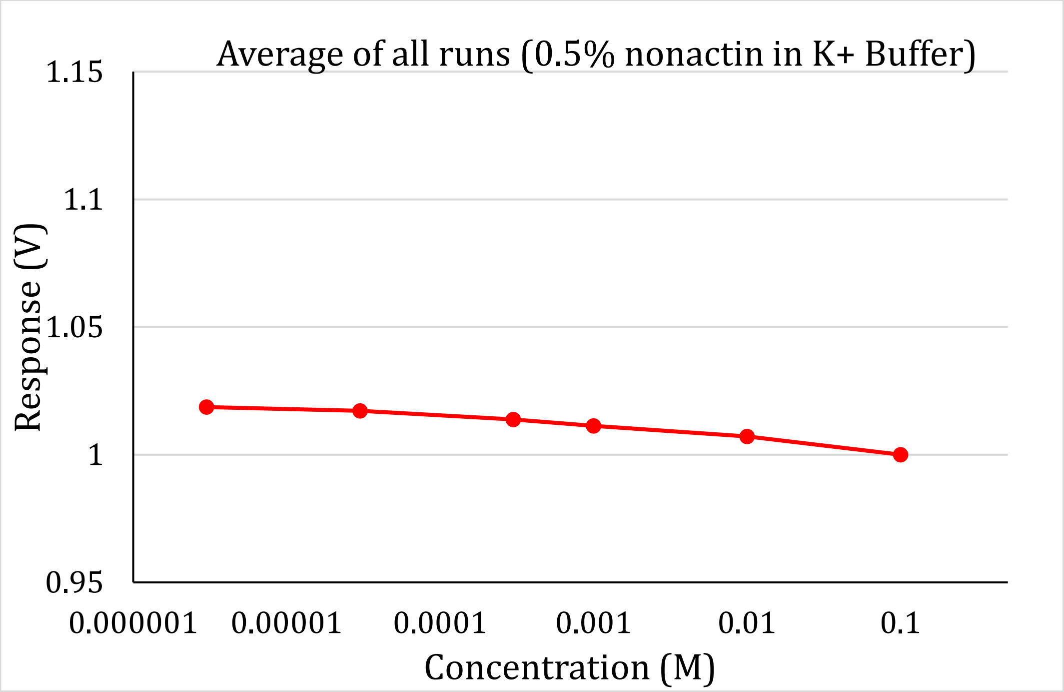
Solvent: A liquid used to dissolve a substance to form a solution.

Terminals: The endpoint in a circuit or a connector between two circuits.

Vias: An electrical connection between different layers in an electronic circuit. These usually look like small holes.

Supporting Figures

1



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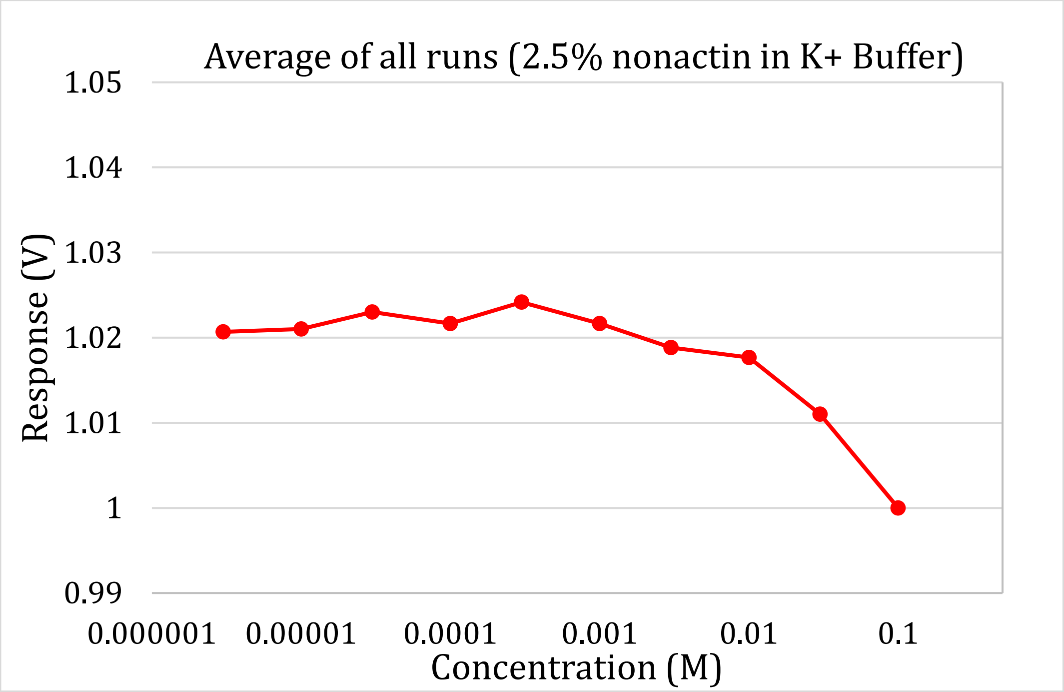

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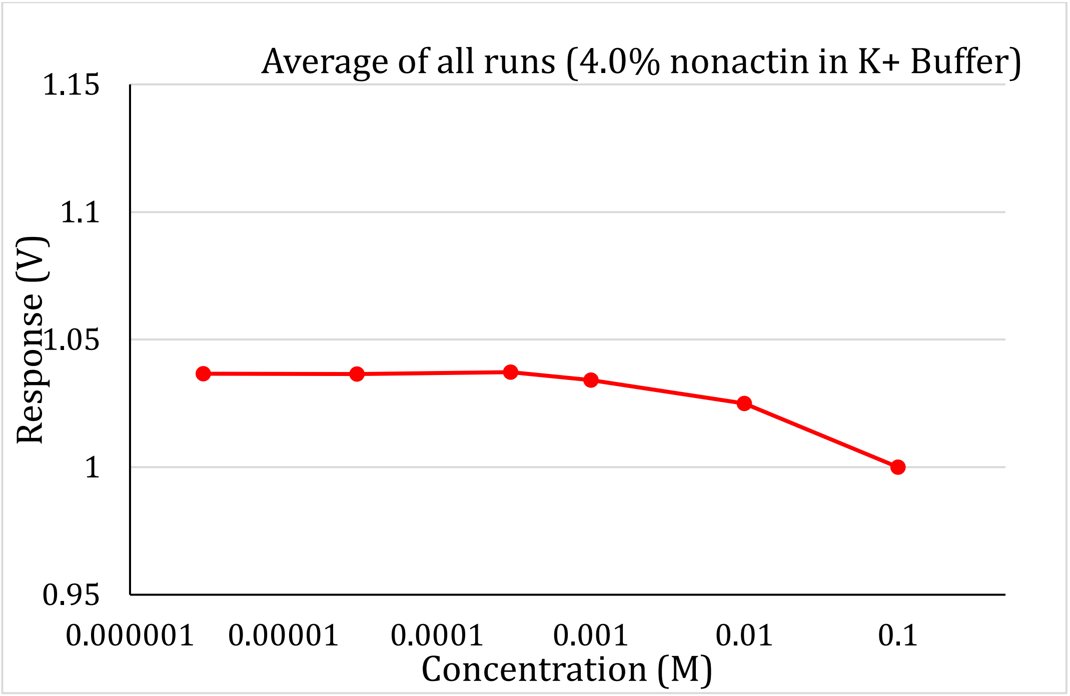
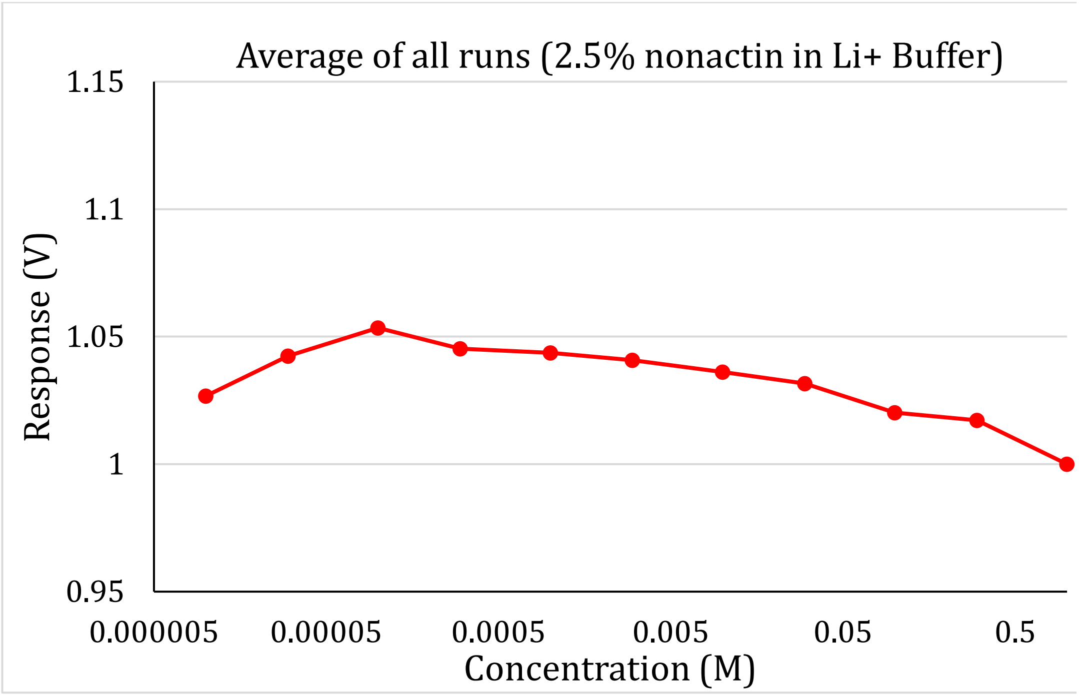

Average of all runs (4.0% nonactin in K+ Buffer)
Response (V)
1.15
1.1
1.05
1
0.95
0.000001
0.00001
0.0001
0.001
0.01
0.1
Concentration (M)

4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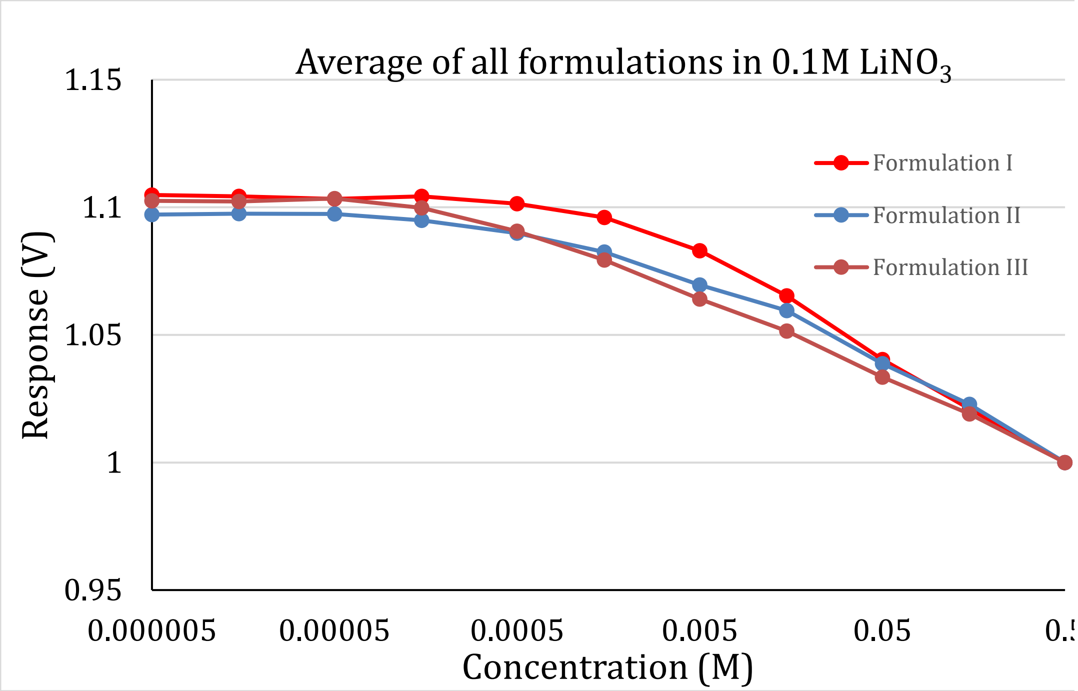
0.005
0.05
0.5
Concentration (M)

5


Average of all formulations in 0.1M $LiNO_3$
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1.1
1.05
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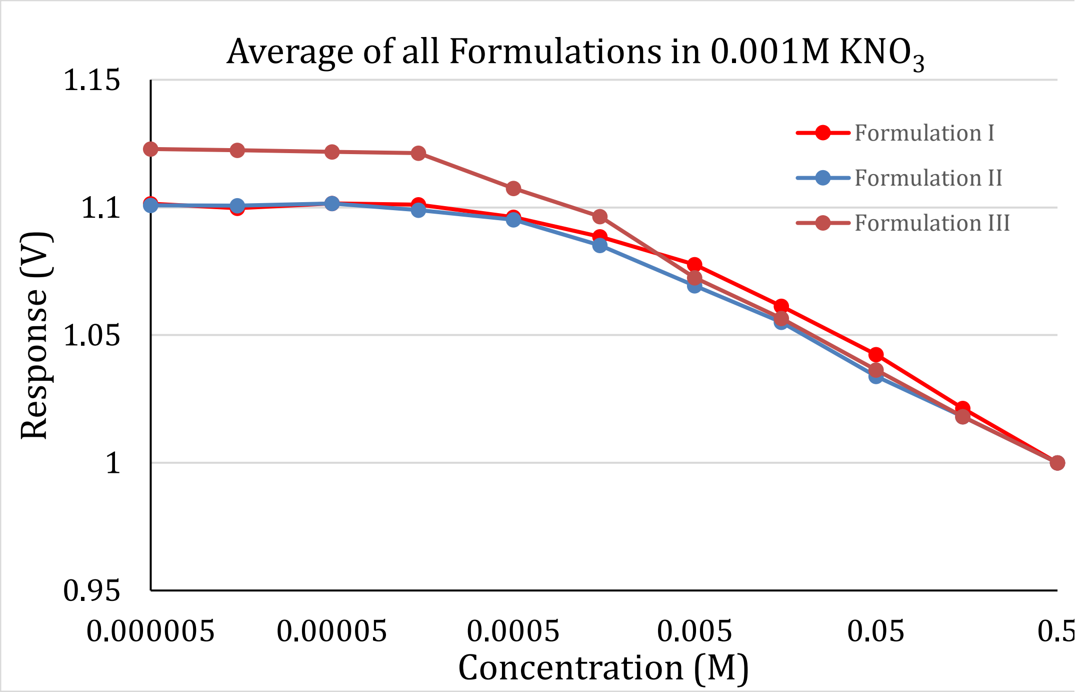
0.00005
0.0005
0.005
0.05
Concentration (M)
Formulation I
Formulation II
Formulation III

6


Average of all Formulations in 0.001M KNO_3
Response (V)
1.15
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Concentration (M)
Formulation I
Formulation II
Formulation III

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