THE USE OF SMART DEVICES FOR THE DETECTION OF AFLATOXIN IN GRINDED CORN FEEDS

The Angelo King Institute for Economic and Business Studies

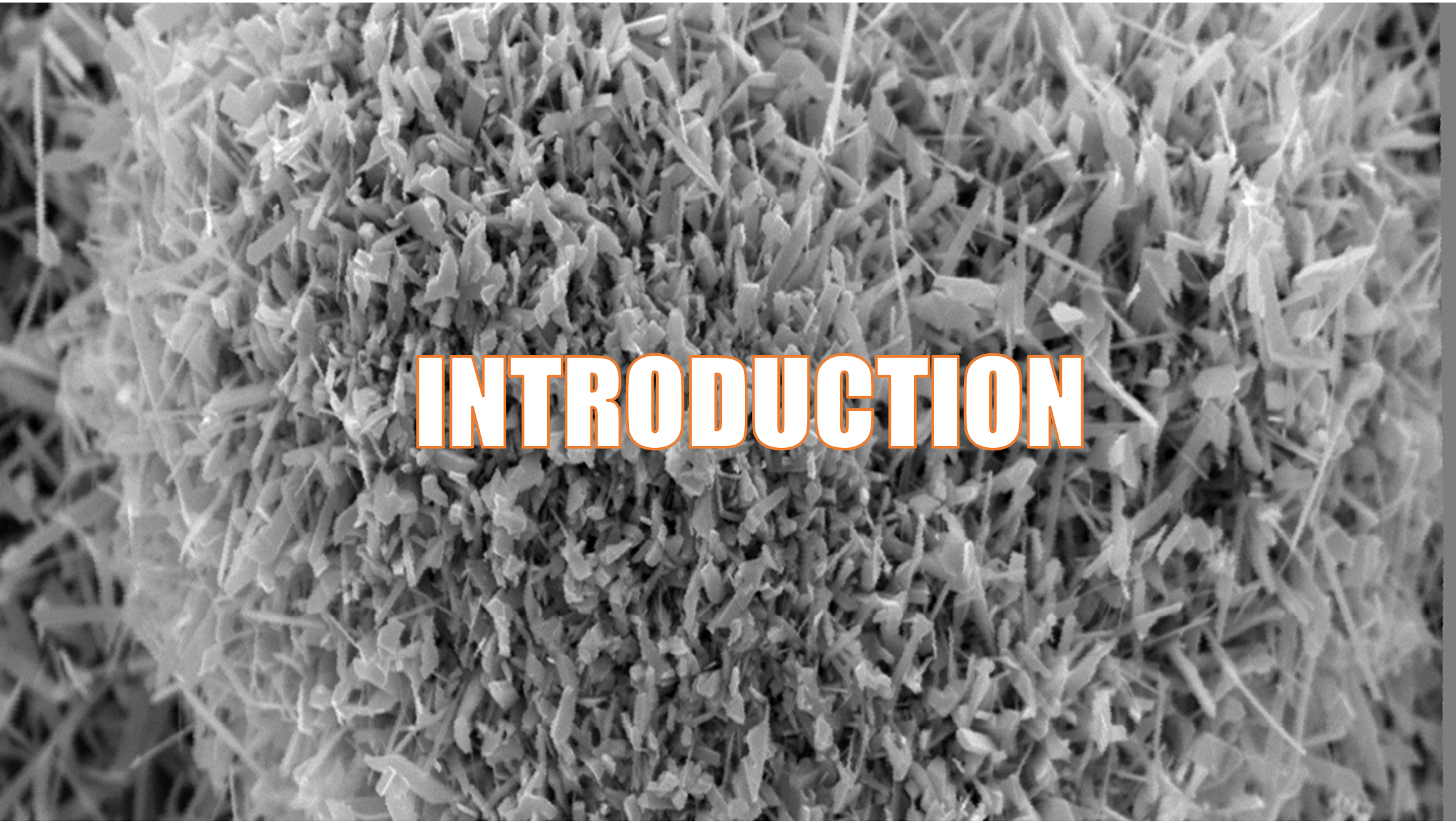
DR. GIL NONATO C. SANTOS

Abstract

Aflatoxin contaminates agricultural commodities, plants or animal derived food, in warm and humid conditions primarily in tropical countries such as the Philippines. Although the type and degree of contamination is dependent on its concentration, its effect becomes critical when biomagnified. In this study, a rapid, simple and portable detection was developed.

Abstract

A smart-device sensor was used to measure the pH of the samples with aflatoxin and compared side by side with the pH of pure samples; Concentrations in parts per billion (ppb) were calculated for each of the samples from the obtained pH readings; Cyclic voltammetry was also conducted to further study the electrochemical properties of the mixture with aflatoxin.


INTRODUCTION

introduction

Aflatoxins are toxic carcinogenic secondary metabolites produced predominantly by two fungal species: *Aspergillus flavus and Aspergillus parasiticus.* These fungal species are contaminants of food crops as well as animal feeds and are responsible for aflatoxin contamination of these agricultural products. The toxicity and potency of aflatoxins make them the primary health hazard as well as responsible for losses associated with contaminations of processed foods and feeds *[Gourama, H. et al., 1995].* Determination of aflatoxins concentration in food crops and animal feeds is thus very important to create policies to be made by Food Safety Regulatory Agencies (FRSA). However, the current mechanism of aflatoxin detection does not provide immediate result, requires technical expertise and are costly *[Paniel. N. et al., 2010]*.

introduction

In several literatures, determination of aflatoxin requires a variety of complex sample preparations, characterization and analysis. Such methods include High-Performance Liquid Chromatography (HPLC), thin layer chromatography, fluorescence, and immunoenzymatic assays. However, detection using a smart device has not been fully explored when it comes to detecting toxins.

introduction

The use of a smart devices received relative attention in the research field due to its simple and abrupt mechanism of detection with its application as a real-time evaluation instrument and currently being explored in different fields. This research developed a simple method for aflatoxin extraction and detection in grinded corn feeds obtained from the Bureau of Animal Industry (BAI). However, the study was limited to the detection of Aflatoxin B1 (*C* 17H12O6).

significanc

Detection using a smart device sensor has been currently explored du to its application as a real-time evaluation instrument of aflatoxin presence in agricultural food materials which benefits several agencies such as the Department of Health (DOH), Department of Agriculture, Bureau of Food and Drugs (BFAD), Local government units, etc. In addition, Having a simple and abrupt detection of such food contaminants are significant to creation of policies which are handled by the Food Safety Regulatory Agencies (FRSA).


OBJECTIVES

Objectives

This research study aims to develop a simple technique for aflatoxin detection.

Specifically, this study aims to:

1. Create a simple procedure in preparing aflatoxin samples
2. Utilize carbon and silver material as sensor for aflatoxin detection
3. Determine the response of the sensors Through capacitance voltage measurements

Objectives

4. Determine the significant difference of the response of the sensors to the target liquid and background/interferents
5. Determine the sensitivity, specificity, and accuracy of the training sets.
6. Identify Concentration of aflatoxin though smart device pH sensor

Scope & Limitations

The study is limited to the detection of Aflatoxin B1 ($C_{17}H_{12}O_6$) with the parameters set as follows:

- 100 mL of solution will be used for all samples.
- 1:4 ratio of water to methanol will be used. Other type of alcohol will not be tested as a solution.
- Experiments will done at room temperature under normal lighting.

However, the percentage composition of the B1 type compared to the other type of aflatoxins will not be discussed as well as the specific properties of the analyte.


THEORIES AND RELATED
LITERATURES

Aflatoxin

- Aflatoxins are a group of secondary metabolites produced by fungi. Different aflatoxins exist, including aflatoxins B1, B2, G1 and G2. Aflatoxin B1 is mainly produced by two fungi, Aspergillus flavus and Aspergillus parasiticus *(Creppy, E.E. , 2002)*
- Aflatoxins, when ingested, inhaled or adsorbed through the skin, have carcinogenic, hepatotoxic, teratogenic and mutagenic effects in human and animals (rats, ferrets, ducks, trout, dogs, turkeys, cattle and pigs even at very small concentrations). *(Anwar-Ul_Haq & Iqbal, 2004)*
- Among all types of aflatoxin, B1 is the most toxic and commonly present in feeds *(Waliyas, F.; Reddy, S.V. , 2009)*

Aflatoxin

- There are a lot of explored and well studied techniques that has been used to detect aflatoxins *(Torres-Pacheco, I. , 2011).* To name a few:
  - ✓High Performance Liquid Chromatography
  - ✓Electrochemical Immunosensors
  - ✓Fluorescence
  - ✓Ultra-violet absorption
  - ✓Spectrometry
  - ✓Biosensors
  - ✓Voltammetry

Using pH as a basis of detection of aflatoxin is somehow not been fully explored.

Aflatoxin

- Recent development of the mechanism of aflatoxin detection and extraction involves the use of organic solvents such as either methanol or acetonitrile or acetone mixed in different proportion with small amounts of water
- Aflatoxin determination based on immunoassay technique requires extraction using mixture of methanol-water (8 + 2 v/v)

(Wacoo, A., et.al., 2014)


METHODOLOGY

TEST

- Measure the pH of each set up using the SPARVUE software.
- Make sure to submerge the tip of the pH sensor to acetone and let it dry before using the sensor to another set up.
- Make 5 trials and take the average measurements.
- Perform cyclic voltammetry using the same set up. Avoid using the same chip for the different set up with aflatoxin.

PREPARATION OF MATERIALS

- Aflatoxin samples are obtained from BAI and is fully sealed in a plastic container.
- 100 mL of solution will be used for all set ups.
- Preparation of corn feeds with aflatoxin should be done under the fume hood.

ANALYSIS

- Calculate concentration from pH readings.
- Plot the concentration and pH to compare.
- Compare results with the CV graph.
- Compare concentration results from other standardized measurements of other agencies.
- Characterize sensing device.

Note: Safety measurements and procedures should be observed in handling and disposing aflatoxin samples.

Prepare 100 ml of solution

- Pure distilled water
- Water-Methanol (1: 4)
- 3 set ups with Water-Methanol plus Aflatoxin

Add 2 tbsp. of aflatoxin to the water-methanol solution.

Corn Feed samples with different amount of Aflatoxin.

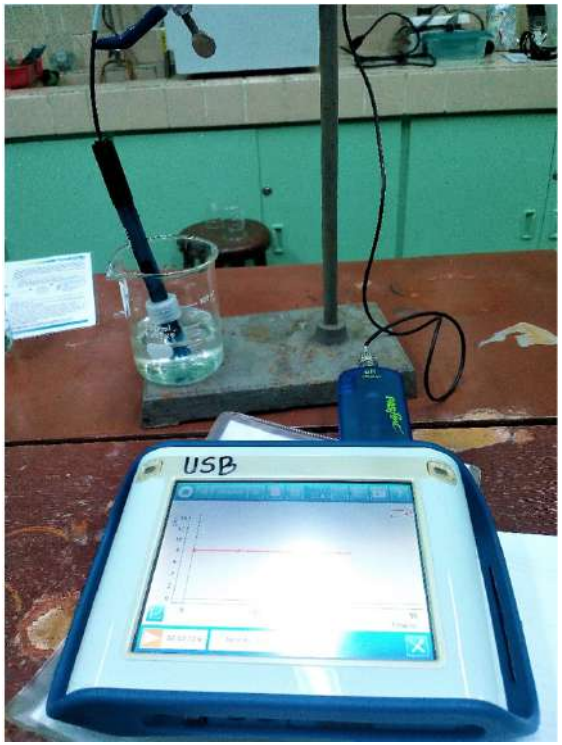
2 tbsps. of each of the samples were placed in a 100ml water-methanol solution

Get pH of each setup

Calculate ppb concentration from pH readings

Plot pH vs ppb concentration

pH SPARKVUE Sensor

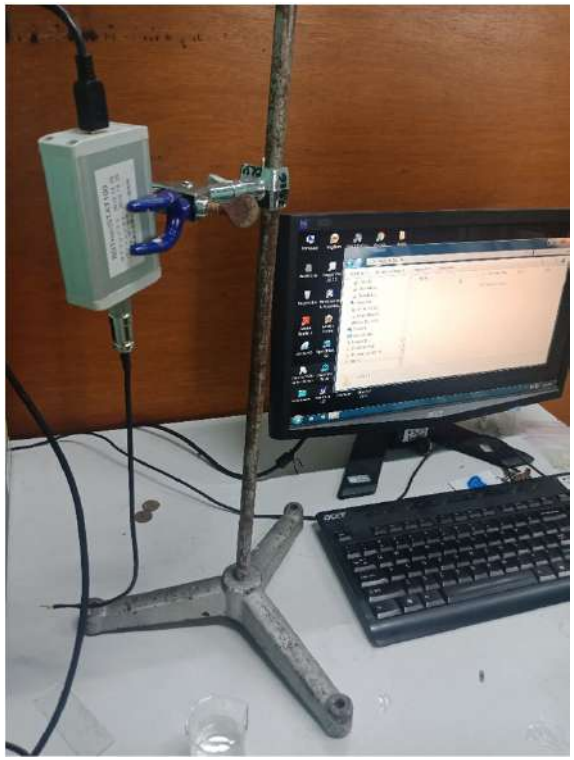


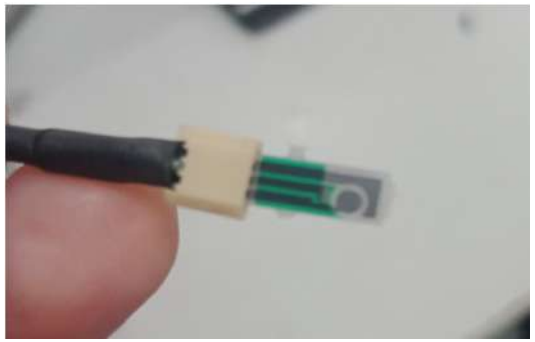
Set up in measuring pH

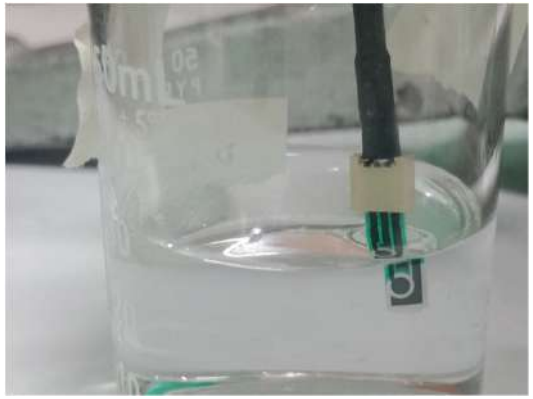
Measure current – voltage response (CV)

Characterization of sensor

Analysis

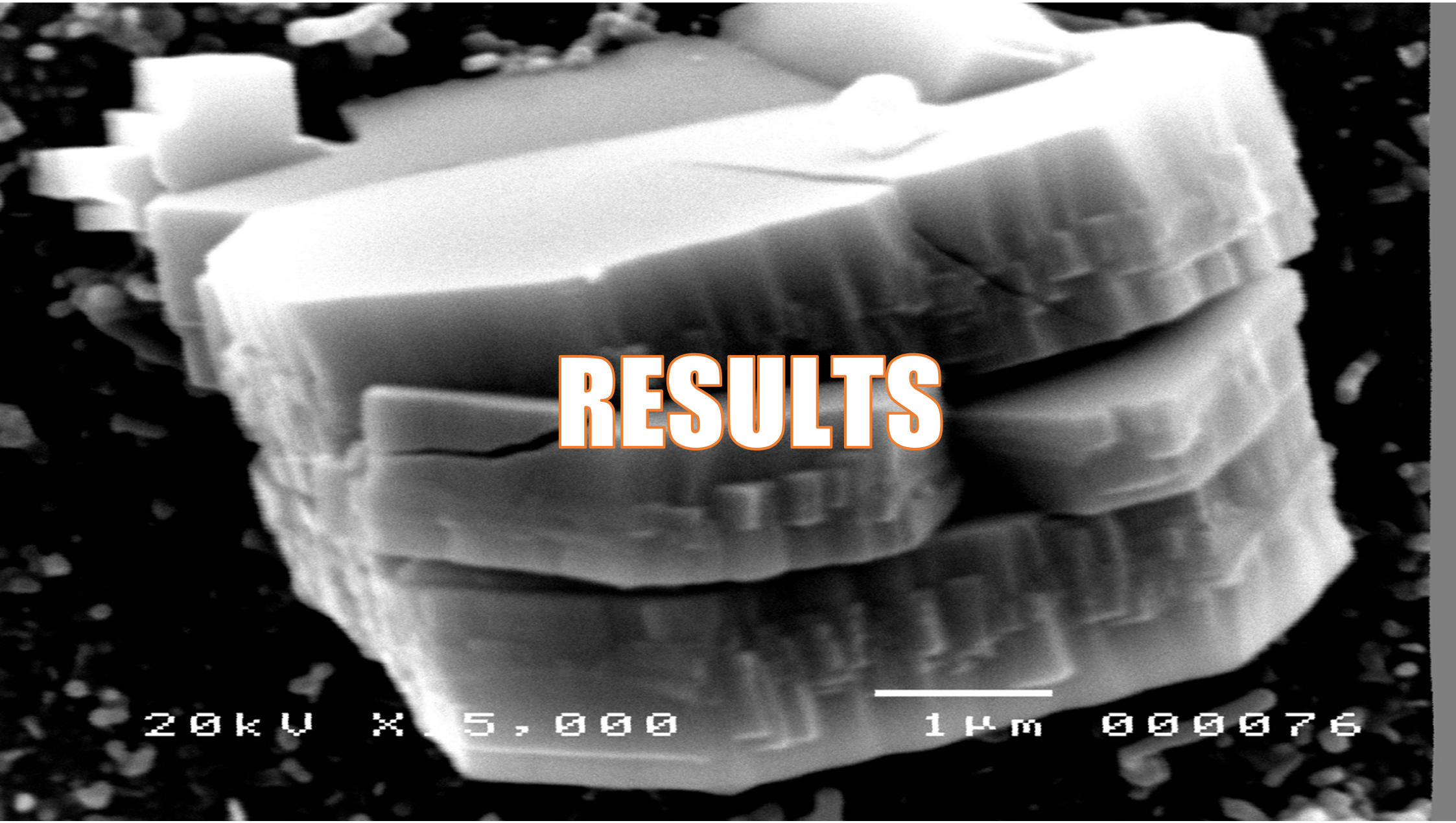






Cyclic Voltammetry Set up

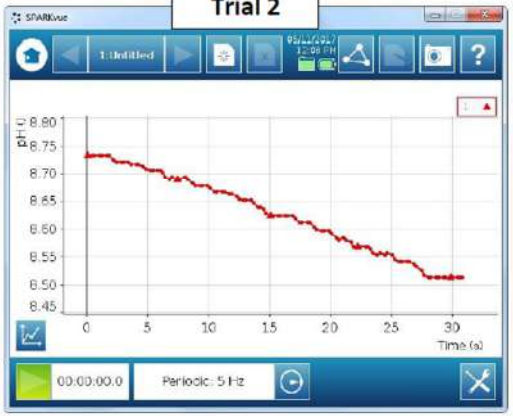
Phenom SEM/EDX


RESULTS
20kV X 5,000
1µm 000076

Pure Distilled Water (100 mL)
Running Time: 30 seconds

Trial 1

Trial 2



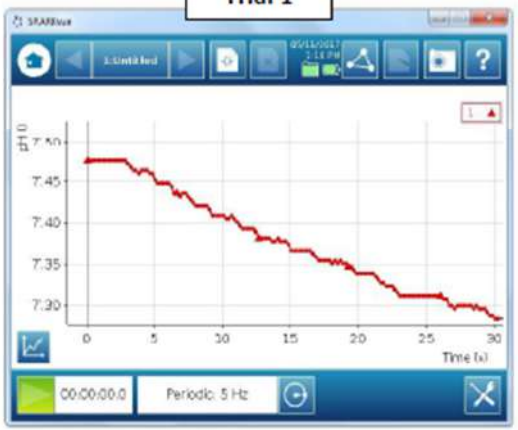
Trial 3

Trial 4

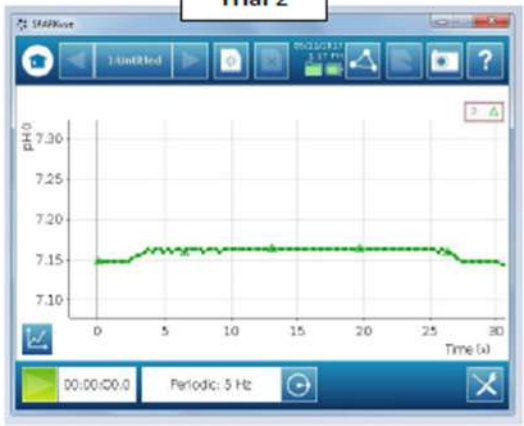
Trial 5

Distilled Water with Methanol (1:4 ratio, 100 Ml)
Running Time: 30 seconds

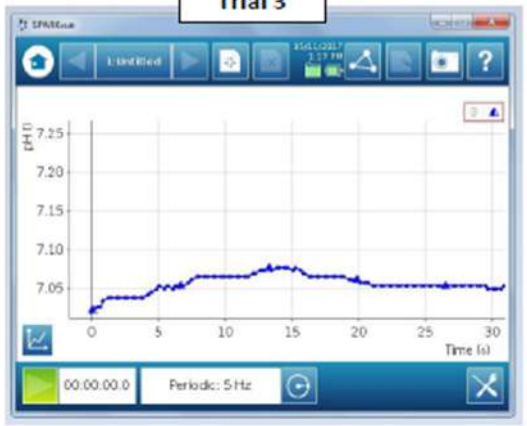
Trial 1



Trial 2

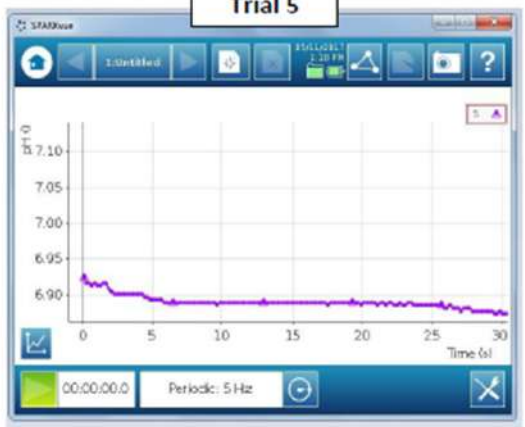


Trial 3



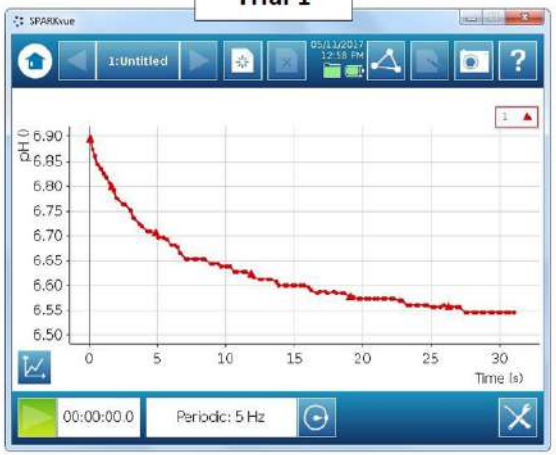
Trial 4

Trial 5



Distilled Water with Methanol (1:4 ratio, 100 Ml) and Aflatoxin (Bottle 1)
Running Time: 30 seconds

Trial 1

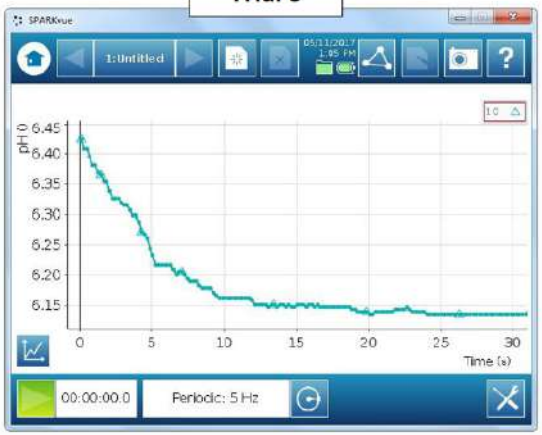


Trial 2

Trial 3

Trial 4

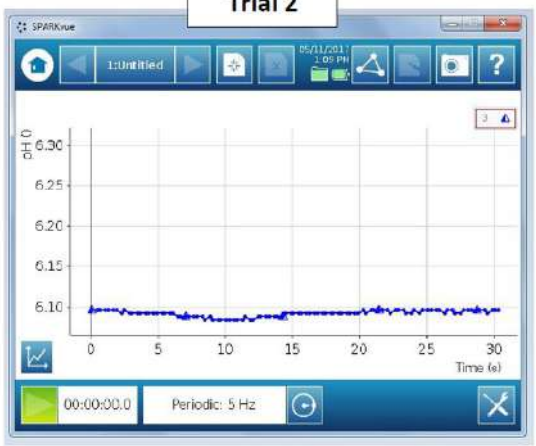
Trial 5



Distilled Water with Methanol (1:4 ratio, 100 Ml) and Aflatoxin (Bottle 2)
Running Time: 30 seconds

Trial 1

Trial 2



Trial 3

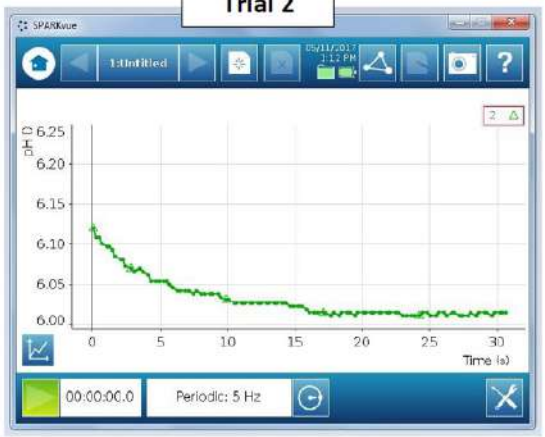
Trial 4

Trial 5

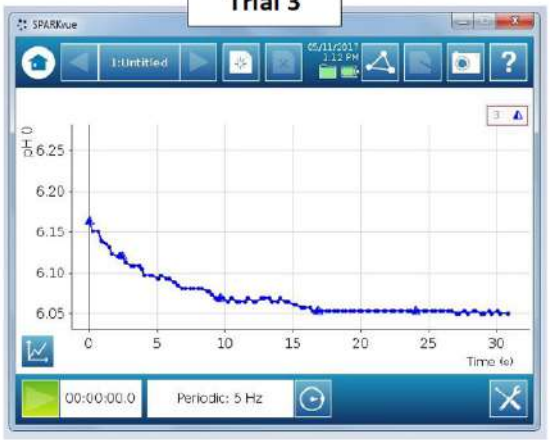
Distilled Water with Methanol (1:4 ratio, 100 Ml) and Aflatoxin (Bottle 3)
Running Time: 30 seconds

Trial 1

Trial 2

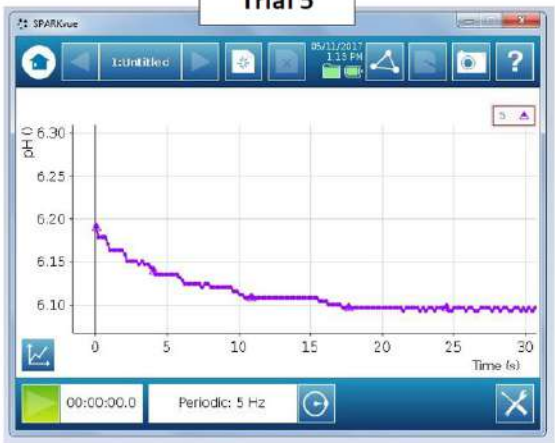


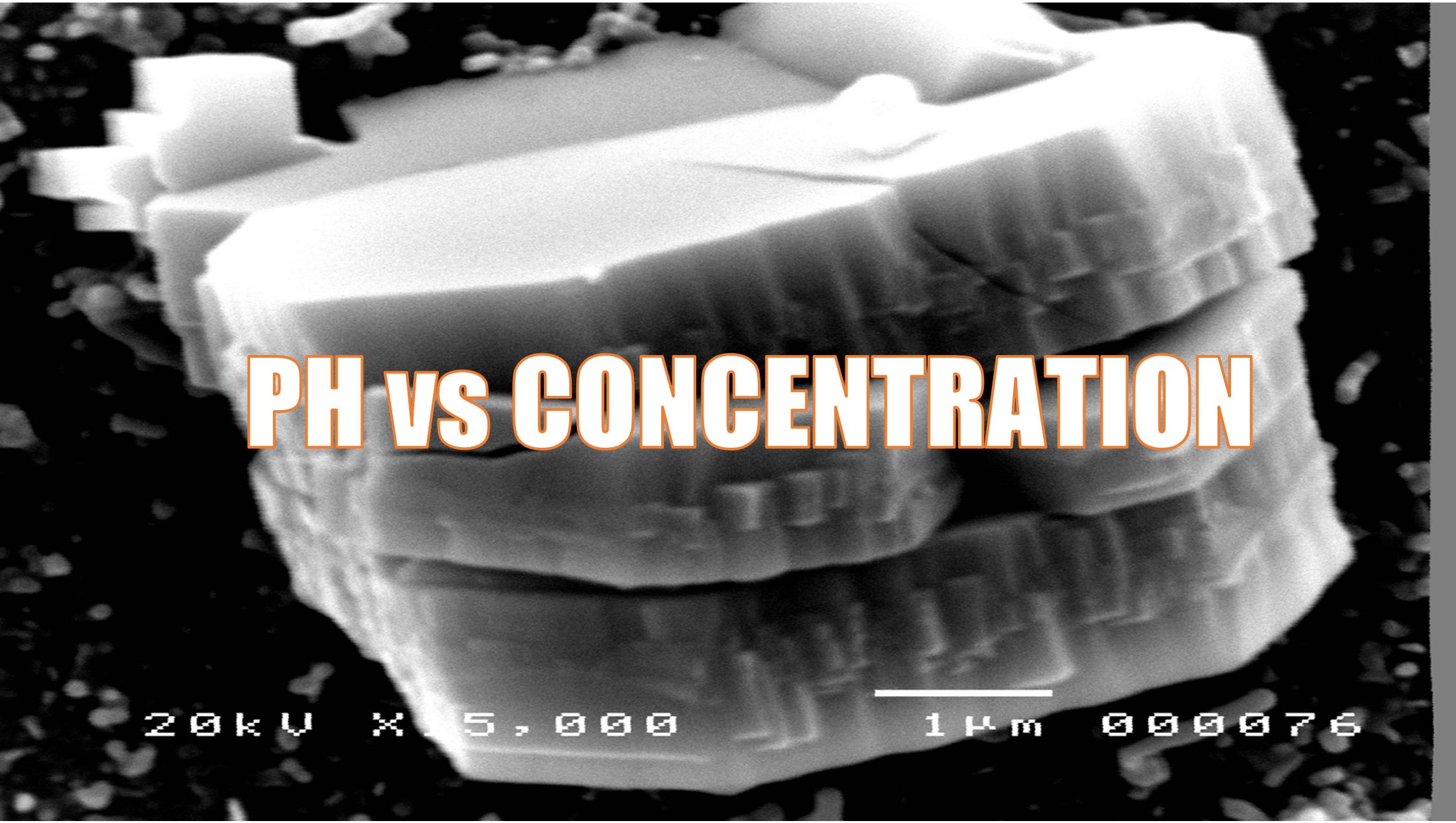
Trial 3



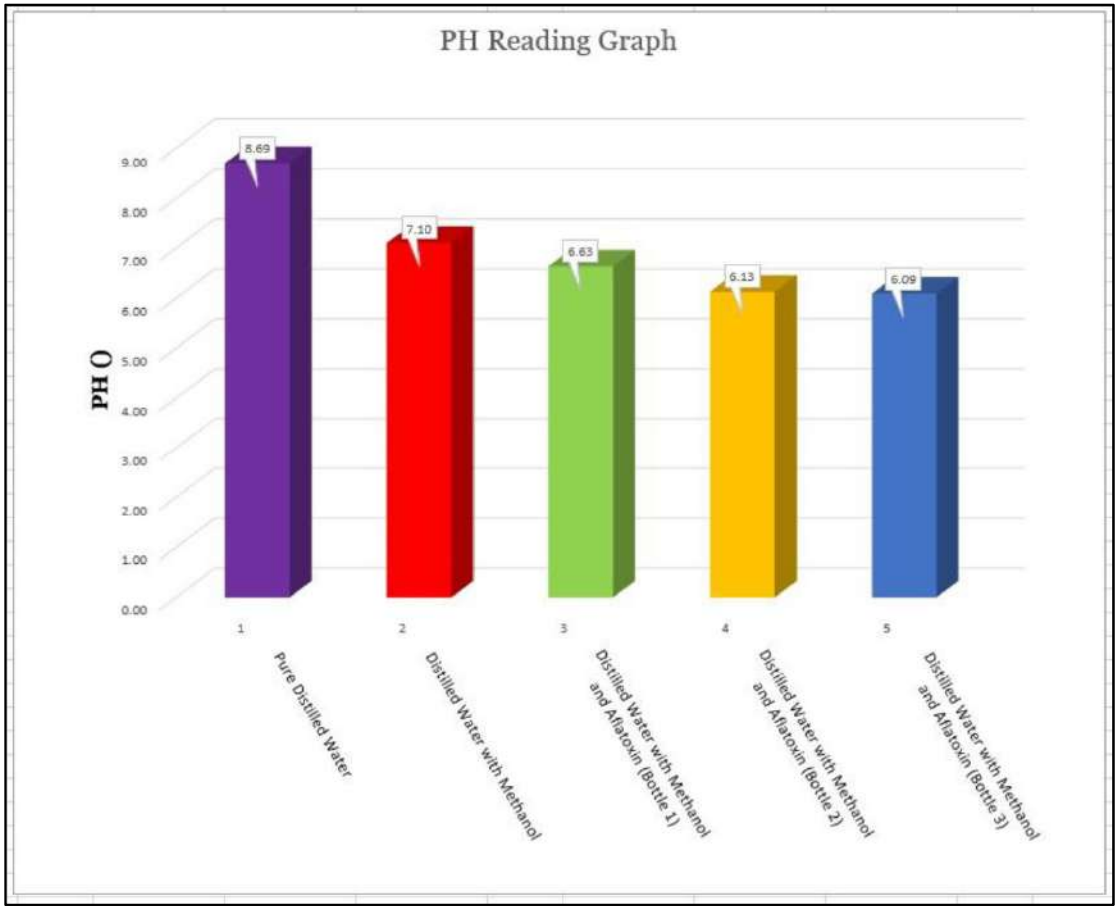
Trial 4

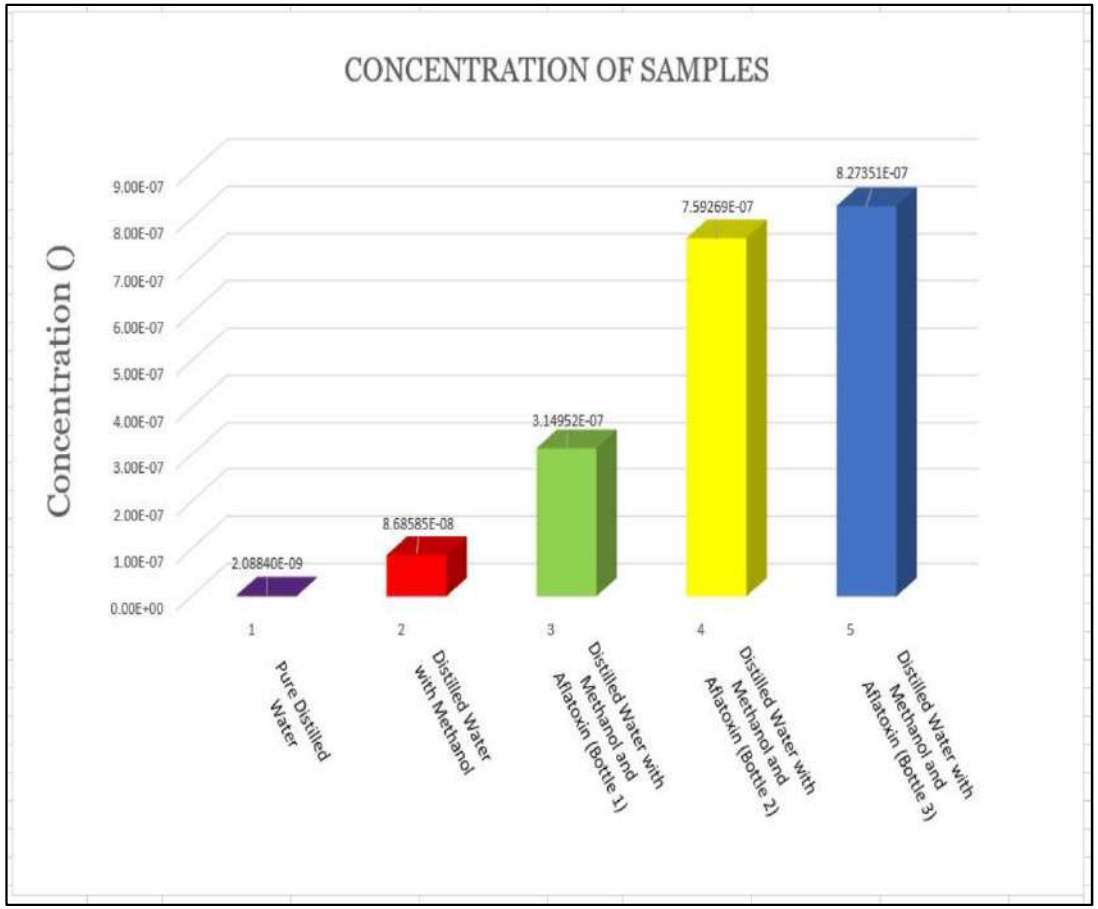
Trial 5

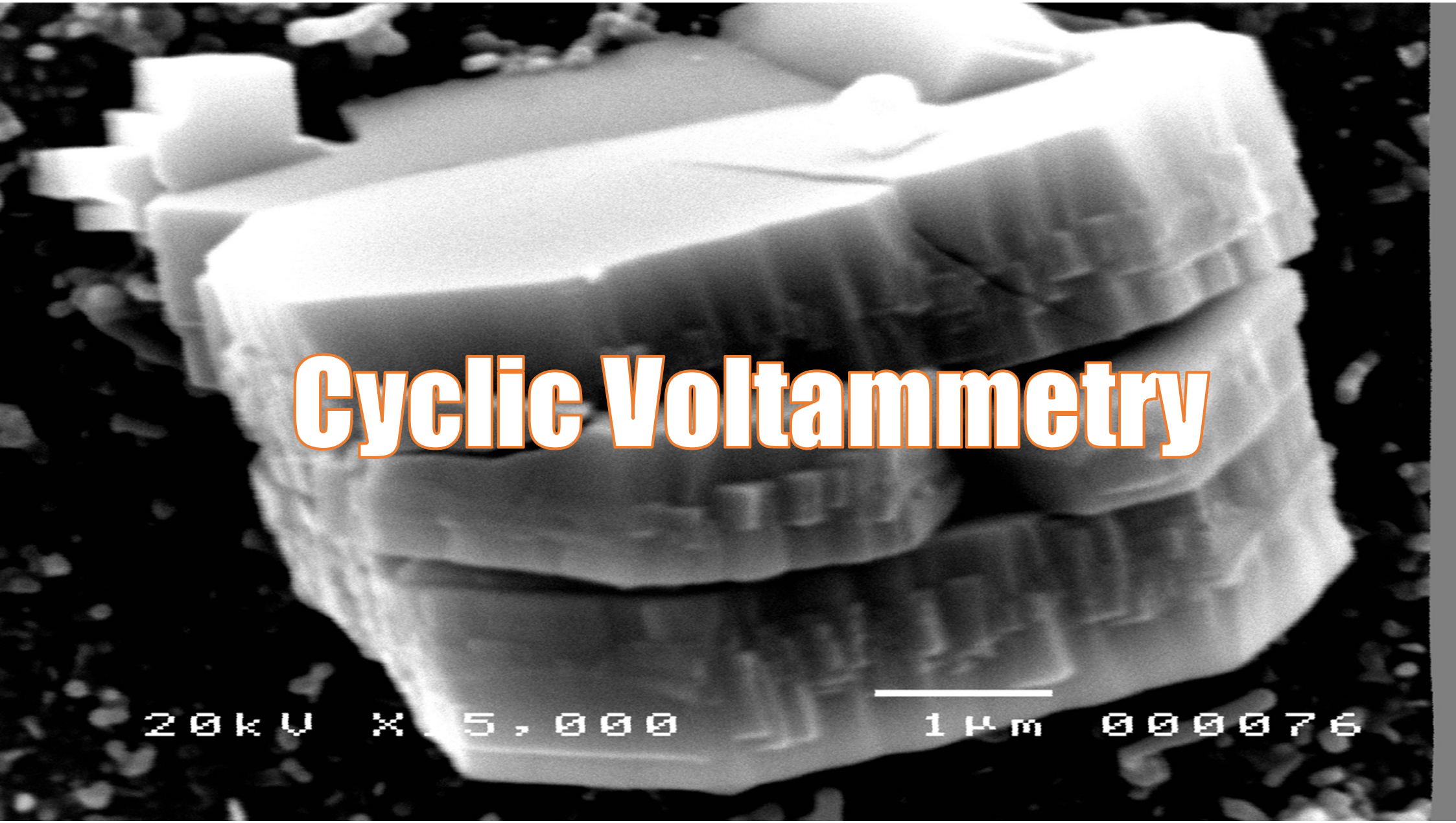



PH vs CONCENTRATION
20kV X 5,000 1μm 000076

Concentration Vs PH Graph






Cyclic Voltammetry
20kV X5,000
1μm 000076

Cyclic Voltammetry

Cyclic Voltammetry (CV) is an electrochemical technique which measures the current that develops in an electrochemical cell under conditions where voltage is in excess of that predicted by the Nernst equation. CV is performed by cycling the potential of a working electrode, and measuring the resulting current.

Water

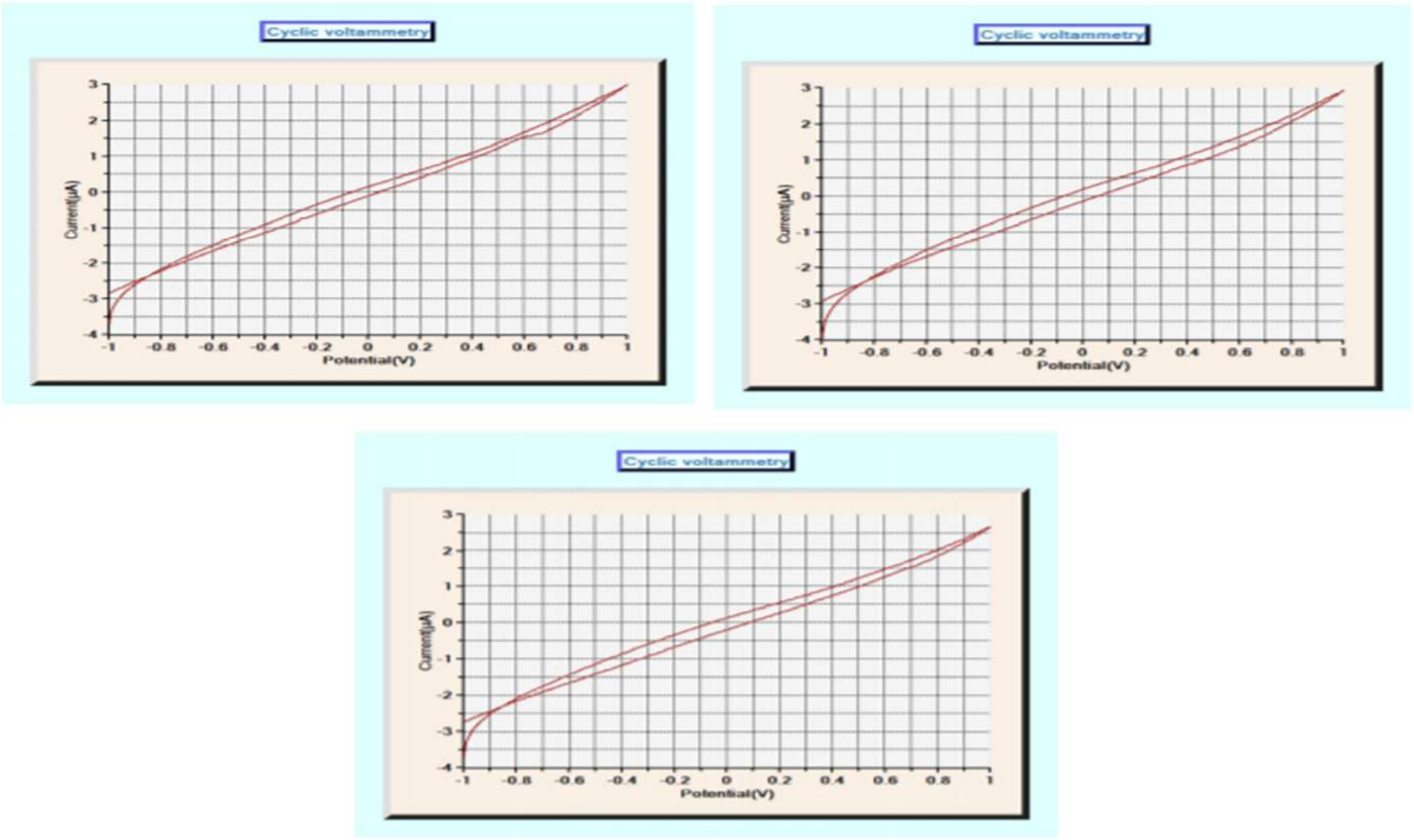


Figure 1: Three trials graph of cyclic voltammetry using BDminstat with Triple Distilled Water as the analyte.

Water with Methanol

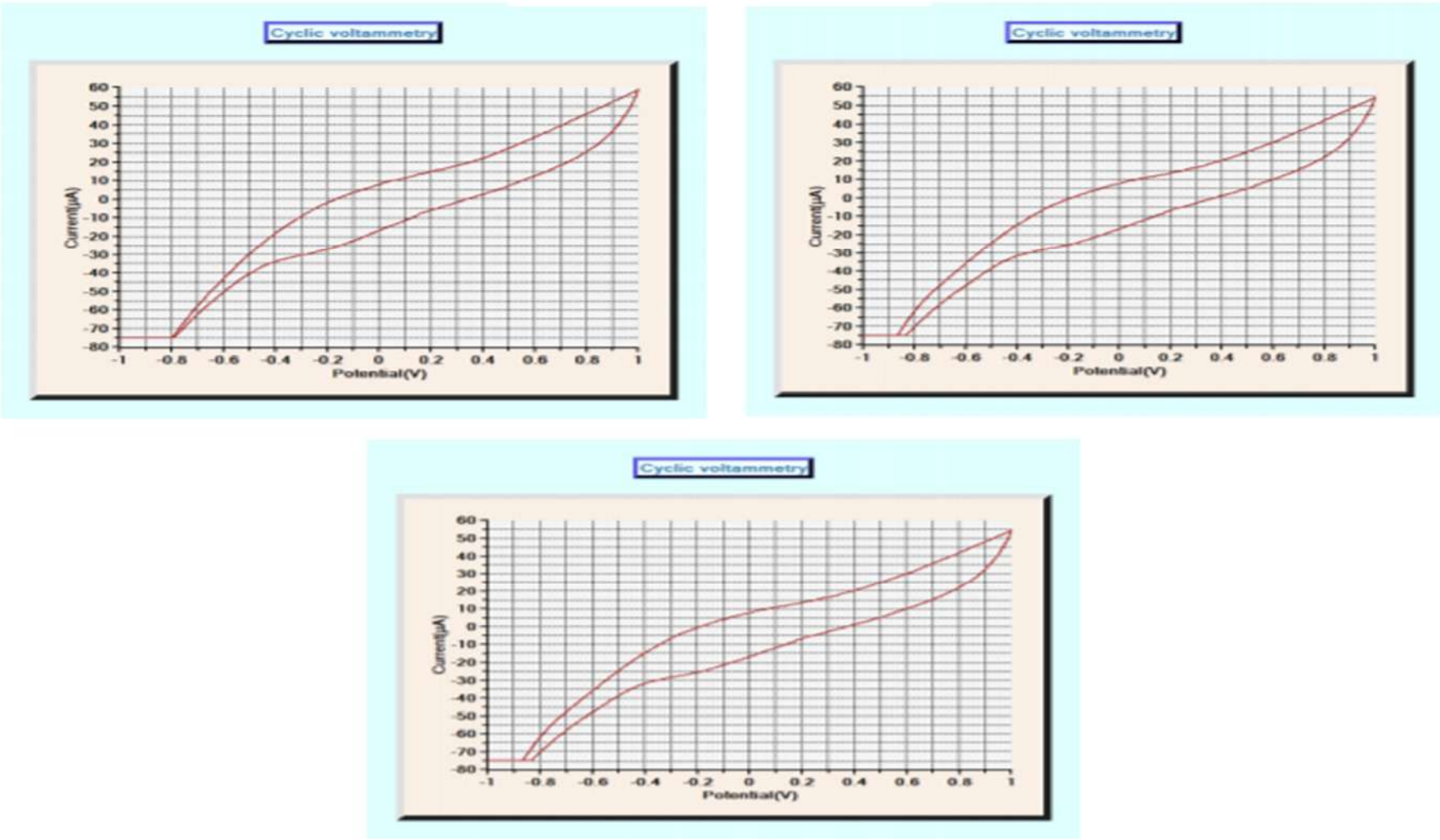


Figure 2: Three trials graph of cyclic voltammetry using BDminstat with Triple Distilled Water mixed with methanol as the analyte.

Aflatoxin Mix (Bottle 1)

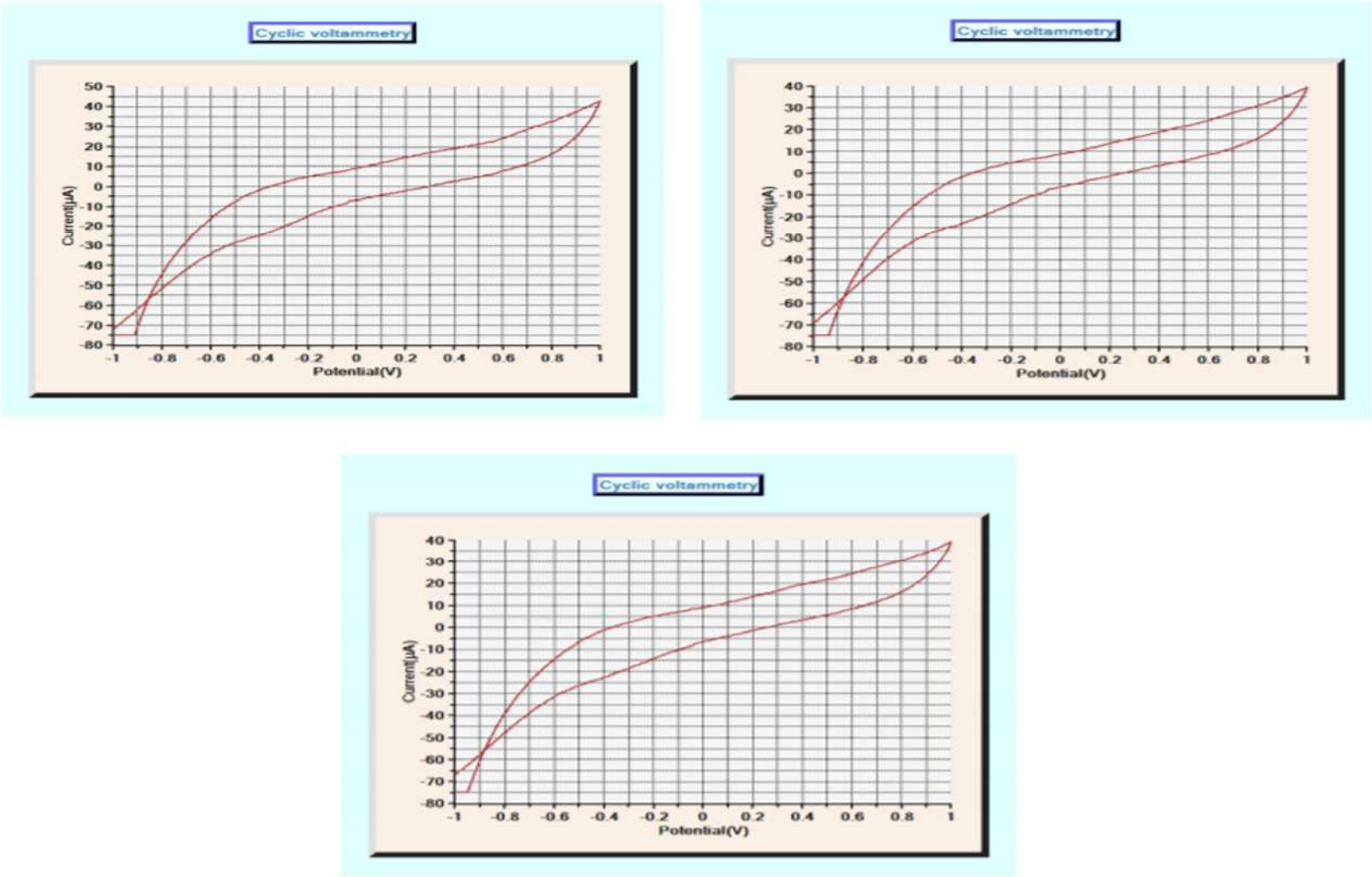


Figure 3: Three trials graph of cyclic voltammetry using BDminstat with Aflatoxin mixed in water-methanol solution as the analyte. Aflatoxin is from Bottle No. 1 which contains an unknown amount of toxin.

Aflatoxin Mix (Bottle 2)

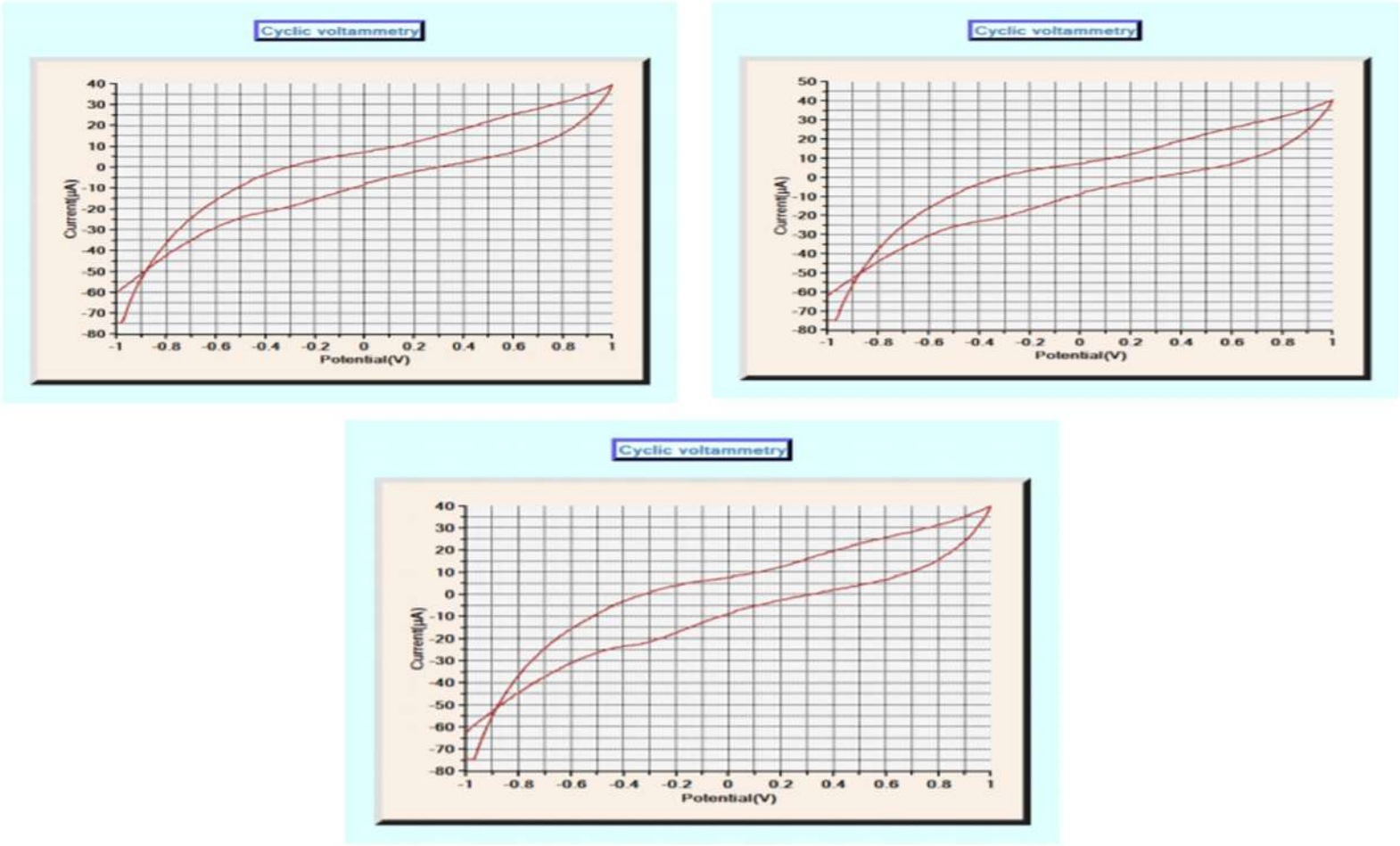


Figure 4: Three trials graph of cyclic voltammetry using BDminstat with Aflatoxin mixed in water-methanol solution as the analyte. Aflatoxin is from Bottle No. 2 which contains an unknown amount of toxin.

Aflatoxin Mix (Bottle 3)

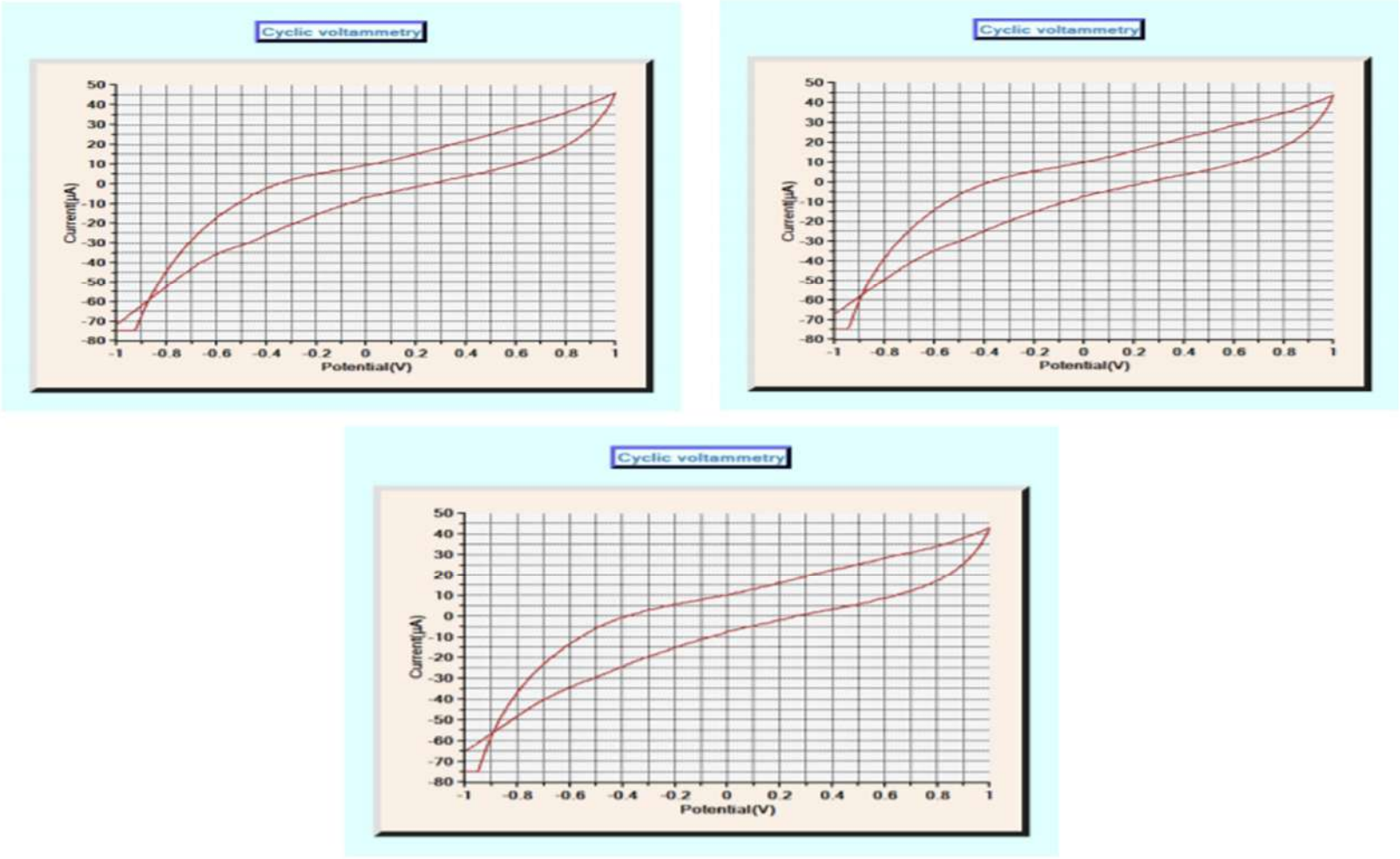


Figure 5: Three trials graph of cyclic voltammetry using BDminstat with Aflatoxin mixed in water-methanol solution as the analyte. Aflatoxin is from Bottle No. 3 which contains an unknown amount of toxin.

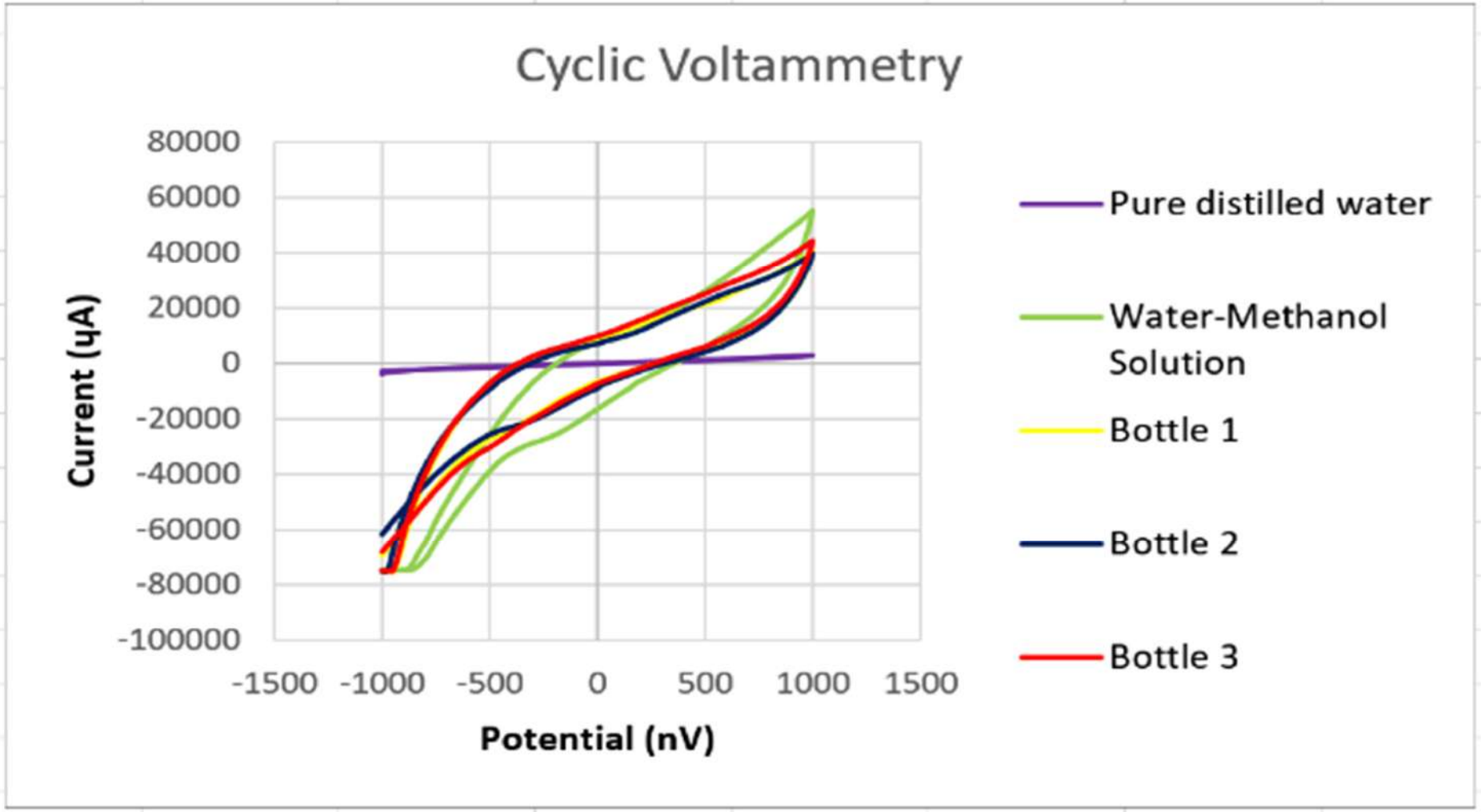
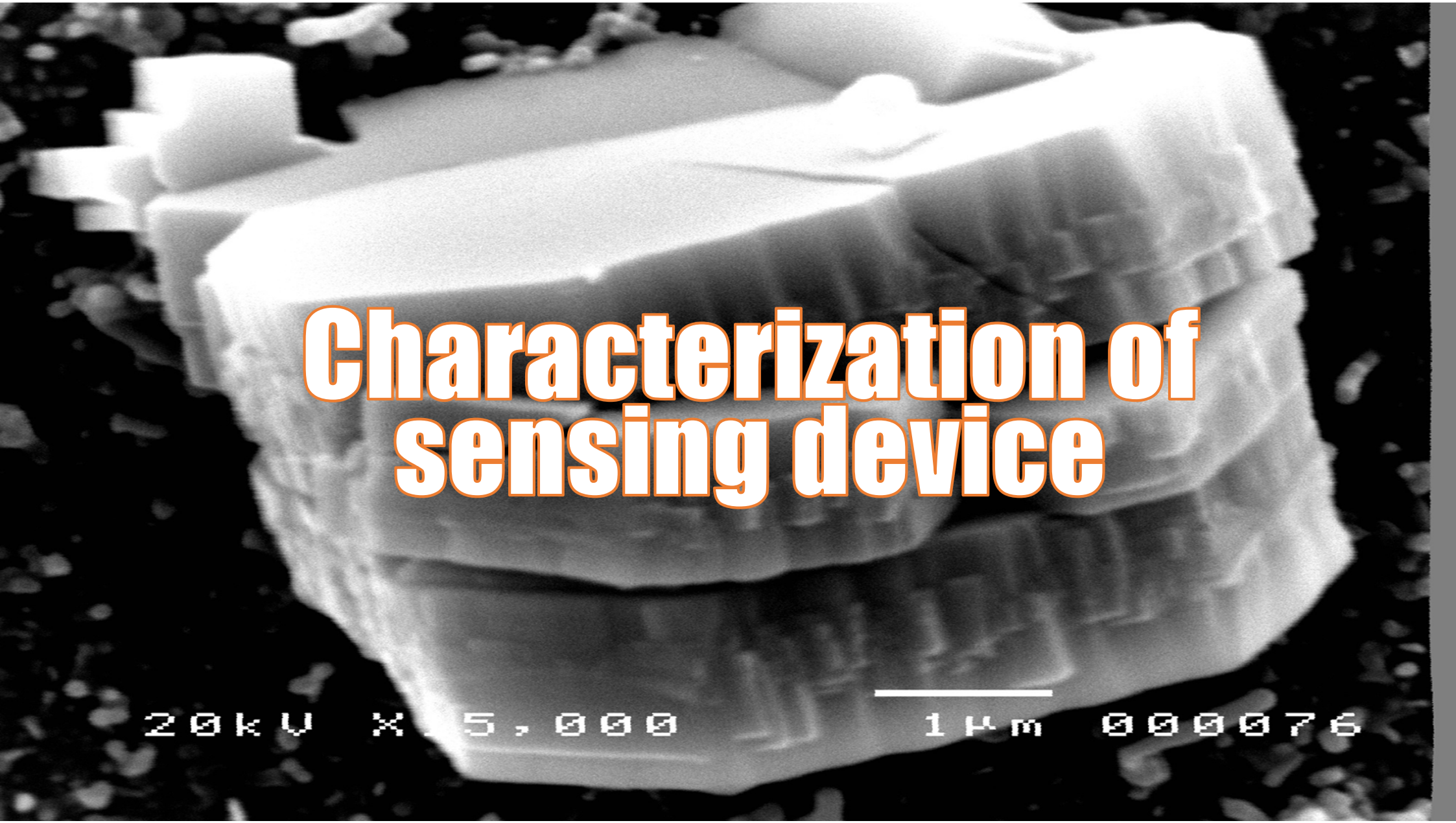
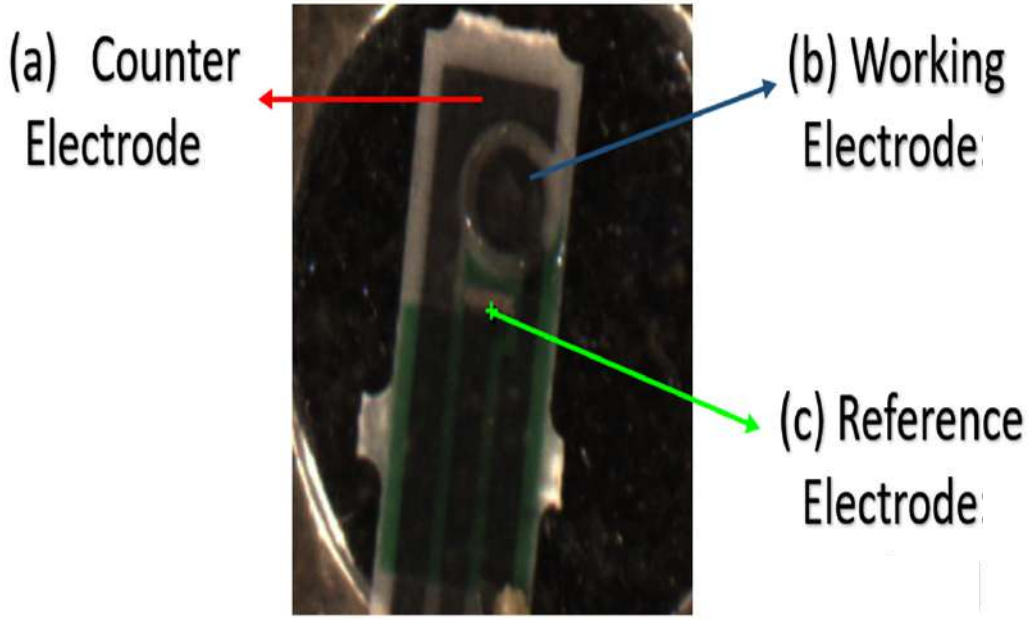


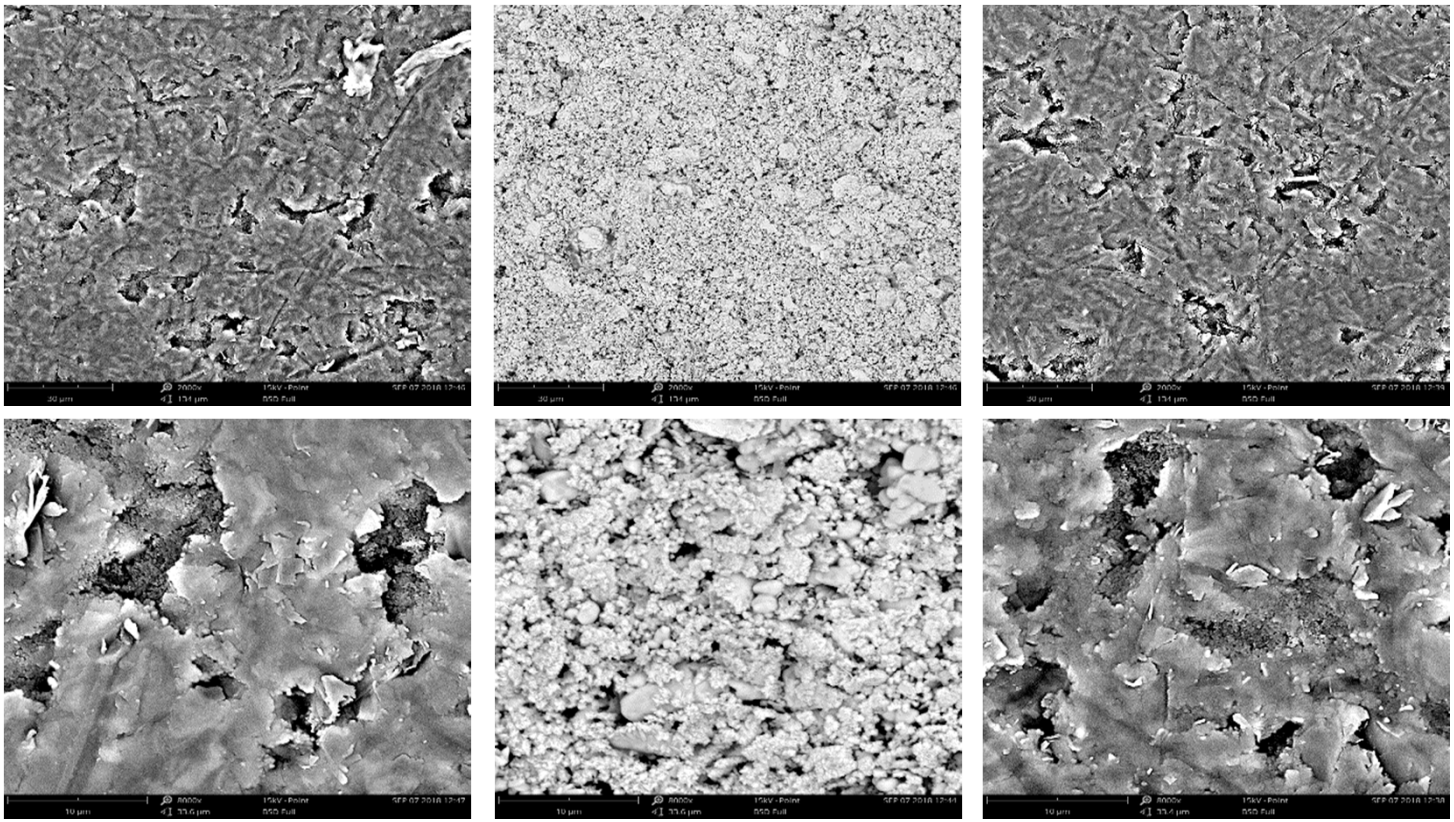
Figure 6: Average Current vs. Voltage Cyclic Voltammetry plot of all samples.


Characterization of sensing device
20kV X5,000
1μm 000076

To confirm observations and results, a cyclic voltammetry (CV) test was conducted; A CV plot provided the electrochemical properties of the sample analyte. The CV device is composed of three working electrodes; counter, reference and the working electrode.



The morphological structure of the three electrodes were examined using the Double Unit Phenom Pro with Advance Imaging System for Scanning Electron Microscopy (SEM).



(a) (b) (c)

Figur 7: *Scanning Electron Microscopy (SEM) images of (a) counter electrode, (b) reference electrode and (c) working electrode. Top row shows SEM images with 2000 magnification and bottom row shows 8000 magnification.*

The elemental composition of the electrodes was characterized using Energy Dispersive X-Ray (EDX).

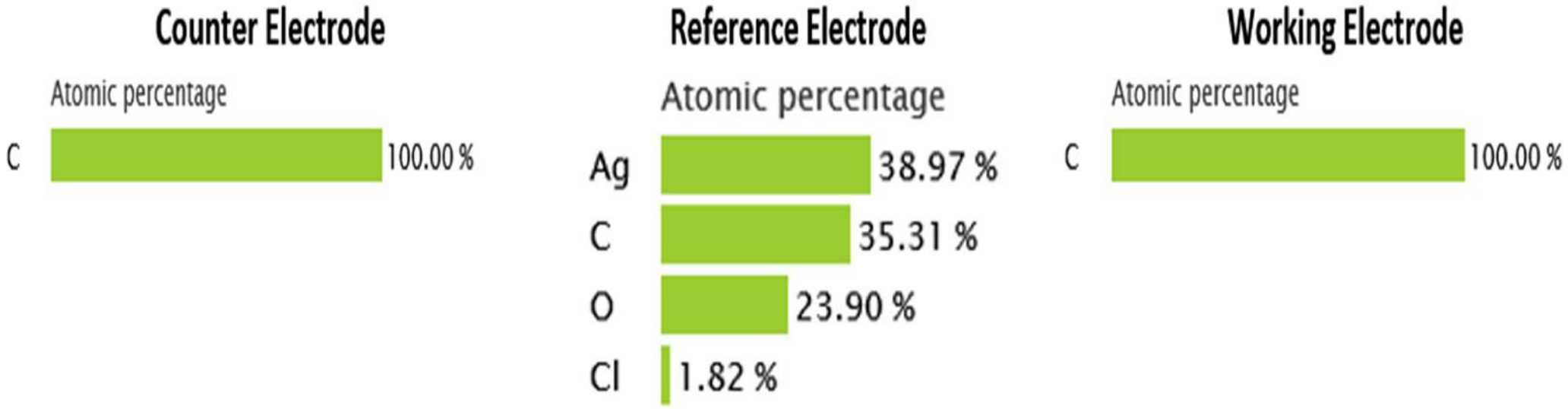


Figure 8: Atomic percent composition of the three electrodes using Energy Dispersive X-Ray (EDX).

FINDINGS AND CONCLUSIONS

Findings & Conclusions

- The concentration of aflatoxin in corn feed samples can be obtained using a conversion of the pH reading of the samples to its equivalent parts per billion using the formulas below:

$$[\,concentration] = 10^{-pH}$$

To get the concentration to ppb:

$$[\,concentration] = \frac{moles}{L}\ x\ \frac{molar\ mass\ (\frac{g}{mol})}{1}\ x\ \frac{1000\ mg}{1g}\ x\ \frac{1ppm}{1mg/L}\ x\ \frac{1000\ ppb}{1\ ppm}$$

Molar Mass or Aflatoxin B1 ($C_{17}H_{12}O_6$) $= 312\ \frac{g}{mol}$

please see excel file for the calculations

Findings & Conclusions

- The calculated ppb concentrations are close to values reported by The United States Food and Drug Administration (USFDA)

SET UP	pH	CONCENTRATION (ppb)
Pure Distilled Water	8.69	0.65
Distilled Water with Methanol	7.10	27.10
Distilled Water with Methanol and	6.63	98.27
Distilled Water with Methanol and	6.13	236.89
Distilled Water with Methanol and	6.09	258.13

Figure on the left: Results gathered from the experimental set up.

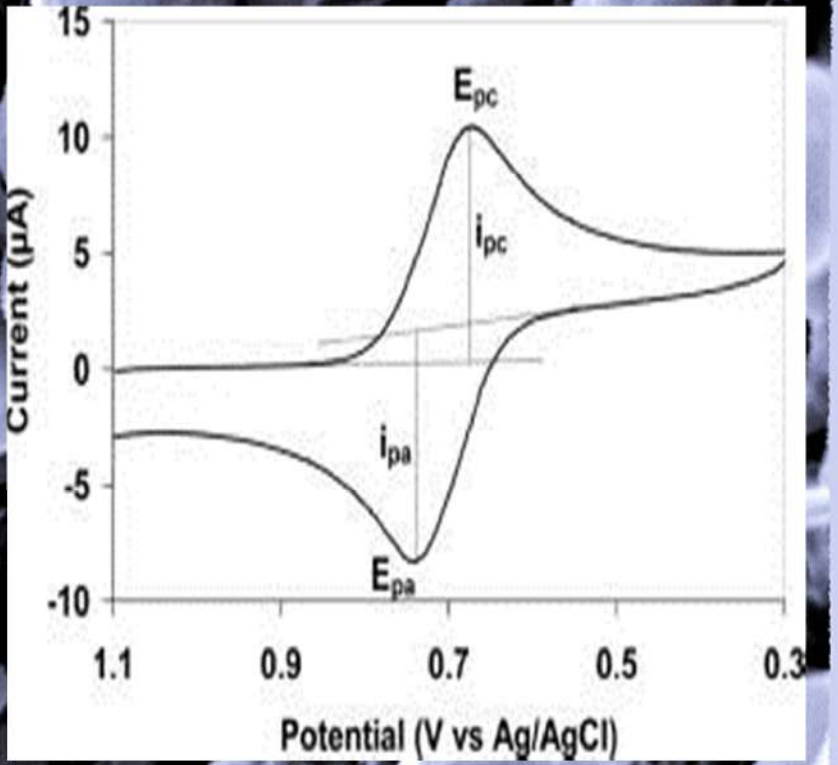
Figure on the right: Results gathered from the USFDA.

Aflatoxin leve (in parts per billion)	Commodities and species
10	All products, except milk, designated for humans
0.5	Milk
20	Corn for immature animals and dairy cattle
100	Corn for breeding beef cattle, swine and mature poultry
200	Corn for finishing swine
300	Corn for finishing beef cattle
300	Cottonseed meal (as a feed ingredient)
20	All feedstuff other than corn

Findings & Conclusions

- Based on the presented results from previous slides, the mixture of water-methanol with or without aflatoxin is IRREVERSIBLE (transfer of electrons from the analyte to the electrodes are slow).
- The potential also dropped when the aflatoxin sample was added to the mixture of water and methanol. This is possibly due to the breakage of the intermolecular forces of the aflatoxin molecules. Thus extraction of aflatoxin is possible using the water-methanol solution.

Figure A: Standard CV graph for a reversible REDOX reaction.



De La Salle University
i-NANO
RESEARCH FACILITY
AKI
Angelo King Institute
for Economic and Business Studies

The Angelo King Institute for Economic and Business Studies

DR. GIL NONATO C. SANTOS