Electrochemical Detection of Fenthion for Forensic Analysis

by
Sydney Robbins

A thesis presented to the Honors College of Middle Tennessee State
University in partial fulfillment of the requirements for graduation
from the University Honors College

Fall 2023

Thesis Committee:

Dr. Charles Chusuei, Thesis Director

Dr. Mengliang Zhang, Second Reader

Dr. Amy Phelps, Thesis Committee Chair

Electrochemical Detection of Fenthion for Forensic Analysis

by Sydney Robbins

APPROVED:

Dr. Charles Chusuei, Thesis Director
Associate Professor, Chemistry

Dr. Amy Phelps, Thesis Committee Chair
Chairperson, Chemistry

Dr. Mengliang Zhang, Second Reader
Associate Professor, Chemistry

Acknowledgements

There is so much gratitude I owe to the MTSU Chemistry Department for supporting me, giving me opportunities I never would have dreamed of, and believing in me even as a shy, little undergraduate freshman. Every faculty and staff member has taken such good care of me, and I hope to never let their time and effort go unnoticed. I want to thank Dr. Chusuei for stepping out of his area of expertise and exploring the world of forensics with me. His guidance, advice, encouragement, and movie recommendations made this experience an invaluable one. Thank you Dr. Zhang for being an amazing second reader, Joyce Miller for helping me collect SEM and EDX data, and Ram Nepal for helping with the Raman data. I want to thank my parents for homeschooling me since day one, for buying me a chemistry set as a child, and for teaching me how to glorify God through science. Most importantly, I want to thank God for His creation, His science, and His truth.

Abstract

Fenthion is an insecticide that has been widely used to protect crops against pests, however its toxicity has led to environmental and health concerns. Because of the harms fenthion presents, the ability to rapidly and accurately assay this pesticide is essential for forensic investigations of suicides, environmental violations, and poisonings. Electroanalytical techniques have been shown to be advantageous in detecting pesticides. Cyclic voltammetry is an electrochemical technique used to determine the reduction and oxidation of analytes in order to identify unknown compounds. The efficiency of cyclic voltammetry to detect trace amounts of fenthion is reportedly increased with the use of nanoparticles to modify the electrode used. The electrocatalytic capabilities of various nanomaterials drop-casted onto glassy carbon electrodes for fenthion detection will be presented to provide a non-destructive, cost efficient, time sensitive, and reliable method of fenthion detection that can be applied to forensic investigation, environmental protection, and public health efforts.

Table of Contents

List of Figures

CHAPTER I: INTRODUCTION

A. Background

Fenthion (*O*,*O*-dimethyl *O*-4-methylthio-*m*-tolyl phosphorothioate) is an organophosphate insecticide that has been widely used to protect crops against pests like mosquitos, flies, mites, and birds. Due to its toxicity, the U.S. Environmental Protection Agency (EPA) has labeled fenthion as a Class II insecticide and restricted its use.[1] The structure of the fenthion is shown in the figure below. It is a synthetic organic thiophosphate that is typically either colorless or a yellow/tan color. Fenthion acts as an insecticide, acaricide, agrochemical, avicide, and acetylcholinesterase inhibitor.[2]

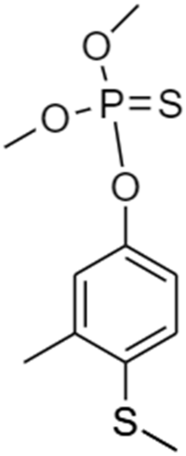


Figure 1: Fenthion (*O*,*O*-dimethyl *O*-4-methylthio-*m*-tolyl phosphorothioate)

Adverse effects of fenthion on the environment and human health have been reported following accidental and intentional exposure.[1] Fenthion, like all organophosphates, inhibits the neurotransmitter acetyl-cholinesterase. While it is extremely effective in deterring insects and birds, fenthion is also harmful to the human nervous system. Acute cholinergic crisis, delayed neurotoxicity, and intermediate syndrome have been linked to fenthion exposure.[3]

Because pesticides like fenthion are inexpensive and accessible, they are often used in suicide attempts. A study done in 2019 concluded that an estimated 14,000,000 individuals globally from 1960-2018 had committed suicide by intentionally consuming pesticides.[4] Unfortunately these millions of individuals are not the only victims of pesticides. In the United States alone, 8,000 people are exposed to toxic organophosphate pesticides like fenthion each year. The vast majority of these cases are purely accidental exposure.[5] Research has found a direct link between organophosphate exposure and ADHD in children.[6] Because of the harms fenthion presents, the ability to rapidly and accurately assay this pesticide is important for forensic investigations of suicides, environmental safety violations, and accidental poisonings.

B. Current Methods

Point of care diagnostic tests (POCT) like calorimetric immunoassays are typically administered by forensic scientists and criminal investigators to detect compounds like drugs, poisons, and pesticides. While these methods are convenient, they commonly present false positives and are not definite. Further confirmatory tests like gas chromatography-mass spectrometry or liquid chromatography tandem mass spectrometry are needed. These methods are reliable and accurate, but substantial sample pre-treatment, a large sample size, and costly reagents are required. The confirmatory tests are destructive, and the sheer number of forensic samples sent to crime labs has caused a massive backlog.[7]

C. Electrochemical Analysis

Electroanalytical techniques have been shown to be advantageous in detecting pesticides. These methods are more time efficient, require less preparation, and are non-destructive. Cyclic Voltammetry (CV) is an electrochemical technique used to determine the reduction and oxidation of analytes. It will be the main analytical tool in this study. CV produces data in graphs called voltammograms which plot the applied potential (in volts) on the x-axis and current (in microamps) on the y-axis. The curve that is shown on the voltammogram indicates the reduction-oxidation cycle of the analyte.[8] In the figure below, reduction is shown as beginning at point *a* and moving towards point *d*. The potential is negative due to reduction. Oxidation then occurs from point *d* to point *g*, and the potential is positive. Point *c* indicates the cathodic peak potential (reduction peak) while point *f* indicates the anodic peak potential (oxidation peak).[9]

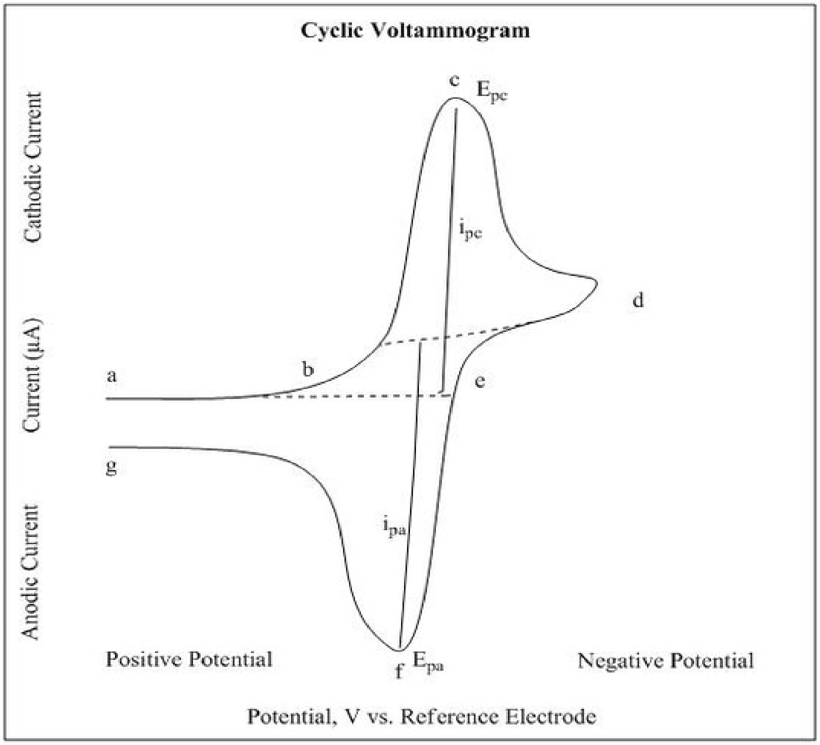


Figure 2: Cyclic voltammogram[9]

Chronoamperometry (CA) is another electrochemical technique that commonly follows cyclic voltammetry. CA is performed with the same electrochemical cell as CV, but measures current against time, and the applied potential is constant.[10] CA is a more rapid test that extends the lifetime of the composite. The purpose of performing chronoamperometry is to establish the sensitivity and concentration range for the analyte.[11]

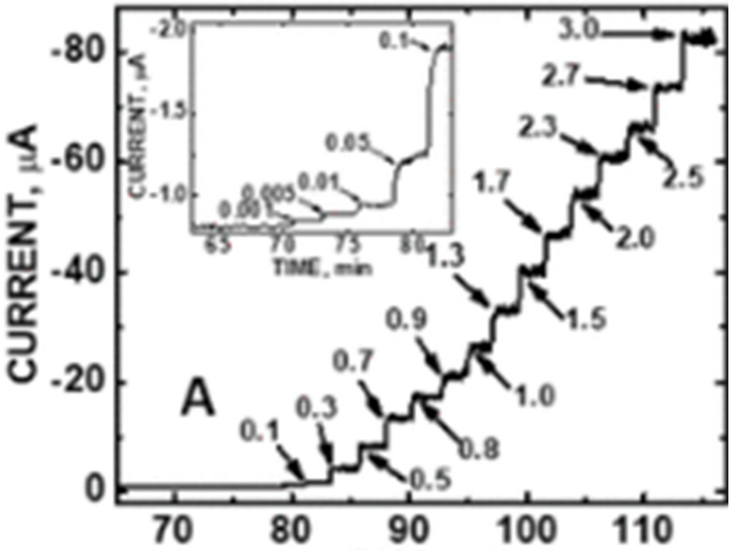


Figure 3: Typical data from chronoamperometry[11]

Nanoparticles can be utilized to modify electrodes for CV and CA tests. Multiwalled carbon nanotubes (MWNT) are commonly used to enhance the efficacy of detection because of their conductivity. MWNT themselves do not interact with the molecules of an analyte without first being functionalized. Functionalized MWNT are less cytotoxic, have increased dispersion and solubility, and do not conglomerate. They also have the ability to be tethered to metal oxide nanoparticles. Together, metal oxides and functionalized MWNT can be applied to glassy carbon electrodes to increase their sensitivity for electrochemical detection.[12]

D. Research Focus

The goal of this project was to provide a non-destructive, cost efficient, time sensitive, and reliable method of fenthion detection that can be applied to forensic investigation, environmental protection, and public health efforts. To accomplish this, multiple composites of nanomaterials were tested as modifications to glassy carbon electrodes. These electrodes were used in cyclic voltammetry experiments to detect trace amounts of fenthion. The first tested composite was created with carboxylic acid functionalized multiwalled carbon nanotubes tethered to cupric oxide (CuO) nanoparticles. The decision to use CuO nanoparticles follows research that successfully detected the organophosphate malathion by using a CuO modified electrode in cyclic voltammetry. Malathion and fenthion have similar chemical structures. While the study was successful in detecting malathion, the authors recommended further research be done to thoroughly explore the capabilities of CuO as an electrode modifier for the electrochemical detection of organophosphate pesticides.[13] In addition, composites of cupric oxide alone, carboxylic acid functionalized multiwalled carbon nanotubes, elemental copper nanoparticles, and copper nanoparticles tethered to carboxylic acid functionalized multiwalled carbon nanotubes were tested for sensitivity and reliability.

CHAPTER II: MATERIALS AND METHODS

A. Materials

In order to accomplish the goal of detecting fenthion, a composite of metal oxide nanoparticles (CuO) tethered to carboxylic acid functionalized multiwalled carbon nanotubes (COOH-MWNT) was applied to a glassy carbon electrode to optimize its efficacy. This modified glassy carbon electrode (GCE) was used for cyclic voltammetry and chronoamperometry tests to detect the presence of fenthion.

Three control tests were done with cyclic voltammetry (CV) using an unmodified glassy carbon electrode, a GCE modified with only COOH-MWNT, and a GCE modified with only CuO nanoparticles to evaluate the reactivity with fenthion. The results of these experiments provided a baseline to later compare the activity and selectivity of CuO/COOH-MWNT nanocomposite.

Every composite that was tested was made by mixing 2.0 milligrams of nanomaterials in 1 mL of ethyl alcohol. In composites where nanoparticles were tethered to multiwalled nanotubes, the composite was sonicated for 30 minutes in order for the materials to be tethered. These composites consisted of 1.0 milligrams of nanoparticles and 1.0 milligrams of multiwalled nanotubes. Throughout the course of the project, the composites tested were cupric oxide nanoparticles (CuO NP's), carboxylic acid functionalized multiwalled carbon nanotubes (COOH-MWNT), cupric oxide nanoparticles tethered to carboxylic acid multiwalled carbon nanotubes (CuO/COOH-MWNT), elemental copper nanoparticles (Cu NP's), and elemental copper nanoparticles tethered to carboxylic acid functionalized multiwalled carbon nanotubes (Cu NP's/COOH-MWNT).

In order to modify the surface of the GCE with the metal oxide nanoparticle composite, a drop-casting technique will be used. A micropipette is used to carefully drop the composite onto the electrode's surface so that the surface tension of the liquid is maintained. The composite is allowed to air dry before Nafion™ is applied in the same way to secure the nanomaterials to the surface of the electrode. Nafion™ is a polymer that creates a membrane that is permeable to electrochemical activity.[14] Chitosan was also tested as a capping agent to secure the composite to the GCE.[15] Figure 4 shows a simplified illustration of the drop casting method.

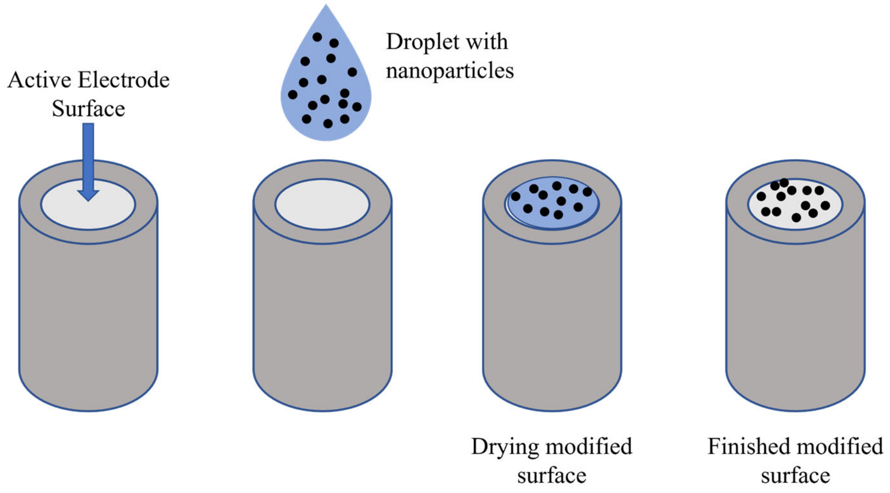


Figure 4: Drop casting technique for modification of glassy carbon electrodes

Once the electrode is modified, it can be tested with CV in a phosphate buffer solution (PBS) with a pH of 7 to mimic physiological conditions. Because fenthion is only partially soluble, the greatest concentration that can be dissolved and detected is 0.201 mM. The sample was prepared by dissolving 1.40 mg of fenthion in 25.0 mL of pH 7 PBS for a 0.201 mM solution. The reduction peak measured through CV was used as the value of applied potential for CA. CA was performed using this value to determine the range of selectivity and concentration of fenthion.

B. Experimental Procedure

a. Cyclic Voltammetry

Cyclic voltammetry (CV) was performed in a glass electrochemical cell, and three electrodes were used in the setup. One was the glassy carbon electrode (GCE) that was modified with a composite. This was the working electrode that carried out the electrochemistry of interest. The second electrode was the reference electrode, which provides a reference to compare the signal from the working electrode against. The reference electrode used in every CV experiment was an AgCl/Ag electrode. The third electrode was a ground electrode, which was a platinum wire in these experiments. The experimental setup is shown below in Figure 5.

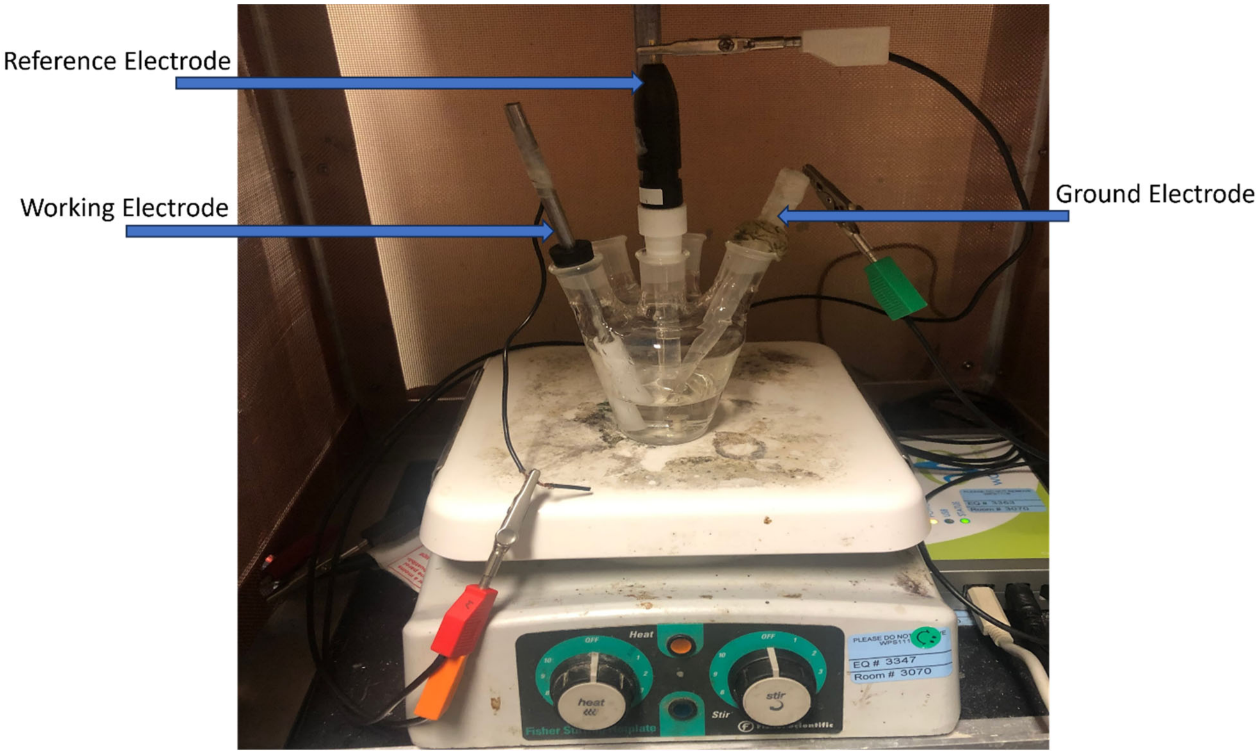


Figure 5: Experimental setup for cyclic voltammetry

The red wire on the left in Figure 5 was connected to the working electrode, the white wire in the middle was connected to the AgCl/Ag reference electrode, and the green wire on the right was connected to the platinum wire ground electrode. These electrodes were connected to a potentiostat that measures the reduction and oxidation activity to plot a graph of voltage vs. current. The CV parameters were set to the following: segments = 2; initial potential = -1 V (vs REF); vertex potential = 1 V (vs REF); final potential = -1 V (vs REF); sweep rate = 50 mV/s; initial range = highest (μ); autorange = up. The cell is filled with 20-30 mL of liquid so that each electrode is submerged. The minimum amount of liquid for the glass cell is 8 mL, and the maximum amount is 100 mL. Each cyclic voltammetry experiment is run in triplicate.

b. Chronoamperometry

The experimental setup for chronoamperometry (CA) used the same electrochemical cell setup as cyclic voltammetry (See Figure 5). The oxidation peak from cyclic voltammetry was used for the potential that was kept constant throughout the entire experiment. The experiment started by collecting the signal of 25 mL of pH 7 phosphate buffer solution (PBS) to collect data on a blank sample. After running the blank solution for 6 minutes, increments of fenthion were added to the PBS to increase the concentration from 0.01 mM to 0.10 mM of fenthion in 10 increments. Three minutes were allowed in between each increment to collect the signal.

c. SEM/EDX

To characterize the composite of cupric oxide nanoparticles tethered to carboxylic acid functionalized carbon nanotubes (CuO/COOH-MWNT), scanning electron microscopy (SEM) was used alongside energy dispersive X-ray spectroscopy (EDX). SEM is commonly used with carbon materials to visualize their size and structure. Scanning electron microscopes have an electron gun that accelerates electrons towards the sample and a detector that detects the electrons scattered from the sample.[12] The sample must be prepared with a coating to generate a high resolution image. EDX is used to identify the individual elements that are present in a sample by the x-rays the elements emit. For both methods of analysis, the CuO/COOH-MWCNT composite was drop casted onto the GCE without the addition of Nafion™. A gold/palladium coating was applied to the top half of the electrode, and copper tape was applied to the sides of the electrode (see Figure 6). These modifications were made to establish grounding to overcome charging that may occur at the surface.

Figure 6: GCE modified with CuO/COOH-MWNT composite, sputter-coated with Au and Pd, and modified with copper tape for SEM and EDX analysis

d. Raman Spectroscopy

In order to prepare the sample for Raman analysis, 10 µL of composite was drop casted onto a small silicon wafer and allowed to air dry. Two composites were tested: CuO/COOH-MWNT and COOH-MWNT. Once the samples were prepared, they were analyzed on the Raman instrument.

CHAPTER III: RESULTS AND DISCUSSION

A. Cyclic Voltammetry

The first test performed was a CV of 0.201 mM fenthion in pH 7 phosphate buffer solution (PBS) with a glassy carbon electrode (GCE) that was not modified with any composites of nanomaterials. This test serves as a control with which each composite can be compared. When an unmodified glassy carbon electrode was used to detect fenthion, the voltammogram did not show any diagnostic peaks. There was also a concern about the reproducibility of the results since only two out of the six tests run produced similar looking voltammograms (see Figure 7).

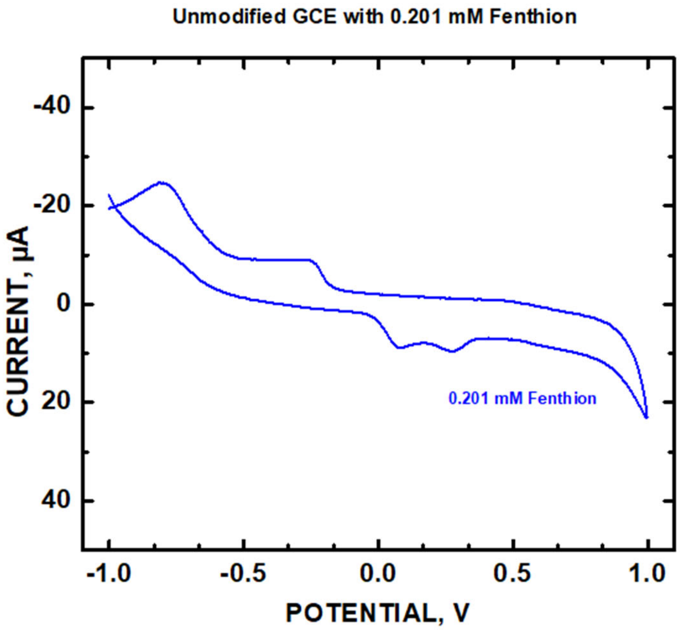


Figure 7: Control experiment using an unmodified glassy carbon electrode to detect 0.201 mM fenthion in pH 7 phosphate buffer solution

Next the GCE modified with the composite of carboxylic acid functionalized multiwalled nanotubes was tested with 0.0201 mM fenthion. Like the first test, when the GCE was modified with a composite of COOH-MWNTs, there were no diagnostic peaks. The reproducibility was better than the unmodified GCE, but this method would not be able to reliably detect fenthion (see Figure 8).

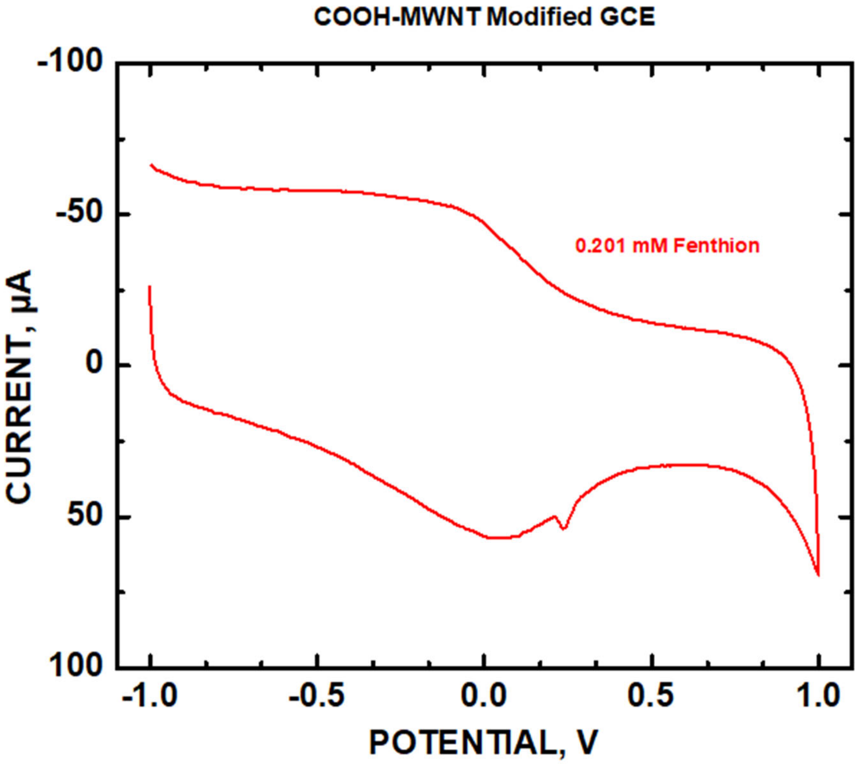


Figure 8: Control experiment using a glassy carbon electrode modified with COOH-MWNTs to detect 0.201 mM fenthion in pH 7 phosphate buffer solution

The next composite that was used to modify the GCE was composed of cupric oxide nanoparticles. When the GCE was modified with CuO nanoparticles, there were two sharp diagnostic peaks. However, six tests were run, and only two of these tests resulted in similar voltammograms. This caused concerns about the reproducibility of the results (see Figure 9).

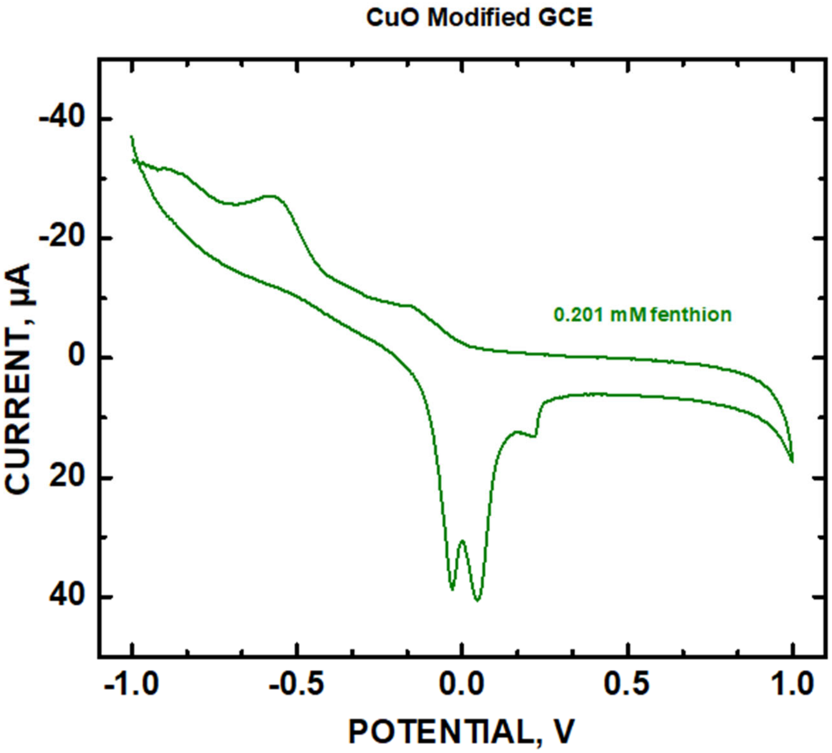


Figure 9: Control experiment using a glassy carbon electrode modified with CuO nanoparticles to detect 0.201 mM fenthion in pH 7 phosphate buffer solution

When the GCE was modified with a composite of CuO/COOH-MWNTs, the signals were much larger than those of the three control tests run. The CV was run three times, and all three voltammograms presented clear and diagnostic peaks with similar measurements of both current and potential. Because of the promising results of this composite, the same electrode was run with pure PBS to ensure the signals were from the fenthion alone. The results of this test showed that the PBS did not interfere with the signal from the fenthion (see Figure 10).

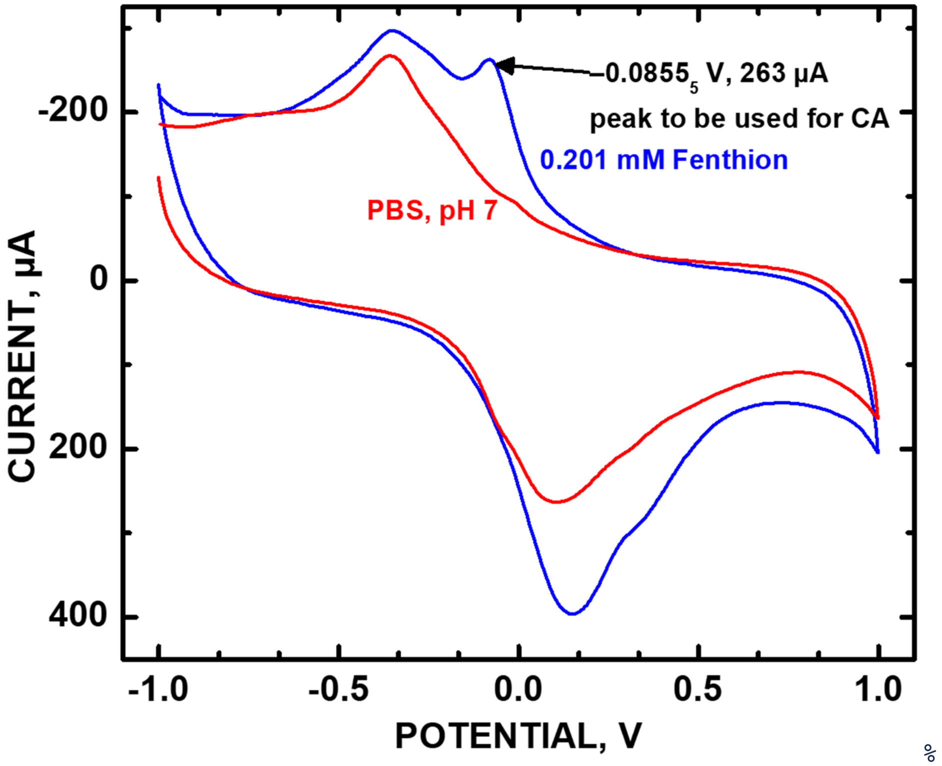


Figure 10: Glassy carbon electrode modified with composite of COOH-MWNTs and CuO nanoparticles to detect 0.201 mM fenthion in pH 7 phosphate buffer solution

The reduction potential was taken from the voltammogram, and this value was used as the constant potential for a chronoamperometry experiment. The chronoamperometry experiment did not yield useful results and is presented in the Appendix (see Figure 1 of Appendix). Composites of elemental copper nanoparticles and copper nanoparticles tethered to carboxylic acid functionalized multiwalled carbon nanotubes were tested as alternatives, and chitosan was tested as an alternative capping agent. None of these methods produced favorable results, and the data from these experiments is presented in the Appendix (see Figure 2 in Appendix).

Since the composite of CuO/COOH-MWNTs produced the most reliable results, the composite was used to test a series of concentrations of fenthion in pH 7 phosphate buffer solution ranging from 0.01 - 0.10 mM fenthion. The results for 10 concentration intervals in this range are shown below in Figure 11, and Figure 12 shows a zoomed in picture of the reduction peak from each concentration.

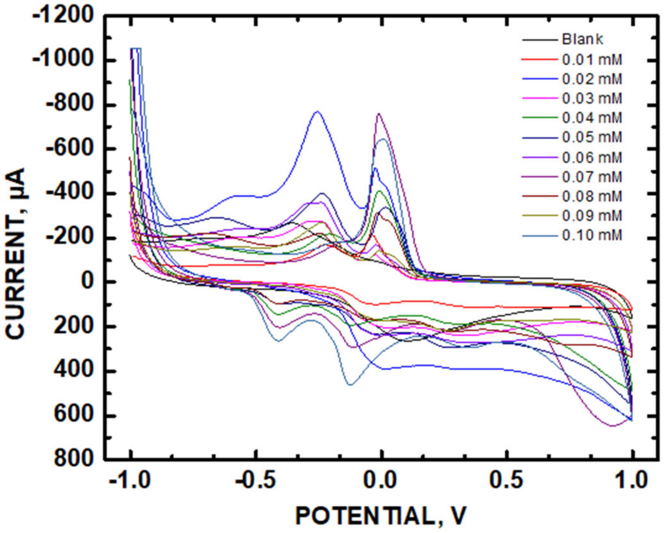


Figure 11: Cyclic voltammogram of fenthion concentrations 0.01 mM - 0.10 mM with GCE modified with CuO/COOH-MWNT

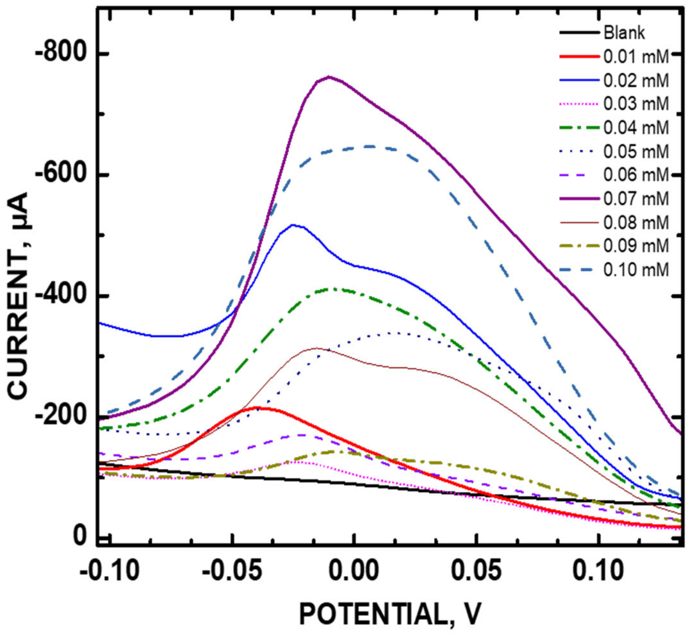


Figure 12: Reduction peak of voltammogram from Figure 11 zoomed in

Figures 11 and 12 show that each concentration presented a voltammogram with a similar shape and reduction peak. Based on these results, fenthion can be qualitatively detected easily within this range. Figure 12 shows that the reduction peaks of the ten concentrations do not follow the expected linear relationship between concentration and peak intensity. The most likely explanation for this is that atmospheric oxygen has an effect on the system (perhaps the CuO nanoparticles, fenthion itself, or both). Another explanation is that the instrument may require some level of calibration before it presents the most accurate readings.

B. Scanning Electron Microscopy

Figure 13 below presents an image of one of ten sites of the cupric oxide nanoparticles that were drop casted onto the GCE. These particles show up as small white "pebble" shapes uniformly throughout the surface of the electrode. Figure 14 shows a histogram of the measured particle diameters that were collected through the software ImageJ. The Gaussian curve shows that the average diameter of the CuNP's was 239 nm ± 2.

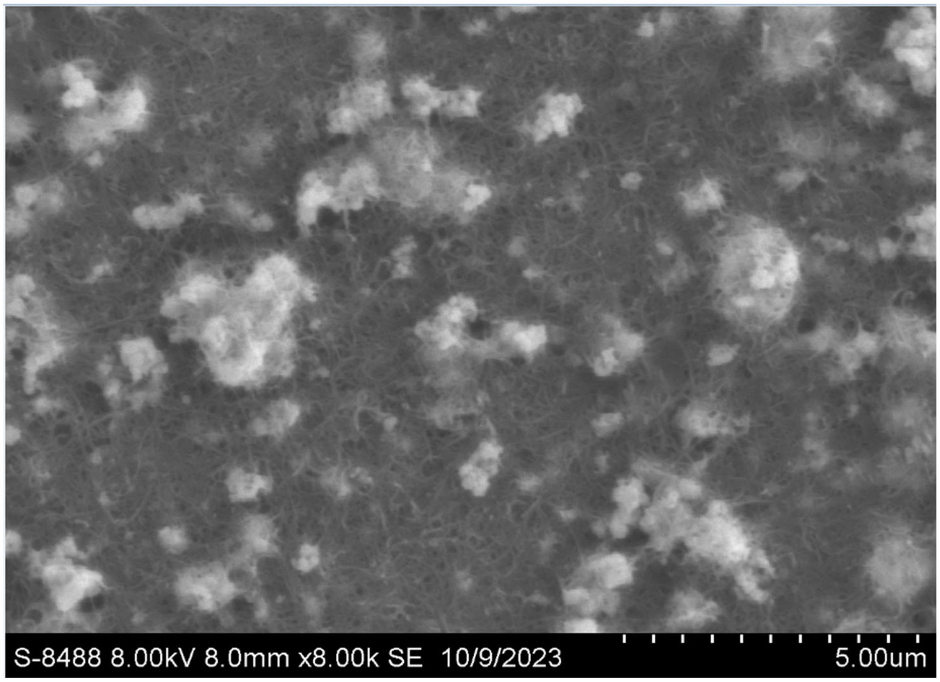


Figure 13: SEM image of CuO/COOH-MWNT composite drop casted on GCE

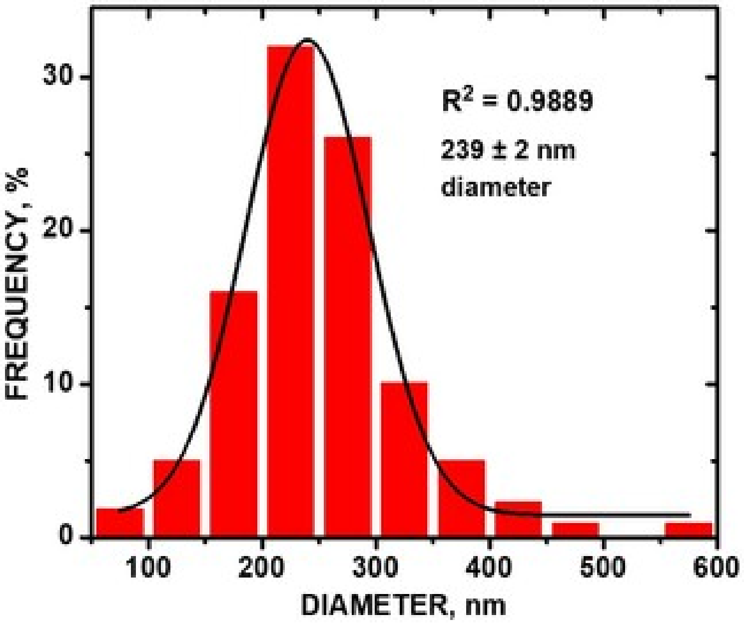
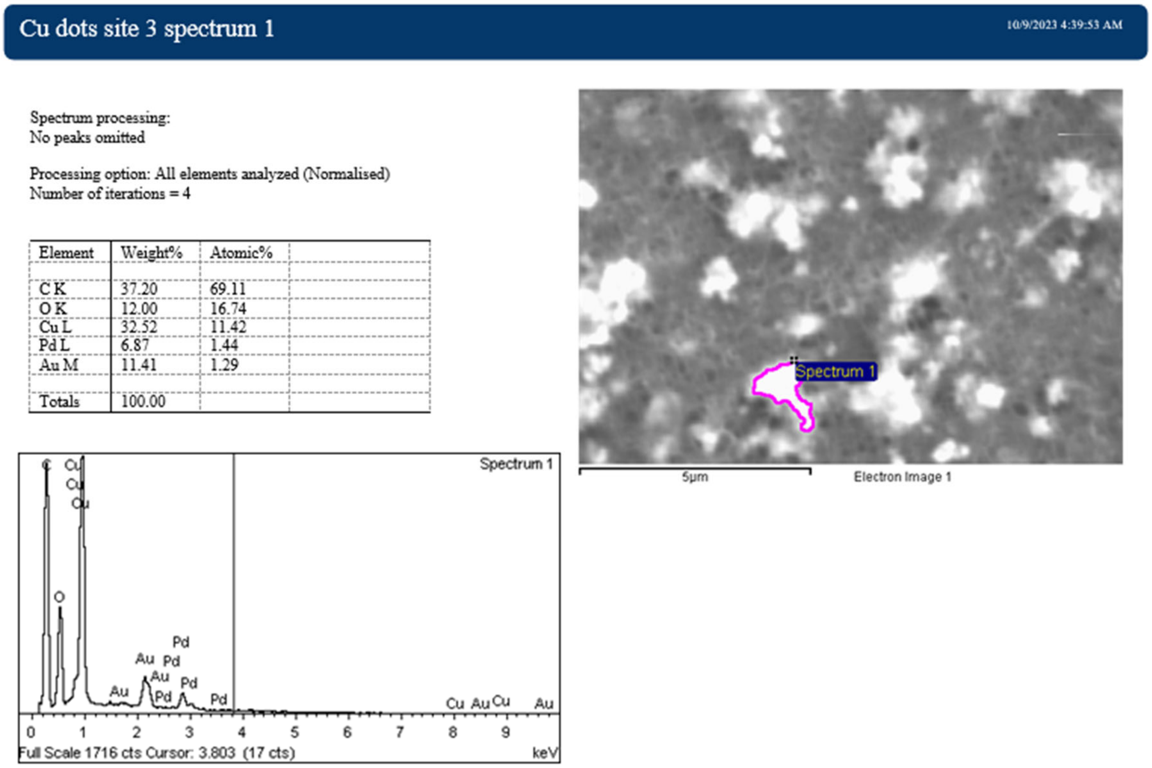


Figure 14: Histogram of particle diameters from SEM data

C. Energy Dispersive X-ray Spectroscopy

Figures 15 and 16 below show the EDX data collected from one site on the surface of the GCE modified with CuO/COOH-MWNT. Figure 15 shows the atomic percentage for a cupric oxide nanoparticle as the ratio for Cu and O is 11.42:16.74 (consistent with the atomic percentage of cupric oxide. Figure 16 shows the atomic percentage for the functionalized carbon nanotubes as the atomic percentage for carbon is 93.53%. The 5.03% oxygen from Figure 16 is from the oxygen on the functionalized groups.



Cu dots site 3 spectrum 1

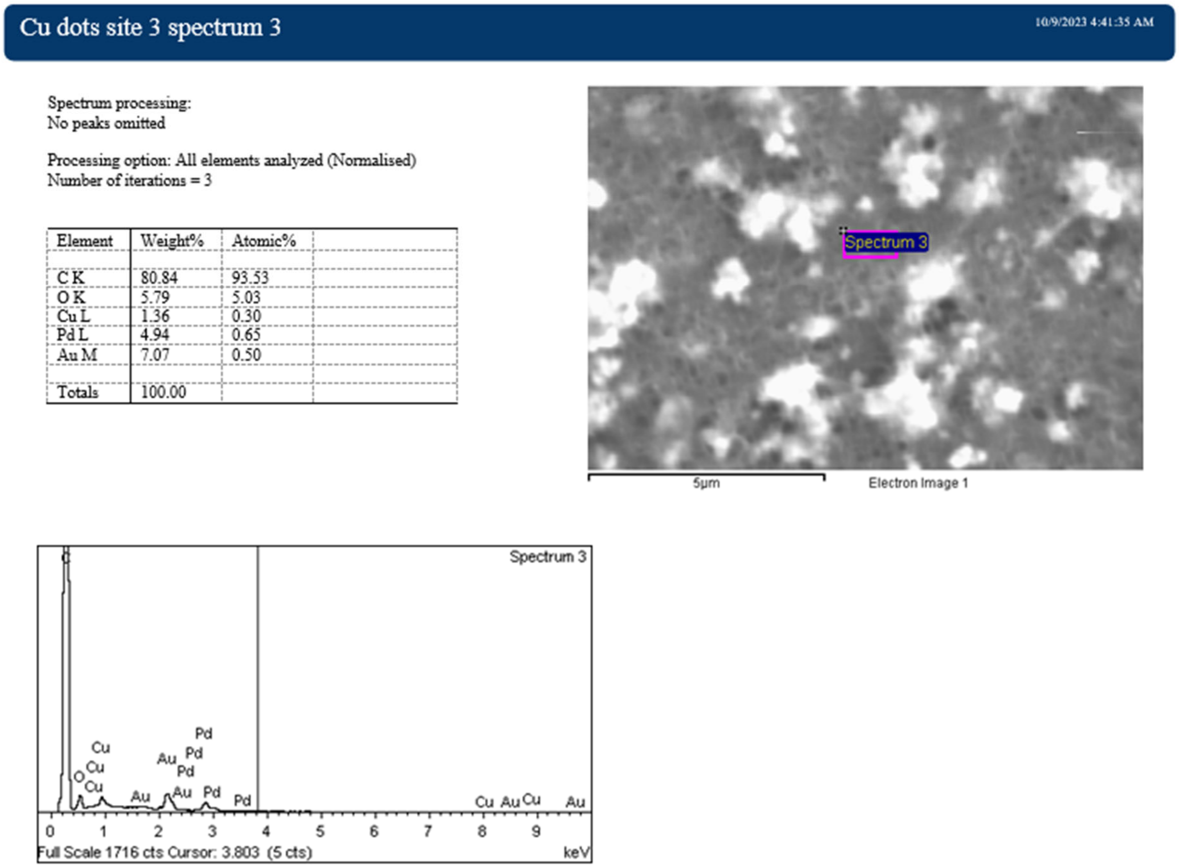
10/9/2023 4:39:53 AM

Spectrum processing:
No peaks omitted

Processing option: All elements analyzed (Normalised)
Number of iterations = 4

Element	Weight%	Atomic%
C K	37.20	69.11
O K	12.00	16.74
Cu L	32.52	11.42
Pd L	6.87	1.44
Au M	11.41	1.29
Totals	100.00	

Figure 15: EDX spectrum 1 from CuO nanoparticle site 3 on GCE



Cu dots site 3 spectrum 3

10/9/2023 4:41:35 AM

Spectrum processing:
No peaks omitted

Processing option: All elements analyzed (Normalised)
Number of iterations = 3

Element	Weight%	Atomic%	
C K	80.84	93.53	
O K	5.79	5.03	
Cu L	1.36	0.30	
Pd L	4.94	0.65	
Au M	7.07	0.50	
Totals	100.00		

Figure 16: EDX spectrum 3 from COOH-MWNT site 3 on GCE

D. Raman Spectroscopy

Figures 17 and 18 below show the data from Raman spectroscopy from samples of COOH-MWNT and CuO/COOH-MWNT. The D-band on both figures corresponds to the sp^3 hybridized carbon atoms which are related to the defects in the graphene sheet. The G-band corresponds to the sp^2 carbons that make up the graphene sheet. The observed trend in the literature is that D-bands with larger peak areas (samples with more defects) produce peaks with more intensity.[17] As Figures 17 and 18 show, the sample with the addition of CuO has a smaller peak area even though its intensity should have been greater. This may be due to the orientation of the CuO nanoparticles underneath the carbon nanotubes.

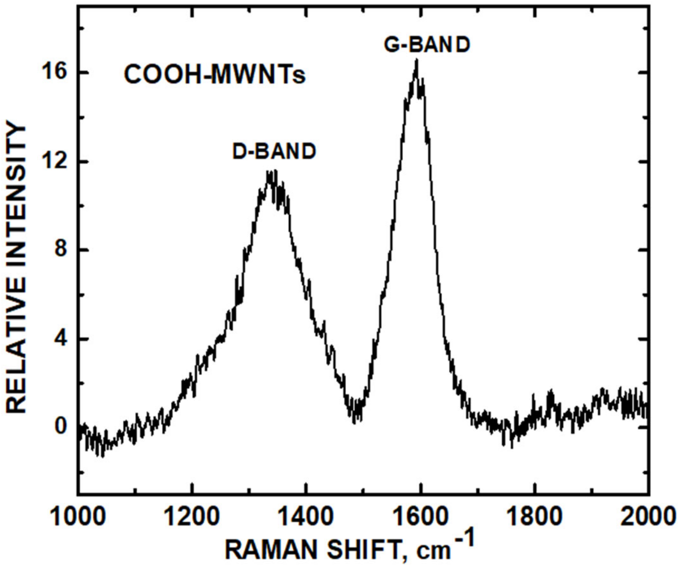


Figure 17: Raman data for COOH-MWNTs

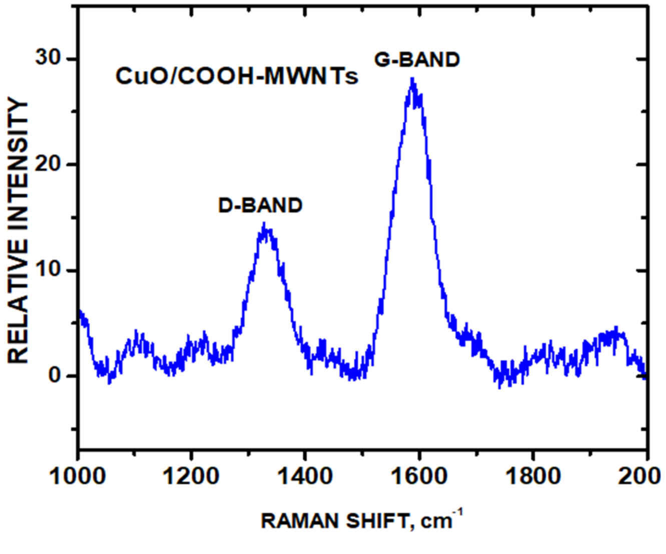


Figure 18: Raman data for CuO/COOH-MWNTs

CHAPTER IV: CONCLUSIONS

The results of cyclic voltammetry concluded that fenthion can be rapidly detected qualitatively at trace amounts by electrochemical methods. The composite that was the most reliable, efficient, and sensitive was the composite of cupric oxide nanoparticles tethered to carboxylic acid functionalized multiwalled carbon nanotubes. The most successful capping agent to secure the composite to the glassy carbon electrode was Nafion™. The lowest concentration of fenthion that was detected in this experiment was 0.01 mM fenthion; however, the results indicate that the technique would likely be able to detect fenthion at even lower concentrations. Determining the limit of detection would be the next step for this research. Additional future directions would include testing potential interferents that may be present in biological and environmental samples with fenthion to determine the selectivity.

While the results of this experiment showed that a composite made of elemental copper nanoparticles degraded quickly and was not a sustainable option for electrochemistry, the composite did show potential at first before the nanoparticles degraded. Further attempts to preserve the copper nanoparticles for use would be promising to increase the efficacy of this technique even more.

The most significant future goal of this project would be to develop a hand-held device with an electrode that could reliably detect trace amounts of fenthion out on the field for forensic and environmental efforts. This would replace the presumptive point of care diagnostic tests with a selective and sensitive analytical technique.

There is a strong need for continued research into the electrochemical detection of fenthion for the sake of the countries harmed by its abundant presence. For forensic analysis, the detection method should be non-destructive because evidentiary samples are often small and protected from destruction by law. For public health applications, hospitals should have options for analysis that are cost effective, rapid, and easy to use.

CHAPTER V: REFERENCES

1. Lee, J.; Kim, J.-H. Simultaneous Analysis of Fenthion and Its Five Metabolites in Produce Using Ultra-High Performance Liquid Chromatography-Tandem Mass Spectrometry. *Molecules* **2020**, *25* (8), 1938. https://doi.org/10.3390/molecules25081938.

2. PubChem. Fenthion https://pubchem.ncbi.nlm.nih.gov/compound/Fenthion (accessed 2022 -09 -18).

3. Tsatsakis, A. M.; Bertsias, G. K.; Liakou, V.; Mammas, I. N.; Stiakakis, I.; Tzanakakis, G. N. Severe Fenthion Intoxications due to Ingestion and Inhalation with Survival Outcome. *Human & Experimental Toxicology* **2002**, 21 (1), 49–54. https://doi.org/10.1191/0960327102ht232cr.

4. Karunarathne, A.; Gunnell, D.; Konradsen, F.; Eddleston, M. How Many Premature Deaths from Pesticide Suicide Have Occurred since the Agricultural Green Revolution? *Clinical Toxicology* **2019**, 58 (4), 227–232. https://doi.org/10.1080/15563650.2019.1662433.

5. Robb, E. L.; Baker, M. B. Organophosphate Toxicity. *Stat Pearls* **2022**. https://www.ncbi.nlm.nih.gov/books/NBK470430/#:~:text=An%20estimated%203%20million%20or (accessed 2022 -09 -18).

6. Chang, C.-H.; Yu, C.-J.; Du, J.-C.; Chiou, H.-C.; Chen, H.-C.; Yang, W.; Chung, M.-Y.; Chen, Y.-S.; Hwang, B.; Mao, I-Fang.; Chen, M.-L. The Interactions among Organophosphate Pesticide Exposure, Oxidative Stress, and Genetic Polymorphisms of Dopamine Receptor D4 Increase the Risk of Attention Deficit/Hyperactivity Disorder in

Children. *Environmental Research* **2018**, 160, 339–346. https://doi.org/10.1016/j.envres.2017.10.011.

7. Al-Hetlani, E.; Amin, M. O.; Madkour, M.; D'Cruz, B. Forensic Determination of Pesticides in Human Serum Using Metal Ferrites Nanoparticles and SALDI-MS. *Talanta* **2021**, 221, 121556. https://doi.org/10.1016/j.talanta.2020.121556.

8. Elgrishi, N.; Rountree, K. J.; McCarthy, B. D.; Rountree, E. S.; Eisenhart, T. T.; Dempsey, J. L. A Practical Beginner's Guide to Cyclic Voltammetry. *Journal of Chemical Education* **2017**, 95 (2), 197–206. https://doi.org/10.1021/acs.jchemed.7b00361.

9. Quiroga, A. Cyclic Voltammetry. *LibreTexts Chemistry* **2020**. https://chem.libretexts.org/Bookshelves/Analytical_Chemistry/Supplemental_Modules_(Analytical_Chemistry)/Instrumental_Analysis/Cyclic_Voltammetry.

10. Das, Shawtik C. Electrochemical detection of uric acid using a refluxed zinc oxide carbon nanotube composite. Master of Science Thesis, *Middle Tennessee State University* **2021**.

11. Pandey, R.R; Guo, Y.; Gao, Y.; Charles C. Chusuei, C.C. A Prussian Blue ZnO Carbon Nanotube Composite for Chronoamperometrically Assaying H2O2 in BT20 and 4T1 Breast Cancer Cells. *American Chemical Society* **2019**. https://doi.org/10.1021/acs.analchem.9b01636.

12. Adeyeye, O. A Prussian Blue/Cobalt Oxide/Carbon Nanotube Composite for Homovanillic Acid Detection. Master of Science Thesis, *Middle Tennessee State University* **2021**.

13. Soomro, R. A.; Hallama, K. R.; Ibupotob, Z. H.; Aneela Tahirab, A.; Sherazic, S. T. H.; Sirajjuddinc; Memonc, S. S.; Willander, M. Amino acid assisted growth of CuO nanostructures and their potential application in electrochemical sensing of organophosphate pesticide. *Electrochimica Acta* **2016**, 190. http://dx.doi.org/10.1016/j.electacta.2015.12.165.

14. Prakash, S.; Mustain, W.E.; Kohl, P.A. Electrolytes for Long-Life, Ultra Low-Power Direct Methanol Fuel Cells. *Academic Press* **2009**, pg. 1-50, https://doi.org/10.1016/B978-0-12-374713-6.00001-9.

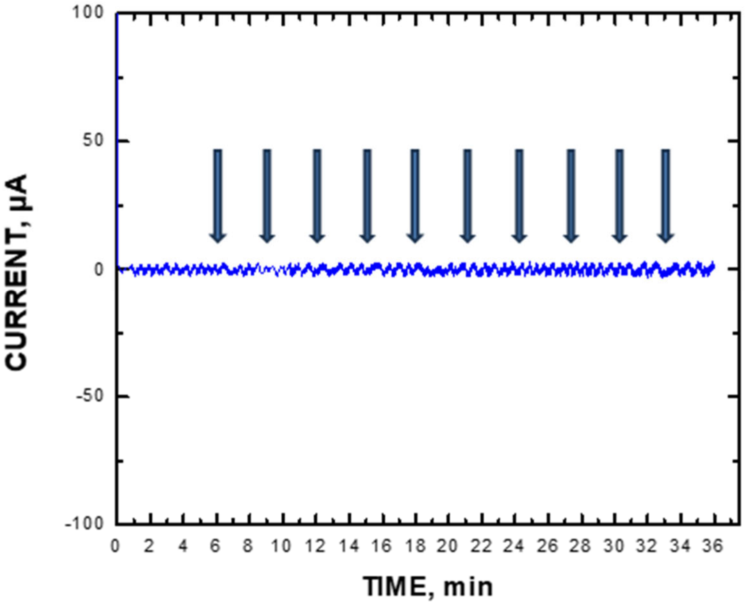
15. Cinteza LO, Scomoroscenco C, Voicu SN, Nistor CL, Nitu SG, Trica B, Jecu ML, Petcu C. Chitosan-Stabilized Ag Nanoparticles with Superior Biocompatibility and Their Synergistic Antibacterial Effect in Mixtures with Essential Oils. Nanomaterials (Basel). *National Center for Biotechnology Information* **2018** Oct 13;8(10):826. doi: 10.3390/nano8100826.

16. França, R. F.; Lima, T. D. S.; Simões, F. R.; Codognoto, L. Electroanalytical Determination of Fenthion in Passiflora Alata Tincture Samples. Orbital: *The Electronic Journal of Chemistry* **2018**, 10 (2). https://doi.org/10.17807/orbital.v10i2.1077.

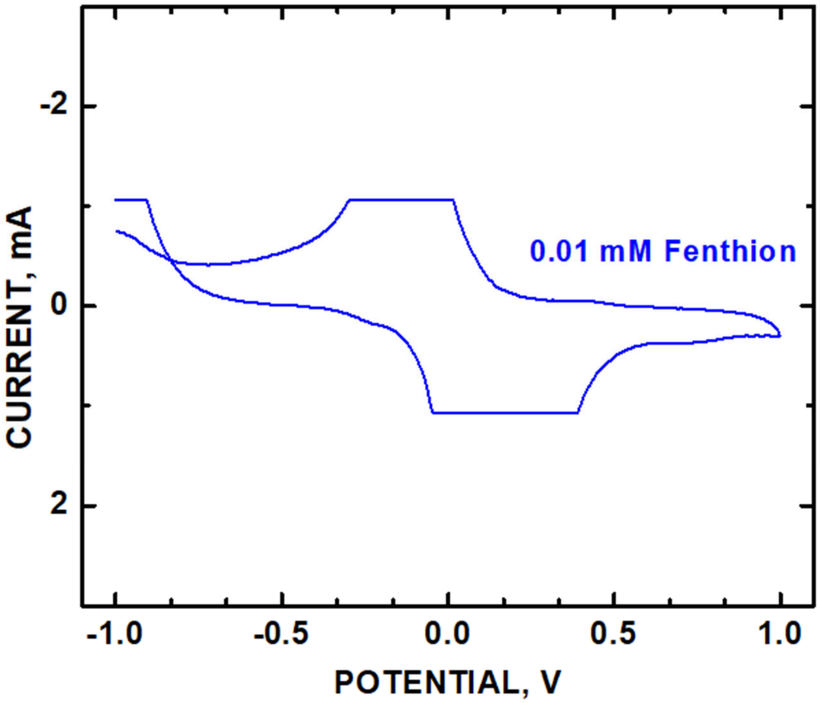
17. Hull, R.V.; Liang Li, L.; Xing, Y.; Chusuei, C.C. Pt Nanoparticle Binding on Functionalized Multiwalled CarbonNanotubes. *American Chemical Society* **2006**, 18, 1780-1788. https://doi.org/10.1021/cm0518978.

CHAPTER VI: APPENDIX

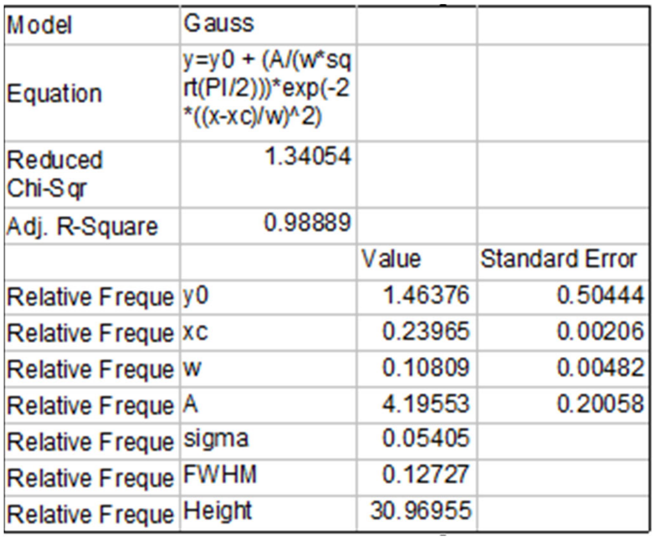
1. Results of chronoamperometry with fenthion concentrations from 0.01 mM - 0.10 mM with CuO/COOH-MWNT composite



2. CV of 0.01 mM fenthion in pH 7 PBS with GCE modified with CuNPs/COOH-MWNTs

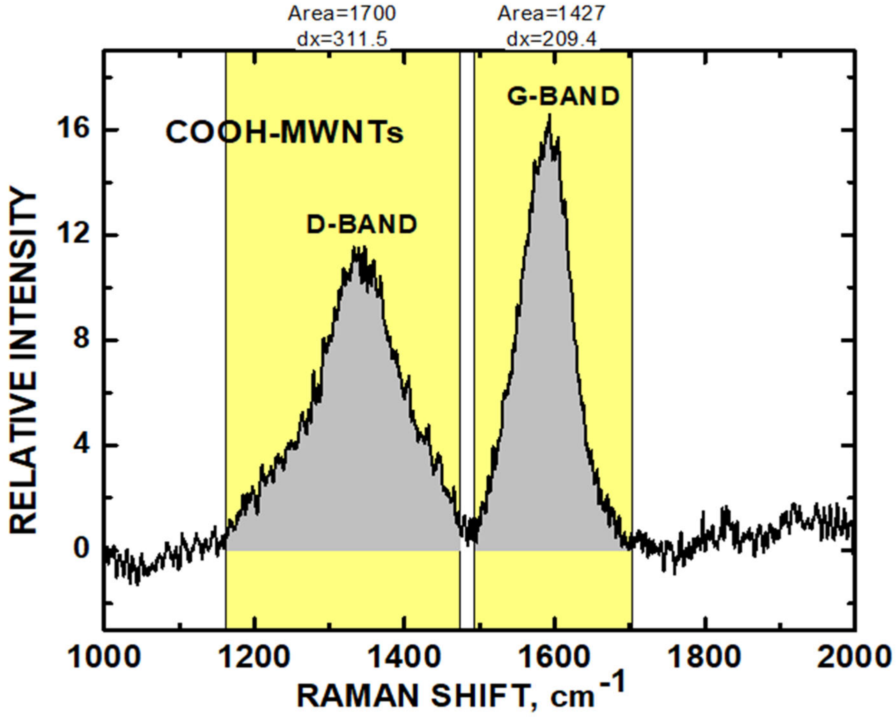


3. Information about Gaussian curve for SEM data histogram

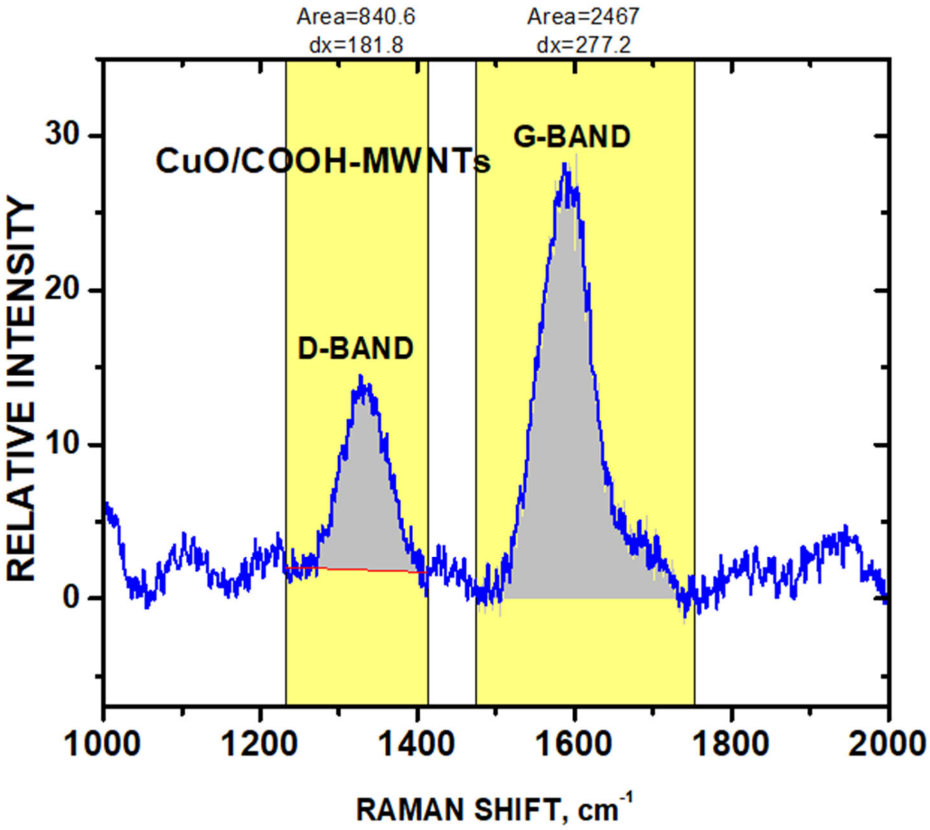


Model	Gauss		
Equation	y=y0 + (A/(w*sqrt(PI/2)))*exp(-2*((x-xc)/w)^2)		
Reduced Chi-Sqr	1.34054		
Adj. R-Square	0.98889		
		Value	Standard Error
Relative Freque	y0	1.46376	0.50444
Relative Freque	xc	0.23965	0.00206
Relative Freque	w	0.10809	0.00482
Relative Freque	A	4.19553	0.20058
Relative Freque	sigma	0.05405	
Relative Freque	FWHM	0.12727	
Relative Freque	Height	30.96955	

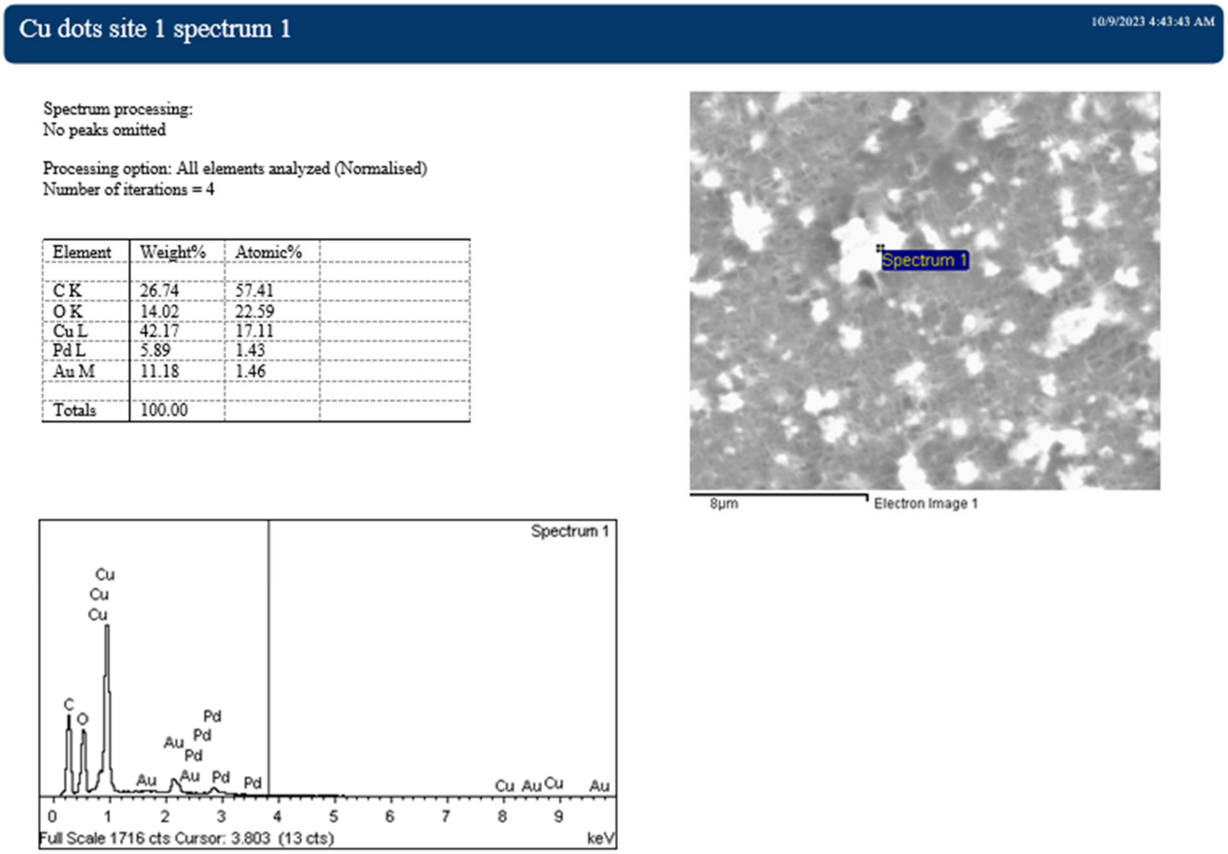
4. Raman data for COOH-MWNTs with peak area



5. Raman data for CuO/COOH-MWNTs with peak area



6. EDX spectrum 1 from CuO nanoparticle site 1 on GCE



Cu dots site 1 spectrum 1

10/9/2023 4:43:43 AM

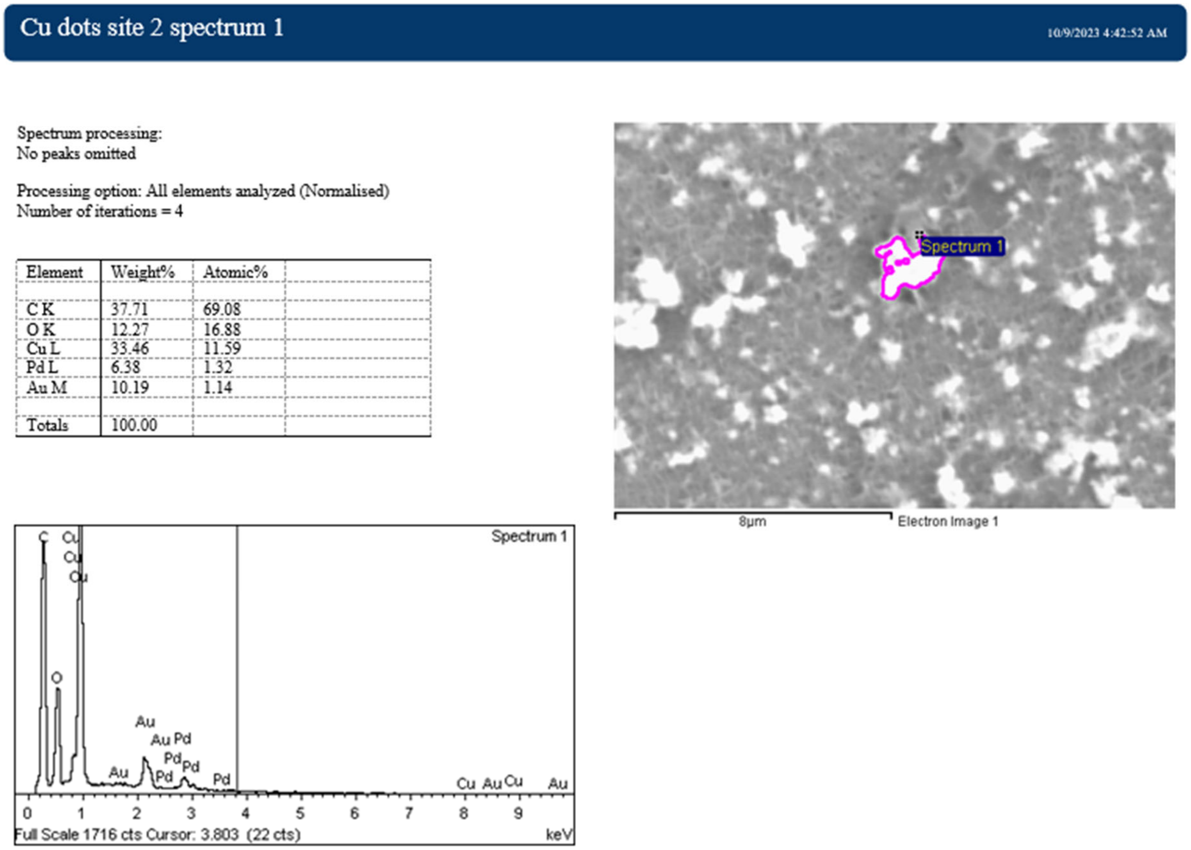
Spectrum processing:
No peaks omitted

Processing option: All elements analyzed (Normalised)
Number of iterations = 4

Element	Weight%	Atomic%	
C K	26.74	57.41	
O K	14.02	22.59	
Cu L	42.17	17.11	
Pd L	5.89	1.43	
Au M	11.18	1.46	
Totals	100.00		

Full Scale 1716 cts Cursor: 3.803 (13 cts)

7. EDX spectrum 1 from CuO nanoparticle site 2 on GCE



Cu dots site 2 spectrum 1

10/9/2023 4:42:52 AM

Spectrum processing:
No peaks omitted

Processing option: All elements analyzed (Normalised)
Number of iterations = 4

Element	Weight%	Atomic%
C K	37.71	69.08
O K	12.27	16.88
Cu L	33.46	11.59
Pd L	6.38	1.32
Au M	10.19	1.14
Totals	100.00	

8. Permission to use Figure 3

12/5/23, 10:54 AM Rightslink® by Copyright Clearance Center

RightsLink

A Prussian Blue ZnO Carbon Nanotube Composite for Chronoamperometrically Assaying H2O2 in BT20 and 4T1 Breast Cancer Cells

ACS Publications
Most Trusted. Most Cited. Most Read.

Author: Raja Ram Pandey, Yuhang Guo, Ying Gao, et al
Publication: Analytical Chemistry
Publisher: American Chemical Society
Date: Aug 1, 2019

Copyright © 2019, American Chemical Society

PERMISSION/LICENSE IS GRANTED FOR YOUR ORDER AT NO CHARGE

This type of permission/license, instead of the standard Terms and Conditions, is sent to you because no fee is being charged for your order. Please note the following:

- Permission is granted for your request in both print and electronic formats, and translations.
- If figures and/or tables were requested, they may be adapted or used in part.
- Please print this page for your records and send a copy of it to your publisher/graduate school.
- Appropriate credit for the requested material should be given as follows: "Reprinted (adapted) with permission from {COMPLETE REFERENCE CITATION}. Copyright {YEAR} American Chemical Society." Insert appropriate information in place of the capitalized words.
- One-time permission is granted only for the use specified in your RightsLink request. No additional uses are granted (such as derivative works or other editions). For any uses, please submit a new request.

If credit is given to another source for the material you requested from RightsLink, permission must be obtained from that source.

© 2023 Copyright - All Rights Reserved | Copyright Clearance Center, Inc. | Privacy statement | Data Security and Privacy | For California Residents | Terms and ConditionsComments? We would like to hear from you. E-mail us at customercare@copyright.com