# ELECTROCHEMICAL CHARACTERIZATION OF FENTANYL FOR FORENSIC ANALYSIS

by

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*To my mom and grandma, who have always believed in me*

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# LIST OF ABBREVIATIONS

CE: Counter electrode

CNO: Carbon nano-onion

CSWV: Cyclic square wave voltammetry

CV: Cyclic voltammetry

DPV: Differential pulse voltammetry

GCE: Glassy carbon electrode

LOD: Limit of detection

PBS: Phosphate buffer saline solution

RE: Reference Electrode

RTIL: Room temperature ionic liquid

SE: Supporting electrolyte

SPE: Screen printed electrode

SWV: Square wave voltammetry

SWAdSV: Square wave adsorptive stripping voltammetry

WE: Working electrode

# ABSTRACT

The use and abuse of fentanyl has risen drastically over the last several decades. The abuse of this substance has created a hazardous situation for law enforcement and first responders because they could arrive at locations and not necessarily know that they will encounter fentanyl or a fentanyl analog. Fentanyl analogs are substances that have a similar structure to fentanyl, and while the analogs may have additional or altered groups on the molecule, the backbone structure remains similar. This work focus on the electrochemical characterization of fentanyl as a stepping stone for the detection of both fentanyl and later fentanyl analogs by electrochemistry. The metabolic reaction of fentanyl is an N-dealkylation to norfentanyl, occurring in the liver, and can be mimicked by electrochemistry through the irreversible oxidation of fentanyl. This electrochemical reaction is hypothesized to generate electroactive metabolites in solution. The combination of the visualization of both the irreversible oxidation with the development of the additional metabolic electrochemical peaks would constitute a unique electrochemical signature for fentanyl and fentanyl analogs towards a universal rapid screening assay.

The electrochemical behavior of fentanyl first needs to be characterized in depth which was done using multiple electrochemical techniques. Cyclic voltammetry was used first (Chapter 3), and permitted the optimization of different conditions such as the choice of supporting electrolyte, adjustments to decrease the background current, and the potential range. Additional electrochemical techniques, square wave voltammetry and differential pulse voltammetry, were then explored for the analysis of fentanyl and the metabolite peaks (Chapter 4). To work towards a more portable system, screen printed electrodes were used (Chapter 5). The observation of the metabolic peaks remained challenging, and different methods were attempted to achieve it (Chapter 6). The quantification of fentanyl was successfully demonstrated using the different electrochemical systems proposed in this work (Chapter 7). The electrochemical characterization of fentanyl and the optimization of multiple experimental parameters were the first step in developing a universal, rapid, electrochemical sensing method for the detection of fentanyl and fentanyl analogs.

# CHAPTER 1. INTRODUCTION

Fentanyl is an opioid that is heavily abused and highly addictive. In the last couple of years, the electrochemical analysis of fentanyl has become an active area of research. The emergence of these new assays are beneficial for forensic analysis, as the electrochemical detection of fentanyl could be used as a preliminary or additional screening for fentanyl samples, and possibly fentanyl analogs samples. The goal of the present work is to electrochemically characterize fentanyl, and determine the optimal parameters and methods for its analysis using unmodified commercial electrodes. Fentanyl can undergo an irreversible oxidation producing metabolites. These metabolites are believed to be electroactive as well. The oxidation of fentanyl corresponds to a N-dealkylation and mimics the enzymatic reaction occurring in the body. The concomitant presence of the irreversible oxidation peak for fentanyl and the redox peaks for the metabolites would established an electrochemical signature positively identifying fentanyl. Numerous fentanyl analogs exist and new ones continue to be synthesized. Chapter 2 introduces fentanyl and fentanyl analogs such as their history, metabolic pathways, and the current state forensic analysis of fentanyl both with standard methods, and electrochemically specifically. Although different substituents are present in various region of the fentanyl molecule, all analogs have the same backbone structure of fentanyl. The long-term goal of our project relies on the hypothesis that since numerous fentanyl analogs undergo a similar metabolic mechanism, that their oxidation peaks, as well as the resulting metabolite peaks would be similarly observed as they are for fentanyl. This combination of redox peak would form a unique electrochemical signature for fentanyl and fentanyl analogs that could be used in preliminary examinations.

To achieve this long-term goal, the electrochemical behavior of fentanyl first needs to be characterized and optimized. The optimization of an electrochemical method involves several elements including type of electrode, the choice of supporting electrolyte, the concentration of that electrolyte, the volume of solution, the potential range, the scan rate, the effect of the pH, and potential treatments to improve the signal-to-noise ratio, which in electrochemistry corresponds to an increase of the faradaic current and decrease of the capacitive current. Chapter 3 focuses on the main electrochemical parameters, such as supporting electrolyte and electrode, and potential range for cyclic voltammetry (CV). Chapter 4 describes additional electrochemical techniques, such as

square wave voltammetry (SWV) and differential pulse voltammetry (DPV), which were optimized and used to analyze fentanyl. Chapter 5 describes both an alternative electrochemical set up that was used to control the volume of solution, as well as the optimization of the use of SPEs for fentanyl. Chapter 6 describes the challenges observed when trying to identify, and improve the metabolite peaks observed for fentanyl. Chapter 7 describes the quantification of fentanyl using CV, SWV, and DPV. Chapter 8 details the future work aspects of this project including a full pH study, the analysis of fentanyl analogs, as well as further investigation of the metabolite peak. This project was done without modifying the electrode surface to analyze the irreversible fentanyl oxidation peak, as well as attempting to study the metabolite peaks with the objective to ensure an easy dissemination and replication of the assay by potentially any laboratory equipped with minimum electrochemical equipment.

# CHAPTER 2. OVERVIEW OF FENTANYL, FENTANYL ANALOGS, AND THEIR ANALYSES

## 2.1 Background of Fentanyl and Fentanyl Analogs

### 2.1.1 Fentanyl

Fentanyl (Figure 1) is a highly potent and addictive opioid that is heavily abused, and has a potency that is 50-100 times greater than morphine.[1] It has several effects on the human body, such as respiratory depression, analgesia, anxiolysis, euphoria, drowsiness, and miosis.[2,3] These side effects are observed because fentanyl is a μ-receptor in the brain, tied to the central nervous system and the peripheral nervous system.[4] Fentanyl itself was originally synthesized in 1960 as a pain treatment drug, by Dr. Paul Janssen and the Janssen company,[3] and was used to treat chronic or acute pain in patients.[5] The FDA originally approved fentanyl in 1968 in the United States, as long as it was mixed with droperidiol, which was another analgesic, to decrease the risk of abuse.[3] The first misuses of the drug was originally reported in the 1980s, and continued to rise into the early 2000s.[6] In the mid-2000s there was a rise of other drugs, especially heroin and cocaine, laced with non-pharmaceutical fentanyl, leading to unintentional overdoses.[6] The respiratory depression and distress, which causes lack of oxygen and hypoxia, are what leads to the lethality of fentanyl.[7] The potency of fentanyl can lead to unintentional overdoses by users of the drug,[8] as well as first responders and care takers who may get into contact with the drug without their knowledge.

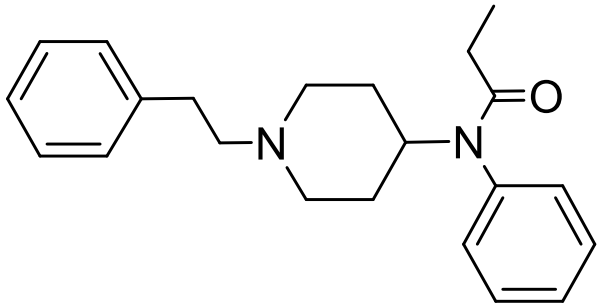



**Figure 1:** Structure of Fentanyl Molecule

Fentanyl is recognized as a Schedule II substance[1,9] which indicates that the drug has a high potential of abuse, but still has a recognized medical use.[10] This drug can also lead to psychological or physical dependence with repeated use.[1] According to the 2020 DEA National Drug Threat Assessment, fentanyl still has high availability across the United States, with 100,378 reports from forensic laboratories in 2019, which was a 12% increase from those reported in 2018.[9] They also noted that the majority of cases for fentanyl were reported as single drug entries, at 58%, which means that fentanyl was not mixed with another illicit substance, and that of the mixtures of fentanyl and other substances, 27% of those cases were fentanyl mixed with heroin.[9]

### 2.1.2 Fentanyl Analogs

#### *Types of Fentanyl Analogs*

Fentanyl analogs were designed and synthesized for two reasons after the synthesis of fentanyl. They were developed for medical and veterinary use[6] or developed for illicit purposes.[11] Fentanyl analogs are synthesized by changing one or more substituents linked to the backbone of the fentanyl structure. Some examples of fentanyl analogs developed for medical or veterinary uses are sufentanil, remifentanil, carfentanil (Figure 2A), and alfentanil (Figure 2B).[6] The fentanyl analogs designed for the illicit drug trade are numerous and is constantly growing in number. Some of these analogs include 3-methyl-fentanyl (Figure 2C), alpha-methylfentanyl (Figure 2D), acetyl fentanyl and several others. The potency of these analogs depends on the specific molecule: some are more potent than fentanyl, whereas others are equally or less potent than fentanyl itself (Table 1). Additional to the potency, the fentanyl analogs can also vary in toxicity compared to fentanyl. Carfentanil is currently one of the most potent analogs of fentanyl, at 30 to 100 times the potency of fentanyl itself, while alfentanil is 5 to 10 times less potent than fentanyl.[12] Alpha-methylfentanyl has a similar potency compared to fentanyl, but has a higher toxicity, and 3-methylfentanyl has varying potencies depending on the isomer of the drug.[12] These differing potencies are dangerous, as in white powder form they appear similar with fentanyl. They pose a risk to the health and safety of both the users and first responders who can arrive at locations with a suspicious white substance and not know its identity.

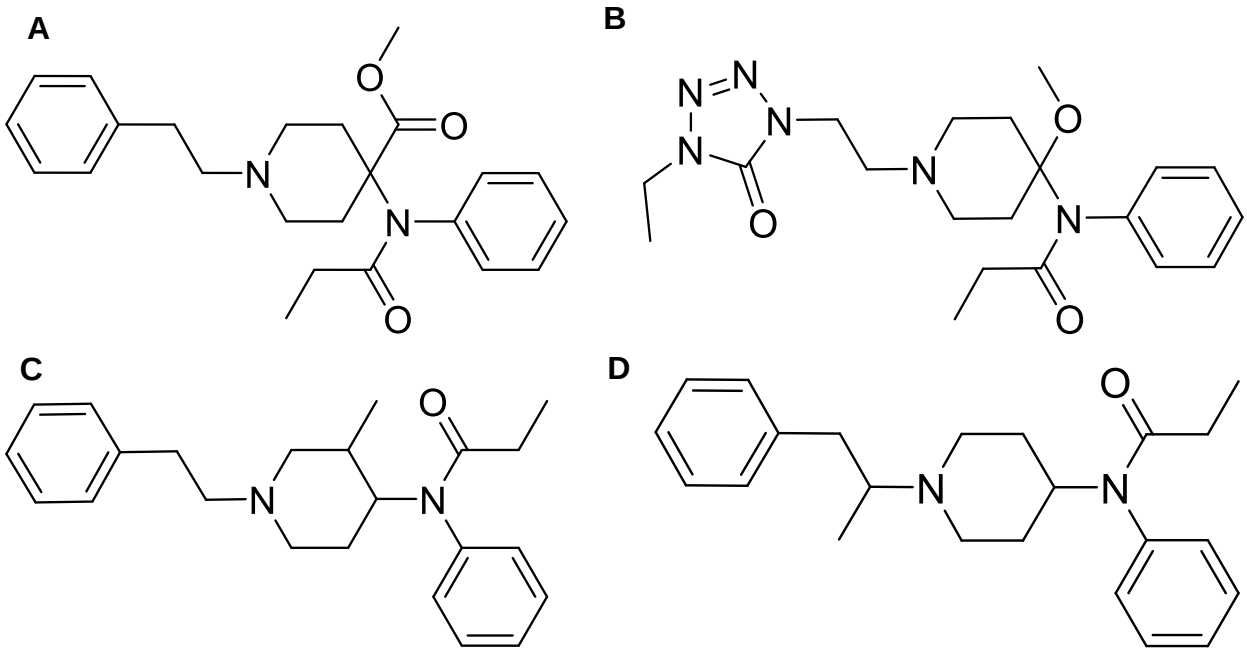



**Figure 2:** Fentanyl analog structures A: Carfentanil, B: Alfentanil, C: 3-methyl fentanyl D: Alpha-methyl fentanyl

**Table 1:** Examples of fentanyl analog potencies

| **Fentanyl Analog** | **Potency Compared to Fentanyl** |
|---|---|
| Alpha methylfentanyl | Same potency[11] |
| Carfentanil | 100 times more potent[11] |
| Butyrfentanyl | 30 times less potent [11] |
| Acetylfentanyl | 20% of potency of fentanyl[11] |
| Furanylfentanyl | 8 times more potent[11] |
| Alfentanil | 5-10 times less potent [11] |
| Acrylofentanyl | 75% of fentanyl potency[12] |
| (+)-cis 3-methylfentanyl | 20 times more potent[12] |
| (-)-cis 3-methylfentanyl | 20% of fentanyl potency[12] |
| (±) trans-3-methylfentanyl | Same potency[12] |

***Scheduling of Fentanyl Analogs***

The scheduling of fentanyl analogs by the Department of Justice depends on the type of fentanyl analogs. The fentanyl analogs that are used for medical and veterinary purposes are

Schedule II substances, like fentanyl itself.[6] The fentanyl analogs that have been developed for more illicit purposes that have been scheduled individually are classified as Schedule I substances.[6,12] This classification means that they are highly addictive, have a high likelihood of abuse, and that they do not have an accepted medical use.[10] Fentanyl analogs that have not been scheduled individually can be subjected to the Controlled Substances Analog Enforcement Act, also known as the Federal Analog Act.[13] The Federal Analog Act states that "A controlled substance analog shall, to the extent intended for human consumption, be treated, for the purposes of any Federal law as a controlled substance in Schedule I."[14] Even though all illicit fentanyl analogs are then considered Schedule I substances, there have been difficulties enforcing this law, due to the burden of proof that the analogs are substantially similar to the original substance, and that they would be marketed for human consumption.[6]

### 2.1.3 Structure of Fentanyl, Fentanyl Analogs, and Metabolites

#### *Structure of Fentanyl and Fentanyl Analogs*

The structure of fentanyl is comprised of multiple functional groups on the molecule. including the piperidine ring, the anilinophenyl ring, the 2-phenyethyl substituent and the carboxamide moiety that is linked to the anilino-nitrogen (Figure 3).[12]

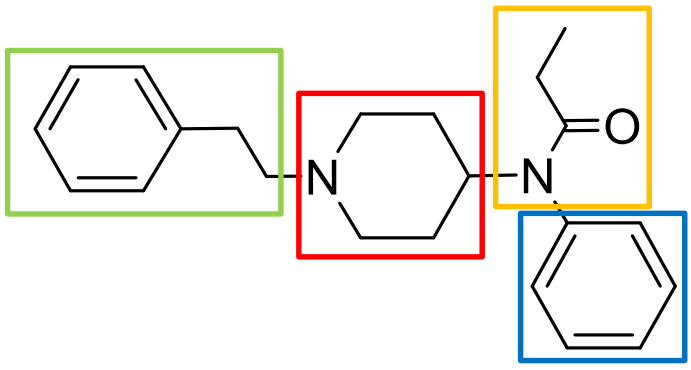



**Figure 3:** Functional groups of fentanyl molecule including the: piperidine ring (red), anilinophenyl ring (blue), 2-phenethyl substituent (green), and the carboxamide moiety linked to anilino-nitrogen (orange)

The analogs of fentanyl have similar structures to fentanyl, with changes typically in the rings or groups along the structure while keeping the backbone fairly intact. The hundreds of variations possible for fentanyl analogs are due to the structure of the fentanyl molecule that lends multiple locations that are chemically easy to structurally modify with other substituents.[12] Some examples of these structural changes can be observed in Figure 4. These similarities between the molecular structure of fentanyl and fentanyl analogs are beneficial to the goal of developing a general electrochemical screening for all fentanyl derivatives.

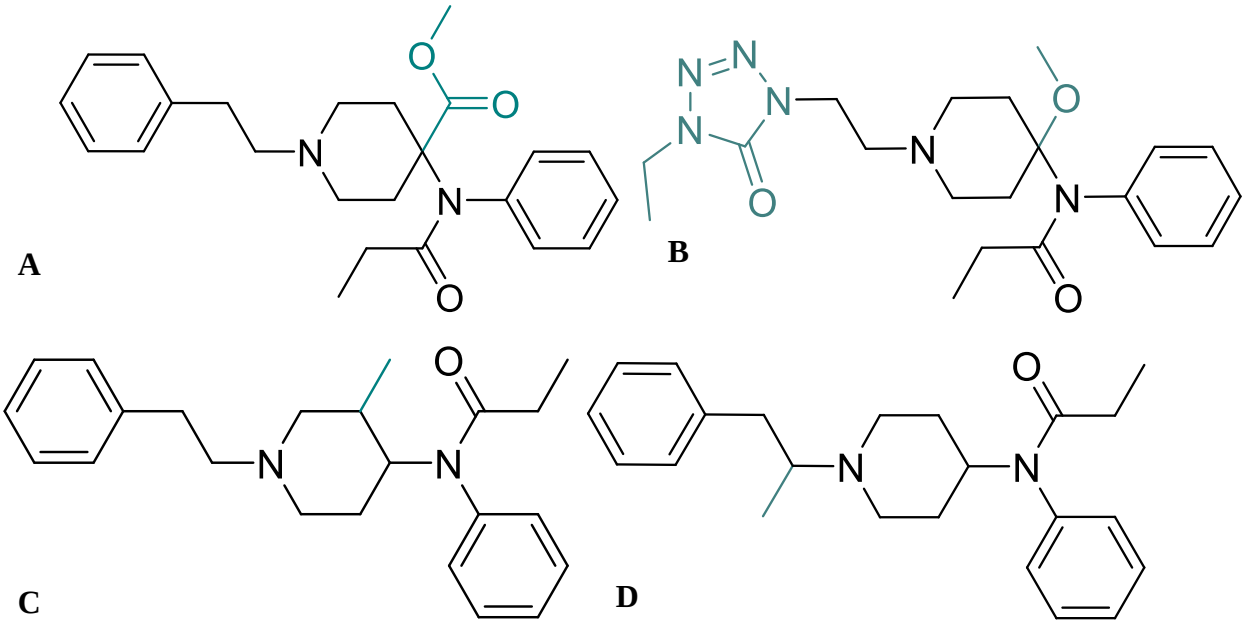
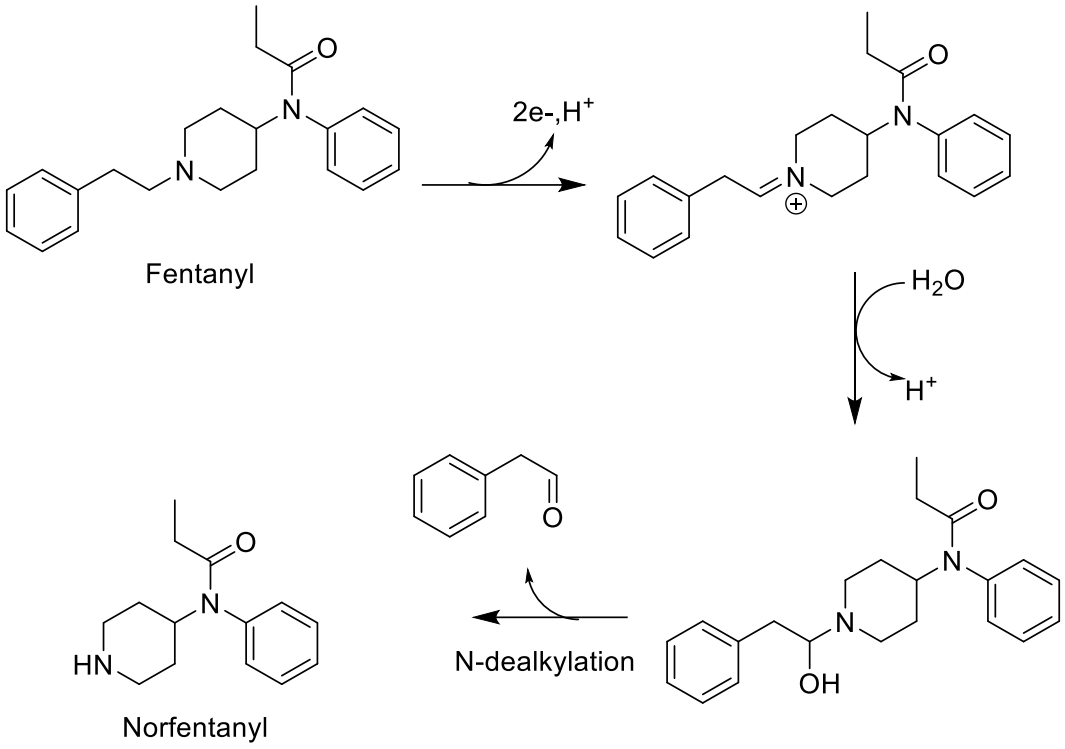



**Figure 4:** Structures of different fentanyl analogs with the varying substituents highlighted in green; A: Carfentanil, B: Alfentanil, C: 3-methyl fentanyl D: Alpha-methyl fentanyl

## 2.2 Metabolic Pathway

The metabolic reaction that occurs in the body to breakdown fentanyl is well known. An enzymatic breakdown of fentanyl by the cytochrome P-450-family occurs in the liver this enzyme family is also responsible for the metabolism of other drugs and carcinogens.[15] The specific enzyme is the CYP3A4 in the liver.[16] The specific reaction is an oxidative N-dealkylation at the piperidine ring of fentanyl (Scheme 1)[16] and leads to the formation of norfentanyl, one of the most common metabolites of fentanyl. An induced electrochemical oxidation of fentanyl can thus mimic

the enzymatic reaction in the body, resulting in metabolites that should be observable thanks to their corresponding redox peaks. [17-18] The observation of the oxidation and reduction peaks for the metabolites while also observing the irreversible oxidation peak of fentanyl add up to an electrochemical signature which can permit the identification of fentanyl.




**Scheme 1:** Hypothetical reaction mechanism for fentanyl[19]

There are several fentanyl analogs that were reported to undergo similar metabolic reactions. Fentanyl analogs like carfentanil, sufentanil, and alfentanil, which were developed for medical and veterinary purposes, all undergo an N-dealkylation reaction to metabolites, similar to fentanyl.[11-12,20] There has been less study of the more illicit fentanyl analogs besides the more common of them, such as alpha-methylfentanyl, 3-methylfentanyl, acetyl fentanyl, butyrfentanyl.[12] Both alpha-methylfentanyl and 3-methyl fentanyl have had studies reporting the N-dealkylated metabolite is present in urine, demonstrating the similar metabolite path to fentanyl.[20-21] The nor-metabolite of acetyl fentanyl has also been observed and studied in urine, indicating a similar metabolite mechanism, but is not the predominant metabolite observed.[22-23] In a post-mortem study

of a butyrfentanyl overdose, norbutyrfentanyl, carboxybutyrfentanyl, and hydroxybutyrfentanyl were all identified as metabolites, demonstrating similar metabolic reactions that fentanyl undergoes, including that of the N-dealkylation.[24] Several other fentanyl analogs are also observed to undergo an N-dealkylation reaction forming the nor-metabolite for the molecule.[12]

**Table 2:** Fentanyl analogs and their resulting nor-metabolites after undergoing an N-dealkylation[12]

| **Fentanyl Analog** | **Corresponding Nor-metabolite** |
|---|---|
| Alfentanil | Noralfentanil |
| Sufentanil | Norsufentanil |
| Acetylfentanyl | Acetyl norfentanyl |
| Acryloylfentanyl | Acryloylnorfentanyl |
| Alpha-methylfentanyl | Norfentanyl |
| Cis-3-methyl fentanyl | Nor-3-methylfentanyl |
| Isofentanyl | Nor-3-methylfentanyl |
| Butyrfentanyl | Norbutyrfentanyl |
| Carfentanil | Norcarfentanil |
| Cyclopropylfentanyl | Norcyclopropylfentayl |
| Cyclobutylfentanyl | Norcyclobutylfentanyl |
| Cyclopentylfentanyl | Norcyclopentylfentanyl |
| Cyclohexylfentanyl | Norcyclohexylfentanyl |
| 4-Fluoroisobutyrfentanyl | Nor-4-fluoroisobutyrfentanyl |
| Furanylfentanyl | Norfuranylfentanyl |
| Methyoxyacetlyfentanyl | Normethoxyacetylfentanyl |
| Ortho-Fluorofentanyl | Nor-ortho-fluorofentanyl |
| Tetrahydrofuranylfentanyl | Nortetrahydrofuranylfentanyl |

Due to the structural similarities between fentanyl and the fentanyl analogs, it is hypothesized that most of the other fentanyl analogs not yet studied will undergo the same N-dealkylation of the piperidine ring,[25] or undergo similar metabolic reactions of fentanyl such as hydroxylation, carboxylation, or dihydroxylation that were also observed. [25] These metabolic pathways have not yet been studied electrochemically, and there would need to be further investigated on whether analogs undergoing these forms of metabolic reaction would have electrochemically detectable metabolites.

## 2.3 Current State of Art for Fentanyl Detection in Forensic Science

### 2.3.1 Clinical/State Laboratory Methods for Analyzing Fentanyl

The current laboratory standards to analyze fentanyl for forensic chemistry purposes are typically GC-MS and LC-MS/MS.[11] These methods are commonly used because they are selective, highly sensitive, and have limits of detection in the nanomolar range.[25] These methods are considered confirmatory for fentanyl analysis because they both provide structural information about the molecule, as well as qualify as two methods under the SWG DRUG standards, as both a category A test in mass spectrometry, and a category B test, in gas and liquid chromatography.[26] This would be unlike the electrochemical detection of fentanyl, which would be considered a presumptive test, as the goal would not be to differentiate between fentanyl and its analogs, but to rapidly determine if any of them could be present in a sample

### 2.3.2 Current Research into Analyzing Fentanyl

While GC-MS and LC-MS/MS are the gold standard in a forensic laboratory for illicit substances, there are also several additional techniques being developed to analyze fentanyl in-field. In regards to fentanyl, the common preliminary test, both in-field and in a laboratory are spot tests, in which a small amount of the suspected fentanyl would be mixed with the reagents that correspond to the color test being performed.[27] In the presence of fentanyl, the color of the reagents would change appropriately. This is considered a preliminary test because no structural or quantitative information can be gained using this test. Color tests are also preliminary because they have chances of false positives and negatives, so their result needs further confirmation. One common color spot test for fentanyl is the Marquis test, which is used for amphetamines and opiates, in which the color response for fentanyl is dark orange to brown.[28]

Another preliminary method to detect fentanyl are immunoassay kits. These tests use an antibody-antigen interaction to induce a color change on the test strip. This method is also a quick and cost effective method to test for fentanyl in-field.[25] These fentanyl test strips, which generate a result within 5 minutes, can identify fentanyl and some fentanyl analogs like acetyl fentanyl, carfentanil, and furanylfentanyl, but can also not distinguish between fentanyl and fentanyl analogs.[8] These tests can be performed by dissolving some of the sample in water and then dipping

the test strip into the solution. Another portable method for detection of fentanyl that is currently used by some law enforcement agencies is a handheld Raman spectrometer.[8] This instrument has the ability to analyze drug samples both within the packaging, as well as when dissolved into a sample stick that can be analyzed by the instrument. This instrument cannot quantify the amount of fentanyl in a sample, but also produces a result within 5 minutes, and has an LOD of 2% weight of the sample. A portable desktop Fourier-transform infrared spectrometer (FTIR) has also been explored as a point of care and harm reduction method to test samples for fentanyl.[8] This instrument proved the most sensitive out of the immunoassay test strips and the Raman spectrometer, but was the bulkiest to move. This instrument could analyze samples with an LOD of 0.15 μg/mL of sample. Another form of in-field testing being developed is an on-site nano-liquid chromatography-electron ionization mass spectrometer (nLC-EI-MS).[29] The nLC-EI-MS would be more of an in-field confirmatory test, as it yields structural information of the sample. Fentanyl, carfentanil, acetyl fentanyl, and butyryl fentanyl were analyzed at ranges of 4 to 80 ng.

These various in-field methods under development for fentanyl have benefits and drawbacks. The color test is quick and cost effective, but its interpretation is subjective in nature. There is also a higher chance of false results and interference from other components of the samples. The immunoassay test strips are low-cost, portable, easy to use and sensitive, but other substances in the matrix could interfere and affect the result of the test. The portable Raman spectrometer, FTIR, and nLC-EI-MS all involve instruments that have a high cost, and while the Raman is hand-held, the FTIR and nLC-EI-MS are not and would require transport of bulky equipment, and then calibration and adjustments to those instruments. Electroanalytical chemistry provides solutions to several of these issues, in that the result is not subjective in nature compared to colorimetric tests. The matrix of the sample would only impact the electrochemistry if there are electrochemically active species present. The advancements in portable potentiostats have already come a long way with instrument that are small, handheld, rugged, affordable and portable. They can often use the Bluetooth signal of a cellphone, tablet or laptop to display results, rendering electrochemistry particularly attractive for in-field test compared to the other reportedly portable methods like the FTIR and nLC-EI-MS.

## 2.4 Electrochemistry in Forensic Science

There are several benefits of using electrochemistry in everyday life and in forensic science. Electrochemistry can easily be made portable, miniaturized, and inexpensive. These advantages benefit in field detection, as electrochemistry can then be taken into the field and used as a preliminary test for various substances such as drugs, explosives, etc. Electrochemistry can offer alternatives to current presumptive tests, which are often color-based tests, which are subjective in nature due to the user reading a color change.

### 2.4.1 Forensic Electrochemistry

The field of forensic electrochemistry is one that has been growing to cover multiple aspects of forensic science, with one of the more highly researched areas being drug and explosive detection using electrochemical methods. In Jong, M. D., *et al*, they worked on an electrochemical sensor for cocaine using a screen printed electrode attached to the finger of a glove.[30] In this case, the glove had the screen printed electrode (SPE) placed on the index finger, while on the thumb a conductive gel was placed, so after the index finger swiped for a sample, the two fingers could be brought together to perform the electrochemical analysis. In this set up, the potentiostat was portable which made in-field work feasible. They were able to detect cocaine in the presence of several adulterants including paracetamol, levamisole, caffeine, lidocaine, and procaine.[30]

Another example of the use of an electrochemical glove device is that of the Bandodkar, A. J., *et al*., [31] in which they used a similar set up to detect gunshot residue, as well as nitro aromatic explosives, such as DNT. Instead of a full glove, they had individual slip gloves just for the index and the thumb. Similar to the set-up used to detect cocaine, the index finger had the SPE components, while the thumb held the conductive gel that allowed for electrochemical analysis. They were able to detect both GSR and explosives within four minutes using the glove and a portable potentiostat.[31]

Electrochemical detection of drugs was also applied to the detection of mephedrone metabolites in both buffer solution and human urine.[32] In this case, they were detecting 4-methylcathinone (4-MC) and dihydromephedrone (4-MMC-R), which are two common metabolites of mephedrone found in urine. They used SPEs, and were able to have a linear concentration range for 4-MC of 40-300 µg/mL in both phosphate buffer and human urine, while

for 4-MMC-R the linear range for phosphate buffer was 15-300 µg/mL, and in human urine was 25-300 µg/mL, all using differential pulse voltammetry as the electrochemical technique.

Benzylpiperazine is another example of a drug that was detected electrochemically by Waddell, S. A., *et al*., using carbon paste electrodes to analyze benzylpiperazine.[33] In this case, they achieved an LOD of 6 µM in KCl. Additionally, they proposed a mechanism for the electrochemical reaction of the substance.

Electrochemistry has also been used to detect sedatives and analgesics in whiskey samples.[34] With a paper-based device, fabricated using a pencil as the graphite source for the working electrode, they detected metamizol, paracetamol, and midazolam maleate to varying limits of detection. The LOD they achieved for was 5 mg/L, 45 mg/L and 20 mg/L for metamizol, paracetamol, and midazolam maleate, respectively. Since the devices were fabricated in lab using inexpensive available materials, it was disposable, simply manufactured, and portable. All of these examples of the use of electrochemistry for the detection and analysis of drugs and explosives demonstrated that this field is rapidly developing and exploring different applications that will be beneficial to both electrochemistry as a field as well as the field of forensic science.

### 2.4.2 Electrochemical Detection of Fentanyl

In the last three years, there has also been an emergence in research focusing on detecting fentanyl electrochemically. In 2019, Goodchild, S. *et al*., explored the electrochemistry of fentanyl using SPEs.[17] They modified the surface of their electrode using RTIL to enhance the conductivity and peak sensitivity using cyclic square wave voltammetry (CSWV). They identified the irreversible fentanyl oxidation peak at 0.56 V vs. Ag/AgCl reference, while also observing the development of a reduction peak at -0.235 V vs. Ag/AgCl, as well as an additional oxidation peak at -0.227 V vs Ag/AgCl. They attributed these two additional peaks to norfentanyl, which was a result of the irreversible oxidation of fentanyl to the metabolite. This was mimicking the reaction that is observed in the liver. The limit of detection was 5 µM, with a linear range of 10 to 100 µM.[17]

The use of single walled carbon nanotubes (SWCNTS) was explored by Wester *et al.* in 2020, in which they developed a disposable electrode using SWCNTs to electrochemically analyze fentanyl.[35] Using differential pulse voltammetry (DPV) they were able to achieve a limit of detection as low as 11 nM, with a linear range of 0.1 to 1 µM using PBS as their supporting

electrolyte. They identified two fentanyl oxidation peaks at 0.866 V as well as 0.967 V at scan rates above 100 mV/s.

Also in 2020, Ott, C.E, *et al.*, explored the electrochemistry of fentanyl using an unmodified screen printed electrode and square wave adsorptive stripping voltammetry (SWAdSV).[36] In this work they achieved a limit of detection of 0.6 µM with a linear range of 2.35 to 20.51 µM for a 100 µL drop of sample on lab-made SPEs and with Tris-HCl, at a pH of 8.5, as supporting electrolyte. They also performed LC-MS-MS on samples of fentanyl prior to and after electrochemical scans, noting the presence of norfentanyl only after the SWAdSV. This work was the second report on the electrochemistry of fentanyl identifying the formation of the norfentanyl metabolite after electrochemical scans of fentanyl.[36]

Sohouli, E. *et al.*, explored the use of carbon nano-onions (CNOs) to analyze fentanyl electrochemically.[37] The use of CNOs allowed for the increase of the current of the fentanyl peaks from 4.5 µA, using an unmodified GCE, to ~ 30 µA with the CNO-modified GCE. With CNO-modified GCE, the potential of the oxidation peak of fentanyl was observed at 0.78 V vs. Ag/AgCl. They also observed the development of additional peaks, a reduction peak at 0.06 V vs. Ag/AgCl after the oxidation of fentanyl, and an additional oxidation peak. They identified the additional oxidation peak as the oxidation of the species responsible for the reduction peak at 0.06 V vs. Ag/AgCl. They did not indicate that these peaks could correspond to a metabolic product of fentanyl. The LOD utilizing DPV as the electrochemical technique was 300 nM, with a linear range of 1 to 60 µM.[37]

Electrochemical analysis of fentanyl using an SPE modified with a MOF on the surface was done by Naghian, E., *et al*., in 2020, in which the modification allowed for an increase in surface area of the electrode, and thus larger currents for fentanyl.[19] They were able to achieve an LOD of 0.3 µM, with a linear range of 1 to 100 µM, and observed the fentanyl oxidation peak at 0.9 V vs. Ag/AgCl using DPV.

These electrochemical methods developed to analyze fentanyl have different advantages and drawbacks. Several of them yielded low limits of detection for fentanyl, even to the nM level, but most of them used greatly modified electrodes to achieve these levels. The group who used a SPE combined it with SWAdSV which is a more laborious electrochemical technique compared to DPV or SWV. Thus far there has not been a study of the use of an unmodified electrode that utilizes DPV or SWV to quantify and characterize fentanyl electrochemically.

## 2.5 Conclusion

Fentanyl is a highly potent synthetic opioid that is currently heavily abused around the world. There have also been numerous fentanyl analogs, designed and synthesized for medical and veterinary uses, and illicit uses. They vary in potency and toxicity depending on the analog itself. The field of forensic electrochemistry has existed for decades, and in the past several years has come to the forefront with new methods and instrumentation to detect illicit substances and evidence such as drugs, explosives or GSR. These electroanalytical methods can detect these substances to low LODs, and are commonly portable, low cost and disposable. The exploration of the electrochemistry of fentanyl has emerged in the last couple of years, using both modified electrodes and SPEs. Several of the proposed methods yielded low limits of detection for fentanyl, even to the nM level, but most of them used greatly modified electrodes to achieve these levels. The only group who used a SPE, combined it with SWAdSV which is a more laborious electrochemical technique compared to DPV or SWV.

To this date, there has not been a study of the use of an unmodified electrode that utilizes DPV or SWV to quantify and characterize fentanyl electrochemically. In the following chapters the exploration of fentanyl using CV, SWV, and DPV as electrochemical methods will be optimized, as well as the use of screen printed electrodes and the quantification of fentanyl using the optimized methods explored.

# CHAPTER 3. CYCLIC VOLTAMMETRY ANALYSIS OF FENTANYL

## 3.1 Introduction

A common method utilized in electroanalytical chemistry for characterization purposes is cyclic voltammetry (CV). This method only requires an easy set-up, is adaptable to many configurations of electrodes and electrochemical cells, and yields information on multiple aspects of the system. For example, CV can determine and quantify the presence of an analyte, but also if the analyte is adsorbed or its diffusing behavior, the size of the active working area, the Nernstian behavior of a species and the electrodes, etc. This technique is very versatile for multiple applications and has been used to analyze analytes from illicit substances[38] to biological samples,[39] to detecting amounts of ascorbic acid in fruit juice[40] to countless other samples. Therefore, we first electrochemically characterized fentanyl using CV.

This electrochemical technique is a sweeping method, that scans the potential linearly in one direction, and then scans the potentials in the opposite direction (Figure 5).[41] This sweep forward and backward generates a cycle, and allows for the visualization of both the oxidation and the reduction peaks separately. In voltammetry in general, the potential is the controlled parameter and the resulting current is measured. In the IUPAC convention of presenting the electrochemical scans, the oxidation peaks are represented as positive current, while the reduction peaks are represented as negative currents along the y-axis.[42] The CV of fentanyl, however, necessitated some optimizations to obtain observable peaks. First, working electrodes (WE) made of various material were tested. Then, different supporting electrolytes (SE) were explored while also determining the optimal potential range and concentrations in electrolytes. During the optimization phase of the SE, phosphates were observed to impact the oxidation of fentanyl and thus this effect was further explored. The effect of the pH was also briefly studied to ensure small changes would not impact the redox reactions of fentanyl, but a more in-depth study is proposed in Chapter 8: Future work. To further improve the visualization of the peaks corresponding the electrochemical activity of fentanyl, different techniques aimed at decreasing the background current were also attempted.

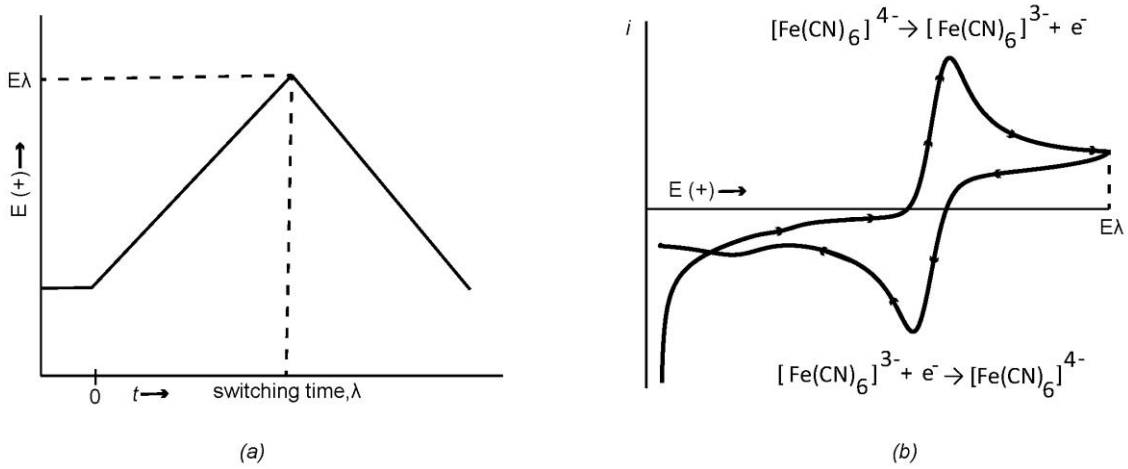



**Figure 5**: Cyclic voltammetry diagram

## 3.2 Experimental

### 3.2.1 Materials and Reagents

Fentanyl ($C_{22}H_{28}N_2O$) (1 mg/mL) was purchased from Fisher. Potassium chloride (KCl) was purchased from Fisher BioReagents. Phosphate buffered saline solution ($Cl_2H_3K_2Na_3O_8P_2$) (PBS) was purchased from Fisher. Potassium ferrocyanide trihydrate ($K_4[Fe(CN)_6]\cdot 3H_2O$) was purchased from Acros Organics. Glassy carbon electrodes, Ag/AgCl reference electrodes, and Pt wire electrodes were purchased from CHI Instruments. Sodium chloride (NaCl) was purchased from Fisher Chemical. Sodium phosphate monobasic anhydrous ($NaH_2PO_4$) was purchased from Fisher Bioreagents. Sodium phosphate dibasic anhydrous ($Na_2HPO_4$) was purchased from Fisher Scientific. Sodium hydroxide (NaOH) was purchased from Fisher Scientific.

### 3.2.2 Solution Preparation

A solution of 100 µM fentanyl was prepared using 1 mg/mL fentanyl, which equates to 2.9 mM fentanyl, then prepared with the desired supporting electrolyte. This solution preparation was followed for each 100 µM solution of fentanyl unless otherwise specified. PBS 1 X was made by diluting the 10 X stock with milliQ water. Solutions of 1.0 M KCl, 0.1 M KCl, 1.0 M NaCl, and 0.1 M NaCl were prepared with milliQ water. 5 mM potassium ferrocyanide trihydrate was

prepared using 0.1 M KCl as the supporting electrolyte unless otherwise specified. Solutions of 1.0 M KCl/0.1 M NaCl and 0.1 M KCl/0.1 M NaCl were prepared with milliQ water. Solutions of 1.0 M KCl/ 0.1 M NaCl/0.0018 M $NaH_2PO_4$/0.01 M $Na_2HPO_4$ and 0.1 M KCl/0.1 M NaCl/0.0018 M $NaH_2PO_4$/ 0.01 M $Na_2HPO_4$ were prepared with milliQ water. 0.1 M NaOH was prepared with milliQ water. All weight measurements were taken using a Mettler Toledo XPE105 analytical balance. Any pH measurements performed were done using Mettler Toledo Sven Compact modular pH meter.

### 3.2.3 Electrochemical Measurements

The electrochemical method of CV was performed using an Autolab PGSTAT204 potentiostat with Electrochemical Impedance Spectroscopy FRA2 module, with Nova 2.0 software. The potential ranges used for fentanyl for the CV were 1.2 to -0.5 to 1.2 V and 1.2 to -0.3 to 1.2 V for PBS. The potential ranges for fentanyl for NaCl and KCl were 1.0 to -0.5 to 1.0 V, and 1.0 to -0.3 to 1.0 V. The potential range used for potassium ferrocyanide trihydrate was -0.85 to 0.9 to -0.85 V. The potential ranges for phosphate buffer solutions used were 1.2 to -0.5 to 1.2 V and 1.0 to -0.5 to 1.0 V. The scan rate for all CVs was 50 mV/s unless otherwise indicated. The working electrode was glassy carbon, with a reference electrode of Ag/AgCl, and a counter electrode Pt wire unless otherwise specified.

## 3.3 Preliminary Trials with Cyclic Voltammetry

To obtain preliminary data in electrochemistry, the first thing that is necessary is to have an electrochemical cell. The electrochemical cell is comprised of the supporting electrolyte, the analyte, the working electrode (WE), the counter electrode (CE), and the reference electrode (RE) (Figure 6).[41] The supporting electrolyte provides additional ions in the solution that decrease the resistance, and carry charge, while the working electrode is the electrode where the half reactions of interest occur.[41] The RE provides a fixed potential, that does not change as the redox reactions occur at the working electrode, and this indicates that any changes in the cell are due to the WE, which is why the potentials are recorded against the RE.[41] The purpose of the CE is to allow the completion of the electronic circuit with the WE while avoiding passing large intensity of current through the RE.[43] Thus CEs are made of inert material that will not interact electrochemcially with

the cell. In standard CV, convection is added by using a magnetic stirrer, however in sensing development and miniaturized system, the convection is often not required. With all of these aspects in place, the electrochemical measurement can then be taken.

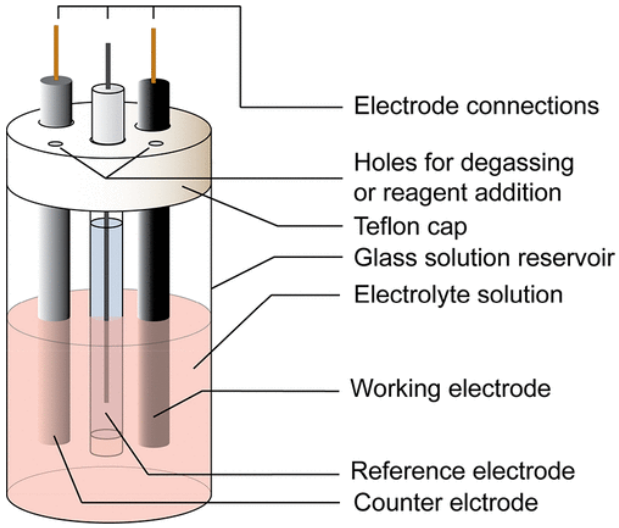



**Figure 6:** Electrochemical cell diagram

Figure 7a shows a preliminary CV of fentanyl without any optimization. The irreversible oxidation peak of fentanyl is observed at at 0.85 V vs. Ag/AgCl in both the first and the fiftieth scan. The oxidation is confirmed to be irreversible because there is no corresponding reduction peak. The definition of the peak, its current intensity, and the overall shape of the CV are far from ideal. The peaks for the metabolites of fentanyl are not clearly observed at ~ -0.1 V vs. Ag/AgCl (Figure 7b).

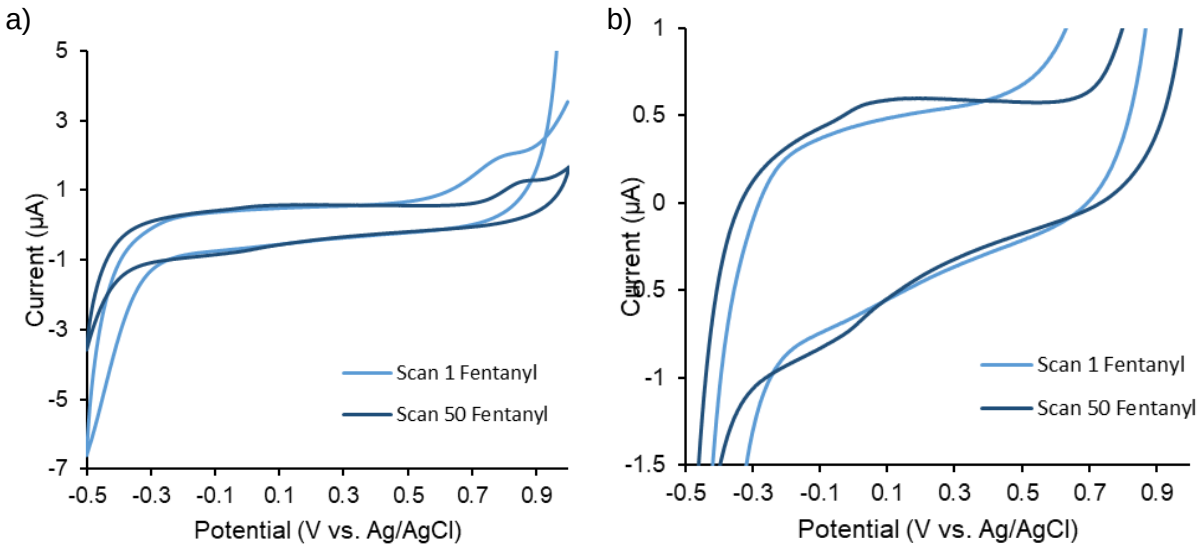



**Figure 7:** a) 50 scan CV of fentanyl at a concentration 100 µM in 0.1 M KCl using a GCE as the working electrode, b) zoom of metabolite region 50 scan CV of fentanyl

## 3.4 Optimization of Electrochemical Cell Conditions

### 3.4.1 Selection of WE

The working electrode in an electrochemical cell is the most important electrode in the system because it is the location where the half reaction of interest is occurring that is being monitored by the system.[41] Working electrodes are typically solid metal, liquid metal, carbon or semi-conductors.[41] There are several choices and options for the working electrode of an electrochemical system, but three of the most common are platinum (Pt), gold (Au), carbon (C), and mercury (Hg).[44] The working electrodes attempted for the analysis of fentanyl were Pt, Au, and C. Both Au and Pt working electrodes have more limited potential windows than carbon electrodes due to the oxidation of the metal of the electrode itself (Au), or the reduction of hydrogen in solution, causing an additional reduction peak that can hinder desired peak visualization (Pt).[44] Out of carbon-based working electrodes, glassy carbon working electrodes (GCE) are one of the most common to use in electroanalytical chemistry.

Since the working electrode choice for fentanyl can impact the appearance and potential of the fentanyl oxidation peak, the selection of the best working electrode will help the characterization of fentanyl moving forward. Figure 8 demonstrates that neither Pt nor Au were

suitable working electrodes for fentanyl, and that GCE was the optimal choice. Figure 8a shows that a fentanyl oxidation peak is not clear for the Pt working electrode, and has additional reduction and oxidation peaks at ~ 0 V vs. Ag/AgCl and ~0.2 V vs. Ag/AgCl, respectively. The Au working electrode was not suitable because the potential range needed to observe the fentanyl peaks encompassed the potential range in which the oxidation of the gold electrode itself takes place.[45] The observed reduction peak at ~0.3 V vs. Ag/AgCl, as well as both oxidation peaks at ~1.0 V vs. Ag/AgCl and ~1.4 V vs. Ag/AgCl are all due to the oxidation of the gold electrode. Figure 8b demonstrates that the fentanyl oxidation peak, using the GCE as the working electrode, has a clear fentanyl oxidation peak at 0.85 V vs. Ag/AgCl, with no other major additional or interfering peaks. The best choice of working electrode for fentanyl is the GCE due to lack of additional peaks and the adapted window of potential of the electrode itself.

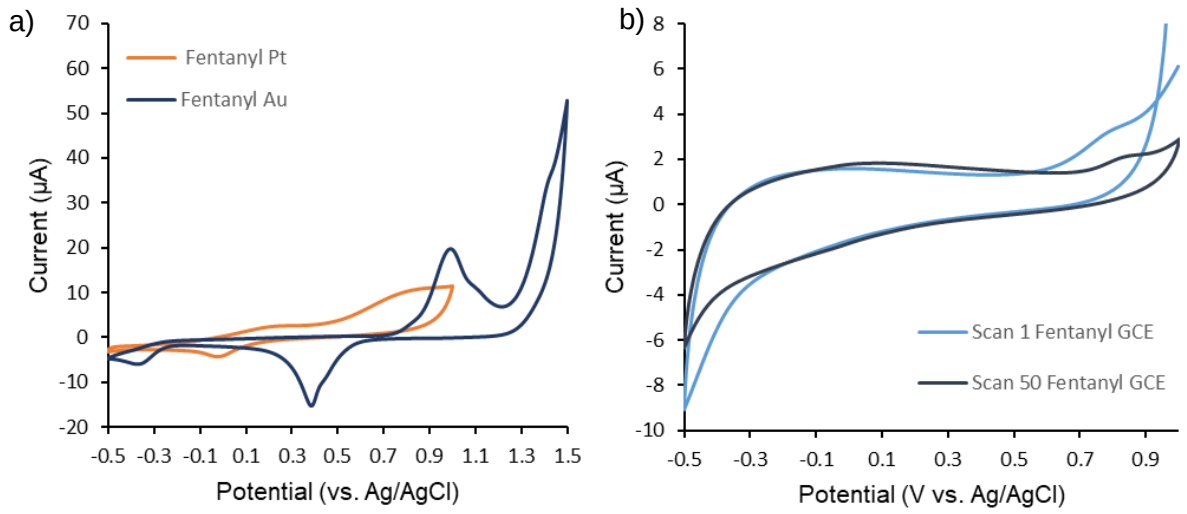



**Figure 8:** a) CV of fentanyl at a concentration of 100 µM using an Au working electrode (orange) and a Pt working electrode (navy), b) 50 scan CV of fentanyl at a concentration of 100 µM using a GCE as the working electrode

### 3.4.2 Effect of pH on Fentanyl

The pH of the solution can have an impact on the potential of the fentanyl oxidation peak. Multiple literature reports noted that at lower pH values the potential of fentanyl oxidation peak as at higher oxidative potentials, while higher pH values result in lower oxidative potentials.[19, 35-

[36] In these publications, the optimal pH value varied, but was between values of 7-9, with specific values of a pH of 7[19], 8.5[36], and 8[35]. The measured pH values for the fentanyl solutions used in this work were typically between 6.5 and 8, which was in range of the optimal potentials based on these publications. The pH of the solution for fentanyl based on other studies could impact the location of the fentanyl oxidation peak, so ensuring that small changes in the pH of the solution did not drastically impact the appearance of the fentanyl peak was necessary. Figure 9 shows that a slight change in the pH of the solution for fentanyl will not result in a drastic change of definition of potential for the fentanyl oxidation peak.

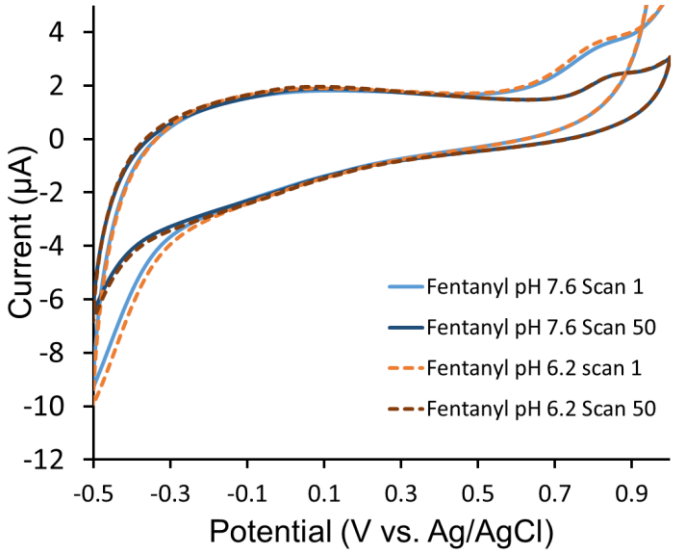



**Figure 9:** CVs of fentanyl at pH 7.6 as well as a pH of 6.2

### 3.4.3 Effect on Concentration of Supporting Electrolyte

The supporting electrolyte is critical in electrochemistry, because it aids in the movement of the charge through the solution.[41] Common supporting electrolytes typically contain $Na^+$, $H^+$ or $Cl^-$ ions, but can contain others as well.[41] The supporting electrolyte also decreases the resistance in the electrochemical cell by increasing the conductivity, and helps eliminate the contributions of the mitigation of the charge away from the electrodes, by carrying the current between them as needed.[41] These conditions also help the double layer at the surface of the working electrode to

remain thin, and gives a uniform ionic strength throughout the solution. The supporting electrolyte should be selected such that there are no additional electrochemical reactions in the conditions of the electrochemical cell, in particular the chosen range of potentials, and not interferences with the redox reactions of the analyte.

For the analysis of fentanyl, we tested different common supporting electrolytes; NaCl, KCl, and PBS. Figure 10 shows that in the case of KCl the concentration of the supporting electrolyte was having some unexpected impacts on the oxidation peak of fentanyl, which lead to exploration of additional supporting electrolytes in NaCl and PBS. Figure 10a demonstrates that 100 µM fentanyl has a more defined fentanyl oxidation peak at 0.85 V vs. Ag/AgCl in 1.0 M KCl than in 0.1 M KCl. With the concentration of fentanyl being in the micromolar range, 0.1 M KCl should be sufficiently concentrated enough to work as a supporting electrolyte, and thus no changes should have been observed when the concentration is increasing further above this value. Due to this observation, the two concentrations of 0.1 M and 1.0 M were attempted for NaCl (Figure 10b), which yielded the expected results. Both for 0.1 M NaCl and 1.0 M NaCl, the fentanyl oxidation peak is observed at 0.85 V vs. Ag/AgCl, and both have similar definition and peak current. Figure 10c shows that changing the concentration of PBS between PBS 1X and PBS 10X did not change the peak definition observed for fentanyl, however the fentanyl oxidation peak shifted from 0.85 V vs. Ag/AgCl in NaCl and KCl to 0.9 V vs. Ag/AgCl. The change in concentration between 0.1 M KCl and 1.0 M KCl affecting the peak definition meant that KCl was not an optimal supporting electrolyte for the analysis of fentanyl. PBS resulting in a larger fentanyl oxidation peak made it the optimal choice of supporting electrolyte.

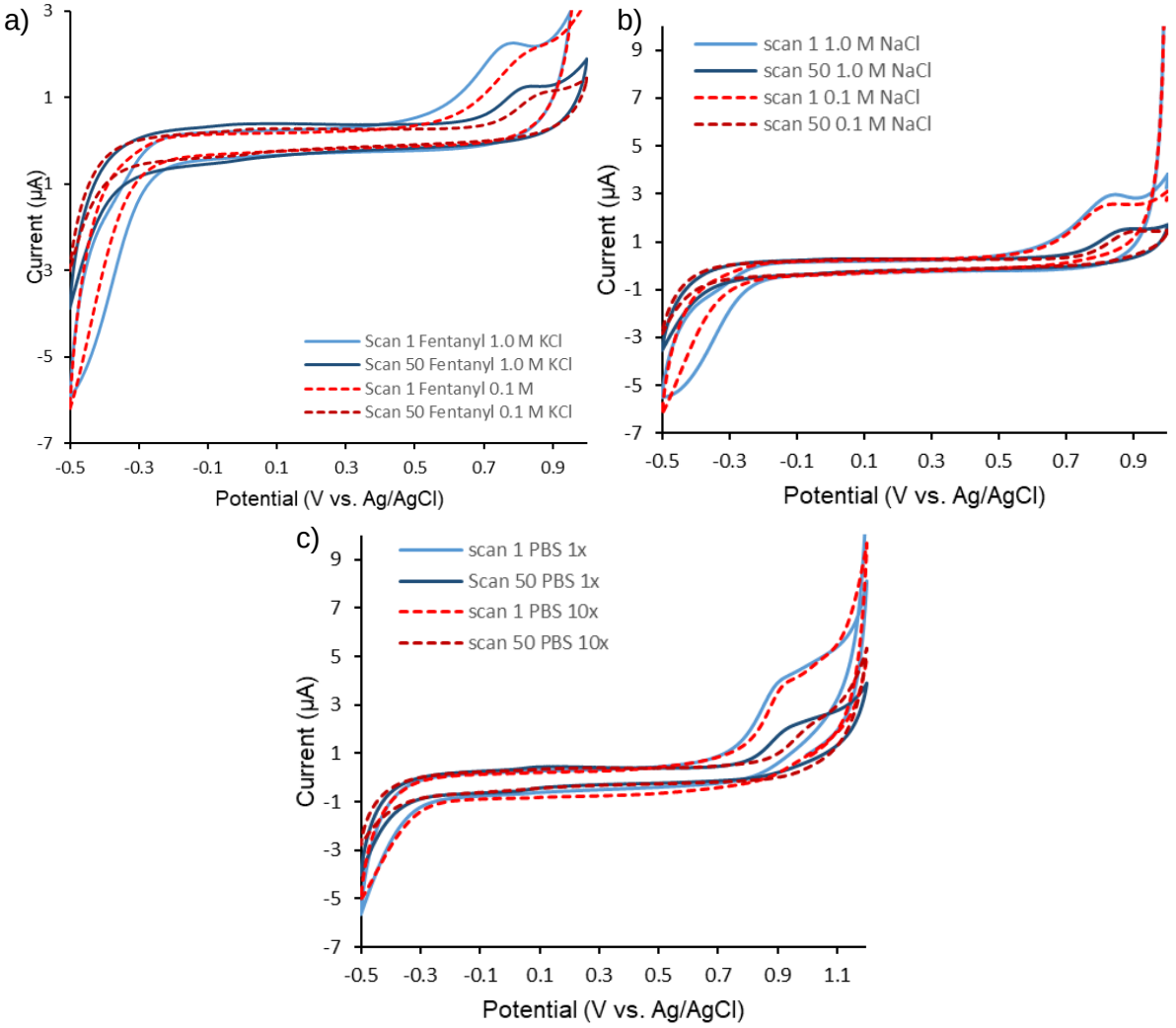



**Figure 10:** CV of 50 scans of fentanyl at a concentration of 100 µM with supporting electrolytes of a) KCl at concentration of 1.0 M (blue) and 0.1 M KCl (red), b) NaCl at concentrations of 1.0 M NaCl (blue) and 0.1 M NaCl (red), and c) PBS at concentrations of PBS 10x (blue) and PBS 1x (red)

### 3.4.4 Effect of Potential range

The potential range in electrochemistry is the window of potentials scanned using an electrochemical method. The potential range can vary depending on the choice of working electrode[44], as well as the restrictions of the supporting electrolyte.[41] The limits of the potential range are typically the potentials at which the intensity of current sharply increase corresponding to the redox reactions of the solvent; they are known as solvent walls or background limits.[41] Outside of these restrictions of the electrode and the SE, changing the potential range should not

drastically impact the appearance of the peaks, as long as the potential range still encompasses them.

The potential range used for fentanyl should encompass the fentanyl oxidation peak and metabolite peaks, and the changing of the potential range should not impact the appearance of the fentanyl oxidation peak. Figure 11 demonstrates, however, that in the case of fentanyl, the potential range did affect the definition of the irreversible oxidation peak in both KCl and NaCl. Figure 11a shows that a change in the potential range from a longer range (1.0 V to -0.5 to 1.0 V (blue)) to a slightly shorter range (1.0 V to -0.3 V to 1.0 V (orange)) in 1.0 M KCl had a decrease in the current as well as the definition of the fentanyl oxidation peak. Figure 11b shows that the peak current was also decreased when shortening the potential range in 1.0 M NaCl. This effect was not observed in PBS, for both potential ranges: 1.2 to -0.5 to 1.2 V (blue) and 1.2 to -0.3 to 1.2 V (orange) (Figure 11c). Indeed, the definition of the peak, as well as the peak current were the same, with no distinguishable difference. This result signified that the optimal SE for fentanyl would be PBS because it was not affected by the change in the potential range, unlike NaCl and KCl.

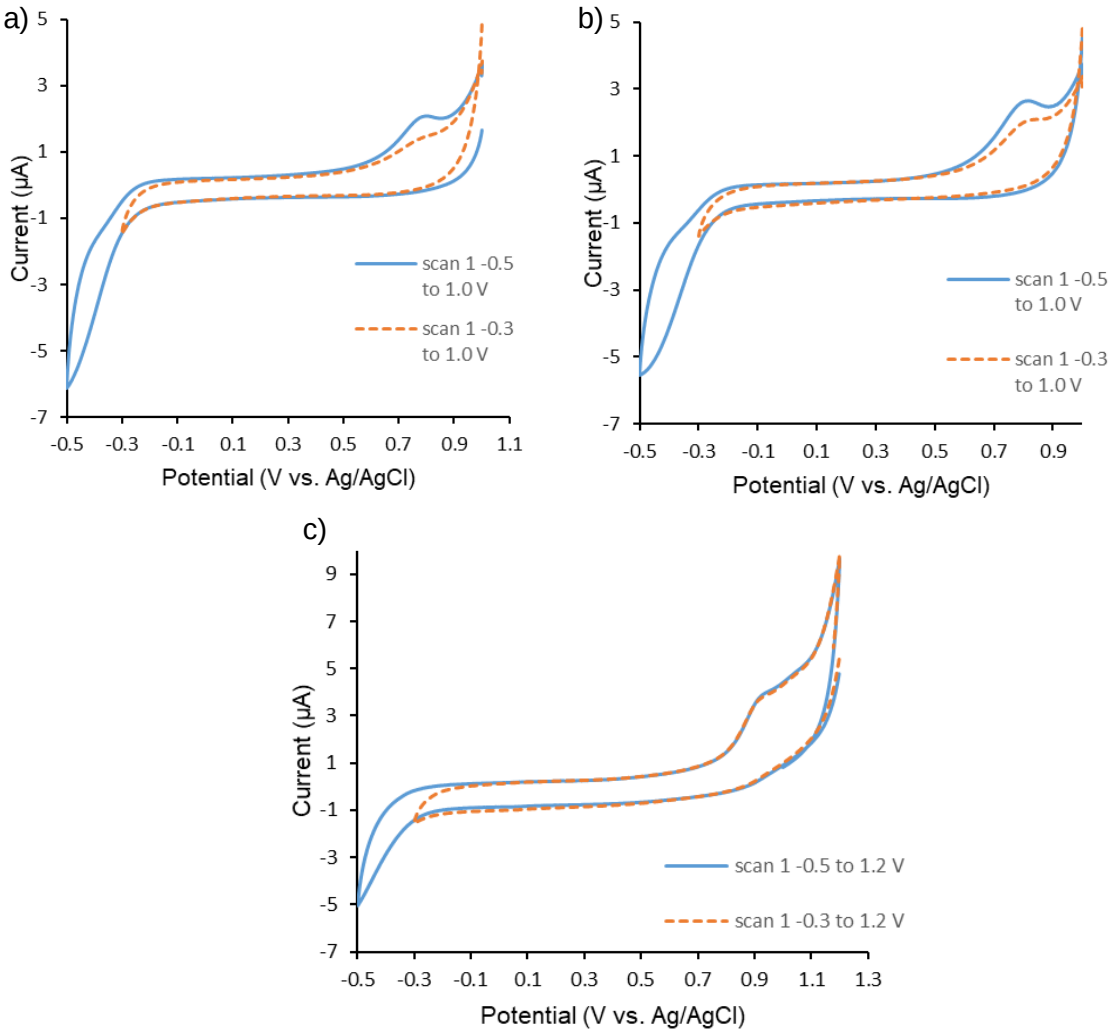



**Figure 11:** CVs of fentanyl at a concentration of 100 µM at different potential ranges in a) KCl 1.0 M, b) NaCl 1.0 M, c) PBS

### 3.4.5 Effect of Phosphates

As originally observed in section 3.4.3, the potential of the oxidation peak of fentanyl shifted in PBS to a more oxidative potential than the potential observed for the peak in KCl and NaCl (Figure 12). The fentanyl oxidation peak in both KCl and NaCl is 0.85 V vs. Ag/AgCl, while in PBS it is observed at 0.9 V vs. Ag/AgCl. The cause of this shift was not known especially as all three solutions are commonly used interchangeably as supporting electrolytes in electrochemistry and NaCl and KCl are part of the composition of PBS. The components of PBS are NaCl, KCl, $NaH_2PO_4$, and $Na_2HPO_4$ (Table 3). Briefly, the main differences between PBS, and NaCl and KCl were the mixture of NaCl and KCl together and the presence of phosphates. A combination of 1.0

M KCl and 0.1 M NaCl, as well as a combination of 0.1 M KCl and 0.1 M NaCl were tested. Both mixtures of NaCl and KCl resulted in fentanyl oxidation peaks at 0.85 V vs. Ag/AgCl (Figure 13a-b) The difference in the definition of the peaks in Figure 13a compared to 13b was another example of the effect of the higher concentration of KCl leading to a better definition of the oxidation peak of fentanyl. Figures 13c-d shows that the addition of phosphates to both mixtures of NaCl and KCl shifted the fentanyl oxidation peak from 0.85 to 0.9 V vs. Ag/AgCl. These results indicate that the shift in the potential of the fentanyl oxidation peaks is due to the presence of phosphate in the PBS, even at low concentrations compared to the other salts present at much larger concentrations in the supporting electrolyte.

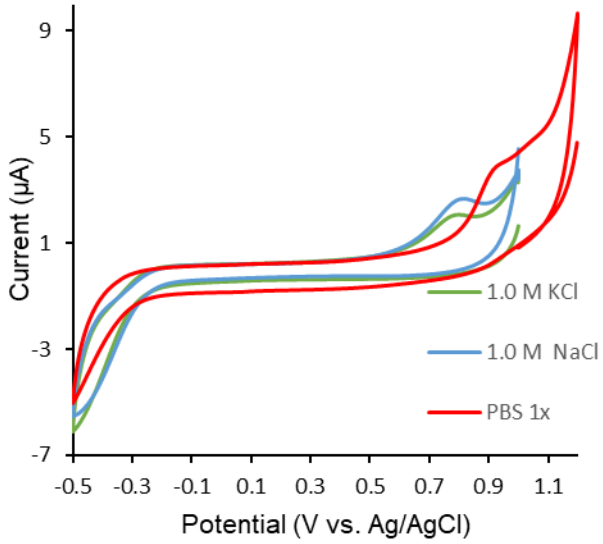



**Figure 12:** Overlay of fentanyl at a concentration of 100 µM in PBS (red), 1.0 M KCl (green dash), and 1.0 M NaCl (blue)

**Table 3:** Concentrations of Components in PBS 10x

| **Chemical** | **Concentration in PBS 10x** |
|---|---|
| NaCl | 1.37 M |
| KCl | 0.1 M |
| $NaH_2PO_4$ | 0.0018 M |
| $Na_2HPO_4$ | 0.01 M |

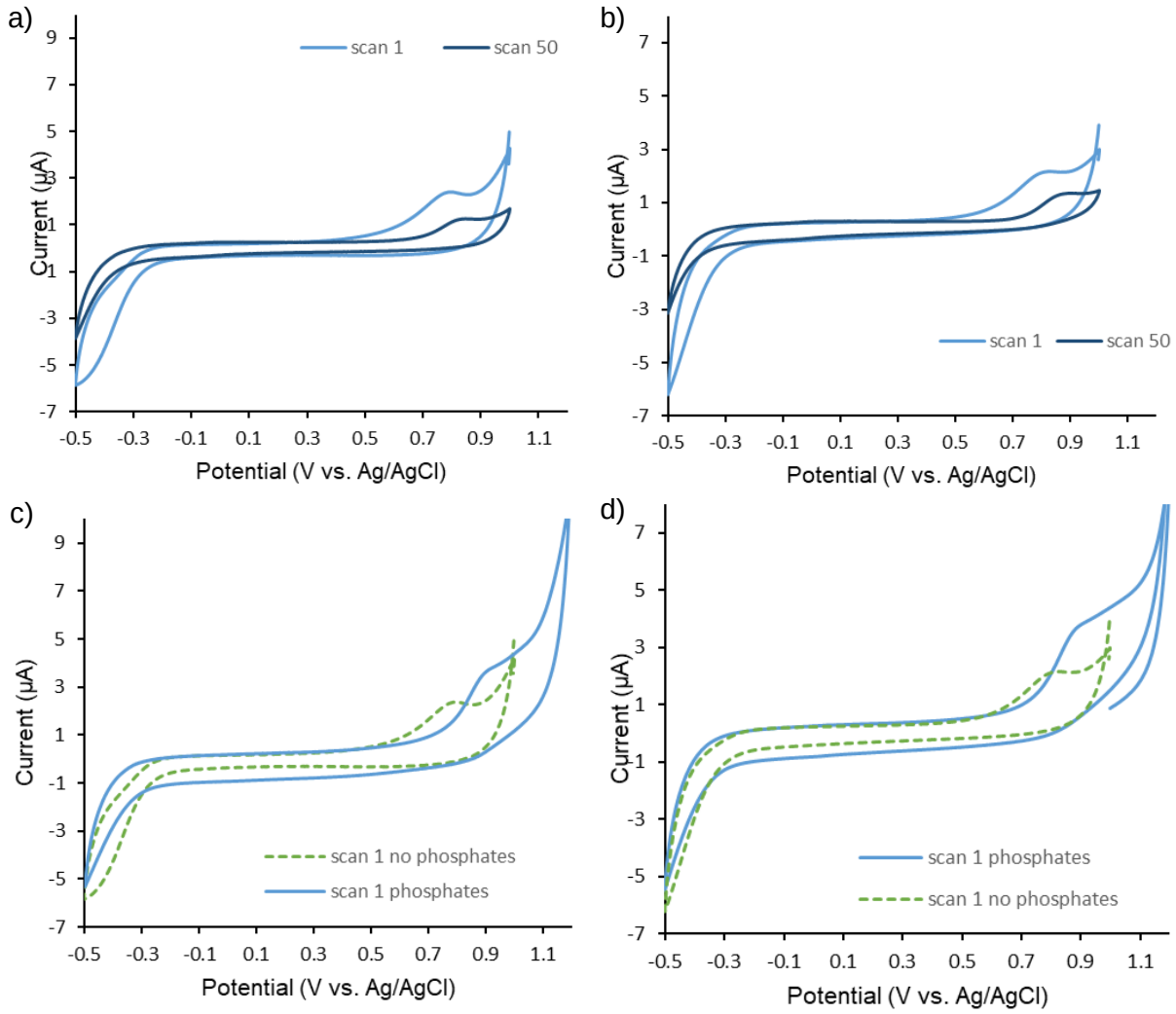



**Figure 13:** CV of 50 scans of fentanyl at a concentration of 100 µM with a) 1.0 M KCl/0.1 M NaCl, and b) 0.1 M KCl/0.1 M NaCl as supporting electrolytes, c) 1.0 M KCl/0.1 M NaCl/0.00018 M $NaH_2PO_4$/0.001 $Na_2HPO_4$ and d) 0.1 M KCl/0.1 M NaCl/0.00018 M $NaH_2PO_4$/0.001 $Na_2HPO_4$ as supporting electrolytes

### 3.5 Effect of Stirring and Nitrogen Bubbling

The oxidation peak of fentanyl was observable in all tested supporting electrolytes, but the peak was still poorly defined, and the metabolite peaks were difficult to observe using CV. One way to help with the observation of redox peaks is to decrease the capacitive current of a system. In an electrochemical system, the measured current is the sum of two types of current:, the faradic current, and the non-faradic or capacitive current.[41] The faradic current is the current generated by

the observed redox reaction of the analyte at the working electrode, while the capacitive current is a sort of background current and is due to the rest of the electrochemical cell, i.e., the system of the electrodes, the solutions, the connection and cables, etc.[46] One way to decrease this capacitive current is through adding convection to the system. The convection will impact the double layer and therefore the capacitive current. The double layer are the layers at the interface of the working electrode. They are comprised of the inner layer, which are molecules and ions adsorbed to the surface of the working electrode, and a diffuse layer, which are ions that are not specifically adsorbed.[41] The thickness of the double layer impact on the electrochemistry and the current observed for the system, and in particular the capacitive current of the system.

The typical way to add convection to the system is to stir the solution. Figure 14 shows that stirring the solution while taking electrochemical scans can result in noisy data, due to too much movement of the solution in the configuration of the small volumes used. Figure 14a is a control scan of potassium ferrocyanide in which the solution was not being stirred with the stir bar and stir plate, while Figure 14b represents the same set-up and electrode positioning while the solution is stirred. The capacitive current is decreased in the potential range of -0.5 to -0.1 V, but the faradic current of reduction and oxidation peaks ~ 0.15 V vs. Ag/AgCl and 0.25 V vs. Ag/AgCl respectively remained similar. The signal is unsteady between the potentials of 0.3 to 0.9 V. This noise is likely due to the solution disturbing too much the surface of the electrodes or the electrodes themselves as it is stirred. To try to avoid these negative effects, the position of the electrodes was adjusted at different levels in the beaker (Figure 14d and 14f). Figure 14c shows the CV taken when the electrodes were high in the solution, as shown in figure 14d, and even at this height the noise and unsteadiness of the current was still observed on the CV. When the electrodes were deeper in the solution as was the case for figure 14e-f, the disturbance in the potential range of 0.3 V to 0.9 V vs. Ag/AgCl increased. As the noise was observed in all CVs taken while attempting to decrease the background current by stirring the solution, and thus the stirring was not used further.

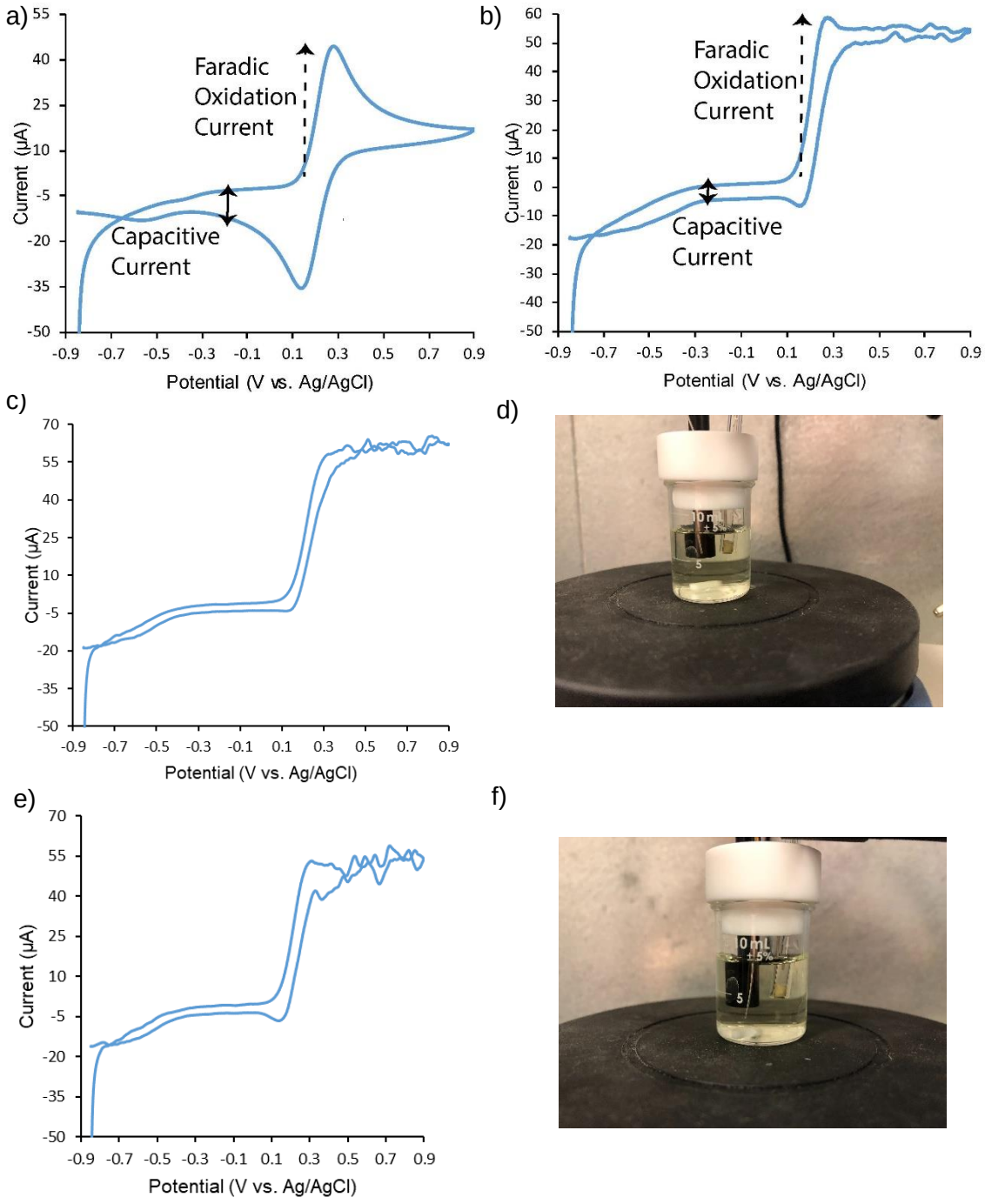



**Figure 14:** Stirring potassium ferrocyanide at a concentration of 5 mM in 0.1 M KCl, a) no stirring control, b) stirring same set up as (a), c) stirring electrodes higher in solution, d) high placement of electrodes, e) stirring electrodes lower in solution, f) low placement of electrodes

Another method that is commonly used to attempt to decrease the capacitive current is to bubble an inert gas through the solution prior to taking electrochemical scans. As oxygen is an electrochemically[47] active species, it can result in additional redox peaks, as well as broaden the capacitive current. The removal of oxygen from the solution prior to taking scans could decrease the current, and thus allow for better visualization of the fentanyl peaks. Figure 15 shows that bubbling nitrogen in the solution improved the capacitive current for the first scan, but by the fiftieth scan the effect was negligible. This result was likely due to the time it took to perform 50 scans of a CV, which with a potential window of 1 V in both directions, and a scan rate of 50 mV/s, was approximately 33 minutes. In this time, the effect of the nitrogen bubbling could have dissipated. As after the first several scans the effect of nitrogen bubbling was not as prominent, and was a relatively inconvenient added step, it did not get pursued further either.

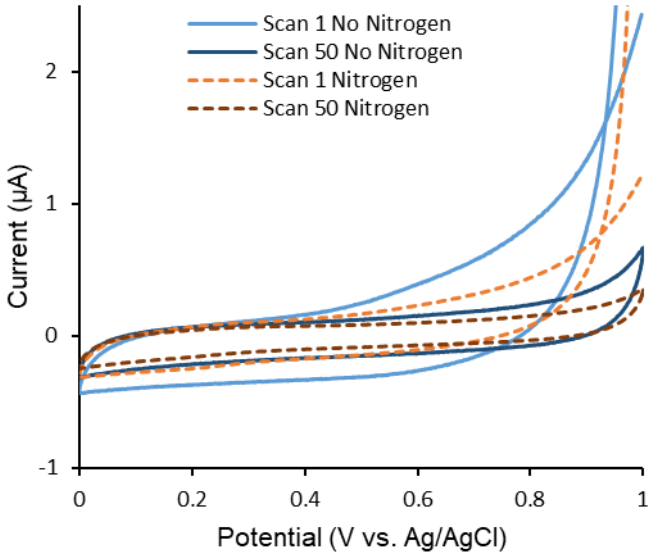



**Figure 15:** CV of 50 scans of PBS both with (blue) and without (orange) bubbling nitrogen prior for 10 minutes

## 3.6 Conclusion

After determining that GCE was the optimal WE, several aspects of the SE and the cell were optimized. PBS was determined to be the best choice of SE because it was not impacted by the concentration or the potential range used, despite the shift in the fentanyl oxidation peak from 0.85 V vs. Ag/AgCl in KCl and NaCl to 0.90 V vs. Ag/AgCl due to the presence of phosphates. Since

the metabolite peaks were not clearly visible, attempts to decrease the capacitive current were made by stirring the solution and bubbling nitrogen prior to obtaining data, but neither of these methods had the desired effect and were not further pursued.

# CHAPTER 4. ANALYSIS OF FENTANYL BY ALTERNATIVE VOLTAMMETRIC TECHNIQUES

## 4.1 Introduction

While cyclic voltammetry is a method that is common, especially for initial electrochemical investigation, there are other additional electrochemical techniques that are considered more sensitive, due to less background current interference. These methods include square wave voltammetry (SWV) and differential pulse voltammetry (DPV). Both SWV and DPV are also widely used in electrochemistry, especially for quantification because of the sensitivity of these methods.[41] Both SWV and DPV allow for low limits of detection in quantification, and also due to the sensitivity, allow for better peak visualization for analytes, and take less time due to the speed of the scans.[48] SWV and DPV have been used broadly, just like CV has, to analyze many types of samples, for example SWV has been used for illicit substances, such as cocaine mixing with levamisole, caffeine, and paracetamol,[49] as well as measure the quercetin in tea samples,[50] and detect levels of theophylline for health care applications[51] and numerous other types of samples. DPV is also widely used for analysis of countless analytes and different applications, such as being used to detect and quantify acetaminophen and L-ascorbic acid,[52] as well as lead ions in water.[53] Both SWV and DPV were useful methods to analyze fentanyl because they are more sensitive than CV and thus required fewer scans to observe the redox peaks of fentanyl and its metabolites. The fast analysis could also make them more applicable than CV for the long-term goal of the project, i.e., to develop an in-field screening assay to detect fentanyl. They however necessitated significant adjustments to achieve optimized results.

## 4.2 Experimental

### 4.2.1 Materials and Reagents

Fentanyl ($C_{22}H_{28}N_2O$) (1 mg/mL) was purchased from Fisher. Potassium chloride (KCl) was purchased from Fisher BioReagents. Phosphate buffered saline solution ($Cl_2H_3K_2Na_3O_8P_2$) (PBS) was purchased from Fisher. Glassy carbon electrodes, Ag/AgCl reference electrodes, and Pt wire electrodes were purchased from CHI Instruments. Potassium ferrocyanide trihydrate

($K_4[Fe(CN)_6]\cdot 3H_2O$) was purchased from Acros Organics. Sodium chloride (NaCl) was purchased from Fisher Chemical.

### 4.2.2 Solution Preparation

A solution of 100 µM fentanyl was prepared using 1 mg/mL fentanyl, which equates to 2.9 mM fentanyl, then prepared with the desired supporting electrolyte. This solution preparation was followed for each 100 µM solution of fentanyl unless otherwise specified. PBS 1 X was made by diluting the 10 X stock with milliQ water. Solutions of 1.0 M KCl, 0.1 M KCl, 1.0 M NaCl, and 0.1 M NaCl were prepared with milliQ water.

### 4.2.3 Electrochemical Measurements

The electrochemical methods of SWV and DPV were undertaken using an Autolab PGSTAT204 potentiostat with Electrochemical Impedance Spectroscopy FRA2 module, with Nova 2.0 software. The potential range for SWV and DPV for fentanyl was -0.5 to 1.2 V for PBS unless otherwise specified. The potential range for SWV and DPV for -0.5 to 1.0 V unless otherwise specified. The optimized parameters for SWV were a step size of 0.004 V, an amplitude of 30 mV, a frequency of 15 Hz and a calculated scan rate of 59 mV/s. Other attempted parameters for optimization included variation of the step size between 0.001 V, 0.002 V, 0.003 V, 0.004 V and 0.005 V. The other attempted frequencies were 10 Hz, 15 Hz, 20 Hz, 30 Hz, and 40 Hz which correspond to scan rates of 39 mV/s, 79 mV/s, 11 mV/s and 159 mV/s respectively. The other attempted amplitudes for SWV were 20 mV, 40 mV, and 50 mV. The parameters optimized for DPV were a step size of 0.005 V, a modulation time of 0.05 s, an interval time of 0.2 s, a modulation amplitude of 50 mV, and a calculated scan rate of 25 mV/s. The other attempted interval times were 0.08 s, and 0.1 s. The other tested amplitudes for DPV were 30 mV and 40 mV. The working electrode was glassy carbon, with a reference electrode of Ag/AgCl, and a counter electrode Pt wire unless otherwise specified.

## 4.3 Square Wave Voltammetry (SWV)

Square wave voltammetry is a form of pulse voltammetry that is extremely versatile, in which there is a combination of pulse methods and a staircase scan.[41] In this electrochemical

method, samples are taken at the end of the top pulse and then again at the end of the bottom pulse as seen in Figure 16. This method of taking the current, results in simultaneously taking the forward and the backward current.[41] The forward and the backward current will correspond to the oxidative and reductive current, depending on the direction of the scan. If the scan begins at more reductive potentials and scans towards oxidative potentials, then the forward scan would be the oxidation, while the backward scan would be the reduction, and vice versa. The difference between these two currents is determined, and given as the delta current. Taking both scans simultaneously decreases the time needed to obtain both scans, unlike in CV where both directions are scanned separately. There are three main parameters involved in SWV. They are the frequency (Hz), the amplitude (V) represented in Figure 16 as $\Delta E_p$, and the step size (V) represented in Figure 16 as $\Delta E_s$. The frequency in SWV is related to the pulse width ($t_p$) shows in Equation 1,[41] which indicates frequency is inversely related to pulse width. The scan rate (v) for SWV is dependent on the step size as well as the frequency, represented in Equation 2.[41] This indicates that the scan rate for SWV is directly related to the step size, and directly related to the frequency.

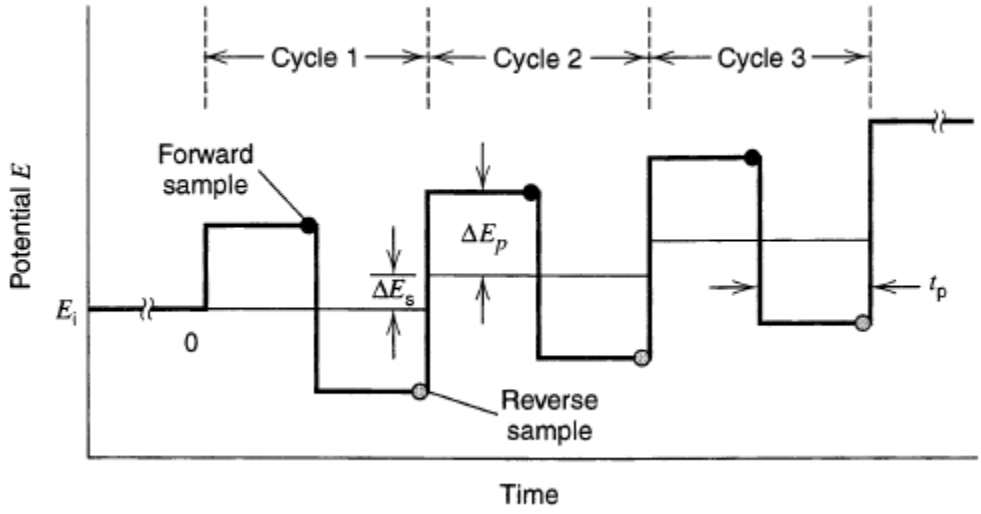



**Figure 16:** Square wave voltammetry diagram[41]

$$f = \frac{1}{2t_p}$$

**Equation 1:** Frequency and pulse width relationship

$$v = \frac{\Delta \mathrm{Es}}{2t_p} = f\Delta \mathrm{Es}$$

**Equation 2:** Scan rate for SWV

### 4.3.1 Optimization of SWV Parameters

In order to best visualize the fentanyl peaks using SWV, the parameters that can affect the appearance, and shape of the peaks need to be optimized, namely frequency, step size, and amplitude. Figure 17 shows the optimization of the parameters for SWV in KCl. The amplitude that resulted in the best peak definition for the fentanyl oxidation peak at 0.85 V vs. Ag/AgCl was 30 mV, when using a frequency of 25 Hz and a step size of 0.004 V, in the first and the fourth scans of fentanyl (Figure 17a-b). Figures 17c-d correspond to the optimization of the frequency for SWV and fentanyl and was performed using an amplitude of 30 mV, and a step size of 0.004 V. The frequencies tested were 10 Hz, 15 Hz, 20 Hz, 30 Hz and 40 Hz, which correspond to calculated scan rates of 39 mV/s, 59 mV/s, 79 mv/s, 119 mV/s, and 158 mV/s, respectively. Figure 17c shows that, for the first scan of fentanyl at multiple frequencies, the fentanyl oxidation peak at 0.85 V vs. Ag/AgCl has similar appearance and definition for 10 Hz, 15 Hz, 20 Hz, and 30 Hz. The background current increases as the frequency increases, but the definition of the peaks is similar. Figure 17d, which shows the fourth scan of the frequency optimizations, 10 Hz, 15 Hz and 20 Hz all maintain good peak shape for the fentanyl oxidation peak. The frequency chosen as the optimal thus far was 15 Hz, which is the closest calculated scan rate to the scan rate used in CV (50 mV/s). The optimization of the step size included a range from 0.001 V to 0.005 V (Figure 17e). The optimal step size was 0.004 V, due to the appearance of the peak at 0.85 V vs. Ag/AgCl in Figure 17e. This step size presented the best definition of peak of all attempted step sizes. The optimization of the parameters for SWV thus far has resulted in an optimal amplitude of 30 mV, an optimal frequency of 15 Hz, and an optimal step size of 0.004 V. These parameters were all optimized using KCl as the supporting electrolyte. Another student is currently verifying that these parameters are still optimal when using for PBS, which preliminary appears to be the case.

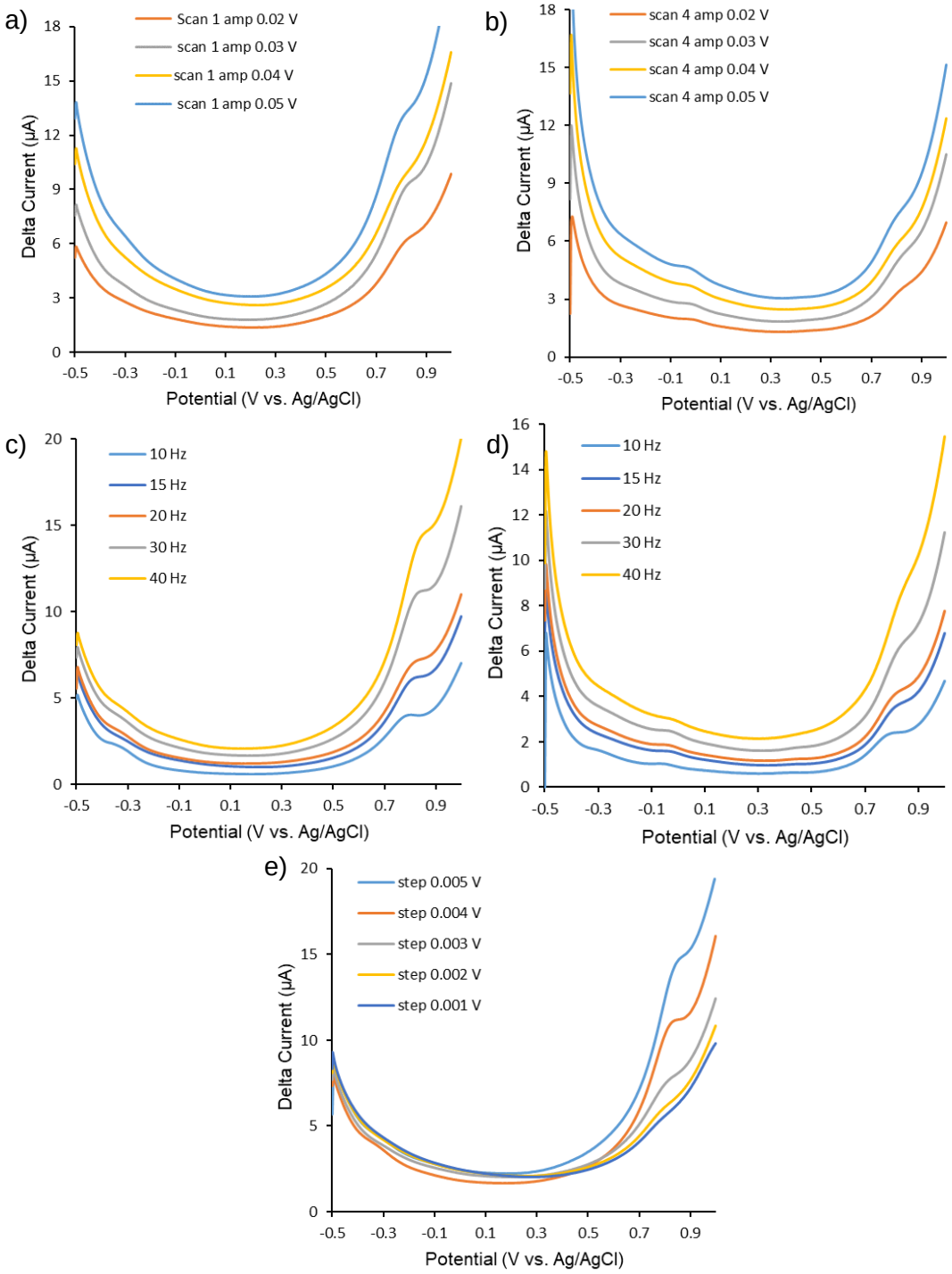



**Figure 17:** SWVs of fentanyl optimization for varying parameters: a) first scan, amplitude optimization, b) second scan, amplitude optimization, c) first scan, frequency optimization, d) fourth scan, frequency optimization, e) first scan, optimization of the step size

Since PBS has been determined as the optimal SE for CV, we verified PBS was also optimal for SWV. Figure 18 shows that the fentanyl oxidation peak in SWV is also shifted to more

oxidative potentials in PBS compared to SWV in KCl. The fentanyl peak in PBS, like in CV, is observed at 0.9 V vs. Ag/AgCl, while in KCl and NaCl, the peak is observed at 0.85 V vs. Ag/AgCl. The fentanyl oxidation peak also had a larger peak current in PBS than in KCl and NaCl, and did not have the additional shoulder peak observed at -0.3 V vs. Ag/AgCl in both NaCl and KCl. The metabolite peak observed for PBS also has a better definition than in NaCl, and KCl, and has also shifted from a potential of ~-0.1 V vs. Ag/AgCl in KCl and NaCl to ~ 0 V vs. Ag/AgCl in PBS. These results demonstrate that PBS is the optimal SE for SWV as well.

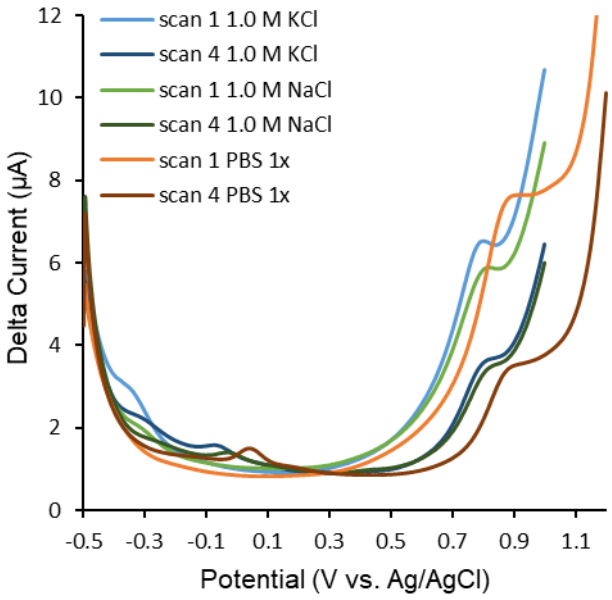



**Figure 18:** SWV of fentanyl at a concentration of 100 µM in 1.0 M KCl, 1.0 M NaCl, and PBS

### 4.3.2 Stirring Solution and Effect on Fentanyl Peaks

After optimization of the SWV parameters, the definition of the oxidation peak of fentanyl at 0.85 V vs. Ag/AgCl for NaCl and KCl, and 0.9 V vs. Ag/AgCl for PBS was satisfactory, but the intensity of peak current for the first scan was still higher than the intensity of peak current for the scans 2-4. Additionally, as the scans progressed, the definition of the oxidation peak decreased. The diffusion of the species from the bulk of the solution to the electrode and in the double layer could be different at the initial scan, after the system was voltage-free, than for the subsequent scans, when the voltage was controlled and redox reactions started occurring. Resetting the profile

of concentrations of the species from bulk to the electrode can be done easily by adding convection (stirring) between each sweep in potential. To test this hypothesis, we performed four individual SWV of one scan and mixed the solutions between each SWV. Before this experiment, however, it was important to verify that performing scans individually compared to by-batch did not impact. Collecting individually means that the same solution was used, without polishing the electrode between voltammograms and that on the instrument itself, the SWV was initiated four individual times. The four scans c by-batch, or together, were collected consecutively during one SWV trial of four scans with only one initiation from the user. Figure 19 shows that there was no significant difference in the oxidation peak of fentanyl observed at 0.85 V vs. Ag/AgCl when taking four SWVs individually (blue) or four scans by-batch in one SWV (orange).

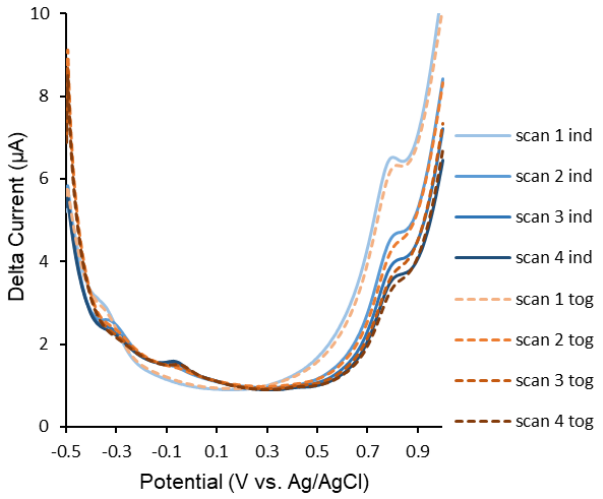



**Figure 19:** SWVs of fentanyl at a concentration of 100 µM collected as four individual voltammograms (blue) and as four scans of one voltammogram (orange).

Stirring the solution between taking scans of fentanyl could improve the separation and definition of the peaks, because as discussed in Chapter 3, Section 3.5, the use of convection can decrease the background current, as well as impact the double layer.[41] Figure 20 shows that mixing between the individual voltammograms of SWV resulted in better definition of the fentanyl oxidation peak than no mixing. Additionally, the mixing resulted in the subsequent scans, scan 2-4, having an intensity of current for the oxidation peak greater than without mixing. This trend was observed for KCl (Figure 20a), PBS (Figure 20b), and NaCl (Figure 20c). The current measured

for the first scan, however, was still largely distinct of the following three scans. The metabolite peaks were also of similar appearance for both mixing and non-mixing Taken into account these results, and the inconvenience and time spent to mix, which would not be adapted for any form of in-field use, the mixing between scans was not implemented further for the analysis of fentanyl by SWV.

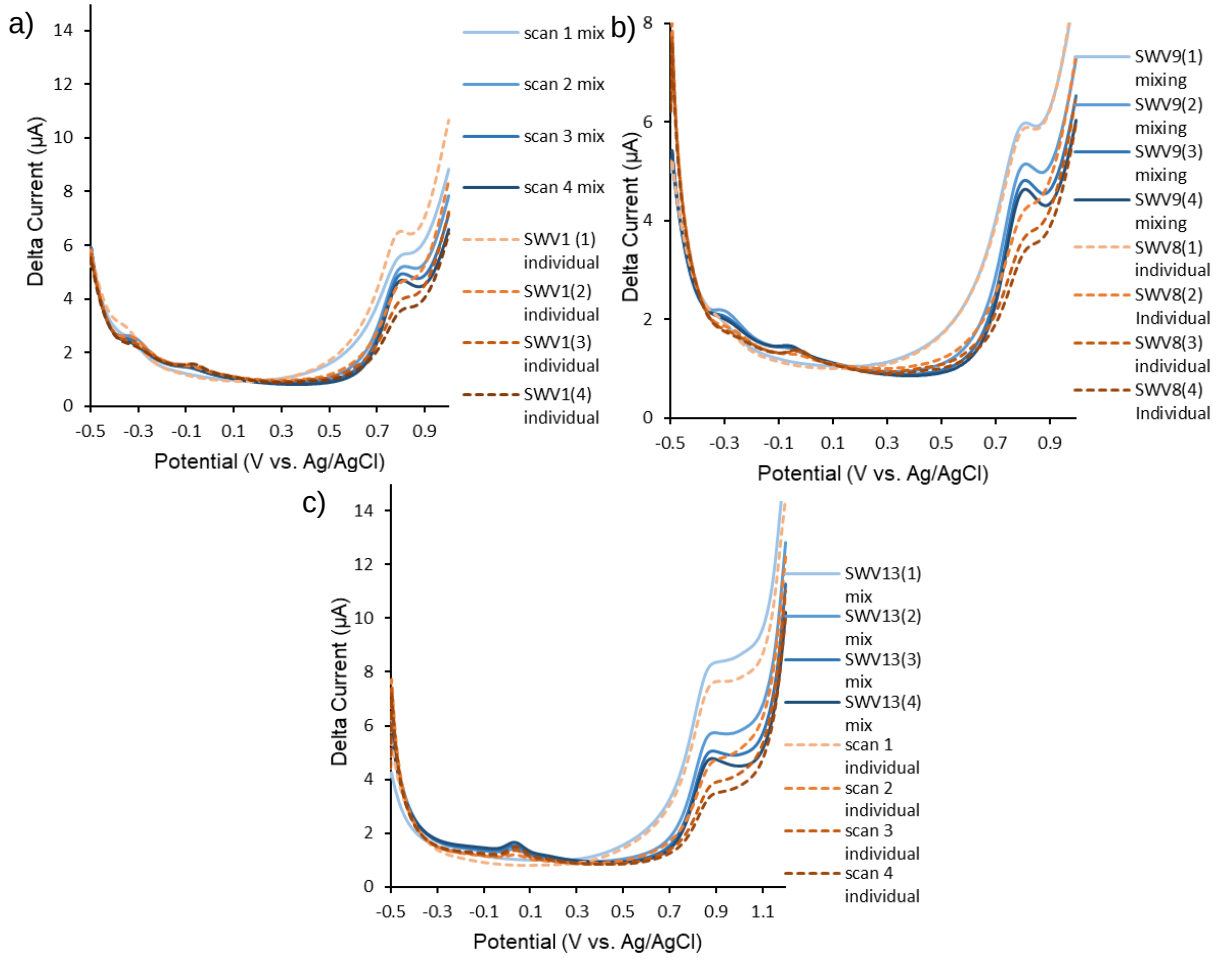



**Figure 20**: Four successive SWVs collected while mixing the solution between each trial (blue) or without mixing (orange). The solution of fentanyl was at a concentration of 100 µM in 1.0 M KCl (a), in PBS (b), and in 1.0 M NaCl (c).

## 4.4 Differential Pulse Voltammetry (DPV)

Differential pulse voltammetry is an electrochemical pulse technique. This technique involves applying pulses of potential over time while measuring the resulting current (Figure 21).[41] There are several parameters in DPV that can impact the resulting shape of the DPV. These parameters include the modulation time, the interval time, the amplitude, and the step size. The modulation time is the amount of time the pulse is applied for in seconds, also named pulse width. The interval time is the amount of time in-between the application of the pulses. The pulse amplitude is the size of the pulse applied, given in V, and the step size is the difference of voltage between each pulse.[41] The sample periods on the figure are when the current is measured, once before the application of the pulse, and once before the drop of the pulse. The difference of these two measured currents is referred to as the delta current.[41] This delta current is the main current displayed by the instrument, but both the pulse current and the base current can also be retrieved, as those currents are the measurements truly recorded by the instrument.

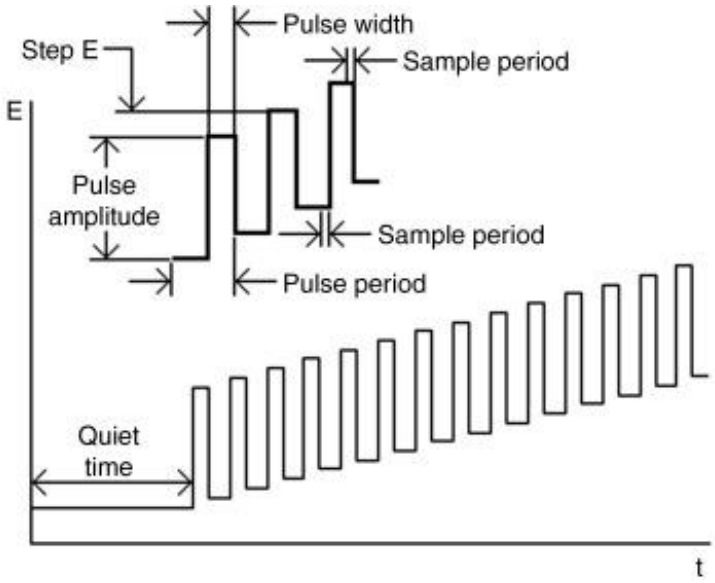



**Figure 21**: Diagram of differential pulse voltammetry[54]

### 4.4.1 Optimization of DPV Parameters

To achieve the best definition and current for the peaks of fentanyl that will be observed for DPV, the parameters that can impact the peak need to be optimized such as the interval time, and amplitude. Figure 22 shows that the optimization of two of the parameters for DPV were already performed. The interval times of 0.1 s, 0.2 s and 0.08 s were all tested, using a 30 mV amplitude, and in Figure 22a, the first of four scans, shows that all three interval times displayed peaks with sufficient definition at 0. 9 V vs. Ag/AgCl. Therefore, 0.2 s was the optimal interval time chosen, because it yielded the best return to baseline of all trials. This was also true for the fourth scan in Figure 22b, where the interval time of 0.2 s again had the best return to baseline, indicating it was the optimal interval time of those attempted. The amplitude of the pulse was also explored between the values of 20 to 50 mV, using 0.2 s as the interval time. Figure 22c shows that for the first scan, the best definition and peak current for the fentanyl oxidation at 0.9 V vs. Ag/AgCl is the result of an amplitude of 50 mV. The fourth scan also demonstrates that the greater peak current and better shape for the fentanyl oxidation peak at 0.9 V vs. Ag/AgCl is maintained best for an amplitude of 50 mV (Figure 22d). The metabolite peaks in Figure 22d at ~0 V vs. Ag/AgCl all are clearly visible for all attempted amplitudes, but at 50 mV they had a greater current, which was also beneficial. The optimization of these two parameters for DPV already show a better definition to the peak than what was observed in SWV for fentanyl in PBS, as well as fentanyl in KCl. The optimal parameters were an interval time of 0.2 s with an amplitude of 50 mV. DPV appears as promising as SWV, or potentially even better than SWV if further optimized.

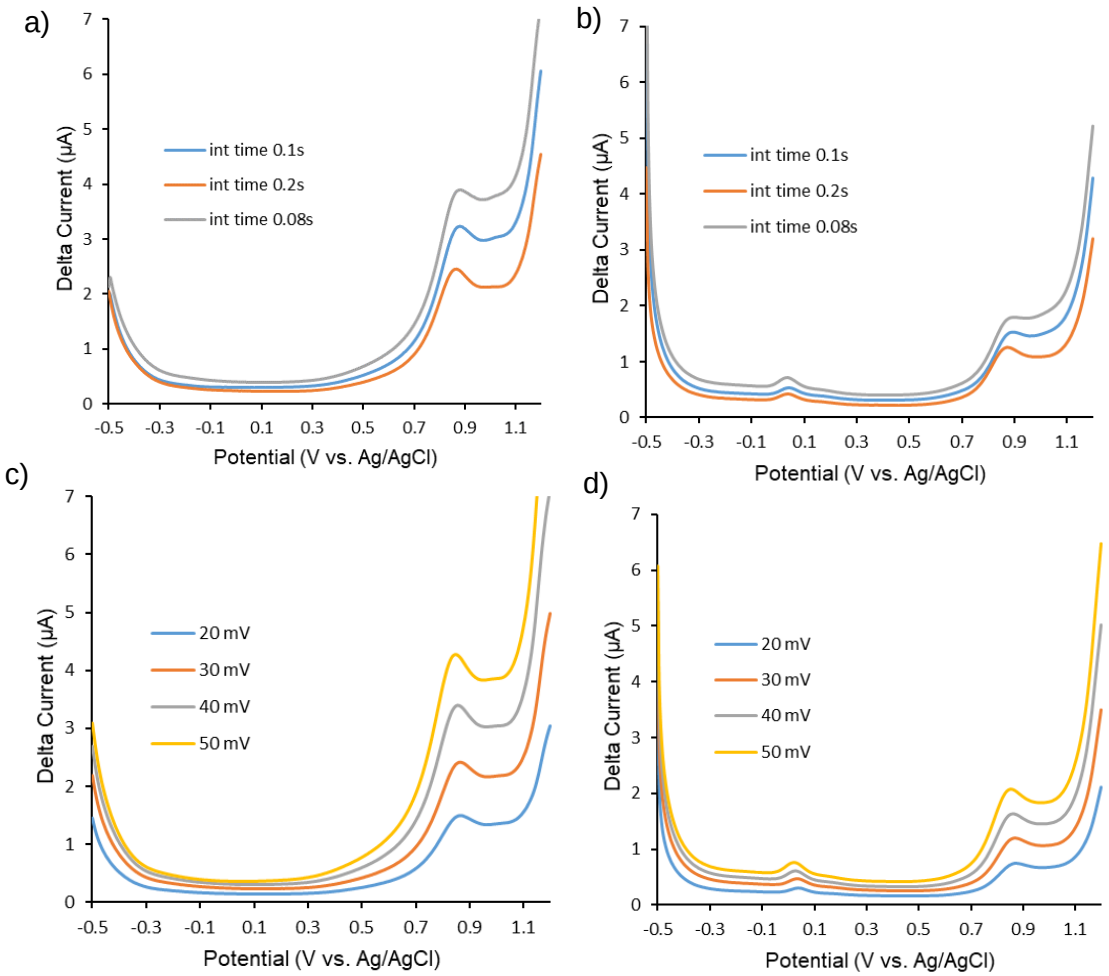



**Figure 22:** Optimization of the parameters of DPV for fentanyl in PBS, a) interval time, first scan, b) interval time fourth scan, c) pulse amplitude first scan, d) pulse amplitude fourth scan

## 4.5 Conclusion

In regards to the detection of fentanyl by SWV, the optimal step size was determined to be 0.004 V, the optimal amplitude was 30 mV, and the optimal frequency thus far was 15 Hz. Stirring the solution in a beaker between SWV scans resulted in better definition for peaks in scans 2 to 4, but did not improve the first scan, and is a method that is not very portable, and thus not pursued. The optimal parameters for DPV to detect fentanyl were an interval time was 0.2 s, with an optimal amplitude of 50 mV. Both electrochemical methods were promising in particular regarding the low number of scans required to see the appearance and disappearance of the peaks. Further parameter optimization will be pursued, especially for DPV.

# CHAPTER 5. ALTERNATIVE ELECTROCHEMICAL SET UPS

## 5.1 Introduction

In the previous chapters of this work, the electrochemical setup used the typical semi-micro electrodes, with glassy carbon as the WE, Ag/AgCl as the RE and Pt wire as the CE, and utilizes a 2-mL beaker filled with the solution to be analyzed. This set-up would not be convenient and applicable to in-field forensic work. There are several portable systems in electrochemistry, including hand held potentiostats,[55] that are easy to maneuver and transport to multiple locations. There has also been a boost in the development and commercialization of Bluetooth handheld potentiostats that interact with cellphones to communicate and interpret the results.[56-58] These portable systems typically use small microfluidic and electrochemical plastic strips. These strips can range from screen printed electrodes (SPEs) that are purchased commercially to electrochemical devices made in house.[59] As a smaller volume of solution is used with SPE, the three electroanalytical techniques were first performed on an intermediate unconventional set-up with the typical semi-micro electrodes and a drop of solutions. These results allowed for easy comparison between data on SPE and on semi-microelectrodes. The preliminary trials with the SPE displayed some additional redox peaks and different tests were performed to understand their cause. A cleaning step was designed to remove the species linked to the problematic peaks. Finally, some additional data processing allowed for an optimal observation of the redox peaks relevant to the detection of fentanyl.

## 5.2 Experimental

### 5.2.1 Materials and Reagents

Fentanyl ($C_{22}H_{28}N_2O$) (1 mg/mL) was purchased from Fisher. Potassium chloride (KCl) was purchased from Fisher BioReagents. Phosphate buffered saline solution ($Cl_2H_3K_2Na_3O_8P_2$) (PBS) was purchased from Fisher. Carbon screen printed electrodes were purchased from CHI Instruments Glassy carbon electrodes, Ag/AgCl reference electrodes, and Pt wire electrodes were purchased from CHI Instruments. Potassium ferrocyanide trihydrate ($K_4[Fe(CN)_6]\cdot 3H_2O$) was purchased from Acros Organics.

### 5.2.2 Solution Preparation

A solution of 100 µM fentanyl was prepared using 1 mg/mL fentanyl, which equates to 2.9 mM fentanyl, then prepared with the desired supporting electrolyte. This solution preparation was followed for each 100 µM solution of fentanyl unless otherwise specified. PBS 1 X was made by diluting the 10 X stock with milliQ water. A solution of 0.1 M KCl, were prepared with milliQ water. 5 mM potassium ferrocyanide trihydrate was prepared using 0.1 M KCl as the supporting electrolyte unless otherwise specified.

### 5.2.3 Electrochemical Measurements

The electrochemical methods of CV, SWV, and DPV were all undertaken using an Autolab PGSTAT204 potentiostat with Electrochemical Impedance Spectroscopy FRA2 module, with Nova 2.0 software. The potential range used for fentanyl for the CV was 1.2 to -0.5 to 1.2 V for PBS. The scan rate was 50 mV/s. The parameters for SWV were a step size of 0.004 V, an amplitude of 30 mV, a frequency of 15 Hz and a calculated scan rate of 59 mV/s. The parameters for DPV were a step size of 0.005 V, a modulation time of 0.05 s, an interval time of 0.2 s, a modulation amplitude of 30 mV, and a calculated scan rate of 25 mV/s. The working electrode was glassy carbon, with a reference electrode of Ag/AgCl, and a counter electrode Pt wire for the drop method set up. Screen printed electrodes were utilized otherwise. The cleaning scan for the SPE was optimized for SWV over the potential range of -1.5 to 1.5 V, with varying smaller potential windows tested as well.

## 5.3 Voltammetry in Smaller Volumes: The Drop Method

As SPEs would ideally be used in a later stage of the project, the test volume will be smaller. To compare and use the electroanalytical measurements proposed so far in this work and the future measurements on SPEs, an intermediate system permitting to analyze smaller volumes of solution, while still using the standard three electrodes was developed. In this set-up, referred to as the “drop method’, the electrodes were positioned in a manner such that a drop of solution could be placed on the section of the WE, while forming contact with the RE positioned above (Figure 23a). The drop of solution (e.g., 60 µL) is extended to a cylindrical shape instead of the typical hemispherical shape expected on a surface by capillary action with the base of the RE. The

drop can then be pulled vertically to generate enough space for the CE to be placed through the center of the drop, without touching either of the other electrodes (Figure 23b).

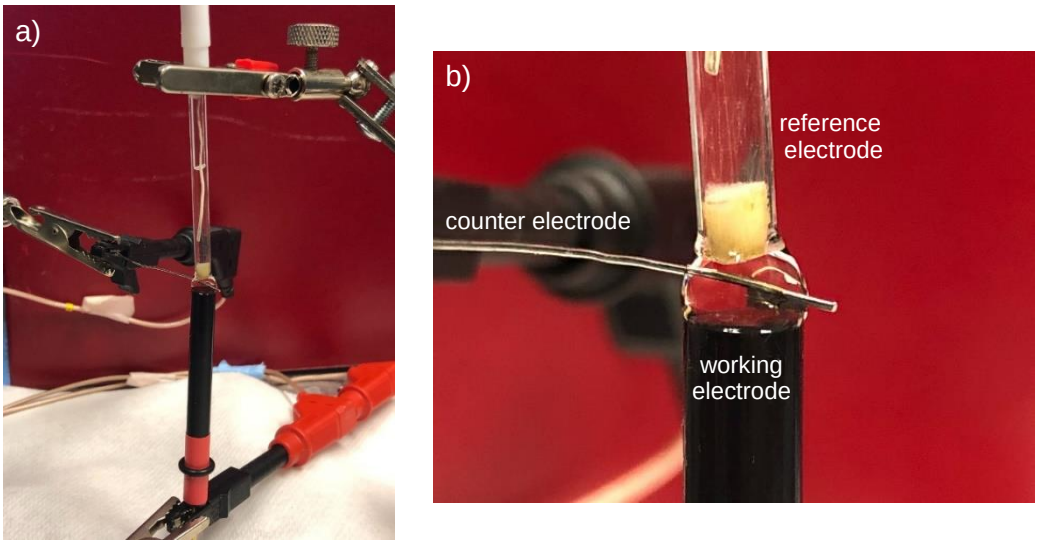



**Figure 23:** Photographs of the set-up to perform analysis in drop using the three standard electrodes showing the positioning of the connected electrodes (a) and a close up of the drop of solution between the WE and the RE with the CE running through the drop, without touching the other electrodes (b).

To validate the drop method, a solution of potassium ferrocyanide at a concentration of 5 mM in 0.1 M KCl was analyzed by CV using a 60 µL drop and with the standard set-up of the three electrodes dipped in a beaker containing 1 mL of the same solution (Figure 24). The two CV overlap and shows the expected peaks for potassium ferrocyanide, with an oxidation peak at ~0.3 V vs Ag/AgCl and a reduction peak at ~0.15 V vs Ag/AgCl. There was a slight increase in current and a smaller difference between the potential of the reduction peak and the potential of the oxidation peak, corresponding to a more optimal electrochemical behavior, with $E_{ox}$-$E_{red}$ being 0.14 V for the drop compared to 0.16 V for the beaker. This improvement could be attributed to the proximity and alignment of the three electrodes conferred by the drop method set-up, which was considered an optimal geometry. These results confirmed that the small volume and the non-obvious set-up did not impact the electrochemical analysis, and thus the drop method can be used to compare prior results with the new measurements performed on SPE.

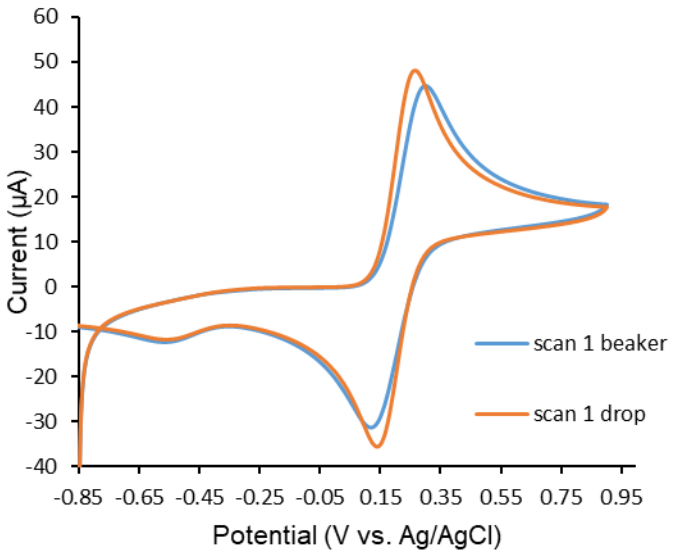



**Figure 24**: CV of potassium ferrocyanide in a 60-µL drop (orange) and in a beaker with 1 mL of solution (blue). Solution of potassium ferrocyanide at a concentration of 5 mM in 0.1 M KCl.

The three voltammetric techniques of interest in this work, CV, SWV, and DPV were then evaluated using a standard solution of 100 µM fentanyl, to compare the drop method with the typical measurements performed in a beaker (Figure 25). For all three electrochemical methods, there was no significant difference between the results obtained using the 60 µL drop of fentanyl compared to the larger volume with electrodes dipped in the beaker. The fentanyl oxidation peak was still observed at ~0.90 V vs Ag/AgCl for all three electrochemical methods no matter the set-up. These results further confirmed that the drop method allowing for a small volume to be analyzed is a viable intermediate system to bridge the standard voltammetric measurements and the measurements on SPE.

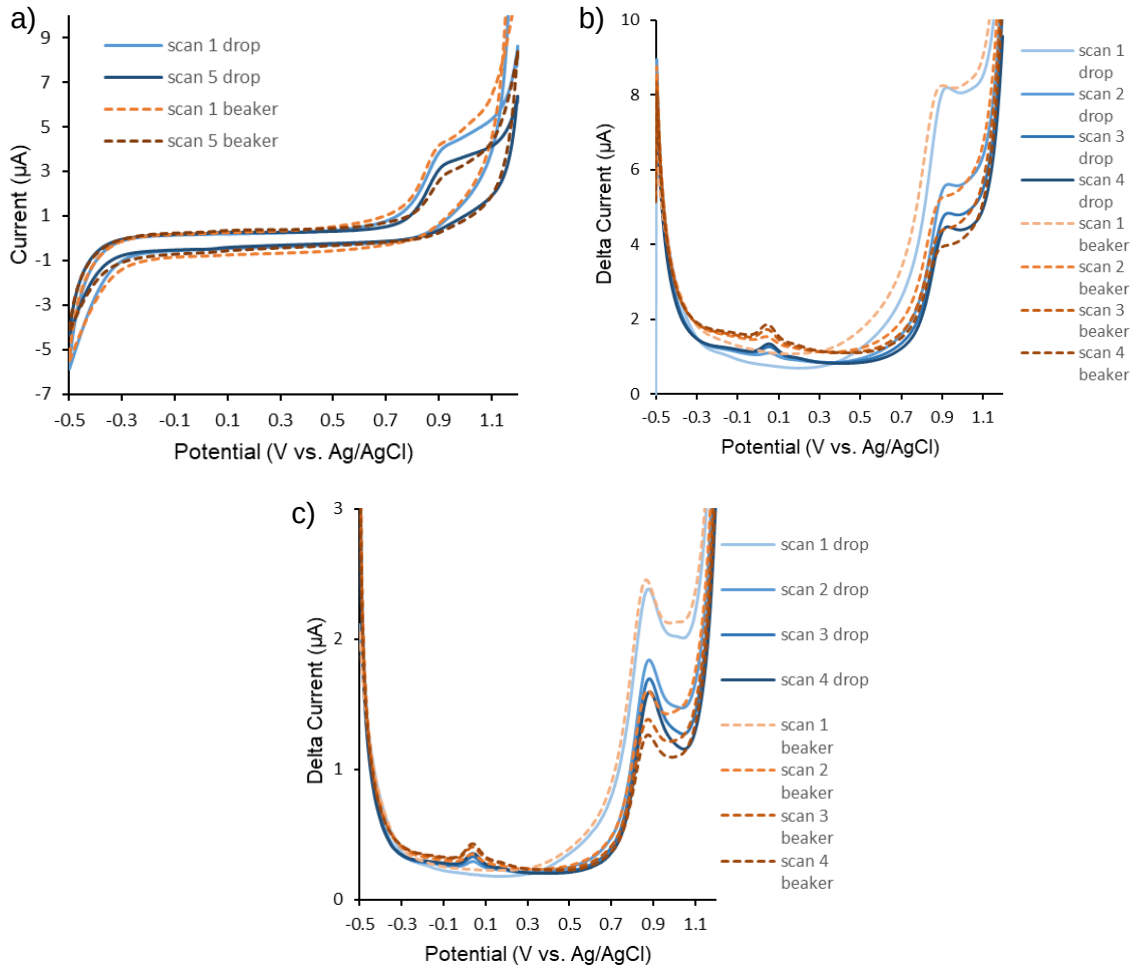



**Figure 25:** Comparison of voltammetric measurements using a beaker (dashed lines) or the drop method (solid lines): CV (a), SWV (b), and DPV (c). Solution of fentanyl at a concentration of 100 µM in PBS, 60 µL for the drop and 1 mL in the beaker.

## 5.4 Voltammetric Analysis with Screen Printed Electrodes

### 5.4.1 Preliminary experiments using SPEs

Screen printed electrodes (SPEs) are nowadays commonly used in electroanalytical chemistry in particular for sensing applications[60-61] and when small volumes of samples are required, and disposability is beneficial, such as for biological analyses.[62-63] The interest for using SPE in this project relies on both the portability of the analysis and the ability to test smaller volumes. The screen printed electrode (Figure 26a) is a device that has all three necessary

electrodes, being the WE, the RE, and the CE, printed on the surface, aiding in the portability and disposability of the device. Both the working and the counter electrode are comprised of graphite paint, while the reference is done in Ag/AgCl paint. The Deiss group has been developing a particular type of those electrodes on tape and paper with added functionality (Figure 26b), and routinely compares results to commercial SPEs. Typically, carbon-based SPEs yield similar voltammograms than GCE. The CVs of fentanyl on SPE, however, show an unexpected reduction peak at 0.1 V vs Ag/AgCl and a small oxidation peak at 0.35 V vs Ag/AgCl developing over the successive scans (Figure 27a) that were not visible on the scan 1. Performing a CV in only PBS confirmed that this peak is due to the supporting electrolyte/electrode system and not the fentanyl itself, as Figure 27b shows the same peak also developed in PBS on SPE whereas it was not present on GCE, with the peak developing as the five scans in PBS progressed (Figure 27c). The presence of these peaks can be problematic, as they develop in the same range of potentials, -0.1 to 0.1 V vs Ag/AgCl, where the metabolite peaks for fentanyl would be expected, rendering their visualization difficult. Additionally, the peak at 0.85 V vs Ag/AgCl corresponding to the oxidation of fentanyl is not as well defined on SPE (Figure 27a) as previously observed on GCE (Figure 10c in Chapter 3).

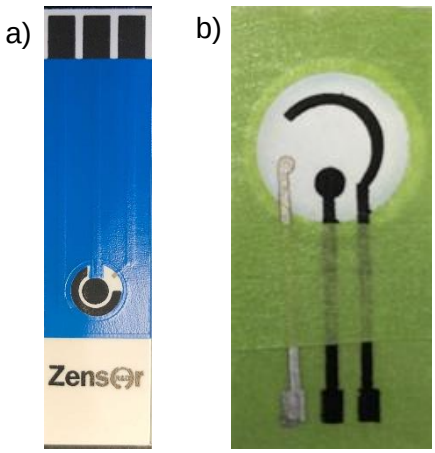



**Figure 26**: a) Screen Printed Electrode, b) paper-based device

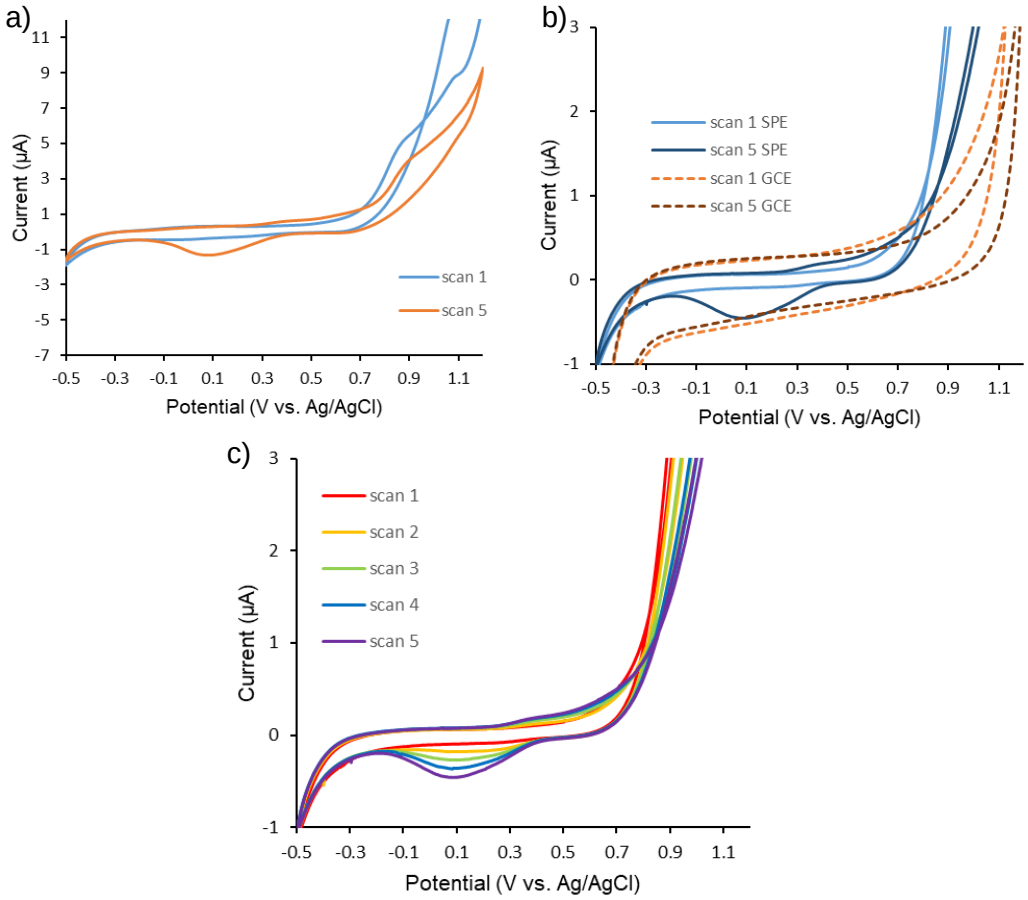



**Figure 27:** a) CVs on SPE (solid lines) and on GCE (dashed lines) of PBS (blank), and b) CV on SPE of 100 µM fentanyl in PBS. c) PBS scans 1-5 as peak develops

Focusing on the major unexpected peak at 0.1 V vs Ag/AgCl on the reduction wave, its absence in the first scan indicated that it was not correlated to an electroactive species originally present in the solution or on the SPE, but rather an electroactive species formed as scans progressed. To help determining which element of the electrochemical cell participate in the generation of the observed reduction, a lab-made stencil-painted paper-based device was utilized. These paper-based devices are designed and fabricated in the Deiss group, and this particular device was run by an experienced user. Similar to the SPE, the paper-based device consisted of three electrodes: a working electrode and a counter electrode made of graphite, and a pseudo-reference electrode made of silver-silver chloride. The electrodes are painted using a stencil on a paper-and-tape substrate. By using a lab-made device over the commercial SPE, the components of the electrochemical system and in particular the electrodes are well known. Figure 28 shows

that the electroanalytical paper-based device, similarly to the GCE, did not yield an additional reduction peak at 0.1 V vs. Ag/AgCl observed on the SPE. This result indicated that the additional reduction peak observed when using PBS as a solvent for analysis was particular to the commercial SPE and not general to any painted/printed carbon and silver-silver chloride electrodes.

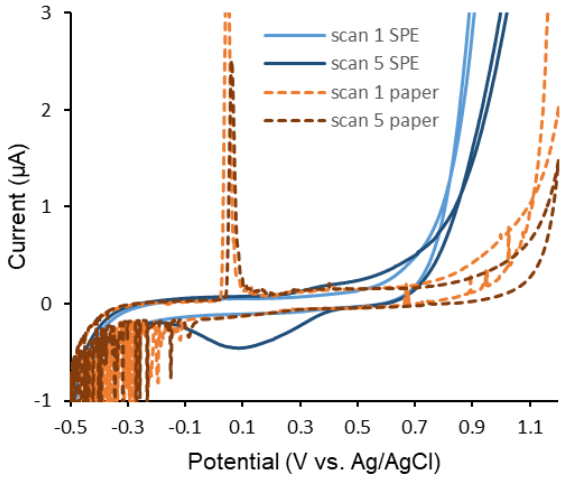



**Figure 28:** CVs on SPE (solid lines) and on paper-based devices (dashed lines) of PBS.

It was necessary to determine if this additional peak would also develop when using SWV or DPV instead of CV. SWV and DPV are often considered electroanalytical method of choice for the end application of sensitive quantification, because standard CV can be considered a slow voltammetric technique relative to SWV or DPV. Indeed, scanning over the range 1.2 to -0.5 V and back, takes around 68 s with a scan rate of 50 mV/s, whereas in SWV with a scan rate of 59 mV/s the same range would be covered in 28 s. As SWV also typically requires a smaller number of scans, the duration of the experiment is greatly decreased. As seen in previous chapters, SWV and DPV are useful techniques to lesser the background effects of the electrochemical cell such as capacitive current. Figure 29 shows that for SWV, no peak appeared at 0.1 V vs. Ag/AgCl, but, two other electrochemical peaks were observed at 0.7 V vs. Ag/AgCl and 1.1 V vs. Ag/AgCl, both for fentanyl in PBS and only PBS. The peak at 0.7 V vs. Ag/AgCl was present only in the first scan, indicating that it could be due to a residual chemical on the electrode that is easily removed by the potential sweep. The peak at 1.1 V vs. Ag/AgCl was observed for all three scans, but with a decreasing current at each subsequent scan. As both peaks displayed a decreasing or disappearing

current at each successive scan, the electroactive species associated to the observed redox reactions must be residual species adsorbed on the electrodes, and thus could potentially be removed by pre-treatment of the electrodes. Those peaks were not clearly observed by CV as they most likely overlapped with the oxidation peak of fentanyl, yielding the poorly defined oxidation "wave" observed in Figure 27b. SWV typically allows for a better resolution and decorrelation of close peaks, which is observed in these results with the oxidation peak of fentanyl at 0.85 V vs Ag/AgCl distinguishable from the two residual redox peaks. SWV can thus be used to analyze fentanyl solutions on commercial SPE.

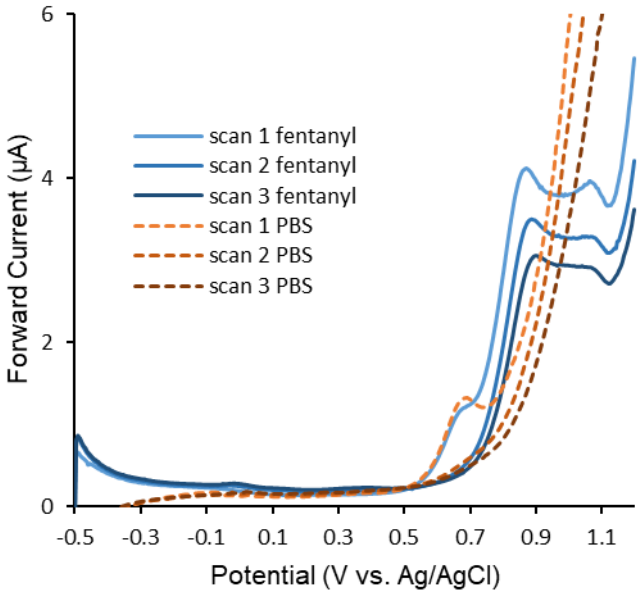



**Figure 29:** SWV (delta current), scan 1 to scan 3, of 100 µM fentanyl in PBS (dashed lines) and PBS (solid lines) on SPEs

### 5.4.2 Optimizing the Cleaning Step for the SPE

Since the additional peaks on the SPE are present from the initial scans and could be due to residual chemicals, performing a cleaning scan, also called preconditioning scan, of the SPE in the supporting electrolyte could help. Figure 30 demonstrates that utilizing the same SPE for sequential SWVs in PBS decreases or removes the additional peaks observed in the first SWV on a specific SPE. When three SWVs were taken on the same SPE, the additional peak at 0.7 V vs.

Ag/AgCl was not present in the following three scans of the first SWV, nor in any of the scans in the subsequent two additional SWVs collected with that SPE. The additional peak at 1.1 V vs. Ag/AgCl was present in all four of the scans of the first SWV, but decreased in intensity as the scans progressed, and then disappeared for the two additional SWVs collected with the same SPE.

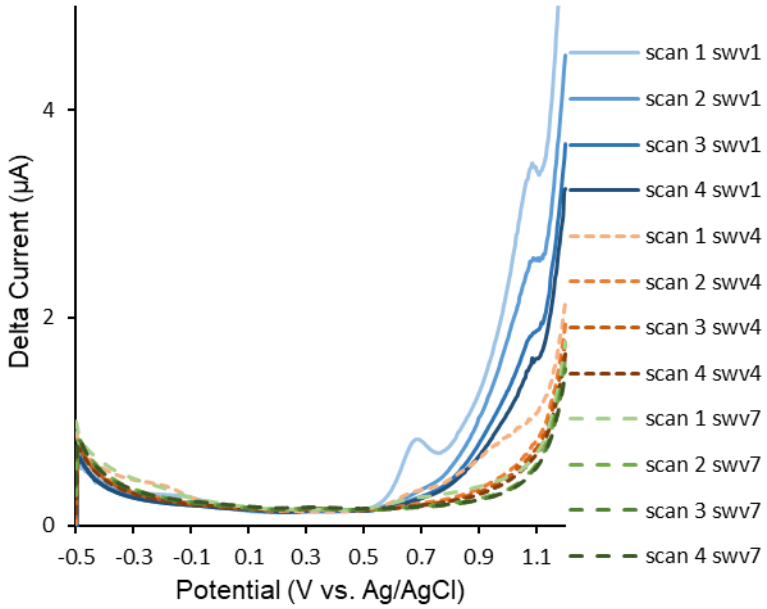



**Figure 30**: Three SWVs for PBS, SWV1 (blue) first SWV on the SPE, SWV4 (orange) second SWV on the same SPE, SWV7 (green) third SWV on same SPE

This study was repeated in triplicate using three individual SPEs in PBS, and similar results were obtained (Figure 31). Additionally, the overlay of the different SWV for each SPE demonstrates that the resulting currents from the SPEs is very reproducible across multiple devices. It was demonstrated that after the first scan of the first SWV the peak at 0.7 V vs. Ag/AgCl is largely decreased and no longer a concern, and after the first SWV, the peak at 1.1 V vs. Ag/AgCl is no longer present in subsequent SWVs.

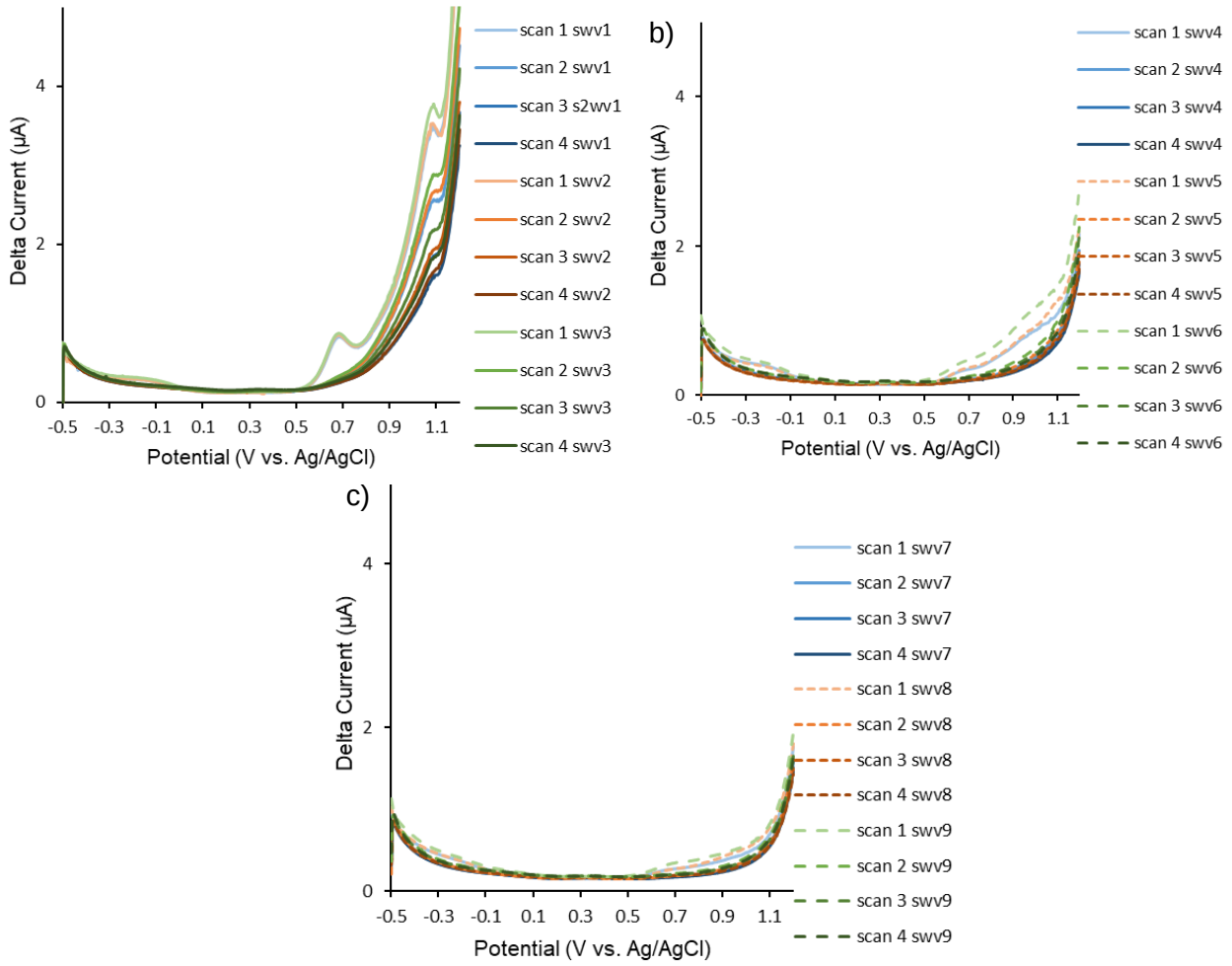



**Figure 31:** a) overlay of the first SWV on three individual SPEs, b) overlay of second SWV on same SPEs as (a), c) overlay of third SWV on same SPEs as (a,b), blue-singular SPE, orange-singular SPE, green-singular SPE

Since the peak present on the SPE at 0.7 V vs. Ag/AgCl is removed after the first scan on the SPE, performing a single scan cleaning step on the SPE compared to a four scan SWV would be more time beneficial for removing the additional peaks. Figure 32 demonstrates that instead of performing an entire SWV of four scans on the SPE prior to using it to obtain additional data, that a single cleaning scan can be utilized to remove the additional peaks at 0.7 V vs. Ag/AgCl and 1.1 V vs. Ag/AgCl. Figure 32a shows the initial potential range used for the cleaning step with a sweep from -1.5 to 1.5 V vs. Ag/AgCl in SWV using PBS. Figure 32b shows that after a cleaning step on an SPE, the additional peaks are removed for both PBS (orange) and fentanyl (green). The removal of the additional peaks in fentanyl, leave a clearly defined fentanyl oxidation peak at 0.87 V. vs.

Ag/AgCl. This oxidation peak is clearly visible, and less merged with the additional peak at 1.1 V vs. Ag/AgCl (Figure 32 c).

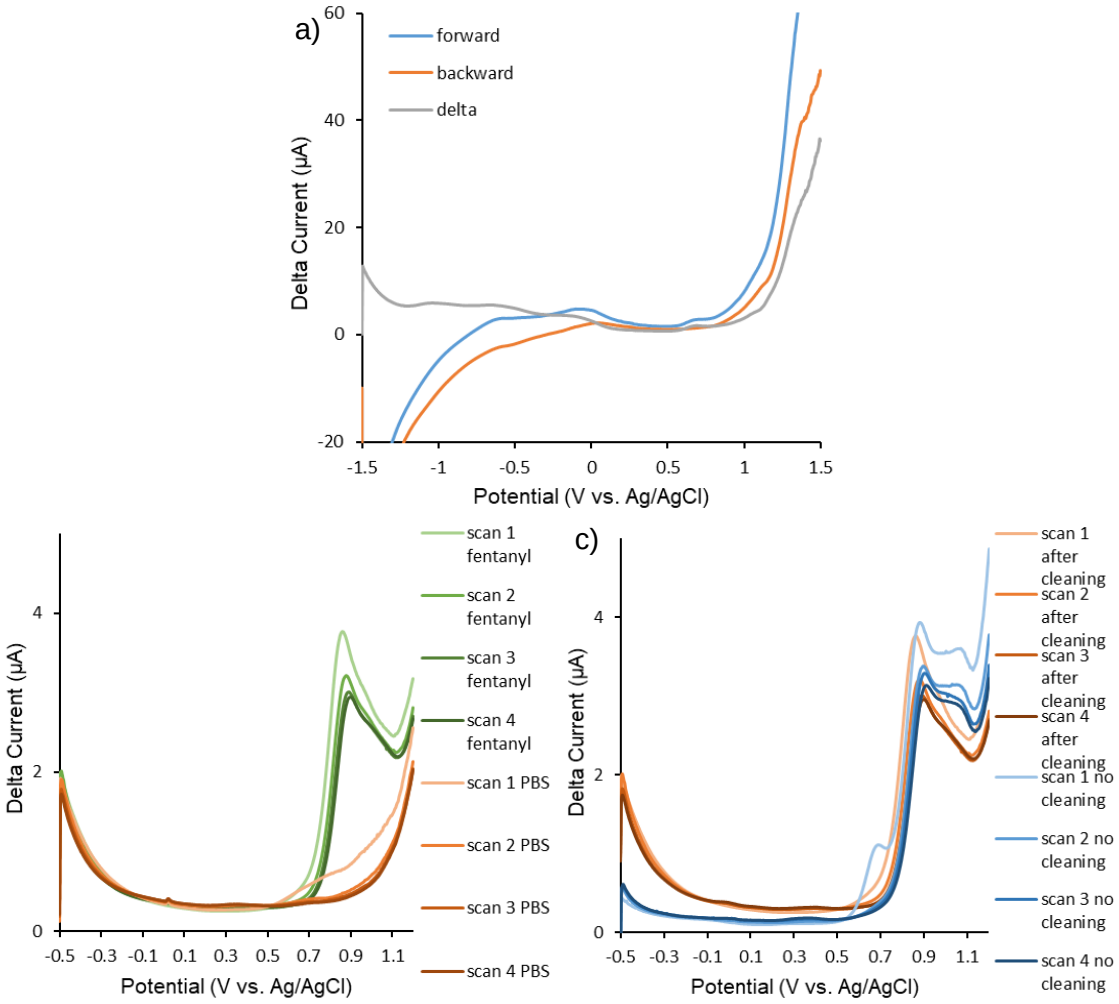



**Figure 32:** a) PBS cleaning step from -1.5 V to 1.5 V, b) fentanyl at a concentration of 100 µM after a cleaning step (green) overlaid with PBS after a cleaning step (orange) on two individual SPEs, c) comparison of fentanyl on SPE with (orange) and without cleaning (blue)

The cleaning step was then optimized to the best potential range, i.e., the shortest sweep in potential still efficient to prepare the electrodes. To remove the additional peak at 1.1 V vs. Ag/AgCl, the potential range for the cleaning step needs to reach the potential of 1.5 V (Figure 33). Figure 34 shows that when removing the additional peak at 0.7 V vs. Ag/AgCl, the lower potential does not need to be specified, as any cleaning step range attempted decreased this peak greatly compared to no cleaning step (black). In summary, the cleaning step of a single scan in

SWV removed the peak at 1.1 V vs. Ag/AgCl as long as the upper oxidative potential reached 1.5 V, while the peak at 0.7 V vs. Ag/AgCl was removed with any potential applied.

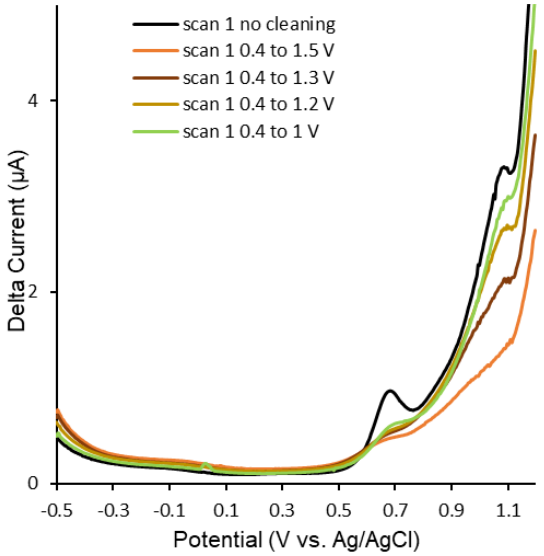



**Figure 33:** Optimization of the upper potential for cleaning step for SPE in PBS to remove peak at 1.1 V vs. Ag/AgCl

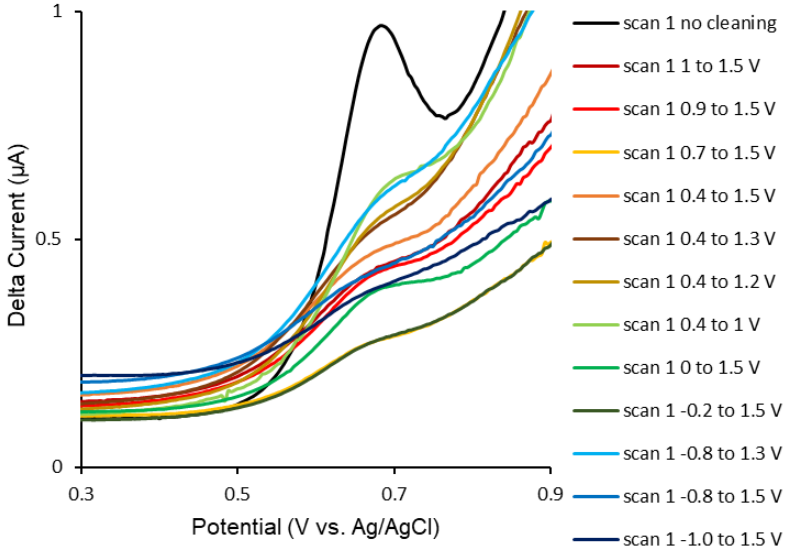



**Figure 34:** Optimizing of the cleaning step to remove peak at 0.7 V vs Ag/AgCl in PBS

### 5.4.3 Increasing the Number of Scans for Metabolite Visualization

The peaks which are thought to correspond to the metabolites from the oxidation of fentanyl which were visible after four scans of SWV on the GCE were not as clearly defined after three scans on the SPE. Increasing the amount of scans utilized for SWV on the SPE could help observe the metabolite peaks for fentanyl. Figure 35a demonstrates that the baseline current for both fentanyl and PBS are consistent with each other on SPE. This confirmation allowed us to subtract the current for the PBS from the current of fentanyl, and plot Figure 35b. Figure 35b displays a large defined peak for fentanyl at 0.87 V vs. Ag/AgCl, as well as small potentially metabolite peaks at 0 V vs. Ag/AgCl.

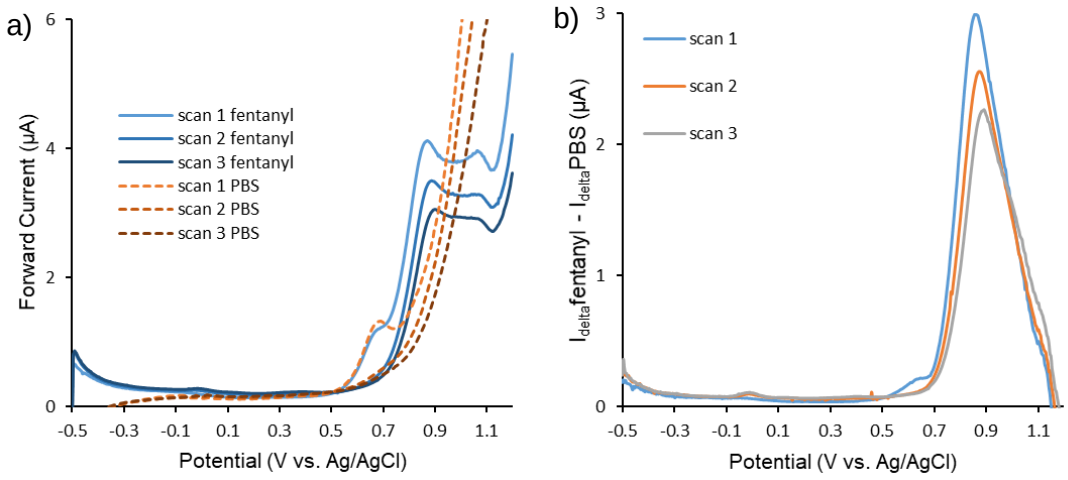



**Figure 35:** a) SWV three scans of fentanyl at a concentration of 100 µM overlaid with PBS blank, b) current of fentanyl with current of PBS subtracted from it

As the metabolite peaks were very small and difficult to visualize, the number of scans was increased. Figure 36a demonstrates that increasing the number of scans to 10 for both fentanyl and PBS did not make the metabolite peaks more visible on the SPE. Figure 36b shows that subtracting the PBS current from the fentanyl current shows a large fentanyl oxidation peak at 0.87 V vs. Ag/AgCl, similar to after three scans, and figure 36c, which is a magnified plot of the metabolite region for the subtraction of the PBS current from the fentanyl current, shows that a small peak is indeed visible at 0 V vs. Ag/AgCl. This small peak appeared to increase in current intensity as scans progressed. This was also observed previously on GCE but at a lesser scale.

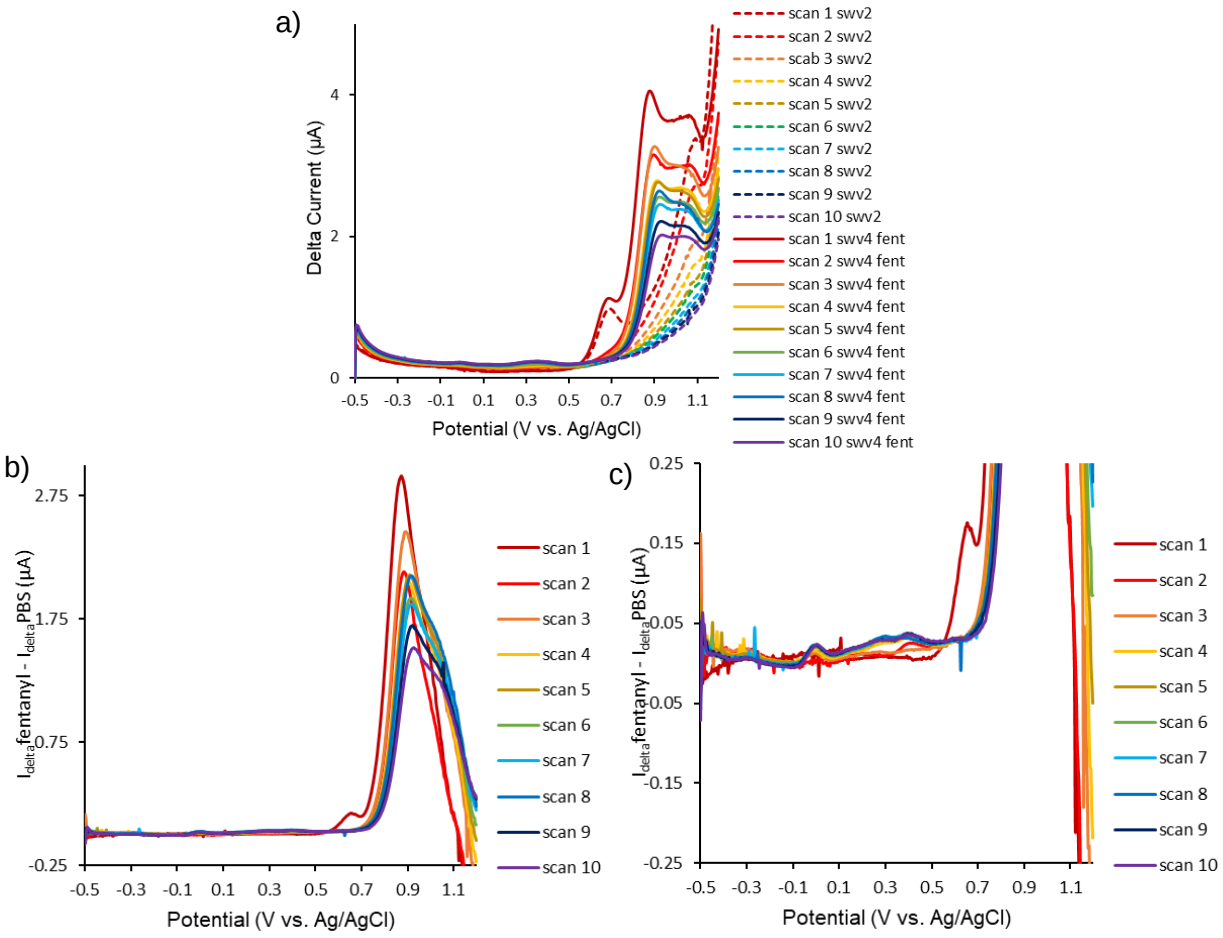



**Figure 36:** a) SWV on SPE fentanyl at a concentration of 100 µM in PBS overlaid with PBS blank, b) Current of fentanyl with current of PBS subtracted from it, c) zoom of metabolite region for (b)

Figure 37 shows that using the cleaning step prior to both fentanyl and PBS may improve the visualization of the metabolite peak without needing to subtract the current of the blank from the current of fentanyl. Figure 37a, which is a 10 scan SWV of fentanyl overlaid with PBS, shows the clear fentanyl oxidation peak at 0.87 V vs. Ag/AgCl without the additional peaks, and a magnified plot of the metabolite region (Figure 37b) shows that at 0 V vs. Ag/AgCl a clear peak is developing which was not present in the first scan of fentanyl or PBS. This peak also does not develop at all for PBS. Figure 37c shows the fentanyl current with the current of PBS removed from it, showing the clear fentanyl oxidation peak, similar to what was observed in figure 37b. The zoom of the metabolite region in figure 37d shows that at 0 V vs. Ag/AgCl a peak is present, which is not in the first scan, indicating that the metabolite peak is visible on the SPE. The

metabolite peak for fentanyl that was clearly observable on the GCE in SWV has not been as observable on the SPE for SWV. Increasing the number of scans taken in SWV, as well as utilizing a cleaning step to remove additional unwanted peaks slightly helped in the visualization of the metabolite peaks on SPE, but there is still a need for optimization for better visualization.

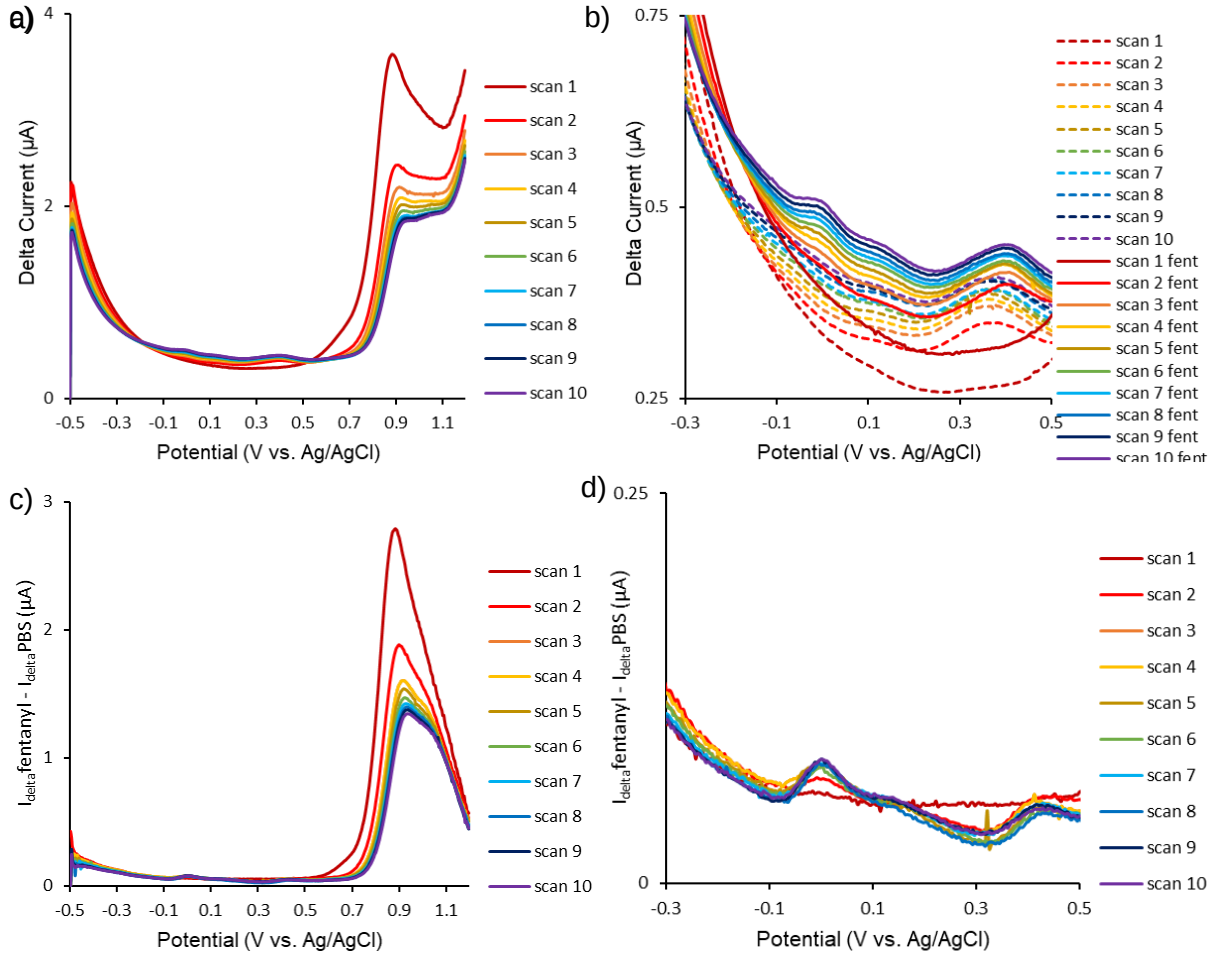



**Figure 37:** a) SWV 10 scans of fentanyl at a concentration of 100 µM overlaid with PBS after a cleaning step, b) zoom of metabolite region for (a), c) current of fentanyl with current of PBS subtracted d) zoom of metabolite region for (c)

## 5.5 Conclusion

Two types of set-up were used to analyze fentanyl for a smaller volume of solution (~60 µL) and demonstrated that the change in volume would not drastically alter the shape or definition of

the fentanyl oxidation peak or metabolite peaks. For SPE a cleaning step was necessary to remove residual species that were causing additional unwanted peaks in the SWV. The supposed metabolite peaks that were clearly visible in SWV when using the GCE were not as visible on the SPE, but using ten scans instead of four improved slightly the visualization. Further optimization is still needed to characterize the metabolite peaks.

# CHAPTER 6. CHALLENGES TO OBSERVE METABOLITE PEAKS

## 6.1 Introduction

The additional electrochemical peaks that develop after the initial irreversible fentanyl oxidation are thought to be the peaks of norfentanyl, one of the metabolites of fentanyl. As discussed in Chapter 2, the metabolic reaction of fentanyl is an irreversible N-dealkylation[12] to form norfentanyl, and could be mimicked by the galvanic oxidation of fentanyl. Goodchild S, *et al.*[17] and Sohouli, E. *et al.*,[37] have both reported additional peaks in their electrochemical analysis of fentanyl that were not observed in the initial scan. Goodchild, S. *et al.* identified these additional peaks as the metabolite norfentanyl. [17] Ott, C.E, *et al.* performed LC-MS-MS analysis on samples taken after they were used electrochemically, and identified norfentanyl in the solution, lending to the belief that the electrochemical reaction of fentanyl forms norfentanyl in solution.[36] When we tried to observe the redox peaks of norfentanyl, however, we encountered some difficulties. The initial norfentanyl derivative attempted did not display any redox peaks. After not observing these expected peaks for norfentanyl, multiple other derivatives were tested with similar results. Some reports in literature identified norfentanyl as the redox species produced by the oxidation of fentanyl. Several methods to confirm this result in our experiments were performed. They involved (i) exploring the effect of polishing the electrode to determine if the species were developed near the WE surface, (ii) using the same aliquots of fentanyl multiple times to assess if the metabolite would be observed in a re-used solution, (iii) verifying which additional peaks also develop with other analytes and thus determining if the redox peaks were due to the electrochemical system itself or the presence of norfentanyl. Furthermore, additional methods were attempted to improve the peak visualization, such as using room temperature ionic liquids, but did not yield the desired response.

## 6.2 Experimental

### 6.2.1 Materials and Reagents

Fentanyl ($C_{22}H_{28}N_2O$) (1 mg/mL) and Nor-fentanyl-D5 oxalate ($C_{16}D_5H_{17}N_2O_5$) were purchased from Fisher. Potassium chloride (KCl) was purchased from Fisher BioReagents.

Phosphate buffered saline solution ($Cl_2H_3K_2Na_3O_8P_2$) (PBS) was purchased from Fisher. Norfentanyl monohydrate ($C_{14}H_{20}N_2O\cdot H_2O$) and Norfentanyl oxalate ($C_{14}H_{20}N_2O\cdot C_2H_2O_4$) were purchased from Sigma Aldrich. N-phenyl-N-(4-piperidinyl)propanamide ($C_{14}H_{20}N_2O$) mixture with HCl salt was purchased from Acros Organics. Glassy carbon electrodes, Ag/AgCl reference electrodes, and Pt wire electrodes were purchased from CHI Instruments. Screen printed carbon electrodes were purchased from CHI Instruments. Potassium ferrocyanide trihydrate ($K_4[Fe(CN)_6]\cdot 3H_2O$) was purchased from Acros Organics. Hexamine ruthenium(III) chloride ($Ru(NH_3)_6Cl_3$) was purchased from Acros Organics. 1-Butyl-1-methylpyrrolidinium Bis-9-trifluormethanesulfonyl)imide ($C_{11}H_{20}F_6N_2O_4S_2$) was purchased from TCI Chemicals. Methanol ($CH_3OH$) was purchased from Fisher Scientific.

### 6.2.2 Solution Preparation

A solution of 100 µM fentanyl was prepared using 1 mg/mL fentanyl, which equates to 2.9 mM fentanyl, then prepared with the desired supporting electrolyte. This solution preparation was followed for each 100 µM solution of fentanyl unless otherwise specified. PBS 1 X was made by diluting the 10 X stock with milliQ water. A solution of 0.1 M KCl was prepared with milliQ water. Norfentanyl-D5 oxalate was also a 100 µM solution prepared from the 100 µg/mL ampules in a with milliQ water. Solutions of Norfentanyl monohydrate and N-phenyl-N-(4-piperidinyl)propanamide were prepared from powder to a 1 mg/mL ampule with methanol. From the 1 mL volume, the calculated volume for a 100 µM solution was prepared with milliQ water. Norfentanyl oxalate solutions were prepared using purchased ampules of 1 mg/mL norfentanyl oxalate in methanol. The correct volume for the desired concentration was prepared with milliQ water. Solutions of varying concentrations of ($Ru(NH_3)_6Cl_3$) were prepared using solid ($Ru(NH_3)_6Cl_3$) dissolved in the desired supporting electrolyte. Both 0.1 M KCl and 1.0 M KCl were prepared using milliQ water. The 100 µM solution of fentanyl prepared directly in ($C_{11}H_{20}F_6N_2O_4S_2$) was a 1 mL solution prepared with a micropipette in a vial. The solution of 5 mM ($K_4[Fe(CN)_6]\cdot 3H_2O$) prepared directly in ($C_{11}H_{20}F_6N_2O_4S_2$) was also a 1 mL solution of 1 mL RTIL. The ferrocyanide did not fully dissolve, true concentration is thus unknown. The varying ratios of ($C_{11}H_{20}F_6N_2O_4S_2$)/$CH_3OH$ were diluted with methanol, depending on the desired ratio v/v. The ratios tested were 1:100, 1:10, 1:50, pure RTIL, and 0.01% v/v RTIL to methanol. The solution of ($K_4[Fe(CN)_6]\cdot 3H_2O$) directly in the RTIL was also sonicated.

### 6.2.3 Electrochemical Measurements

The electrochemical methods of CV and SWV were undertaken using an Autolab PGSTAT204 potentiostat with Electrochemical Impedance Spectroscopy FRA2 module, and Nova 2.0 software. The potential range for CV varied depending on the supporting electrolyte, but was typically 1.0 to -0.5 to 1.0 V when the supporting electrolyte was KCl or NaCl, and 1.2 to -0.5 to 1.2 V when the supporting electrolyte was PBS unless otherwise stated. The potential range for ($Ru(NH_3)_6Cl_3$) varied depending on the experiment between 0.5 to -0.5 to 0.5 V, or the range used for fentanyl and norfentanyl for the supporting electrolyte. The scan rate was 50 mV/s unless otherwise indicated. The potential range for the SWV of norfentanyl varied depending on the experiment and also depended on the supporting electrolyte. The range of -0.5 to 1.2 V was used when the supporting electrolyte was PBS unless otherwise indicated. The potential range of -0.5 to 1.0 V was used when the supporting electrolyte was KCl or NaCl, unless otherwise indicated. The parameters for SWV were a step size of 0.004 V, an amplitude of 30 mV, a frequency of 15 Hz, with a calculated scan rate of 59 mV/s unless otherwise indicated. The drop method set up using the GCE working electrode, the Ag/AgCl reference electrode, and the Pt counter electrode was utilized for the solutions of fentanyl and potassium ferrocyanide in the RTIL alone. SPEs were utilized when a varying ratio of RTIL in 1 µL volumes was applied to the surface of the WE. The potential range used for potassium ferrocyanide trihydrate was -0.85 to 0.9 to -0.85 V with a scan rate of 50 mV/s. For SWV, the potential range used for potassium ferrocyanide was -0.85 V to 0.90 V, with a step size of 0.004 V, an amplitude of 30 mV, a frequency of 15 Hz, and a calculated scan rate of 59 mV/s unless otherwise specified. The working electrode was glassy carbon, with a reference electrode of Ag/AgCl, and a counter electrode Pt wire, except in the use of the RTIL which utilized an SPE.

## 6.3 Electrochemical Results Norfentanyl

### 6.3.1 Preliminary Analysis of Norfentanyl

If the peaks observed at ~ -0.1 V vs. Ag/AgCl after the initial scan of fentanyl would have been due to norfentanyl, then those peaks should have been observable by CV of a solution of norfentanyl-d5-oxalate at a concentration of 100 µM for any scans, including the initial scan. There

were no identifiable peaks around -0.1 V vs. Ag/AgCl present when analyzing norfentanyl-d5-oxalate (Figure 38).

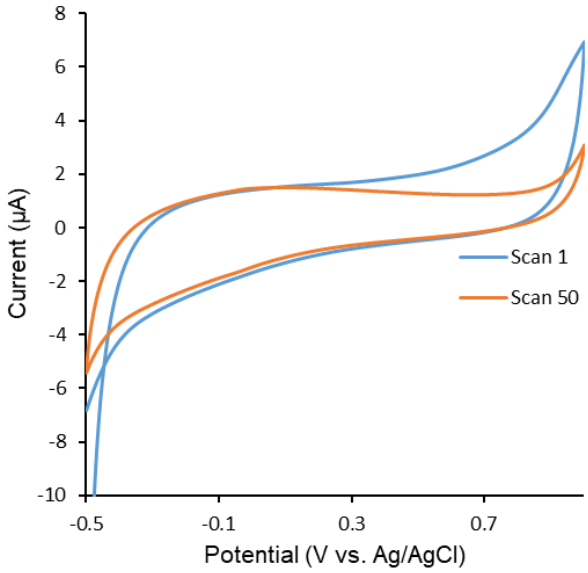



**Figure 38:** 50 µM norfentanyl-D5-Oxalate in 0.1 M KCl on GCE

### 6.3.2 Testing Different Norfentanyl Derivatives

The lack of peaks could indicate that the form of norfentanyl (d5-oxalate) used may not have been the expected form of the metabolites produced by the oxidation of fentanyl. Therefore, other derivatives of norfentanyl should be attempted. Figure 39 shows that the use of other norfentanyl derivatives resulted in the development of additional peaks: Norfentanyl oxalate (blue), norfentanyl monohydrate (orange), and norfentanyl with HCl salt (green) all resulted in the development of an additional peak at ~ 0 V vs. Ag/AgCl in PBS. This potential was closed to the potentials of the peaks corresponding to the supposed metabolite of fentanyl observed previously. These peaks, however, are not present in the initial scan, but develop as subsequent scans are performed (Table 4). If the peaks were associated to norfentanyl itself, they would be expected to be observed from the initial scan. All the derivatives were displaying similar behavior and that the identity of the species corresponding to those peaks is still unknown and more peculiar. The species

could be a product of an additional reaction undergone by norfentanyl or another unidentified reaction in solution, or at the surface of the working electrode.

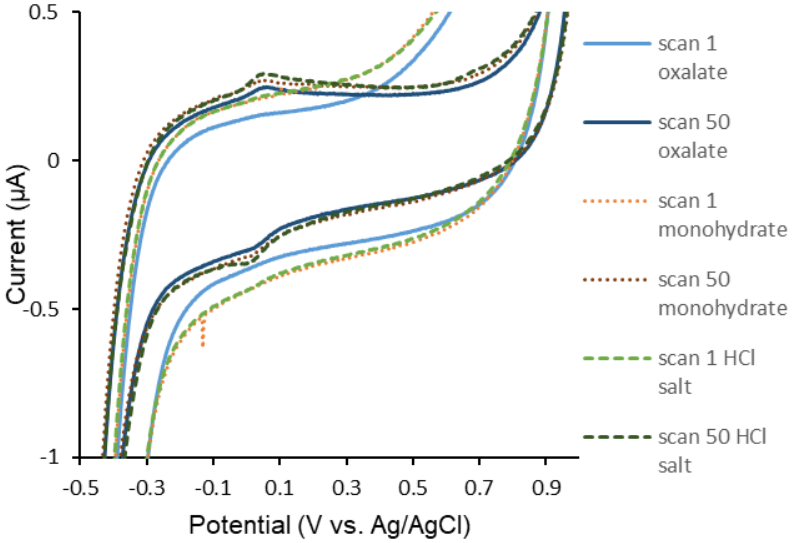



**Figure 39:** Norfentanyl oxalate (blue), norfentanyl monohydrate (orange), and norfentanyl HCl salt (green) all at concentrations of 100 µM in PBS on the GCE

### 6.3.3 How Does Polishing Affect the Metabolite Peak?

In electrochemistry the surface of the working electrode is where the relevant half reactions occur, so it is critical that this surface be free of defects, and avoid fouling as they would impact the electrochemical response. One of the main methods to prepare the working electrode between runs is to polish the surface.[42] This polishing is typically done by moving the working electrode parallel to a polishing pads with an alumina slurry or paste, moving the electrode in a figure eight configuration to even the surface of the electrode. The step of polishing removes substances that may have adhered or adsorbed to the surface of the WE during electrochemical analysis and renew the bare surface of the electrode.[42] Since the additional peaks that develop for the norfentanyl derivatives are not present in the initial scan, but develop as the scans progress, this could be an indication that something is adhering or affecting the surface of the electrode.

We thus compared multiple scans of norfentanyl taken in succession and multiple scans of norfentanyl taken individually, while polishing between each scan. Figure 40 shows that when taking the scans together without polishing (orange) the additional peak does develop at ~ 0 V vs. Ag/AgCl. When the four scans are taken individually while polishing the WE between each scan,

the peak does not develop (blue). This result indicates that the peak developing may be due to a species developing at the surface or in close proximity of the WE as the scans progressed (Table 4).

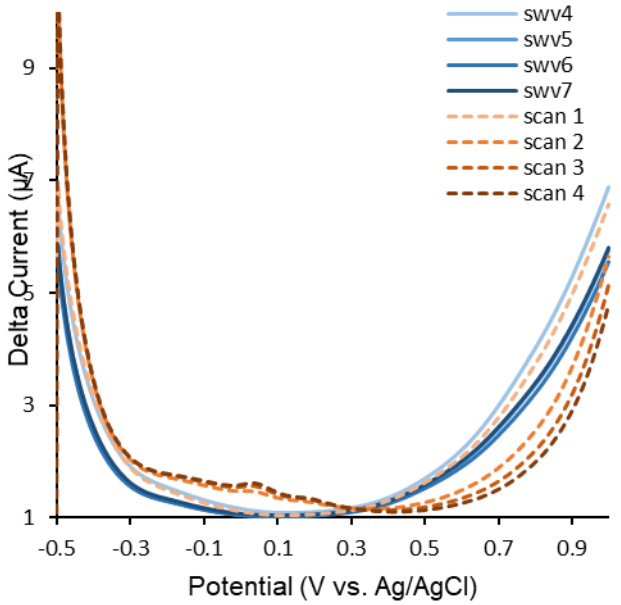



**Figure 40:** SWV of norfentanyl HCl salt at a concentration of 100 µM in PBS, taken consecutively (orange), and with polishing between each scan (blue)

To test if the additional peaks were produced in solution by the oxidation of fentanyl, the same aliquot of fentanyl was used for muliptle successive SWV. The hypothesis was that the trial should result in a smaller current of the fentanyl oxidation for the initial scan of the second SWV than the first SWV, as well as the presence of a small metabolite peak in the initial scan of the second SWV. Figure 41 shows that across mulitple trials of re-using the same aliquot of fentnayl, the initial scan of the second SWV in the aliquot did not have a metabolite peak (Figure 41a-c) at 0 V vs. Ag/AgCl. This result indicated that the peak developing at 0 V vs. Ag/AgCl would most likely be related to a species formed at the interface of the working electrode, and that polishing the WE between the two SWVs would remove it (Table 4).

Furthermore, only one of the trials (Figure 41a) displayed a lower current for the fentanyl peak in the initial scan of the second SWV than for the initial scan of the first SWV. In another trial the initial scan of the second SWV had a greater peak current than the initial scan of the first SWV (Figure 41b), and in a third trial, the initial scans of both SWVs had the same peak current

(Figure 41c). These results indicated that the amount of fentanyl being oxidized in the solution was not signficantly changing the concentration of fentanyl in the overall aliquot.

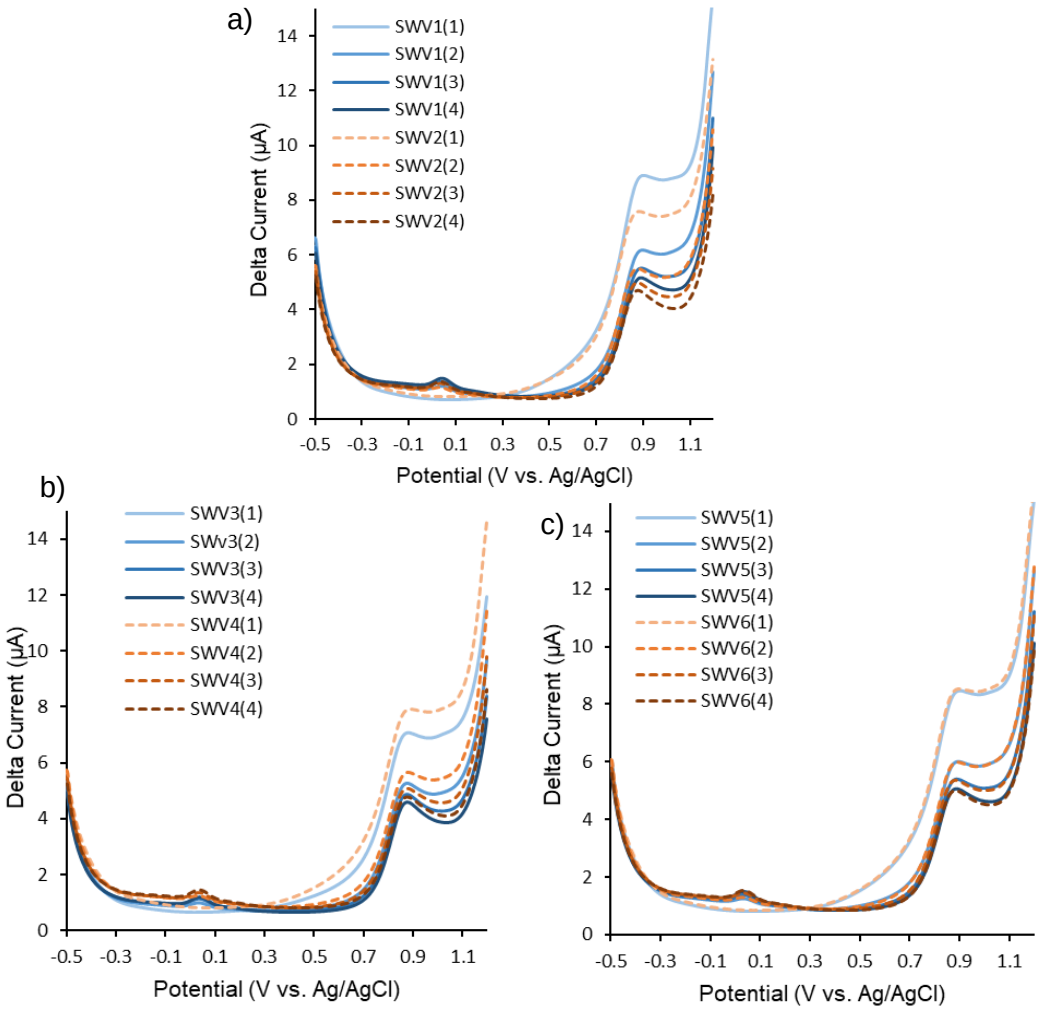



**Figure 41:** SWV of fentanyl at a concentration of 100 µM in PBS, two SWVs taken in same aliquot of solution after polishing WE, initial SWV (blue), second SWV (orange)

### 6.3.4 Ruthenium Hexamine Trichloride Experiments with Norfentanyl

If the development of the additional peaks would be due to the adsorption to the surface of the WE of species present in the solution besides fentanyl or norfentanyl, they would theoretically also develop when using other analytes as well. Ruthenium hexamine is a well-studied electrochemical standard such as ferrocyanide[64]. The reason that ruthenium hexamine was chosen

was that the redox peaks occur at lower potentials than potassium ferrocyanide. The redox peaks for ruthenium hexamine occur at approximately -0.25 V vs. Ag/AgCl and -0.1 V vs. Ag/AgCl for the reduction and oxidation, respectively. In potassium ferrocyanide, the peaks occur at 0.3 V vs Ag/AgCl for the oxidation and ~0.15 V vs Ag/AgCl for the reduction peak. Thus the redox peaks of ruthenium hexamine would be less prompt to overlap with the additional peaks forming at 0 V vs. Ag/AgCl that we are studying. Figure 42 a-b shows that both the solution of 50 µM ruthenium hexamine and the solution of 50 µM ruthenium hexamine with 50 µM norfentanyl HCl salt did not result in observable additional redox peaks around 0 V vs. Ag/AgCl neither in CV nor SWV.

The potential range used for ruthenium hexamine was, however, smaller than that of typical voltammograms in samples of fentanyl. When increasing the potential range to that typically used for fentanyl the additional peaks were observed, both in the solution of 50 µM ruthenium hexamine and in the mixture of 50 µM ruthenium hexamine with 50 µM norfentanyl (Figure 42c-d). In the ruthenium hexamine solution alone, for both CV and SWV, an additional peak ~ 0.2 V vs. Ag/AgCl was observed. For the mixture of norfentanyl and ruthenium hexamine two additional peaks were observed in SWV at the potentials of ~ 0 V vs. Ag/AgCl, and ~ 0.2 V vs. Ag/AgCl and in CV, three additional peaks were observed with two oxidation peaks at ~ 0 V vs. Ag/AgCl and ~ 0.2 V vs. Ag/AgCl, and a reduction peak at ~ 0 V vs. Ag/AgCl.

The observation of these additional peaks observed when the potential range was extended when analyzing either the standard or the mixture indicate that the peak at 0.2 V vs. Ag/AgCl was indeed due to an identified species of the electrochemical system independent of norfentanyl. Furthermore, that the peaks at 0 V vs. Ag/AgCl were indeed correlated to the presence of norfentanyl and that the presence of these electroactive species was dependent on the application of an oxidative potential between 0.5 and 1 V vs. Ag/AgCl (Table 4).

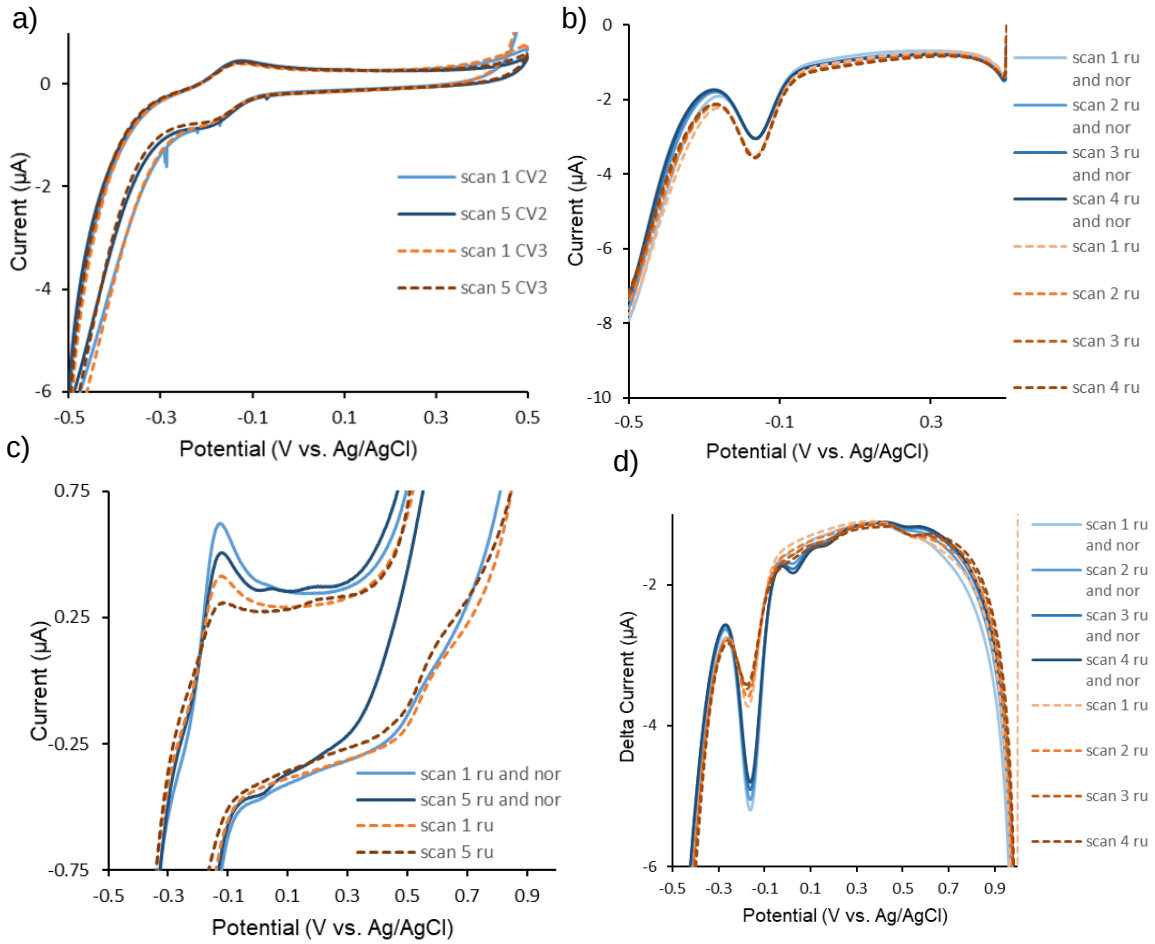



**Figure 42:** Mixture of ruthenium hexamine and norfentanyl HCl salt at concentrations of 50 µM in PBS, a) CV with shorter potential range -0.5 to 0.5 V, b) SWV with shorter potential range, -0.5 to 0.3 V, c) CV with longer potential range, -0.5 to 1.0 V, d) SWV longer potential range -0.5 to 1.0 V

## 6.4 Room Temperature Ionic Liquids

Besides identifying the additional peaks produced when electrochemically analyzing fentanyl, another important aspect was to improve the actual detection of these peaks. When analyzing fentanyl electrochemically in CV, these peaks were difficult to observe even after 50 scans. In SWV and DPV, they were observable after four scans, but still displayed a small peak current. One method attempted to improve the visualization of the metabolite peaks was to use a room temperature ionic liquid (RTIL) both as the SE and as a conducting gel applied to the surface of an electrode. The premise behind using RTIL was that they were known to improve the sensitivity of electrochemical sensors due to their augmented conductivity, and wider potential

windows.[65] The increased performance is because ionic liquids are pure ionic salts liquid at room temperature, thus, as a supporting electrolyte, it is easier for them to carry charge, which then drastically decrease the resistance of the electrochemical cell.[41] Their other application as a film on SPEs stemmed from a previous publication on the electrochemistry of fentanyl in which they used an RTIL, (1-Butyl-1-methylpyrrolidinium Bis-9-trifluormethanesulfonyl) imide), as a conducting gel on the surface of their SPEs to concentrate the analyte at proximity of the electrodes and reported observing convincingly the peaks for the metabolite that they identified as norfentanyl.[17]

### 6.4.1 Using RTIL as Supporting Electrolyte

The use of the RTIL as the supporting electrolyte would allow for the solution containing fentanyl to have a high conductivity, which could increase the peak current of the metabolite peaks and aid in visualization. Figure 43 demonstrates that using the RTIL as a supporting electrolyte did not result in the expected electrochemical peaks for potassium ferrocyanide or fentanyl. Figure 43a shows that there were no oxidation or reduction peaks where they typically occur for potassium ferrocyanide, 0.3 V vs Ag/AgCl for the oxidation and ~0.15 V vs Ag/AgCl for the reduction peak. The couple of peaks observed at ~ -0.9 V vs. Ag/AgCl was determined not to be due to potassium ferrocyanide because they were also present in the blank. Figure 43b also shows that the expected fentanyl oxidation peak was not observed in the first scan, and in the fifth scan a possible fentanyl oxidation peak might have been observed at ~ 0.85 V vs. Ag/AgCl. The presence of this peak in the fifth scan but not in the first scan could indicate that the viscosity of the RTIL was impacting the ability of the analyte to access the surface of the electrode. This could have also been a factor in the inability to observe potassium ferrocyanide peaks, as it was difficult to determine if any of the potassium ferrocyanide had truly dissolved in the RTIL. In an attempt to increase the amount of potassium ferrocyanide that was dissolved, the solution was sonicated for an hour. This sonication did not aid in the visualization of the ferrocyanide peaks (Figure 44). All of these results lead to the determination that the pure RTIL was too viscous to be pursued as a SE for the analysis of fentanyl in our conditions (Table 4).

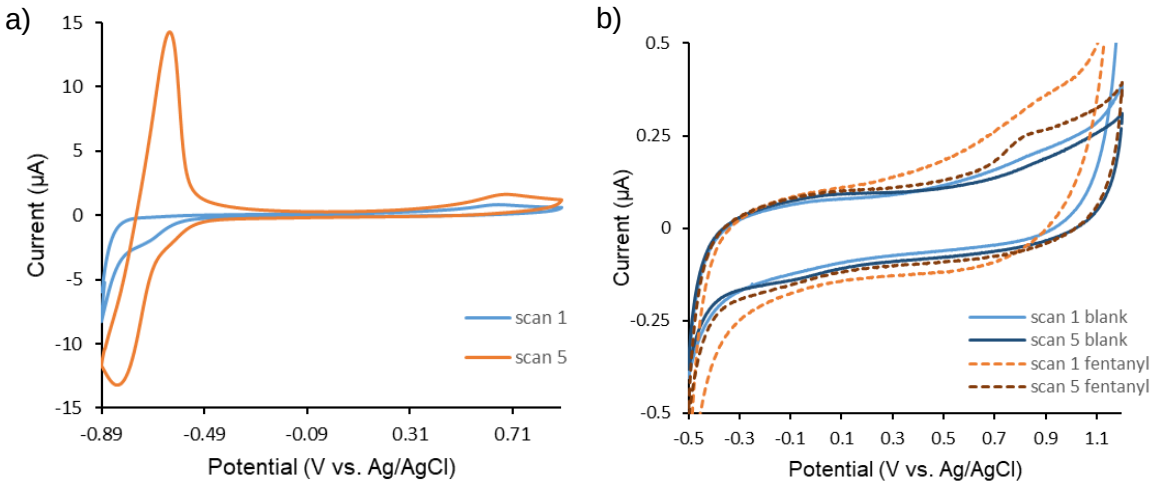



**Figure 43:** a) CV of potassium ferrocyanide with 1-Butyl-1-methylpyrrolidinium Bis-9-trifluormethanesulfonyl)imide ($C_{11}H_{20}F_6N_2O_4S_2$) as an SE on SPE, b) CV of fentanyl at a concentration of 100 µM with 1-Butyl-1-methylpyrrolidinium Bis-9-trifluormethanesulfonyl)imide ($C_{11}H_{20}F_6N_2O_4S_2$) as SE on SPE

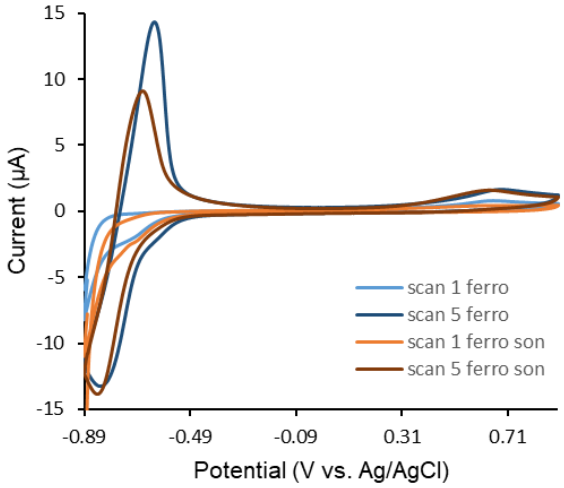



**Figure 44**: Potassium ferrocyanide with 1-Butyl-1-methylpyrrolidinium Bis-9-trifluormethanesulfonyl)imide ($C_{11}H_{20}F_6N_2O_4S_2$) as SE, without (blue) and with (orange) sonication for one hour

### 6.4.2 Applying RTIL to the Surface of SPE

The first necessary step to use RTIL as a film on the SPE was to determine the ratio of RTIL to solvent, here methanol, that was needed to drop cast a film. These trials were performed using potassium ferrocyanide as standard redox. Figure 45 shows that both pure RTIL and 50 %

RTIL in methanol when deposited as a 1 µL drop on the surface of the WE of an SPE did not result in desired peaks for potassium ferrocyanide in SWV (Figure 45a) or CV (Figure 45b). The drops were placed on the WE of a stationary SPE, as well as on an SPE that was rotating to aid in creation of an even film on the surface. The lack of potassium ferrocyanide peaks indicated that smaller ratios of RTIL in methanol should be attempted to try to visualize the desired peaks. Figure 46a shows that a ratio of 10% RTIL to methanol applied to the WE of an SPE also did not result in desired potassium ferrocyanide peaks. There were no peaks in both the first scan of the blank, as well as the first scan of 5 mM potassium ferrocyanide in PBS. The peak developed as the scans progressed for both the blank and potassium ferrocyanide at ~ 0.4 V vs. Ag/AgCl, indicating that the peaks were not due to the analyte itself but to some reactions with the RTIL, the SPE or the solution. Figure 46b shows that a ratio of 1% RTIL and methanol yielded peaks corresponding to potassium ferrocyanide at ~ 0.2 V vs. Ag/AgCl, when drops of either 1 µL or 5 µL were applied to the WE. There was also the additional shoulder peak observed at ~ 0.4 V vs. Ag/AgCl as observed in the 10 % RTIL system. This result indicated that even at smaller ratios of the RTIL the additional peaks would still be observed and could interfere with the observation of the relevant peaks from fentanyl and its metabolites (Table 4). Due to these challenges and lack of any improvement in the detection of the redox peaks the use of RTIL as a film on the SPE was not further pursued.

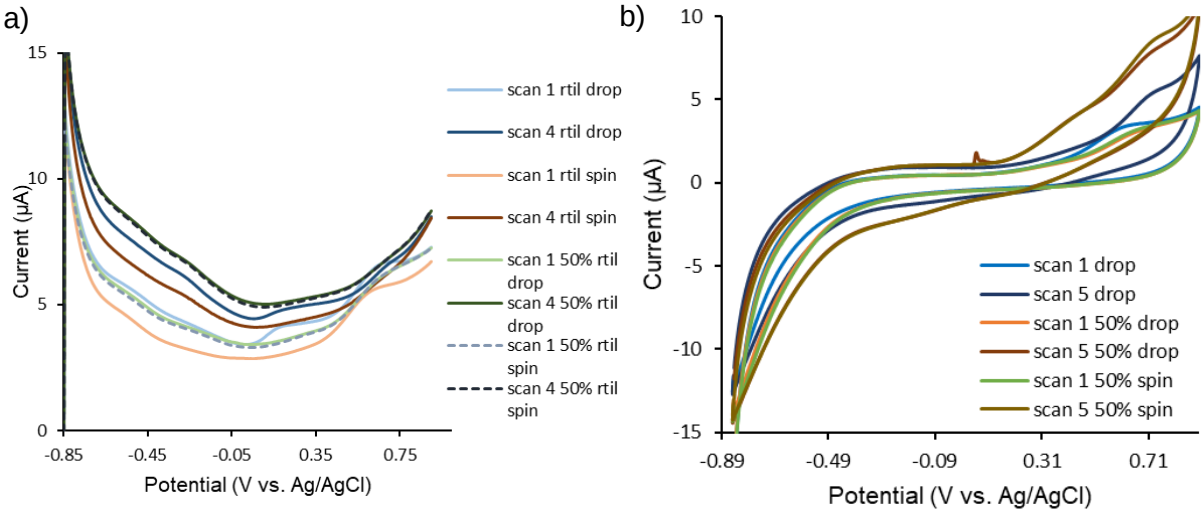



**Figure 45**: Drop cast RTIL on SPE, analyzing 5 mM potassium ferrocyanide in 0.1 M KCl a) SWV with pure RTIL and a stationary electrode (blue) and a spinning electrode (orange), and 50 % RTIL to methanol on a stationary electrode (green) and a spinning electrode (dash) b) CV with pure RTIL on a stationary electrode (blue), and 50% RTIL on a stationary electrode (orange) and a spinning electrode (green)

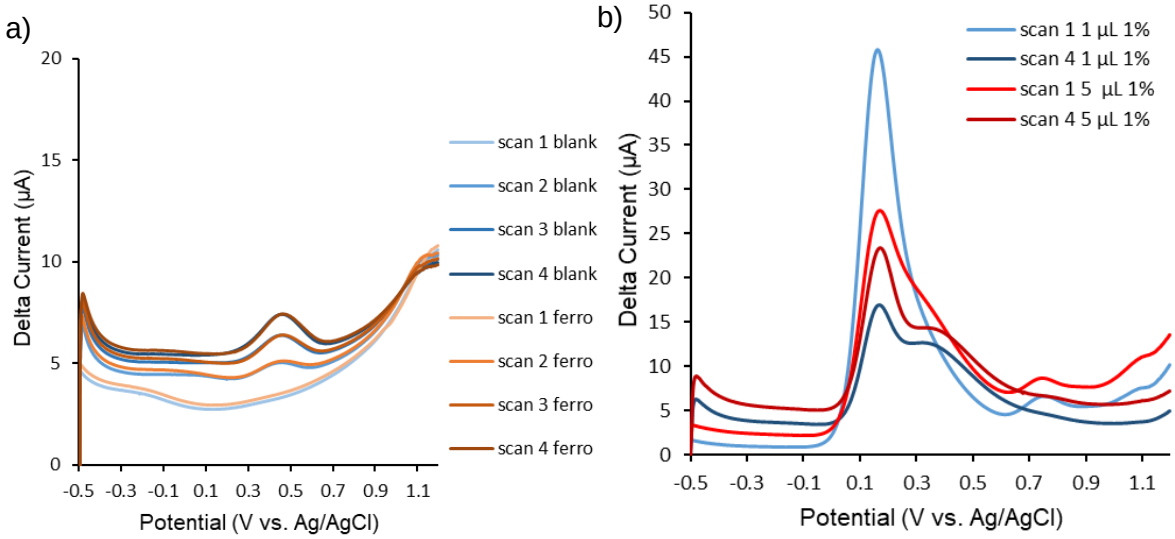



**Figure 46:** SWVs of 5 mM potassium ferrocyanide in PBS, with RTIL drop cast on WE, a) 10% RTIL in methanol 1 µL volume, b) 1% RTIL in methanol on WE 1 µL volume

**Table 4:** Conclusions of experiments related to the metabolite and its redox peaks

| Conclusions for Metabolite and its Peaks | Experiments |
|---|---|
| None of the derivatives of norfentanyl had redox peaks in the initial scans | Testing of multiple norfentanyl derivatives |
| The developing peak may be a species that adsorbed or was developed near the surface of the WE | Polishing electrodes between scans and monitor if the observed peak was still present |
| Product formed by oxidation of fentanyl was not detected in solution afterwards | Testing multiple times the same aliquot of fentanyl sample |
| The peak at ~0 V vs. Ag/AgCl was due to norfentanyl (other peaks forming are due to the system) | Peak developed only if norfentanyl was present in a sample (tested in mixture with ruthenium hexamine) |
| Using room temperature ionic liquids did not improve visualization of additional peak | Use of RTIL as SE and as film on SPE to increase conductivity, tested with ferrocyanide |

## 6.5 Conclusion

The peaks produced after the initial irreversible oxidation of fentanyl and supposedly linked to the formed metabolites have proven difficult to observe and unambiguous to identify. Multiple norfentanyl derivatives including norfentanyl-d5-oxalate, norfentanyl oxalate, norfentanyl monohydrate, and norfentanyl HCl salt were also tested and did not display an electrochemical peak in their initial scans. Norfentanyl oxalate, norfentanyl monohydrate, and norfentanyl HCl salt all developed a peak at ~ 0 V vs. Ag/AgCl in PBS over multiple scans, which potential-wise corresponded to the expected "metabolite" peaks. Study of individual vs batch scans with or without polishing in between scans proved that the species are formed at the surface of the working electrode. Additional experiments demonstrated that an oxidative potential between 0.5 to 1 V vs Ag/AgCl was necessary to the observation of these peaks. The use of RTIL was also explored to enhance the detection of these peaks and facilitate their characterization, but was unsuccessful. Further exploration to improve the detection of the peaks as well as the clear identification of the species involved is discussed in Chapter 8, Future Work.

# CHAPTER 7. QUANTIFICATION OF FENTANYL

## 7.1 Introduction

Quantification is the ultimate goal of most analytical measurement and characterization of substances. Electrochemical quantification has multiple advantages such as inherent selectivity, sensitivity, and a high degree of repeatability. Additionally, thanks to the miniaturization and portability of the electrochemical instrumentation, the electroanalytical assays can easily be adapted for in-field or point-of-care measurements. Multiple electroanalytical techniques can be applied to quantifications, including all those techniques utilized in previous chapters: SWV, CV, and DPV. There are different benefits to use each method for quantification, in CV the peaks are easy to visualize and provide additional information on the electrochemical system, whereas DPV and SWV are considered more sensitive methods because as discussed in previous chapters, they have less interference from the background current, allowing quantification at low concentrations.[48] All three methods have previously reported the ability to quantify multiple different analytes, including illicit substances such as cocaine[30, 36], atropine[66], xylazine[38], and phenethylamine derivatives[67]. In this chapter, CV, SWV, and DPV were all tested for the quantification of fentanyl using both GCE and SPEs and the optimized parameters described in previous chapters, to determine the optimal quantification method for fentanyl.

## 7.2 Experimental

### 7.2.1 Materials and Reagents

Fentanyl ($C_{22}H_{28}N_2O$) (1 mg/mL) was purchased from Cerilliant. Phosphate buffered saline solution ($Cl_2H_3K_2Na_3O_8P_2$) (PBS) was purchased from Fisher. Carbon screen printed electrodes were purchased from CHI Instruments. Glassy carbon electrodes, Ag/AgCl reference electrodes, and Pt wire electrodes were purchased from CHI Instruments.

### 7.2.2 Solution Preparation

Solutions of varying concentrations of fentanyl were prepared by using 1 mg/mL fentanyl, which equates to 2.9 mM. A series of dilutions was then performed for 80 µM, 60 µM, 40 µM, 20

µM, 15 µM, 10 µM, and 5 µM solutions of fentanyl from the 100 µM solution of fentanyl. The volumes were measured using a micropipette and appropriate pipette tips. PBS 1x was made by diluting the 10x stock with milliQ water.

### 7.2.3 Electrochemical Measurements

The electrochemical methods of CV, SWV, and DPV were all undertaken using an Autolab PGSTAT204 potentiostat with Electrochemical Impedance Spectroscopy FRA2 module, with Nova 2.0 software. The potential range used for fentanyl for the CV was 1.2 to -0.5 to 1.2 V for PBS. The scan rate was 50 mV/s. The potential range for SWV and DPV for fentanyl was -0.5 to 1.2 V for PBS. A cleaning step prior to the use of each individual SPE was necessary prior to use in fentanyl. The CV cleaning step for the SPE range was 1.5 to 0 to 1.5 V. The cleaning step for SWV was 0 to 1.5 V using a SWV method, and the cleaning step for DPV was 0 to 1.5 V using a DPV method. The parameters for SWV were a step size of 0.004 V, an amplitude of 30 mV, a frequency of 15 Hz and a calculated scan rate of 59 mV/s. The parameters for DPV were a step size of 0.005 V, a modulation time of 0.05 s, an interval time of 0.1 s, a modulation amplitude of 30 mV, and a calculated scan rate of 50 mV/s.

## 7.3 Cyclic Voltammetry

The use of CV for quantification purposes in electrochemistry has been used for numerous analytes including several in more forensic applications, such as the quantification of Rohypnol in buffer solution as well as coca cola[68], as well as synthetic cathinone derivatives.[69] For the detection of Rohypnol, as well as the synthetic cathinone, SPEs were utilized for the analysis as well as the quantification. In the quantification of Rohypnol, they determined there was no need for a preconditioning step, and had a linear range of 1-95.24 µM/mL. For the quantification of the synthetic cathinone derivatives, they explored three different molecules including methcathinone, 4-methylmethcathinone (4-MMC), and 4-methyl-N-ethylcathinone (4-MEC). In this work they were able to obtain linear concentration ranges of 16-200 µg/mL for methcathinone, and linear ranges of 16-350 µg/mL for 4-MMC and 4-MEC.

The electrochemical quantification of fentanyl over the range of concentration 0 to 100 µM was performed using cyclic voltammetry in both a beaker with 1 mL solution of fentanyl on the standard GCE working electrode, as well as in a 100 µL drop of solution on an SPE. Figure 47 shows the comparisons of the quantification of fentanyl for both the GCE system as well as with the SPE. When comparing the two types of electrodes used for fentanyl quantification, the sensitivity of both systems was similar as shown by the similarity in the slopes, with 0.0200 µA/µM for the GCE and 0.0213 µA/µM for the SPE. Both systems displayed a linear correlation, however, SPE had a larger $R^2$ value of 0.9975 compared to the $R^2$ value for GCE at 0.9898, while also displaying that linearity for a wider concentration range. When utilizing the GCE as the WE, the concentration range that displayed a linear correlation was 10 to 100 µM fentanyl, while on the SPE the linear range was from 5 to 100 µM fentanyl. This linear concentration range indicates that the SPE performed slightly better than the GCE for the quantification of fentanyl. Table 5 demonstrates that both systems had values for the average standard deviation that were less than a tenth of a µA, with the average standard deviation for GCE being 0.0724 µA, and 0.0875 for SPE. It is notable that even though the SPE had larger standard deviations for some of the concentrations, especially for both 40 µM and 100 µM, where both standard deviations were 0.20 µA or larger, these values were taken with a new SPE each trial. The standard deviations indicate that there is good repeatability across the use of multiple SPEs, not just focusing on one GCE. The quantification of fentanyl using CV as the electrochemical method using both the GCE, as well as the SPE resulted in a strong linear correlation for both systems, as well as strong repeatability, demonstrated by the standard deviation values. The SPE also performed slightly at the quantification of fentanyl, with a wider linear concentration range.

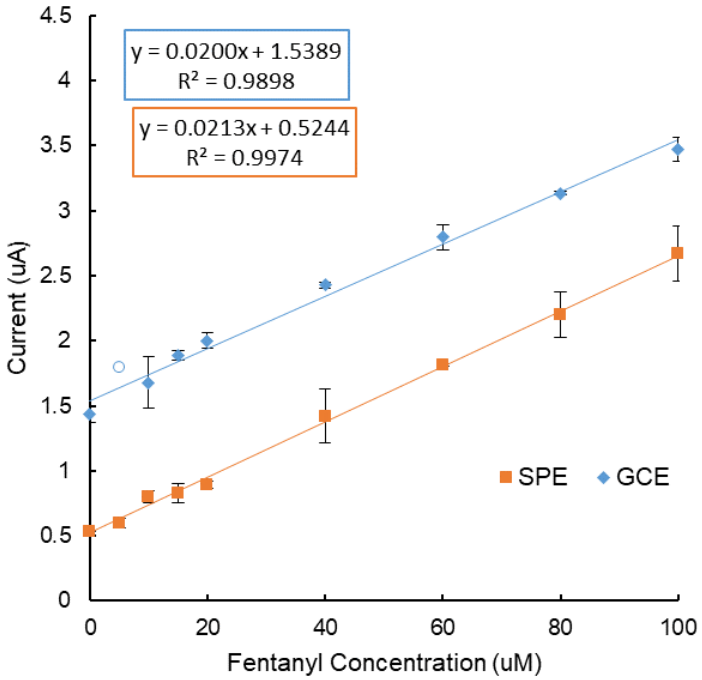



**Figure 47**: Calibration Curve of Fentanyl CV for GCE (Blue) and SPE (orange), blue triangle representing 5 µM on GCE

**Table 5:** Average peak current and standard deviations for CV calibration of fentanyl on GCE and SPE

| GCE | | | | SPE | | | |
|---|---|---|---|---|---|---|---|
| **Conc. µM** | **Average Current at 0.9 VµA** | **St. Dev µA** | **RSD %** | **Conc. µM** | **Average Current at 0.87 V µA** | **St. Dev µA** | **RSD %** |
| 0 | 1.432 | 0.056 | 3.92 | 0 | 0.537 | 0.004 | 0.74 |
| 5 | 1.793 | 0.185 | 10.29 | 5 | 0.598 | 0.032 | 6.49 |
| 10 | 1.679 | 0.195 | 11.61 | 10 | 0.801 | 0.044 | 5.511 |
| 15 | 1.886 | 0.040 | 2.14 | 15 | 0.826 | 0.071 | 8.58 |
| 20 | 2.001 | 0.058 | 2.92 | 20 | 0.893 | 0.027 | 2.98 |
| 40 | 2.427 | 0.026 | 1.06 | 40 | 1.420 | 0.208 | 14.66 |
| 60 | 2.796 | 0.096 | 3.44 | 60 | 1.812 | 0.010 | 0.52 |
| 80 | 3.133 | 0.015 | 0.48 | 80 | 2.198 | 0.174 | 7.91 |
| 100 | 3.467 | 0.093 | 2.68 | 100 | 2.667 | 0.211 | 7.92 |
| **Average** | | 0.072 | | | | 0.087 | |

## 7.4 Square Wave Voltammetry

The use of SWV for quantification in electrochemistry is widespread due to the sensitivity of the method, has been utilized multiple times for the quantification of drugs in samples. The use of SWV for quantification has been demonstrated in an on-site method to detect scopolamine, which is used in drug facilitated sexual assults,[70] in urine. In this case they used a portable potentiostat, which utilized a boron doped diamond electrode, and were able to use a volume as small as 50 µL to achieve an LOD of 0.18 µM. Another example of quantification using SWV is the detection and quantification of benzocaine in the presence of antipyrine.[71] The quantification of benzocaine and antipyrine was undertaken using carbon paste electrodes modified with a combination of titanium dioxide nanoparticles and a graphene oxide nanosheet. This increased their sensitivity, and they achieved a linear range for benzocaine of 1 to 100 µM, and 12 nM to 80 µM for antipyrine.

SWV if often considered a more sensitive method than CV for analytes at low concentrations in complex matrices, thus we performed the quantification of fentanyl using SWV. Both systems of standard electrodes in beaker and SPE with drop method yielded calibration curves. Figure 48 shows that the SPE demonstrated a higher sensitivity than the GCE for the quantification of fentanyl for both the delta and forward current. In the delta current, the SPE had a slope of 0.0222 µA/µM, while for the forward current the slope was 0.0328 µA/µM. These slopes compared to the delta current slope on the GCE of 0.0158 µA/µM, and 0.0269 µA/µM for the forward current. The greater sensitivity is also reflected in the larger concentration range in which the fentanyl quantification had a linear correlation on the SPE, which was from 5 to 100 µM, compared to the GCE with a concentration range of 20 to 80 µM. The lower concentrations of 5-15 µM were attempted for the GCE, but as observed on Figure 48 they did not follow the linear trend, and appear to plateau. For both the delta and the forward current, the SPE had larger $R^2$ values with 0.9968 for the forward current, and 0.9968 as well for the delta current compared to the GCE with 0.9895 for the forward current and 0.9649 for the delta current. Table 6 demonstrates that the average standard deviations for the forward and delta current on the SPE were smaller than those observed for the GCE, with the forward average being 0.075 µA, and the delta being 0.121 µA, while the forward for the GCE was 0.168 µA, and the delta value was 0.153 µA. These average standard deviations again are a reflection of the repeatability with the SPES, even though it is a new electrode every trial. The quantification of fentanyl using SWV as the electrochemical method

demonstrated good linear correlation for both systems, but especially for the SPE, which had better $R^2$ values over a larger concentration range, with a smaller average standard deviation than GCE.

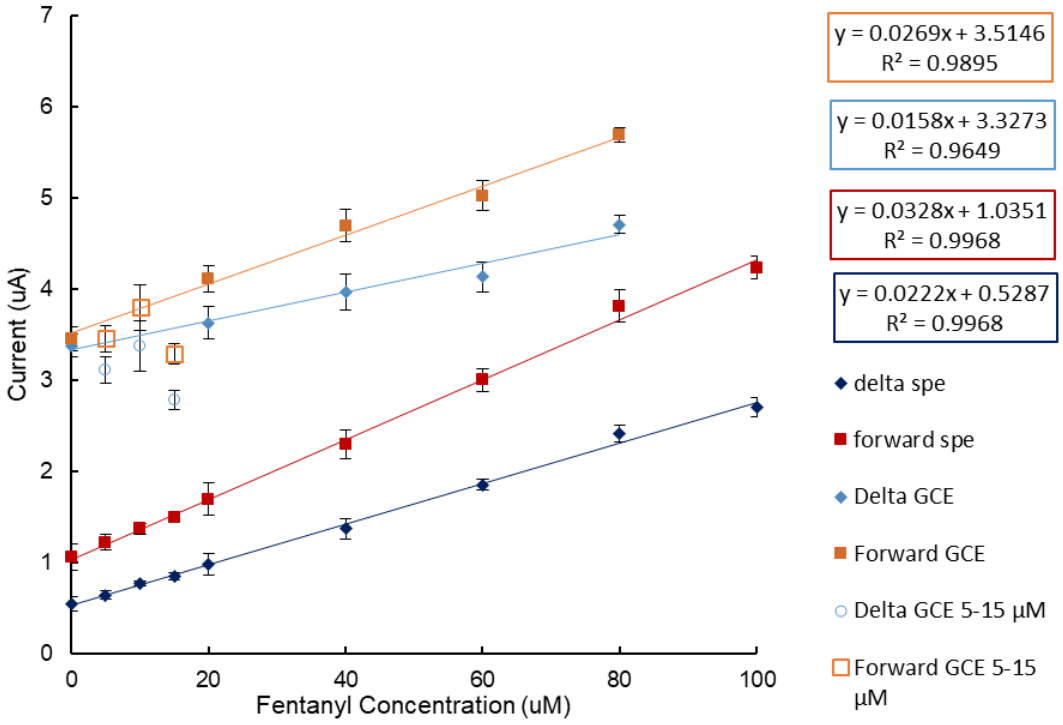



**Figure 48**: Calibration Curves SWV of Forward and Delta current for SPE and GCE for fentanyl, second scan

**Table 6:** Average peak current and standard deviations on SWV for the delta and forward current for both GCE and SPE

| **Delta GCE** | | | | **Forward GCE** | | | |
|---|---|---|---|---|---|---|---|
| **Conc. µM** | **Average Current at 0.9 V µA** | **St. Dev. µA** | **RSD %** | **Conc. µM** | **Average Current at 0.9 V µA** | **St. Dev. µA** | **RSD %** |
| 0 | 3.37 | 0.128 | 3.81 | 0 | 3.448 | 0.127 | 3.69 |
| 5 | 3.107 | 0.149 | 4.81 | 5 | 3.453 | 0.143 | 4.16 |
| 10 | 3.368 | 0.276 | 8.22 | 10 | 3.793 | 0.246 | 6.50 |
| 15 | 2.781 | 0.103 | 3.72 | 15 | 3.286 | 0.107 | 3.25 |
| 20 | 3.627 | 0.175 | 4.82 | 20 | 4.110 | 0.144 | 3.52 |
| 40 | 3.965 | 0.195 | 4.93 | 40 | 4.691 | 0.182 | 3.87 |
| 60 | 4.131 | 0.162 | 3.92 | 60 | 5.016 | 0.165 | 3.29 |
| 80 | 4.706 | 0.099 | 2.11 | 80 | 5.683 | 0.083 | 1.45 |
| 100 | 4.668 | 0.250 | 5.35 | 100 | 5.854 | 0.219 | 3.75 |
| Average | | 0.168 | | | | 0.153 | |
| **Delta SPE** | | | | **Forward SPE** | | | |
| **Conc. µM** | **Average potential at 0.87 V µA** | **St. Dev. µM** | **RSD %** | **Conc. µM** | **Average potential at 0.87 V µA** | **St. Dev. µM** | **RSD %** |
| 0 | 0.541 | 0.081 | 14.884 | 0 | 1.054 | 0.147 | 13.93 |
| 5 | 0.640 | 0.042 | 6.52 | 5 | 1.219 | 0.090 | 7.38 |
| 10 | 0.767 | 0.026 | 3.45 | 10 | 1.367 | 0.056 | 4.10 |
| 15 | 0.847 | 0.034 | 4.05 | 15 | 1.487 | 0.035 | 2.34 |
| 20 | 0.972 | 0.119 | 12.25 | 20 | 1.692 | 0.178 | 10.52 |
| 40 | 1.371 | 0.111 | 8.11 | 40 | 2.290 | 0.160 | 7.01 |
| 60 | 1.849 | 0.063 | 3.39 | 60 | 2.996 | 0.127 | 4.22 |
| 80 | 2.408 | 0.092 | 3.84 | 80 | 3.809 | 0.175 | 4.59 |
| 100 | 2.697 | 0.108 | 4.00 | 100 | 4.230 | 0.122 | 2.89 |
| Average | | 0.075 | | | | 0.121 | |

## 7.5 Differential Pulse Voltammetry

Differential pulse voltammetry is another technique that has widely been used for quantification in electrochemistry, including into more forensic applications. This electrochemical method has been utilized to detect and quantify fentanyl[19, 35, 37] as described in Chapter 2, in which

LODs as low as 0.3 µM were obtained using modified electrodes. DPV has also been used as an electrochemical technique to quantify codeine, acetaminophen, and caffeine,[72] tradamol[73], as well as MDMA and PMA.[74] The quantification of codeine, acetaminophen, and caffeine were undertaken using a cerium oxide nanoparticle modified SPE, and were able to quantify all three substances simultaneously. The linear concentration range for codeine was 0.09-50 µM with an LOD of 0.043 µM, 5-286 µM with an LOD of 2.4 µM for caffeine, and for acetaminophen the linear range was 0.09-7 µM with an LOD of 0.051 µM. The quantification of tramadol was undertaken using a graphene oxide multi-walled carbon nanotube composites on a carbon paste electrode. The limit of detection in this case was $1.5 \times 10^{-10}$ M, with a linear range of $2 \times 10^{-9}$ to $1.10 \times 10^{-3}$ M. The quantification of MDMA and PMA was done on an unmodified SPE, and they were able to simultaneously detect MDMA and PMA, as well as detect and quantify them individually. The linear concentration ranges of MDMA and PMA individually were 0.5 to 4.99 µg/mL, while the mixture of the two had a range of 2 to 19.60 µg/mL for each drug.

Similar to SWV, DPV is another alternative electrochemical technique for sensitive quantification when analyzing low concentrations of analytes in real samples. For comparison, we thus also performed the quantification of fentanyl by DPV over the same range and with both GCE and SPE systems described in the previous sections. As observed in Figure 49, similar to that of SWV, in DPV the sensitivity was greater for the SPE than the GCE, with the slope for the pulse current at 0.0245 µA/µM, and the slope for the delta current at 0.0100 µA/µM. The GCE had a slope for the pulse current of 0.0186 µA/M, and 0.0058 µA/µM for the delta current. This greater sensitivity on the SPE than the GCE is also represented in the fact that the linear range for SPE was 15 to 100 µM, and on GCE the range was 20 to 80 µM. The $R^2$ values for SPE were more consistent than those for GCE, with an $R^2$ for the pulse current of 0.9803, and 0.9851 for the delta. On the GCE, the pulse current had a very strong linear correlation with an $R^2$ value of 0.9953, but the delta current had a lower $R^2$ value of 0.9299. Table 7 demonstrates that the average standard deviation for the pulse current was greater for the GCE, with a value of 0.100 µA, were SPE had a value of 0.121 µA, but for the delta current the SPE had a smaller average standard deviation at 0.041 µA, while the GCE had a value of 0.052 µA. Both average standard deviations for the delta current in DPV are the smallest average standard deviations observed for SWV, CV and DPV. The low standard deviations demonstrate good repeatability with both the GCE as well as between devices for the SPEs. The quantification of fentanyl using DPV as the electrochemical method

demonstrated good linear correlation for both GCE and SPE, with a slightly larger concentration range for the SPE. The standard deviations for both electrode systems were some of the smallest observed, and demonstrated strong repeatability in this method, though the concentration ranges were not as large as those observed for CV and SWV using the SPE.

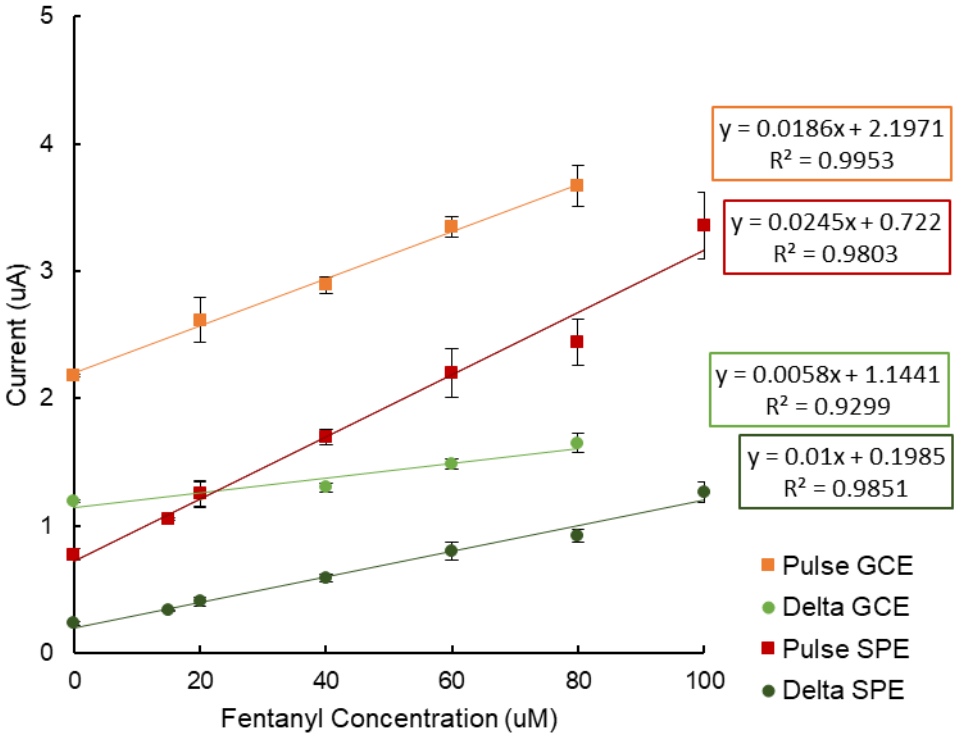



**Figure 49:** Calibration curve of fentanyl using DPV, pulse and delta current of GCE and SPE, second scan

**Table 7:** Average fentanyl oxidation peak current and standard deviations for DPV calibration

| **Pulse GCE** | | | | **Delta GCE** | | | |
|---|---|---|---|---|---|---|---|
| **Conc. µM** | **Average Current at 0.9 V (µA)** | **St. Dev. µA** | **RSD %** | **Conc. µM** | **Average Current at 0.9 V (µA)** | **St. Dev. µA** | **RSD %** |
| 0 | 2.178 | 0.009 | 0.43 | 0 | 1.193 | 0.010 | 0.87 |
| 20 | 2.615 | 0.180 | 6.87 | 20 | 1.244 | 0.098 | 7.90 |
| 40 | 2.889 | 0.065 | 2.26 | 40 | 1.301 | 0.034 | 2.63 |
| 60 | 3.345 | 0.083 | 2.48 | 60 | 1.486 | 0.042 | 2.83 |
| 80 | 3.669 | 0.164 | 4.46 | 80 | 1.649 | 0.074 | 4.50 |
| Average | | 0.100 | | | | 0.052 | |
| **Pulse SPE** | | | | **Delta SPE** | | | |
| **Conc. µM** | **Average Current at 0.87 V (µA)** | **St. Dev. µA** | **RSD %** | **Conc. µM** | **Average Current at 0.87 V (µA)** | **St. Dev. µA** | **RSD %** |
| 0 | 0.770 | 0.046 | 6.01 | 0 | 0.230 | 0.015 | 6.44 |
| 15 | 1.052 | 0.007 | 0.70 | 15 | 0.331 | 0.003 | 0.91 |
| 20 | 1.249 | 0.102 | 8.16 | 20 | 0.402 | 0.034 | 8.50 |
| 40 | 1.697 | 0.057 | 3.38 | 40 | 0.589 | 0.027 | 4.53 |
| 60 | 2.197 | 0.188 | 8.56 | 60 | 0.801 | 0.072 | 9.03 |
| 80 | 2.439 | 0.183 | 7.49 | 80 | 0.920 | 0.053 | 5.76 |
| 100 | 3.353 | 0.263 | 7.85 | 100 | 1.266 | 0.080 | 6.34 |
| Average | | 0.121 | | | | 0.041 | |

### 7.6 Comparison of Quantification Methods

The performances for the quantification of fentanyl by the three electrochemical techniques were compared separately for the systems using the SPE and for the systems using the GCE. In regards to the SPE, for all three electrochemical techniques, the range of concentrations with a linear correlation was wider than that of the GCE. Table 8 shows that the linear range for CV was 5 to 100 µM, for SWV the linear range was also 5 to 100 µM, while using DPV the linear range was 15 to 100 µM for the SPE. The smaller linear concentration range for DPV on the SPE could be resolved with further optimization of the method used for DPV, and thus improve the linear range.

**Table 8:** Comparison quantification for DPV, SWV, and CV on GCE and SPE

| Electrochemical Method | Current | scan | Equation of line | $R^2$ | Tested Linear Range |
|---|---|---|---|---|---|
| DPV | base | scan 1 | y = 0.0191x+1.8305 | 0.9667 | 20-80 µM |
| DPV | base | scan 2 | y = 0.0128x + 1.0529 | 0.9793 | 20-80 µM |
| DPV | pulse | scan 1 | y = 0.0285x + 3.6769 | 0.9843 | 20-80 µM |
| DPV | pulse | scan 2 | y = 0.0186x + 2.1971 | 0.9953 | 20-80 µM |
| DPV | delta | scan 1 | y = 0.0094x + 1.8464 | 0.9600 | 20-80 µM |
| DPV | delta | scan 2 | y = 0.0058x + 1.1441 | 0.9299 | 20-80 µM |
| DPV SPE | base | scan 1 | y = 0.0177x + 0.8781 | 0.9652 | 15-100 µM |
| DPV SPE | base | scan 2 | y = 0.0146x + 0.5105 | 0.9772 | 15-100 µM |
| DPV SPE | pulse | scan 1 | y = 0.0294x + 1.2373 | 0.9729 | 15-100 µM |
| DPV SPE | pulse | scan 2 | y = 0.0245x + 0.722 | 0.9803 | 15-100 µM |
| DPV SPE | delta | scan 1 | y = 0.0117x + 0.3592 | 0.9822 | 15-100 µM |
| DPV SPE | delta | scan 2 | y = 0.0100x + 0.1985 | 0.9851 | 15-100 µM |
| SWV | forward | scan 1 | y = 0.0416x + 5.8456 | 0.9634 | 20-80 µM |
| SWV | forward | scan 2 | y = 0.0269x + 3.5146 | 0.9895 | 20-80 µM |
| SWV | backward | scan 1 | y = 0.0162x + 0.5496 | 0.9537 | 20-80 µM |
| SWV | backward | scan 2 | y = 0.0119x + 0.1709 | 0.9574 | 20-80 µM |
| SWV | delta | scan 1 | y = 0.0254x + 5.2959 | 0.9605 | 20-80 µM |
| SWV | delta | scan 2 | y = 0.0158x + 3.3273 | 0.9649 | 20-80 µM |
| SWV SPE | forward | scan 1 | y = 0.0383x + 1.7828 | 0.9853 | 5-100 µM |
| SWV SPE | forward | scan 2 | y = 0.0328x + 1.0351 | 0.9968 | 5-100 µM |
| SWV SPE | backward | scan 1 | y = 0.0123x + 0.8675 | 0.9731 | 5-100 µM |
| SWV SPE | backward | scan 2 | y = 0.0106x + 0.5064 | 0.9960 | 5-100 µM |
| SWV SPE | delta | scan 1 | y = 0.0259x + 0.9141 | 0.9913 | 5-100 µM |
| SWV SPE | delta | scan 2 | y= 0.0222x + 0.5287 | 0.9968 | 5-100 µM |
| CV | | scan 1 | y = 0.0233x + 1.8416 | 0.9873 | 10-100 µM |
| CV | | scan 2 | y = 0.02x + 1.5389 | 0.9898 | 10-100 µM |
| CV SPE | | scan 1 | y = 0.0241x + 0.5948 | 0.9928 | 5-100 µM |
| CV SPE | | scan 2 | y = 0.0213x + 0.5244 | 0.9974 | 5-100 µM |

For all electrochemical methods on SPE, the forward current of SWV had the highest sensitivity, with a slope of 0.0383 µA/µM for the first scan, and 0.0328 µA/µM for the second scan. The second scan for taken for fentanyl for all SPE methods had a higher linear correlation than the first scan, likely because by the second scan the system has had more time to equilibrate, resulting in a more consistent response. The quantification of fentanyl using the forward current of the SWV displayed the greatest sensitivity of all the electrochemical techniques on the SPE, but

also had the largest LODs due to the high standard deviations for the blank. The quantification of fentanyl on the SPE using CV had the lowest calculated limit of detection (Table 9), and largest linear range (Table 8), indicating that currently this quantification method performs the best out of the techniques and electrodes tested.

**Table 9:** Comparison of best results for fentanyl quantification

| Electrochemical Technique | Sensitivity µA/µM | $R^2$ | Tested Linear Range µM | St. Dev. Blank µA | LOD µM |
|---|---|---|---|---|---|
| **DPV Pulse GCE** | 0.019 | 0.9953 | 20-80 | 0.009 | 1.5 |
| **DPV Delta GCE** | 0.006 | 0.9299 | 20-80 | 0.010 | 5.4 |
| **DPV Pulse SPE** | 0.025 | 0.9803 | 15-100 | 0.033 | 4.0 |
| **DPV Delta SPE** | 0.010 | 0.9851 | 15-100 | 0.014 | 4.1 |
| **SWV Forward GCE** | 0.027 | 0.9895 | 20-80 | 0.127 | 1.4 |
| **SWV Delta GCE** | 0.016 | 0.9649 | 20-80 | 0.128 | 24.4 |
| **SWV Forward SPE** | 0.033 | 0.9968 | 5-100 | 0.147 | 13.4 |
| **SWV Delta SPE** | 0.022 | 0.9968 | 5-100 | 0.081 | 10.9 |
| **CV GCE** | 0.020 | 0.9898 | 10-100 | 0.056 | 8.4 |
| **CV SPE** | 0.021 | 0.9974 | 5-100 | 0.004 | 0.6 |

In regard to the GCE, the linear concentration ranges for both SWV and DPV were the same at 20-80 µM, while when using CV, the range was 10 to 100 µM (Table 8). This larger linear concentration range is likely due to the smaller standard deviations observed for CV on the GCE. The sensitivity, similar to SPE, was greatest for the forward current of the SWV on the GCE, with a slope of 0.0416 µA/µM on the first scan, and 0.0269 µA/µM on the second scan. The first scan of the pulse current on GCE had a higher sensitivity with a slope of 0.0285 µA/µM. The highest linearity was observed for the pulse current second scan with an $R^2$ value of 0.9953 µA, but the linearity for CV on the GCE was most consistent between the two scans with an $R^2$ value of 0.9873 µA for the first scan and 0.9898 µA for the second scan. For all three electrochemical methods on the GCE, CV has thus far performed the best, due to the larger linear concentration range, and even though it is not the most sensitive method, it has high $R^2$ values, and small standard deviations.

When comparing the quantification of fentanyl to other examples of quantification found in literature for fentanyl using electrochemistry, thus far our LOD is still larger than those reported. This could potentially be resolved with further optimization of the electrochemical methods used,

but it is also important to note that according to the 2020 National Drug Assessment by the Drug Enforcement Administration, that noted a method for ingesting fentanyl that is becoming more common is fentanyl-containing pills.[9] In the 2018 National Drug Assessment by the Drug Enforcement Administration noted that the amount of fentanyl in these pills ranged on average from 0.03 mg to 1.99 mg, with 1.1 mg being the average.[75] If half of the fentanyl containing pill was dissolved in 1 mL of solvent, the resulting concentrations of fentanyl would be 44.5 µM, 2950 µM, and 1646 µM, respectively. If $1/10^{th}$ of the fentanyl containing pill was dissolved in 1 mL of solvent, the resulting concentrations would be 8.9 µM, 320 µM, and 590 µM respectively for pills containing 0.03 mg, 1.1 mg, and 1.99 mg of fentanyl. The average concentration exceeds the current linear range for fentanyl, and thus could be measured by further dilution of the sample. On the other hand, the concentration in 1 mL of solution for the lowest reported amount of fentanyl would be at a concentration above the LODs as calculated for the quantification methods identified as the best (Table 9). This statement excluded the delta current for SWV on the GCE, and the forward and delta current for SWV on the SPE. Indeed, the larger LODs for those techniques can be attributed to the larger standard deviations of the blanks, as the LOD was calculated using Equation 3, and thus the standard deviation of the blank and the limit of detection are directly proportional. This issue could be mitigated by using a smaller volume of solution to dissolve the pill, as decreasing the volume while keeping the amount of pill to dissolve would increase the concentration, and a volume of 1 mL is not needed with more portable methods such as SPE, which as seen in Chapter 5, can be used with concentrations of 100 µL, and lower.

$$LOD = \frac{3 \times (st.dev.blank)}{(slope\ calibration\ curve)}$$

**Equation 3:** Equation to calculate limit of detection (LOD)

## 7.7 Conclusion

All three electrochemical techniques optimized throughout this work were explored for the quantification of fentanyl, and all three displayed good linear correlations. The use of SPEs resulted in better linear correlation than the use of the standard GCE for all three electrochemical techniques, likely due to it being a new electrode with a smaller volume of solution each time. Both SWV and CV on SPE demonstrated linear ranges of 5 to 100 µM, while CV on the GCE

demonstrated a linear range of 10 to 100 µM, and SWV on the GCE, and DPV on both the GCE and SPE had linear ranges from 20 to 80 µM. The standard deviations of all methods were small and demonstrated good repeatability within the triplicates for each concentration. The linear concentration ranges observed for all the methods were considered suitable for typical measurements as they could be used to detect the concentrations of fentanyl observed in fentanyl-containing pills that are becoming a common method of consumption.

# CHAPTER 8. FUTURE WORK

## 8.1 pH Study of Fentanyl

The pH of the fentanyl solution can affect its electrochemical behavior as shown in several publications on the electrochemistry of fentanyl, which performed pH studies and determined different optimal pH values for their solutions[19, 35-36]. The pH of PBS, which was used in most of the presented work, is 7.4, and is in the range of pH values other researchers reported as their optimal pH. As shown in Section 3.2.4, small variations in the values of the pH did not greatly impact the fentanyl oxidation peak. Performing a more in-depth pH study, would however be pertinent to demonstrate the optimal pH for our described system. The expected optimal pH would be within the range of 7-9. We would focus our study on solution of fentanyl with adjusted pH values between 4-10, as it has been shown in literature that there was no oxidation peak present for solutions with a pH below 4, and barely observable for solutions with a pH above 10.[35] Through this pH study, we could also quantify the impact of the pH on the electrochemical detection of fentanyl for different pH values, and thus determine whether the pH of a sample should be critically adjusted prior to analysis. .

## 8.2 Preliminary Testing of Fentanyl Analogs

The premise of this project is that the electrochemistry of the fentanyl analogs would be similar to that of fentanyl, which would lead to universal detection of fentanyl and fentanyl analogs. There have been only few studies on the electrochemistry of fentanyl analogs with one of the only examples being the electrochemical detection of sufentanil on SPEs.[76] In this case an SPE modified with multi-walled carbon nanotubes (MWCNTS) was used to detect sufentanil at 0.13 mM, and observed the oxidation peak at ~0.5 V vs. Ag/AgCl.

Sufentanil will be our first analog target to test if the optimized conditions established in this work for fentanyl are indeed also adapted for a fentanyl analog. One interesting aspect would be to determine if the oxidation of sufentanil was occurring for Ahmar, *et al*. at a lower potential due to the design and set up of their specialized electrodes, or if the lower potential was due to sufentanil. This exploration will be performed using both SPE and GCE, with the optimized parameters for CV which would be comparable to the literature report as CV was also used.

Multiple scans will be performed to determine if additional peaks due to possible electroactive metabolites would be formed, as sufentanil was believed to undergo an N-dealkylation reaction similar to that of fentanyl when metabolized.[12] After electrochemically characterizing sufentanil as a proof-of-concept that fentanyl analogs can present similar electrochemical signature than fentanyl, other fentanyl analogs will also be explored, both those illicit in nature, as well as other medical fentanyl analogs. Due to the high and growing number of fentanyl analogs, we will group them by the region of the fentanyl skeleton that has been substituted, and select two or three analogs from each group considering the electrochemical nature of the substituents they present and test them similarly to the study for sufentanil study. Once this demonstration would be done for sufficient representative fentanyl analogs, a single measurement screening in low volume will be done using CDC Traceable Opioid Material® in particular the Fentanyl Analog Screening Kit (FAS Kit), distributed by Cayman Chemical which currently contains over 210 fentanyl analogs.

## 8.3 Characterizing the Electrochemically-Active Metabolite of Fentanyl

As described in Chapter 6, the detection and analysis of the fentanyl metabolite peaks encountered several obstacles and remained challenging. The formed metabolite is supposedly norfentanyl, based on both Wang and coworkers who reported briefly the electrochemical observation of norfentanyl[17], and Ott *et al.*, who performed LC-MS-MS analyses of samples containing fentanyl before and after square wave voltammetry and reported the presence of norfentanyl only in the "after" samples[36]. Despite these literature reports of norfentanyl as the identified metabolites of the irreversible oxidation of fentanyl, using the current optimized parameters presented in this work, the electrochemical response of norfentanyl has yet to be confirmed. There are several steps to satisfyingly demonstrate that the formed metabolite(s) could be electrochemically detected and that the metabolite is in fact norfentanyl. One of the first steps would be to use an additional analytical technique such as LC-MS to determine if there is norfentanyl in the sample of solution after performing electrochemical analyses in particular after a large number of scans.

More critically, if the production of norfentanyl during the electrochemical oxidation of fentanyl is confirmed, additional optimizations for the electrochemical observation of norfentanyl would be necessary. Several electrochemical methods and parameters have already been attempted, as discussed in Chapter 6, to observe the peaks for norfentanyl. One future method that could be

used to analyze metabolic products will be DPV. Indeed, this electrochemical technique yielded the best definition for the fentanyl oxidation and metabolite peaks, so it could possibly give the best response for norfentanyl.

Another aspect that needs to be explored further is that when polishing the working electrode between scans, the peaks expected to be from the metabolites did not develop. This result could indicate that the forming peaks were caused by a reaction occurring at or close to the surface of the electrode. Although an amount of produced metabolites could explain why it could not be detected in the bulk of the fentanyl solution, this hypothesis did not explain why no peaks were visible in the initial scan of the norfentanyl standards. One hypothesis could be that it is not norfentanyl itself that is electroactive but some derivatives produced during the first scan, explaining why they are not detected at the initial scan. An in-depth electrochemical characterization of norfentanyl should uncover the optimal parameters for its detection and the electro-oxidative mechanism(s) involved.

### 8.4 Detection of Fentanyl and Fentanyl Analogs in Presence of Other Compounds

Fentanyl and its analogs are often found mixed with other adulterants and drugs in samples, thus it will be important to demonstrate that the screening method developed here will be selective to our targets. Different mixtures will be prepared with other drugs such as heroin and cocaine[6, 9] and adulterants such as caffeine, diphenhydramine, or cutting agents. With the help of collaborators, other realistic samples, or seized drugs could also be tested. The electrochemical detection method for fentanyl will be applied to those mixtures and establish if there are some potential interferents. With the whole signature, the method will focus on four different locations of the voltammograms and different behaviors: decrease of the oxidative peak of fentanyl, absence of a concomitant reduction peak, formation of the reduction and oxidative peak of the metabolites. Thus, even if other peaks are present from interfering electroactive species, the signature of fentanyl should be distinguishable.

# CHAPTER 9. CONCLUSIONS

The focus of the research discussed in this work was centered on the electrochemical characterization of fentanyl over multiple aspects including the electrochemical method, and the optimization of those parameters for the methods. The initial exploration with the electrochemistry of fentanyl utilized cyclic voltammetry the irreversible fentanyl oxidation peak, as well as the development of metabolite peaks in subsequent scans were observed. The best working electrode was a GCE and SPE, while PBS was considered the best supporting electrolyte. Through these optimization, f the impact of the concentration of KCl in the solution on the current intensity and definition of the fentanyl oxidative peak was observed, as well as a shift in potential of the fentanyl oxidative peak in presence of phosphates from 0.85 V vs. Ag/AgCl to 0.9 V vs. Ag/AgCl. SWV and DPV were also optimized for the detection of fentanyl. Both of these methods are considered more sensitive, and displayed a fentanyl oxidation peak, as well as metabolite peaks after only four scans, instead of the fifty used in CV. Commercial SPEs were also used for fentanyl, for their low-cost, portability, disposability, and small-volume. We demonstrated that a cleaning step was necessary prior to use the SPE and greatly improved the observations of the targeted peaks. Additional studies are still needed to electrochemically detect the formation of the metabolites. Overall, the electrochemical characterization of fentanyl resulted in the optimization of multiple electrochemical techniques, as well as the electrochemical conditions such as pH, ions present in supporting electrolyte, types of electrodes, or volume of solution. Using the optimized parameters for CV, SWV, and DPV with both the SPEs and the GCE, quantification of fentanyl was successfully performed. The linear ranges and correlation for the quantification of fentanyl with SPE was better than with GCE. The linear range for CV and SWV on the SPE was 5 to 100 µM, while when using DPV the linear range was 15 to 100 µM. The use of SPEs was explored successfully to visualize the electrochemical peaks of fentanyl, and the quantification was performed successfully with good linear correlation for concentrations of fentanyl that would be observed in forensic chemistry cases.

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# CURRICULUM VITAE

**NATALIE SELLNAU**

## EDUCATIONAL BACKGROUND

**Masters in Forensic and Investigative Sciences** **Aug 2019 – Present**

Chemistry Concentration

Indiana University - Purdue University Indianapolis (IUPUI)

Anticipated Graduation, Summer 2021

**Bachelor of Science with a Major in Chemistry** **May 2019**

Minor: Spanish

GPA: 3.59

University of Wisconsin Stevens Point (UWSP)

## PROFESSIONAL EXPERIENCE

**Research Assistant, Department of Chemistry and Chemical Biology, IUPUI Aug 2019-Present**

Indianapolis, Indiana

- Developing an electrochemical assay for the detection of fentanyl
- Using electrochemical techniques such as cyclic voltammetry and square wave voltammetry to analyze fentanyl and optimize parameters for detection

**Teaching Assistant, Forensic and Investigative Sciences, IUPUI January 2020-May 2020**

Indianapolis, Indiana

- Forensic Chemistry II-Lab, FIS: 40401
- Undergraduate Trace analysis lab, teaching use of gas chromatography-mass spectrometry (GC-MS), Fourier-transform infrared spectroscopy (FTIR), microspectrophotometer (MSP), Raman instruments to analyze trace evidence such as fibers, adhesives of tape, fire debris, and explosives

**Research Assistant, Chemistry Department, UW-Stevens Point** **Sept 2017- May 2019**

Stevens Point, Wisconsin

- Electrochemical analysis of THC (tetrahydrocannabinol) and CBD (cannabidiol)
- Using electrochemical techniques such as cyclic voltammetry and square wave voltammetry to optimize parameters for detection

## EXTRACARICCULAR ACTIVITIES

**Co-Chair of Suites Leadership Team** **Sept 2018-May 2019**

- Led Leadership Team meetings
- Helped mentor other members on leading programs, and organizing creative activities to engage students in residence hall

**General Member of Suites Leadership Team** **Sept 2017-May 2018**

- Contributed Ideas
- Collaborated with Suites Leadership Team to facilitate events to contribute to the community in the residence hall

## STUDENT ORGANIZATIONS

- American Chemical Society Student Affiliate (ACS), ACS member: September 2017- May 2019, general group member, participated in volunteer events and outreach activities
- National Residence Hall Honorary (NRHH), Inducted May 2019, Top 1% of student leadership in residence halls, general member

## PROFESSIONAL ORGANIZATIONS

- Society for Electroanalytical Chemistry (SEAC), general member **June 2020**
- International Society of Electrochemistry (ISE), general member **March 2020**

## VOLUNTEER WORK

- Alternative Spring Break 2019, Memphis, Tennessee March 17$^{th}$- March 23$^{rd}$, 2019
- STEAM Point Day for Girls, Stevens Point, Wisconsin February 2019
- STEAM Point Day for Boys, Stevens Point, Wisconsin November 2018

- National Chemistry Week, Stevens Point, Wisconsin October 2018
- Boys and Girls Club Chemistry Outreach, Stevens Point, Wisconsin October 2018
- Pointer Pals, Stevens Point, Wisconsin September 2018
- Steiner Hall Alcohol Awareness Run, Stevens Point, Wisconsin April 2018
- Earth Day at Farmshed, Stevens Point, Wisconsin April 2018
- Science Extravaganza, Stevens Point, Wisconsin March 2018
- RAVE Recovery Avenue Thanksgiving, La Crosse, Wisconsin November 2016, 2017
- STEM Point Day for Boys, Stevens Point, Wisconsin November 2015
- Community Thanksgiving Dinner, La Crosse Wisconsin November 2012-2016

**LEADERSHIP CONFERENCES**

- Great Lakes Affiliate of College and University Residence Halls (GLACURH), La Crosse, WI, November 17$^{th}$-19$^{th}$, 2018, Develop leadership skills in workshop environment at regional level to utilize in residence hall programs
- National Affiliate of College and University Residence Halls (NACURH), Tempe AZ, May 25$^{th}$-27$^{th}$, 2018, Develop leadership skills in workshop environment at national level to utilize in residence hall programs

**AWARDS**

- Second Place ACS Chair's Award "Think Like a Molecule" 2021 Poster Session, April 2021
- School of Science Forensic and investigative Sciences Program Charles (Chuck) Gould Memorial Scholarship, April 2021
- 2019 Undergraduate Award in Analytical Chemistry, University of Wisconsin-Stevens Point, May 2019
- OSCAR Research & Creative Activity Award, UWSP, awarded $500 for research materials and supplies in undergraduate research for 2018-2019 academic year

**PRESENTATIONS**

- 3rd Annual Chemistry Research Day, IUPUI, May 11th 2021, Indianapolis IN, *Electrochemical Characterization of Fentanyl for Forensic Analysis*, **Natalie Sellnau**, Frédérique Deiss (poster)
- Think Like a Molecule Annual poster session, Local ACS Indiana section, April 10th 2021, Virtual poster session, *Electrochemical Characterization of Fentanyl for Forensic Analysis*, **Natalie Sellnau**, Frédérique Deiss (poster)
- IU200 Multidisciplinary Poster Presentations, IU Beyond Borders Symposium, April 9th 2021, virtual, *Electrochemical Characterization of Fentanyl for Forensic Analysis*, **Natalie Sellnau**, Frédérique Deiss (poster)
- 2021 ACS Indiana Selected Graduate Student Symposium, Local ACS Indiana section, April 9th 2021, *Electrochemical Characterization of Fentanyl for Forensic Analysis*, **Natalie Sellnau**, Frédérique Deiss (oral)
- PITTCON Conference and Expo., March 2021, virtual due to COVID-19, *Electrochemical Analysis of Fentanyl and Fentanyl Analogs Towards a Rapid Screening Assay*, **Natalie Sellnau**, Frédérique Deiss (Oral)
- AAFS 2021 Annual Scientific Meeting, February 2021, Virtual due to COVID-19, *Electrochemical Analysis of Fentanyl and Fentanyl Analogs Towards a Rapid Screening Assay*, **Natalie Sellnau**, Frédérique Deiss (poster)
- FIS Graduate Student Seminar, October 2020, IUPUI, Virtual due to COVID-19, *Electrochemical Analysis of Fentanyl and Fentanyl Analogs Towards a Rapid Screening Assay*, **Natalie Sellnau**, Frédérique Deiss (oral)
- 71st Annual Meeting of the International Society of Electrochemistry, August 30th-September 4th, 2020, Virtual poster session due to COVID-19, *Electrochemical Analysis of Fentanyl and Fentanyl Analogs Towards a Rapid Screening Assay*, **Natalie Sellnau**, Frédérique Deiss (poster)
- Annual poster session, Think Like a Molecule, Local ACS Indiana section, April 18th, 2020, Virtual poster session due to COVID-19, *Electrochemical Analysis of Fentanyl and Fentanyl Analogs Towards a Rapid Screening Assay*, **Natalie Sellnau**, Frédérique Deiss (poster)

- 2nd Annual Chemistry Research Day, IUPUI, January 7th, 2020, Indianapolis, IN, *Electrochemical Analysis of Fentanyl and Fentanyl Analogs for Universal Detection*, **Natalie Sellnau**, Frédérique Deiss (poster)
- 68th Midwestern Universities Analytical Chemistry Conference (MUACC), November 2019, Indianapolis, IN, *Electrochemical Analysis of Fentanyl and Fentanyl Analogs for Universal Detection*, **Natalie Sellnau**, Frédérique Deiss (poster)
- 20th Annual College of Letters and Science Undergraduate Research Symposium, May 3rd,2019, Stevens Point, WI, *Using Electrochemistry to Detect THC Metabolites and CBD*, **Natalie Sellnau**, **Jaden McKiernan**, Shannon Riha (poster)
- 18th Annual UW System Symposium, Undergraduate Research, Scholarly and Creative Activity, April 26th, 2019, Green Bay, WI, *Using Electrochemistry to Detect THC Metabolites and CBD*, **Natalie Sellnau**, Jaden McKiernan, Shannon Riha (poster)
- UWSP Chemistry Student Seminar Series, March 27th, 2019, Stevens Point, WI, *Electrochemical Analysis of THC and CBD*, **Natalie Sellnau**, Shannon Riha (oral)
- 19th Annual College of Letters and Science Undergraduate Research Symposium, May 4th, 2018, Stevens Point, WI, *Using Electrochemistry to Detect THC Metabolites*, **Natalie Sellnau**, Shannon Riha (poster)