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The Use of Electrospun Nanofibers with Gold and Silver Nanostars for the Detection of Fentanyl Using Surface Enhance Raman Spectroscopy, the Modification of Current GC-MS Methods to Increase Separation and Detection of Synthetic Cathinones in Mixtures

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Daniel Rubin

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To: Dean Michael Heithaus
College of Arts, Sciences, and Education

This thesis written by Daniel Rubin and entitled The Use of Electrospun Nanofibers with Gold and Silver Nanostars for the Detection of Fentanyl Using Surface Enhanced Raman Spectroscopy, the Modification of Current GC-MS Methods to Increase Separation and Detection of Synthetic Cathinones in Mixtures, having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this thesis and recommend it be approved.

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The thesis of Daniel Rubin is approved.

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ABSTRACT OF THE THESES

THE USE OF ELECTROSPUN NANOFIBERS WITH GOLD AND SILVER NANOSTARS FOR THE DETECTION OF FENTANYL USING SURFACE ENHANCED RAMAN SPECTROSCOPY, THE MODIFICATION OF CURRENT GC-MS METHODS TO INCREASE SEPARATION AND DETECTION OF SYNTHETIC CATHINONES

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Many criminal cases involve the use or sale of illicit substances. The forensic analyst has many different techniques they can use to analyze samples to determine which illicit substance is present. This project aims at developing electrospun nanofibers with nanoparticles to analyze fentanyl by surface enhanced Raman spectroscopy, as well as modifying current GC-MS parameters to enhance the separation and analysis of synthetic cathinones. Using the fibers with nanoparticles allowed for the detection of fentanyl standards at 1mg/ml while slowing the oven ramp rate and modifying the hold times led to an increase in separation and better fragmentation patterns for determination. Overall, this thesis presents 2 different projects involving methodologies to improve drug detection and analysis. The results of this work can benefit forensic analysis of seized drugs.

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LIST OF ABBREVIATIONS

CNS	Central Nervous System
GPCR	G-Protein Coupled Receptor
PNS	Peripheral Nervous System
GC-MS	Gas Chromatography-Mass Spectrometry
TOF	Time of Flight
MW	Molecular Weight
PVP	Polyvinylpyrrolidone
PVA	Polyvinyl alcohol
SERS	Surface Enhanced Raman Spectroscopy
ng	nanogram
mg	milligram
ml	milliliter
˚C	Degrees Celsius
Hr	Hour
m/z	Mass to charge ratio

Chapter I: Illicit Substances

1.Introduction

Illicit substances are drugs that have negative long-term health effects on the human body. They can be classified into three main groups stimulants, depressants, and hallucinogens. A drug classified as a stimulant will have a stimulating effect on the central nervous system (CNS) inducing an individual to feel more awake or alert, a depressant will reduce CNS activity which causes an individual to relax, or fall asleep, a hallucinogenic will affect the signaling pathway in the brain which alters a person's perception of reality. Due to the serious health issues that may stem from the consumption of these substances the United States federal government passed the controlled substance act of 1970. This act grouped drugs into 5 different schedules based on the drugs' medical use, the potential for abuse, or if the drug induces some type of dependency. Schedule 1 is the highest level a drug can be placed in. For drugs to be placed in this category they have a high potential for abuse, and currently have no medical use. The most famous drugs in this category are heroin, marijuana, LSD, ecstasy, and synthetic cathinones. In schedule 2 the drugs have a high potential for abuse but can be used in certain medical situations. The most famous drugs in this category are cocaine, methamphetamine, and fentanyl. Schedule 3 drugs are classified as having a moderate level of physical or psychological abuse. Some common drugs in this category are ketamine, steroids, and testosterone. Schedule 4 drugs are drugs that are classified as having a low potential for abuse and low risk of dependence. Some common drugs in this category are Xanax, valium, and tramadol. Schedule 5 is the lowest level of scheduling.

In this category, the drugs have the lowest level of abuse and some common drugs are cough syrups, and antidiarrheal medication like Lomotil. The different schedules of drugs will help the courts determine what sentencing if any will be given at trial. For this reason, it is very important for the forensic analyst to have many different tools to analyze the different illicit substances.

2.Fentanyl

Fentanyl is a synthetic opioid that was designed to be used as a pharmaceutical pain killer in the treatment of late-stage cancer. It was originally synthesized by Dr. Janssen in Belgium in December 1960 (Stanley, 2008). Initially, fentanyl was used as an intravenous painkiller to help treat patients with pain in a hospital setting. Fentanyl has been used as an analgesic during surgical operations and is used in most developed countries around the world for that purpose. More recently, fentanyl has also been incorporated into a patch that can be worn by patients for the treatment of chronic cancer pain. While fentanyl is designed to treat pain in hospital patients it also has the ability to induce a feeling of euphoria in people that use the drug recreationally. This sense of euphoria after taking the drug leads people to become addicted to the drug and can lead to long-term term health problems and death by overdose. The best method to treat someone suspected of having a fentanyl overdose is to treat them with naloxone as an intranasal spray, or intramuscular or intravenous injection. Naloxone works as a mu-opioid antagonist that can prevent fentanyl from binding to the receptor, as well as remove fentanyl that has already bound to the receptor. It is this ability to unbind fentanyl that makes naloxone such an effective treatment for opioid overdoses.

Fentanyl is one of the most potent opioids currently being made. It is a synthetic opioid that is 80-100 times more potent than morphine with a therapeutic range of 0.3-3.8 ng/ml (Anderson, 2000). This means that only a small amount of fentanyl is needed to induce the analgesic effects, making it easy to overdose on this drug. Fentanyl is a synthetic opioid that works by binding to the mu-opioid receptor in the brain. The mu-opioid receptor is responsible for the body's interpretation and response to pain. Pain travels throughout the body in two main ways. The first method is known as the ascending pathway. This pathway carries the signal from the sensory neurons in the peripheral nervous system to the central nervous system, and then this signal travels up the spine to the brain. The second pathway is known as the descending pathway. In this pathway, signals are sent from the brain down the spinal to the peripheral nervous system to induce a reflex response to limit interaction with the pain source (Cross, 1994). Fentanyls and other opioids affect both the ascending and descending pathways to limit pain. Fentanyl works as an antagonist in the ascending pain pathway by inhibiting the pain neurotransmitters at the rostral ventral medulla (Elbaridi, 2017). This limits the number of neurotransmitters reaching the brain minimizing the binding of pain neurotransmitters and reducing the amount of pain felt. Fentanyl works as an antagonist in the descending pain pathway by activating the signaling pathway of G-protein coupled receptors (GPCR) (Lueptow, 2018). The activation of the GPCRs in turn reduces the activity of the GABA neurotransmitters reducing the pain signals. These two methods for inhibiting the pain signaling lead to sedation and reduces the emotional response to pain making fentanyl and other opioids such strong pain killers.

3.Cathinones

Cathinones are a constantly changing class of designer drugs that originally derived from the Khat plant (Catha edulis), originating from the horn of Africa (Luqman and Danowski, 1976). The leaves from the Khat plant are chewed by the locals due to the stimulating effects, which they say is similar to drinking a cup of coffee. Due to the stimulating effect of the Khat plant, a lot of research has been done to determine which chemical is responsible for the stimulating effect. The leaves of the khat plants contain over 40 different compounds such as alkaloids, tannins, flavonoids, amino acids, vitamins, minerals, and cathine (Halbach, 1972). It was originally thought that the plant contained caffeine which was leading to the stimulating effects however it was discovered that there was an alkaloid present in the plant that was later called cathine (Fluckiger and Gerock, 1887). In the early 1900s research was done to extract the cathine from the khat plant. During the extraction process, an amorphous base was discovered. It was determined that this base was the sulphate and hydrochlorate form of katine (Beitter, 1900). After the extraction of this katine compound, it was determined that the concentration of this compound was less than 1% in the leaves (Beitter, 1900). Eventually, cathine was extracted from the leaves and the structure was determined to be (+)-norpseudoephedrine (Wolfes, 1930). For many years this was assumed to be the active component in the leaves until 1980 when it was discovered that there was less than 10% cathine in the leaves (Peterson, 1980). Eventually, it was discovered that cathinone is the compound that leads to the stimulating effect specifically the (*S*)-(−) stereoisomer of cathinone, and that it consists of a benzene ring, an alpha-betaa carbonyl, and an amino group (Kalix, 1990). The structure of cathinone allows it to cross the blood-brain barrier

to interact with neurotransmitters. The cathinones will interact with monoamine neurotransmitters leading to the release of neurotransmitters and preventing the reuptake of these neurotransmitters (Simmler, 2014). Cathinones can interact with neurotransmitters to increase the synaptic concentrations of neurotransmitters like dopamine, noradrenaline, or serotonin (Gołembiowska and Kamińska, 2018). Other synthetic cathinones can act as a neurotransmitter blocker, which prevents the reuptake of the neurotransmitters (Gołembiowska and Kamińska, 2018). This blocking mechanism is similar to the way cocaine interacts with neurotransmitters. The increase in the release of these neurotransmitters and the slow reuptake leads to the stimulation of the central nervous system (CNS) as well as the peripheral nervous system (PNS). The stimulation of the PNS leads to an increase in the body temperature, heart rate, blood pressure, and respiration (Patel, 2009). The stimulation of the CNS leads to a feeling of euphoria if enough drug is consumed, along with the individual being more alert to their surroundings (Patel,2009). Some of the more common side effects of this are insomnia, anorexia, as well as some manic behavior such as schizophrenia, which have been reported (Patel,2009). It is due to these unwanted side effects that lead cathinone to be classified as a schedule 1 drug by the drug enforcement agency.

4.Drug Scheduling

Once a drug becomes scheduled by the DEA the risks involved increase for the drug dealers. In order to minimize the risks taken on by the drug dealers, they have begun synthesizing designer drugs. These designer drugs are designed to have a similar structure to illicit substances in order to have the same or similar effects to currently

scheduled substances. These designer drugs are designed to be different enough from the scheduled drug in order to circumvent drug laws. Fentanyl and cathinone are two illicit drugs that are prime candidates to be used as a base for designer drugs due to their popularity among recreational users, as well as the ease at which they can be modified into designer drugs. Fentanyl has a propanamide, a piperidine, and two benzene rings. These functional groups make fentanyl reactive with many other compounds allowing for modification for many points on the molecule. Cathinone has a benzene ring, an alpha-beta carbonyl, and an amino group. These functional groups are reactive and allow for the modification of cathinones into designer drugs.

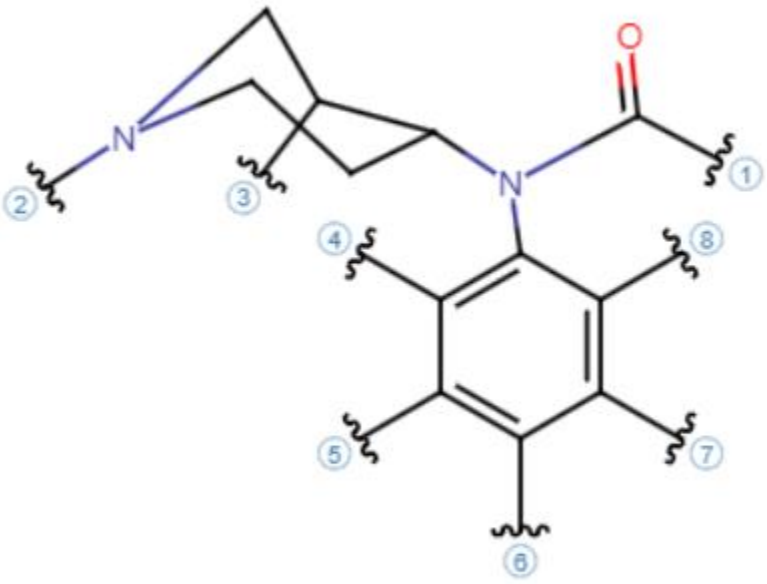

Figure 1. The reactive sites for fentanyl, and where the modifications are made to make fentanyl a designer drug.

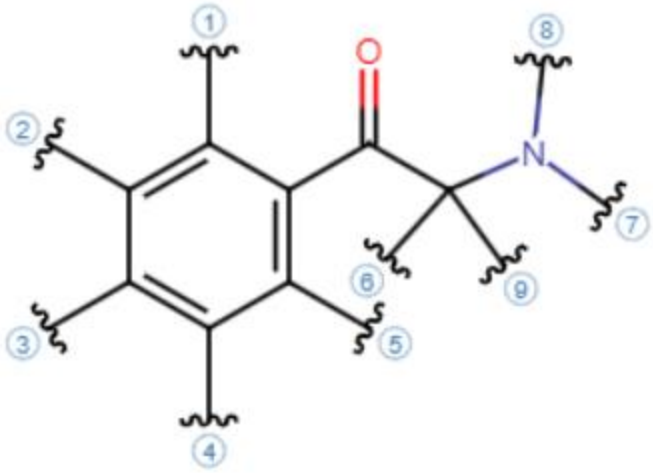

Figure 2. The reactive sites for cathinone, and where the modifications are made.

Drug arrests in the United States have been decreasing over the past several years, while drug-related deaths have increased (DEA, 2020). One explanation for the decrease in the number of arrests nationally for illegal substances is because of the legalization of marijuana in certain states. Another possible explanation for the decrease in drug arrests is that people have begun to decrease their consumption of certain types of illicit substances. This is best shown by looking at the trends for cathinone abuse. From 2015 to the present, the number of people abusing synthetic cathinones has decreased. This is shown in data from records from the American Association of Poison Control Centers. Where in 2016 there were 382 cases of people requesting assistance with cathinone poisoning. In 2018 the number of cases decreased to 290 and then in 2019, the cases decreased to 268 cases. One possible explanation for the increase in the number of drug-related deaths is due to the fact that people are using more potent forms of drugs than they have used in the past. This can be seen in the increase in fentanyl use in the U.S.

from 2013-2019. In 2013 there were close to zero cases of fentanyl reported while in 2019 the number of fentanyl cases increased to over 100,000 cases (NFLIS, 2019). The death rate for opioids has been steadily increasing in the U.S., and in 2019 there were more than 36,000 deaths by synthetic opioids alone (Mattson, 2019). The number of deaths by opiate abuse in 2019 is estimated to be 12 times higher than it was in 2013 (Mattson, 2019). Another possible explanation for the increase in opioid deaths is drug dealers are selling opioids like heroin with fentanyl mixed in to try and increase potency and increase profits. The individuals that are purchasing these drugs believe that they are purchasing heroin and will accidentally overdose on fentanyl not knowing that it was present in their sample.

Chapter II Detection Methods

1.Minimum Criteria for Confirmation

There are many different analytical tests that can be done to determine if an illicit drug is present. These tests can be divided into presumptive or confirmatory tests. Presumptive tests are qualitative tests that are used to identify the presence of certain compounds. The most common types of presumptive tests in forensic science are the color spot tests. In the spot test, a small amount of sample is mixed in a known solution. If the solution changes color, then it is presumed that an illicit substance is present. In the event that there is a color change then the analyst will move on to the second phase of analysis, which is the confirmatory test. The confirmatory test is used to positively identify the unknown compound that had a positive presumptive test. These presumptive and confirmatory tests are broken down into three different categories. Category C contains all the presumptive tests such as the color tests, immunoassay, melting point, UV spectroscopy, and immune assay. Category B contains all the separation techniques, microscopic analysis, and crystal tests. Category A contains the detection methods for confirmation and consists of mass spectrometry, Raman spectroscopy, and nuclear magnetic resonance analysis. When analyzing samples for illicit substances multiple methods must be used from the different categories. The minimum criteria for the determination of a controlled substance will consist of two methods from category C and 1 method from category B or A, or it will consist of two methods with one method being from category B and the other method being from category A.

Table 1. The three different categories of analysis and the different techniques that fit the criteria.

Category A	Category B	Category C
Infrared Spectroscopy	Capillary Electrophoresis	Color Tests
Mass Spectrometry	Gas Chromatography	Fluorescence Spectroscopy
Nuclear Magnetic Resonance Spectroscopy	Ion Mobility Spectrometry	Immunoassay
Raman Spectroscopy	Liquid Chromatography	Melting Point
X-ray Diffractometry	Microcrystalline Tests	Ultraviolet Spectroscopy
	Pharmaceutical Identifiers	
	Thin Layer Chromatography	
	Cannabis only: Macroscopic Examination Microscopic Examination	

2.Raman Spectroscopy

Raman Spectroscopy is a spectroscopic technique that works by detecting the interaction between inelastically scattered light and the polarizability of molecular vibrational bonds. The scattered light interacts with these molecular bonds resulting in a unique spectrum for each compound. The inelastically scattered light may be higher (anti-Stokes scattering) or lower (Stokes scattering) in energy than the Raleigh scattered light resulting in two separate but equivalent spectra (Kauffmann, 2018). The problem with this method is that only a small proportion of the scattered light is inelastically

scattered (~ 0.000001%). Thus, Raman spectroscopy is not sensitive enough to detect drugs at trace concentrations. To combat this relatively low sensitivity, gold, or silver metallic nanoparticles can be added to the surface to enhance the signal from the analyte. The metallic nanoparticles help enhance the signal through an interaction between the localized surface plasmon field of the metal, the adsorbed molecules, and the scattered light. This surface Enhanced Raman Spectroscopy technique has been shown to increase an analyte signal by a factor of up to 10^{10} which means theoretically it is possible to measure a single molecule in solution.

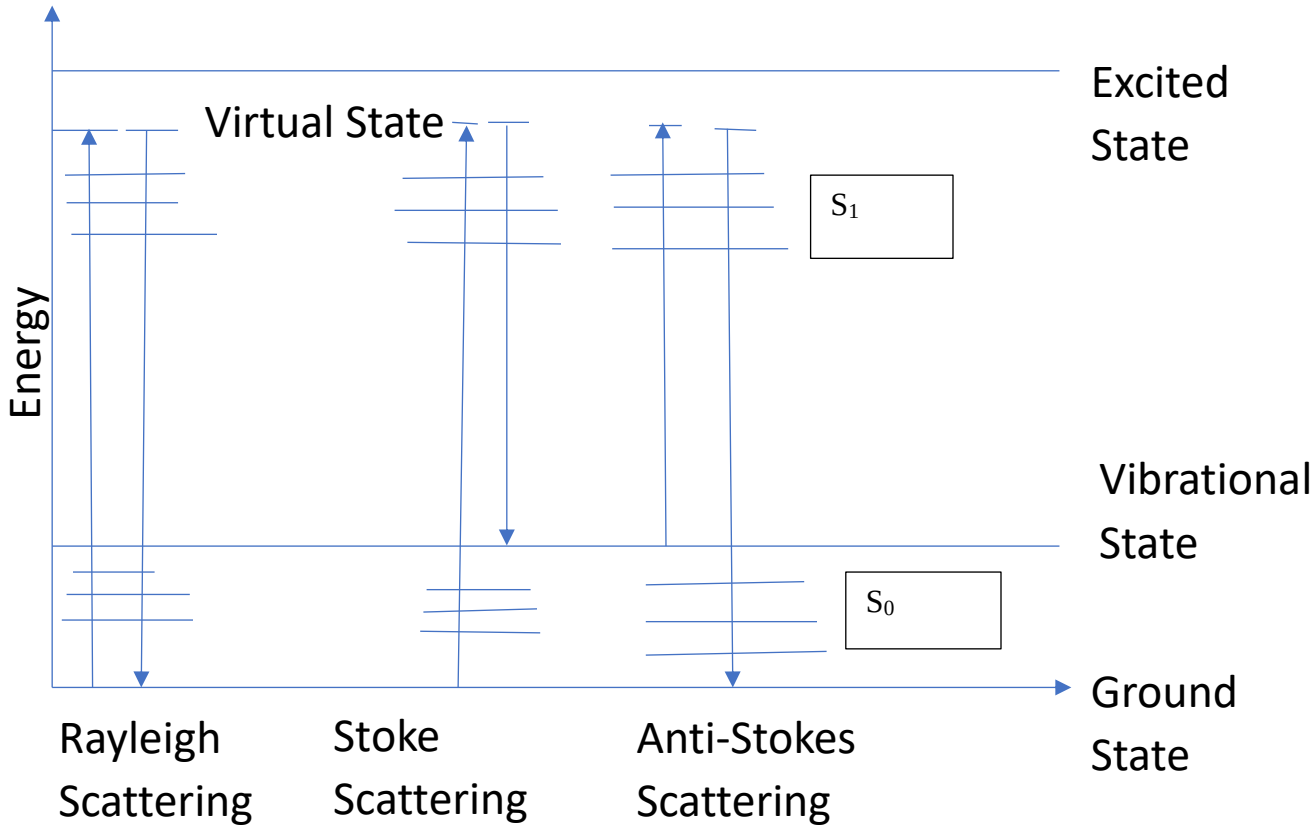

Figure 3. A Jablonski diagram of the energy of scattering light from a Raman system

3.Nanoparticles

The metallic nanoparticles can have different sizes and shapes which can affect their ability to enhance Raman signals. Nanoparticle size influences surface plasmon adsorption frequencies and affects signal enhancement, depending on the molecular distance from the particle and laser frequency (Joseph, 2011). Two common shapes for nanoparticles are spherical and star-shaped. It has been experimentally shown that star-shaped nanoparticles with more rough edges lead to an improved electromagnetic field enhancement effect, presumably due to lighting rod effects and the orientation between the molecule and the electric field (Gersten,1980).

Nanoparticles can be synthesized in a variety of methods. One method involves crushing a larger particle into smaller pieces. This method is relatively quick to perform, but it gives very little control over the final shape of the particle. A second method of synthesizing nanoparticles involves building up nanoparticles from smaller particles through a synthetic process and crystallization. In this method, there is more control over the final shape of the particle, but it takes more time (Arora, 2003). An example of a bottom-up pathway is the seeded development of silver nanoparticles. silver seeds can be made from an aqueous solution of $AgNO_3$, 2-Hydroxy-4′-(2-hydroxyethoxy)-2-methylpropiophenone, and trisodium citrate that is irradiated for 5 minutes with long-range ultraviolet light rays. Once this is done L-ascorbic acid is slowly added to the solution, gradually increasing the size of the silver nanoparticles (Stamplecoskie, 2011). Gold nanoparticles can be synthesized in a similar way. For example, $HAuCl_4$ can be

reduced with trisodium citrate to make the seeded form of the nanoparticle. Once this is done hydroxylamine can be added to the solution to increase the size of the nanoparticle (Hong, 2012). An example of a top-down synthesis is shown in the formation of multifunctional iron oxide magnetic particles. In this method bulk iron is placed in a container with distilled water and a high-powered laser from a laser ablation system is fired at the bulk iron sample (Amendola, 2011). In this top-down method, the strength of the laser pulses and the radius of the focus lens determine the size of the nanoparticles, and the amount of time the laser is allowed to pulse determines how many nanoparticles are produced.

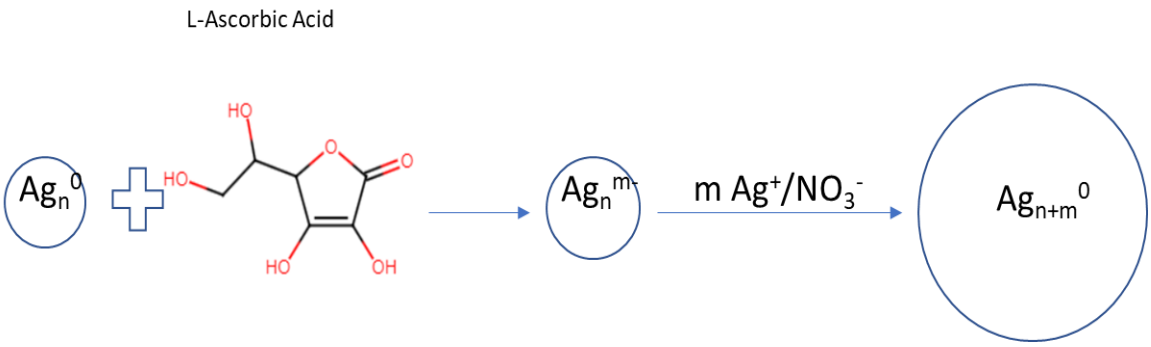

Figure 4. The chemical reaction of a silver nanoparticle reacting with L-ascorbic acid to create a larger silver nanoparticle.

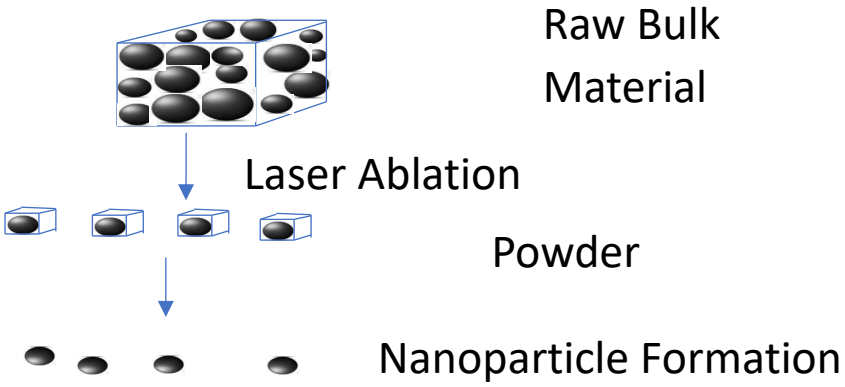

Figure 5. A schematic of how to make nanoparticles from a top-down approach.

Once the nanoparticles have been made, they can be used to enhance the signal of different organic compounds in Raman Spectroscopy. In order to get the greatest enhancement of the signal, an aggregating agent should be used. The aggregating agent is used to bring the nanoparticles closer together in a cluster. This clustering of the nanoparticles allows for an increase in hotspots formed allowing for a greater enhancement of the signal. To induce aggregation a chloride salt is typically used as the chloride will displace the stabilizing agent and the chloride will modify the ionic strength of the solution changing the surface charge of the substrate and leading to increased intensity (Doctor, 2013). When using aggregating agents time is required to allow the aggregating agent to interact with the nanoparticles. Once this has been done the target analyte can be added to the solution. After this addition, more time is required to allow for the analyte to interact with the created hot spots. Using this method allows analysts to detect illicit substances at very low levels. Previous work shows fentanyl can be detected at the ng/ml level (Wang, 2021). This method of aggregation requires at least 10 minutes of interaction between the analyte and the aggregates. Improved methods are needed to reduce this time.

4.GC-MS

Gas chromatography-mass spectroscopy (GC-MS) is the gold standard for forensic analysis because it combines a category B technique with a category A technique which satisfies the minimum criteria for the identification of a controlled substance. This technique can also be used to analyze a variety of different compounds while only needing to modify the oven parameters. This technique combines gas chromatography

which is a separation technique with mass spectrometry which is a determination technique. The GC instrument consists of an oven, an injector, the carrier gas, and the separation column. GC is a useful technique that can be used to separate compounds that can be volatilized. The injector is used to collect the liquid sample from the vial and inject into the column. The injector is important because it allows for more consistent injection volumes run after run. The compounds are injected into the GC and the oven is used to increase the temperature to volatilize the analyte. Once the analyte has been volatilized the carrier gas is used to carry the analyte through the column. The carrier gas should be an inert gas that will not interfere with the analysis. For this reason, helium is the most commonly used carrier gas. The column is the main separating agent that separates compounds based on how long the analyte interacts with the stationary phase. Compounds that are strongly attracted to the column will take longer to elute from the end of the column while compounds that are not attracted to the column will elute much quicker. This difference in attraction is what leads to the separation of the different compounds in the GC system. Once the analytes have been separated the analytes can then be immediately analyzed via mass spectrometry.

Mass spectrometry is one of the most used detection methods in forensic analysis. In this method, the detector measures the mass to charge ratio of the analyte. There are two main ways the MS can separate the mass to charge ratio of the analyte fragments. The first method is to use the time of flight (TOF) separation, and the second method is to use quadrupoles to filter out ions one at a time. These methods are important to prevent multiple ions from hitting the detector at the same time, in order to prevent the detector from being overwhelmed. TOF uses distance traveled to separate charged ions. The

instrument has an electric field with a known strength that imparts the same amount of kinetic energy to the ions. Since all the ions have the same kinetic energy, the lighter ions will travel faster than the heavier ions leading to separation based on mass to charge ratio. This separation of light and heavy ions can be increased even further by increasing the length of the instrument or by using a reflectron to increase the distance traveled by the ions. This increased travel distance allows for greater separation of ions with similar mass to charge ratios.

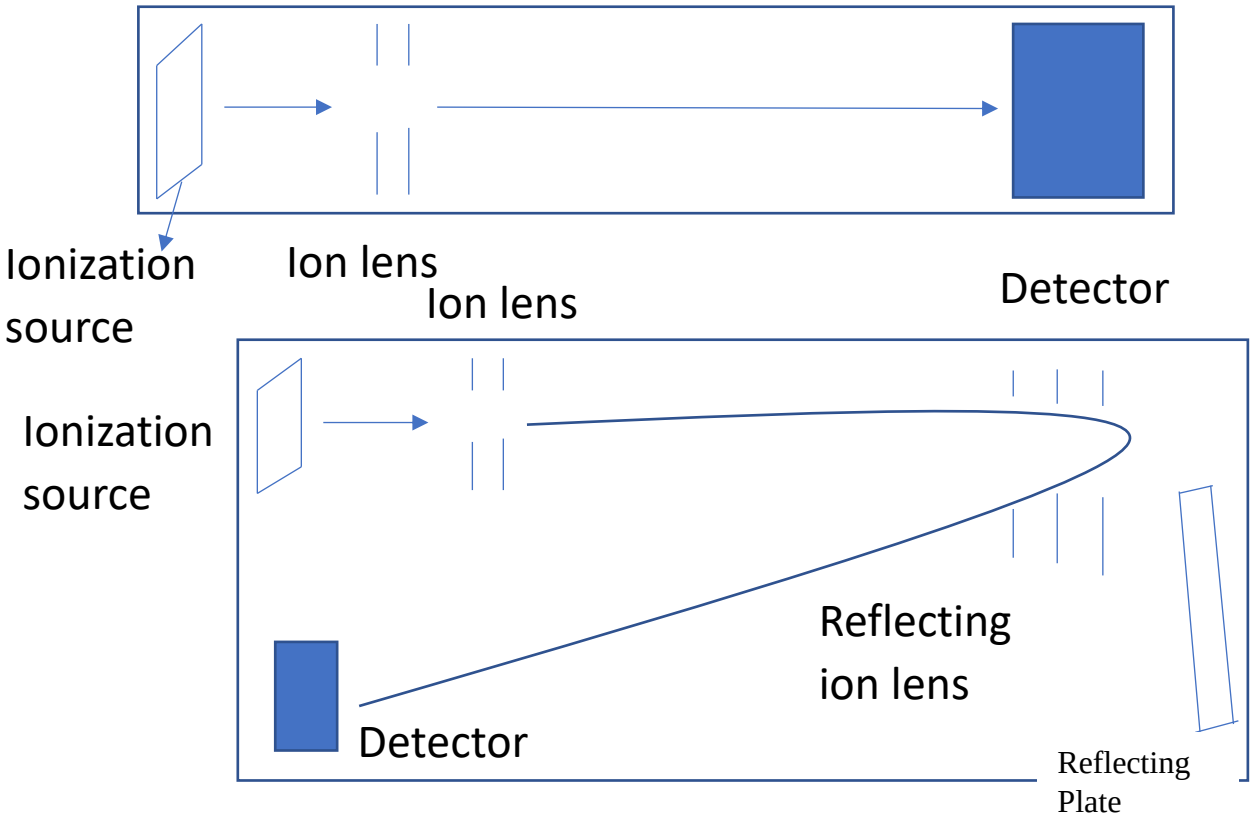

Figure 6. A schematic of a TOF MS. Top is a linear TOF Bottom is a reflectron TOF

Quadrupole separation uses four current-carrying rods to separate the ions based on their mass to charge ratio. The current flows through the four rods creating a magnetic field. The strength of the magnetic field is specific enough to allow one ion to pass through the entire quadrupole while causing all other ions to crash into the rods preventing them from reaching the detector. Once the ion reaches the detector the current flowing through the rod can be changed, altering the strength of the magnetic field and permitting a different ion to travel through the magnetic field to reach the detector. This is step is repeated multiple times until the entire sample has been analyzed.

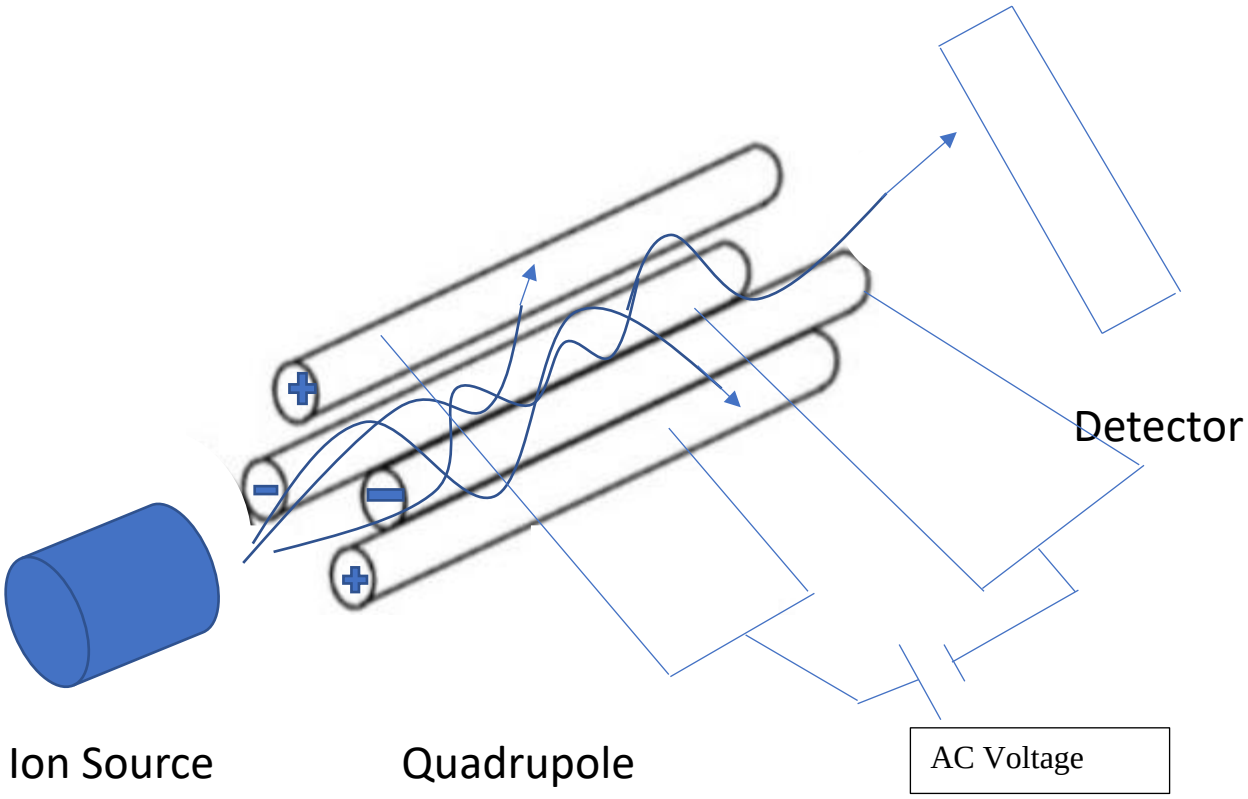

Figure 7. A schematic of a quadrupole MS.

GC-MS is a staple in the forensic lab and has been used to analyze many different substances. The importance of this instrument can be clearly seen when used to identify

new designer drugs with similar morphology. When analyzing the data generated by GC-MS it can be difficult to identify compounds with similar structures, therefore it is important to optimize the GC conditions in order to get better separation to assist in the analysis (Carlsson, 2018). The best way to get better separation using a GC instrument is to use a longer column, slow the oven ramp temperatures, and use a thinner column to increase interaction between the target analyte and the stationary phase.

Raman spectroscopy and GC-MS are two excellent tests used in forensic science that can be used to analyze a wide range of analytes. Raman spectroscopy alone is considered a presumptive test but can be confirmatory when combined with other analytical methods. GC-MS is a confirmatory method as it combines a separation technique with a detection method all in one. The benefits of using Raman spectroscopy are it is a non-destructive technique, and the spectra obtained are unique to each compound. The downsides to this method are it is a newer method with a smaller library of references, and it can only be used for Raman active compounds. The benefits of using GC-MS are it is a sensitive technique with a wide library to reference. The downside to this detection technique is it is a destructive technique, so samples cannot be reanalyzed.

Chapter III: Electrospinning

1.Introduction

Electrospinning is a technique that is used to make a continuous fiber with a diameter in the submicron to the nanometer range. To prepare these fibers, a high voltage is applied to a solution of water-soluble polymers at the tip of a needle. As the liquid leaves the syringe the electrostatic repulsion forces overpower the surface tension forces of the liquid and the droplets are stretched to create a Taylor cone. As the liquid travels from the needle tip to the collecting plate the liquid dries creating a fiber that is then collected onto a grounded plate. The process gets its name from the fact that the applied voltage creates a perpendicular magnetic field and when the polymer is pulled through a fine needle the viscous liquid spins and dries creating nanofibers. The spinning of the liquid looks similar to a ribbon being spun. The electrospinning process is incredibly versatile and can be used to make many different types of nanofibers

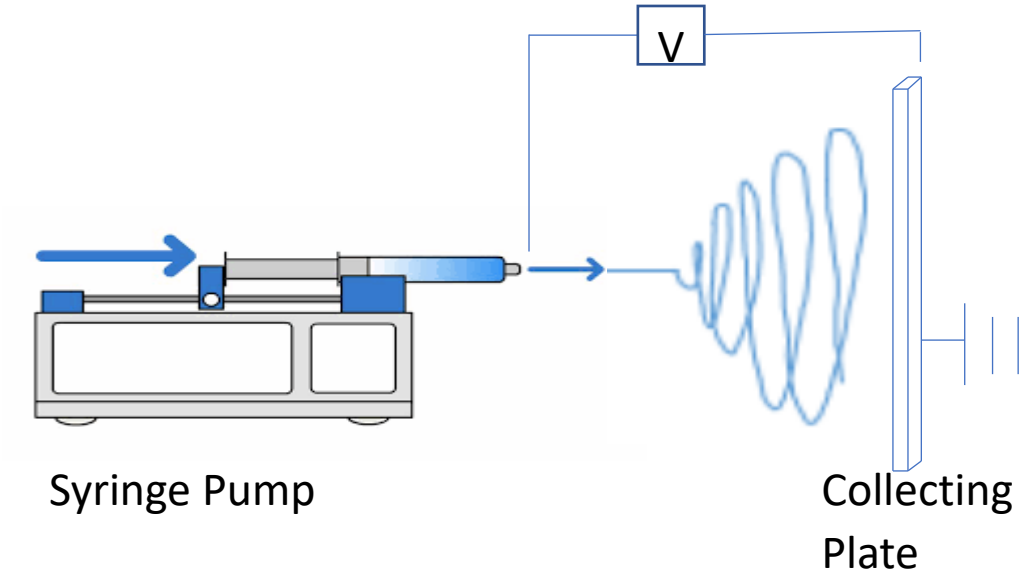

Figure 8. A schematic of the electrospinning process.

When using the electrospinning technique there are many different parameters that need to be controlled to successfully create fibers. These factors are the viscosity of the liquid, the voltage applied, the collecting plate used, the flow rate of the syringe pump, and the percent humidity in the room.

2.Viscosity

Viscosity is the resistance of a fluid to change shape and denotes the fluid's opposition to flow. The lower a solution's viscosity the easier the liquid will flow, while the higher the viscosity the stickier the solution is. A solutions viscosity is one of the most important factors to consider when using the electrospinning procedure. If the viscosity is too low the fibers created will not be continuous and the procedure essentially becomes an electrospray technique (Amariei, 2017). In the event that this occurs, the collected sample will end up evaporating if it is not stored in the proper conditions. If the solution is too viscous then the needle will become clogged, and the solution will not come out of the tip of the needle. In general, the optimum viscosity of the solution will be from 1-20 poise rating (Amariei, 2017). If the viscosity is on the lower end of this range, then the diameter of the created nanofibers will be on the lower end while if the viscosity is on the upper level of this range, then the diameter of the created nanofiber will be larger.

3.Voltage

Voltage is a measurement of the energy contained in an electrical field, or in an electric circuit at a given point. The voltage is related to the current and resistance in the circuit and can be calculated using the V=IR equation. Essentially the voltage indicates

the electrical potential energy at that point. Voltage plays a major role in the electrospinning process as it plays a role in the shape and diameter of the created fiber. Using a low voltage during the electrospinning process reduces the strength of the electric field reducing the jet speed and increasing the flight time (Zhao, 2004). If the applied voltage is increased then the flight of the jet increases, leading to an increase in the stretch of the fibers, decreasing the diameter of the created nanofiber (Mo, 2004). Using a higher voltage during the electrospinning process also has the added benefit of drying the fibers quicker, meaning the fibers will be usable quicker with a high voltage compared to a low voltage (Pawlowski, 2003). Essentially this means that using a higher voltage will lead to thinner diameter fibers while using a lower voltage will lead to thicker fibers. The lowest voltage that can be used in the electrospinning procedure is 6Kv while the highest voltage that can be used will depend on the polymer used and the viscosity of the solution (Taylor, 1964). The typical range of voltage used in this procedure is 6-50 kilovolts.

4.Collection Method

The collecting plate used also plays a major factor in the way nanofibers are produced. When collecting the nanofibers there are two types of collecting plates that can be used. The first one is a rotating plate, and the second type is a flat stationary collector. The rotating collecting plate allows for the collection of aligned nanofibers. The degree of the alignment will depend on the speed of rotation of the collecting plate (Baji, 2010). If the collecting plate rotates at a fast rate the fibers will be completely aligned with the other fibers formed while if the plate rotates at a slow rate, then the fibers will be

collected with a more random orientation. When using a stationary collecting plate there is no alignment of the fibers and as a result, this method creates the most random alignment of the fibers. Since there is no alignment of the fibers created different layers of fibers will form on top of the previous layers. When using a stationary collecting plate, the distance from the tip of the needle to the collecting plate plays a very important role in the morphology of the fibers. The distance from the needle to the collector allows for the solvent to dry out and the solid nanofibers to form. If the distance between the needle to the collector is too short then the solvent will not have enough time to evaporate, and the fibers will become fused together (Ghelich, 2015). This fusing of the fibers is not ideal and as a result of this the shortest distance that should be used when creating nanofibers is 10 centimeters. The diameter of the nanofibers is also related to the distance from the needle tip to the collecting plate. The elongation of the fibers created can be calculated by $\varepsilon = \frac{vspin - vsol}{H}$ (Baji, 2010). Where ε is the elongation of the fiber, v_{spin} is the spinning velocity of the Taylor cone, v_{sol} is the ejecting velocity of the solution, and H is the distance. Using this equation, it can be determined that the closer the collecting plate is to the needle the smaller in diameter the fibers are while the closer the collecting plate is the thinner the fibers are. This is why the collecting plate distance plays an important role in the formation of the nanofibers.

5.Humidity

Humidity is the amount of water vapor in the atmosphere or in a gas. The amount of humidity in the air has a major impact on the morphology and mechanical properties of the nanofibers. As the humidity increases the nanofibers become less uniform and

more unstable (Raksa, 2021). This instability occurs because as the amount of water in the air increases the longer it takes for the electrospun solution to dry out making the solidification process take longer. When the time for the drying process increases the layers of nanofibers coalesce and form beads. These beads have a negative impact on the formation of the electrospun nanofibers and bead formation needs to be reduced as much as possible. The optimum humidity for uniform nanofiber shape and diameter is between 40-50% humidity (Raksa, 2021). When the percent humidity is in the 60% range the fibers formed have more inconsistencies in the shape and diameter and when the humidity gets any higher than that the fibers do not form. It is for these reasons that the humidity is tightly controlled during the electrospinning process.

6.Crosslinking

Electrospun nanofibers are incredibly small and as a result they are very delicate. In order to increase the strength of the nanofibers, the fibers can be cross linked together. Crosslinking is the process of linking one polymer chain to another polymer chain by chemical, physical, or enzymatic means (Zahra, 2018). For chemical crosslinking the links can be either covalent or ionic bonds. Chemical crosslinking typically occurs using a chemical reaction such as condensation, ring closures, or standard addition of the polymer in order to create an irreversible change (Rosato, 2003). Another subtype of chemical crosslinking is thermal crosslinking. Thermal crosslinking involves heating the polymer to impart energy onto the reactive sites in the polymer to induce crosslinking (Jin, 2018). Thermal crosslinking is the easiest and safest method to induce crosslinking as it does not rely on any harsh chemicals, enzymes, or radioactive material. The

downsides of using this method to crosslink is it is less specific than the other methods. Enzymatic crosslinking typically occurs during the process of protein synthesis in order to ensure proteins have the correct 3-dimensional conformation of the protein (Heck, 2012). The enzymes interact with the amino acids in a cite specific manner making it so that the protein will have the correct shape and structure to perform its function. The advantage of using this method of crosslinking is the crosslinking will be done at specific sites, and the downside of using this method is it is very difficult to modify this method for other uses. In physical crosslinking the polymers are exposed to some type of radiation source or electron beams to induce free radical formation, making the polymers react together (Walsh, 2011). The free radicals make it more likely that the polymers will become crosslinked and become physically entwined with each other. The crosslinking of polymers with each other results in greater tensile strength of the polymer as well as making the polymers significantly less soluble. The crosslinking of electrospun nanofibers is of utmost importance to make it less soluble for future uses.

7.Fiber Uses

The types of electrospun nanofibers created can vary based on the solution used, and whether the collecting plate is stationary or rotatable (Leach, 2011). If the collecting plate is rotatable the nanofibers will be aligned, while if the collection plate is stationary the fibers will align randomly (Leach, 2011). When nanofibers are formed, they typically have a solid structure. However, it may be beneficial to induce pores in the nanofibers to increase the surface area of the nanofibers. To induce pores in the nanofibers they are rapidly cooled before solidification or certain sections of the fiber can be removed

through the process of calcination (Xue,2017). Nanofibers can also be formed in a way that a tubular structure will form. This structure is formed by using two different immiscible liquids (McCann, 2005). This procedure of making hollow nanofibers can be useful because the outer and inner surfaces can be separately functionalized to adsorb different types of particles (McCann, 2005). Once the nanofibers are formed, they can be collected and stored for later use in different experimental setups.

The creation of nanofibers is important in many different scientific disciplines, and as such the process of electrospinning has many broad applications and has been used in many different fields to solve a variety of problems. For example, electrospinning has been used in the medical field as a way of enhancing tissue regeneration and drug release (Liu, 2020), as well as being used as a highly efficient filter in certain engineering processes (Qin, 2006).

Recently it has been proposed that nanofibers can be bound to metallic nanoparticles to be used as a surface to enhance the signal of organic compounds (Singh,2017). In this paper, the researchers used Ta-doped titanium oxide nanofibers to detect methylene blue with SERS. When using SERS, they mention that there were more peaks discernable by SERS and enhancement of the characteristic peaks at 1390 and 1634 inverse centimeters. In another paper researchers incorporated silver nanoparticles into an electrospun polyvinyl alcohol solution to detect 4-mercaptobenzoic acid (He, 2009). This paper showed an enhancement factor of 10^9 which allowed them to detect the acid at a concentration of 10^{-6} molar (He, 2009). Another group of researchers used SERS to detect multiple different pesticides (Chamuah, 2018). In this paper they created a PVA

electrospun nanofiber and spray-coated the fiber with gold beads in order to detect deltamethrin, quinalphos , and thiacloprid. In this paper, they mention that they were able to detect these common pesticides at a lower concentration than was required by the USDA foreign agricultural service (Chamuah, 2018). Electrospinning nanofibers with nanoparticles have been used to detect organic compounds in different scientific fields. More research needs to be done to determine if electrospun nanofibers with nanoparticles can be used in forensic samples.

Chapter IV: Experimental Design

1.Raman

Materials

Sodium carbonate monohydrate was acquired from Alfa Aesar. Silver nitrate was obtained from Fischer chemical. Polyvinylpyrrolidone (MW 1,000,000) was obtained from poly sciences. polyvinyl alcohol (MW 89,000-98,000) was obtained from Aldrich. L-Ascorbic Acid was obtained from Fischer, Tetra chloroauric (III) acid trihydrate was obtained from Arcos Organics. Benzylfentanyl (ng/ml) and Acrylfentanyl (mg/ml) were purchased from Cayman Chemical.

Apparatus

An electrospinning apparatus was developed in-house. Six sheets of plexiglass were used to create a square box in order to isolate high voltage. The humidity inside the container was controlled by flowing oxygen into the container to dry the air. A Harvard Apparatus 11 plus syringe pump was used to control the flow rate of the syringe. Plastic 3CC syringes from Becton Dickinson were used with a flat-tipped metal needle with a diameter of 0.1cm. A Spellman CZE 1000R high voltage power supply was used to apply a high voltage between the needle and the collector. A stationary metal plate was grounded, and aluminum foil was used to collect the electrospun nanofibers. The Raman spectrums were obtained with a Jasco (NRS 4500) benchtop Raman system.

Electrospinning Procedure

Different polymer solutions were tested to determine the optimum conditions for nanofiber fabrication. PVP solutions were prepared with different weight percentages, different flow rates, and different voltages. The optimum conditions for the PVP solutions were found to be 8% PVP by weight in ethanol, with 8kV, with a flow rate of 0.6ml/Hr. For the PVA solution, it was dissolved in water with a ratio of 5% PVA by weight. The optimum electrospinning parameters were to flow the PVA solution at a flow rate of 0.3ml/Hr. with an applied voltage of 12kV.

Nanostar Preparation

The nanoparticles were prepared using a method previously developed in our lab (Wang, 2019). Briefly, the $HAuCl_4$ and $AgNO_3$ were prepared at a molar concentration of 10^{-2} M, the L-AA was prepared at a molar concentration of 10^{-1} M, and the Na_2CO_3 was prepared at a molar concentration of 1 M. 1 ml of water was mixed with 36 µl of the $HAuCl_4$ and 2 µl of $AgNO_3$. This mixture was vortexed for 10 seconds. Next 6 µl of L-AA was added to the mixture and vortexed for 20 seconds. Last 1 µl of Na_2CO_3 was added to the mixture and vortexed for 5 seconds. The entire vortexing procedure was completed in under a minute in order to create gold and silver nanostars.

Raman Experimental Procedures

The grounded metal plate was placed 10 cm from the tip of the needle and the fibers were collected for 1 hour. The collected fibers were heated in an oven at 180˚C for two hours to induce crosslinking of the multiple layers to reduce solubility. The heated

fibers were allowed to cool and placed in the nanoparticle solution overnight. The nanofibers were then allowed to dry for 1 hour in a dark container as the nanoparticles are light sensitive. Two microliters of acrylfentanyl drug standard (1mg/ml) were drop cast onto the nanofibers with the nanoparticles. The sample was then placed under the Raman system for data collection. The sample was analyzed by focusing the 100X on the sample. The Raman system was set to 785nm, and spectrum were collected from 15-45 seconds. Data analysis was done using Origin for baseline corrections and to determine peak overlap.

2.GC-MS

An Agilent 5977B GC-MS was used with a reverse-phase non-polar column for the separation of different synthetic cathinones. The standard conditions for separation are as follows:

Oven temperature to 180°C and hold it there for 3 minutes. Then the Oven will heat up at a rate of 10°C/Min up to 200°C. At 200°C there will be a hold of 1 minute before a 25°C/minute ramp will commence. This ramp will continue until the oven reaches 300°C and at this temperature, there will be a 3-minute hold. This method has a hold at 180°C to help with the detection of amphetamines that may be mixed with cathinones. The hold at 300°C is done in order to burn out any additional residue. Overall, this programming takes a total of 13 minutes and leads to variable separation of synthetic cathinones. In order to increase the separation of synthetic cathinones, we modified the oven temperatures, the injection volume, and the split ratio. These parameters were modified to try and increase the separation of synthetic cathinones so that there would be 0.1 minutes

of separation. This degree of separation was the goal as this degree of separation that is the standard in the lab when performing GC retention time comparison with reference standards. The optimum modified method parameters consisted of 180°C hold for 2 minutes, ramp at 2.5°C/min to 195°C, ramp 5°C/min from 195°C-200°C, hold 200°C 1 min, Ramp5°C/min from 200°C-220°C, ramp 25°C/min from 220-300°C, hold 2 minutes

Materials and methods

eutylone, alpha-PVT, alpha-PPP, N-ethyl hexedrone, 4-chloro-N, N dimethyl cathinone, 4-methyl pentedrone, 4-MEAP, ethylone, alpha-PVP, 3,4 methylenedioxy PV8, N-butyl pentylone, N, N diethyl pentylone, N-propyl hexylone, N-ethyl hexylone, 4-fluoro-3-methyl-alpha PVP, alpha-ethyl aminopentiophenone, 3,4 methylenedioxy-N-benzylcathinone, pentylone, methylone, 4-chloro ethcathinone, mephedrone, methedrone, dibutylone, buphedrone, 4-chloro-alpha PVP, methylenedioxy-N-benzyl cathinone, PV8, methylone were obtained from Cayman Scientific and Sigma Aldrich.

Each drug was analyzed individually to determine their retention times, and to create the library. Next, a set of 8 different mixtures were made with 2 different compounds in them. The 8 mixtures consisted of

1. alpha-PVP and PV8
2. alpha-PVP and 4-methoxy alpha-PPP
3. 3-methoxymethcathinone and mephedrone
4. 3-methoxymethcathinone and pentedrone
5. pentylone and dibutylone
6. pentylone and methylone
7. mephedrone and buphedrone
8. mephedrone and 4-MEC

These mixtures were made to compare whether the molecular weight (MW) or the polarity of the compound had a greater impact on retention time. Next, a mixture of 6 compounds was created and analyzed via GC-MS to determine the degree of separation. The 6 compound mixture contained 4-chloror N, N dimethyl cathinone, N-ethyl hexedrone, alpha-PVP, alpha-PVT, eutylone, and 4-fluoro-3-methyl-alpha-PVP. Once separation was achieved alpha-ethyl aminopentiophenone, 3 methoxy methcathinone, alpha PPP, 4-MEAP, methylone, ethylone, N, N-diethyl pentylone, N-ethyl hexylone, N-propyl hexylone, and N-butyl pentylone were added to the 6 compound mixture to create a mixture of 16 different compounds. The oven parameters were modified to obtain the best separation possible while remaining under 20 minutes.

Chapter V: Results and Discussion

1.Raman Results

This study predicted that electrospun nanofibers could be used as the support for gold and silver nanoparticles to enhance the Raman signal of fentanyl and its analogs. The idea behind this is that the nanofibers would create a larger surface area for the nanoparticles to aggregate on and allow the nanoparticles to interact with the incoming incident beam more efficiently. This interaction would allow for greater enhancement of the Raman signal making this technique a viable method for testing illicit drugs at the trace level required by forensic labs. Initially, PVP was used as the electrospun nanofiber support for the created nanoparticles. The analysis of standard fentanyl analogs was accomplished using electrospun nanofibers imbedded and coated with gold and silver nanostars. The nanofiber synthesis was first optimized by electrospinning 8%PVP by weight with the collecting plate 10 cm from the needle tip, with an applied voltage of 8kV, with a flow rate of 0.6ml/hr and heated at 200˚C for 2 hours. The nanoparticles were incorporated into the PVP with a 1:1 ratio of PVP to nanostars. The standard 1mg/ml sample of benzylfentanyl was drop cast onto the fiber for analysis.

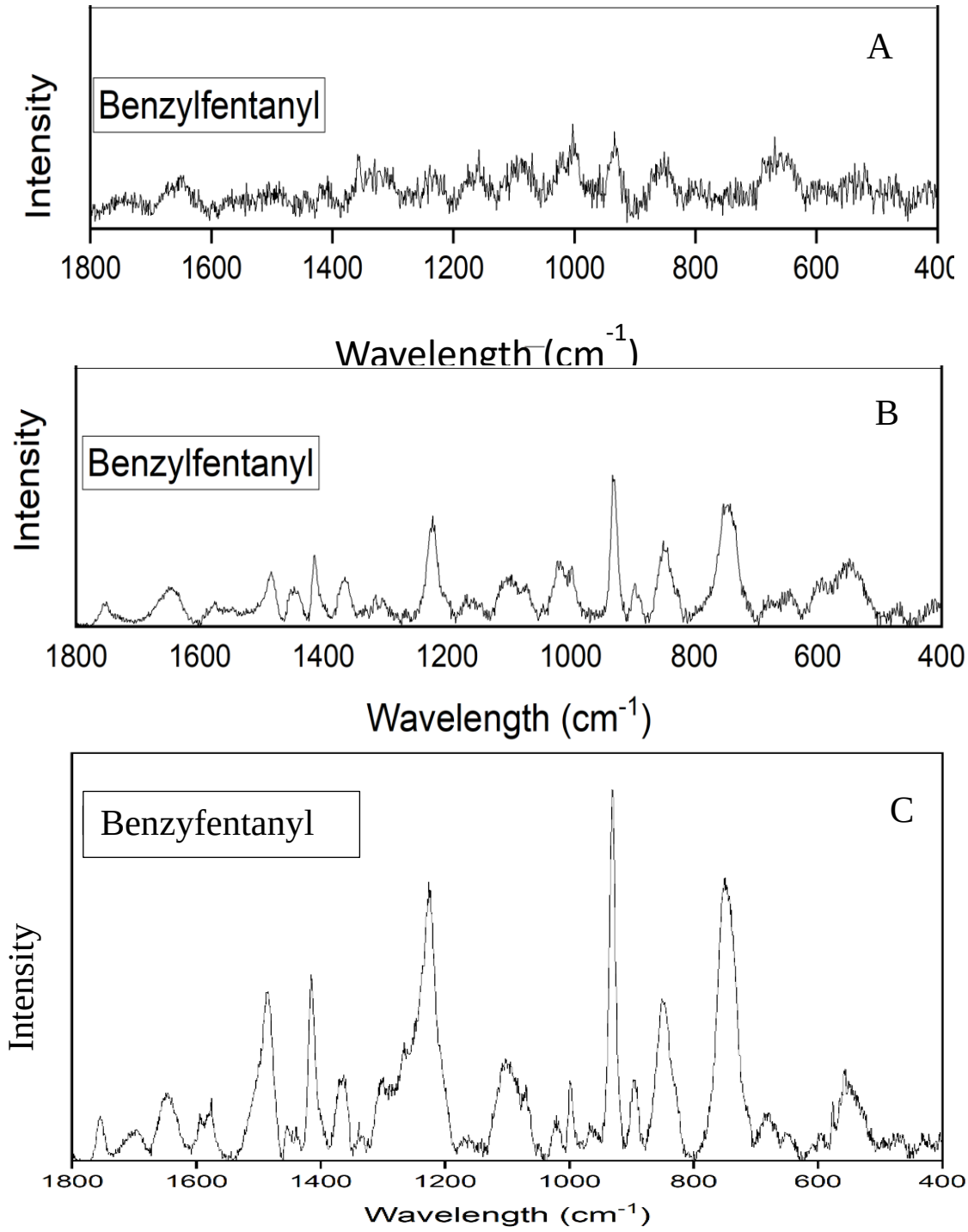

Figure 9. Shows the different Raman spectra for benzylfentanyl under different experimental conditions. A.) The Raman spectra of benzylfentanyl after drop-casting the standard onto the fiber with nanoparticles imbedded B.) The Raman spectra after optimizing the fiber synthesis C.) The Raman spectra after optimizing fiber synthesis and instrumental parameters.

Figure 9 part A shows that there are small peaks at 1000 cm^{-1} which are the characteristic peaks of fentanyl. Comparing the characteristic peak at 1000 cm^{-1} with the noise from the background gives a signal-to-noise ratio of 1.2, which is too low to confirm the presence of our standard. However, this implied that after optimizing the methodology we would be able to obtain better enhancement for our analyte. The first step in optimizing our experimental design was to optimize the fiber synthesis. The electrospinning process was optimized to obtain better enhancement. The optimized fiber formation involved dissolving PVP in ethanol at 8% PVP by weight, with a flow rate of 0.6ml/hr, an applied voltage of 8kV, and heated at 200˚C for 2 hours. Then 2µl of the sample was drop cast onto the fiber and analyzed immediately. When comparing the signal for the peaks at 1000 cm^{-1} to the background noise we find that the signal-to-noise ratio is 4.3, which is 4 times the initial ratio. Once we were able to obtain Raman spectra consistently, we began to further optimize our experimental design by optimizing the instrumental parameters. In order to optimize the instrumental parameters, we focused the 5X, 20X, and 100X objectives onto different spots of the fibers and collected the Raman spectra over the course of 15-90seconds. After these tests, it was determined that the optimum instrument parameters were to focus on the fibers with the 100X objective and focus the 785 nm laser on the sample for 90 seconds. Using these parameters, we obtained a signal-to-noise ratio of around 4 for the peak at 1000 cm^{-1}. After acquiring the spectra, we noticed the peaks did not exactly match with the peaks in the literature as the peak at 1000 cm^{-1} should be the highest peak. This led us to attempt background corrections and to remove interferent peaks from the polymer and the solvent

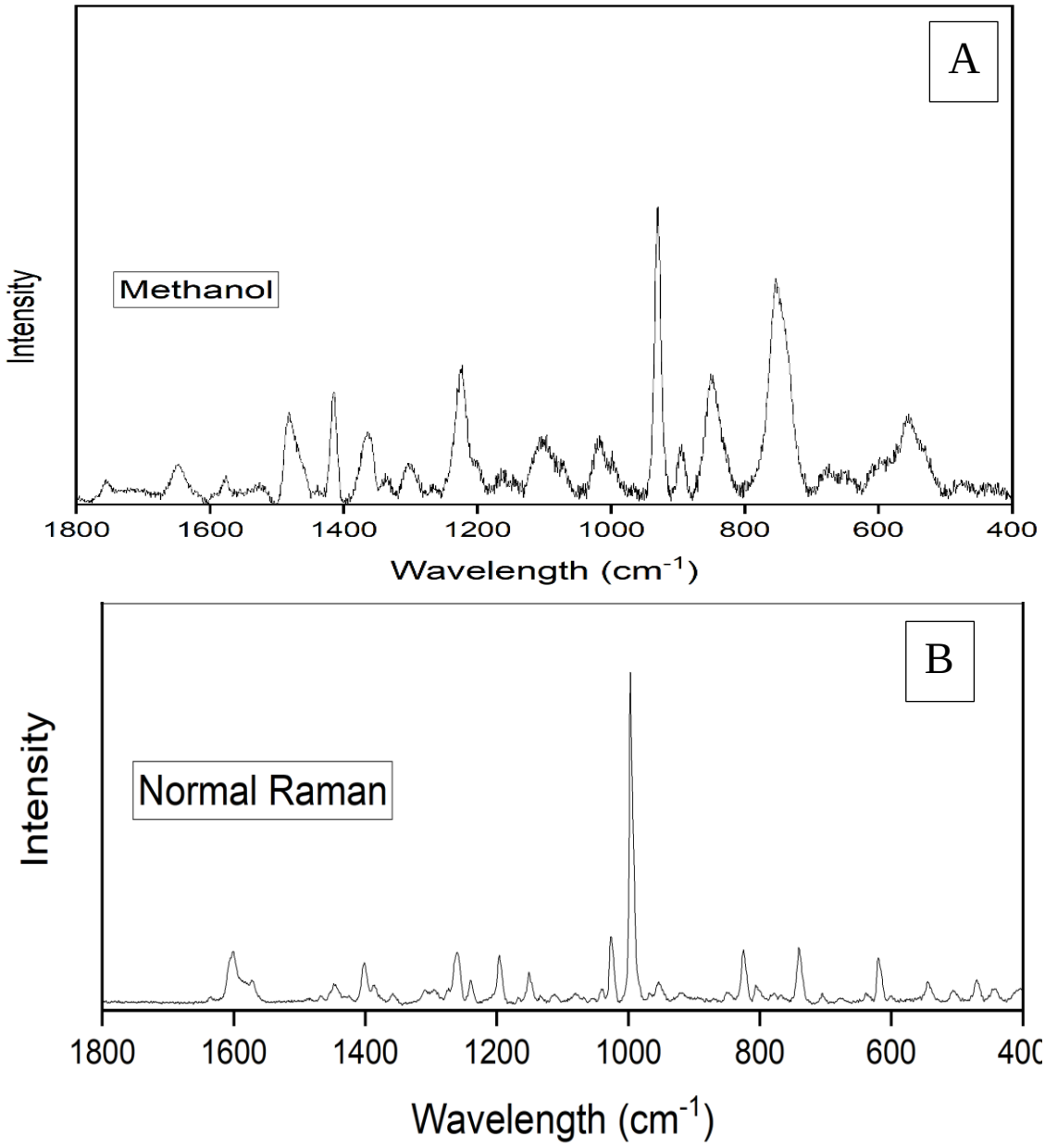

Figure 10. Shows the data collected to remove the background noise. A.) The SERS spectra for methanol B.) The normal Raman spectra for benzylfentanyl.

The purpose of obtaining the spectra in figure 10 was to determine which peaks were part of the background noise and which peaks were from the benzylfentanyl. The methanol peaks were obtained because methanol is the solvent used to dissolve the solute.

Methanol has characteristic peaks at 750cm^{-1}, 900cm^{-1}, 1200cm^{-1}, 1400cm^{-1}, and 1450cm^{-1}. While benzylfentnayl has a main characteristic peak at 1000 cm^{-1} with other smaller peaks at 600cm^{-1}, 750cm^{-1}, 800cm^{-1}, 1030cm^{-1}, 1200cm^{-1}, 1400cm^{-1}, and 1600cm^{-1}. Due to the difference in the characteristic peaks for these two compounds, it would be possible to remove the background noise peaks during baseline correction during the data analysis process. Before removing the background noise, we created a figure with all the spectra obtained to determine if there was overlap between the different spectra.

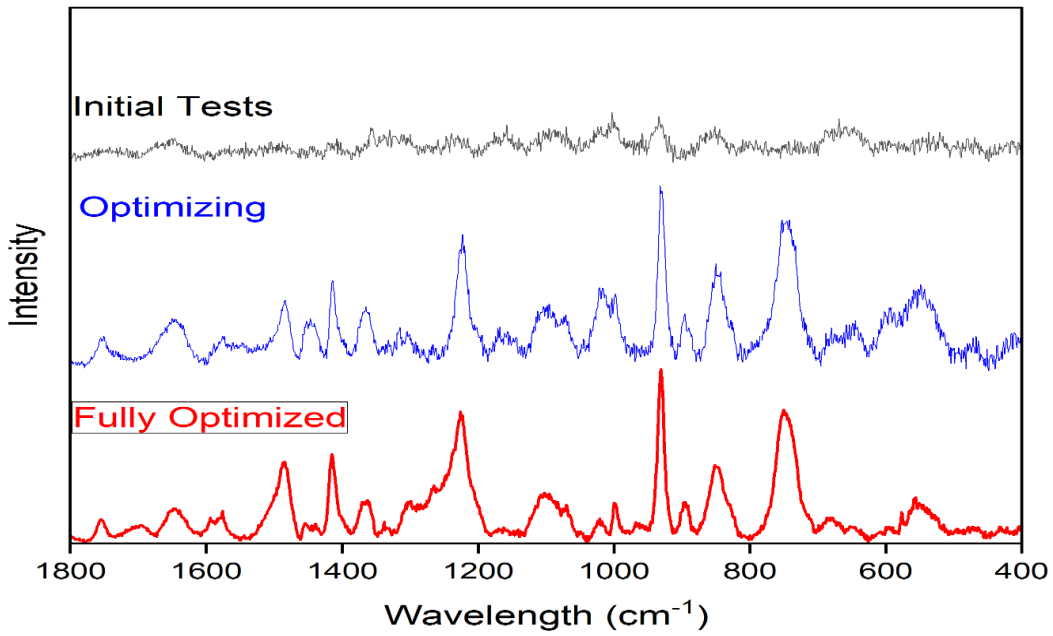

Figure 11. The comparison of the different optimizing methods for analyzing benzylfentanyl on PVP electrospun nanofibers with nanoparticles imbedded in the fibers.

Figure 11 showed that there was an improvement in the enhancement of the sample that was drop cast onto the nanofibers with nanoparticles imbedded in the fiber. The initial

test had a signal-to-noise ratio of 1.3 while the optimizing and fully optimized method had a signal-to-noise ratio above 4, which is well above the ratio needed to determine if a peak is present. The figure also shows that the enhancement for the peaks increases with more optimization, and the peaks line up with the other peaks obtained from the previous tests. This shows that our experimental design is reproducible and gives enhancement of Raman peaks even with the nanoparticles imbedded inside of the electrospun nanofibers. Once it was determined that our method was reproducible, and the peaks lined up we began the process of trying to remove the background noise. To remove the background noise, we combined the Raman spectra from the fully optimized spectra, the spectra from just methanol drop cast onto the fibers with nanoparticles, and the normal Raman spectra for benzylfentanyl.

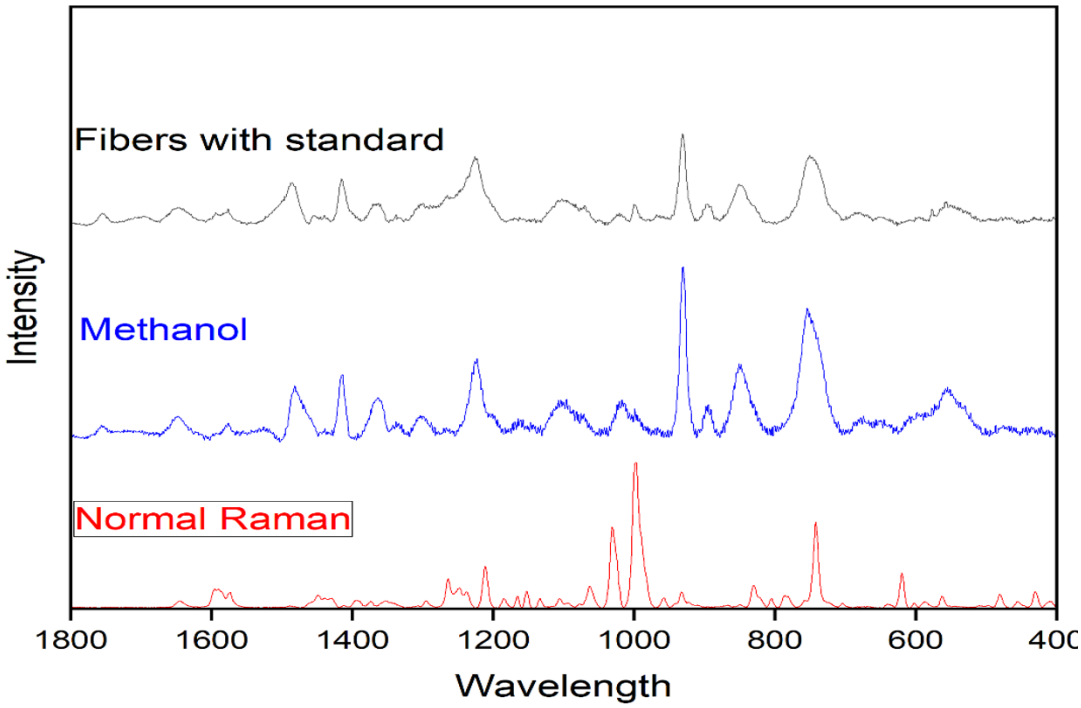

Figure 12. Normal Raman spectra of Benzylfentanyl, SERS spectra for methanol, and the fiber with the standard.

This figure was obtained to determine which peaks could be attributed to background noise and which peaks could be attributed to the benzylfentanyl in the standard. While determining which peaks were from the background, we realized the fibers with the standard matched almost identically with the standard methanol solution. The overlap of the fibers with standard and the methanol combined with the miss alignment of the normal Raman spectra led us to conclude that we were obtaining enhancement from the methanol solvent instead of the fentanyl solute. To overcome this issue, we switched from the PVP polymer to the PVA polymer as there were other papers in the literature that used PVA as a polymer as the base for SERS. As well as switching from benzylfentanyl standard to acrylfentanyl standard due to the fact that we were running low on the benzylfentanyl standard.

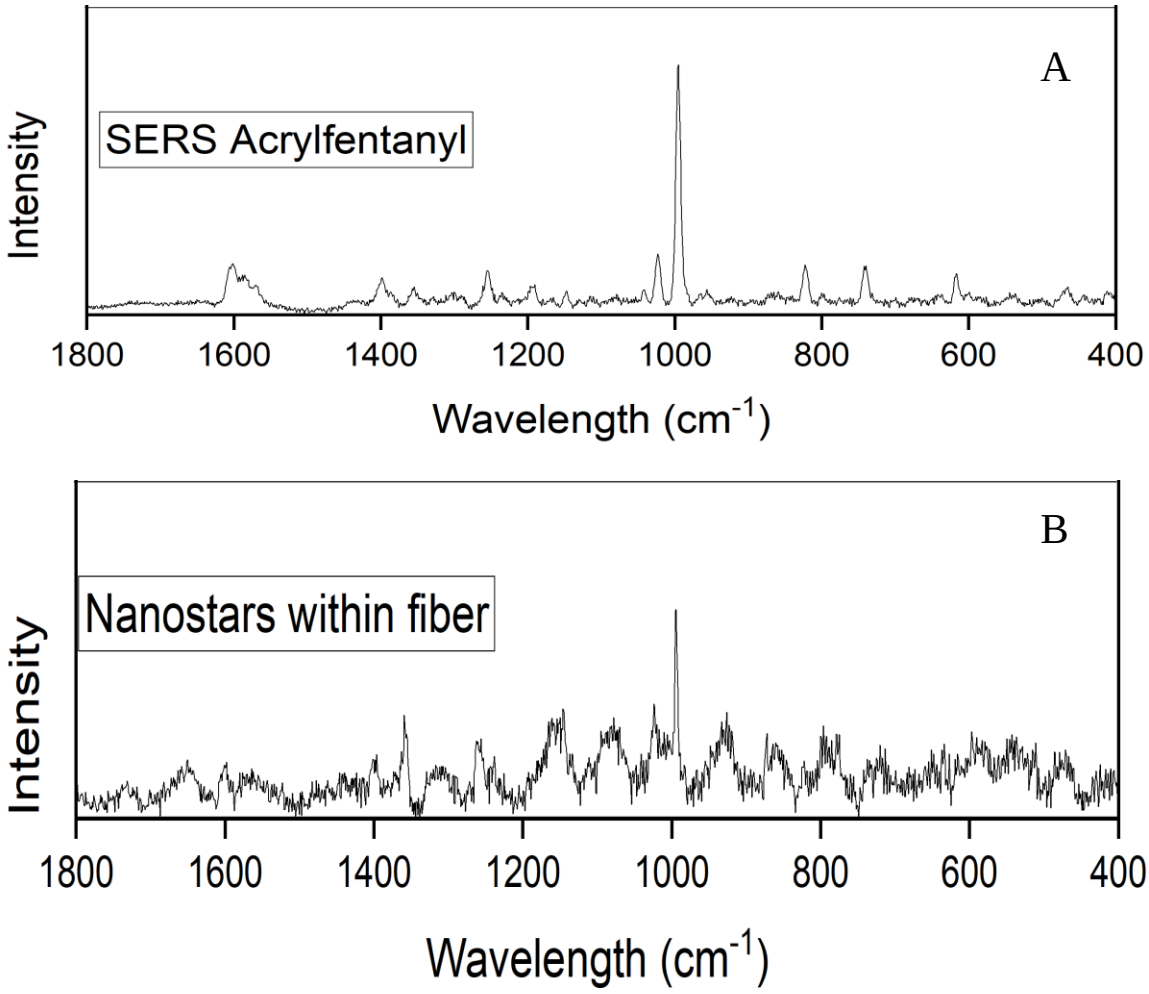

Figure 13. Shows the Raman spectra of acrylfentanyl. A.) Shows the normal SERS spectra for acrylfentanyl. B.) The SERS spectra for acrylfentanyl after being drop cast onto the nanofibers with nanostars imbedded in the fiber.

The SERS spectrum for acrylfentanyl was obtained to test our standard acrylfentanyl sample and to verify that the nanoparticles were working as intended. The enhanced peaks at 1000cm^{-1} confirmed the presence of acrylfentanyl in our standard and gave a point of reference for further tests with our electrospun nanofibers. The PVA fibers with nanoparticles were made by electrospinning a 5% by weight PVA solution dissolved in water. The solution was electrospun with 12kV, with a collection distance of 10cm, a flow

rate of 0.3ml/hr, and heated in the oven at 180˚C for 2 hours. The nanoparticles were mixed in PVA solution at a 1:1 ratio prior to electrospinning to create fibers with nanoparticles imbedded in the fibers. 2µl of our drug standard was drop cast onto the fibers and the spectra were collected and shown in figure 17 above. The figure shows two peaks at 1000 cm^{-1} which was consistent with the SERS spectra shown in figure 16. The characteristic peak at 1000 cm^{-1} had a signal-to-noise ratio of around 2.3, which was an improvement from the PVP fiber but needed more optimization in order to confirm the presence of acrylfentanyl. In order to determine how to further optimize our method we took SEM images of our fibers with nanoparticles in order to visualize the ratio of fiber to particle and to see what changes would be needed to make to get a better signal.

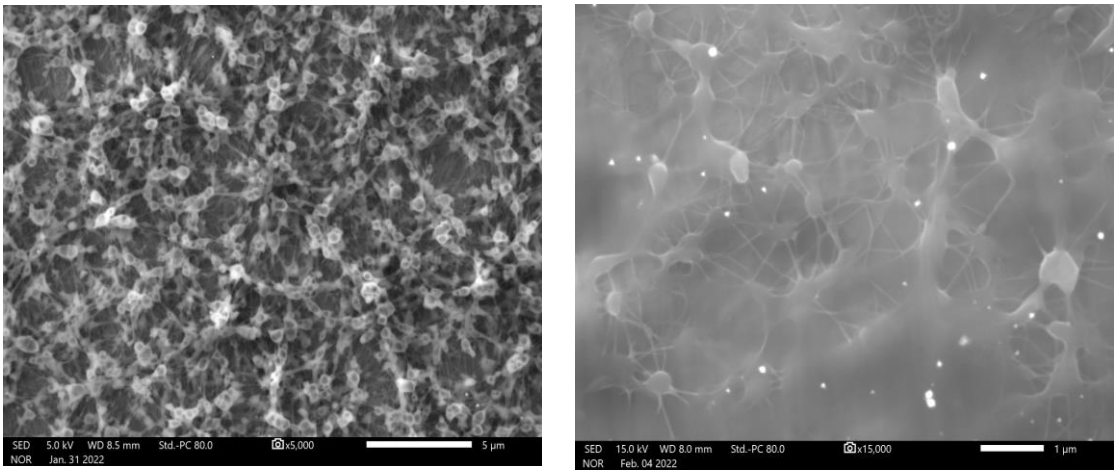

Figure 14. SEM images of the electrospun nanofibers with nanoparticles imbedded. Left is 5,000X, Right is 15,000X.

The images show the fibers in dark gray and the particles in light gray. The image on the right shows that the nanoparticles were too spaced out and were not close enough to create the hotspots required to enhance the Raman signal. This is the most likely cause as to why the signal-to-noise ratio in figure 13 B.) was just barely high enough to detect the

characteristic peak for fentanyl. In order to try and increase the enhancement of the acrylfentanyl, we tried adding more nanoparticles to the electrospinning solution. When trying to increase the concentration of the nanoparticles within the fibers the viscosity of the solution was decreased, and nanofibers were no longer formed. To overcome this issue, we tried coating the surface of the nanofibers with the nanoparticles to increase the number of nanoparticles and obtain better enhancement. In order to coat the nanofibers with the nanoparticles, the nanofibers were soaked in 10ml of the nanoparticle solution overnight. The soaking of the fibers allowed for an increase in the nanoparticles on the fibers, which allowed for greater enhancement of the characteristic fentanyl peaks at 1000 cm^{-1}. Using this technique, we were able to increase the signal-to-noise ratio to 3 which is shown in figure 15. The two spectra obtained in figure 15 were done in order to show how much better the enhancement of fentanyl could be and to show the reproducibility of the method by showing that different areas of the fibers could be analyzed and would lead to similar spectra being obtained. Once we were able to get better enhancement, we began to test allowing the fibers to dry for longer periods of time to test how this impacted the signal enhancement. This can be seen in figure 16.

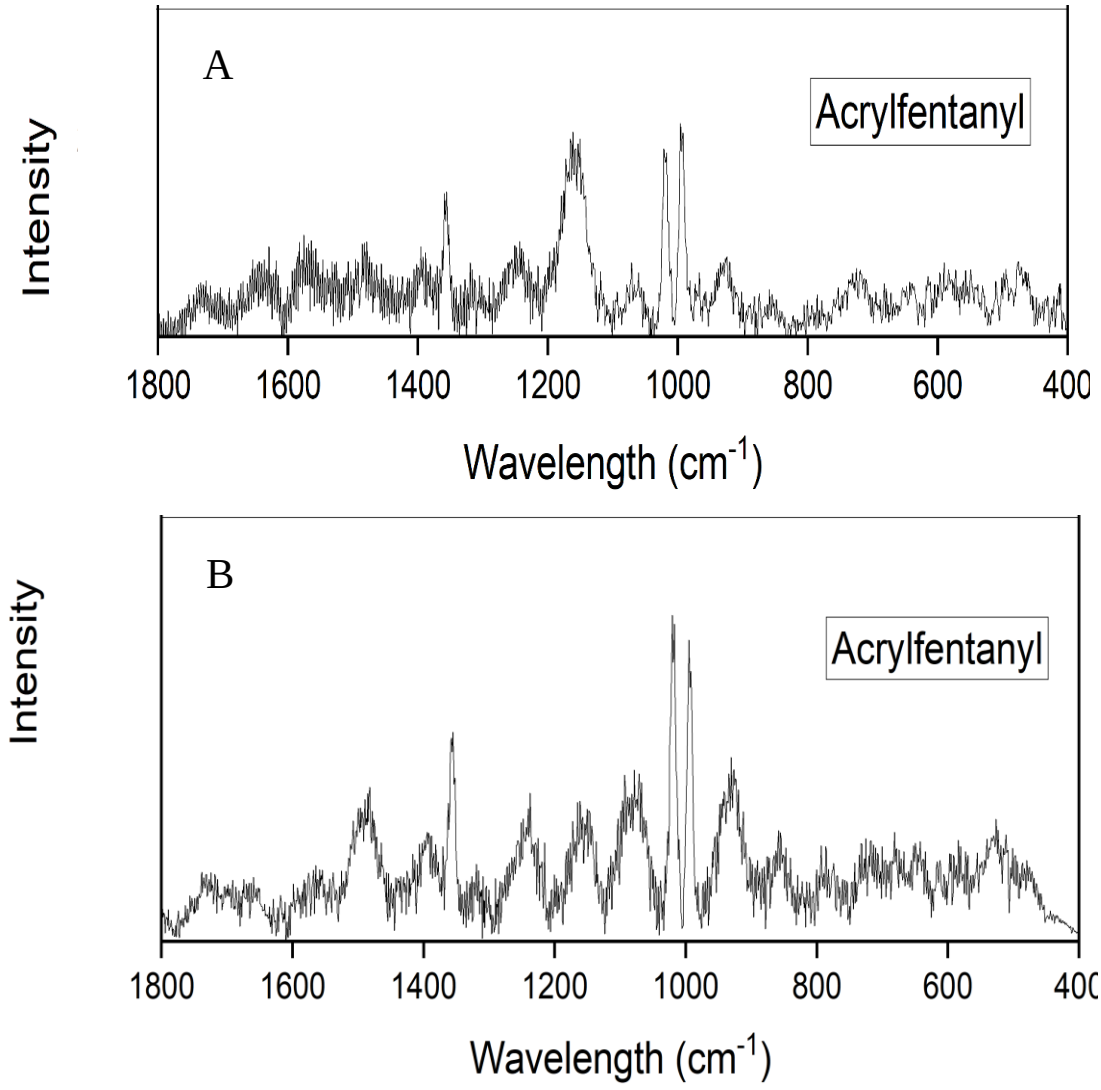

Figure 15. Shows the Raman spectra of the acrylfentanyl after being drop-cast onto the fibers that were coated with the nanoparticles. A.) The Raman spectra were obtained for 15 seconds B.) spectra obtained for 30 seconds.

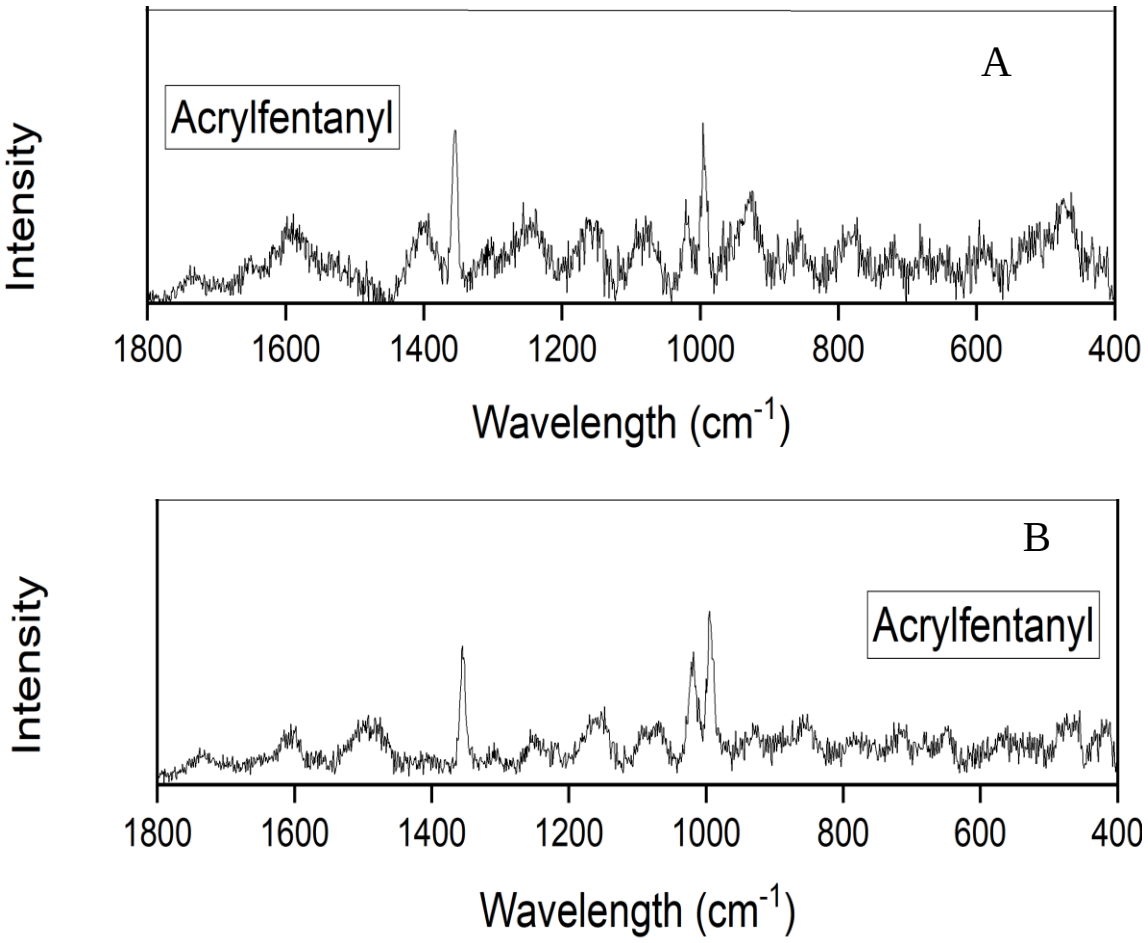

Figure 16. Shows the Raman spectra with nanoparticles on the PVA fibers after soaking the fibers in the nanoparticle solution overnight and air drying for 24 hours. A.) The spectrum was collected over the course of 45 seconds B.) Spectrum collected for 30 seconds.

Allowing the fibers to dry for a longer period of time had minimal impact on the enhancement of the fentanyl standard. The intensity of the characteristic peaks was slightly decreased after allowing the fibers to dry longer and had a signal-to-noise ratio of 2 as shown in part A and a signal-to-noise ratio of 2.7 as shown in part B. The results from the drying tests show that coating the electrospun nanofibers leads to better enhancement of the Raman signal, and the signal is fairly consistent regardless of where the laser strikes the sample.

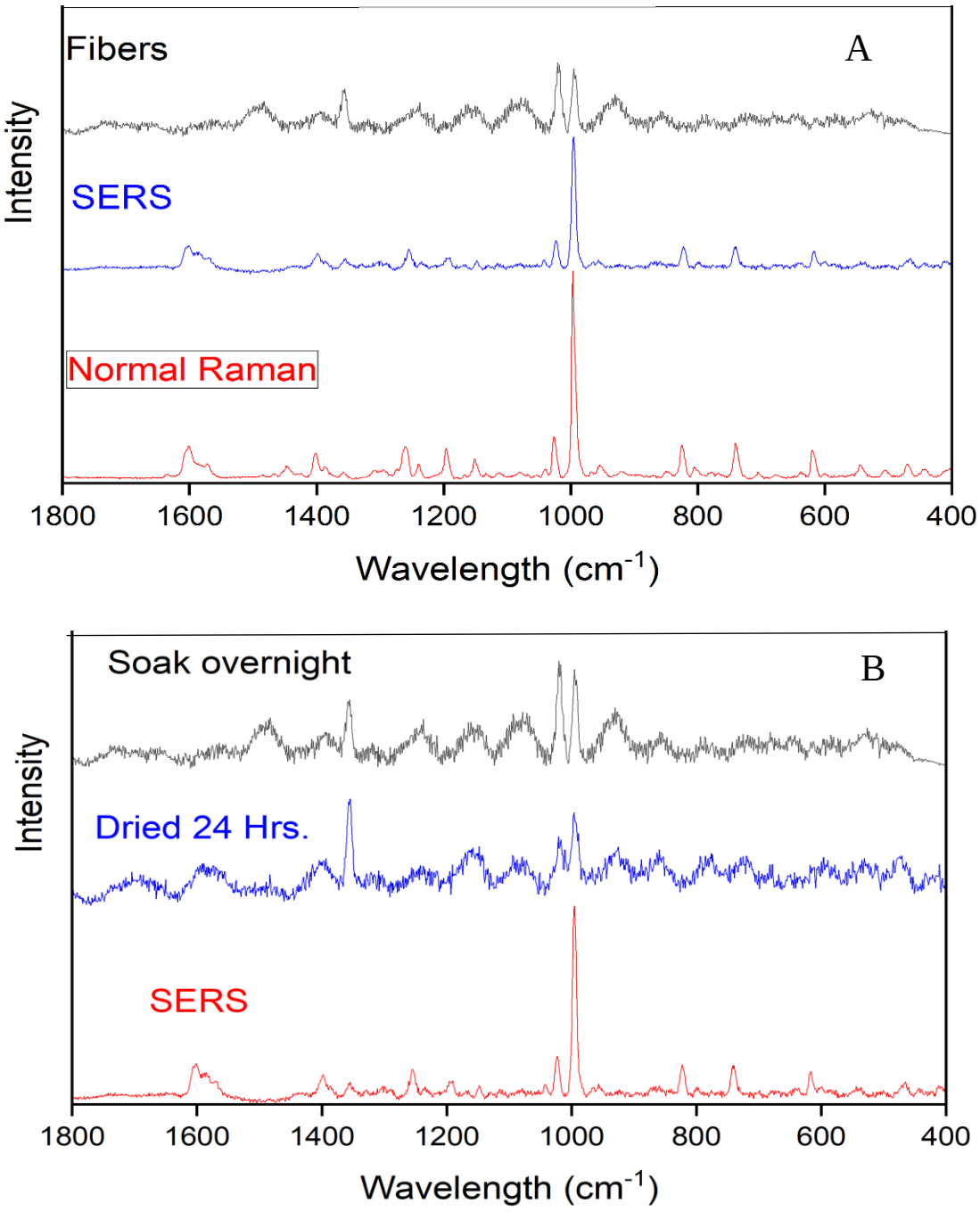

Figure 17. Shows the comparison of the different Raman spectra obtained during this study. A.) The normal Raman, SERS, and fiber spectra for acryl fentanyl match the characteristic peaks. B.) The SERS spectra and the coated fibers match the characteristic peaks for acrylfentanyl.

The results from figure 17 shows that when the fibers are coated with nanoparticles, we obtained enhancement of our acryfentanyl standard. Initially, PVP electrospun nanofibers with nanoparticles imbedded in the fibers were used as the surface for SERS detection of benzylfentanyl, the initial results are shown in figure 9. Using this information, the parameters for electrospinning were better optimized to obtain better results. The voltage applied to the tip of the needle was varied from 5kV to 10kV, PVP was dissolved in ethanol at 5-10% by weight, the flow rate was changed from 0.3ml/hr to 0.6 ml/hr, tested different needle diameters, and tested different oven parameters to induce crosslinking of the multiple fiber layers. The results of this optimization can be seen in Figure 10. Next, the instrument parameters were optimized by testing the 5X, 20X, and 100X objectives at different collecting times to determine the optimum conditions for enhancement. The results from this optimization are shown in Figure 11. The optimum conditions were determined, and data analysis began. Initial SERS spectra were taken of methanol as that is the solvent in which the analyte was dissolved. The results from this test are shown in Figure 12. This figure showed that using PVP as the fiber we were obtaining enhancement of the solvent and not the solute. To overcome this issue, we began using PVA as the source for our nanofibers. PVA was dissolved in water at 5-10% by weight, the flow rate was varied from 0.3-0.6ml/hr and heated in the oven for varying lengths of time to determine the optimum conditions. The results from this optimization are shown in Figure 11. The SERS spectra were then compared to the SERS signal for acrylfentanyl to verify the peaks overlap. This overlap is shown in Figure 23. SEM images were obtained of the fibers imbedded with nanoparticles to determine the morphology of the fiber/nanoparticle aggregate. The images are shown in Figure 18. The images showed the

nanoparticle coverage was relatively low. Thus, different ratios of nanoparticles were incorporated into the electrospinning solution. This change led to a reduction in the viscosity and the fibers no longer formed from the solution. To overcome this issue the fibers were soaked in 10ml of the nanoparticle solution overnight and allowed to dry before analysis. This methodology worked the best and can be seen in Figure 17.

The use of electrospinning to create nanofibers with nanoparticles as a flexible and freestanding surface to enhance an analytes signal is a new idea that has been gaining traction over the past 10 years or so. The nanoparticles are either imbedded in the fibers during the electrospinning procedure, or the nanoparticles are used to coat the outside of the fibers to enhance the Raman signal. The types of fibers and types of nanoparticles used can be varied and more tests need to be done to determine the optimum conditions to detect complex molecules. Electrospun PMMA/P4VP nanofibers were coated with gold sea urchin-shaped nanoparticles to detect 4-mercaptobenzoic acid (Sang, 2021). In order to coat the outside of the fibers, the fibers were placed in the nanoparticle solution and swirled for 5 minutes, and then let sit for another 5 minutes. The now created fibers with the nanoparticles were then allowed to soak in a mercaptobenzoic acid solution for 30 minutes, rinsed with deionized water, and then dried using compressed air. When using this polymer in combination with the nanoparticles they were able to detect 4-mercaptobenzoic acid at a concentration below 10^{-6} M. The nanoparticles can also be imbedded in the electrospun nanofibers (He, 2009). In order to imbed the nanoparticles in the fibers, the created nanoparticles were mixed in the PVA solution after shaking at 40ºC for 5 hours before electrospinning the solution. Then 4-mercaptobenzoic acid was absorbed by the fiber so the analyte could interact with the hot spots of the silver

nanoparticles in the fiber. Using this method of creating electrospun nanofibers with silver nanoparticles imbedded in the fibers 4-mercaptobenzoic acid was detectable at 10^{-6} M. When detecting different analytes, the parameters of the experimental procedures can be changed to achieve greater enhancement (Zhang, 2016). The silver nanoparticles were incorporated into the PVA solution and then allowed to sonicate for 30 minutes before the electrospinning procedure leading the silver nanoparticles to be imbedded in the fiber. Using these methods, the researchers were able to detect 4-mecraptophenol at $2X10^{-8}$ M. The novelty of our method from this study was using gold and silver nanostars as the nanoparticle, incorporating the nanoparticles onto the surface of the fibers, and detecting fentanyl which is a much more complex compound compared to the 4-mercapto species in the previous studies. Based on the results from previous studies and our results, it is possible that after some more optimization fentanyl can be detected using electrospun nanofibers with nanoparticles.

GC-MS Results

The analysis of standard synthetic cathinones was done on a new Agilent GC-MS instrument. Initially, 29 standards were run to add them to the instrument library and to determine the retention time for the different compounds. Using this information, we created multiple different mixtures to determine the optimum oven programming for the best separation of synthetic cathinones.

Table 2. Shows the 29 drug standards tested with their retention times

Drug	Retention Time (minutes)
eutylone	3.87
alpha-PVT	3.77
alpha-PPP	3.10
N-ethyl hexedrone	3.04
4-chloro-N, N dimethyl cathinone	2.74
4-methyl pentedrone	2.97
4-MEAP	3.14
ethylone	3.60
alpha-PVP	3.63
3,4 methylenedioxy PV8	5.78
N-butyl pentylone	4.87
N,N diethyl pentylone	4.54
N-propyl hexylone	4.85
N-ethyl hexylone	4.52
4-fluoro-3-methyl-alpha PVP	3.87
alpha-ethyl aminopentiophenone	2.71
3,4 methylenedioxy, N-benzylcathinone	5.92
pentylone	4.02
N-ethyl pentylone	4.17
methylone	3.41
4-chloro ethcathinone	2.83
mephedrone	2.46
methedrone	2.81
dibutylone	3.80
buphedrone	2.30
4-chloro-alpha PVP	4.32
methylenedioxy-N- benzyl cathinone	5.92
PV8	4.37
methylone	3.40

Table 3. Shows the 2 compound mixtures and their separation.

Mixture	Separation (minutes)
alpha PVP & PV8	0.734
alpha PVP & 4-methoxy alpha-PPP	0.623
3-methoxymethcathinone and mephedrone	0.375
3-methoxymethcathinone and pentedrone	0.27
pentylone and dibutylone	0.207
pentylone and methylone	0.605
mephedrone and buphedrone	0.148
mephedrone and 4-MEC	0.178

The two compound mixtures were created to test whether having a different structure or having a different MW leads to better separation.

Table 4. Shows the molecular weight of the compounds in the mixture.

Compound	Molecular weight
alpha PVP	231.331
PV8	260
4-methoxy alpha PPP	234
3-methoxymethcathinone	194
mephedrone	178
pentedrone	192
pentylone	236
dibutylone	236
methylone	208
buphedrone	178
4-MEC	192

Based on the information from table 3 and 4 it shows that when synthetic cathinones have similar MW then the compounds will have similar retention times. The gas chromatograms and the fragmentation patterns for the compounds in the large mixture are shown over the next few pages.

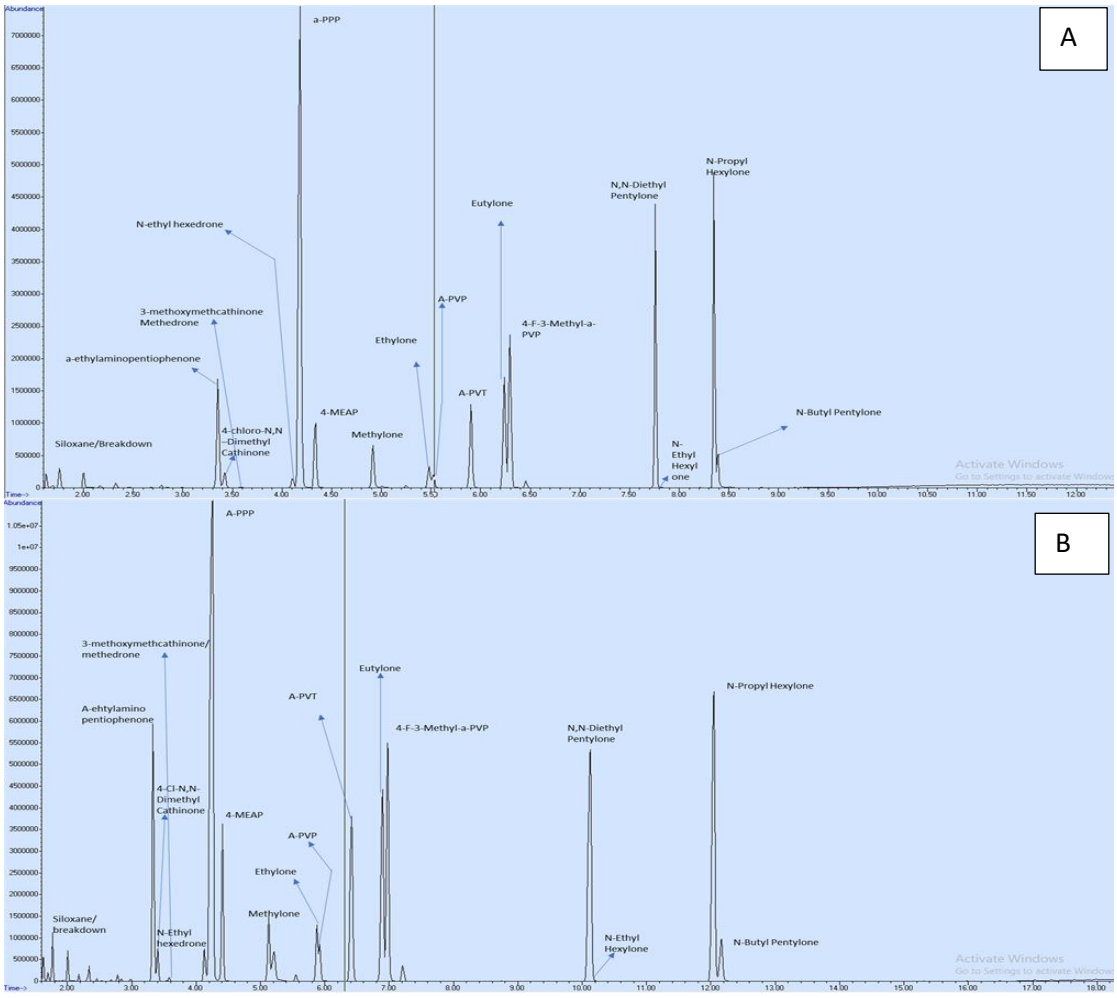

Figure 18. Chromatogram for the separation of the 16-compound mixture. A shows the total chromatogram for the standard method which consisted of an initial oven temperature of 180°C hold for 3 minutes, ramp at 10°C/Min to 200°C, hold at 200°C for 1 minute, ramp at 25°C/minute to 300°C, hold for 3-minutes. B shows the total chromatogram of the modified method which consisted of a a180°C hold for 2 minutes, ramp at 2.5°C/min to 195°C, ramp 5°C/min from 195°C-200°C, hold 200°C 1 min, Ramp5°C/min from 200°C-220°C, ramp 25°C/min from 220-300°C, hold 2 minutes

When using method B we were able to obtain better separation compared to method A.

The enhanced separation is shown best in the chromatogram between N-propylhexylone and N-butyl pentylone, eutylone, and the 4-fluoro-3-methyl alpha PVP, and alpha ethylaminopentiophenone and N-ethyl hexedrone. The new method was slower than the

previous method however it was still shorter than 20 minutes which was the original goal.

The fragmentation pattern for the more difficult compounds are shown below.

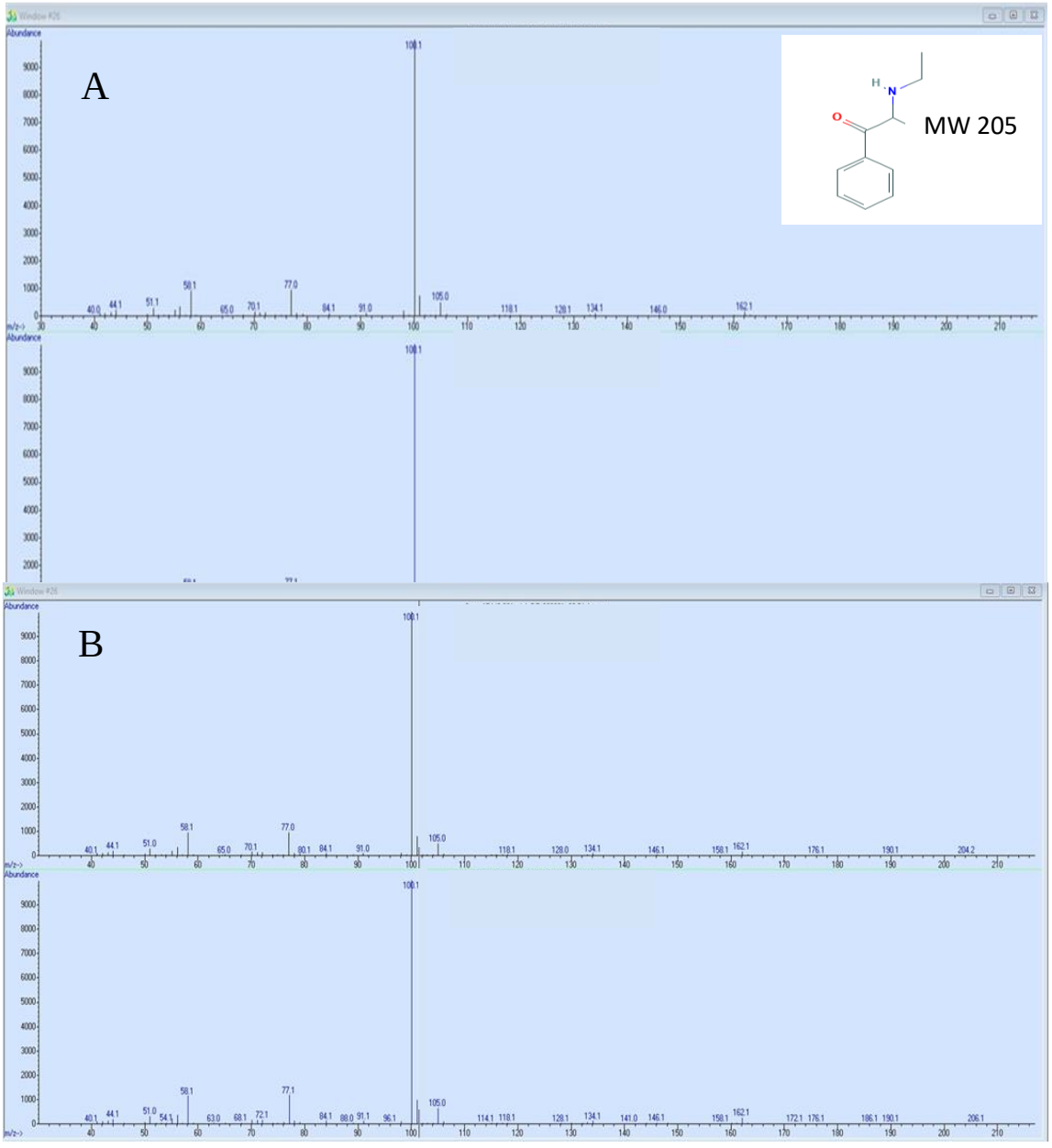

Figure 19. Shows The mass spectra for alpha-ethylaminopentiophenone A.) under the standard cathinone mixture method B.) under the modified method

The characteristic peaks for alpha-ethyllaminopentiophenone are 44, 58, 77, 100, and 205m/z. Under the modified method the molecular ion was more obtainable leading to a better percent match with the library. This led to the algorithm to correctly determine when this compound was present in the mixture.

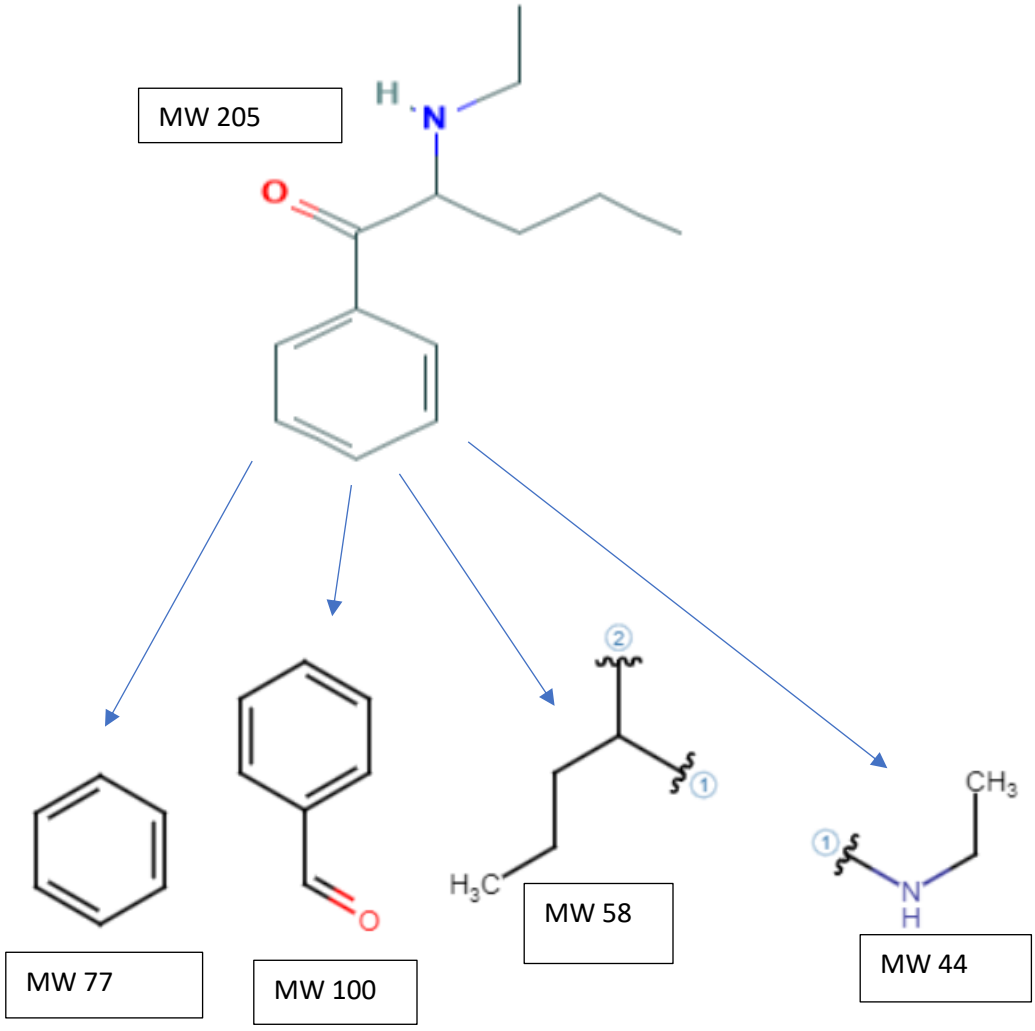

Figure 20. MS fragmentation of alpha-ehtylaminopentiophenine. The figure shows the MW for the molecular ion, as well as the characteristic fragments that form during the ionization process.

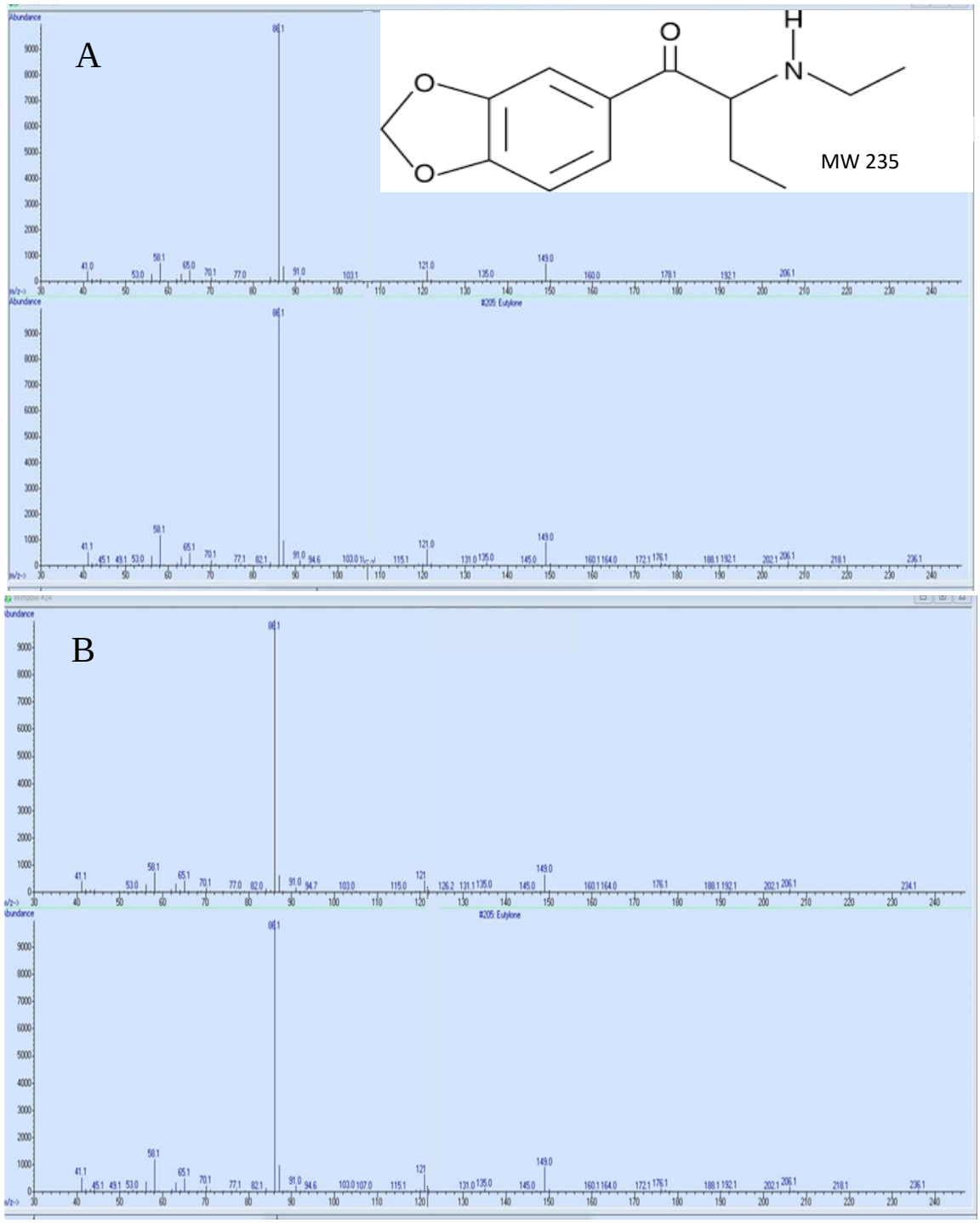

Figure 21. Shows The mass spectra for eutylone A.) under the standard cathinone mixture method B.) under the modified method

The characteristic fragments for eutylone are 42, 44, 86, 121, 149, and 235m/z. The molecular ion at 235 is one of the most difficult ions to obtain under the standard method

and was obtainable in the modified method. This in combination with the increased number of characteristic peaks allowed the algorithm to correctly determine the presence of eutylone.

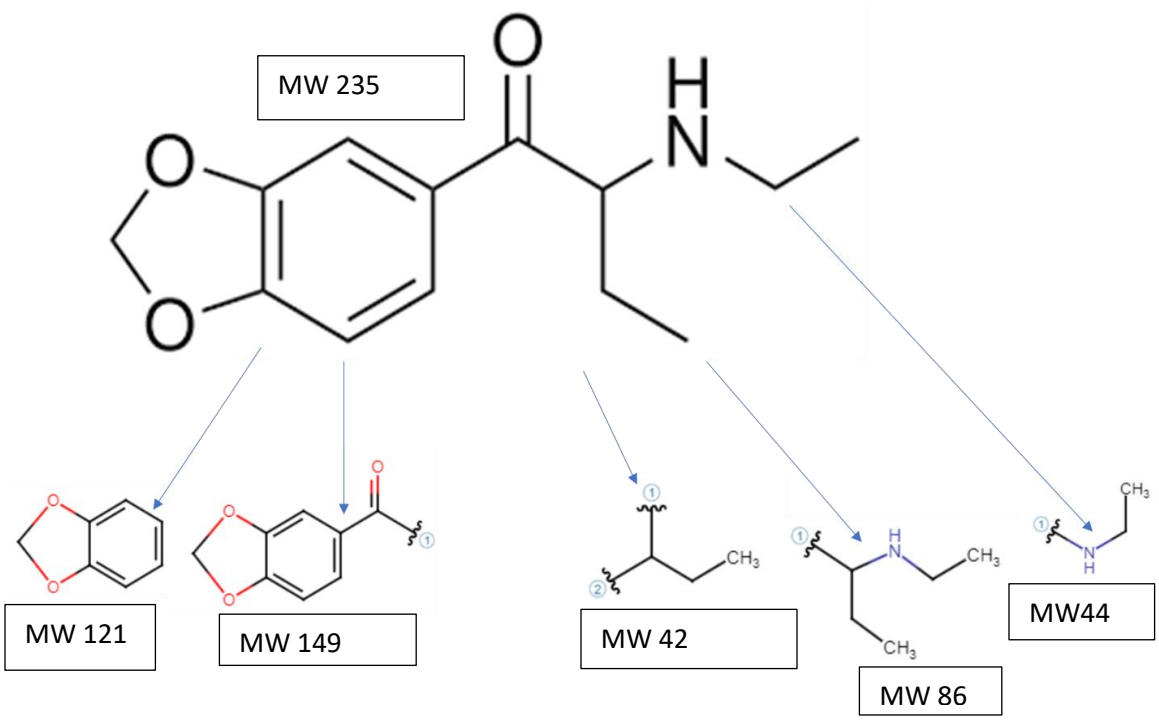

Figure 22. MS fragmentation of eutylone. The figure shows the molecular ion as well as the characteristic fragments that form during the ionization process.

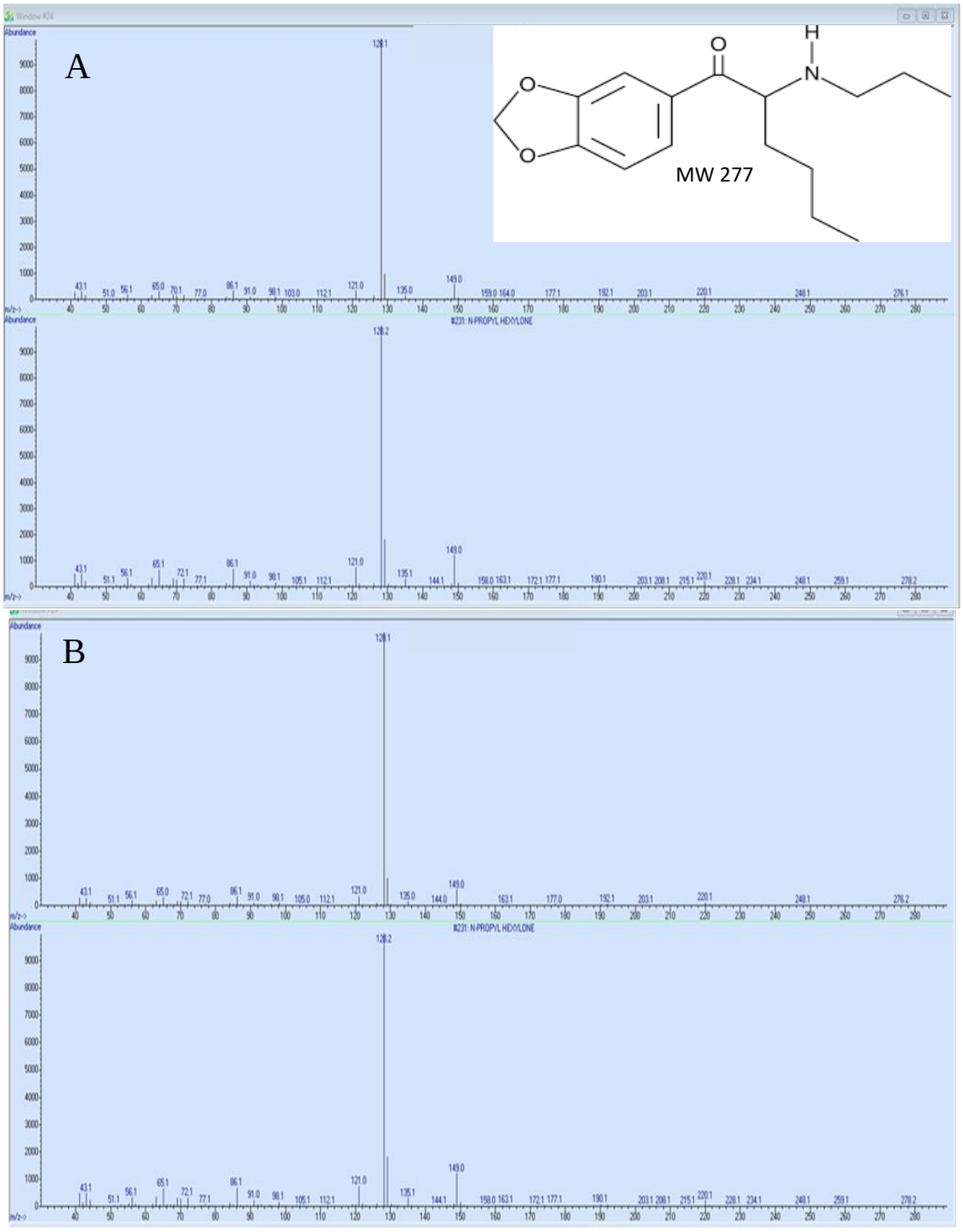

Figure 23. Shows the mass spectra for N-propyl hexylone A.) under the standardcathinone mixture method B.) under the modified method

The characteristic fragments for N-propyl hexylone are 43, 57, 58, 121, 127, and 277.

The fragmentation pattern under the modified method and the standard method were

more or less identical. This showed that in the worst-case scenario the new method gave equivalent mass spectra to the standard method. However, in the best-case scenario, we were able to obtain more characteristic ions for the cathinones present.

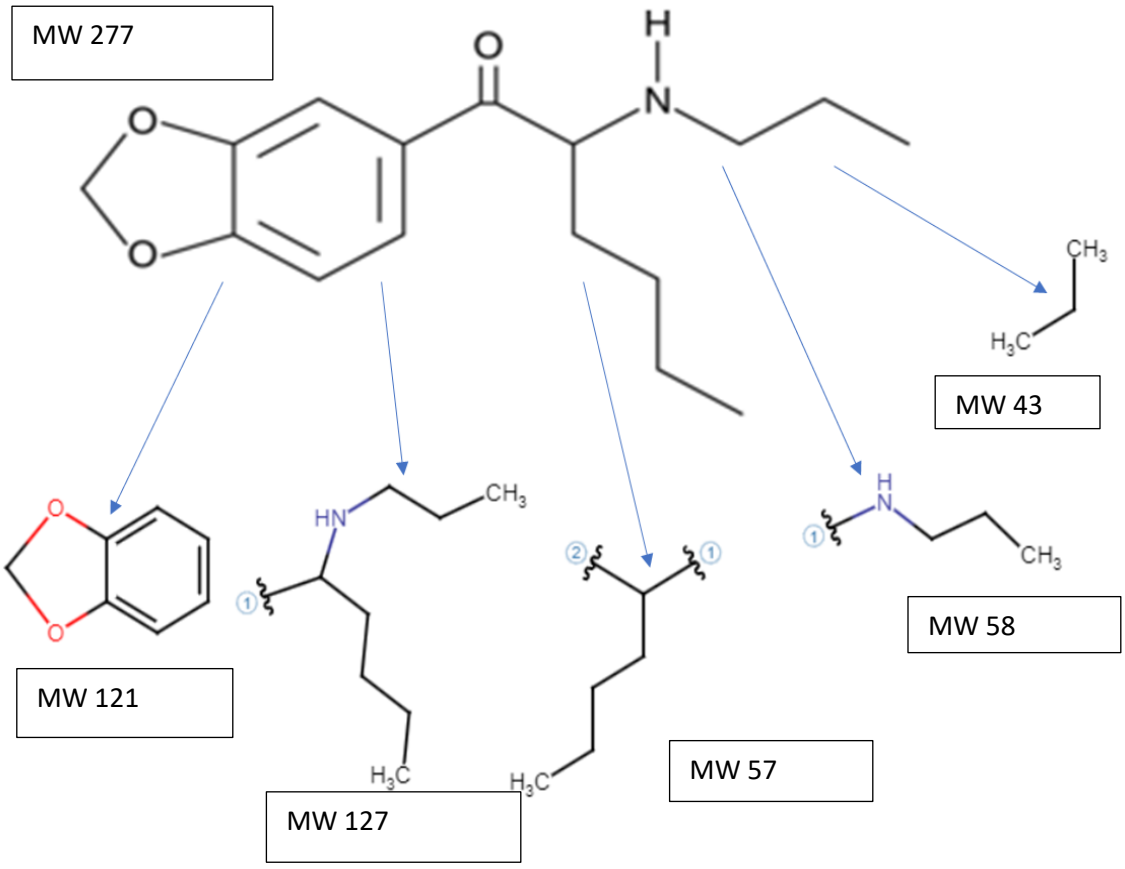

Figure 24. MS fragmentation of N-propyl hexylone. The figure shows the molecular ion and the characteristic ions that form during the ionization process.

This section of the thesis was designed to solve the common problem of determining which illicit compound is present in mixtures when the compounds have very similar structures. In order to get the best separation of compounds with similar structures using gas chromatography, the selectivity of the column should be increased. There are two

ways to increase the selectivity in gas chromatography. The first way is to use a specific column that will specifically interact with the compound of interest and ignore the other compounds in the mixture. This method leads to the best separation however the downside to this method is the column would need to be changed in between runs when testing for different compounds of interest. The second way of increasing the selectivity of a GC column is to slow the oven ramping. Slowing the oven ramp changes the equilibrium expression of the analyte in the mobile phase and the stationary phase. This change in equilibrium allows compounds to stay in the stationary phase longer depending on the structure of the compound. This increase in retention time allows for an increase in separation for compounds with similar structures. The only downside to this method of increasing sensitivity is that it increases the run time of the experiment. In this experiment, we opted to go with the second method of slowing the oven ramping as it is the quickest and least expensive way of increasing the separation of compounds.

To ensure the modified method was reproducible multiple runs were done consecutively and the results were analyzed. The current oven parameters were run three times in succession, followed by running the modified method three times in succession. The chromatograms and mass spectra data obtained were compared to determine the consistency of the retention times and the fragmentation pattern. This setup was tested twice over the course of two days. The two methods were determined to be reproducible as the inter-run retention times and fragmentation patterns matched. Once this was completed the current GC-MS parameters were compared to the modified GC-MS parameters to determine which method gave the best separation and fragmentation pattern. The mass spectra showed that the new methodology increased the number of

fragments obtained for most compounds and leads to the molecular ion being detected more frequently. This is shown in the spectra for alpha-ethylaminopentiophenone, and eutylone. In these two spectra, our modified methodology gave more fragments, and we were able to obtain the molecular ion in our method. Issues were noted in the mass spectra for N-propyl hexylone. In this spectrum, we got mostly the same fragmentation however the cathinone mixture method had a peak at 103, 159, and 164 while our modified method had a peak at 105, 158, and 163. This showed that in the worst-case scenario the modified method gave a similar fragmentation pattern, while in the best-case scenario the modified method gave better fragmentation patterns with the molecular ion present. Using this method, we ran different street samples to verify that this new method works on standards as well as samples used in actual case work.

Chapter VI: Conclusion

Two different projects are described in this thesis. The first project was undertaken to develop a new electrospinning technique to detect fentanyl by Surface Enhanced Raman Spectroscopy (SERS). The second project was to develop a modification of a GC-MS method in order to increase the separation and detection of synthetic cathinones.

The present techniques for analyzing fentanyls by SERS require the addition of an aggregating agent before the addition of the analyte. This procedure can take 10 minutes or more before the results will be known. Using the electrospun nanofibers with gold and silver nanostars on the surface of the fiber the analyte can be drop cast onto the fiber and analyzed immediately. This shortens the method by 10 minutes meaning results will be known quicker using this method. The drawback to this method is there is background noise making it difficult to detect analytes. Further optimization is needed to increase the nanoparticle concentration on the nanofibers. This should improve spectra and increase the signal-to-noise ratio.

A previously used technique for analyzing synthetic cathinones by GC-MS took 13 minutes for separation and analysis via GC-MS. The drawback with this run time is that it was not long enough to get complete separation of synthetic cathinones with similar structures. In order to increase the separation, the ramp temperature was reduced, and the hold times were modified to increase the separation in the chromatograms. This increased the run time to 19.2 minutes making the run time 6.2 minutes longer. When this was done the separation of the different compounds in the mixture increased, the peak

intensity increased, and there were more molecular fragments available for use in identifying unknowns. This method was shown to be reproducible among instruments of the same make and model suggesting, it can be used in a variety of different labs. The ability of this method to increase the number of ion fragments present will help the analyst to determine unknown cathinones quicker, improving the quality of results and the ability to detect these compounds when present in seized drugs.

Chapter VII: Future Work

With the increasing number of new novel psychoactive substances and designer drugs being created, it is important for the analyst to have as many techniques as possible to detect these compounds. Future goals for the fentanyl project include increasing the signal to make it easier to detect different analytes, and to determine the limits of detection for fentanyls using this method. Once this has been accomplished more tests can be done in order to develop a library of Raman spectra to determine which fentanyl analog is present. After a library has been created mixtures can be created and tested to determine which compounds are present in mixtures.

Future goals for the GC-MS project include testing the method on more GC-MS systems made by other companies to verify that the method works on a variety of different instruments. Testing the method on instruments with a more polar column may increase the interaction of the polar groups of the synthetic cathinones with the stationary phase, improving resolution. Overall, these two projects show how important it is to have multiple techniques available to attack specific problems in a forensic laboratory. These new techniques can be easily incorporated into any forensic lab and should prove useful in many forensic laboratories.

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