ABSTRACT

Title of Thesis:	CONCENTRATION AND DETECTION OF HYDROPHOBIC ANALYTES USING MODIFIED PAPER-SERS
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Surface Enhanced Raman Spectroscopy (SERS) has been used to measure trace quantities of analytes and cost-effective paper-based SERS substrates have been used in the field to detect heroin and fentanyl in low-resource settings. However, due to the inherent properties of paper and the manufacturing process, these SERS substrates have limited capabilities in detection of lipophilic and hydrophobic analytes. This is of critical importance in markets such as the detection of delta9-THC, which is found in trace amounts (parts per billion) in saliva of intoxicated drivers. In this defense, I will show the how the paper-based SERS substrate manufacturing process can be modified to allow for the detection of hydrophobic analytes. Then, I will show how an alternative detection protocol can be applied to concentrate and measure larger volumes of hydrophobic analytes in an aqueous solution.

CONCENTRATION AND DETECTION OF HYDROPHOBIC ANALYTES USING MODIFIED PAPER-SERS

by

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Chapter 1: Introduction & Motivation

Ever since *Star Trek*'s tricorder, there has been a drive to build a device that could detect anything and everything at the push of a button. This dream has continued into the modern day, from *CSI*'s instantaneous forensic results to Theranos's quest to perform hundreds of tests with a drop of blood in the patient's own home. However, the furthest science has arrived at a portable medical device is a glucometer; forensics still relies on imprecise colorimetric roadside tests; and most diagnostic tests still require a pathology lab.

All of these cases have the same core scientific issue: given an unknown sample, determine what analytes are present and quantify them. Surface Enhanced Raman Spectroscopy (SERS) presents the optimal solution. SERS presents a number of advantages to achieve this goal: high sensitivity, specificity, and the ability to multiplex. Whereas the typical blood glucose concentration is on the millimolar scale, SERS can provide the requisite micromolar to nanomolar detection limits. Many colorimetric and label-based test have a significant number of false positives, but as SERS is measuring an intrinsic property of the analyte, it is label-free and virtually eliminates false positives. Finally, SERS is not only quantitative but allows for multiple analytes to be detected in the same sample.

These qualities of SERS solve a critical need in the market—a sensitive, specific, and portable test for analytes outside of lab and in low-resource settings. Previously developed paper-based SERS substrates have begun to solve that need. As sold by Diagnostic anSERS (now a part of Metrohm Raman), they have not only been used in research labs but in the field to aid police officers in detecting heroin and

fentanyl in the field. But, the hydrophilic nature of paper and charged silver nanoparticles render it ineffective in detecting nonpolar analytes.

Detection of certain nonpolar analytes is critical outside the lab today in a few broadly applicable forensics and medical use cases. As marijuana legalization has spread throughout the United States, it is of critical importance that marijuana-intoxicated drivers are detected by police. However, there is no "breathalyzer" for Δ^9-THC, marijuana's psychoactive ingredient. That leaves police to depend on other, less effective and more expensive techniques to attain probable cause. A roadside saliva test using SERS would immediately solve this issue. On the medical side, many medications have a narrow therapeutic range, thus making them difficult for doctors to accurately dose. The patients are required to get frequent blood tests, but delay in results leads to difficulty in dosing. A portable test, similar to a glucometer, would greatly improve the doctor's ability to provide optimal care. And from explosives to environmental contamination detection, there are a multitude of other nonpolar analytes that require trace detection outside the lab.

In this thesis, we explore the use of hydrophobically modified paper SERS sensors for the enrichment and detection on non-polar molecules in aqueous solution. Specifically, we compare methods for forming hydrophobic SERS sensors by inkjet printing silver nanoparticle ink and by filter-based deposition of silver nanoparticles. We show that using a hydrophobic membrane patterned with silver nanoparticles by the filtering method delivers the best sensing performance, and that using the SERS sensor in a filtering format for capturing analytes from an aqueous sample is a viable approach for the detection of non-polar targets in the field.

Chapter 2: Background

2.1 Raman Shift

When a photon impacts an analyte, it excites the analyte to a higher state energy state and a photon is emitted upon subsequent relaxation. Usually, this excitation and relaxation return the analyte to its original energy state, meaning the photon released has the same energy and wavelength as the original incoming photon in an elastic process called Rayleigh scattering. In rare events, the analyte relaxes to an energy state either higher or lower than its original energy state, resulting in a non-elastic shift in energy and wavelength of the emitted photon, deemed a Stokes or Anti-Stokes shift, respectively (See Figure 1).

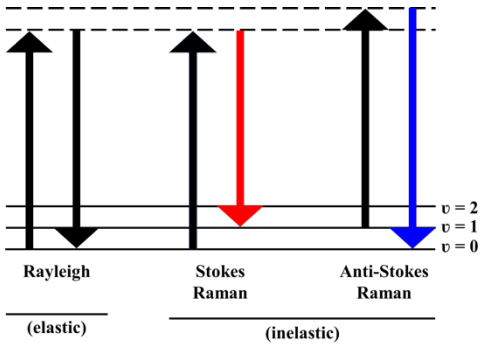

Figure 1: Jablonski diagram of excitation and relaxation of a molecule, resulting in Rayleigh or Raman scattering

Similar to IR, Raman scattering is unique for each analyte resulting in label-free and multiplex-capable sensing. As the excited virtual states of the analyte are dependent on the molecular structure with a probabilistic frequency of each state, collecting a large number of Raman scattering events results in a series of scattering events dependent on that molecular structure, resulting in a unique Raman spectrum

(See Figure 2). Hence, each analyte has a unique Raman scattering diagram, provided enough scattering events can be recorded to ascertain each peak [1].

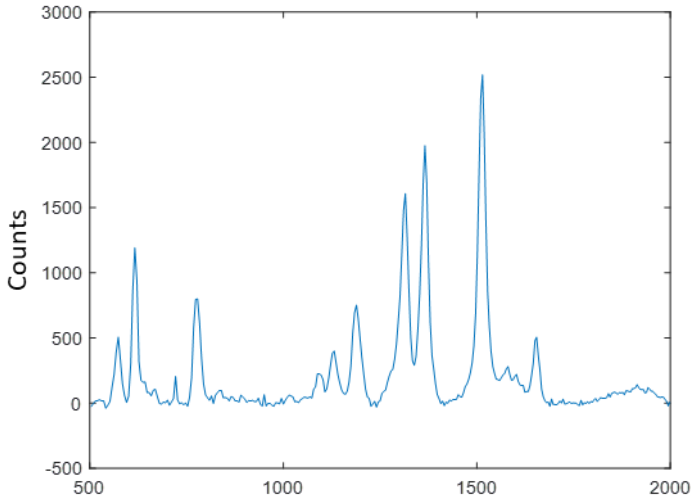

Figure 2: Example Raman spectra, R6G

2.2 Surface Enhanced Raman Spectroscopy (SERS)

Compared to Rayleigh scattering, Raman scattering events are extremely rare occurrences and thus it is not appropriate for trace detection. This can be remedied by leveraging localized surface plasmon resonances. First discovered with roughened silver, these areas of localized surface plasmons create an oscillating electromagnetic field around noble metallic nanostructures structure that increases the frequency of Raman scattering events by increasing the number of Raman excitation events [2]. By placing the analyte in a localized surface plasmon "hotspot", Raman scattering events could be increased by up to 11 orders of magnitude, with single-molecule detection reported [3].

While SERS was initially discovered using roughened silver, nanostructures have been designed to have higher electromagnetic fields, which naturally occurs at sharp points of individual nanostructures and between two nearby structures [4], [5]. Nanofabrication on silicon chips allowed for creation of precisely patterned nanostructures on silicon wafers, such the classic inverted pyramids of the Klarite SERS substrates, as well as porous silicon substrates coated in silver, as developed by Intel [6][7] and Ocean Optics [8]. Each SERS substrate follows a similar clean-room process. First, a precise pattern is rendered. For Klarite, a series of inverted pyramids is etched into a silicon wafer. Intel etched pores into a silicon wafer. After this patterning step, this surface design is then coated in a layer of gold (or silver, in the case of Intel), usually via sputtering. (Intel subsequently loaded their porous silicon substrates with silver nanoparticles [6].) This results in the same principle as the roughened silver; by creating hot-spots on the silicon wafer, the Raman spectra is enhanced by ~10^8 [9]. In the same vein as the precise patterning, researchers have shown that nano-cubes, nano-rods, and nano-stars provide similarly significant enhancement factors of 10^8 due to their shape [9].

However, in all of these rigid SERS substrates, the complex fabrication techniques result in a price-point that is too high for general use outside of the research setting. In the case of Klarite, sales were so anemic due to this high price point of $100+ that the product line was discontinued. A new approach to SERS substrate manufacture is needed to achieve a low price point that is commercially viable.

2.3 Flexible SERS Substrates

To achieve a similar result without the need for expensive clean room nanofabrication, clusters of gold or silver nanoparticles can achieve the same result upon aggregation in a droplet of liquid. However, as nanoparticle fabrication is particular, over-aggregation results in quenching, and the test is time-dependent, this technique is relegated to laboratory analysis. As a way to avoid these issues and create a low-cost SERS substrate that is stable over time, nanoparticle embedded flexible SERS substrates were created; this creates the additional advantage that paper allows for easier-to-use sample acquisition techniques, such as dipping into a liquid or swabbing a solid surface.

Initial fabrication techniques included dipping paper into a nanoparticle solution [10] and repurposing an off-the-shelf, consumer-grade printer to ink-jet print a nanoparticle ink onto paper [11]. This led to the development of similar SERS substrates using techniques such as screen printing [12], spraying,[13] electrospinning [14], and *in situ* nanoparticle growth [15]. In each of these cases, the end-result is similar: a flexible SERS substrate with sample wicking and swabbing capabilities in addition to a similar enhancement factor as colloids and rigid substrates. As the manufacturing techniques vary, a few fall in the same trap as the traditional rigid SERS substrates; because of low assembly throughput, they are simply too expensive to mass produce to be competitive in the market. Hence, the flexible SERS substrates that will gain success in the marketplace are the ones with the lowest production cost. So far, ink-jet printing continues to be the market leader in flexible SERS substrates via

Diagnostic anSERS' P-SERS sensors [16], which was acquired by Metrohm Raman in late 2017 [17].

2.4 Ink-Jet Printed SERS Substrates

Yu and White created flexible paper-based SERS substrates by ink-jet printing nanoparticles onto untreated cellulose paper. By creating a custom ink consisting of SERS-active silver nanoparticles and glycerol or propylene glycol as viscosity-enhancing agents, an off-the-shelf ink-jet printer was capable of printing the nanoparticles onto untreated cellulose paper. Altering the number of print cycles allowed the quantity and location of the randomly aggregated silver nanoparticles to be precisely controlled [11].

2.5 Use Cases for Flexible SERS Substrates

While traditional, rigid SERS substrates are limited to simply pipetting the sample onto the surface—a task difficult to manage even by experts in the lab, let alone impossible by non-experts outside of the lab—flexible SERS substrates have additional properties that make them easier to use both in the lab and the field. These advantages include a larger non-sensing surface area, wicking capabilities, and flexibility.

Commercial rigid SERS substrates, due to their small size (often less than 1 cm^2) are simply too hard to handle; they require tweezers to manipulate without contaminating the surface, and a pipette to precisely load the sample properly, all of which is practically impossible in a low resource setting. Anecdotally, Raman experts refuse to employ these sensors because of poor usability.

Flexible SERS substrates overcome these challenges. By utilizing less expensive manufacturing techniques, they can afford to have an adjacent non-sensing region so the substrate can be grasped more easily without contaminating the sensing region as well as allow a larger sensing region so sample loading can be performed more easily. In addition, the wicking properties of paper allow the SERS substrates to be used as a dipstick, a swab [18], or a filter [19], as initially shown by Yu and Hoppmann. In the case of a dipstick, the SERS sensing region is dipped into an aqueous sample where the analyte is then attracted to the sensing region [18]; alternatively, the dipstick could be placed upside down in an aqueous sample for a longer period of time to allow for the sample to wick up the dipstick and concentrate at the tip [20][21]. For the swab, the sensing region is wiped across a solid surface containing the analyte. As a filter, a syringe is used to force an aqueous sample through the SERS sensing region. In all of these cases, the unique porous and wicking properties of paper allow it to not only provide an easier method of sample collection, but to concentrate the analyte, as well [19].

2.6 Remaining Challenge: Non-polar targets

While flexible SERS substrates provide clear cost and ease-of-use advantages in the field, they are traditionally tested with only a few different analytes to aid in the comparison between them, namely rhodamine-6G and BPE. These analytes have two key advantages: (1) The thiol group on BPE allows the analyte to bind to the silver nanoparticles. (2) The charge on the citrate-capped silver nanoparticles allow them to attract polar, hydrophilic analytes. This has resulted in a dearth of literature on analytes

that are critical to issues outside the lab but do not fit these criteria, namely non-polar analytes, such as THC [22].

Detection of hydrophobic and lipophilic compounds using cost-effective SERS substrates has remained elusive. This has drastically limited its market potential, as most biologically relevant small molecules are hydrophobic. A cost-effective SERS substrate has the potential to address three key issues: (1) Roadside detection of marijuana intoxication, (2) field detection of the explosive Acetone Peroxide (TATP), and (3) Therapeutic Drug Montoring (TDM) of drugs with a narrow therapeutic range.

2.7 Driving Under the Influence of Drugs

Annually, more than 1.4 million people are arrested for Driving Under the Influence (DUI); but over 110 million DUI drivers don't get caught [23], [24]. Since Colorado and Washington states legalized recreational marijuana in 2012, the rates of drivers operating a vehicle while under the influence of marijuana has skyrocketed from 1 in 11 weekend-night drivers in 2007 to 1 in 8 in 2014 [25]. Additionally motor vehicle accidents due to marijuana intoxication have increase 4%-14% in Oregon, Washington, and Colorado following legalization of recreational marijuana [26]. As marijuana legalization has spread across the US, with recreational use legal in 10 states and medical in an additional 23, police departments are increasingly concerned about combating this rise in drugged driving. While intoxication levels have yet to be determined scientifically (i.e. a 0.08% blood alcohol equivalent), preliminary studies suggest a limit of 13 ng/mL of Δ^9-THC in blood [27]. As the blood concentration is directly correlated to the amount of THC in saliva, this provides a goal for the detection

limit of a THC test [28]; however current research is still in its infancy, primarily due to two key factors: (1) marijuana-impaired drivers and alcohol-impaired drivers are *differently* impaired and (2) the incredible difficulty in performing research on human subjects using marijuana plants due to it still being federally classified as a Schedule I drug.

During the course of a traffic stop, police officers rely on a series of observations and tests to ultimate arrest a driver for DUI (alcohol) or DUID (Driving Under the Influence of Drugs). For DUI, first the officer observes the vehicle behaving in an unsafe manner, such as weaving, driving too fast or slow, not stopping at red lights or stop signs. This gives them probable cause to pull over the driver. Next, the officer observes the driver; if he notices signs of alcohol impairment, such as smelling alcohol on the breath or an open container, that gives him probable cause to administer a roadside portable alcohol breathalyzer. A positive result of 0.08% gives the officer

Figure 3: The chain of probable cause for a DUI (top) or DUID (bottom) arrest

probable cause to arrest the driver and take him to the hospital for a blood test. Only the blood test is used in court, however if there is a break in the chain of probable cause, the blood test evidence is inadmissible in court [29]. (See Figure 3)

However, in the case of DUID, the officer observes the driver showing signs of drug use instead of alcohol use. But there is no reliable roadside test for marijuana or

other drug use. This causes a Probable Cause Gap, which does not allow officers to arrest suspected DUID drivers and bring them to the hospital for a blood test. One option is to use a Drug Recognition Experts (DRE), which are specially trained police officers whose job is to determine drug impairment in drivers [29]. However, DREs are not a sustainable solution due for two key reasons: (1) The testing protocol DREs use to determine impairment is scientifically flawed. Doctors have shown that this testing, such as horizontal gaze nystagmus and pupil size, are not diagnostic of drug use. Furthermore, DRE accuracy is limited, resulting in 10-30% false positives and 50-90% false negatives for various drugs (cannabis, alprazolam, codeine, amphetamine) when using this protocol [30], [31]. (2) As they are specially trained, the high cost of their salary makes police departments unable to employ enough DREs to adequately serve the public, especially precincts in rural areas [32].

Not having a DRE causes this gap in probable cause that SERS can fill. However, as noted above, Δ^9-THC being extremely nonpolar (logP = 5.6) and present at very low concentrations (10s of ppb), conventional hydrophilic SERS substrates do not work.

2.8 THC Metabolism and Location

Upon drying and heating (i.e. smoking) the tetrahydrocannabinolic acid (THCA) in the plant is converted to Δ^9-THC, the psychoactive ingredient in marijuana. Upon ingestion, the Δ^9-THC enters the bloodstream and is quickly compartmentalized to fatty tissues, maintaining a low concentration in the blood, lowering levels from hundreds of ppb upon inhalation to tens of ppb. CYP 450 enzymes metabolize Δ^9-THC

to 11-OH-THC (11-Hydroxy-Tetrahydrocannabinol), which is still psychoactive but is then quickly oxidized to COOH-THC (Carboxy-Tetrahydrocannabinol), the non-psychoactive metabolite. COOH-THC is then filtered by the kidneys and excreted [33].

As COOH-THC is non-psychoactive, a test for THC intoxication must specifically measure Δ^9-THC, which is not found in urine [34]. While Beck (from the Karolinska Institute in Sweden) has done multiple studies on quantifying Δ^9-THC in breath [35]–[37], his results have come into question due to saliva contamination [38]. Concentrations of THC in saliva a few hours after smoking marijuana are in the one to tens of nanograms per milliliter range [39]–[41], while in breath THC concentrations are so low that *only one of nine breath samples tested positive* in a mass spectrometry analysis with a 10 minute breath collection time and a LOD of one pictogram [35]. As illicit drugs have been shown to cross the blood/saliva barrier and achieves an approximate 1:1 ratio, saliva remains as the best avenue for detection [42].

As preliminary studies show that an approximate limit of 13 ppb of Δ^9-THC [27] as the threshold for intoxication, this must be the required detection limit for devices (ideally one order of magnitude lower) on the market and equivalent to a 0.08% alcohol breathalyzer result. These intoxication limits are not set scientifically, but legally with input from the scientific community; thus, it is possible to be physically impaired below this number but not legally impaired.

Regulatory Requirements

Since the results of a roadside marijuana intoxication test would be used in the legal system and not to provide medical care, FDA guidelines for biosensors do not

apply. For any forensic test, the findings have to pass a Frye or Daubert Hearing in state or federal court. In these hearings a judge decides whether a piece of evidence (or expert witness) can be heard at trial. Commonly used tests that have already been used in multiple courts are exempted as a robust precedent has been established. However, in the case of a new technique without established precedent in the current court, the first step a judge will look for is a body of scientific literature and forensic experts to testify to the test's method and reliability. This bar is considerably higher if it is an evidentiary test. However, if it's used presumptively (i.e. in the chain of probable cause with a later test used evidentiary), the bar is considerably lower. As the US has a piecemeal judicial system, there is no *regulatory* body to prevent sales; rather, any roadside test can be marketed to police. However, if a test repeatedly fails to pass the Frye and Daubert Hearings, it would quickly be blacklisted by police; and if it results in false positives, it opens up the manufacturer to civil liability, as has happened frequently to Safariland Group and Sirchie, the makers of commonly used color-change field tests for narcotics [43].

2.9 Requirements for a portable device

The European Union released the DRUID Report [44] which set standards for and evaluated existing roadside drug-impairment detection devices. These standards included portability; ease of use; roadside results; and 80% sensitivity, specificity, and accuracy (i.e. correctly identified cases) [44]. After evaluating a number of devices on the market, none were found to achieve all of these criteria, especially the sensitivity and specificity, varying from 10%-59%. Additionally, none of the devices posited a

detection limit of 10 ppb. (See Figure 4) While multiple startup companies have posited working technology, none has released scientific findings achieving these requirements.

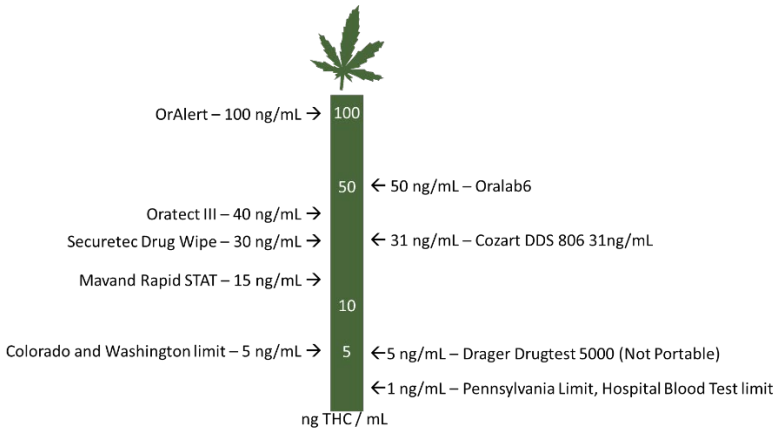

Figure 4: Detection and legal limits of Δ9 -THC

2.10 Other Applications – TATP & Therapeutic Blood Monitoring

Tri-Acetone Tri-Peroxide (TATP) is a highly explosive compound that can easily be made in a non-labatory using conventional household ingredients that are not controlled. Nicknamed the "Mother of Satan", TATP is highly unstable and is over 60 times more powerful than TNT [45]. For these reasons, it is commonly used in terrorist attacks (including the 2016 Brussels attack and as the detonator in Richard Reid's "shoe bombing" attempt). Due to its hydrophobicity and lack of nitrate groups, it has been traditionally hard to detect. While there are new training aids for canine detection and current products now hitting the market, mainly for stationary checkpoint security queues, portable detection devices that can directly detect TATP, rather than synthesis byproducts, is still a work in progress [46]–[48].

For pharmaceuticals with a narrow therapeutic range, continuous monitoring of medication levels in blood is vital. In extreme cases, such as cancer or sever infections,

some medications frequent blood testing to ensure that the dosage is still within the therapeutic window. However, this is limited due to the high cost and central pathology laboratory requirements of the frequent blood testing [49], [50].

While conventional pathology laboratory testing is capable of measuring drug concentration in the blood, the results often take hours to days to be received; however, the physician needs periodic results immediately in order to provide adequate care. Paper SERS has been shown to work with polar medications [51], a low-cost SERS substrate capable of measuring hydrophobic analytes would allow for the testing of a wider variety of medications; however more research would need to be done in blood filtration before a product can be finalized, in addition to FDA trials as this would be a medical device (unlike in the case of THC).

Chapter 3: Methods

3.1 Materials

- Chemicals
    - Propylene Glycol, ≥99.5% (Sigma Aldrich)
    - Glycerol, ≥99.5% (Sigma Aldrich)
    - Silver nitrate (Sigma Aldrich)
    - Sodium citrate tribasic dehydrate (Sigma Aldrich)
    - Poly(styrene-co-maleic anhydride), cumene terminated (Sigma Aldrich)
    - Rhodamine 590 Chloride (Exciton)
- Other
    - Deskjet 1010 Ink-jet Printer and Ink Cartridges (HP)
    - Silhouette Cameo Electronic Cutting Machine (Silhouette)
    - Whatman Chromatography Paper, 1CHR (GE)
    - 1mL & 10mL Luer-lock Syringes (BD)
    - Re-usable Syringe Filter Holders (Sartorius Stedim)

3.2 Silver Nanoparticle Formation

Silver nanoparticles (AgNPs), which were used to generate the SERS sensors (via printing and filtering), were synthesized. AgNPs were formed by the Lee-Miesel method [52]. Briefly, 80 mg sodium citrate was added to 72 mg silver nitrate at a vigorous boil (at 450ºC) in a 400 mL volume of DI water in a narrow-mouthed Erlenmeyer flask. After 10 minutes, the solution was allowed to cool a dark grey color.

The nanoparticles were subsequently concentrated by a factor of 100x via centrifugation at 2000xg for 30 minutes; after spinning, 99% of the supernatant was removed.

3.3 Ink Jet Printing

Two different nanoparticle ink formulations were used. Either glycerol or propylene glycol was added to the 100x colloid in order to enhance the viscosity for printing. For the glycerol ink, a ratio of 2:1 glycerol to 100x silver nanoparticles was used. For the propylene glycol ink, a ratio of 1:1 propylene glycol to 100x silver nanoparticles was used.

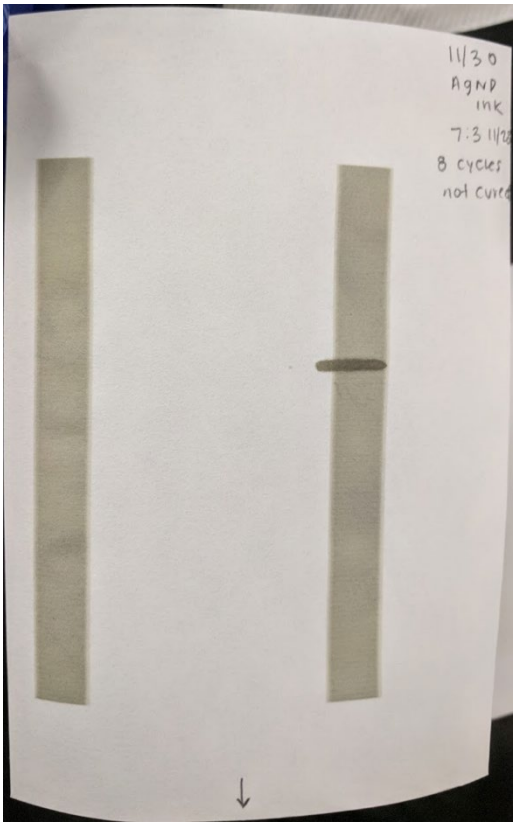

Figure 5: Example of ink-jet printed silver nanoparticles

To print, a 4”x6” piece of Whatman filter paper was loaded into the ink-jet printer. The requisite ink was loaded into an emptied black ink cartridge and the appropriate design was printed. Two different printing protocols were used: (1) 5 cycles of propylene glycol ink, followed by 5 cycles of glycerol ink or (2) 10 cycles of propylene glycol ink. Every 3 cycles (for hydrophobic paper) or 5 cycles (hydrophilic), the paper was allowed to dry for approximately 10 minutes (See Figure 5).

3.4 Hydrophobic Paper Modification

Paper sensors were first rendered hydrophobic before silver-ink deposition to enable enrichment of non-polar molecules onto the sensor. Styrene maleic anhydride enables covalent crosslinking via a dehydration reaction. Styrene maleic anhydride was dissolved in acetone at 10% w/v. The solution was then applied to Whatman Filter Paper Type 1. The paper was then placed between a hot plate at 225 ^{o}C and glass heated to the same temperature for 10 minutes. The paper was then soaked in an acetone bath for 10 minutes to remove unbound styrene. An automated craft cutter was then used to cut 15 mm discs in the paper. These discs were washed again in an acetone bath for 10 minutes and allowed to dry. The discs were then placed in water to ensure complete hydrophobicity; discs with partial hydrophilicity were discarded.

3.5 SERS-active Filter Production

Silver was deposited onto the paper membrane discs by a filtering method. For both hydrophilic and hydrophobic paper, the discs were placed in a reusable syringe filter holder. (See Figure 6). Then, 100 µL of 100x silver nanoparticle solution was passed through the paper using a 1mL syringe by hand at an approximate speed of 10 µL/second; a 10mL syringe was then used to pass 10mL of air through the filter at approximately 1 mL/second to ensure the solution passed through the filter. The paper was removed and allowed to dry.

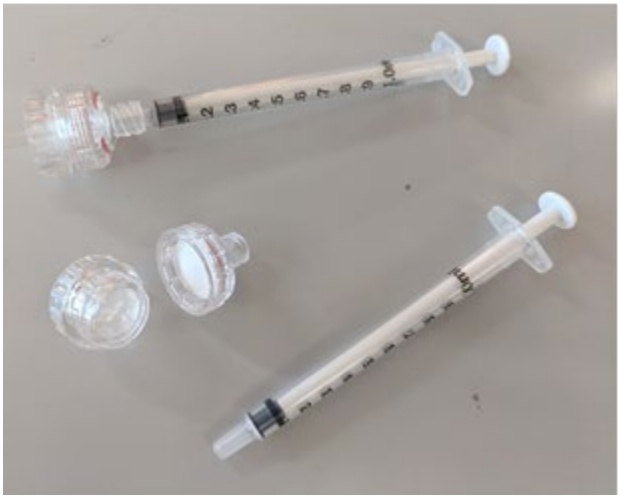

Figure 6: Reusable Filter Preparation

3.6 Sample Filtration

Target analytes were diluted in an aqueous solution (to mimic field use applications); the solution was passed through the SERS disc, enabling the hydrophobic substrate to capture and enrich the non-polar targets through a filtering mechanism. To create an aqueous stock solution, rhodamine-6G (R6G) was first dissolved in methanol at 2 mM. That 2 mM stock solution was subsequently diluted 1:50 in water to generate a 100 µM aqueous stock solution and subsequently diluted 1:10 in water to achieve

appropriate concentrations. These aqueous solutions were then filtered through the SERS discs using a reusable filter holder and luer-lock syringe, as above.

3.7 SERS Measurements

The SERS substrates were measured with a USB-2000 Spectrometer (Ocean Optics), a small and portable spectrometer, with a 785 nm laser (Inphotonics) and probe (Inphotonics) (See Figure 7). At a laser power of approximately 17 mW and 1 second integration time, multiple measurements were carried out on each disc at local maxima (i.e. locations that produced the largest signal) and averaged together. The spectra was background subtracted using an asymmetric least squares analysis to remove the background in MATLAB [53].

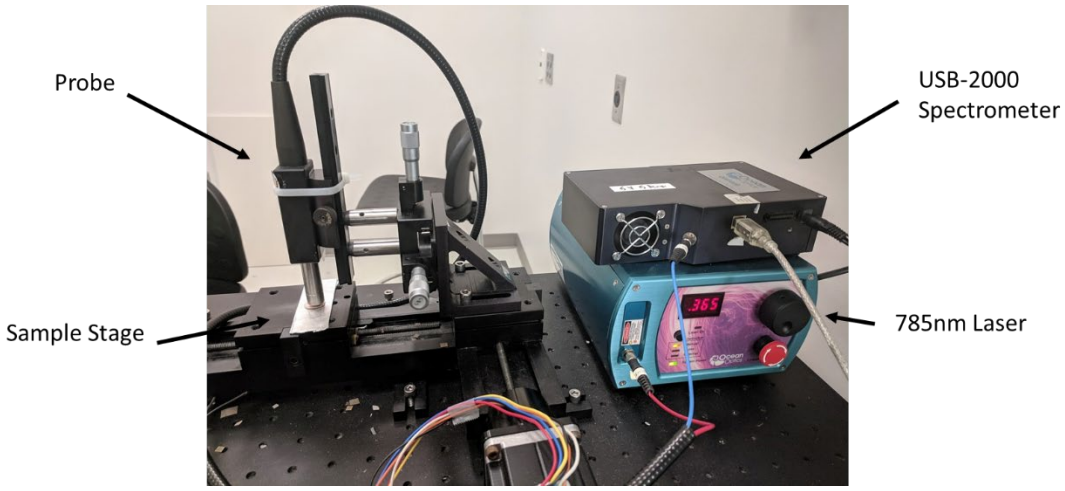

Figure 7: Raman Spectroscopy Bench

Chapter 4: Results

While the end result was a method to concentrate hydrophobic analytes onto a SERS sensor, multiple designs and iterations led to the development of the final technique. As the lab has a history with ink-jet printed SERS substrates, we started with printed substrates. Difficulties in printing and hydrophobic modification ultimately led to the abandonment of printing in favor of a filter-based production technique. Ultimately, we showed that using a hydrophobic membrane patterned with silver nanoparticles by the filtering method delivers the best sensing performance, and that using the SERS sensor in a filtering format for capturing analytes from an aqueous sample is a viable approach for the detection of non-polar targets in the field.

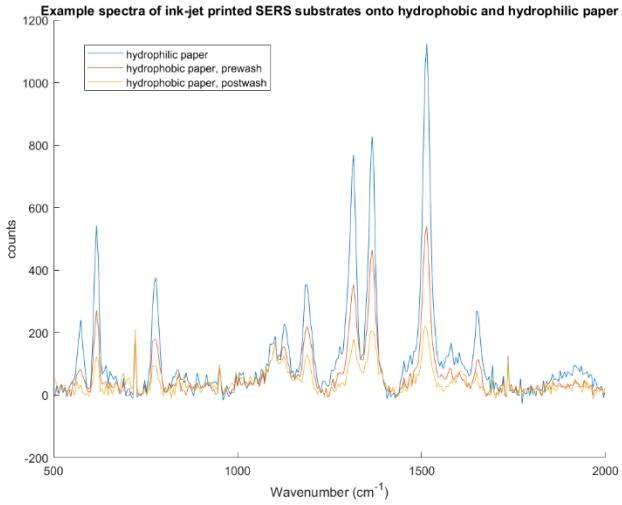

Figure 8: Example spectra of ink-jet printed SERS substrates onto hydrophilic and hydrophobic paper

In order to get a baseline of how hydrophobicity impacts the ink jet printing process and SERS measurements, silver nanoparticles were printed onto hydrophilic and hydrophobic paper using the standard propylene glycol / glycerol ink formulations; R6G was then pipetted onto the substrates and measured. (See Figure 8) When

acquiring data in future experiments, it's best to refer to this as a relative baseline. Importantly, this showed that not ink-jet printing onto hydrophobic paper was feasible, however the 20x decrease in signal provided room for improved. (Note that 100 μM R6G is used for the hydrophobic paper, while 10 μM was used for hydrophilic.)

4.1 Hydrophobic Modification using SMA

Styrene maleic anhydride (SMA) modification ultimately results in a hydrophobic modification of the paper through a dehydration reaction with the cellulose fibers upon heating. This requires heating paper between 200ºC and 250ºC for approximately 10 minutes, which is enough time for the acetone to evaporate. Lower temperatures resulted in inconsistent hydrophobicity while higher temperatures resulted in the paper becoming too charred and brittle. Following the heating step, it was critical to wash the paper in a bath of acetone to remove the unbonded styrene. If the styrene is not heated, it provides initial hydrophobicity but easily falls off over time due to physical handling of the paper, rendering that section hydrophilic. Thus the wash step is required. A subsequent dip into water provides a visual confirmation that

the paper is indeed hydrophobic as it remains opaque, while any areas that turn semi-translucent are hydrophilic. (See Figure 9)

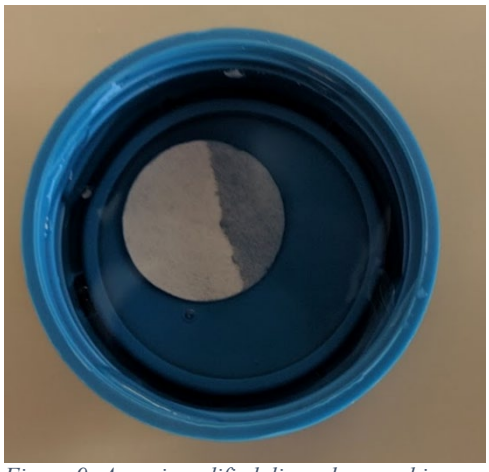

Figure 9: A semi-modified disc submerged in water

4.2 Organic Layer Separation and Extraction

As THC and other nonpolar analytes have a high partition coeffiecient (i.e. a logP greater than 1), they will prefer an organic phase over an aqueous phase. Thus, nonpolar analytes in large volumes of aqueous solutions could be concentrated through the addition of small volumes of octanol. Subsequently, the octanol layer could be extracted using hydrophobic paper, as it repels the aqueous phase and absorbs the organic phase. To demonstrate this effect, hydrophobic and hydrophilic paper was added to 10 ppm (21 μM) R6G in water as well as a 9:1 10 ppm R6G:Octanol mixture. In both cases, the hydrophobic paper readily absorbed the R6G, as seen in Figure 10. However, at this large concentration, there's only a factor of 2 increase in extraction. This enhancement increases as total concentration of R6G decreases, as higher amounts saturate the paper. Thus, a small piece of hydrophobic paper is capable of extracting a significant fraction of nonpolar analyte from a large volume of water. The next step is

to measure using SERS, as it is a more sensitive technique and applicable analytes other than dyes.

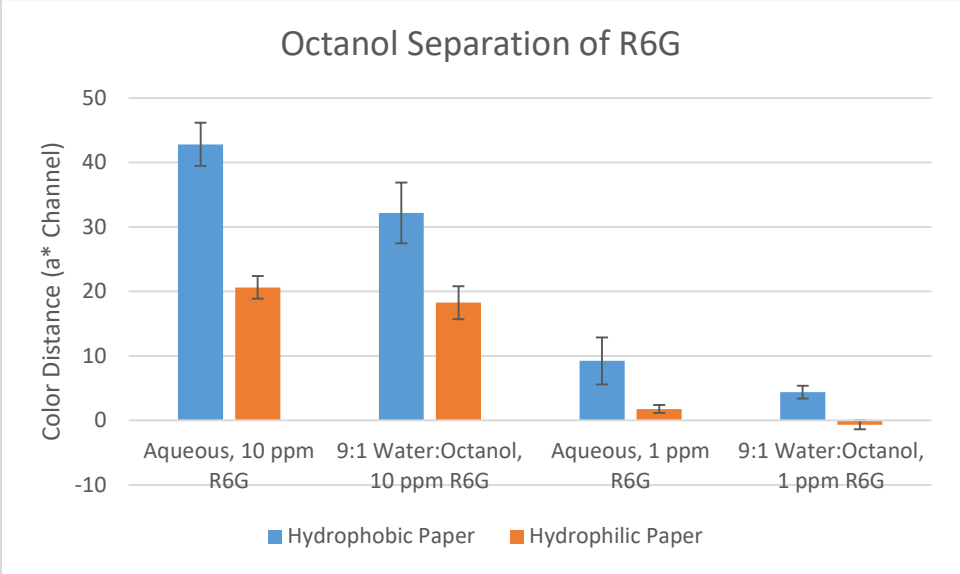

*Figure 10: Octanol Extraction of R6G onto Paper. Quantification was done via L*a*b* analysis of the a* channel.*

4.3 Hydrophobic Patterning

In order to selectively pattern paper so that there are clear hydrophobic and hydrophilic areas, multiple methods were tested. Aside from SMA, multiple methods of hydrophobic modification were tested, namely using ASA and SMA. The ultimate goal of the patterning was to be able to selectively wick the octanol layer from the water/octanol extraction method and concentrate it at the tip of a SERS sensor.

First, alkenylsuccinic anhydride (ASA) was tested with the goal of ink jet printing the ASA onto paper. This yielded multiple issues. The bond was not permanent and the paper lost hydrophobicity within 24 hours. The new HP printers also did not consistently print the ASA onto paper. And when patterned onto paper,

the ASA did not consistently cure to produce a consistently hydrophobic region. For these reasons, ASA was rejected.

SMA was used to replace ASA; however it, too, cannot be printed, since the SMA is dissolved in acetone which would destroy the plastic ink cartridge. Instead, an aluminum block approximately 1 cm tall was milled in the design of the pattern. By heating this block to 250 ºC, it served as a heat stamp. After stamping the paper soaked in SMA, the paper was bathed in acetone and the uncured SMA washed off.

While this SMA stamping fabrication technique worked, it did not end up being beneficial to concentration of nonpolar analytes for two reasons. First, while the SMA effectively repelled the water, the hydrophilic regions did not repel the octanol. Thus, the octanol was allowed to flow freely throughout the paper. More importantly, the area of the hydrophobic region was too large; as there needed to be a small amount of octanol in order to concentrate the R6G, there was not enough octanol present act as a wick. Thus, the idea of patterning a hydrophobic layer onto paper was nixed in favor of using smaller sized SERS sensors to absorb all of the octanol present.

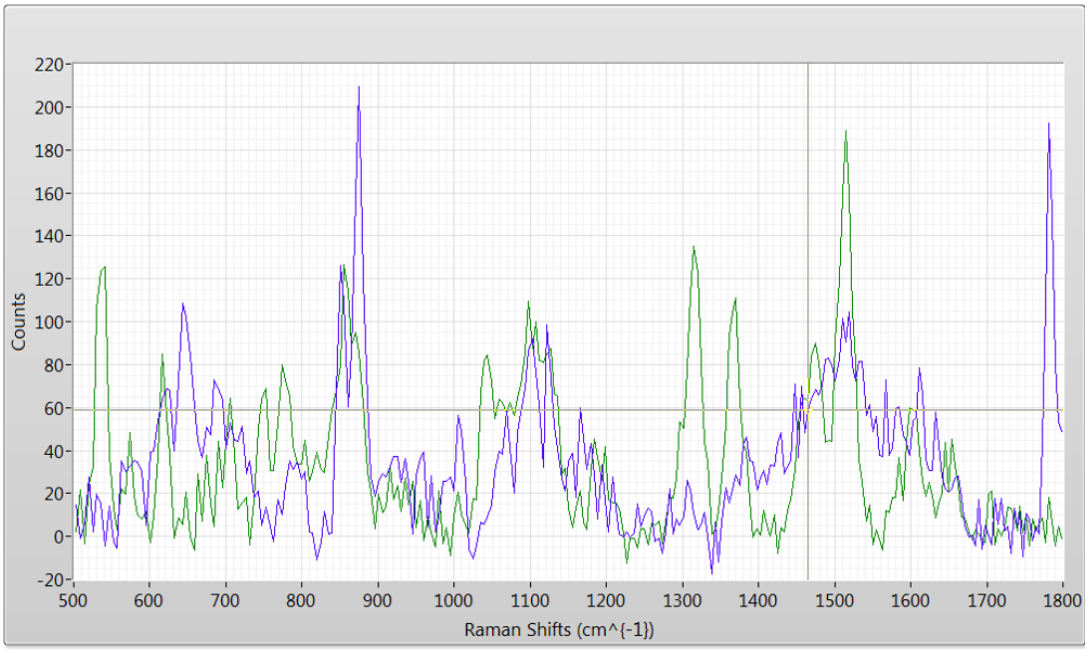

Figure 11: Loss of SERS activity of styrene-modified SERS substrates after drying. (Before: green, After: blue)

4.4 Hydrophobic modification of ink-jet printed substrates

Once reliable production of hydrophobic-modified paper was complete, it was ready for nanoparticle printing. As the original ink-jet printing recipe called for five layers of propylene glycol ink followed by glycerol ink, we started with this protocol, printing onto the hydrophobic paper. As glycerol and propylene glycol are *humectants*, they render the printed areas hydrophilic; hence the next step was to remove them following printing. As seen in Figure 10, not only is the R6G SERS spectra significantly less than the normal thousands of counts, most likely due to the ink not absorbing into the hydrophobic paper as it does in hydrophilic paper; but there is a complete loss of signal after the humectant is removed. As shown in Figure 11, a simple droplet of water onto a printed region wicks into the paper, albeit slower than normal, while a similar droplet remains on top of a non-printed region. As heating the paper

would eliminate the SERS activity of the nanoparticles (as shown in Figure 10), the paper was washed in solvents (i.e. acetone, ethanol, or methanol). However, this resulted in the mobility of the nanoparticles. Their shifting during this wash step changed the way they aggregate on paper, eliminating the SERS activity. This was primarily due to the glycerol. On unmodified, hydrophilic paper, the glycerol is able to seep into the pores of the paper, but on the hydrophobic paper, it rests on top of the propylene glycol layer. Hence, the glycerol layer was simply washing off, and taking the nanoparticles with it, as exhibited in Figure 12 where a drop of water removes the nanoparticles from the top glycerol layers.

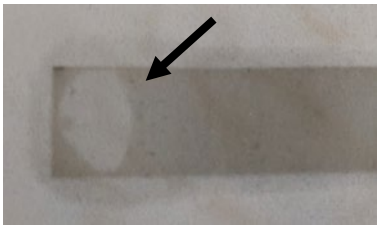

Figure 13: Water washing away glycerol layers in ink-jet printed SERS substrates

For this reason, we switched to using only propylene glycol ink and using it to print all ten cycles. Upon washing in methanol, the nanoparticles remained, however the signal was still weak and approximately 100x lower than on similarly printed hydrophilic paper. After trying a number of printing modifications (more print cycles,

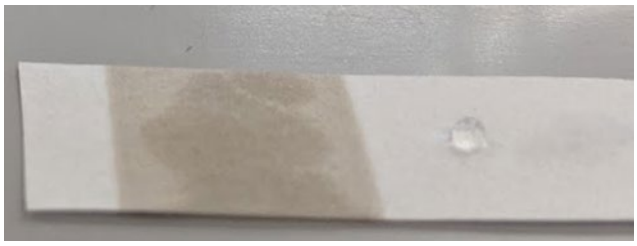

Figure 12: Ink-jet printing rendering hydrophobic-modified paper hydrophilic

altering the washing methods, etc.), the end results were the same; removing the

humectants was altering the nanoparticles too much, resulting in too steep of a loss of signal.

4.5 Filter SERS using Hydrophobic Paper

The benefits of using the filter technique is that glycerol, propylene glycol, or any other humectant are not needed. By eliminating this wash step, the stability of nanoparticles was preserved. The protocol was the same as proposed by this lab before [19]. As described above, the same technique was used to both manufacture the filter SERS substrates and load the sample. To first demonstrate the protocol, R6G was filtered through filter paper without first depositing nanpoarticles. This showed a significant improvement above background. (See Figure 13) Notably, there was no need for an ethanol prewash, as previously suggested.

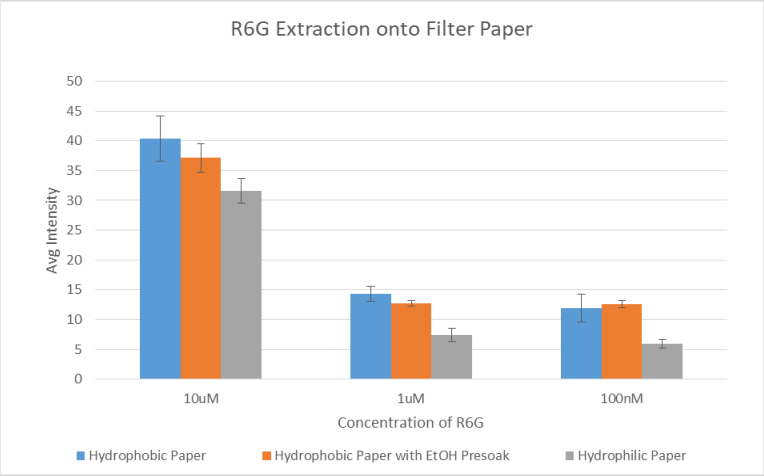

Figure 14: R6G Extraction

However, filtration of both R6G and silver nanoparticles through both hydrophilic and hydrophobic paper exhibited a few intricacies. First, the paper is sided

and care has to be taken to ensure that the same orientation is maintained when reloading the filter SERS paper into the holder to maximize the signal. But more importantly, the filter process results in blotches, especially on the hydrophobic paper, resulting in a variation of ~15% standard deviation in variation of deposition. Even with that issue, the SERS results still show a marked increase in signal intensity for R6G, showing a dramatic increase in signal intensity when compared to the traditional SERS test where a 2 µL spot is pipetted onto a substrate. (See Figure 14) While the larger volume alone greatly increases the magnitude of the signal, a traditional spot test simply is not feasible for a filter-based approach. Additionally this filter approach more closely resembles the final use case for this technique.

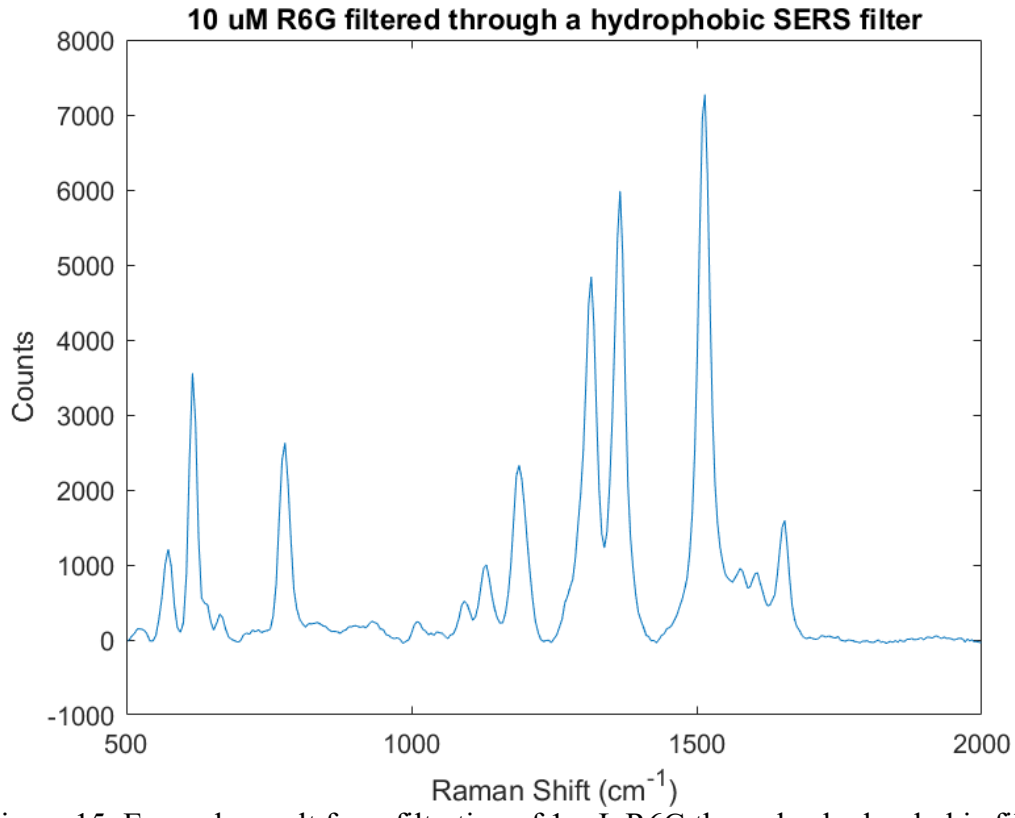

Figure 15: Example result from filtration of 1 mL R6G through a hydrophobic filter

Chapter 5: Summary and Future Work

Detection of trace amounts of nonpolar analytes remains a challenge, and a solution is much needed in the market today, especially in the areas of THC intoxication and therapeutic drug monitoring. While SERS presents an excellent modality for trace chemical detection, paper-based SERS substrates, specifically ink-jet printed ones, remain the only cost-effective version. However, as ink-jet printing requires the nanoparticles to contain citrate caps, these charged nanoparticle surfaces repel nonpolar analytes and make their detection more difficult.

In this thesis, we've shown the need for a SERS substrate to detect nonpolar analytes. From THC detection in the field to therapeutic drug monitoring to explosives detection, there is a critical necessity for a portable, inexpensive, easy to use test. As traditional ink-jet printed SERS substrates were not sensitive enough for these nonpolar analytes, we set out to develop a modified paper-based SERS substrate that could concentrate non-polar analytes. We showed how ink-jet printing results in loss of hydrophobicity due to the humectants in the ink formulations. Thus, styrene-modified filter SERS was introduced and shown to concentrate nonpolar analytes from large-volume aqueous samples. And we've shown the filtration technique using the hydrophobic paper not only concentrated the analyte onto the paper but also aids in SERS detection. While these results only show a minimal increase in concentration, they show that the technique works, and with optimization can lead to a successful solution.

There are a few remaining directions for the research before a usable roadside THC test can be developed. First, the nanoparticle filtration needs to be optimized. As

there are variances in sample loading and frequent device failures due to the design of the filter holders, a few modifications need to be made; altering the size of the discs and the size, surface chemistry, volume, and concentration of the nanoparticles would yield in a more reproducible technique. Second, the effects of the saliva matrix on SERS substrates needs to be explored; proteins, food particles, and other components of saliva would need to be filtered out to reduce their impacts on detection without removing the analyte. Finally, other applications for this device need to be explored, whether that's as a similar filtration unit for therapeutic drug monitoring or a wipe for explosive detection.

Chapter 6: Contributions

- Introduced new and tweaked existing techniques in the lab, including styrene-modified hydrophobic paper and hydrophobic filter SERS.
- Proposed a locking probe coupled with a polynomial rolling circle amplification (RCA) scheme for single nucleotide polymorphism (SNP) detection.
- Commercialized ink-jet printed SERS substrate technology through co-founding Diagnostic anSERS, marketing, business development, product development, patent prosecution, and selling the company to Metrohm Raman. This resulted in the research-focused P-SERS product line and a white-labeled kit for police detection of heroin and fentanyl in the field.
- Emphasized and explored real-world applications to current laboratory techniques, including forensics and Tuberculosis detection.
- Trained 2 undergraduates and 1 high school student in lab safety and SERS techniques.
- Mentored numerous UMD students in entrepreneurship and technology commercialization through the Smith School's Dingman Center for Entrepreneurship and the Clark School of Engineering's MTECH.

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