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The Creation and Evaluation of Novel Canine Training Aids for Cocaine Using Molecularly Encapsulated Sol-Gel Polymers and an Investigation of Canine Field Accuracy

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FLORIDA INTERNATIONAL UNIVERSITY

Miami, Florida

THE CREATION AND EVALUATION OF NOVEL CANINE TRAINING AIDS FOR
COCAINE USING MOLECULARLY ENCAPSULATED SOL-GEL POLYMERS
AND AN INVESTIGATION OF CANINE FIELD ACCURACY

A dissertation submitted in partial fulfillment of

the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

CHEMISTRY

by

Michelle Cerreta

2015

To: Dean Michael R. Heithaus
College of Arts and Sciences

This dissertation, written by Michelle Cerreta, and entitled The Creation and Evaluation of Novel Canine Training Aids for Cocaine Using Molecularly Encapsulated Sol-Gel Polymers and an Investigation of Canine Field Accuracy, having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this dissertation and recommend that it be approved.

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Anthony DeCaprio

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Date of Defense: March 2, 2015

The dissertation of Michelle Cerreta is approved.

Dean Michael R. Heithaus
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Dean Lakshmi N. Reddi
University Graduate School

Florida International University, 2015

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DEDICATION

This dissertation, and the 5 years of the work that helped produce it, is dedicated in memory of my dad, Kenneth Cerreta Jr. At a young age, my dad always taught me the value of hard work and assured me that I could have whichever career I wanted; I just needed to give it my all. He inspired and encouraged me to keep pushing even when I wanted to quit. Even after he passed away, I still felt his words and strength pushing me through this PhD program and supporting me when I stumbled. My dad always said he had two loves of his life - his family and his garden. I have a feeling that the reason I was able to grow cool-weather plants in Miami was because he gave me a little help. Dad, you are forever on my mind and in my heart and I thank you for every lesson you taught me, which shaped me into who I am today.

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ABSTRACT OF THE DISSERTATION

THE CREATION AND EVALUATION OF NOVEL CANINE TRAINING AIDS FOR
COCAINE USING MOLECULARLY ENCAPSULATED SOL-
GEL POLYMERS AND AN INVESTIGATION OF CANINE FIELD ACCURACY

by

Michelle Cerreta

Florida International University, 2015

Miami, Florida

Professor Kenneth G. Furton, Major Professor

Biological detectors, such as canines, are valuable tools used for the rapid identification of illicit materials. However, recent increased scrutiny over the reliability, field accuracy, and the capabilities of each detection canine is currently being evaluated in the legal system. For example, the Supreme Court case, *State of Florida v. Harris*, discussed the need for continuous monitoring of canine abilities, thresholds, and search capabilities. As a result, the fallibility of canines for detection was brought to light, as well as a need for further research and understanding of canine detection. This study is two-fold, as it looks to not only create new training aids for canines that can be manipulated for dissipation control, but also investigates canine field accuracy to objects with similar odors to illicit materials.

It was the goal of this research to improve upon current canine training aid mimics. Sol-gel polymer training aids, imprinted with the active odor of cocaine, were developed. This novel training aid improved upon the longevity of currently existing training aids, while also provided a way to manipulate the polymer network to alter the

dissipation rate of the imprinted active odors. The manipulation of the polymer network could allow handlers to control the abundance of odors presented to their canines, familiarizing themselves to their canine's capabilities and thresholds, thereby increasing the canines' strength in court.

The field accuracy of detection canines was recently called into question during the Supreme Court case, *State of Florida v. Jardines*, where it was argued that if cocaine's active odor, methyl benzoate, was found to be produced by the popular landscaping flower, snapdragons, canines will false alert to said flowers. Therefore, snapdragon flowers were grown and tested both in the laboratory and in the field to determine the odors produced by snapdragon flowers; the persistence of these odors once flowers have been cut; and whether detection canines will alert to both growing and cut flowers during a blind search scenario. Results revealed that although methyl benzoate is produced by snapdragon flowers, certified narcotics detection canines can distinguish cocaine's odor profile from that of snapdragon flowers and will not alert.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
ATP	Adenine Triphosphate
cAMP	Cyclic Adenine Monophosphate
CAR/PDMS	Carboxen/Polydimethoxysilane
CNCA	California Narcotic Canine Association
CNS	Central Nervous System
COMPS	Controlled Mimic Permeation System
CSA	Controlled Substance Act
DA	Dopamine
DVB/CAR/PDMS	Divinylbenzene/Carboxen/Polydimethoxysilane
EI	Electron-Impact
GC	Gas chromatograph/gas chromatography
GC-MS	Gas chromatography - mass spectrometry
HCl	Hydrochloric Acid
HPLC	High performance liquid chromatography
HS/GC-MS	Headspace/Gas chromatography - mass spectrometry

IFRI	International Forensic Research Institute
IMS	Ion Mobility Spectrometry
LFIA	Lateral Flow Immunoassay
MDMA	3,4-methylenedioxy-N-methylamphetamine
MIP	Molecular Imprinting
MS	Mass Spectrometer/Mass Spectrometry
NFSTC	National Forensic Science Technology Center
NNDDA	National Narcotic Detection Dog Association
NPV	Negative Predictive value
NSDUH	National Survey of Drug Use and Health
PA	Polyacrylate
PCA	Principal components analysis
PCP	Phencyclidine
PDMS	Polydimethylsiloxane
PDMS/DVB	Polydimethylsiloxane/ divinylbenzene
PEG	Polyethylene glycol
PPV	Positive predictive value
SEM	Scanning Electron Microscope
SPME	Solid-phase microextraction
TEOS	Tetraethyl Orthosilicate
TIC	Total ion chromatogram
USPCA	United States Police Canine Association
VOCs	Volatile organic compounds

1. INTRODUCTION

On June 24, 2006, Officer William Wheatley of the Liberty County, Florida, Sheriff's Office, pulled over Mr. Clayton Harris after noticing that Harris was driving a vehicle with an expired license plate. When Wheatley approached the driver's side door, he noticed that Harris was visibly shaking and fidgeting inside the vehicle. After seeing an open can of beer in the cup holder, Wheatley asked Harris if he could search his truck, upon which Harris refused. Wheatley retrieved his trained detection canine, Aldo, from his patrol car and had the dog search the truck perimeter. Aldo alerted to the driver's side door handle. Based on Aldo's alert, the behavior of Harris, the expired license plate, and the opened container of beer, Wheatley concluded that he had probable cause to search the vehicle for evidence of illegal drugs. The search revealed 200 pseudoephedrine pills, matches, muriatic (hydrochloric) acid, antifreeze, and iodine crystals- all ingredients used for making methamphetamine. After being arrested and read his Miranda rights, Harris admitted to Wheatley that he routinely cooked methamphetamine in his house and was an addicted user.¹

After Harris was charged in a state court, he moved to suppress the evidence seized from his truck on the grounds that Aldo was "insufficiently reliable to provide a basis for probable cause to search a vehicle."¹ It was then required that Officer Wheatley testify about Aldo's training in drug detection, including his hours of training/training courses and certifications. The court concluded that there was probable cause to search the vehicle and denied Harris' suppression notion, sentencing him to 24 months of imprisonment, followed by five years of probation.¹

Harris sought review by the Supreme Court, which led to the 2013 case, *State of Florida v. Harris*. The Florida Supreme Court reversed the initial ruling, stating that the evidence from Harris' truck should have been suppressed. While it is recognized that a canine "sniff" at a vehicle is not considered a "search" and that probable cause to search without a warrant can be subjective and not always set by legal rules, probable cause to search from a canine can only be achieved if dog's reliability is determined. Specifically, a dog's trainings and certifications must be presented as well as thorough documentation of a canine's field performance at detecting illicit materials. Evidence that the canine is trained and certified is not considered legally sufficient. The Supreme Court ruled that there was insufficient probable cause to search Harris's vehicle, as "Aldo's training and certification [was] insufficiently thorough."^{1,2}

The *State of Florida v. Harris* marked the first Supreme Court case to challenge a detection canine's reliability, which was considering infallible in previous legal proceedings. The ruling brought to light the many unanswered questions associated with detection canine research and training. Because of this case, and others, such as the *State of Florida v. Jardines*, which argued about the accuracy of canines in the field, a growing need in the scientific and legal community has emerged to determine and monitor specific capabilities and thresholds of detection canines.¹⁻³ Therefore, the goal of the present research was to create novel training aids for narcotics that allows for odor dissipation manipulation to gauge the ranges of odor concentrations to which canines alert. In addition, the accuracy of detection canines was evaluated in the presence of household objects with similar odors as illicit substances. The development and assessment of these training aids provides canine trainers with quantitative support

regarding the accuracy and reliability of a specific canine's ability to detect illicit substances.

2. BACKGROUND INFORMATION

2.1. Narcotics/Illicit Materials

Pharmacologically, drugs are defined as any chemical agent that alters the biochemical or physiochemical processes of organs or tissues.⁴ Drugs, and in some cases, the chemicals used to make them, are classified into five categories, as dictated by the Controlled Substance Act (CSA). The CSA schedules drugs according to their acceptable medical use and potential for abuse and addiction (Table 1).⁵

Table 1: List of drug schedules in accordance with the Controlled Substance Act ⁵

Schedule	Definition	Example
Schedule I	High potential for abuse and addiction; no medical use	Heroin
Schedule II	High potential for abuse and addiction; regulated medical use	Cocaine
Schedule III	Moderate potential for abuse and addiction; regulated medical use	Codeine
Schedule IV	Low potential for abuse and addiction; guarded medical use	Xanax
Schedule V	Low potential for abuse and addiction; accepted medical use	Cough Medicine (less than 200 mg codeine)

According to the 2012 National Survey of Drug Use and Health (NSDUH), an estimated 22.2 million Americans, aged 12 and older, were classified with substance dependence in the past year. Of those, 2.8 million people were classified as dependent on

both alcohol and illicit materials, while 4.5 million were considered dependent on just illicit drugs. Additionally, 23.9 million Americans were classified as current users, or used within the past month, making up 9.2% of the population. The popularity of abused illicit substances can be seen in Figure 1, with results derived from individuals that were classified as dependent users in 2012.⁶ On the basis of the study, marijuana, pain relievers, and cocaine were determined to be the three most commonly abused drugs in the United States, with 4.3 million, 2.1 million, and 1.1 million users, respectively.⁶ Because of the popularity of cocaine, its high scheduling (schedule II), and having no intention of legalizing its recreational use, as is the case for marijuana, cocaine was the major focus of this study.⁷

Figure 1: Specific illicit drug dependence in 2012 among persons aged 12 or older ⁶

2.1.1. Cocaine

Cocaine is an alkaloid found in *Erythroxylum coca*, a plant principally found in South America, India, Africa, and Indonesia. In the mid-19th century, cocaine was first isolated from this coca plant by Albert Niemann and initially used in tonics and elixirs, believing it had “magical properties.” In 1884 Carl Koller became the first physician to use cocaine for medicinal purposes- as a topical anesthetic for ophthalmologic surgeries. Early reports of cocaine toxicity and abuse among the population have led it to be labeled a narcotic under the Harrison Narcotic Act in 1914. In 1970, under the Controlled Substance Act (CSA), cocaine was characterized as a schedule II drug and has remained as such ever since.⁸

Figure 2: Chemical structure of cocaine ⁸

Cocaine is an ester of benzoic acid and the amino alcohol, methylecgonine (Figure 2). It is commercially synthesized from ecgonine in the presence of methanol and benzoic acid after hydrolysis of the ester alkaloids extracted from the plant material. Cocaine, more colloquially referred to as “blow”, “dust”, “flake”, and “snow”, is sold illegally on the street in two forms: the hydrochloride salt and the freebase form. The salt

is typically cut or mixed with numerous agents and stimulants, such as caffeine or pseudoephedrine, and is usually inhaled through snorting. The freebase form of cocaine, called crack, is prepared by adding baking soda to aqueous cocaine hydrochloride and heating. Once filtered, the cocaine precipitates into small “rocks” that when heated becomes a volatile vapor that can be inhaled by the user.⁸

Cocaine is a strong central nervous system (CNS) stimulant that produces short term euphoria coupled with increased awareness and concentration. Acute users have been known to experience ventricular arrhythmias, hypertension, and respiratory dysfunction and paralysis. In addition to those above, chronic users experience tachycardia, tremors, violent behavior, dyspnea, epilepsy, rupturing of cerebral vessels, and heart disease.^{8,9} In acute doses, euphoric and stimulatory affects are as a result of the fact that cocaine prevents the re-uptake of dopamine (DA), the neurotransmitter responsible for regulating pleasure and movement, by binding to the DA re-uptake transporter in the CNS. Because of this, there is an increased concentration of DA and norepinephrine in the synapses, causing the experienced “high” (Figure 3).

Figure 3: Effects of cocaine on the central nervous system (CNS), where cocaine blocks the dopamine re-uptake transporter, preventing the re-uptake of dopamine ^{8,9}

Cocaine metabolism occurs via hepatic metabolism to the primary metabolites, benzoylecgonine and ecgonine methyl ester, by altering the functional groups on the parent drug, causing an increase in the drugs' solubility for eventual clearance in urine (Figure 4).^{8,10} While cocaine is rapidly eliminated from the body, with a biological half-life of about one hour, the metabolites, benzoylecgonine and ecgonine methyl ester, are eliminated more slowly, with half-lives of five and eight hours, respectively. This is convenient for drug analysis, as 1-5 percent of the parent drug is eliminated unchanged, while benzoylecgonine and ecgonine methyl ester make up a combined 75-90% of the eliminated drug product.¹¹

Figure 4: Cocaine major metabolites: benzoylecgonine (A) and ecgonine methyl ester (B) ^{8,10}

2.2. Narcotics On-Site Detection

In recent years, there has been much research regarding on-site narcotics detection. In most cases, detection methods have been developed for on-site, roadside drug screening tests. For example, Drugwipe, a non-instrumental immunoassay, has the ability to detect amphetamines, cannabis, opiates, cocaine, and benzodiazepines from sweat and oral fluids.^{12,13} Other immunoassays, such as the Pro-Multi Drug Screening Test, can test for amphetamine, opiates, phencyclidine (PCP), and marijuana from urine, using a chromatographic absorbent testing device.¹⁴ On-site drug testing for suspicious powders, however, is more limited, but is commonly performed using color tests and some solid immunoassay kits to screen for drugs of abuse. More advanced technology, such as the use of ion mobility spectrometry (IMS) can be coupled to portable detectors for both screening and confirmatory results.¹⁵⁻¹⁷ In addition to these methods, biological detectors, such as canines are useful for rapid screening and are capable of detecting narcotics, even before the suspicious powders are visibly present.¹⁸

2.2.1. Non-Biological Detectors

2.2.1.1. Color/Spot Tests

One of the most common field tests for the detection of illicit substances is using color, or spot tests. Color tests are presumptive tests, used primarily for screening purposes. Despite the lack of confirmatory information, these tests serve as a means to give probable cause to law enforcement officials in order to make arrests or issue search warrants.^{15,16} Color tests are commonly used because they are portable, require minimal materials, and produce rapid results. Most commonly, color tests employ a spot plate, in which samples are placed in the depression or well of the plate. Once the sample is added to the plate, specific reagents are added and, depending on the reagent used and the presence of a particular drug, the drug solution will turn a specific color. Color tests can also be completed in open test tubes, bags, or on filter paper or test strips. The common field color tests, along with the reagents used, color changes, and substances identified can be seen in Table 2.^{15,16,19} Despite the many advantages of using these tests, there is some dispute over the reliability of these tests. First, color tests are destructive; therefore if a trace amount of sample is found, the whole sample must be sent to the lab for confirmatory results, and therefore forego presumptive screening.^{15,16} Second, these tests are considered subjective because of potential color biases. While a positive result should only be called if the color change experienced matches the color key chart provided, results are at times debated because of personal interpretations, especially in the case of color blindness. Additionally, the supposed color indicated by the test kit is, at times, not precise, especially when the controlled substances are mixed with other

materials.¹⁶ Finally, the appropriate sample size must be placed in the spot place, as a variation in this amount may cause false positives or false negatives. An example of these disadvantages was observed by Tsumura et al. in the assessment of the Scott Test, a common color test for cocaine detection. Tsumura et al. determined that testing more cocaine than the recommended amount showed persisting precipitates after the addition of the second reagent, which indicated a false negative. When too much heroin was placed in the spot plate, the color change indicated a false positive for cocaine.^{16,20}

Table 2: Color Tests, including reagents, color changes, and substance identification^{15,16,19}

Test	Reagents	Color Change	Substance Detected
Marquis	9:1 Sulfuric Acid and 37% Formaldehyde	Purple –Violet Violet to reddish purple Black Orange	Opium Morphine, Codeine, Heroin MDMA Amphetamine or Methamphetamine
Ferric Sulfate	Ferric Sulfate Solution	Brownish Purple Red	Opium Morphine
Fast Blue B Salt	(1) Fast Blue Salt in Sodium Sulfate (2) Chloroform (3) Sodium Hydroxide Solution	Purple-Red	Cannabis
Duquenois-Levine	(1) Vanillin in Ethanol (2) Acetaldehyde (3) HCL (4) Chloroform	Violet	Cannabis
Cobalt Thiocyanate	(1) 16% Hydrochloric Acid (HCl)(2) Cobalt (II) thiocyanate	Blue	Cocaine
Scott	(1) Cobalt (II) thiocyanate (2) HCl (3) Chloroform	Blue reappearance	Cocaine
Simon	(1) 10% Acetaldehyde (2) 10% Sodium nitroprusside	Blue	Methamphetamine

2.2.1.2. Immunoassays

An alternate method for drug screening is through the use of immunoassays. Immunoassays employ antibodies, which are proteins that recognize, bind to, and elicit defenses against foreign objects. Antibodies bind to specific antigens with varying specificity analogous to a lock and key, where the antibody is the lock and the antigen is the key. As mentioned above, immunoassays are mostly used when detecting drugs from various biological specimens, such as urine and saliva, but they can be used for the

screening of suspicious solid powders. One such example is the Cozart solids test, which has been used to detect amphetamines, cannabis, cocaine, heroin, and methamphetamines.¹⁷ Such tests are considered lateral flow immunoassays (LFIA), utilizing chromatography to complete detection (Figure 5). The Cozart solids kit, resembling a pregnancy test, comes with a collection swab to collect the sample. Following collection, the swab is placed in a solution bottle containing a solvent or buffer, where the analyte is completely dissolved. The solution bottle is mixed and the sample is applied to a portable test cartridge containing chromatography paper. The sample migrates along the chromatography paper to a conjugate pad, where a particulate conjugate has been immobilized. The particulate conjugate can be a colored or fluorescently labeled antigen, for example, of biological specificity to the assay. Next, the analyte and conjugate particle migrate to the reaction matrix, where they encounter the other biological component of the assay, the antibody, for example. The antibodies are immobilized on two lines, an analysis line and a control line. If there are no drugs present, the antigen particulates will bind to the antibodies on both the analysis and control line, showing two lines to appear in the analysis window. If drugs are present, the drugs will bind to the antibodies on the analysis line, blocking the binding of the antigen particle. However, the particulates will still bind to the control line, causing only one line to appear in the analysis window. It is important to note that for these tests, the location of the antibody and antigen can be alternated, depending on the format of the assay used.^{15,21} These types of immunoassays are very sensitive, able to detect the presence of drugs at parts per million levels, rapid, accurate, non-hazardous, and easy to use.¹⁷ While more sensitive than color/spot test, these tests are more costly in comparison, and are

therefore used less often for routine screening. In addition, strongly acidic and basic materials inhibit antibody binding, yielding false positive results.¹⁵

Figure 5: Lateral flow immunoassay, where the left image shows when drugs are present and the right shows the absence of drugs. In the presence of drugs, the drug and antigens will migrate along the test cartridge, where the drugs will bind to the immobilized antibodies, taking the place of antigens. When drugs are not present, antigens will bind to all antibodies.^{15,21}

2.2.1.3. Ion Mobility Spectrometry

Ion Mobility Spectrometry (IMS) refers to an analytical instrument used for characterizing substances based on the speed of gaseous ions derived from the analyte in an electric field through a supporting gas atmosphere.²² Ion mobility spectrometry involves the introduction of a sample, usually through thermal desorption, into the ion source of an atmospheric gas ionizer, typically a radio nuclei isotope. Once the sample is ionized, ions are pushed through a drift region where the time it takes to reach the detector is measured. Contrary to typical mass spectrometry, which detects analytes according to their mass to charge ratios, IMS utilizes the mobility of the ions for

identification, which is directly related to the size and shape of the ion. The IMS technology offers many advantages over traditional mass spectrometry. These advantages include the selectivity of IMS based on the ionization and mobility of ions, or drift time; sensitivity that rivals traditional mass spectrometry; and the ability to operate without a vacuum, allowing for the analysis to be done using a small, inexpensive, handheld device at ambient conditions.¹⁵ Ion mobility spectrometry has proven to be useful for on-site drug detection and has been implemented in major international airports as well as military and law enforcement agencies. Samples can be easily collected through swabbing many surface, including luggage, electronics, skin, and clothes, allowing for the detection of illicit substances from many surfaces as well as human sweat.²³ The instrumentation has been successful with the detection of cocaine in sweat and in its powder form, and has shown to be a rapid and non-evasive technique with minimal sample preparation.^{24,25} One major disadvantage of this technique, however, is that IMS is extremely sensitive to contamination, causing interferences for the detection of target analytes.²⁶

2.2.2. Biological Detectors

A biological detector, by definition, positively identifies a substance through living processes.²⁷ To date, numerous biological detectors have been used for forensic and medical applications. For example, insects such as flies, parasitic wasps, and honey bees were initially shown to possess a highly sensitive and accurate olfactory system, and as a result, some have been used for the detection of explosives and toxins.^{28,29} Pigs and rats have also been shown to positively detect land mines.³⁰ While options exists, the

use of canines (*Canis familiaris*) has become highly popular because they represent one of the most reliable, versatile, rapid, and cost effective real-time detection devices for many substances, including explosives, drugs, arson products, and human remains detection.^{18,31-33}

2.2.2.1. Canines

The domestication of canines, and therefore their use for providing protection and assistance for humans, started as long as 100,000 years ago.³⁴ There has been evidence of canines used as tracking dogs by ancient Egyptians (496-406 B.C.) and as guard dogs and hunting dogs by the Romans (A.D. 23-79). Throughout the Middle Ages (15th century), canines were used to track and trail thieves, which may be the first time that canines were used for forensic/legal purposes. Tracking by law enforcement agencies for both criminals and missing persons continued to be used through the centuries, but it was not until the 1890s when canines were trained to accompany officers during patrols. These canines were trained to bark when observing suspicious behavior, find his master through aid of scent, and search for objects either belonging to the criminal, or those stolen by the criminal. Realizing the immense value of these canines in the field, they became permanent additions to the police force in the 1930s across Europe. In the early 1900s, the United States began training dogs for routine patrols; however, due to lack of funding, canines, and trainers, the program was abandoned. Following the Korean War, canine training in the U.S began to rise again, with the first effective canine unit developed in Baltimore, MD in 1957.³⁴⁻³⁶

It was not until the latter part of the 1960s that the former U.S. Customs Service carried out studies to determine the feasibility of using detection canines for the purposes of drug detection. Canine trainers from the U.S. military were recruited and on April 1, 1970, the U.S Customs narcotic detector dog training program was established at the Lackland Air Force Base in San Antonio, Texas. The initial success of the canine's abilities to detect marijuana led to the training of additional popular narcotics, including heroin and cocaine. In 1974, the training operation was relocated to its current location in Front Royal, Virginia, where it was renovated to include administrative offices, classrooms, and kennels. As a result of funding granted by Congress to the facility in the 1990s, the training facility was expanded, leading to the production of canines trained in narcotics, currency, firearms, and explosives. The success of training canines for the detection of illicit substances has led to their use amongst law enforcement agencies throughout the world.³⁷

Canines have become popular for their use in the detection of illicit material because of their impressive olfactory capabilities, demonstrating higher sensitivities to odors than humans. Both canines and humans have receptor areas for scent, as well as olfactory nerves, which carry signals to the brain where odors are recognized. Nearly one eighth of a canine's brain and over 50% of the internal nasal area is committed to olfaction, while humans have much smaller olfactory lobes in the brain and only contain one square inch of olfactory cells. For humans, the number of olfactory sensory cells within the nose is said to be around 5 million, while German Shepherds, for example, have 220 million, indicating that a canine's olfaction abilities are at least 44 times greater than a human's, while some studies have shown canines' abilities to be 100 fold.³⁸

Canines also contain approximately 1300 genes in their olfactory repertoire, nearly 20 times more than humans.³⁹ This increase in sensory cells and genes allows for canines to be trained on many diverse odors with enhanced sensitivity compared to humans. Additionally, pigmentation in the olfactory areas is said to be associated with the ability to smell. Macrosomatic animals (animals with a high sense of smell), such as dogs, have a richer brown pigmentation in their olfactory areas while microsomatic animals (animals with a low olfaction function), such as humans have a yellowish pigment, further explaining canines' increased odor sensitivity.^{38,39}

Canine detection and the theory of olfactory activation is a multistep process, very similar to human olfaction. When searching, a canine inhales, or sniffs, a sharp breath at a frequency of 5-8 Hz, equivalent to 300-480 breaths per minute. The sniffing leads to differences in air pressure in the nose and allows odorant molecules to enter deeply into the nasal cavity.^{34,40,41} To enter the nasal cavity, odorants must pass the external nares of vertebrates where humidification occurs. The odorants then pass through the turbines, or cartilaginous flaps, that serve to increase the surface area of the epithelium. Within the nose there are two types of epithelium, the respiratory epithelium and the olfactory epithelium. The respiratory epithelium has small hairs, or cilia, and is coated with mucus. It functions as a means to clean incoming air and warm and moisten it to body temperature, helping to continue the breathing cycle. The olfactory epithelium, on the other hand, is located deeper inside the nose and is covered with cilia, mucous, and continuously regenerative neurons. Odorants bind to odor-transport proteins, which transport the molecules through the mucous and cilia, to the neuron receptors.^{34,41,42} The interaction between the odorant and the neuron receptor activates a G protein, which in

turn activates adenine cylase, converting adenine triphosphate (ATP) to cyclic adenine monophosphate (cAMP). Cyclic adenine monophosphate binds to a cyclic nucleotide-gates ion channel, which enables it to conduct cations. The influx of cations increases the membrane potential, generating an action potential that propagates the olfactory sensory neuron along the axon, through the cribiform gate, to the glomerulus and mitral cells, and finally travels along the olfactory tract to the brain where the odor is interpreted (Figure 6).^{41,43-45}

Figure 6: Canine olfaction: When odorants pass through the nasal cavity, they bind to odor transport proteins and are transported to a neuron receptor. This activates a G protein, which in turn activates adenine cylase, converting ATP to cAMP, which binds to a cyclic nucleotide-gate ion channel. An influx of cations is created which generates an active potential that propagates the olfactory sensory neuron to the brain.^{41,43-45}

2.2.2.2. Canine Training

When considering and selecting a canine for a job as a biological detector, there are many reactions to look for. It is very important that a canine detector portrays a strong “drive” - without drive, the canine will have no motivation to perform the daily detection work. Three types of drive are *hunt*, *retrieval*, and *play* behavioral

characteristics. The *hunt* drive is the dog's persistent desire to find something, despite fatigue or other difficulties. The *retrieval* drive is the dog's sense of will and loyalty to find a specific substance and bring his findings to his master. Finally, the *play* drive is the desire for fun and positive reinforcement, used as a reward or motivator for the canine to work and succeed. Additionally, a detection canine should pay no heed to environmental changes-such as changes in temperature and precipitation and variations in floor surfaces, and room lighting- and carry on with a task, even when distractions or problems arise.^{46,47}

Once a canine is chosen to be trained as a biological detector, the canine must be imprinted with the target odor. Through this process, the canine draws a relationship between the target substance and his reward, whether it be verbal reinforcements or the use of a toy. In other words, if an alert from a canine, usually represented by a canine sitting in front of the substance, is a correct response, the canine will be rewarded. Following the imprinting process, canines must continuously train to ensure the satisfactory level of detection. The Scientific Working Group on Dog and Orthogonal Detector Guidelines (SWGDOG), has been developed to provide a consensus for canine training guidelines to ensure the most consistent and reliable method throughout the canine and forensic community. According to SWGDOG, the training canines must be exposed to a variety of environments, locations, and material weights in order to ensure reliable detection in all field scenarios. SWGDOG has also implemented a canine certification program, which tests the canine's ability to detect all odors trained upon in various scenarios resembling normal operational searches. The certification of a canine

provides evidence as to the specific canine's detection capabilities on the same scale as all other detection canines across the country, improving their strength in court.^{47,48}

As previously mentioned, canines have the ability to be trained on numerous odors, but studies have shown that canines typically respond to the most volatile compounds, or volatile organic compounds (VOCs) contained within the headspace of the material. This fact has led researchers to determine a dominant signature odor or active odor for various narcotics and explosives in which will elicit a positive response from a detection canine.^{18,49} For example, studies show that the dominant signature odor of cocaine is methyl benzoate, meaning that the canine alerts, specifically, to methyl benzoate and not cocaine itself.⁵⁰ This advancement in canine research and biological detection theory has led to the creation of canine training kits, which are currently commercially available through Florida International University, Sigma Aldrich, and ScentLogixs.⁵¹⁻⁵³ By definition, training aids are any kind of training material used as a positive control for detection canine training and maintenance. Ideally, a training aid should be effective, harmless to canines, have high longevity, detectable at low levels, and easily obtainable. There are three types of training aids most often referenced and used by canine trainers and handlers. The first type uses the actual illicit material in the training aid. While often viewed as the most efficient method for training, this type of aid poses a risk to both canines and their handlers and typically requires a license to train with the actual illicit substance. The second type of training aid uses diluted illicit material. Because of the use of solvents for dilution, this training method is more expensive. Additionally, its effectiveness, especially in comparison to using the actual illicit material, is questionable. The last type of training aid is the mimic, which serves to

“mimic” the odor of the illicit substance by substituting the controlled substance with the dominant signature odor of the compound. Mimics are advantageous in the sense that they are nonhazardous, easy to obtain, and effective in the field.^{52,54,55}

Mimics, which are the main focus of the present study, have been produced using a variety of different methods, but one such example, Controlled Odor Mimic Permeation System (COMPS) (Figure 7), has proven to be effective; safe; easy to make and maintain; and provides a reliable, measurable, and controllable delivery system of the target odor.

Figure 7: COMPS layout, which utilizes a permeable polymer container to deliver a target odor. The permeable containers are packaged inside an impermeable aluminum bag

The COMPS devices use a permeable polymer container, such as a polyethylene bag, for the delivery of the target odor. The dominant signature odor of a specific illicit substance is coated on a vehicle, such as cellulose. The odor and vehicle mixture is placed and sealed in the polymer bag, which provides consistent permeation of the odor. Odor permeation can be controlled and manipulated by changing the polymer type and bag

thickness, as well as the surface area of the bag used. Polymer bags are then packaged in impermeable aluminum bags, which not only allows for minimal odor loss when shipping and packaging to canine facilities, but also allows for the volatile component in the inner container to reach equilibrium between the space between the inner and outer container. As a result, once the COMPS is received and the inner, polymer container is removed from the outer aluminum container, the training aid is ready for use, permeating at a constant rate.^{56,57} While COMPS have proven to be effective in the field for a variety of narcotics and explosives, they do suffer from a few drawbacks. For one, the dissipation rate from the polymer bags is determined by polymer bag availability. Therefore, a specific limit of longevity is reached, and in most cases, cannot be improved upon. Since some VOCs dissipate faster than others, this is especially problematic for the shelf-life of specific training aids. Further discussion of COMPS, as well as improving upon this concept, will be explored in the dissertation.

2.2.2.3. Canine Controversy

While detection canines have proved their worth and success over the years, their reliability is continuously scrutinized. Some claims against canines and their handlers are that handlers were influencing their canines, causing canines to falsely alert to specific locations. Additionally, variations in canine training, testing, and searching raised much doubt in the reliability of canines in the field.^{58,59} The formation of SWGDOG sought to improve on these problems by helping to standardize canine training and testing across the law enforcement and canine community, in addition to implementation guidelines for canine certifications.³¹ In spite of demonstrated successes, claims surrounding a canine's

accuracy have been recently brought forward, specifically whether a detection canine would false alert to substances with similar odor profiles. It has been noted that many household items contain some of the active signature odors of illicit substances. This specific controversial claim was referenced in the Supreme Court case, *State of Florida v. Joelis Jardines*.⁶⁰

2.2.2.3.1. State of Florida v. Joelis Jardines

On November 3, 2006, Detective Pedraja of the Miami-Dade Police Department received an anonymous, unverified “crime stoppers” tip that marijuana was being grown at the residence of Joelis Jardines. One month later, the Miami-Dade Police Department, Narcotics Bureau and agents of the Drug Enforcement Administration (DEA) conducted a surveillance of the home. After a 15 minute surveillance period, in which it was determined that there was no observable activity at the residence, Detective Bartlet arrived with his drug detector canine, Franky, and approached the residence. Franky began “wildly” tracking an airborne odor, and alerted, by assuming the sitting position, once sniffing the base of the front door. Detective Pedraja also claimed he smelled marijuana at the doorway. As a result of the canine alert, a search warrant was obtained. Upon searching the residence of Jardines, 179 marijuana plants were found. Consequentially, Jardines was arrested and charged with trafficking in marijuana.^{61,62}

Following the arrest, Jardines filed a motion to suppress the evidence seized from his residence claiming that law enforcement’s use of a detection dog at his front door was an illegal search. This argument led to the Supreme Court case in 2013, *State of Florida v. Joelis Jardines*. Specifically, this case debated whether a warrantless “sniff” from a

detection canine performed at the doorstep of a private residence violated the Fourth Amendment of the United States Constitution, which prohibits unlawful search and seizure.^{60,62} During this case, an *amici curiae* wrote a Brief in favor of the respondent's position, believing the scenario leading to Jardines's arrest was, in fact, unconstitutional, providing scientific evidence to support their view. The Brief stated that since canines have been scientifically proven to alert to the active signature odor of the contraband and not the contraband itself, canine selectivity and accuracy is called into question since certain active odors are sometimes found in common household items. Specifically, methyl benzoate, the active odor of cocaine has been recently found to be the most abundant odor produced by snapdragon flowers (*Antirrhinums*), a popular landscaping flower. The Brief claimed that the amount of methyl benzoate emitted by the flower in an hour period was well above the drug-detection dog's threshold, therefore concluding that substances containing a substantial source of methyl benzoate, such as the snapdragon flower, will trigger a positive response from the detection canine. In this scenario, there is no way for the handler to know whether his canine alerts to a front door because they smell an illicit substance or the active odor from a flower arrangement.^{60,63}

On May 26, 2013, the Supreme Court arrived at their decision, affirming the suppression of evidence, claiming that an unwarranted canine sniff in front of a private residence is unconstitutional and against the Fourth Amendment.³ The decision and the Brief written in defense of the respondent position discredited the use of canines as a method for detection, specifically for their accuracy in the field. Although extensive research has been done on the odors produced from snapdragon flowers, there is no current literature on whether those flowers producing methyl benzoate would elicit a

response from a detector canine.^{63,64} The research described herein seeks to answer this question as well as determine a canine's discrimination power when presented with similar pools of odor.

2.2.2.3.2. Snapdragon Flowers

Snapdragon flowers (Figure 8), belonging to the genus *Antirrhinum* and family Scrophulariaceae, owe their name to their petals resembling that of a dragon's mouth. They are cool-weather plants, native to southern Europe, that have recently begun to spread in popularity in the United States for decorative and landscaping purposes. Snapdragons, ranging from 10-120 cm in height, are classified on the basis of their total growth in height- tall (1 m-1.2 m), medium (.6 m- 1 m), dwarf (~ 0.3 m), and carpet type (.1 m - .15 m).⁶⁵

Figure 8: Snapdragon flower (*Antirrhinum*)

Most flowers, including the snapdragon flower, use visual and tactile cues in addition to a unique floral scent, to achieve reproductive success by attracting pollinators.^{64,66,67} The scent produced is mainly determined by the type of pollinator they wish to attract, such as bees, beetles, and flies, as these pollinators are capable of distinguishing between complex odor mixtures, allowing for enhanced pollinator specificity.^{63,64,67,68} For example, snapdragon flowers emit a sweet smelling scent to attract bees, their major pollinator. Fragrances produced by all flowers consist of volatile organic compounds (VOCs) that are species specific in terms of the compounds, as well as the relative abundances of the compounds produced.^{63,64} The composition of the flowers' odor profile as well as the quantity of fragrance changes throughout the plants' life cycle. Newly opened flowers have under developed anthers and therefore produce less scent, as they are not quite ready for pollination. Odor production reaches its maximal emission when the flower is ready for pollination. Once the flower has been pollinated, the scent emitted decreases, and continues to decrease until the end of the flower's life cycle.^{63,64,69} It has also been cited that after pollination, not only does odor emission decrease, but the compounds emitted are altered in order to deter pollinators from coming back to the same, already pollinated flower.^{70,71}

In 2000, Dudareva et al. conducted research monitoring the production of methyl benzoate from snapdragon flowers. It was these studies that were specifically cited during the Supreme Court Case, *State of Florida v. Joelis Jardines*.^{60,63} Volatile organic compounds were extracted and analyzed from 37 different *Antirrhinum* cultivars using headspace gas chromatography- mass spectrometry (HS/GC-MS) and results showed that the Maryland True Pink cultivar emitted the most methyl benzoate, making up 60% of

the total volatiles. It was also found that unopened buds release no methyl benzoate, and only begin to emit once the flower opens (anthesis), albeit at very low levels. The production of methyl benzoate reaches its peak 5-6 days after anthesis, and then declines thereafter.⁶³ Additionally, methyl benzoate production was highest between the daylight hours between 9 am and 4 pm, which coincides with the high activity of pollinators to ensure the highest chance of successful reproduction.^{63,64}

2.3. Odor Encapsulation

A new type of training aid will be developed that utilizes odor encapsulation. The method of odor encapsulation is very similar to that of molecular imprinting (MIP), in which a polymer is prepared in the presence of a “guest” or “print” molecule such that specific chemical interactions occur between the functional monomers (building blocks of the polymer) and the print molecule, creating a polymer that is highly selective to the initial print molecule.^{72,73} The first approach to molecularly imprinted silica-based materials began in 1949 by Frank H. Dickey, who demonstrated that after preparing a silica gel in the presence of a methanol orange dye, he found that the imprinted gel absorbed the orange dye more efficiently than a non-imprinted silica gel.^{74,75} While Dickey’s study was successful, the use of molecularly imprinting silica polymers did not become popular until the 1970s, and has now become quite useful in the production of adsorbents, extraction materials, and biosensors, to name a few^{76,77} For MIP, the print molecule, or template, is mixed with polymer precursors in a suitable solvent. The monomers are chosen on the basis of their ability to form specific and definable interactions with the template. During the process of “pre-arrangement” or “self-

assembly” the polymer precursors assemble around the template forming either covalent or non-covalent bonds with the template molecule. Following “pre-arrangement”, a polymerization reaction occurs, in which the polymer solidifies around the template, trapping the template inside of the polymer and forming “imprints,” which are complementary in both shape and chemical functionality to the template. The print molecule is then removed through solvent extraction, leaving behind recognition sites which possess an affinity for the template molecule, for purposes of re-uptake and extraction.⁷⁸ For the process of odor encapsulation, which has been utilized for this research, the same process for MIP has been employed, except that the template, or odor molecule in this case, is not washed out, and is instead left bound in the polymer where the odor can slowly dissipate over time (Figure 9).⁷⁹

Figure 9: Process of odor encapsulation: The target molecule meant for encapsulation is mixed with polymer precursors. During self-assembly the polymer precursors assemble around the target molecule, forming covalent and noncovalent bonds. Polymerization occurs, in which the polymer solidifies around the template, trapping the template within the polymer⁷⁹

Both MIP and encapsulation require a suitable matrix. The ideal matrix has to be able to physically entrap and stabilize the template molecule without altering it through the entrapment process or from outside environmental conditions.⁸⁰⁻⁸² Both MIP and encapsulation has utilized polymeric matrices to suit the needs of the specific applications. The polymer chosen for MIP and encapsulation is usually dependent on the backbone of the polymer matrix. Organic polymers, such as acrylics, form a densely packed network structure, making their structure and porosity hard to manipulate, and therefore difficult to optimize for a variety of applications. More commonly, inorganic polymers, such as sol-gel polymers, have been utilized for both imprinting and encapsulation because they are more thermally stable and easier to prepare than organic polymers. In addition, a sol-gel polymer's thickness, porosity, and surface area can easily be manipulated, increasing its versatility for a variety of applications.^{75,83}

Sol-gel encapsulation, or encapsulation within a sol-gel polymer, has recently been used for the entrapment of proteins and biosensors successfully.^{81,82} Sol-gel polymers have become popular for encapsulation not only because of thermal stability and pore size distribution and manipulation, but also because of their variety of forms that are producible, such as glass, fibers, films, and powders. The ability to make these polymers at room temperature is ideal, most especially for applications involving the encapsulation of proteins and odors. Proteins, specifically, are unstable at high temperatures and odors experience an increased dissipation rate at high temperatures risking complete loss of the analyte. The porosity of the sol-gel polymer is ideal because it allows for large molecules, such as proteins, to be trapped, while small molecules, such as odorants, are able to diffuse out of the matrix, a characteristic that is beneficial for the

creation of a canine training aid. Inorganic polymers suffer from a rigid and brittle texture, causing network cracking and therefore a less rugged product. In addition, when making inorganic polymers, there are limited chemical interactions between the guest molecule and the polymer network, decreasing the staying power of the template within the network. A sol-gel polymer can be optimized to solve these problems as a highly durable and rugged polymer can be formed with precursors containing organic substituents for increased chemical binding.^{81,82,84-86} The process of the sol-gel polymerization, which was used in this research, as well as its creation and optimization will be explored in the next section.

2.4. Sol Gel Polymers

The interest of sol-gel processing of inorganic materials began in the mid-1800s with Edelman and Graham's early investigations of silica gels. Early studies showed that the hydrolysis of tetraethyl orthosilicate (TEOS), $\text{Si}(\text{OC}_2\text{H}_5)_4$, under acidic conditions, yielded SiO_2 , a glass-like structure.^{87,88} Because of challenges with long drying times for this type of polymer production as well as their fragility, research surrounding sol-gel polymers went dormant from the late 1800s through the 1920s. It was not until the mid-1970s that sol-gel chemistry began to be studied extensively and has been found to be a successful method to form homogenous, high purity inorganic products finding use as various products including molded gels, thin films, coatings, and molecular cages, to name a few.^{82,83,89-92}

The sol-gel chemical process describes the evolution of a sol-solution into a gel. A sol is a colloidal dispersion of small particles in a liquid. A gel is a substance

composed of a continuous network characterized by a continuous liquid phase. The sol-gel process, therefore promotes the growth of colloidal particles into the formation of a gel-like network and is completed through a dual reaction process-hydrolysis followed by condensation. More specifically, sol-gel precursors, commonly metal alkoxides, are first mixed with a solvent. The solvent is needed before the addition of water because the sol-gel precursor is not miscible in water, and therefore is necessary to produce homogeneous polymers. Water is added, which is the driving force that promotes hydrolysis. Through the hydrolysis reaction, the -OR alkoxide substituents reacts with the water, under catalytic conditions, and produces hydroxyl groups in the place of the initial alkoxide substituents. Following hydrolysis, silanol groups undergo condensation with either another silanol group or an alkoxide, resulting in the formation of strong siloxane linkages with the production of water or an alcohol as a by-product (Figure 10). As Si-O-Si bridges form, siloxane particles aggregate into a sol. The gelation begins when the sol particles undergo sufficient condensation reactions, such that a gel-network forms, trapping the aqueous and alcohol by-products.^{87,89,93,94} The next step of the sol-gel process is aging, or syneresis, in which the sol-gel remains in its container for an extended period of time, usually hours to days, completely immersed in a siloxane solution. The purpose of the aging process is to allow for condensation to continue, increasing the stiffness and strength of the gel network.^{87,95,96} Following aging, the sol-gel polymer undergoes drying, usually utilizing heat or vacuum to remove the initially trapped by-products from the interconnected pore network. While the drying process causes stress to the gel and may cause it to crack, proper drying techniques can produce a sol-gel polymer with low density and high thermal insulation.^{87,89,97}

Figure 10: Sol-gel polymer dual-reaction process, hydrolysis followed by a condensation reaction⁸⁷

As previously mentioned, one of the major advantages of using sol-gel polymers is that their network can be easily modified. There are several factors that influence the sol-gel reaction and therefore modifies the network, consistency, and utility of the specific polymer. The following factors can be modified during the sol-gel process: sol gel precursor, water-to-alkoxide ratio, and catalyst chosen.^{89,93-95} The precursor chosen is, in most cases, a silicon alkoxide producing a strong three-dimensional inorganic network. The simplest silicon alkoxide, tetramethyl orthosilicate (TMOS) (Figure 11, left), bears the formula $\text{Si}(\text{OCH}_3)_4$ while the similar, TEOS (Figure 11, right), bears the formula $\text{Si}(\text{OC}_2\text{H}_5)_4$. The simple addition of extra carbon within the substituent chain greatly affects the network formed during sol-gel polymer solidification; TMOS therefore, forming a tighter network than TEOS. Polymers created with TMOS as a precursor also produce narrow, uniform pores with higher surface areas than TEOS.^{95,98}

The use of Organically Modified Silicates (ORMOSILS) in the sol-gel process involves the introduction of sol-gel precursors with inorganic silica backbones that are bonded to organic functional groups or a combination of an organic monomer with an inorganic monomer. The addition of the organic substituents not only produces a less rigid, bulkier sol-gel network, but can produce materials containing specifically designed chemical functionalities.^{89,94,95,99,100}

Figure 11: TMOS (A) and TEOS (B)

The amount of water added during the sol-gel process is an extremely important factor since water is required for hydrolysis to occur.⁸⁹ Generally speaking, as the ratio of water-to-alkoxide increases, so does the SiO₂ content. Therefore, to be sure that complete hydrolysis has occurred and a complete network has formed, there must be at least one mole of water for every alkoxide group. While often debated, some researchers have stated that if more than one mole of water per alkoxide group is added during sol-gel production, the reverse hydrolysis reaction- or re-esterification- will occur faster than the forward reaction. Contrarily, recent studies have shown that there is no correlation between the water:alkoxide ratio and the achievement of complete hydrolysis, especially

since water is a byproduct of the sol-gel reaction, and therefore self-propagates the reaction.^{89,94,95,101}

Since water is a byproduct of the hydrolysis reaction, determining the precise concentration of catalyst needed is a challenge. Studies have shown that while there is no correlation between the final sol-gel polymeric structure and the *concentration* of catalyst used, the *type* of catalyst has an effect on the properties of the final sol-gel product. During the sol-gel solidification process, it has been shown that using an acid catalyst increases the rate of the hydrolysis reaction but using a base catalyst increases the rate of condensation. While it is ideal to use a dual-catalyst system, in which both catalysts are used to speed up both reactions in a stepwise fashion, creating sol-gel polymers in strictly acidic conditions creates a linear network, with unreacted silanols within the network. Conversely, polymers created in a solely basic environment, yields a more dense material and a more stable network.^{89,95,101}

The optimization of the sol-gel process and therefore the creation of the ideal polymeric network was vital in the current research to ensure that a strong, stable network was created that would slowly dissipate the target odorant while still being reproducible and rugged for field use.

2.5. Instrumental Analysis

The extraction of volatile organic compounds from various specimens can be achieved through the use of solid-phase microextraction (SPME). Thereafter, these compounds can be separated and detected with gas chromatography- mass spectrometry (GC-MS).

2.5.1. Solid-Phase Microextraction

Solid-phase microextraction (SPME), first reported by Arthur and Pawliszyn in 1990, was developed as an extraction method to address the need for a simple, efficient, rapid, and inexpensive sample preparation technique that could be used in the laboratory or on-site.¹⁰²⁻¹⁰⁴ Solid-phase microextraction utilizes a fine, fused silica fiber, coated with a thin layer of selective coating or polymer. The coated fiber (SPME fiber) is mounted to a metal rod, both of which are protected by a metal sheath or needle that houses the fiber when not in use. The assembly is placed in a fiber holder, resembling a syringe-like apparatus (Figure 12).¹⁰⁵⁻¹⁰⁷

Figure 12: Solid-phase microextraction (SPME) fiber with holder¹⁰⁵⁻¹⁰⁷

The technology of SPME relies on two processes. First, analytes partition between the sample and the final coating, and second, the concentrated analytes are desorbed from the fiber coating, at high temperatures, into an analytical instrument.^{105,108} To perform sample extraction using SPME, the sample is placed in a vial and sealed with a cap and septum. The needle housing the SPME fiber pierces the septum and the plunger is lowered inside, immersing the SPME fiber directly into an aqueous solution or exposing it to the gaseous headspace of the sample. Target analytes are extracted based on their affinity to the polymeric fiber coating.¹⁰⁵ Once analytes are extracted, the SPME fiber can be removed from the vial and directly injected into a gas chromatograph (GC) where analytes are thermally desorbed from the fiber using high temperatures.^{105,108}

The chemical nature of the analytes extracted is determined by the SPME fiber coating used. Generally speaking, the rule “like dissolves like” can be applied, in which the fiber is chosen on the basis of its selectivity for certain target analytes. Selecting a fiber/coating is primarily determined by the polarity and volatility of the analytes of interest.^{105,107} The affinity for the fiber coating to the analyte is vital since both the sample matrix and the fiber are competing for the analytes; therefore, if the affinity for the fiber is higher than the matrix, the analyte will be extracted. In terms of the specific fiber coating, a fiber coated with polydimethylsiloxane (PDMS), a nonpolar coating, will extract nonpolar analytes, such as aromatics, most efficiently, while polyacrylate (PA), a polar coating, efficiently extracts polar analytes, such as phenols. Table 3 shows the common commercially available SPME fibers, as well as the polarity of each fiber.^{105,109} Coating thickness determines the sensitivity of the extraction method and extraction time. Thick coatings offer increased sensitivity, but much longer equilibration times.

Conversely, thinner coatings speed up the sampling process at the cost of the sensitivity, but are well suited for less volatile analytes.^{105,107}

Table 3: Commercially available SPME fibers and their polarities^{105,107}

Acronym	Name	Polarity
CAR/PDMS	Carboxen/Polydimethylsiloxane	Bi-polar
DVB/CAR/PDMS	Divinylbenzene/Carboxen/Polydimethoxysilane	Bi-polar
PA	Polyacrylate	Polar
PDMS	Polydimethylsiloxane	Nonpolar
PDMS/DVB	Polydimethylsiloxane/ divinylbenzene	Bi-polar
PEG	Polyethylene glycol	Polar

Solid-phase microextraction is an equilibrium technique, meaning that the extraction process is complete only when the analyte concentration has reached equilibrium with the fiber coating and the sample matrix. As a result, SPME is considered non-exhaustive, as analytes are not completely extracted from the matrix. When the sample is placed in a vial, equilibrium is achieved between the following phases: (1) the fiber coating and the matrix, (2) the headspace and the matrix, and (3) the fiber coating and the headspace. The analyte recovered is determined by the overall equilibrium of these three phases, seen in Equation 1, where C_0 is the initial concentration of the analyte in the matrix; C_h^∞ , C_s^∞ , and C_f^∞ are equilibrium concentrations of the

analyte in the headspace, sample, and fiber coating, respectively; and V_h , V_s , and V_f are the volumes of the headspace, sample, and fiber coating, respectively.

$$C_o V_s = C_h^\infty V_h + C_s^\infty V_s + C_f^\infty V_f$$

Equation 1

For direct liquid sampling, which is a type of SPME technique in which the matrix is an aqueous solution and the SPME fiber is directly immersed in said liquid, the amount, in moles (n_f), that is extracted by the fiber coating is described in Equation 2, where C_o is the initial concentration of the analyte in solution, V_f and V_s are the volumes of the fiber coating and aqueous solution, respectively, and K_{fs} is the partition coefficient describing the partitioning between the fiber and the aqueous phase.

$$n_f = \frac{K_{fs} V_f V_s C_o}{K_{fs} V_f + V_s}$$

Equation 2

In scenarios where the volume of the aqueous sample is much larger than the fiber coating, Equation 3 shows the amount extracted by the fiber coating. This modification is as a result of the fact that the sample volume (V_s) is relatively infinite, meaning that it does not need to be known.^{105,110}

$$n_f = K_{fs} V_f C_o$$

Equation 3

For SPME headspace sampling, the SPME fiber is not directly immersed in the sample, but instead exposed to the gas phase above the sample, where volatile organic compounds

(VOCs) can be extracted.¹⁰⁷ When performing headspace sampling, the denominator for Equation 2, which represents the analyte capacity for each phase, has the addition of the analyte capacity within the headspace ($K_{hs}V_h$), where K_{hs} represents the partition coefficient of an analyte between the headspace and matrix, and V_h represents the volume of the headspace (Equation 4). Equation 4 suggests that the analyte extracted by the fiber is independent of the fiber's location, whether the fiber is placed in the headspace or directly in the aqueous solution, as long as the volumes of the fiber coating, headspace, and matrix are kept constant.¹⁰⁵

$$n_f = \frac{K_{fs}V_fV_sC_o}{K_{fs}V_f + K_{hs}V_h + V_s}$$

Equation 4

Solid-phase microextraction has been used for a number of applications, especially those of forensic significance. For example, SPME has been successful with the detection of accelerants associated with fire debris, drugs from biological matrices, such as urine, and pesticides from food and drinks.¹¹¹⁻¹¹³ Additionally, SPME has been used to characterize odors produced from drugs, explosives, human remains, and for human scent analysis, which have aided in the development and improvement of canine detection training aids.^{18,32,44,114} SPME will be the primary sample preparation technique for this study.

2.5.2. Gas Chromatography-Mass Spectrometry

Gas Chromatography (GC), a method first introduced by Martin and Synge in 1942, is an analytical technique in which volatile analytes can be separated before detection. As shown in Figure 13, once the sample is injected through a heated injection port, the analytes quickly begin to evaporate. The vapors are swept through the column by a carrier gas (mobile phase), where they are separated based on their affinity for the stationary phase lined or packed within the column. The column is housed within a thermostated oven in order to provide sufficient vapor pressure for the analytes to elute from the column and into the detector.^{115,116}

Figure 13: Gas chromatography schematic¹¹⁵

For gas chromatography, the separation of complex mixtures depends on the affinity of the analyte for the stationary phase as the mobile phase moves the analytes through the column. The affinity of the analyte for the stationary phase governs the elution time for each compound. Carrier gases commonly used for GC are helium,

nitrogen, and hydrogen. Helium, however, is most commonly used because it is inert; compatible with most detectors; safe, as it does not form explosive mixtures as is the case with hydrogen; and achieves fast separations at a higher resolution than nitrogen. For most GC analyses, an open tubular column is used, in which the stationary phase is lined on the inner wall of the column. As opposed to packed columns, which are filled with fine solid particles coated with a stationary phase, open tubular columns offer higher resolution, shorter analysis time, and greater sensitivity, albeit a lower sample capacity. As with SPME, the choice of the stationary phase depends on the principle “like dissolves like,” as polar columns are best for polar analytes and nonpolar columns are best for nonpolar analytes. The retention time, or the time at which the analyte reaches the detector, is determined by the affinity of the analyte to the stationary phase. For example, when using a polar column, polar analytes will be strongly retained by the polar column and therefore elute, or reach the detector, after the more nonpolar analytes.^{115,116} Some common commercially available columns, with their stationary phases and polarities can be seen in Table 4.^{117,118} Once analytes elute from the column, they are swept into the detector where they qualitatively and quantitatively analyzed.¹¹⁶

Table 4: Commercially available GC columns and their polarities ^{117,118}

Name	Stationary Phase	Polarity
DB-1ms	100% Dimethoxypolysilane	Nonpolar
DB-5ms	(5% -Phenyl)-methylpolysiloxane	Nonpolar
DB-35ms	(35%-Phenyl)-methylpolysiloxane	Mid-polarity
DB-17ms	(50%-Phenyl)-methylpolysiloxane	Mid-polarity
VF-17ms	50% phenyl/50%dimethylpolysiloxane	Mid-polarity
DB-Wax	Polyethylene glycol	Polar
SolGel-Wax	Polyethylene Glycol in Sol Gel Matrix	Polar

Mass spectrometry (MS), a technique commonly used in conjunction with GC, is a detection method in which a sample's ions are identified on the basis of their mass to charge ratio (m/z). A mass spectrometer consists of the following components: an inlet system, ionization source, mass analyzer, and detector. The ionization source, mass analyzer, and detector are contained under vacuum conditions to prevent ion collision during detection. Following detection, the signal is processed and a readout is produced, showing a fragmentation pattern characteristic of a specific compound (Figure 14).^{116,119,120}

Figure 14: Components of mass spectrometry¹¹⁶

After separated analytes elute from the GC column, they enter an inlet system, which guides a small amount of sample into the ion source with minimal loss of vacuum. In the ionization source, the sample is converted into gaseous ions by bombarding the sample with electrons, photons, or ions or through thermal or electrical energy. The ion source produces charged, gaseous ions that enter the mass analyzer.^{116,119,120} The present study utilized an electron-impact (EI) ionization source, in which the sample is brought to a high temperature to produce a molecular vapor which is ionized by bombarding the molecules with a beam of energetic electrons.¹¹⁹ The mass analyzers, such as quadrupoles and ion traps, both used in this study, detect the ions based on their m/z . The mass to charge ratio is determined by measuring the resonant oscillations of the ions in electric or magnetic fields. Those ions that are not of the specific m/z ratio, set by either the user or determined by the type of analyzer used, are removed.^{121,122} Ions of the target range m/z reach the detector, containing a transducer, such as an electron multiplier, that

converts the beam of ions to an electrical signal, which is then processed and displayed into a compatible format; a total ion chromatogram (TIC) is produced from GC-MS, for example. The mass spectrum within the TIC displays a graph of the detector response, or signal intensity of each ion, versus the m/z for each peak, which can be used to identify the specific compound that eluted at that specific retention time. The identity of each compound is often identified through a library database, and then confirmed with reference standards.^{116,119,120,123}

Gas chromatography-mass spectrometry has been used for countless applications because of its high selectivity and sensitivity leading it to become the “gold standard” for forensic substance identification, especially for drug screening/forensic toxicology, fire debris analysis and human scent determination.¹²⁴⁻¹²⁶ Because of the popularity of this instrumentation in analytical laboratories, as well as its crucial role in forensic applications, GC-MS was used as the primary instrument for the present research.

2.6. Statistical Analysis

Most chromatographic and spectroscopic techniques yield data on many components of a single specimen. In this case, multivariate statistical analyses are used to provide a method in which all variables are considered simultaneously and compared as a whole. Principal Components Analysis (PCA) is an example of such a statistical tool. When statistically analyzing data containing one variable, a univariate statistical method is required. Box-and-whisker plots and predictive values are both examples of a univariate statistical tool. More specifically, box-and whisker plots are further classified as non-parametric methods. Non-parametric, or distribution-free methods are statistical

methods in which no assumptions about data shapes and distributions are made. Such methods are highly appealing because they utilize simple calculations and are therefore easy to use.¹²⁷ Another statistical tool during analytical analysis is using significance tests, in which two results can be compared in order to determine whether there is a significant difference between the two, or whether their difference can be accounted for by random variations.¹²⁷ PCA, box-and-whisker plots, predictive values, and significance tests have all been utilized in this research and will be further explained in the upcoming sections.

2.6.1. Principal Components Analysis

Principal components analysis (PCA) is a statistical technique that substantially reduces data when variables are correlated.¹²⁷ Principal components analysis was originally introduced independently by both Pearson in 1901 and Hotelling in 1933 as a way to describe the set of multivariate data in terms of uncorrelated variables.¹²⁸ To use PCA, principal components, or linear combinations of the original data values, are obtained such that the first principal component has the highest variation in the data set, while the second accounts for the second largest. Mathematically speaking, principle components are eigenvectors, which corresponds to an eigenvalue. Eigenvalues give a number which corresponds to the amount of variance within a data set, while eigenvectors show directionality of said eigenvalue. Eigenvectors are determined through eigenanalysis of the covariance matrix, or matrix obtained through a measure of the joint variance of two variables. Graphically, upon completion of PCA, a plot is obtained in which principal components are portrayed orthogonally, or at a right angle

from each other, and data are graphed in terms of the principal components. Through this visual representation, PCA reveals clusters of data points, showing data sets that are similar as being closer together and these data sets that are different as being further apart (Figure 15).^{127,129}

Figure 15: PCA plot showing data clusters

2.6.2. Box-And-Whisker Plot

Box-and-whisker plots were first introduced by the statistician, Tukey, as a means of rapidly visualizing groups of data.^{130,131} Box-plots have been described as a form of “exploratory data analysis” since they are used to observe the behavior of the data set as a whole, thereby uncovering interrelationships amongst multiple data sets.^{131,132} A box-and-whisker plot consists of a rectangle (the box) and two lines (whiskers) extending from opposite sides the box (Figure 16). The ends of the whiskers represent the range of

data, with each end portraying the lowest and highest value, respectively. The line inside the box running perpendicular to the whiskers represents the data's median, while the edges of the box, from which the whiskers protrude, represents the lower and upper quartiles.¹²⁷ More specifically, the lower quartile signifies that 25% of the data are less than this point and the upper quartile signifies that 25% of the data are greater than this point.¹³³ The overall importance of using a box-and-whisker plot is to show, at a glance, the spread and symmetry of data, revealing “typical” values of a data set.^{127,131}

Figure 16: Box-and-whisker plot¹²⁷

2.6.3. Predictive Values

To statistically analyze data gathered from canine trials, positive predictive value (PPV) and negative predictive value (NPV) can be used. The PPV gives the probability that a positive result is the correct answer or are “true positives.” In terms of canine alert rates, the PPV is the probability that when a canine alerts to a sample, the alert is correct. The NPV, alternatively, gives the probability that the negative results, or the lack of responses from a canine, are “true negatives,” and therefore does not contain the odor of interest.^{32,134} The calculations used to determine both statistical values, can be seen in Equations 5 and 6, in which PPV is calculated by the number of true positives divided by

the summation of the true and false positives, while NPV is calculated by the number of true negatives divided by the summation of true and false positives.¹³⁴ In both cases, the closer the results are to 100%, the more likely that the results obtained from canines are the true result.^{32,134}

$$PPV = \frac{\text{True Positive}}{(\text{True Positive} + \text{False Positive})} \quad NPV = \frac{\text{True Negative}}{(\text{True Negative} + \text{False Positive})}$$

Equations 5 and 6: Positive predictive value and negative predictive value

2.6.4. Significance Testing

As mentioned above, significance tests are statistical analyses in which two results can be compared to determine if they differ significantly. In other words, the test serves to conclude whether the difference in values can be accounted for by just random variation or due to a change in analytical technique. One such example is a t-test, which is mostly used when comparing two means from independent groups. In this research specifically, the t-test is used to compare parameters obtained during method optimization to determine if changing one parameter will have a significant change to the produced results. The t-test works under two assumptions, that the populations sampled are normally distributed and that the population variances are equal. The t-value can be calculated from Equation 6, where the numerator represents the mean difference between two groups, while the denominator represents the common population variance.^{127,135}

$$t = \frac{\bar{y}_1 - \bar{y}_2}{s \sqrt{1/n_1 + 1/n_s}}$$

Equation 6: Formula for t-test where s is the sample variance, n is the number of trials, and y is the sample average

Another significance test is analysis of variance (ANOVA) in which, contrary to t-test that can only statistically compare two values, more than two means are able to be compared at once. This type of method is used, for example, when comparing mean results of analyte concentrations obtained from using multiple methods, as is the case with this research. Therefore ANOVA is a powerful statistical tool used to test whether altering a controlled experimental factor leads to significant difference between the mean values obtained. Results can be obtained by using Equation 7 where the numerator represents the variation between trials and the denominator represents the variation amongst replicates.¹²⁷

$$\frac{\frac{n \sum (\bar{x}_1 - \bar{x}_2)^2}{h - 1}}{\frac{\sum_i \sum_j (x_{ij} - \bar{x})^2}{h(n - 1)}}$$

Equation 7: Formula for ANOVA, where n is the number of replicates within a trial, h is the number of trials, and x is the trial values

3. SIGNIFICANCE OF STUDY AND RESEARCH OBJECTIVES

Research surrounding detection canines has been ongoing for many years, but despite all the knowledge obtained through previous research, detection canines and their capabilities are still questioned in court. Thus, this research sought to develop a novel training aid that will improve currently existing, commercially available training aids. It was the goal of this study to develop new training aids that improve upon the existing aids by elongating its shelf-life and develop a way to manipulate the dissipation of the odorants within. Through this manipulation, the handlers and trainers could get a better sense of the capabilities of their specific canine. In addition, the accuracy of canines in the field was evaluated on the basis of their ability to differentiate between objects dissipating similar odors.

This dissertation presents the results of the laboratory and field work used to satisfy the above objectives. The tasks of this project can be seen below:

1. The creation and optimization of a novel, sol-gel polymer to be used as a canine training aid
 - a. Development of polymers through variations in molar ratios between precursor, solvent, water, and catalyst
 - b. Capabilities of odor encapsulation within sol-gel polymer network
 - c. Ruggedness of sol-gel polymer for effective field use
2. A comparison of the newly developed sol-gel training aids with the existing training aid, COMPS
 - a. Direct comparison between dissipation rate of sol-gel training aids and COMPS

- b. Sol-gel training aid dissipation manipulation based on COMPS technology
 - c. Contamination study between sol-gel training aids and COMPS
- 3. The determination of dissipation manipulation of sol-gel training aids through network variations
 - a. Determination of the effects of dissipation when varying specific ingredients of sol-gel polymer production
 - i. Precursor
 - ii. Type of solvent (i.e. polar or nonpolar)
 - iii. Water molarity
 - b. Scanning Electron Microscope (SEM) analysis of network changes and how they affect odor dissipation
- 4. The assessment of sol-gel training aid effectiveness through canine trials
- 5. An exploration of canine field accuracy to flowers producing methyl benzoate
 - a. Laboratory experiments of odors dissipating from freshly grown snapdragon flowers
 - i. Odors released immediately after cutting
 - ii. Persistence of odors over time, simulating flower bouquets
 - b. Field experiments of canine alert rates when presented to snapdragon flowers
 - i. Potted, growing flowers
 - ii. Cut flowers

4. MATERIALS AND METHODOLOGY

4.1. Task 1: Creation of a Novel, Rugged Sol-Gel Polymer

The purpose of this task was to create a novel, sol-gel polymer that can be used for the encapsulation of a signature odorant in order to create an efficient training aid for narcotics detection canines. Commercially available training aids, such as COMPS, are an effective and safe canine training aid option that utilizes a signature odor to train canines instead of the actual illicit substance. While proven successful in the field, COMPS may still be improved by better odor dissipation rate manipulation and increased shelf life for specific odorants, such as methyl benzoate, cocaine's signature odor. This task sought to produce a polymer that was effective, reproducible, and rugged in the field, with the hopes that it could improve upon training aid longevity.

4.1.1. Materials

4.1.1.1. Optimization of a Novel, Sol-Gel Recipe

An optimal sol-gel polymer recipe was created and optimized using the ingredients shown in Table 5 purchased from Gelest (Morrisville, PA), Fisher Scientific (Pittsburg, PA), or Sigma-Aldrich (St. Louis, MO). Deionized water (DI), required to initiate the hydrolysis reaction, was purified using a Nanopure Diamond Ultrapure water system from Barnstead (Dubuque, IA). All polymer ingredients were mixed using a Digital Vortex Mixer from Fisher Scientific (Pittsburg, PA). During the solidification process, polymers were placed in a desiccator containing 10-20 mesh Drierite with calcium sulfate and cobalt (II) chloride (Fisher Scientific, Pittsburg, PA).

Table 5: Sol-gel polymer ingredients for optimization, including compound, CAS #, batch/lot #, and purpose

Compound	CAS #	Batch/Lot #	Purpose
Tetramethyl orthosilicate (TMOS)	681-84-5	BCBC4877V	Precursor
Tetraethyl orthosilicate (TEOS)	78-10-4	MKBF6044V	Precursor
Methyltrimethoxysilane (MTMOS)	1185-55-3	BCBC3453V	Precursor
Phenethyltrimethoxysilane (PeTMS)	N/A	4D-4786	Precursor
HPLC Grade Methanol	67-56-1	136943	Solvent
HPLC Grade Ethanol	64-17-5	04314EH	Solvent
Ammonium Hydroxide (NH ₄ OH)	1336-21-6	SHED7421V	Catalyst
Sodium Hydroxide (NaOH)	1310-73-2	110M0066V	Catalyst
Hydrochloric Acid (HCl)	7647-01-0	092603	Catalyst
Trifluoroacetic Acid	76-05-1	SHED3981V	Catalyst
Methyl Benzoate	93-58-3	STED1140V	Template

4.1.1.2. Ruggedness of Sol-Gel Polymer

The ruggedness of the sol-gel polymers were tested to ensure field utility. In the case of canine training aids, polymers must be able to withstand shipping and handling without breaking or loss of product. In order to test the polymers' ability to be shipped without damage, polymers were shipped to three locations, New Orleans, LA; Baltimore, MD; and Old Bridge, NJ and then sent back to Miami, FL through the United States Postal Service. Sol-gel polymers were also exposed to extreme temperature conditions, using an Isotemp Digital and Analog Dry Bath Incubator set at 80°C (Fisher Scientific; Pittsburg, PA) for hot conditions and a Kenmore Freezer set at -4°C for cold conditions. Additionally, polymers were placed in dry ice (FIU, Miami, FL) to determine if it can withstand extreme low temperatures (-78.5°C) for shipping and odor preservation purposes. Polymers were also exposed to various solvents commonly encountered in the

field, including acetone (CAS # 67-64-1; Sigma-Aldrich, St. Louis, MO), methanol (CAS # 67-56-1; Fisher Scientific; Pittsburg, PA), commercially available rubbing alcohol, and water. After exposing polymers to each condition, polymers were weighed using a Mettler Toledo XPE 205 analytical balance (Columbus, OH).

4.1.2. Methodology

4.1.2.1. Optimization of a Novel Sol-Gel Recipe

An optimized sol gel polymer recipe was determined through a trial and error method, varying the molar ratios of the following sol-gel ingredients: precursors, solvents, catalysts, and water ratios (Table 5). The goal was to determine a recipe that would create a solidified polymer in a timely manner with complete and rugged sol-gel network formation, while still efficient enough for rapid mass production. Moreover, polymer solidification had to be reproducible, even under varying laboratory conditions, including changes in temperature and humidity often experienced. It was found, through extensive testing, that the best method for the creation of sol-gel polymers for this specific application was through the following procedure: Inside a 10 mL clear, screw top vial with PTFE/Silicone septa the precursors were first added, followed by the solvent, and thoroughly mixed using a Digital Vortex Mixer. Next, the purified DI water and acid catalyst was added and again, thoroughly mixed. Polymers were allowed to sit for 2 hours to ensure complete hydrolysis. After, the base catalysis, diluted in the initial solvent, was added, 10 μ L at a time, with mixing between each increment. Immediately following, the pre-polymer solution was placed in a desiccator containing 10-20 mesh Drierite with calcium sulfate and cobalt (II) chloride to dry out excess solvent as well as

to trap out environmental humidity, keeping consistent temperature conditions. The specific ingredients, including the chosen precursor, solvent, and catalysts will be discussed in section 5.1.1 of the results.

Once a reproducible polymer recipe was determined and optimized, the polymers' ability to solidify around the template, methyl benzoate, was sought, as well as the polymer's loading capacity. Methyl benzoate was added to the sol-gel solution post-hydrolysis, right before the addition of the base catalyst, in varying increments, starting with 1 mg (diluted in solvent) to 54 mg (50 μ L pure methyl benzoate). The polymer's ability to solidify was observed. The limit of the amount of methyl benzoate that could be added was determined by if the pre-polymer solution remained liquid after a 12 hour drying period.

The amount of methyl benzoate that was able to be trapped inside the sol-gel polymer was determined. Because methyl benzoate, as well as many other odorants, is extremely volatile, it was possible that much of the sample could be lost in the solidification process. While keeping the polymers shut inside a sealed desiccator sought to minimize sample loss, it was still inevitable. Therefore the amount of methyl benzoate successfully encapsulated in the sol-gel polymer was determined through weight. Nine - 10 mL screw top vial with PTFE/Silicone septa were obtained. Within three of the vials, imprinted sol gel polymers were made following the usual process, with the addition of the template, methyl benzoate. In another three vials, blank sol-gel polymers were made, replacing the volume of methyl benzoate with the addition of solvent, allowing for the sol-gel polymer to be made with the same volume, without the addition of a template. Following solidification, sol-gel polymers were weighed. The last three vials were filled

only with the amount of methyl benzoate used to make the imprinted polymers and weighed immediately. The average blank sol-gel polymer weights were subtracted from the average imprinted sol-gel polymer weights to determine the amount of template within the solidified polymer. The percent recovery of methyl benzoate was calculated by the following formula:

$$\left(\frac{(\text{aver. wt. of imprinted polymers} - \text{aver. wt. of blank polymers})}{\text{Aver. wt. of methyl benzoate only}} \right) \times 100$$

Because the amount of template added to the polymer was known, the percent recovery of methyl benzoate was used to determine the amount encapsulated within the polymer:

$$\begin{aligned} & \% \text{ recovery} \times \text{starting concentration of methyl benzoate} \\ & = \text{Total encapsulated template} \end{aligned}$$

4.1.2.2. Ruggedness of Sol-Gel Polymer

The ruggedness of the sol-gel polymer was tested to determine their use in the field. Specifically, to be used as a canine training aid, the imprinted sol-gel polymer must be able to withstand rough physical treatment, including handling during the shipping process, and refrain from cracking/shattering within the screw top vial. In the field, these training aids will remain within the 10 mL screw top vials. If the polymer shatters, pieces of the polymer could be lost upon opening the aid, or worse, be consumed by the training canine creating a choking hazard by ingesting small sharp objects. Therefore, it is

extremely important to ensure that the polymers were unbreakable. Blank polymers were created following the optimized recipes, weighed, and shipped to three locations, Old Bridge, New Jersey; Baltimore, Maryland; and New Orleans, Louisiana, through standard United States Postal Service Shipping from Miami Florida. The recipient opened the package, handled the vials, and then sent the polymers back to Miami, using the same standard shipping. Upon their return, polymers were visually observed and weighed to determine loss of sample. Triplicate samples were given to each recipient at the three locations.

The ability for sol-gel polymers to withstand extreme temperature conditions was important to assess because the training aids should be able to be used in various locations that experience extreme highs and lows in temperature. Therefore, blank sol-gel polymers were created, again following the optimized procedure, and weighed. Then three polymers were placed in a freezer (-4°C), three on a heater (80°C), and three placed inside a Styrofoam container containing dry ice (-78.5°C). Samples were left in their respected locations for 24 hours, followed by a 2 hour equilibration period, where samples could reach room temperature. Visual observations were recorded, as well as final weights after the experiment. The weights of the polymers were recorded daily for 5 additional days following the initial experimental treatment.

Sol-gel polymers were also exposed to common solvents frequently encountered in the field, including water, acetone, and rubbing alcohol. While water is obviously experienced during rainy environmental conditions, acetone and rubbing alcohol are often encountered when cleaning and decontaminating equipment used during canine training to ensure that target odors do not experience cross-contamination. Again, blank

sol-gel polymers were created and weighed. Within three polymer-containing vials, 1 mL of water was added. The same was repeated for acetone and rubbing alcohol. Solvents were exposed to the polymers for six hours, followed by removal of excess solvent, and a 12 hour drying period in the desiccator. Samples were weighed following this 12 hour period. For the rubbing alcohol containing polymer-vials, the solvents appeared to dissolve the polymer, and so the solvent was not emptied, and instead just placed into the desiccator for 12 hours prior to weighing. Visual observations were also recorded. The weights of the polymers were recorded recording daily for 5 additional days following their experimental treatment.

4.2. Task 2: Comparison of Sol-Gel Training Aids to IFRI COMPS

The purpose of this task was twofold. It first sought to directly compare the dissipation rates and the percent of odor loss over time of the COMPS and the newly developed, sol-gel polymer training aids. An additional objective of this task was to attempt to manipulate the dissipation rate of the sol-gel polymer training aids using similar methods that have proven successful for COMPS. The dissipation rate of odors from COMPS can be varied by changing the polyethylene bag that the COMPS are made in. Using different polymer bags to further control the dissipation from the polymers was attempted. The dissipation rates of COMPS also change in extreme temperatures and environmental conditions. Whether the sol-gel polymers will behave the same way when exposed to varying temperatures was determined, with the hopes of using this as an advantage.

4.2.1. Materials

4.2.1.1. Method Optimization

To determine which SPME fiber had the highest extraction efficiency for the template odorant, six SPME fibers, seen in Table 6, were purchased from Supelco (Bellefonte, PA). The template, methyl benzoate (CAS # 93-58-3; Sigma-Aldrich; St. Louis, MO), was contained within 10 mL clear, screw top vials with PTFE/Silicone septa (Supelco, Bellefonte, PA), and was extracted from the headspace.

Table 6: SPME fibers tested for method optimization

Acronym	Name	Size	Polarity
PA	Polyacrylate	85 μm	Polar
PEG	Polyethylene Glycol	60 μm	Polar
PDMS	Polydimethylsiloxane	7 μm	Nonpolar
PDMS	Polydimethylsiloxane	100 μm	Nonpolar
DVB/PDMS	Divinylbenzene/Polydimethylsiloxane	65 μm	Bi-polar
PDMS/CAR	Polydimethylsiloxane/Carboxen	75 μm	Bi-polar
DVB/CAR/PDMS	Divinylbenzene/Carboxen/Polydimethylsiloxane	50/30 μm	Bi-polar

A containment system that would equally fit both the sol-gel polymer training aid and the COMPS was determined. The following three containers were tested and compared: 16 oz wide mouth Ball mason jars (Daleville, IN), commercially available 1 qt metal paint cans, and clear glass jars (5.5 in high, 2.5 in diam.); see Figure 17. The tops of all three containment systems were punctured with one hole in the center and fitted with a septa/stopper to allow for SPME fiber exposure. Both the mason jars and paint cans were fitted with a 1.4 cm Suba-Seal rubber septa (Sigma-Aldrich, St. Louis,

MO), while the glass jars were fitted with an 11 mm sleeve stopper (Wheaton; Millville, NJ).

Figure 17: Containments systems tested: Mason jar (A), paint can (B), glass jar (C)

The abundances of methyl benzoate were detected and identified by GC-MS using a Varian 3800 Gas Chromatograph and a Saturn 2000 Ion Trap Mass Spectrometer (Walnut Creek, CA) fitted with a SGE SolGel-Wax capillary column of 30 m x 0.25 mm (i.d.) with a film thickness of 0.25 μ m (Fisher Scientific, Pittsburgh, PA). The concentrations of methyl benzoate were determined using an external calibration curve of the standard, diluted in methylene chloride (CAS # 75-09-2; Fisher Scientific, Pittsburg, PA), and injected into the GC-MS using a Combipal autosampler (CTC Analytics, Zwingen, Switzerland).

4.2.1.2. Dissipation Comparison between Sol-Gel Training Aids and COMPS

Sol-gel polymers were made in 10 mL clear, screw top vial with PTFE/Silicone septa (Supelco, Bellefonte, PA) using the optimized ingredients shown in Table 7, purchased from Gelest (Morrisville, PA), Fisher Scientific (Pittsburg, PA), or Sigma-Aldrich (St. Louis, MO). Deionized water (DI) was purified using a Nanopure Diamond

Ultrapure water system from Barnstead (Dubuque, IA). All polymer ingredients were mixed using a Digital Vortex Mixer from Fisher Scientific (Pittsburg, PA). During the solidification process, polymers were placed in a desiccator containing 10-20 mesh Drierite containing calcium sulfate and cobalt (II) chloride (Fisher Scientific, Pittsburg, PA).

Table 7: Sol-gel polymer ingredients, including compound, CAS #, batch/lot #, and purpose

Compound	CAS #	Batch/Lot #	Purpose
Phenethyltrimethoxysilane (PeTMS)	N/A	4D-4786	Precursor
HPLC Grade Methanol	67-56-1	136943	Solvent
Hydrochloric Acid (HCl)	7647-01-0	092603	Catalyst
Ammonium Hydroxide (NH ₄ OH)	1336-21-6	SHBD7421V	Catalyst
Methyl Benzoate	93-58-3	STBD114OV	Template

Controlled Odor Mimic Permeation Systems (COMPS) for cocaine were made by spiking methyl benzoate onto cellulose microcrystalline powder (CAS # 9004-34-6; Sigma-Aldrich, St. Louis, MO). Approximately 10 g of the spiked cellulose (54 mg methyl benzoate) was placed in 3 inch x 3 inch low-density polyethylene bags (4 mil thickness) and heat sealed using a Midwest Pacific MP-8 Impulse Heat Sealer (J.J. Elemer Co., St. Louis, MO).

Both the sol-gel training aids and the COMPS were contained in the clear glass jars (5.5 in high, 2.5 in diam.) fitted with an 11 mm sleeve stopper. Methyl benzoate was extracted overtime using the DVB/CAR/PDMS SPME fiber (Supelco, Bellefonte, PA). Duplicate trials were performed and results were statistically compared using a t-test

generated by Microsoft Excel 2010 (Microsoft, Redmond, WA). Throughout the course of this experiment, the temperature in the lab was monitored using Micro-t loggers (Nexsens Technology, Fairborn, OH).

4.2.1.3. Dissipation Manipulation of Sol-Gel Training Aids

Sol-gel polymers were created using the same materials from 4.2.1.2 and wrapped in 3 inch x 3 inch low-density polyethylene bags of various thicknesses (2 mil and 4 mil). When ready for testing, sol-gel polymers were contained in the clear glass jars, fitted with 11 mm sleeve stopper. Methyl benzoate was extracted overtime using the DVB/CAR/PDMS SPME fiber, followed by GC/MS analysis. The effects of temperature changes on the dissipation rates were done using an Isotemp Digital and Analog Dry Bath Incubator for heated temperatures. Additionally, the ability to halt dissipation for odor conservation during shipping purposes utilized exposure to Dry Ice (FIU, Miami, FL). Dissipation by weight was completed using a Mettler Toledo XPE 205 analytical balance. Again, Micro-t loggers monitored lab temperatures throughout this study.

4.2.1.4. Contamination Study for Sol-Gel Training Aid v. COMPS

To determine the risk of outside odor contamination on the sol-gel training aid compared to that of the COMPS, blank sol-gel training aids and COMPS were prepared using the same ingredients as stated in section 4.2.1.2, except without the addition of the template, methyl benzoate. Training aids were confined within a 10 gallon fish tank (20 inch x 10 inch x 12 inch). Methyl benzoate was contained in a 50 mL beaker (Fisher Scientific, Bellefonte, PA) and was used to “contaminate” the blank training aids. The

odor that contaminated the training aids was tested by placing the training aids in the clear glass jars fitted with the 11 mm sleeve stopper. The methyl benzoate was extracted overtime using the DVB/CAR/PDMS SPME fiber, followed by GC/MS analysis. Micro-
t loggers monitored lab temperatures throughout this study.

4.2.2. Methodology

4.2.2.1. Method Optimization

4.2.2.1.1. Volatile Extraction Optimization

To determine the most effective SPME fiber to extract the methyl benzoate from the training aids, 5 μ L (5.4 mg) of methyl benzoate was spiked into seven- 40 mL clear, screw top vials with PTFE/Silicone septa and allowed to equilibrate for 10 minutes. Following this equilibration period, headspace SPME was performed for 5 minutes using the SPME fibers from Table 6. Following initial testing and elimination of certain fibers, 100 μ m PDMS, 75 μ m CAR/PDMS, and 50/30 μ m DVB/CAR/PDMS were retested using smaller concentrations of methyl benzoate, simulating experimental conditions. For this experiment, 200 ng of methyl benzoate was placed in two, 10 mL screw top vials with PTFE/Silicone septa. Odors were allowed to accumulate in the headspace for 15 min. Once accumulated, a 15 min and a 30 min extraction was performed on each vial, respectively, with the three remaining SPME fibers. Samples were analyzed via GC-MS, using a 10 min desorption time. The present study was completed in triplicate. Concurrently, another study was completed to determine how long the fibers need to be desorbed in the GC inlet before they have completely expelled the extracted odor; the fiber that would require the least amount cleaning between uses

would be the most efficient for the study. Therefore, the same fiber used for the 30 min extraction above was analyzed again after its initial GC-MS analysis and desorbed for an additional 5 min. The GC-MS runs were continued with a 5 min desorption period until methyl benzoate was no longer detected.

Once the optimal SPME fiber was selected, a time study was completed to determine the point at which the SPME fiber reaches equilibrium with the sample. The time study was completed in the 10 mL screw top vials in order to have a consistent method for Tasks 2 and 3. For this experiment, 200 ng of methyl benzoate was placed in eight, 10 mL screw top vials with PTFE/Silicone septa. Odors were left to equilibrate within the vials for 24 hours. Following, each vial was designed a specific extraction time: 1 min, 15 min, 30 min, 45 min, 60 min, 2 hrs, 3 hrs, and 4 hrs. After each extraction period, samples were run in the GC-MS. The experiment was completed in triplicate.

Finally, the determination of the appropriate containment system was necessary. In order to appropriately compare the sol-gel polymers to the currently utilized COMPS, a contained system that could house both the COMPS and the sol-gel polymers was required. A total of 200 ng of methyl benzoate was added the three containment systems: a mason jar, a paint can, and a clear glass jar. Using the optimized method determined previously, odors were allowed to accumulate and were extracted using the chosen SPME fiber. The experiment was completed in triplicate and the relative standard deviation between the triplicates was determined and compared.

4.2.2.1.2. GC-MS Analysis

Once the methyl benzoate was extracted from the training aids using SPME, samples were analyzed using GC-MS. An optimized GC-MS method was developed using a Varian 3800 Gas Chromatograph coupled to a Saturn 2000 Ion Trap Mass Spectrometer. The GC was fitted with a SGE SolGel-Wax capillary column of 30 m x 0.25 mm (i.d.) and a film thickness of 0.25 μm . Helium was used as the carrier gas at a flow rate of 1.0 mL/min. The injection port was operated in split mode, 100:1, and maintained at a temperature of 250°C. The GC column initial temperature was set to 60°C. Upon initial sample injection, the GC column temperature was increased to 80°C at a rate of 10°C/min, followed by an increase to 150°C at a rate of 10°C/min, where it was held for 1 min, and a final increase to 260°C at a rate of 20°C/min, for a total runtime of 15.50 minutes. The mass spectrometer transfer line was maintained at a temperature of 250°C, the manifold at a temperature of 80°C, and the ion trap at a temperature of 150°C. The mass spectra were continuously scanned from 20 to 300 amu. Methyl benzoate was identified, confirmed, and quantifies using the Varian MS Workstation Software, Version 6.6 (Service Pack 1).

4.2.2.2. Dissipation Comparison between Sol-Gel Training Aids and COMPS

Twelve COMPS were created by placing 10 g of cellulose, spiked with methyl benzoate (approximately 15 mg), inside 3 in x 3 in low density polyethylene bags, 4 mil thick and heat sealed, following the standard operating procedures of Florida International University's International Forensic Science Institute.^{56,57} Previous research has shown that when optimizing COMPS with piperonal, the signature odor of 3,4-

methylenedioxy-N-methylamphetamine (MDMA), odor dissipates from COMPS at the slowest rate when 11 mg of odor was placed inside the polyethylene bags. Additionally, the thicker the bag, the slower the dissipation rate of the odor out of the bag (Figure 18).⁵⁶

Table 16 - Piperonal permeation rate summary						
COMPS	Permeation Rate (ng/sec)					
	10-20	20-50	50-100	100-150	150-200	200-250
11mg 3x3 2mil LDPE	X					
50mg 3x3 2mil LDPE		X				
100mg 3x3 2mil LDPE		X				
200mg 3x3 2mil LDPE			X			
500mg 3x3 2mil LDPE				X		
2000mg 3x3 1.5mil LDPE						X

Figure 18: Previous work on the dissipation of piperonal from COMPS by Macias, M. (2009) ⁵⁶

Therefore, the cocaine COMPS were prepared in a similar fashion by adding about 15 mg of methyl benzoate to 4 mil polyethylene bags. Immediately following the generation of the COMPS, three of the 15 COMPS bags were placed inside clear screw top jars fitted with an 11 mm sleeve stopper, with one bag per jar. Jars were sealed and odor was allowed to accumulate for 30 min. After this accumulation period, odors were extracted for 30 min using DVB/CAR/PDMS SPME fiber and analyzed using the optimized GC-MS method from section 4.2.2.1.2 to determine the percent loss of methyl benzoate over time. Once these COMPS were tested, they were disposed of. The remaining nine

COMPS were suspended inside a fume hood and flipped hourly to simulate training aid handling. Four hours later, three more COMPS were tested within the clear jars following the same procedure as the first three COMPS. In total, for Day 1 of testing, nine COMPS were tested: three at the initial time at 9 am, three tested 4 hours later at 1 pm, and the final three tested 8 hours following the initial testing time (5 pm). This left three prepared COMPS left for the following days' analysis. Day 2 and Day 3 of testing used the final three COMPS with the same procedure from the previous day except the COMPS were reused and tested twice that day, at 9 am and at 5 pm. On Days 4-8, again, the same COMPS were tested, but were only tested once a day, at 9 am. The percent lost, starting from the initial test at Day 1, until the final test at Day 8 was calculated. This procedure was repeated a second time, with a t-test being employed to compare the results between the two trials to determine the dissipation rate reproducibility of the training aid. Three additional COMPS were created, following the above procedure, and weighed every half hour to determine the dissipation rate through gravimetric analysis. Blank COMPS containing no methyl benzoate were also weighed hourly and subtracted from the gravimetric results obtained from the methyl benzoate containing COMPS

Twelve sol-gel polymers, imprinted with approximately 1 mg of methyl benzoate were created in 10 mL screw top vials following the procedure from section 4.1.1.1, using the recipe stated in section 4.1.2.1, developed upon the completion of Task 1. The percent loss from the sol-gel polymer training aids were determined following the same procedure and time table used for testing the COMPS, except that when the polymers were not being tested, the jars were placed inside the hood and not suspended or flipped hourly. This was because the polymers are solid and remained coated to the bottom of

the 10 mL vials, even when handled. As with the analysis of the COMPS, two trials were completed and compared with a t-test to determine dissipation variation. The dissipation rate through gravimetric analysis was also completed for the sol-gel polymers, except that approximately 50 mg of methyl benzoate was imprinted inside the sol-gel polymer in order to see a change in weight over time. The percent loss of methyl benzoate over time as well as the dissipation rates of the COMPS and sol-gel polymers were compared and statistically analyzed using ANOVA. The temperature and humidity inside the lab was monitored throughout the entirety of this experiment.

4.2.2.3. Dissipation Manipulation of Sol-Gel Training Aids

This portion of the study sought to manipulate the dissipation of the sol-gel polymer training aid through two different methods: through the addition of a polyethylene bag and through temperature changes. In an attempt to manipulate the sol-gel polymer dissipation rate through the use of low-density polyethylene bags, in the same fashion as COMPS, three sol-gel polymers were created, imprinted with approximately 1 mg of methyl benzoate, in 10 mL screw top vials following the procedure from section 4.1.1.1. Following solidification, a 2 mil, low-density polyethylene bag was placed over the opening of the 10 mL vials and parafilm around the sides to keep the bag securely in place. The percent loss of the methyl benzoate was determined following the same procedure and time table from 4.2.2.2. The same procedure was repeated using a 4 mil low-density polyethylene bag, as well. As with the previous experiments, the dissipation rates were determined through gravimetric analysis by weighing sol-gel polymers

imprinted with approximately 50 mg methyl benzoate hourly. The temperature and humidity was monitored throughout this experiment.

To determine the change in dissipation when subjected to various temperatures, three sol-gel polymers, imprinted with approximately 1 mg of methyl benzoate were created in 10 mL screw top vials following the procedure from section 4.1.1.1. Immediately following creation (Day 0), the vials containing the polymers were capped. Odor accumulation was allowed to take place for 30 min. Following this accumulation period, odors were extracted for 30 min using DVB/CAR/PDMS SPME fiber and analyzed using the optimized GC-MS method from section 4.2.2.1.2. Following analysis, vials containing polymers were opened and placed on an Isotemp Digital and Analog Dry Bath Incubator set at 50°C. Polymers were tested following the above procedure from Day 0 - Day 3, twice daily, at 9 am and again at 5 pm. On the last day of testing (Day 4), polymers were just tested once, at 9 am. The percent loss of methyl benzoate over time was calculated. The same procedure was repeated at 40°C and 30°C (using the same Dry Bath Incubator) and at room temperature (monitored using Micro-t loggers) and compared. The percent loss was also determined when placing polymers in dry ice, in an attempt to halt dissipation for shipping purposes. The same procedure was repeated for the dry ice experiment except that polymers were only tested from Day 0 to Day 3. Additional sol-gel polymers were created, imprinted with approximately 50 mg of methyl benzoate, and weighed hourly when exposed to each temperature in order to determine the dissipation rate through gravimetric analysis.

4.2.2.4. Contamination Study for Sol-Gel Training Aid vs. COMPS

Two blank sol-gel polymers (without the addition of the methyl benzoate template) were created in 10 mL screw top vials following the procedure from section 4.1.1.1, using the recipe stated in section 4.1.2.1, developed upon the completion of Task 1. Approximately 10 mL of methyl benzoate, or 10.8 g, was placed in a 50 mL beaker. Inside a fish tank, the two sol-gel polymers were placed 25.5 centimeters apart, with the beaker of methyl benzoate placed in between the two, equidistant from the two polymers (Figure 19 - A). The fish tank was then covered and samples were left for 1.5 hours. After the allotted time, one sol-gel polymer vial was removed and placed inside the clear glass jar, and covered with the lid fitted with an 11 mm sleeve stopper. The sample was then left for 30 min, followed by a 30 min SPME extraction time using the DVB/CAR/PDMS SPME fiber, and injected into the GC-MS for analysis. The second sol-gel polymer left inside the fish tank was tested following the same method after a total time of 3 hours inside the fish tank. The assigned times of testing, 1.5 and 3 hours, were chosen because during canine trials, samples are usually left out in their hidden locations for roughly 2 hours before samples are collected and stored for later use. The above procedure was repeated in triplicate, over the span of 3 days.

The same procedure as above was completed for COMPS in order to directly compare the risk of contamination for both the COMPS and sol-gel training aids. To do this, two blank COMPS (created without the addition of methyl benzoate) were made by placing 10 g of blank cellulose inside 3 inch x 3 inch low-density polyethylene bags, 4 mil thick. Using twine, COMPS were suspended from the top of the fish tank, ten inches apart, such that the bags brushed the bottom of the tank (Figure 19 - B). Samples were

removed after 1.5 and 3 hours and tested following the same procedure used for the sol-gel polymers. Similarly, this was completed in triplicate over the span of 3 days. The temperature changes within the fish tank were monitored throughout this experiment.

Figure 19: Contamination study set-up: sol-gel polymers (A) and COMPS (B)

4.3. Task 3: Dissipation Manipulation through Sol Gel Network Variations

One of the short comings of COMPS is that the dissipation of the odorants from the COMPS can only be manipulated through the use of different polymer bags. As a result, COMPS dissipation manipulation is contingent on the availability of said bag. It has already been tested whether the same polymer bags can be used to manipulate the dissipation of the sol-gel polymer training aid, but the purpose of this task was to determine if the sol-gel network could be manipulation through changing the recipe of its creation. With this change of network, the dissipation would be control inherently, and not through the use of “outside influences.” The success of this task allows for more options for canine handlers during training to control the amount of odors presented to their canines during a specific training session. Moreover, the canines’ strength in court is improved as the handler will be more aware of the capabilities of his specific canine.

This task involves sol-gel network manipulation through changes in precursor type, solvent type, and amount of water added. It is important to note, that catalyst changes were not attempted in this study. While sol-gel polymers with an acid-catalyzed reaction yields more linear networks, and base-catalyzed reactions yields more dense materials, it has been found that the precise concentration of catalyst only changes the kinetics of the sol-gel reaction and not the basic structure of the overall network.^{89,95,101}

4.3.1. Materials

Sol-gel polymers were made in 10 mL clear, screw top vials with PTFE/Silicone septa (Supelco, Bellefonte, PA) using the ingredients shown in Table 8, purchased from Gelest (Morrisville, PA), Fisher Scientific (Pittsburg, PA), or Sigma-Aldrich (St. Louis, MO). Deionized water (DI) was purified using a Nanopure Diamond Ultrapure water system from Barnstead (Dubuque, IA). Table 8 includes all of the optimized sol-gel ingredients as well as the additional precursors and solvents used to manipulate the dissipation of methyl benzoate. All polymer ingredients were mixed using a Digital Vortex Mixer from Fisher Scientific (Pittsburg, PA). During the solidification process, polymers were placed in a desiccator containing 10-20 mesh Drierite containing calcium sulfate and cobalt (II) chloride (Fisher Scientific, Pittsburg, PA). Odors were extracted from the polymers using a DVB/CAR/PDMS SPME fiber (Supelco, Bellefonte, PA) and run using a Varian 3800 Gas Chromatograph and a Saturn 2000 Ion Trap Mass Spectrometer (Walnut Creek, CA) fitted with a SGE SolGel-Wax capillary column of 30 m x 0.25 mm (i.d.) and a film thickness of 0.25 μ m (Fisher Scientific, Pittsburgh, PA). The concentrations of methyl benzoate were determined using an external calibration

curve of the standard, diluted in methylene chloride (CAS # 75-09-2; Fisher Scientific, Pittsburg, PA), and injected into the GC-MS using a Combipal autosampler. Throughout the course of this experiment, the temperature in the lab was monitored using Micro-t loggers (Nexsens Technology, Fairborn, OH). The morphology of the sol-gel polymers was analyzed using a Philips XL30 Scanning Electron Microscope (SEM), provided by the Trace Evidence Analysis Facility (FIU, Miami, FL), and a JEOL JSM 5900IV SEM using a SPI-Module sputter coater, for sample treatment, provided by the Florida Center for Analytical Electron Microscopy (FIU, Miami, FL).

Table 8: Compounds used for the variation of the sol-gel polymer network, including CAS #, batch/lot #, and purpose

Compound	CAS #	Batch/Lot #	Purpose
Tetramethyl orthosilicate (TMOS)	681-84-5	BCBC4877V	Precursor
Tetraethyl orthosilicate (TEOS)	78-10-4	MKBF6044V	Precursor
Phenethyltrimethoxysilane (PeTMS)	N/A	4D-4786	Precursor
HPLC Grade Methanol	67-56-1	136943	Solvent
Hexanes	73513-42-5	962305	Solvent
Ammonium Hydroxide (NH ₄ OH)	1336-21-6	SHBD7421V	Catalyst
Hydrochloric Acid (HCl)	7647-01-0	092603	Catalyst
Methyl Benzoate	93-58-3	STBD1140V	Template

4.3.2. Methodology

4.3.2.1. Manipulation through Changes in Precursor

Literature shows that when making sol-gel polymers, the starting material or precursor dictates the network. The sol-gel polymer recipe that has been used throughout this project, utilized the precursor, phenyltrimethoxysilane (PeTMS) (Figure 20 (A)).

This precursor, while containing a bulky R group, creating a weaker network, also contains a phenyl group. Since methyl benzoate also contains a phenyl group, it was assumed that *pi-pi* interactions would occur between the template and the polymer, increasing the affinity of the template odor for the polymer, therefore producing a slower dissipation rate. However, two other precursors, tetramethyl orthosilicate (TMOS) and Tetraethyl orthosilicate (TEOS) were attempted to test whether creating a tighter network would help to manipulate the dissipation rate of the sol-gel polymer training aid (Figure 20 (B and C)).⁸⁷

Figure 20: Sol-gel polymer precursors: PtTMS (A), TMOS (B), and TEOS (C)

To compare the dissipation rates of the odor template when changing the precursor of the sol-gel polymer, three sol-gel polymers were created, imprinted with approximately 1 mg of methyl benzoate, in 10 mL screw top vials. Polymers were created following the procedure from section 4.1.1.1, using the recipe stated in section 4.1.2.1, developed upon the completion of Task 1. This recipe was the “original” sol-gel polymer containing the following: the PtTMS precursor, polar methanol solvent, and 5 moles of water (molar ratio 1 mole of precursor to 5 moles of water). The recipe was

used as the standard when comparing recipe variations in each portion of the task. Following the creation of this sol-gel polymer vials were capped and odors were allowed to accumulate for 30 min. After, odors were extracted using a DVB/CAR/PDMS SPME fiber and analyzed using a GC-MS. Following analysis, samples were uncapped and left under the hood until the following day. Twenty-four hours later, the same analysis was repeated. This was continued for 5 total days of analysis.

The same procedure was completed for using TEOS and TMOS precursors, except the original sol-gel recipe was altered during creation, using 1 mole of TEOS or TMOS, in replace of PeTMS. It is important to note that the recipe for the sol-gel polymers was altered such that the molar ratio between the precursors and the remaining ingredients remained consistent throughout this experiment.

In addition to just changing the precursor, sol-gel polymers were tested that were made from a combination of two different precursors, thereby increasing the inorganic species within the polymer. It has been stated in previous work that increasing the inorganic nature of the network increases the rigidity of the sol-gel polymer, and therefore increase the staying power of the template.⁹⁹ To test how this affects the staying power of odorants, sol-gel polymers were created using the following precursor combinations: 70% PeTMS/ 30% TMOS, 60% PeTMS/ 40% TMOS, and 50% PeTMS/ 50% TMOS. The amounts of the other ingredients remained same, but the PeTMS and TMOS mixtures were kept separately through the hydrolysis step of the reaction. For example, when creating the 50% PeTMS/ 50% TMOS polymer, the PeTMS and TMOS solutions were placed into 2 different vials. After, 50% of the total amount of solvent was placed in the vial containing the PeTMS and 50% was placed in the vial containing

the TMOS. The same was done when adding the water and acid catalyst. These mixtures were allowed to hydrolyze for one hour before the two vials were combined. Additional hydrolysis was allowed to occur for another hour before the template and base catalyst was added to the sol solution.⁹⁹ Following the solidification of these polymers, samples were tested over 5 days in the same fashion as the polymers with the changed precursors.

With all of these experiments, three additional sol-gel polymers were created following the same recipes/changes in recipes as above, except with an addition of approximately 50 mg of methyl benzoate. Samples were weighed hourly to determine the dissipation rate through gravimetric analysis. As with previous experiments, all temperature variations within the lab were monitored.

4.3.2.2. Manipulation through Changes in Solvent Polarity

The use of a solvent in the sol-gel polymer recipe is mostly due to the fact that the sol-gel precursors are immiscible in water. In most cases, a polar solvent, such as methanol or ethanol are used as a solvent, allowing for a miscible solution between the precursor and the water for hydrolysis.⁹³ However, the purpose of this experiment was to determine whether the use of a non-polar solvent, such as hexanes, would change the dissipation of the template from the polymer. The same procedure from 4.3.2.1 was repeated, except hexanes were used as a solvent instead of methanol. In this experiment, the amount of hexanes that was added was such that the molar ratios between all of the sol-gel ingredients were kept the same as the sol-gel recipe developed in Task 1.

4.3.2.3. Manipulation through Changes in Moles of Water Added

The amount of water added for the sol-gel reaction is very important, since it is the force that drives the hydrolysis reaction. Research has shown that it is desirable to have a mole of water for every R group of the silica precursor. A decreased ratio of water to precursor will result in incomplete hydrolysis, whereas too much water may result in reverse esterification, causing the reaction to reverse back to the starting material.⁸⁹ When optimizing the sol-gel recipe in Task 1, the recipe was most successful when five moles of water was used. Since the precursor, PeTMS , has three R groups, the network strength should improve significantly if five moles of water was decreased to three. Therefore, for this portion of the task, using three moles and one mole of water was compared to the dissipation results when using five moles of water to initiate hydrolysis. To complete this, the methodology from 4.3.2.1 was repeated with varying the molar ratios of water when creating the sol-gel polymers. The ratios of all other ingredients remained consistent throughout this experiment. Dissipation rates through gravimetric analysis were also obtained following the procedure from 4.3.2.1.

4.3.2.4. SEM Analysis for Sol-Gel Polymer Morphology

A scanning electron microscope (SEM) provides information on the external morphology or physical nature of the surface of a solid sample at high resolution. To obtain an SEM image, a finely focused beam of electrons is aimed and scanned across a sample. Signals are generated from the sample when the electron beam interacts with solid. The signal may be from an elastic interaction, in which the trajectory of the electrons is altered without altering their energy (backscattered electrons) or through

inelastic interactions, which causes a transfer of energy to the solid sample, which then, in turn, causes the excited solid to emit secondary electrons or X-rays, for example.¹³⁶ A detector is required to pick up the electrons within the produced signal, amplifies them and then converts them into an electrical voltage, allowing for a surface image to be viewed.¹³⁷ Because the sol-gel polymers created for this project do not undergo a full drying process, interferences were observed when imaging the wet samples. As a result, samples were imaged using two different methods, one using a low vacuum system and the other, using a gold coated pre-treatment system. The SEM analysis of all samples created in sections 4.3.2.1, 4.3.2.2, and 4.3.2.3 were attempted at the Trace Evidence Analysis Facility and the Florida Center for Analytical Electron Microscopy.

4.4. Task 4: Canine Detection of Sol Gel Training Aids

The purpose of the canine trials in this task were to determine whether trained detection canines would alert to the newly made sol-gel polymer training aids and not a blank sol-gel polymer. The task served as a proof of concept as to whether this method of mimic production would be useful in the field and whether this method could be applied to other narcotics or explosives for canine training. The goal of these trials was to achieve comparable positive results to cocaine COMPS that have already proven to be successful for canine training.

4.4.1. Materials

Sol gel polymers were made in 10 mL clear, screw top vial with PTFE/Silicone septa. Polymers were created using the same ingredients shown in Table 7 as well as the

same materials mentioned in section 4.2.1.2. The COMPSs were used during the canine trials to compare alert rate results against the experimental sol-gel polymer training aids. The COMPS for cocaine were made by spiking methyl benzoate onto cellulose microcrystalline powder. The spiked cellulose was placed in 3 in x 3 in low-density polyethylene bags (2 mil thickness) and heat sealed using a Midwest Pacific MP-8 Impulse Heat Sealer. Filled polymer bags were placed inside a 6 in x 5-1/2 in I.D. Barrier Foil Ziploc Bags from Ted Pella, Inc. (Redding, CA). During the canine trials, cocaine was used as a positive control, obtained from the director of the Drug Enforcement Administration's (DEA) K-9 Academy of Miami (Miami, FL). These cocaine samples have been tested to ensure a purity of over 90% and are used in the training of law enforcement detection canines in Miami. Canine trials were completed using certified canines within south Florida coordinated by the director of the US K-9 Academy.

4.4.2. Methodology

4.4.2.1. Creation of Sol-Gel Training aid for Dog Trials

For each canine trial, a total of three sol gel polymers were made. Polymers were made in 10 mL screw top vials following the procedure from section 4.1.1.1 and using the recipe stated in section 4.1.2.1, developed upon the completion of Task 1. The only variation to this procedure was the amount of template added to each polymer. Of the three polymers created for each trial, two were imprinted with methyl benzoate, while one was a blank sample, containing no methyl benzoate. Of the two imprinted samples, one polymer was made with 1100 µg of methyl benzoate, diluted in the solvent,

methanol. The second imprinted polymer contained 50 μL of pure methyl benzoate, the equivalent of 54 mg. The blank sol-gel polymer involved no addition of a methyl benzoate template, and therefore the equivalent volume was added to the sol-gel as methanol. As stated in section 4.1.2.1, as the sol-solution was combined, samples were left overnight in the desiccator for solvent and network solidification. Imprinted samples were placed in a different desiccator than those of the blank polymers, to prevent possible contamination. On the morning of the canine trial, polymers were removed from the desiccators and immediately capped to allow for odor accumulation in the vial without the VOCs escaping.

4.4.2.2. Creation of COMPS for Canine Trials

For each canine trial, one COMPS was made following the methyl benzoate proportions and procedures set by the standard operating procedure of Florida International University's International Forensic Science Institute that have been proven successful in the field.^{56,57} To make these training aids, cellulose powder was spiked with methyl benzoate and mixed thoroughly. Following, 10 g of the cellulose/methyl benzoate mixture was added to a 3 in x 3 in low-density polyethylene bags (2 mil thickness) and heat sealed. The filled polyethylene bags were then placed inside a 6 in x 5-1/2 in I.D. Barrier Foil Ziploc Bag (Figure 7), which creates equilibrium between the bag and outer foil container, while ensuring that constant dissipation from the polymer bag is achieved.

4.4.2.3. Canine Trials

Canine trials were completed using trained law enforcement detection dogs and their canine handlers from various police departments throughout South Florida. For each of the trials performed, the three sol-gel polymers made (two imprinted and one blank) were hidden in familiar search patterns to the detection canines. Additionally, for each trial, one COMPS containing 10 g of spiked cellulose was hidden, as well as 15-20 g of cocaine, used as a positive control. For all samples hidden during the trial, at least 30 minutes was allowed to pass from the time the samples were placed out until the time the canines' searched the area to ensure that an ample amount of odor dissipated from the samples and were available for possible canine detection. The canines' responses, as indicated by their handlers, were documented. In total, 32 single blind canine trials were completed with 18 difference canine teams. For all canine trials the positive and negative predictive values were determined.

4.5. Task 5: Canine Field Accuracy to Flowers Producing Methyl Benzoate

The purpose of this task was to examine the VOCs released from various types of newly grown snapdragon flowers, primarily methyl benzoate, and assess its potential at eliciting a false alert from specially trained detector canines. This was done in order to evaluate the accuracy and reliability of detector canines, especially in scenarios where odors similar to those of narcotics are present, as in the case of snapdragon flowers.

4.5.1. Materials

4.5.1.1. Snapdragon Planting and Growth

Four types of snapdragon flowers were obtained and analyzed during this study. Seeds belonging to the following flowers were purchased: Maryland True Pink (PanAmerican Seed, West Chicago, IL), Blank Prince, Twinny Peach, and Rembrandt (Thompson & Morgan, Ipswich, England). Flowers were planted and maintained within a greenhouse located at Florida International University (Miami, FL). Flowers were initially planted in fertilized soil in a 72-cell planting tray. After plants had sprouted about 2 inches high, they were transplanted into plastic flower pots. All planting supplies, including soil, fertilizer, planting trays, flower pots, and water were provided by the Florida International University Greenhouse and Department of Biological Sciences (Miami, FL). Temperature and relative humidity were continuously monitored during the growth process using an environmental meter (Extech, Wilmington, NC). In order to track the life cycle of each bloomed flower, flowers were tagged using Fisherbrand Colored Label Tape (Fisher Scientific, Pittsburg, PA) on the day of anthesis.

4.5.1.2. Extraction of VOCs from Snapdragon Flowers and Cocaine Samples

The extraction method used for the collection of VOCs from snapdragon flowers was solid-phase microextraction (SPME). The SPME method was optimized to determine which type of SPME fiber would efficiently extract the highest amount of VOCs produced by the flowers. The SPME fibers, seen in Table 6, were purchased from Supelco.

Two methods of flower containment for SPME extraction were explored. The first used two- 1 gallon paint cans stacked on top of each other and sealed together using duct tape. The bottom can had both the top and bottom of the can removed, while just the lid from the top can was removed in order to create a cylindrical containment system to be placed around the snapdragon flower pot. The top of the cylinder was punctured with 3 holes, where Suba-Seal silicone rubber septa with an i.d. of 14 mm (Sigma-Aldrich, St. Louis, MO) were placed within each hole for triplicate SPME extractions (Figure 21). The second flower containment system explored was to place cut flowers into 40 mL clear, screw top vial with PTFE/Silicone septa (Supelco, Bellefonte, PA).

Figure 21: Possible snapdragon flower containment system

Odor profiles of the snapdragon flowers were compared to those of cocaine samples obtained from a local canine trainer with a DEA license (Miami, FL). These cocaine samples have been tested to ensure a purity of over 90% and have been used in the training of law enforcement detection canines in Miami. Using a Mainstays digital kitchen scale, 1 gram of cocaine powder was scraped into 40 mL clear, screw top vials with PTFE/Silicone septa.

4.5.1.3. Gas Chromatography-Mass Spectrometry Analysis

Instrumental analysis was performed using an Agilent 6890 gas chromatograph (GC) coupled to an Agilent 5973 quadrupole mass spectrometer (MS). The GC was fitted with an Agilent J&W HP-5-MS column, measuring 30 m x .25 mm (i.d.) with a film thickness of 0.25 μm . For liquid injections, an Agilent 7683 series autosampler injector was employed. The GC, MS, GC column, and autosampler were all purchased from Agilent Technologies, Inc (Santa Clara, CA).

4.5.1.4. Reference Chemical Standards

The reference chemical standards that were used for this portion of research are listed in Table 9. These chemicals, purchased from Sigma-Aldrich (St. Louis, MO), were used to confirm instrumental results obtained from the reference library as well as to create calibration curves for quantification. High performance liquid chromatography (HPLC) grade methylene chloride, purchased from Fischer Scientific (Pittsburgh, PA), was used for chemical dilutions of calibrants.

Table 9: List of chemical standards used for Task 5

Compound	CAS #	Batch/Lot #
β -Myrcene	123-35-3	BCBG1825V
β -cis-Ocimene 2,6-Dimethyl-2,4,6-octatriene	13877-91-3	MKBK9668
Acetophenone	96-86-2	MKBL2078V
Methyl Benzoate	93-58-3	STBD1140V
β -Linalool	78-70-6	BCBG7490V
3,5-Dimethoxytoluene	4179-19-5	MKBG3130V
Cinnamic acid, methyl ester	140-10-3	MKBL2079V
α -Farnesene	502-61-4	00496AJ
trans-Nerolidol	7212-44-4	11914KRV
Methyl 3,5-dimethoxybenzoate	2150-37-0	S33586V
Benzoic Acid	65-85-0	MKBG9317V

4.5.1.5. Canine Trials

Canine trials were completed using certified canines within south Florida coordinated by the director of the US K-9 Academy. When cut flowers were presented to detection canines, flowers were placed in 16 ounce Ball mason jars.

4.5.1.6. Statistical Analysis

Principal Components Analysis (PCA) was completed using Minitab 15 English (Minitab, State College, PA). Box-and-Whisker plots were generated and the negative predictive value (NPV) was calculated using Microsoft Excel 2010 (Microsoft, Redmond, WA).

4.5.2. Methodology

4.5.2.1. Snapdragon Planting and Growth

Four different snapdragon flowers (Table 10), varying in species, size, and color were selected for this study. During a period of cooler climates (January), approximately 3-5 seeds of Maryland True Pink, Blank Prince, Twinny Peach, and Rembrandt snapdragons were planted. Since snapdragon flowers require sunlight for germination, seeds were placed on top of fertilized soil in each cell of a 72-cell planting tray; eighteen cells were designated for each type of snapdragon (Figure 22). The flowers were maintained in a greenhouse located at Florida International University.

Figure 22: 72-cell planting tray distribution for snapdragon planting

Generally, germination began after 1 week and after 3-4 weeks, 5 cm sprouts were present. One month after planting, the snapdragon sprouts were transplanted into round, plastic flower pots (6.5 inch diameter, 5 inch. deep) using only 1 sprout per pot, to allow ample room for flower growth, as well as to minimize competition for nutrients and sunlight. All planting supplies, including soil, planting trays, fertilizer, and flower pots,

were provided by the Florida International University Greenhouse and Department of Biological Sciences. Throughout the growth process, the flowers were watered daily and routinely pruned for optimal growth and plant survival. Temperature and relative humidity were continuously monitored using an environmental meter, with values ranging from 21.9 - 28.8°C and 37.5 - 78.1% respectively. Plants started to flower 2 months after planting and were utilized for testing.

Table 10: Types of snapdragons used in this study including name, genus, species, size classification, and color

Name	Genus	Species	Size Classification	Color
Maryland True Pink	Antirrhinum	majus	Tall (24-48 inches)	Light pink
Black Prince	Antirrhinum	nanum	Medium (12-24 inches)	Dark crimson
Twinny Peach	Antirrhinum	nanum	Dwarf (6-12 inches)	Light yellow
Rembrandt	Antirrhinum	majus nanum	Medium (12-24 inches)	White with red

4.5.2.2. Extraction of VOCs from Snapdragon Flowers and Cocaine Samples

4.5.2.2.1. Method Optimization

Before VOCs could be collected from snapdragon flowers, an optimized method for collection had to be developed. It was first necessary to determine which SPME fiber coating would be most efficient for the collection of snapdragon volatiles. In order to assess which SPME fiber should be used, it was imperative to determine which fiber extracted methyl benzoate most consistently at the highest abundance. Therefore, 5 µL of methyl benzoate was spiked into 7- 40 mL clear, screw top vials with PTFE/Silicone septa and allowed to equilibrate for 10 minutes. Following this equilibration period, headspace SPME was performed for 5 minutes using the SPME fibers from Table 6

After the extraction, samples were run on the GC/MS. The process was repeated two additional times, resulting in triplicate trials. The adequate fiber was chosen based on which fiber coating chemistry resulted in the highest abundance of extracted methyl benzoate. A t-test was also employed to determine whether using specific fibers were produce statistically different results.

It was next necessary to determine how flowers would be sampled. The goal of this optimization process was to develop a flower containment system that not only allowed for ample VOC accumulation and collection, but also minimized the amount of time flowers remained capped and therefore derived of necessary nutrients. In an effort to keep the flowers alive and thriving throughout the sampling process, stacked paint cans were used (Figure 21) in an effort to keep the flowers tact. At mid-day, or the high of odor production, stacked cans were placed on top of one of the flower pots belonging to the previously cited Maryland True Pink snapdragon flowers.⁶³ Odor accumulation was allowed to occur for a half an hour, followed by an hour long extraction period using the optimized SPME fiber. Triplicate samples were run on the GC-MS using the optimized method found in section 4.5.2.3. It is important to note that while flowers are covered with the paint cans, they are cut off from both carbon dioxide and sunlight, necessary for survival and growth. Therefore, the accumulation and extraction times could not be extended.

An alternate containment system was attempted and compared to that of the paint cans. For this method, three Maryland True Pink flowers were cut and placed in one 40 mL clear, screw top vial with PTFE/Silicone septa and capped immediately. Odor accumulation was allowed to occur for a half an hour, followed by an hour long

extraction using the optimized SPME fiber. Triplicate samples were run on the GC-MS using the optimized method found in section 4.5.2.3.

It was determined that the optimal method for flower sampling was to use 40 mL clear, screw top vial with PTFE/Silicone septa to house cut flowers. The method allowed for a suitable abundance of accumulated VOCs to be collected as well as provided a method to obtain a snapshot of the odors being produced during various times throughout the flowers' life cycle. With a flower containment system chosen, it was next necessary to complete a time study to determine the suitable SPME extraction time in which equilibrium is reached between the SPME fiber and the sample. One Maryland True Pink flower was cut and placed inside five- 40 mL clear, screw top vial. Odors were allowed to accumulate for 30 minutes. Following the accumulation period, SPME extraction was performed on each vial, with each vial representing a different extraction time. The following extraction times were tested: 1 minute, 15 minutes, 30 minutes, 45 minutes, and 1 hour. Triplicate samples were run on the GC-MS using the optimized method found in section 4.5.2.3.

4.5.2.2.2. Collection of VOCs

Each day, the growing snapdragons were inspected for newly bloomed flowers. On the day of anthesis, the flower was flagged using Fisherbrand Colored Label Tape. Since the study conducted by Dudareva et al. found that odor emission peaked 5-8 days after anthesis, the flowers were cut and immediately placed into a 40-mL clear, screw top vial with PTFE/Silicone septa 2, 6, and 10 days after anthesis in order to obtain a snapshot of the odors being produced during that time of the flower's life cycle.⁶³ The

optimized SPME method was used to extract VOCs from snapdragon flowers. The SPME fiber samples were analyzed via GC-MS using the optimized method explained in section 4.5.2.3.

After the flowers were sampled, the vials were opened, filled with water until the bottom stem of the flower was completely submerged, and then left opened overnight. This was done to simulate the odor released from flowers cut for floral arrangements/bouquets over an extended period of time. The following day, the vials were again capped for 30 minutes, sampled, and analyzed using SPME/GC-MS. This process continued until methyl benzoate, the target odor, was no longer present in the odor profiles. For this experiment, triplicates were run for each of the snapdragon flower types at each of the 3 designated points of the flower's life cycle (2,6, and 10 days after anthesis).

In order to compare the scent profiles of the snapdragon flowers to that of cocaine, samples of cocaine (90% purity) were obtained from a local canine trainer in Miami, FL with a DEA license. Once samples were obtained, about 1 gram of cocaine powder was scraped into three clean, 40 mL clear, screw top vial with PTFE/Silicone septa. Using the same method as the snapdragon exactions, the cocaine samples were capped for 30 minutes and VOCs were extracted using SPME. SPME fibers were taken back to the laboratory for GC-MS analysis.

4.5.2.3. Gas Chromatography-Mass Spectrometry Analysis

Once the VOCs were extracted from the snapdragon flowers and cocaine samples using SPME, samples were analyzed using GC-MS. An optimized GC-MS method was

developed using an Agilent 6890 gas chromatograph coupled to an Agilent 5973 quadrupole mass spectrometer. The GC was fitted with a 0.25 mm x 30 m HP-5-MS column with a film thickness of 0.25 μ m. Helium was used as the carrier gas at a flow rate of 1.2 mL/min. The injection port was maintained at a temperature of 280⁰C and the GC column initial temperature was set to 40⁰C. Upon initial sample injection, the GC column temperature was increased to 100⁰C at a rate of 7⁰C/min, followed by an increase to 110⁰C at a rate of 2⁰C/min, and a final increase to 260⁰C at a rate of 25⁰C/min, for a total runtime of 19.57 minutes. The mass spectrometer transfer line and source were maintained at 280⁰C and 230⁰C respectively. The mass spectra were continuously scanned from 40 to 440 amu. Compounds were identified using the NIST 98 mass spectral reference library and confirmed and quantified using reference standards (Table 9) and an external calibration curve.

4.5.2.4. Canine Trials

Canine-handler teams from various police departments throughout South Florida trained for narcotics detection took part in this study. Two different canine trials were used to assess the canine's alert rate to snapdragon flowers. For all trials completed, the flowers were placed in familiar search patterns commonly encountered by canines during regular training sessions. Blank soil samples, as well as approximately 15 - 20 g of cocaine (positive control), were used in the study. The canines' responses, as indicated by their handlers, were documented.

First, the canines potential to alert to potted, growing flowers was determined. Each type of potted snapdragon plant, consisting of multiple stalks with 15-20 bloomed

flowers per stalk, as well as a blank soil sample and a positive control, were placed in plain sight in a manner that was familiar to the canines' search patterns. The purpose of this trial was to determine if the trained detection canines would alert to growing snapdragon flowers by presenting, at minimum, 1 μg of methyl benzoate, an amount well above the cited detection limit for methyl benzoate.⁵⁰ This study evaluated 14 canine teams over the span of 1 month resulting in a total of 30 runs.

For the next canine trial, cut snapdragon flowers were hidden to create a blind testing situation. The morning before the trials, twenty flowers from each snapdragon were placed into their own respective 16-ounce mason jar to ensure that the canines were presented with a minimum of 1 μg of methyl benzoate. Jars were sealed for approximately one hour, which allowed for odor accumulation during transport to the testing site. At the test site, the jars were opened and hidden in search patterns used in previous training scenarios. Again, blanks and positive controls were used, and appropriately hidden for this trial. Six canine teams were tested over a span of 3 weeks resulting in a total of 14 runs.

4.5.2.5. Statistical Analysis

Each of the four snapdragon flowers' VOCs, as well as their abundances, were statistically compared against those of cocaine through Principle Components Analysis (PCA) to determine the similarity or difference between each of the odor profiles. In addition to PCA, a box-and-whisker plot was used to highlight the percent differences of methyl benzoate contained in both the snapdragon and cocaine samples. To analyze the

results of the canine trials, the negative predictive value (NPV) was calculated to determine the likelihood that the negative response from the canine was a “true negative.”

5. RESULTS AND DISCUSSION

5.1. Task 1: Creation of a Novel, Rugged Sol-Gel Polymer

Task 1 sought to create a polymer that could be used to encapsulate a signature odorant in order to create a training aid for narcotics canine detection using sol-gel technology. The created polymer must be effective in the field, be produced timely and reproducibly, be able to withstand rough use and shipping without damage, as well as endure extreme conditions commonly encountered in the field, including temperature changes and solvent exposure. The creation of the sol-gel polymer required the optimization of the following ingredients: (1) the precursor, commonly a metal alkoxide, which dictates the network of the polymer, (2) the solvent which improves miscibility between the precursor and remaining reactants, allowing for a homogeneous polymer, (3) water, which drives the hydrolysis reaction, and (4) a catalyst, which quickens the hydrolysis and condensation reactions.^{87,89,93,94}

5.1.1. Optimization of a Novel Sol-Gel Recipe

The optimization process began by determining the best ingredients for this specific application. Three different commonly used sol-gel precursors, tetraethyl orthosilicate (TEOS), tetramethyl orthosilicate (TMOS), and methyltrimethoxysilane (MTMOS) (Figure 23), were tested to determine solidification success. While studies

have shown that the ideal amount of water used is equivalent to 1 mole per precursors' - OR substituent, 1-4 moles of water were initially tested.^{89,94} Finally, four types of catalysts were tested, two bases and two acids. Acid catalysts have been shown to speed up hydrolysis and produce polymers with a linear structure, while basic catalysts create a more branched, dense network.^{89,95,101} Therefore the acids, hydrochloric acid (HCl) and trifluoroacetic acid (TFA), and the bases, sodium hydroxide (NaOH) and ammonium hydroxide (NH₄OH) were attempted in the initial optimization process. For this initial experiment, 200 μ L of solvent and precursor, and 0.1 M of the catalyst in water, were continuously used. For each recipe, the precursor, followed by the solvent, and finally the water/catalysts mixture were added, mixing thoroughly between each addition. Samples were left overnight and following, were inspected for solidified polymers.

Figure 23: Initial sol-gel precursors used for optimization: (A) TEOS, (B) TMOS, and (C) MTMOS

The results of the initial optimization study are summarized in Figure 24, with the molar ratios and full formulas, including all optimization recipes during this process shown in Table 18 of the appendix. Figure 24 shows lists all recipes when altering the precursor, water amount, and catalyst used. Those formulas highlighted in green

represent the recipes that produced a solidified polymer. These results suggest that the TMOS precursor produces the most success in sol-gel solidification when using a base catalyst. It is interesting to note that solidification never occurred when only an acid catalyst was used, most likely because without the base catalyst, the condensation reaction is unable to complete in the allotted time. While using the precursor, MTMOS, did produce a solidified polymer, albeit only when using NaOH. MTMOS was not further investigated because the polymers produced were not clear, as they are when using TMOS (Figure 25 A), but a murky, white, symbolizing the production of a less homogeneous polymer (Figure 25 B).

	Precursor	Catalyst		Precursor	Catalyst		Precursor	Catalyst
Water: 1 mol	TEOS	NaOH	Water: 1 mol	TMOS	NaOH	Water: 1 mol	MTMOS	NaOH
	TEOS	NH ₄ OH		TMOS	NH ₄ OH		MTMOS	NH ₄ OH
	TEOS	HCl		TMOS	HCl		MTMOS	HCl
	TEOS	TFA		TMOS	TFA		MTMOS	TFA
Water: 2 mol	TEOS	NaOH	Water: 2 mol	TMOS	NaOH	Water: 2 mol	MTMOS	NaOH
	TEOS	NH ₄ OH		TMOS	NH ₄ OH		MTMOS	NH ₄ OH
	TEOS	HCl		TMOS	HCl		MTMOS	HCl
	TEOS	TFA		TMOS	TFA		MTMOS	TFA
Water: 3 mol	TEOS	NaOH	Water: 3 mol	TMOS	NaOH	Water: 3 mol	MTMOS	NaOH
	TEOS	NH ₄ OH		TMOS	NH ₄ OH		MTMOS	NH ₄ OH
	TEOS	HCl		TMOS	HCl		MTMOS	HCl
	TEOS	TFA		TMOS	TFA		MTMOS	TFA
Water: 4 mol	TEOS	NaOH	Water: 4 mol	TMOS	NaOH	Water: 3 mol	MTMOS	NaOH
	TEOS	NH ₄ OH		TMOS	NH ₄ OH		MTMOS	NH ₄ OH
	TEOS	HCl		TMOS	HCl		MTMOS	HCl
	TEOS	TFA		TMOS	TFA		MTMOS	TFA

Figure 24: Results of initial sol-gel optimization, where the highlighted portions represent the solidified sol-gel polymer recipes.

Figure 25: Solidified sol-gel polymers using (A) TMOS and (B) MTMOS

The results in Figure 24 suggest that sol-gel solidification does not occur in a timely manner when just an acid catalyst is used. The risk of not using an acid catalyst is that some –OR substituents on the precursor may remain unreacted before condensation begins, creating a less fully formed sol-gel network. Reactions with only a base catalyst may also create a less fully formed network with unreacted silanols.^{89,95,101} In order to address this problem, a dual-catalyst system was implemented. For this trial, varying molar ratios of HCl were used in conjunction with either NaOH and NH₄OH, while keeping the molar ratios of the precursor (TMOS) and solvent (MeOH) the same. Since the acid catalyst was made in a water solution, the acid was varied based on adding 1-4 moles of water. To create these polymers, the precursor, followed by the solvent and then the acid catalyst/water mixture were added, and mixed thoroughly between each ingredient. After two hours, to ensure full hydrolysis, the base catalyst (dissolved in the chosen solvent) was added to the sol-solution. The exact recipes, highlighting the molar ratios of each ingredient can be seen in Table 19 in the appendix. While solidification did occur, it was occurring immediately after the base catalyst was added to the sol-solution. The immediate solidification

suggests that there was not enough time for the sol-solution to completely undergo condensation, and therefore further optimization was required.

Possible solutions to immediate solidification of the sol-gel polymers was either to add more solvent, thereby increasing the sol-solution volume, or adding less base catalyst. Results obtained during this optimization, including the molar ratios of the ingredients used can be found in Table 20 in the appendix. The results show that varying the amount of solvent added has little to no effect on the solidification time of the polymer. Varying the amount of base added during the creation of the polymer was next attempted. In addition, adding the base piece-wise, or 10 μL at a time, while mixing between each addition was also attempted. Table 21 in the appendix highlights the recipes attempted during this optimization process. Adding the base piece-wise slowed down the solidification considerably, especially with the Recipes 114, 115, and 117 (Table 21). Unfortunately, these results failed to be reproducible interday.

Two theories as to why the sol-gels were not solidifying reproducibly were from temperature variations in the lab or from pipetting errors. To test the first theory, a SPARK Science Learning System (Pasco, Roseville, CA) was used to track the temperature changes within the lab. Figure 26 shows the results for the temperature changes over three different days. On the first day (A), the temperature varied from 20.3°C - 21.3°C, on the second day (B), the temperature varied from 21.6°C - 23.8°C, and on the last day (C), the temperature varied from 22.9°C – 24.6°C. These values show that the 3 day temperature range in the lab equaled 4.3°C.

Figure 26: Temperature changes within the lab as measured by the SPARK Science Learning System, where (A), (B), and (C) represent measurements over 3 different days

Concurrently, an exploration of whether variations in pipetting could be causing a lack of reproducibility for the sol-gel solidification. To test this, 12 sol-gel polymers were created using recipe 114 from Table 21, therefore the same volumes. Following the addition of each ingredient, the sol-solution was weighed to determine the variation, in terms of the mass, for each reagent that was pipetted. Once all ingredients were added, the solidification times for each polymer were monitored. Interestingly, a trend between the base addition and the time of solidification was seen, shown in Figure 27. These results show an inverse relationship between the addition of base catalyst solution and the time of solidification when the *same* amount of volume was pipetted.

Figure 27: Trend showing that when pipetting the same amount of base catalyst, there is an inverse relationship between the error in pipetting and the time of solidification

Once determining that the temperature changes in the lab as well as the systematic error from variations in pipetting may be causing a lack of reproducibility during sol-gel polymer production, changes in polymer preparation was initiated to counterbalance the issues. First, stock solutions containing the acid and base catalysts were created in larger volumes with the hope of introducing less error when pipetting these amounts. Also, polymer solidification was completed in a refrigerated centrifuge, Eppendorf Centrifuge 5804 (Hamburg, Germany). Table 22 and Table 23 in the appendix highlights all recipes, including a catalyst optimization, focusing on the concentrations of catalyst stock solutions needed to create an ideal sol-gel polymer. Also, extended solidification times were seen when implanting the use of a refrigerated centrifuge. Recipe 135 in Table 22 showed the most promising results, and therefore this recipe was further explored.

The ruggedness of Recipe 135 was first explored. Initially 12 sol-gel polymers were made over four days (three per day). Of the 12 sol-gels, only 7 solidified, giving a solidification rate of 58.3%. In addition, the sol-gel polymers that did solidify were not visually the same. Figure 28 shows this variation, where polymers from Trial 1 and Trial 2 were created using the same recipe under the same conditions, except on different days.

Figure 28: Variation in sol-gel polymer, showing an undamaged polymer (Trial 1) and a cracked polymer (Trial 2)

Both polymers shrunk in size during the solidification process due to solvent evaporation, but shattering of the right image is disconcerting, as it poses a risk for canines training and it shows its lack of ruggedness, most likely further breaking during shipping or rough handling.

Despite the lack of reproducibility explained above, the dissipation of the methyl benzoate odorant from the sol-gel polymer was tested and compared to the COMPS. This was done using the DVB/CAR/PDMS SPME fiber with a 20 min equilibration time and a 20 sec extraction time (these parameters were re-optimized in Task 2). Samples were allowed to dissipate for an hour at a time in between testing. Hourly weight changes were also monitored to determine the dissipation rate, which was compared against the dissipate rate of a COMPS, also through gravimetric analysis. The results, in Figure 29, show that methyl benzoate dissipated completely from the polymer after 10

hours, a result comparable to COMPS, later shown in Task 2. It is also important to note the bell-shaped curve seen in Figure 29. While the reason for this curve is unknown, after sol-gel optimization, this trend was never seen again, and instead, results showed a more linear decrease in odor. The dissipation rate through gravimetric analysis proved to be 300 ng/s for the sol-gel polymer and 360 ng/s for the COMPS (Figure 41). Because of the fact that these specific sol-gel polymers were not reproducible nor rugged and showed little improvement over the COMPS, the sol-gel recipe needed to be further optimized and improved.

Figure 29: Dissipation of methyl benzoate from initial sol-gel polymer over time, using SPME

From the above results, the problems that needed to be addressed in the initial sol-gel polymer recipe were the shattering/shrinking of the polymer network and the quick dissipation rate of the methyl benzoate template. The shrinking was occurring as the excess solvent was evaporating over time. This excess solvent exists not only because it

was initially added in the recipe to promote miscibility between precursor and water, but also because it is produced as a byproduct of the hydrolysis reaction.^{87,89} Usually, sol-gel polymers are heated and dried to avoid this problem, but because heating will drive out the volatile template, heating could not be implemented during this process.⁸⁷ As an alternate solution, during solidification, polymers were left in a desiccator with drierite to not only promote this drying of the excess solvent, but to also lock out additional moisture from the environment. Polymer shrinking was also occurring because the network was too rigid. To solve this problem, a different, bulkier precursor was implemented.⁹⁹ The precursor chosen, phenyltrimethoxysilane (PeTMS), can be seen in Figure 30. The reason for this selection was two-fold. First, the addition of the bulkier organic substituent should create a less rigid polymer network. Second, the addition of the benzene ring on the precursor should create pi-pi interactions between the precursor and the template, methyl benzoate, increasing the staying power of the template within the polymer, thereby decreasing the dissipation rate.

Figure 30: Newly chosen sol-gel polymer precursor and its interaction with the template methyl benzoate

Both theories proved to be successful, creating a polymer that resembles more of a gel consistency, instead of the previous glass-like consistency. The full formula for this sol-gel polymer can be seen in Table 11.

Table 11: Final sol-gel polymer formula, in terms of molar ratios, utilized for all future experiments

PeTMS	Methanol	NH ₄ OH (x10 ⁻³)	HCl (x10 ⁻³)	Water	Temp (°C)	Time	Conc Base	Conc Acid
1	5.42 + 2.5	9.21	6.54	5.76	RT	Overnight	0.09	0.063

Once the final sol-gel recipe had been developed, the loading capacity of the template, methyl benzoate was determined. Through a series of trial and error based tests, it was determined that no more than 50 µL of methyl benzoate could be added during the sol-gel creation process. This was determined through simple observation, as any amount higher than that resulted in a more liquid, less solid polymer. The loading capacity, taking into account the loss of the template over time while inside the desiccator, was determined to be 52.9 mg, as a $98.9 \pm .08\%$ retention factor was determined by the formula in section 4.1.2.1.

Putting the amounts of methyl benzoate added to the sol-gel polymer in terms of molar ratios, the amount of methyl benzoate that could be added to the polymer ranged from 1 mg to 54 mg, where the 1 mg amount represents the “experimental amounts” tested in Tasks 2 and 3. In terms of the molar ratios of the sol-gel polymer recipe (Table 11), the amount of methyl benzoate added to the sol gel polymer represents 0.0118 to 0.58 moles of methyl benzoate to 1 mole of precursor, PeTMS.

5.1.2. Ruggedness of Sol-Gel Polymer

The ruggedness of the newly developed sol-gel polymer was determined. It is important to first reiterate that because this sol-gel recipe produced a less rigid, more gel-like structure, there was minimal to no shrinking and no cracking of the polymer network. Also, being a gel-like consistency, the polymer remained coated on the bottom of the 10 mL vial; even with extreme shaking and dropping, the polymer remained in place. In addition, after making 12 polymers over four days, as done with the previous sol-gel recipe, there was a 100% solidification rate. To test the polymers' ability to withstand shipping conditions and harsh handling, polymers were shipped to three locations: Old Bridge, New Jersey; Baltimore, Maryland; and New Orleans, Louisiana, through standard United States Postal Service Shipping. Once received, recipients sent the package back to Miami, Florida, where they were visually inspected and weighed to determine loss of sample. Visual inspection of the polymers noted that the polymer remained coated to the bottom of the vials even after shipping and appeared a bit harder and less "gel-like" upon reception, most likely due to further drying over time. Samples were weighed and were determined to have suffered no weight loss/sample loss during the shipping process.

The ability for the sol-gel polymers to withstand extreme conditions, including highs and lows in temperature and commonly encountered solvents in the field was explored. Polymers were placed in a freezer (-4°C), on a heater (80°C), and on dry ice (-78.5°C). Visually speaking, the polymers placed on the dry ice and within the freezer began to harden, as if the polymer, itself froze. After removing the polymer from the freezer/dry ice, and allowing it to thaw, the polymer returned to its original consistency.

There was no noticeable change observed for the heated polymer. The changes in weight throughout the course of this experiment can be seen in Figure 31, where the weight seen at Day 0 is the weight of the polymer before exposing it to the extreme temperature and the weight seen at Day 1 is the weight obtained directly after exposing the polymer to the extreme temperatures.

Figure 31: Weigh changes of polymers exposed to extreme temperature conditions

Upon exposing sol-gel polymers to various solvents, marked changes were observed. Visually, exposing the polymer to water caused it turn a whitish tint. The rubbing alcohol seemed to show no visual change to the polymer, but when attempting to remove excess alcohol, a white substance seemed to be lost as well, most likely some of the polymer. This led to the loss of weight after seven days seen in Figure 32. The addition of the acetone actually dissolved the polymer, but when placing the solution

back in the desiccator to allow the acetone to evaporate, the polymer returned to its original consistency. The results of the weight changes can be seen in Figure 32, where the weight observed on Day 0 is the weight of the polymer before exposing it to the solvent and the weight observed on Day 1 is the weight obtained directly after exposing the polymer to the solvent. The spike at Day 1 that occurs when using both acetone and isopropyl alcohol is most likely a result of not extracting all of the solvent from the polymer before weighing. Over time, there is a loss of weight due to the solvent evaporation.

Figure 32: Weight changes of polymers exposed to solvents

Table 12 shows a summary of the results when exposing the sol-gel polymers to all extreme conditions, highlighting the weight change directly after the experiment and then after a total 6 day period. The highest percent weight change occurred for the isopropyl alcohol, as some of the polymer was most likely lost when pouring out the solvent. The polymers exposed to the extreme hot temperatures also lost a considerable

amount of weight most likely due to the heat further evaporating the excess solvent within the sol-gel network. But, for all other conditions there was a minimal amount of weight change, proving this polymers' ruggedness and utility in the field

Table 12: Summary of % weight changes when exposing polymer to extreme conditions

Condition	% Weight Change after Experiment	% Weight Change after 6 Days
Acetone	8.90%	1.78%
Water	2.38%	1.38%
Rubbing Alcohol	24.5%	9.62%
Freezer (-4°C)	1.91%	1.97%
Dry Ice (-78.5°C)	1.12%	1.98%
Heater (80°C)	4.07%	4.10%

5.2. Task 2: Comparison of Sol-Gel Training Aid to IFRI COMPS

Following the creation and optimization of the sol-gel polymer, completed in Task 1, it was next necessary to directly compare its efficiency to that of commercially available training aids, such as IFRI COMPS, that have already been proven to be effective in the field. The goal of Task 2 was to determine if the sol-gel training aid would improve upon the short comings of the COMPS, which includes their rapid dissipation rate and their limited ability for dissipation manipulation. This task sought to: (1) directly compare the dissipation rate of the newly created sol-gel training aid to the COMPS in order to determine if the polymers would have a greater shelf life, (2) determine whether the dissipation rate could be manipulated through external forces, such

a through the use of the low density polyethylene bags (LDPE) bags, used when making COMPS, or through temperature manipulation, and (3) conduct a contamination study to assess the training aids' susceptibility to contamination in the field.

5.2.1. Method Optimization

In order to extract the methyl benzoate odorant from the training aid samples, it was first necessary to develop an optimized method and extraction technique. The chosen extraction technique, SPME, requires the selection of the optimal fiber coating, based on the affinity of the fiber coating to the target compound. To determine the most effective SPME fiber to be used to extract methyl benzoate, the following fibers were tested: 85 μm PA, 60 μm PEG, 7 μm PDMS, 100 μm PDMS, 65 μm DVB/PDMS, 50/30 μm DVB/CAR/PDMS, and 75 μm CAR/PDMS. The results in Figure 33 show the total amount of methyl benzoate extracted using each fiber coating in terms of peak area. The bipolar coating, 50/30 μm DVB/CAR/PDMS, extracted the most methyl benzoate with the 100 μm PDMS and 75 μm CAR/PDMS extracting with a slightly less efficiency. The three most promising SPME fibers were retested using lower concentration of methyl benzoate with two different extraction times, 15 min and 30 min. Figure 34 shows the results, revealing that in trace samples, both the 75 μm CAR/PDMS and the 50/30 μm DVB/CAR/PDMS outperformed the 100 μm PDMS fiber, most likely because of their bipolar chemistries; the PDMS SPME fiber is nonpolar and therefore would be expected to extract less methyl benzoate, a polar compound. The fact that using a lower concentration of methyl benzoate altered the extraction capabilities of the fiber could be explained by the fact the using higher amounts a methyl benzoate resulted saturation of

the SPME fiber, with the 75 μm CAR/PDMS, 50/30 μm DVB/CAR/PDMS, and the 100 μm PDMS fiber reaching their loading capacity. Using lower concentration a methyl benzoate better indicated the affinity of the fiber coating for the analyte.

Figure 33: Method optimization for the extraction of methyl benzoate: SPME fiber study

Figure 34: Method optimization for the extraction of methyl benzoate: SPME fiber study with trace amounts of methyl benzoate

The final variable for consideration when selecting a SPME fiber depended on the time efficacy of the experiment. When using SPME, following volatile extraction, the fiber is injected directly into the heated GC inlet. The volatiles are desorbed over time into the GC-MS where it is separated and analyzed. If volatiles are not fully desorbed within the allotted time, not only will the data not represent the true value extracted, but fibers will have to be cleaned before the next extraction to prevent carryover from the previous sample. Following the usual 10 min desorption time, the same SPME fibers were injected again in the GC-MS for an additional 5 min desorption period to determine carryover from the previous run. This method was repeated until methyl benzoate was no longer detected by the GC-MS. The results for this experiment can be seen in Figure 35, showing that it required an additional 65 minutes inside the GC inlet for the CAR/PDMS fiber to completely desorb methyl benzoate from the fiber, while it only required an

additional 15 min for the DVB/CAR/PDMS and 10 min for the PDMS fiber. Based on these results, as well as the results obtained in Figure 33 and Figure 34, the 50/30 μm DVB/CAR/PDMS fiber was chosen for the entirety of Task 2 and Task 3.

Figure 35: Method optimization for the extraction of methyl benzoate: additional SPME fiber desorption time in GC inlet

Once the optimal SPME fiber was selected, a time study was completed to determine the time required for the SPME fiber to reach equilibrium with the samples. The time study was completed using 10 mL screw top vials, although a larger containment system will be required for the COMPS comparison. The 10 mL vials were used, however, in order to have a consistent extraction time throughout Task 2 and Task 3, regardless of the containment system used. A total of 200 ng of methyl benzoate (diluted in methanol) was pipetted into the 10 mL vials. Vials were sealed and allowed to equilibrate for 24 hours. Following a 24 hour equilibration time at room temperature, a SPME extraction was completed using the 50/30 μm DVB/CAR/PDMS fiber for the following times: 1 min, 15 min, 30 min, 45 min, 60 min, 2 hr, 3 hr, and 4 hr. Figure 36

(A) shows the results for this study, with the amount of methyl benzoate extracted at each designated time point. The goal was to identify the time required for the SPME fiber to reach equilibrium with the sample and therefore extract a consistent amount of methyl benzoate. However, the point at which equilibrium was reached (3 hr) also revealed to have a high standard deviation, as shown through the error bars. A trend became apparent – as the time of extraction increased, the error increased as well - as seen in Figure 36 (B), where the error is represented in terms of relative standard deviation. The high error rate was not the only concern surrounding this time study. While equilibrium was reached in 3 hr, enclosing the training aids for that extended period of time would most likely effect the constant dissipation, distorting the results meant to simulate constant field use. Therefore, a 30 min SPME extraction time was chosen because of the low error rate associated with this time point, as well as providing a minimal containment period of the continuously dissipating training aid samples.

Figure 36: Method optimization for the extraction of methyl benzoate: fiber exposure study (A) and associated error trends within the exposure study (B)

For Task 2, since a direct comparison was being drawn between the IFRI COMPS and the sol-gel polymer training aids, a containment system needed to be chosen that was large enough to house the COMPS. The containment system chosen was used for both the sol-gel training aids and the COMPS to keep all values constant and comparable.

There were three readily available containment systems that were large enough to fit the COMPS: Mason jars, paint cans, and glass jars (Figure 17). This study sought to find a container that would contain the odors, without leaking, therefore giving comparable results between runs. Figure 37 shows the results comparing the reproducibility when extracting from the three containers, represented by the relative standard deviations. The paint cans had the highest relative standard deviation, therefore having the most variation between samples. Conversely, the glass jars had the lowest relative standard deviation between samples; therefore these jars were chosen as the containment system for testing COMPS and sol-gel polymer training aids. It is important to note that the white capped jars will only be used when directly comparing COMPS to the sol-gel polymer training aids.

Figure 37: Method optimization for the extraction of methyl benzoate: containment system for COMPS and sol-gel training aid extractions

5.2.2. Dissipation Comparison between Sol-Gel Training Aids and COMPS

In order to assess the effectiveness of the sol-gel canine training aids, a direct comparison was completed between the sol-gel training aids and the COMPS. This portion of the task sought to compare both the dissipation rates and the percent lost over time to determine if the longevity of canine training aids could be improved upon. To do this, sol-gel training aids were created in 10 mL vials and imprinted with approximately 1 mg of methyl benzoate. These samples were continuously left opened and analyzed using SPME/GC-MS at various points over a 7 day period (See 4.2.2.2 for full procedure). This procedure was completed twice, over two different weeks, to determine the reproducibility of the methyl benzoate dissipation inter-week, with Trial 1 and Trial 2 representing the same experiment completed on two different weeks. The results from this study are shown in Figure 38, where the percent lost over time is highlighted for each trial. The results show that for both trials, after 24 hours, the Trial 1 showed a percent loss of approximately 60%, while Trial 2 showed a loss of approximately 40%. Though these results do show some initial variation, the variation did normalize after 48 hours. Trial 1 lost approximately 75% of methyl benzoate, while Trial 2 lost approximately 68%. After 7 days, both sol-gel training aids still contained methyl benzoate with both trials having lost approximately 86% of the total odorant. Because of the slight variation between the values obtained from this study (most evident from Days 0-2), a two-sample t-test was conducted to compare the results obtained when performing the study over two different weeks. It was found that there was no significant difference between both trials, as $t_{\text{calc}} = 0.729$ was less than the $t_{\text{crit}} = 2.086$, at $p = 0.05$. Therefore, for the rest of this study, one trial was used to characterize the dissipation of methyl benzoate over time.

Figure 38: The percent loss of methyl benzoate over time for sol-gel training aids with trials 1 and 2 representing repeated experiments on two different weeks

In addition to the percent loss of methyl benzoate, determined through the use of SPME/GC-MS, the dissipation rate was also determined through gravimetric analysis. Imprinted sol-gel training aid samples were created and weighed hourly to determine the rate of methyl benzoate dissipating over time. Blank sol-gel samples were also weighed hourly to account for any weight lost from excess solvent evaporation or environmental humidity absorption. As with the previous experiment, two trials were completed over two different weeks. The results for this study are shown in Figure 39, where Figure 39A represents the weight change over time for Trial 1 and Figure 39B represents the same for Trial 2. Using the slope obtained from these graphs, the dissipation rate was calculated. Both trials had the same slope, and therefore the same dissipating rate of methyl

benzoate, 55 ng/s. It is also important to note the R^2 values of the linear trend lines in these two graphs. The R^2 for Trial 1 equaled 0.9959, while the R^2 value for Trial 2 equaled 0.9964. Such linearity reveals a constant dissipation rate, one of the main advantages of the COMPS.

Figure 39: Dissipation rate of methyl benzoate determined through gravimetric analysis from sol-gel training aids: Trial 1 (A) and Trial 2 (B)

The same procedure completed for the previous sol-gel training aid study was completed for the COMPS, following the procedure from Section 4.2.2.2. The results from this study can be seen in Figure 40, where the methyl benzoate percent loss over time is highlighted for two trials, conducted over two different weeks. Upon initial inspection, the results from Figure 40 show that there is much less variation between trials for the COMPS compared to that of the sol-gel training aids (Figure 38). However, a notable difference was observed regarding the percent loss of methyl benzoate over time. After 24 hours, both trials demonstrated approximately 96% loss of methyl benzoate. After 48 hours, approximately 100% of methyl benzoate was lost, with no significant methyl benzoate dissipating thereafter. This shows the rapid dissipation of methyl benzoate from the COMPS, causing a short shelf life. For this study, a two-sample t-test was conducted to compare the results obtained when performing the study over two different weeks. As with the sol-gel training aids, it was found that there was no significant difference between both trials, as $t_{\text{calc}} = 0.134$ was less than the $t_{\text{crit}} = 2.085$, at $p = 0.05$.

Figure 40: The percent loss of methyl benzoate over time for COMPS with trials 1 and 2 representing repeated experiments on two different weeks

As with the sol-gel training aids, the dissipation rate was also determined through gravimetric analysis. COMPS, containing methyl benzoate, were made and weighed every half hour to determine the rate of methyl benzoate dissipation over time. Blank COMPS were also weighed hourly to account for any weight change from environmental humidity absorption. As with the previous experiment, two trials were completed over two different weeks. The results for this study are shown in Figure 41, where Figure 41A represents the weight change over time for Trial 1 and the Figure 41B represents the same for Trial 2. Using the slope obtained from these graphs, the dissipation rate was calculated. Trial 1 had a slightly different slope than Trial 2, providing a calculated dissipation rate of 388 ng/s and 361 ng/s, respectively. As with the sol-gel polymer, the trend line proved to be linear, with R^2 values of 0.9905 and 0.9745, for Trial 1 and Trial

2, respectively, coinciding with the already proven fact of the constant dissipation of COMPS.⁵⁷

Figure 41: Dissipation rate of methyl benzoate determined through gravimetric analysis from COMPS: Trial 1 (A) and Trial 2 (B)

Figure 42 shows the direct comparison of the percent lost from the COMPS versus the sol-gel training, allowing for a clear comparison between the two. Upon initial

inspection, it is clear that the odors contained within the sol-gel polymer are still present even after 7 days of sampling, while the COMPS had lost nearly 100% of total methyl benzoate after 48 hours. Within the first 24 hours, the sol-gel training aid only lost 60% of the methyl benzoate, while the COMPS lost 96%. Such results are in line with the dissipation rates calculated from Figure 39 for the sol-gel training aid and Figure 41 for the COMPS, where the dissipation rates were calculated to be 55 ng/s and 361 ng/s respectively. ANOVA was used to compare methyl benzoate percent lost over time between the two types of training aids. It was determined that there was significant difference between the two training aids, as $F_{\text{calc}} = 6.39$ was greater than the $F_{\text{crit}} = 4.35$, at $p = 0.05$.

Figure 42: Direct comparison of percent loss of methyl benzoate from COMPS v. sol-gel training aid

The results obtained from this portion of Task 2 reveal that the sol-gel training aids are a significant improvement in terms of dissipation rate and training aid longevity over COMPS. The longer lasting sol-gel aids will prove to be more cost effective for canine handlers, requiring handlers to purchase aids less often.

5.2.3. Dissipation Manipulation of Sol-Gel Training Aids

The next portion of Task 2 sought to manipulate the dissipation rate of the sol-gel training aids through the use of “outside forces.” The dissipation of COMPS is controlled through the use of the polymer bag, housing the odorant.⁵⁷ The thickness of the polymer bags directly controls the dissipation rate.⁵⁶ The goal of this portion of the project was to use the polymer, Low Density PolyEthylene (LDPE) bags, commonly used with the production of COMPS, to further manipulate the dissipation rate of the sol-gel training aids. Moreover, a downside of COMPS, as well as many other permeation devices, is that temperature changes can change the rate of dissipation, as odors tend to dissipate at a faster rate at higher temperatures. The ability to manipulate the dissipation rate of sol-gel training aids was determined with the hopes of using this advantage for further dissipation control and for shipping purposes.

Sol-gel polymers, imprinted with methyl benzoate, were created in 10 mL clear, screw top vials. Vials were covered with either a LDPE 2 mil polymer bag or a LDPE 4 mil polymer bag and the dissipation, in terms of percent loss of methyl benzoate over seven days was compared using SPME/GC-MS following the procedure from section 4.2.2.3. The results from the LDPE covered bags were compared to that of an uncovered, “control sol-gel training aid.” Results can be seen in Figure 43. The 2 mil LDPE bag

seemed to dissipate at a slower total rate than the control sol-gel as after 7 days the polymer-covered bag lost 70% of its total methyl benzoate, while the control sol-gel lost 87%. The initial dissipation, from days 0-2 seemed to be similar for both training aids with the exception of the first two data points for the 2 mil LPDE bags, where the percent lost was higher initially and then decreased over time. This translates to an extraction of a lower amount of methyl benzoate on the first days of sampling, followed by an increase. This same scenario occurred with the LDPE 4 mil bags, which could be explained by the fact that when dissipating through a polymer, such as the LDPE bags, there is usually a stabilization period associated with the diffusion rate, where it takes a few days for the diffusion rate to become consistent.¹³⁸ Because of this, the dissipation when using the LDPE bags was less consistent and showed higher variation. However, a clear decrease in the dissipation rate can be seen when implementing the use of the LDPE bags, as seen when determining the dissipation rate through gravimetric analysis.

Methyl Benzoate Percent Lost: LDPE Bags

Figure 43: The percent loss of methyl benzoate over time for sol-gel training aids covered with LDPE 2 mil bags and 4 mil bags compared with Control

The results for the gravimetric portion can be seen in Figure 44, where Figure 44A represents the results for the 2 mil LDPE bags and Figure 44B represents the results for the 4 mil LDPE bags. The results for the 2 mil LDPE bags were not linear, as the previous studies have been, and required a polynomial trend line to determine the slope of the line. Such results are, again, explainable by the need for a stabilization period through a polymer bag before the dissipation will be consistent.¹³⁸ Using the polynomial equation the dissipation was determined to be a changing rate, ranging from 5-40 ng/s. The results for the gravimetric analysis of the 4 mil LDPE bags (Figure 44B) were a bit surprising, as a linear trend line with an R^2 value of 0.9894 was determined. Additionally, the dissipation rate, using the slope, was calculated to be 21 ng/s, which does not coincide with previous research showing that the thicker the bag, the slower the

dissipation rate.⁵⁶ However, the results from Figure 43 show a greater amount of variation when using the 4 mil LDPE polymer bags, and therefore may have unfavorably influenced these dissipation results.

(A) Methyl Benzoate Dissipation Rate: LDPE 2 mil Bags

(B) Methyl Benzoate Dissipation Rate: LDPE 4 mil bags

Figure 44: Dissipation rate of methyl benzoate determined through gravimetric analysis from sol-gel polymers covered with LDPE bags: LDPE 2 mil bags (A) and LDPE 4 mil bags (B)

The dissipation rate of the sol-gel training aids was next manipulated through temperature changes. To complete this experiment, imprinted sol-gels were again made

in 10 mL, screw top vials and following their creation, were placed on either a heated incubator (when testing 30°C, 40°C, or 50°) or on the lab bench (for room temperature) to assess dissipation rates at different temperatures. The dissipation, in terms of methyl benzoate percent lost, was measured using SPME/GC-MS daily for 5 days, following the methodology from section 4.2.2.3. The results can be seen in Figure 45, where Figure 45A represents the percent loss of methyl benzoate at the following temperatures: 30°C, 40°C, 50°C, and room temperature, while Figure 46B shows the results from 30°C, 40°C, and 50°C zoomed in. The results in Figure 45A show a clear difference in the percent loss of methyl benzoate over time at room temperature compared to the heated samples, while all three heated samples behaved similarly. Looking at Figure 45B, a closer look at the heated samples, there is a slight difference where samples left at 50°C appeared to lose methyl benzoate at a faster rate than those left at 40°C and 30°C. After 8 hr, samples left at 30°C lost 60% of methyl benzoate, while those at 40°C and 50°C lost 64% and 74% respectively.

Figure 45: The percent loss of methyl benzoate over time for sol-gel training aids dissipating at different temperatures: Comparing room temperature to 30°C, 40°C and 50°C (A) and zoomed in comparison of 30°C, 40°C and 50°C (B)

The dissipation rates, through gravimetric analysis were evaluated for the sol-gel training aids at each temperature, seen in Figure 46, where Figure 46A represents the

change in mass over time for 30°C, Figure 46B represents 40°C, and Figure 46C represents 50°C. Again, results are linear, proving constant dissipation at these temperatures, with R^2 values of 0.97 or higher for all three temperatures tested. Using the slope of the lines, the dissipation rates were calculated to be 167 ng/s for 30°C, 528 ng/s for 40°C, and 822 ng/s for 50°C. The increase in dissipation rate when raising the temperature proves that the dissipation of methyl benzoate of the sol-gel training aids can be manipulated and controlled using temperature. While the dissipation rate when using other permeation devices, such as COMPS, can also be manipulated through changes in temperatures, sol-gel polymers can be directly exposed to said temperatures, while other devices can be damaged from this direct exposure. Future research may focus on coating imprinted sol-gel polymers on a small heat-able substrate for dissipation control at any environmental temperature.

(A)

(B)

(C)

Figure 46: Dissipation rate of methyl benzoate determined through gravimetric analysis from sol-gel training aids at 30°C (A), 40°C (B), and 50°C (C)

Finally, the dissipation of methyl benzoate for the sol-gel training aids was determined when placing imprinted sol-gel samples on dry ice. The dissipation of methyl benzoate was studied for 3 days using SPME/GC-MS and compared to the dissipation at previously tested temperatures. The results can be seen in Figure 47. It is interesting to note that the percent loss of methyl benzoate of the sol-gel training aid at room temperature steadily increases over time, while the sol-gel left on dry ice increased after the first day, but remained consistent for the remaining two days, meaning that no additional odor was lost for 2 days. These results are promising especially since training aids are commonly shipped to various canine facilities/ police departments throughout the country. Therefore, the results suggest that sol-gel training aids could be shipped or stored on dry ice to preserve the odor imprinted within the aid or halt further dissipation before training aids are ready to be used by the facility.

Figure 47: The percent loss of methyl benzoate over time for sol-gel training aids dissipating at different temperatures: comparing room temperature to 30°C, 40°C, 50°C, and samples left on dry ice

5.2.4. Task 2 Summary

To summarize Task 2, the dissipation rates for each of the sol-gel polymers and the COMPS (all discussed in Trial 2) are compared in Table 13. COMPS have a dissipation rate over six times higher than the sol-gel training dissipating at room temperature. The dissipation of the sol-gel training aid can, in fact, be manipulated through “outside influences,” including through the use of LDPE polymer bags and temperature, with dissipation increasing with a rise in temperature. Additionally, temperature and relative humidity ranges in the lab, during each test of this study can be seen in Table 16 in the appendix.

Table 13: Summary of training aids tested in Task 2 with their corresponding dissipation rate.

Type of Training Aids	Dissipation Rate
COMPS	361 ng/s
RT Sol- Gel	55 ng/s
Sol-Gel w/ LDPE 2 mil Bags	5 - 40 ng/s
Sol-Gel w/ LDPE 4 mil Bags	21 ng/s
Sol-Gel at 30°C	167 ng/s
Sol-Gel at 40°C	528 ng/s
Sol-Gel at 50°C	833 ng/s

5.2.5. Contamination Study for Sol-Gel Training Aid v. COMPS

A contamination study was completed to determine which training aid (the COMPS or the sol-gel training aids) was more susceptible to contamination from outside or environmental odors. During a canine training, training aids are usually hidden in a line-up or other familiar search patterns in a confined location, such as a warehouse. Not only is it nearly impossible to protect training aids from encountering outside odors, either from objects in the testing site or just in the environment, but the training aids are also susceptible to contamination from the other training aids that may be hidden for training purposes. For example, narcotics detection canines will train on multiple targets at once, including cocaine, heroin, MDMA, and methamphetamine. Therefore, the odors dissipating from a heroin training aid, for example, may contaminate the training aid for cocaine, especially if the aids are placed close together. The purpose of this portion of Task 2 was to directly compare the potential of the COMPS contamination to that of the sol-gel training aids by exposing blank training aids to methyl benzoate, for both 1.5 hrs and 3 hrs, in a confined location. Following the allotted time exposure period, samples

were placed in the clear glass jars fitted with 11 mm sleeve stopper and analyzed using SPME/GC-MS. The results from this study are seen in Figure 48, where the amount of methyl benzoate contaminating the blank training aids is highlighted. The results show that the COMPS are exceedingly more susceptible to contamination than the sol-gel training aids as after 1.5 hrs, the COMPS were contaminated with 3064 ng of methyl benzoate, while in comparison, the sol-gel training aids only contained 487 ng of methyl benzoate. After 3 hrs, the COMPS were contaminated with 3836 ng of methyl benzoate, while the sol-gel training aids only contained 626 ng of methyl benzoate. The COMPS suffer from the fact that the entirety of their surface area is porous, because they are made inside the LDPE bags. While this allows for continuous dissipation, it seems to also allow for contaminants to easily enter.⁵⁷ Because the sol-gel polymers are made inside 10 mL clear, screw top vials, the only access point for contaminants are at the opening of the jar. While these results can change if sol-gel polymers were created in a different containment system and not 10 mL vials, for this application, the fact that the sol-gel training aids made inside 10 mL vials are less inclined to absorb outside odors makes them highly effective for field use and even long term storage.

Figure 48: Contamination study results directly comparing exposure of methyl benzoate for 1.5 and 3 hrs to blank COMPS and sol-gel training aids

5.3. Task 3: Dissipation Manipulation through Sol-Gel Network Variations

While COMPS have proven to be effective training aids for field use and canine training, one of the drawbacks they suffer from is the fact that odor dissipation can only be manipulated based on the delivery system (polymer bag), and not the aid itself.⁵⁷ Therefore, dissipation manipulation is limited by the availability/manufacture of bags to enclose the COMPS. While the dissipation of sol-gel training aids can already be manipulated by the same bags as COMPS, as well as through temperature changes (Task 2), Task 3 sought to determine whether the dissipation of methyl benzoate can be manipulated by the sol-gel polymer, itself. By changing the recipe/ingredients during polymer creation, the dissipation rate of the imprinted polymer may change based on

alterations in the sol-gel network that corresponds to such recipe changes. The importance of this is to give more options for handlers and trainers, as well as have more control of the dissipation, since it is based on the inherent properties of the polymer and not “outside influences.” It is the hope that the success of this task and the implementation of these training aids could increase canine strength in court.

5.3.1. Manipulation through Changes in Precursor

As previously mentioned, the sol-gel precursors dictates the network formed during the solidification process. Inorganic precursors, such as TMOS and TEOS, form tight networks, while introducing bulky, organic substituents to the precursor creates a less rigid sol-gel network.^{89,94,98,99} This portion of Task 3 studied how the manipulation of the sol-gel network through the precursor will affect methyl benzoate dissipation. To determine this, sol-gel polymer were created with three different precursors, while keeping all other ingredients/molar ratios the same. The dissipation of methyl benzoate was tested over four days using SPME/GC-MS. Figure 49 shows these results. The sol-gel created with PeTMS, which was the control polymer created in Task 1, lost 41.6% of methyl benzoate after 24 hours and lost 86.9% after four days. TEOS and TMOS behaved similarly to each other, as after 24 hours, TEOS lost 98.4% of methyl benzoate, while TMOS lost 98.7%. After 4 days, both polymers were dissipating zero to minimal amounts of methyl benzoate. While it was theorized that the inorganic precursors would create a tighter network and therefore keep the imprinted odorant tightly bound inside the network, this was not the case. Upon physical observation, polymers created with TEOS and TMOS cracked and shattered over time. It is likely that the breaking of the network

caused rapid dissipation of the odorant, while PeTMS created a gel-like polymer that kept its shape throughout the entirety of the experiment. In addition, PeTMS has the addition of the organic substituent containing a benzene ring. The benzene ring most likely formed pi-pi interactions with the benzene ring in methyl benzoate, therefore increasing the interaction between the two and as a result, the staying power of the odorant within the polymer (Figure 30).¹³⁹

Figure 49: The percent loss of methyl benzoate over time for sol-gel training aids made from three different precursors: TEOS, PeTMS, and TMOS

The dissipation rate for TEOS and TMOS was determined through gravimetric analysis. The dissipation rate for PeTMS was determined to be 55.5 ng/s, as shown previously in Figure 39. Figure 50A shows the results for TEOS, while Figure 50B shows the results for TMOS. Using the slope of the linear trend line for each graph, the dissipation rate of TEOS and TMOS were determined to be 166.7 ng/s and 138.9 ng/s, respectively. The high dissipation rates compared to that of PeTMS coincide to the

results in Figure 49. It is also important to note the high error bars for TEOS and even the lower R^2 values for both TEOS and TMOS, 0.9603 and 0.9566 respectively. This could most likely be due to a less consistent dissipation rate produced when the network began to crack unsystematically.

Figure 50: Dissipation rate of methyl benzoate determined through gravimetric analysis from sol-gel training aids using precursors TEOS (A) and TMOS (B)

The use of TMOS and TEOS as a precursor seemed to produce a polymer that was too fragile, which not only produced a cracked polymer, but also a short-lived training aid. To correct this problem and increase the staying power of the template odor within the sol-gel training aid, training aids were created with varying degrees of TMOS. Polymers were made with: 70% PeTMS and 30% TMOS, 60% PeTMS and 40% TMOS, and 50% PeTMS and TMOS. A previous study had stated that by increasing the rigidity of the sol-gel polymer (by increasing the introduction of inorganic precursors), a dye that was doped on the polymer decomposed at a slower rate, most likely due to the fact that gases diffused through the more rigid polymer at a slower rate.⁹⁹ Therefore, it was hypothesized that if the rigidity of the sol-gel training aid was increased the odor would last longer within the sol-gel matrix and diffuse or dissipate out of the pores at a lower rate. The results, however, showed the opposite to be true, as seen in Figure 51. According to Figure 51, as the percent of TMOS increased, the staying power of methyl benzoate decreased over time. For example, after 24 hrs, the sol-gel polymer made with 0% TMOS lost 41.6% of methyl benzoate, while the polymer created with 30% TMOS lost 53.5%, with 40% TMOS lost 65.6%, and with 50% TMOS lost 74.8%. While visually, the polymer was less rigid, as there was less cracking as more PeTMS was introduced, the rigidity did not prove to improve the staying power or the dissipation rate of the imprinted methyl benzoate.

Figure 51: The percent loss of methyl benzoate over time for sol-gel training aids made with the addition of the TMOS precursor, to increase polymer rigidity

The dissipation rates were calculated through gravimetric analysis for 30% TMOS (Figure 52A), 40% TMOS (Figure 52B), and 50% TMOS (Figure 52C). Based on the slopes generated from the mass lost over time, the dissipation rates were calculated to be 83.3 ng/s, 138.8 ng/s, and 83.3 ng/s, respectively. The dissipation rate for polymers created with 0% TMOS was already determined to be 55.5 ng/s as seen in Figure 39. While the dissipation rate did not steadily increase when varying the amount of TMOS from 30% to 50%, the dissipation rate was well above that calculated for 0% TMOS. The inconsistencies seen for the dissipation rates calculated for 30%, 40%, and 50% could again be due to random cracking in the polymer network, which was still experienced, although at a less extreme than those produced with 100% TMOS. While these results

were the opposite of the hypothesized theory, the results could be explained based on the affinity that the methyl benzoate template experiences with the benzene containing PeTMS precursor. Again, the pi-pi interactions between the precursor and template increases as the amount of TMOS decreases, with could explain the slower dissipation rate when just PeTMS was used as a precursor (Figure 30).¹³⁹

Figure 52: Dissipation rate of methyl benzoate determined through gravimetric analysis from sol-gel training aids made with the addition of the TMOS precursor, to increase polymer rigidity: 30% TMOS (A), 40% TMOS (B), and 50% TMOS (C)

5.3.2. Manipulation through Changes in Solvent Polarity

The next study completed was to determine whether polymers created using a nonpolar solvent would have any effect on the dissipation rate of methyl benzoate. The introduction of a solvent into the sol-gel process in to create miscibility between the precursors and water (needed for hydrolysis). Polar solvents, such as alcohols (methanol and ethanol), are most commonly used and have been found to produce homogenous polymers.⁸⁹ The limited research on nonpolar solvents led to this study. The methyl benzoate percent loss over time was compared between sol-gel training aids made with methanol versus hexanes. The results for experiment could be seen in Figure 53. Results show that polymers produced using hexanes over time lost less methyl benzoate than polymers created using methanol. For examples, after 24 hours, polymers created with methanol lost 41.6% of methyl benzoate while those created with hexanes lost 29.2%. After four days, the sol-gels with methanol lost 86.9%, while the sol-gels with hexanes only lost 73.9%.

Figure 53: The percent loss of methyl benzoate over time for sol-gel training aids made from a polar v. nonpolar solvent

The dissipation rate for hexanes was calculated based on gravimetric analysis; the results can be seen in Figure 54. Using the slope of the linear trend line, the dissipation rate for hexanes was calculated to be 19.4 ng/s, while the sol-gels created with methanol dissipated at a rate of 55.5 ng/s (Figure 39). Visually, the sol-gel training aids made with hexanes were not homogenous, as a separation within the sol solution was visible before solidification. This could have altered the consistency of odor dissipation, as evident from the high error in Figure 54. In addition, the weight of the training aids did not begin to decrease immediately after opening the vials containing the training aids; it was only after five hours that a decrease in weight was apparent.

The slower dissipation rate of the hexane-made sol-gel training aids could possibly be as a result of the evaporation rates of the solvents. Hexanes (boiling point of

68.7°C), evaporates more readily than methanol (boiling point of 64.7°C) despite its higher boiling point because hexanes, a nonpolar solvent, only must overcome dispersion forces to achieve evaporation, while methanol, a polar solvent must overcome hydrogen bonds, a much stronger force. When hexanes quickly evaporate, methyl benzoate may be left behind within the sol-gel matrix because of the *pi-pi* interactions between methyl benzoate and the precursor, PeTMS. Conversely, by the time methanol evaporates, it has already formed stronger hydrogen bonds with methyl benzoate. As a result, when methanol evaporates, methyl benzoate may quickly evaporate as well.¹³⁹⁻¹⁴¹

Figure 54: Dissipation rate of methyl benzoate determined through gravimetric analysis from sol-gel training aids made using a nonpolar solvent

5.3.3. Manipulation through Changes in moles of Water Added

The introduction of water in the sol-gel process is extremely important because it drives the hydrolysis reaction, one of the two reactions that take place during the sol-gel

process. To ensure that complete hydrolysis will occur, and therefore a complete network has formed, there must be at least 1 mole of water for every alkoxide group of the precursor.^{89,94} To test how the moles of water added during the sol-gel process would affect the dissipation of methyl benzoate from the sol-gel training aid, 3 types of sol-gels were made, using 1 mole, 3 moles, and 5 moles of water. The sol-gel using 5 moles of water was the control sol-gel training aid, as the recipe determined in Task 1 contained 5 moles of water. It was theorized that since the precursor contains three alkoxide groups (Figure 30), three moles of water would produce the best results. The percent loss of methyl benzoate over time was compared for the sol-gel created with the three different amounts of water using SPME/GC-MS; the results can be seen in Figure 55. As theorized, the sol-gel training aid containing 3 moles of water was an improvement over the sol-gel training aid made with 5 moles of water. After the sol-gel training aids were left open for 24 hours, 29.7% of methyl benzoate was lost from the polymer made with 3 moles of water, while 41.6% of methyl benzoate was lost from the polymer made from 5 moles. After 4 days, the polymer made with 3 moles lost 79.4% of methyl benzoate, while 86.9% was lost from the control polymer. Contrarily to the previously explained hypothesize, the staying power of the methyl benzoate template further increased when polymers were created using 1 mole of water. After 24 hours of environmental exposure, the sol-gel training aids only lost 18.9% of methyl benzoate, while after 4 days 86.8% was lost.

Figure 55: The percent loss of methyl benzoate over time for sol-gel training aids when varying the moles of water needed for hydrolysis: 1 mole, 3 moles, and 5 moles

The increased staying power of the methyl benzoate in the polymer made with 3 moles compared to 5 moles is plausible, as researchers have argued that adding too much water would cause re-esterification to occur faster than the forward hydrolysis reaction. It was more surprising that 1 mole of water yielded better results, a different result than sol-gel literature. While adding only 1 mole of water would seem to cause incomplete hydrolysis to occur, since there are three alkoxide groups on the precursor, water is a byproduct of the sol-gel reaction. Therefore, the water produced as the byproduct will continue to hydrolyze the remaining alkoxide substituents.^{87,89,94}

As with the previous experiments, the dissipation rates for the sol-gel training aids made with 1 mole, 3 moles, and 5 moles of water were determined through gravimetric analysis. The results for 1 mole and 3 moles of water can be seen in Figure 56A and B,

respectively. Sol-gel training aids created with 5 moles of water, were the control polymers and their dissipation rate was already determined to be 55.5 ng/s (Figure 39). While a somewhat linear trend line was observed, the R^2 values were worse than previous experiments, with the R^2 value for polymers made from 1 mole of water equaling 0.8743 and the R^2 value for the polymers made with 3 moles of water equaled 0.8646. Additionally, the dissipation rates obtained did not coincide with the results seen in Figure 55. The dissipation rate for polymers made with 1 mole of water was calculated to be 83.3 ng/s, while the dissipation rate for polymers made with 3 moles of water was calculated to be 55.5 ng/s. According to Figure 55, the dissipation of polymers made with 1 mole of water should have been much lower. It is important to note that the dissipation rates through gravimetric are not exact measurements. While systematic errors associated with the scale may account for some variation, the more likely explanation is due to the increased volume of methyl benzoate required to register any weight change. As a result, the dissipation may be altered based on the high amount of template fitting in the same sized sol-gel network. Additionally, blanks were made adding the same volume of solvent to compensate for the volume of methyl benzoate added (as the template was usually diluted in solvent before it was added to the sol-solution). Because methanol will evaporate at a different rate than methyl benzoate, the dissipation rate calculated through gravimetric analysis will be altered. But this study does give a general idea of how the dissipation rate is affected by variations in the sol-gel recipe.

Figure 56: Dissipation rate of methyl benzoate determined through gravimetric analysis from sol-gel training aids when varying the moles of water needed for hydrolysis: 1 mole (A) and 3 moles (B)

5.3.4. SEM Analysis for Sol-Gel Polymer Morphology

A scanning electron microscope was used to look at the external morphology of the sol-gel polymers created. The goal of this Task 3 portion was to determine a trend between the external morphology of the polymers created with different recipes/ingredients and the dissipation rate. Unfortunately, this proved to be challenging.

As previously mentioned, the polymers made for this project did not undergo a full drying process because such methods, such as heat, would quickly drive out the methyl benzoate odor from the training aid. Therefore, the polymers were moist, which causes challenges for SEM. Even with the addition of gold to coat the polymer and to improve conduction during imaging, interferences were still observed from water (byproduct of the sol-gel reaction that remains in sol-gel network).⁸⁷ In addition, the electron beam was damaging the polymer and there was no way of knowing if morphological observations were inherent to the polymer or from this damage. Despite these challenges, images of three different types of polymers were obtained, which provided some comparison to the dissipation of methyl benzoate using different precursors. Figure 57A and B shows SEM results for the backscattered electrons at 100x magnification for the precursors TEOS and TMOS, respectively. In comparison, Figure 58A shows the SEM results for the backscattered electrons at 100x magnification when using the precursor, PeTMS, which was the control sol-gel determined from Task 1. Visually, the polymer produced from using TEOS and TMOS (Figure 57) has an apparent flat surface while the polymer made with PeTMS (Figure 58) has more grooves and cavities. Figure 58B shows backscattered SEM results further zoomed in at 500x magnification for PeTMS, where the pores within the polymer are more clearly seen. It seems plausible that the grooves and cavities within the PeTMS polymer allow for the template molecule, methyl benzoate, to remain for a longer period of time within the sol-gel network, buried within the grooves. While all polymers made in Task 3 were not able to be analyzed, the results that were obtained do give a bit of insight as to the behavior of methyl benzoate imprinted within the sol-gel polymer.

Figure 57: Scanning electron microscope results for backscattered electrons at 100x when creating sol-gel training aids using precursors TEOS (A) and TMOS (B)

Figure 58: Scanning electron microscope results for backscattered electrons when creating sol-gel training aids using precursors PtTMS at 100x (A) and zoomed in at 500x (B)

5.3.5. Task 3 Summary

To summarize Task 3, varying the ingredients and molar ratios between the ingredients do, in fact, alter dissipation of methyl benzoate from the sol-gel training aids. While the control sol-gel training aid showed great improvements over COMPS (Task 2), further improvements could be made by using less water or a nonpolar solvent, for

example. Table 14 shows the results from Task 3, highlighting the percent loss of methyl benzoate after 24 hours and 4 days and the dissipation rate calculated through gravimetric analysis. While the results through gravimetric analysis did not perfectly correlate with the instrumental results, it still is clear that altering the recipe of the sol-gel polymer will change the dissipation of the template. This is significant because canine handlers could request specific polymers based on the dissipation rate they want to present to their canines, allowing them to get a better sense of the low and high thresholds that their canine is capable of alerting to. As stated in the Supreme Court Case, *State of Florida v. Harris*, the capabilities of the canine is extremely important to allow their finding to be admissible in court.¹ Therefore it is the hope that the implementation of these sol-gel training aids will increase their strength in court. Temperature and relative humidity ranges in the lab, during each test of this study can be seen in Table 17 in the appendix.

Table 14: Summary of training aids tested in Task 3 highlighting the % Loss at 24 hrs, % Loss after 4 days, and their dissipation rate when manipulating the sol-gel recipe/ingredients

Sol- Gel Manipulations	% Lost after 24 hours	% Lost after 4 days	Dissipation Rate
Control (100% PeTMS, Polar Solvent, 5 moles water)	41.6%	86.9%	55.5 ng/s
100% TMOS Precursor	98.7%	99.4%	166.7 ng/s
100% TEOS Precursor	98.4%	100.%	138.9 ng/s
70% PeTMS and 30% TMOS Precursor	53.5%	85.4%	83.3 ng/s
60% PeTMS and 40% TMOS Precursor	65.6%	93.3%	138.8 ng/s
50% PeTMS and 50% TMOS	74.8%	93.4%	83.3 ng/s
Nonpolar Solvent	29.2%	73.9%	19.4 ng/s
1 moles of water	18.9%	62.8%	83.3 ng/s
3 moles of water	29.7%	79.4%	55.5 ng/s

5.4. Task 4: Canine Detection of Sol-Gel Training Aids

Task 4 served as a proof of concept to test whether trained detection canines would alert to the newly created sol-gel training aid and not the blank polymer. In addition, the goal of this task was to prove if, when conducting a canine trial, the sol-gel training aids would achieve comparable results to the IFRI COMPS, which have already been proven successful in the field. To determine this, canine trials were conducted over the span of four months using both sol-gel training aids and IFRI COMPS. For these tests, two sol-gel training aids were created - one containing 1 mg of methyl benzoate, and the other containing 50 mg of methyl benzoate. The IFRI COMPS used was created such that approximately 16 mg of methyl benzoate was contained within the created training aid. The reason two sol-gels with two different amounts of methyl benzoate were created was that it was desirable to have a comparison to see if canines would alert to varying amounts of methyl benzoate contained within the sol-gel polymers. It is also important to note that, as determined in Task 2, the sol-gel polymers dissipate at a much slower rate than the IFRI COMPS and therefore it was uncertain whether the sol-gel training aids containing 1 mg of methyl benzoate would dissipate enough odor for the canines to alert during the trials; therefore training aids containing 50 mg were added to this canine trial. In addition to the two sol-gel samples and the IFRI COMPS, a blank sol-gel and cocaine, which served as the positive control, were also included in these trials. The canine trials were completed single-blind and 18 canine teams participate in this study. The results of the canine trial can be seen in Figure 59, where both the alert rate and the “interest + alert” rate are highlighted for each sample. Alerts were characterized by canines sitting in front of the hidden sample, while an “interest” was called by the handler if there was a

change in behavior by the canine, such as a change in search pattern or an extended search in a particular area. Of the 32 runs completed by the canine teams, a 100% alert rate was achieved for cocaine, the positive control, and a 3.1% alert rate was determined for the blank sol-gel sample, as one canine falsely alerted to the blank. For the training aid samples, the IFRI COMPS, created with 16 mg of methyl benzoate, achieved a 90.6% “interest + alert” rate. The sol-gel training aids reached an 81.3% “interest + alert” rate for the sol-gel training aid containing 50 mg of methyl benzoate and a 65.6% “interest + alert” rate for the sol-gel training aid containing 1 mg of methyl benzoate. The performance of each canine during the canine trials can be found in Table 24 in the Appendix.

Figure 59: Canine trial results showing the alert rate and interest + alert rate for sol-gel training aids vs. the IFRI COMPS, where N represents the number of runs completed for each trial

The Positive Predictive Value (PPV) was calculated to assess the performances of the sol-gel training aids and the IFRI COMPS using the “interest + alert” results from the canine trial. The Negative Predictive Value (NPV) was calculated for the blank sol-gel

samples as well. These results can be seen in Table 15. These PPV values indicate the probability that when a canine alerts to the sample, it is the correct response. For example, 91.4% of the time, canines will alert correctly to the IFRI COMPS, while only alerting correctly to the sol-gel training aid containing 50 mg of methyl benzoate 84.2% of the time. In the same vain, the calculation of the NPV indicates that a “non-response” from a canine is the true response. The NPV calculated for the blank sol-gel shows that 97% of the time, canines will correctly not alert to the blank sample.

Table 15: Positive and negative predictive values calculated for the results of the canine trials

Sample	Statistical Tool	Result
Sol-Gel Training Aid (1 mg Methyl Benzoate)	Positive Predictive Value	74.4%
Sol-Gel Training Aid (50 mg Methyl Benzoate)	Positive Predictive Value	84.2%
IFRI COMPS (16 mg Methyl Benzoate)	Positive Predictive Value	91.4%
Blank Sol-Gel	Negative Predictive Value	97.0%

While the alert rate of the sol-gel training aids did not quite reach the same performance as the IFRI COMPS, important information was still obtained. First, if the sol-gel training aids are implemented into the canine training system, canines do not have to be trained off the polymer odor, proved by the 97.0% NPV obtained for the blank sol-gel sample. Moreover, any odor produced by the polymer odor does not impede or distract the canine from alerting to the methyl benzoate contained within the polymer.

This is ideal because there will be no time wasted in further canine training for use of this novel training aid, and therefore can be implemented immediately if desired. Next, the fact that canines were alerting to the sol-gel training aids is promising, proving that with the proper creation and polymer optimization, this delivery system can be effective in the field. The improvement in alert rate when imprinting 50 mg of methyl benzoate over 1 mg of methyl benzoate suggests that further improvements could be made by determining the optimal concentration of methyl benzoate required to produce comparable results to the IFRI training aids. It is possible that since the sol-gel polymers dissipate much slower than the COMPS, as shown in Task 2, the odors being produced by these training aids was too low to produce higher alert rates from the canines. Future research should seek to explore if varying the amount of methyl benzoate imprinted within the sol-gel polymer improves the alert rate of the training aid by detection canines.

Finally, the Scientific Working Group on Dog and Orthogonal Detector Guidelines (SWGDOG) has developed best practices for detection canine teams, including a certification process meant to gauge the performance of a canine team and improve their courtroom acceptance. For a canine to be certified, multiple search scenarios are presented to a canine team; a minimal score is required to pass this assessment. While both IFRI and the National Forensic Science Technology Center (NFSTC) require a 90% pass rate, which has been achieved in this study for the IFRI COMPS, with a PPV value of 91.4%, the sol-gel training aids fall short of that. However, the California Narcotics Canine Association (CNCA), the National Narcotic Detection Dog Association (NNDDA), and the United States Police Canine Association (USPCA), all require a 50%, 75%, and 70% passing rate, respectively. Therefore, the PPV rate obtained for the 50 mg

imprinted sol-gel training aid, which was calculated to be 84.2%, is within this threshold and therefore a viable option for training purposes.³¹

5.5. Task 5: Canine Field Accuracy to Flowers Producing Methyl Benzoate

Often, the reliability and accuracy of detection canines is called into question. One such example was in the Supreme Court case, *State of Florida v. Joelis Jardines*, where it was stated that if canines alert to an active odorant, and not the contraband itself, then a canine will alert to any substance containing that odorant. Specifically, snapdragon flowers have been cited to produce methyl benzoate, the active odor of cocaine.^{3,60,63} Therefore, the purpose of Task 5 was to evaluate a detection canine's ability to distinguish between cocaine and snapdragon flowers. The possibility of snapdragon flowers causing canines to false alert was evaluated.

5.5.1. Snapdragon Growth

Weekly photographs were taken, starting at the point of germination through the flowering process, tracking the growth of the snapdragon flowers (Figures 60-66).

Figure 60: Day of germination at 1 week after planting (left) and snapdragon growth after 2 weeks (right)

Figure 62: Snapdragon growth after 3 weeks

Figure 61: Snapdragon growth after 4 weeks

Figure 63: Snapdragon growth after 5 weeks

Figure 64: Snapdragon growth after 6 weeks post plant transplanting (left) and snapdragon growth after 7 weeks (right)

Figure 65: Snapdragon growth after 8 weeks: Full plant (left) and buds (right)

Figure 66: Snapdragon growth after 9 weeks: blooming of flowers

5.5.2. Method Optimization

In order to extract the volatiles produced from the snapdragon flowers, namely methyl benzoate, it was required to develop an optimized method and extraction technique. When using solid-phase microextraction (SPME), the SPME fiber coating needs to have the highest affinity towards the target VOCs. Therefore the efficiency of the following fibers to extract methyl benzoate was studied: 85 μm PA, 60 μm PEG, 7 μm PDMS, 100 μm PDMS, 65 μm DVB/PDMS, 50/30 μm DVB/CAR/PDMS, and 75 μm CAR/PDMS. The results in Figure 67 show the total amount of methyl benzoate, in terms of peak area, for each of the seven SPME fiber coatings. Figure 67 shows that the SPME fiber coated with the bipolar coating DVB/CAR/PDMS extracted the most methyl benzoate with the non-polar coating, 100 μm PDMS, extracting with the second highest efficiency. However, the PDMS coating also showed a lower error rate than when using

the DVB/CAR/PDMS fiber. After performing a two-sample t-test to compare the values obtained when using both fibers, it was found that there was no significant difference when using the PDMS fiber over the DVB/CAR/PDMS fiber, as $t_{\text{calc}} = 1.64$ was less than the $t_{\text{crit}} = 2.77$, at $p = 0.05$. As a result, because of the high extraction efficiency of methyl benzoate and the low error rate, the 100 μm PDMS SPME fiber coating was used for the entirety of Task 5.

Figure 67: Method optimization for the extraction of volatiles from snapdragon flowers: SPME fiber study for methyl benzoate

Next it was next necessary to determine a sampling method for the snapdragon flowers. Two containment systems were tested to determine which would not only provide a way to trap the VOCs and allow for sufficient odor accumulation, but also allow for the preservation of the flowers during sampling. In order to preserve the flowers, the first containment method attempted was the use of two stacked paint cans, which were placed over top of the flower pots. For this initial experiment, stacked cans

were placed over the Maryland True Pink snapdragon at mid-day and odor was allowed to accumulate for a half hour, followed by an hour long SPME extraction using the 100 μ m PDMS fiber coating. The results provided by GC-MS analysis revealed that a half hour odor accumulation period with an hour long extraction time was not enough time for enough odor to build up within the large volume of the can and provide sufficient results. A chromatogram of these results can be seen in Figure 68 (A). Figure 68 (A) shows a standard chromatogram of the results, while Figure 68 (B) shows the same chromatogram zoomed into the time in which methyl benzoate should be present. Figure 68 (B), circled in blue, shows a slight peak for methyl benzoate, but not large enough for quantification and comparison of odor production over time. The obvious solution to this problem would be to cover the flower with the can for a longer period of time, as well as extend the SPME extraction time. Covering the flowers for the extended period of time, however, will cut them off from the proper nutrients required for survival and growth, such as carbon dioxide and sunlight. Moreover, as the flowers began to get bigger and somewhat unruly, containing the flowers under just two cans became challenging, as flower stems began to break under the can confinement.

An alternate method was to use 40 mL clear, screw top vial with PTFE/Silicone septa as the containment system. This method required flowers to be cut and placed inside the vials. Immediately following flower collection, vials were capped and left for a half hour for odor accumulation. As with the previous experiment, odors were extracted using the 100 μ m PDMS SPME for one hour. A sample chromatogram using this method could be seen in Figure 68 (C). Compared to the results when using the stacked paint cans (Figure 68 (A)), a clear peak for methyl benzoate, circled in blue, can

be seen without needing to zoom into the chromatogram. The large amount of methyl benzoate detected allowed for odor quantification as well as provided data of odor depletion over time, which could not be accomplished with such a small amount of odors detected using the paint can containment method. This method requires the cutting of flowers, allowing for a snapshot of the odors produced at that time to be evaluated; however further production of the same flower at a later point in time would be impossible to determine. On the other hand, using this method caused no damage to other flower bushels and never required the covering of multiple flowers for extended periods of time; such treatment could stunt odor production because of the limited nutrients received when covered.¹⁴²

Figure 68: Chromatographic results testing snapdragon containment system: paint can containment system (A), paint can containment system - zoomed in (B), 40 mL vials containment system (C)

Sampling snapdragon flowers in the 40 mL screw top vials allowed for a suitable amount of VOCs to accumulate within the vial as well as provided a snapshot of the volatiles being produced by the flower at that specific time of its life cycle. With the

flower containment system chosen, a time study needed to be performed to determine the suitable SPME fiber extraction time required for the SPME fiber and the sample to be at equilibrium. After cut Maryland Pink flowers were allowed to accumulate odors for 30 min in the 40 mL vials, the following extraction times were tested: 1 minute, 15 minutes, 30 minutes, 45 minutes, and 1 hour. Figure 69 shows the results for this time study, where the average abundances of myrcene, β -cis ocimene, and methyl benzoate, three of the cited VOCs found in snapdragon flowers, were collected at each time of extraction.^{63,64} The abundances of myrcene, β -cis ocimene and methyl benzoate increased when increasing the extraction time from 1 min to 15 min, but the abundances extracted began to level off after 15 minutes, proving that an extraction time of at least 15 minutes is required for equilibrium to be met between the SPME fiber and sample. After conducting a two- sample test, it was proven that there was no significant difference in extraction efficiency when extracting for 30 min versus 45 min, as $t_{\text{calc}} = .270$ was less than the $t_{\text{crit}} = 2.77$, at $p = 0.05$. As a result, a 30 minute extraction time was chosen as a sufficient amount of time to extract the VOCs produced by the snapdragon flowers and therefore was used for the entirety of Task 5. In addition, since equilibrium was met with a 30 min accumulation period, prior to the 30 min SPME extraction time, it was deemed sufficient for all samples to be capped for 30 minutes prior to SPME extraction.

Figure 69: Method optimization for the extraction of volatiles from snapdragon flowers: time study for the 100 μ m PDMS SPME fiber study

5.5.3. Volatile Organic Compounds Released from Snapdragon Flowers

Using the optimized HS-SPME/GC-MS method from sections 0 and 4.5.2.3, the odors released from Black Prince, Maryland True Pink, Rembrandt, and Twinny Peach snapdragon flowers were evaluated in triplicate at 2, 6, and 10 days post anthesis. Figure 70 represents the nine compounds identified for each trial at the three designated days of the flowers' life cycle. In this graph, each color signifies a different compound detected while the length of each bar segment represents the abundance extracted. The odor profiles varied with each type of snapdragon flower, with the Maryland True Pink producing the highest abundance of compounds and the Twinny Peach producing the least. Additionally, as stated by Dudareva et al., the Maryland True Pink produced the

highest abundance of methyl benzoate, compared to the other three flowers tested, but methyl benzoate was not necessarily the most abundance odor produced by each snapdragon flower.⁶³

Figure 70: Odor profiles of each snapdragon (flowers cut 2, 6, and 10 days after blooming)

This observation is more clearly seen in Figure 71, which shows the distribution of the VOCs released from each snapdragon on Day 2 of anthesis. Day 2 was chosen for this comparison because, as seen in Figure 70, on average, Day 2 produced the highest abundance of methyl benzoate. Figure 71 revealed that, in most cases, other compounds contributed more to the snapdragons' signature odor profile than methyl benzoate. For

example, with the Black Prince snapdragon, β -cis-ocimene dominated the odor profile, making up 63%, while both β -myrcene and 2,6-dimethyl-2,4,6-octatriene contributed more to the odor profile, at 14% and 11% respectively, than methyl benzoate at 8%. For the Rembrandt snapdragon, acetophenone contributed 42% to the odor profile, followed by β -cis-ocimene at 30%, and methyl benzoate at 26%. The Maryland True Pink snapdragons' odor profile showed that methyl benzoate and β -cis-ocimene contributed more equally to the odor profile, at 41% and 45% respectively. Alternately, methyl benzoate was the dominant odor at 67% for the Twinny Peach snapdragon, but was the least odiferous type of snapdragon, as seen in Figure 70.

Figure 71: Distribution of the VOCs released from each snapdragon flower 2 days after anthesis

5.5.4. Persistence of Methyl Benzoate from Cut Flowers

In order to test the persistence of methyl benzoate, Black Prince, Maryland True Pink, Rembrandt, and Twinny Peach snapdragon flowers were cut 2, 6, and 10 days after anthesis and tested daily. This experiment was meant to simulate a floral bouquet/flower arrangement in order to assess the methyl benzoate dissipation once flowers have been cut. The results are presented in Figure 72, where $Time_0$ represents the initial flower sampling, and each additional time point represents sampling 24 hours after the previous point. Initial sampling of the flowers showed that the Maryland True Pink snapdragon produced the highest abundance of methyl benzoate (at $Time_0$), while the Black Prince snapdragon produced the least. Although the Twinny Peach snapdragon proved to be the least odiferous, as seen in Figure 70, it produced more methyl benzoate than the Black Prince. Flowers cut at Day 6 and Day 10 after anthesis ceased methyl benzoate dissipation at a faster rate than those cut Day 2. For instance, methyl benzoate was no longer detectable for the Black Prince Snapdragon after 24 hours ($Time_1$) when the flowers were cut at Day 6 and Day 10. Black Prince flowers cut at Day 2 of anthesis, however, showed detectable amount of methyl benzoate 48 hours after cutting ($Time_2$). These findings could be attributed to the time of pollination, as flowers cut at Day 6 and 10 after anthesis, or later in the flowers' life cycle, may have already pollinated resulting in a decreased odor production to deter further pollinators; flowers cut at Day 2 after anthesis, may have not been pollinated yet and therefore are still producing odors at higher abundances.⁶⁴ Moreover, results showed that flowers collected Day 2 after anthesis generally produced the highest abundance of methyl benzoate. A previous study reported that snapdragon flowers produce the most odors when they are at or close to full

bloom. Thus flowers collected at Day 2 were most likely close to or fully opened at the time of collection.⁷¹ This could also explain the initial spike in methyl benzoate production seen at *Time*₂ for the Rembrandt flower cut Day 2 after anthesis. Overall the results indicate that methyl benzoate, on average, persists for approximately 4 days after cutting, with newly bloomed flowers lasting as long as 7 days, as in the case for the Maryland True Pink and the Rembrandt snapdragons. In order to further explore whether a cut bouquet of snapdragon flowers with persisting odors of methyl benzoate would elicit an alert response from a trained detection canine, two flower wholesale companies were contacted to inquire about the time frame in which flowers are cut, incorporated into their bouquets, and received by the recipient. Both companies stated that if the flower ordered is in season, the recipient will receive the flowers one day after they have been cut. However, if the flowers are out of season or unattainable by a local farm, flowers are imported from an outside source, and therefore take approximately 4 days to reach the recipient after cutting. Based upon this information, and the results portrayed in Figure 72, there is a high possibility that the amount of methyl benzoate, if any, dissipating from the flower arrangement containing snapdragon flowers would be minimal, especially when receiving flowers from an outside source. Contrarily, in-season flowers delivered 24 hours after cutting and flowers cut in the early stages of the flowers' life cycle will likely release methyl benzoate upon delivery.

Figure 72: Abundance of methyl benzoate over time after flowers have been cut at 2, 6, and 10 days after anthesis for (A) Black Prince, (B) Maryland True Pink, (C) Rembrandt, and (D) Twinn Peach

5.5.5. Canine Trials

Previous studies have proven that methyl benzoate is the active odorant released from cocaine.⁵⁰ This study, along with Dudareva et al., found that methyl benzoate is one of the odors produced by snapdragon flowers.^{63,64} With these facts in minds, this raises the question: *will narcotics detection canines positively alert to snapdragon flowers?* To answer this question, both potted and cut snapdragon flowers were presented to specially trained narcotics detection canines to assess their accuracy. Fourteen canine teams were evaluated using potted snapdragon flowers, all of which contained a minimum of 15-20 flowers, or at least 1 µg of methyl benzoate. The amount of flowers needed to reach 1 µg of methyl benzoate was estimated based on the abundances detected from Figure 70. To assess the alert rate of canines to cut flowers, 6 canine teams were evaluated, single-blind. For both the potted plant and cut flower evaluation, a blank soil sample was presented to the canines as well as cocaine, which served as the positive control. Presented in Figure 73 are the canines' alert rates for the potted plants and the cut flowers, as well as the blank (soil) and positive control (cocaine). Figure 73 (A) represents the results for the potted plants, while Figure 73 (B) represents the results for the cut flowers. The detection canines did not alert to the blank samples or any of the snapdragon flowers, whether they were potted or cut, but a 100% alert rate was achieved for the positive control. The canine trial results for each canine team can be found in Table 25 for the potted plants and Table 26 for the cut flowers (Found in the Appendix).

Figure 73: Canine trials for the determination of snapdragon alert rate for (A) potted flowers and (B) cut flowers, where N represents the number of runs completed for each trial

The negative predictive value (NPV), which represents the likelihood that the lack of response exhibited during a canine trial was not by chance, was calculated. For both trials, the canines did not alert to any of the snapdragon flowers, and therefore their NPV was calculated to be 100%. As a result, the fact that the canines did not alert to the flowers was not by chance. These results are in agreement with similar studies conducted by Furton et al. in which various perfume samples, known to contain methyl benzoate were presented to narcotics detection canines to determine their false alert rates. As with this study, canines failed to alert to the perfume samples, stating that canines can “readily distinguish these odors” from cocaine samples.⁵⁰

5.5.6. Statistical Analysis

The results from Figure 73 suggests that the canines did not recognize the odor profile emitted from the snapdragon flowers, either because the methyl benzoate produced by the flowers is overshadowed by the other VOCs produced or that the canine is able to differentiate between the pool of odors emitted by the flowers versus that of cocaine. An explanation for the canines’ performance during the trials was sought

through statistical analysis, namely a box-and-whisker plot and principal components analysis (PCA). Figure 74 shows a box-and-whisker plot, in which the percent making up the odor profile of cocaine is compared against that of the snapdragon flowers. This chart shows the range or spread of the data points, while also highlighting the median of each sample.¹²⁷ The results revealed that the percentage of methyl benzoate within the cocaine does not overlap the percentage of methyl benzoate produced by any of the snapdragons evaluated. According to Schoon and Haak, differences in the amount of odors may be perceived as qualitative differences by detection canines; therefore since the percentage of methyl benzoate produced by the flowers was exceedingly higher than the percentage of methyl benzoate dissipating from cocaine, the canines may perceive the snapdragon flowers as an unrecognizable odor, and thus not alert to the snapdragon flowers.³⁴

Figure 74: Box-and-whisker plot of the percentage of methyl benzoate within samples

Principle components analysis was performed using the type and abundances of VOCs detected from cocaine samples as well as from the Black Prince, Maryland True Pink,

Remebrandt, and Twinny Peach snapdragon flowers represented in Figure 70. Each set of triplicates from Figure 70 were averaged and the data was normalized before conducting PCA analysis in order to account for error and give equal weight to each variable. Figure 75 shows the results after conducting PCA. The statistical tool revealed three distinct clusters which are representative the cocaine sample, Twinny Peach cut at Day 10 after anthesis, and the rest of the snapdragon flowers collected Days 2, 6, and 10 days after anthesis. The tight cluster formations reveal similarities within the data set, while spread data points, or distance from clusters reveals dissimilarities. Thus, the cluster of the snapdragon flowers represents that the odor profiles of these snapdragons are statistically similar, but their distance from cocaine proves that they are statistically different from the odor profile of cocaine.

Figure 75: Principal Components Analysis (PCA) of snapdragon flowers and cocaine

Although the odor profile of the Twinny Peach at Day 10 is statistically different from the other snapdragon flowers, it is also statistically distinct from cocaine as well. The statistical dissimilarity of Twinny Peach at Day 10 is also represented in Figure 70, as the Twinny Peach snapdragon was the least odiferous compared to the other three flowers, but Twinny Peach at Day 10, specifically, produced the lowest abundance of odors. This observation is possibly due to early pollination, which causes a decrease in odor production to discourage pollinators or it could also be from an environmental stressor, such as climate or water changes, which has been known to accelerate senescence, or flower death.^{70,143} The significant difference between the cocaine odor profile and that of the snapdragon flowers, along with the fact that the canines did not alert to the flowers, suggests that the pool of odor produced by the flowers aids in the canines' ability to differentiate between the snapdragon flowers and the cocaine samples, even if the active odorant is present in both samples. Since the active odor of illicit materials depends on the canines' olfactory receptor response, and not necessarily the most prominent odor present, it is possible that multiple odors produced by the snapdragon flowers induce an olfactory response, while for cocaine, it is just methyl benzoate. This would allow for canines to interpret the odors produced by the snapdragons as an entirely different scent than that belonging to cocaine.^{44,144}

6. CONCLUSION

While detection canines have proven to be a valuable tool for rapid, on-site detection, their abilities have frequently been called into question. In the Supreme Court

case, *State of Florida v. Harris*, controversy over the capabilities of each specific detection canine was brought to light and the use of continuous monitoring of canines' ability, detection thresholds, and successes in the field are necessary to increase the canines' strength in court.¹ To move towards accomplishing this feat, the goal of this project was to develop new training aids that would not only produce comparable results to the commercially available training aids but also improve upon some of their shortcomings, such as their short shelf-life and their inability for odor dissipation manipulation.

Novel training aids were created using sol-gel technology. The optimization of these polymers was accomplished by varying the molar ratios between the following sol-gel ingredients: precursor, water, solvent, and catalysts. It was found that the most suitable sol-gel polymer for this application was through the use of phenyltrimethoxysilane (PeTMS) as the precursor, because of its creation of a less rigid sol-gel network and because of its ability for *pi-pi* interactions to form between the methyl benzoate template and the precursors' benzene-containing substituent. In addition, a dual catalyst system was used, utilizing hydrochloric acid and ammonium hydroxide to speed up both the hydrolysis and condensation reactions to produce polymers in a timely matter. The created polymers proved to be rugged and reproducible in the field, able to be shipped without breaking and able to withstand extreme temperatures and solvent exposure without loss of sample or polymer shape.

Imprinted sol-gel training aids were directly compared to existing training aids Controlled Odor Mimic Permeation System (COMPS), created by Florida International University.⁵⁷ Using SPME/GC-MS, the percent loss of methyl benzoate from the training

aids were compared over time. In addition, the dissipation rates of both the sol-gel training aids and the COMPS were compared. The sol-gel training aid proved to last longer, dissipating at a rate of 55 ng/s, with COMPS dissipating much more quickly, at a rate of 361 ng/s (Figure 39 and Figure 41). In addition, this portion of the project showed that the dissipation of methyl benzoate from the sol-gel training aids can be manipulated through outside influences, such as with low density polyethylene bags (LDPE), utilized in COMPS technology, and through temperature changes.⁵⁷ Sol-gel training aids covered with a 2 mil LDPE bags dissipated methyl benzoate at a rate of 17 ng/s, while when using 4 mil LDPE bags, methyl benzoate dissipated at a rate 21 ng/s (Figure 44). When exposing the sol-gel training aid to temperature changes, which is an impossible task for COMPS, at 30°C, methyl benzoate dissipated at a rate of 167 ng/s, while at 50°C, methyl benzoate dissipated at a rate of 833 ng/s (Figure 46).⁵⁷ Most notably, when placing sol-gel training aids on dry ice, the dissipation of methyl benzoate ceases, which is highly advantageous for shipping purposes, as it will prevent loss of sample during transit (Figure 47).

Training aids utilizing sol-gel technology also demonstrated the ability to manipulate dissipation rates using modifications to the original sol-gel recipe. Changes to the original sol-gel polymers were performed by varying the recipe ingredients (precursors and solvents) or molar ratios of ingredients (water molarity). Results concluded that the dissipation of methyl benzoate, or any imprinted odor, can be manipulated based on the sol-gel network created. For example, three sol-gel precursors were compared: TMOS, TEOS, and PeTMS. After 24 hours, TEOS and TMOS lost 98% of their total imprinted methyl benzoate, while PeTMS only lost 41.6% (Figure 49).

When mixing TMOS and PeTMS, not only does the rigidity of the network change, but the dissipation of methyl benzoate changes as well. Results also demonstrated that using a nonpolar solvent worked to improve the dissipation, with a 29.2% loss of methyl benzoate after 24 hours (compared to polar solvent, which lost 41.6%) (Figure 53). Finally, varying the moles of water used also changed the dissipation rate of methyl benzoate, with 1 mole of water producing the best results, with an 18.9% loss of methyl benzoate after 24 hours (Figure 55).

The optimized sol-gels created in Task 1 were presented to specially trained narcotics detection canines. The effectivity of these sol-gel training aids was compared against COMPS. Sol-gel training aids imprinted with 50 mg of methyl benzoate achieved an 81.3% “interest + alert rate,” while COMPS achieved a 90.6% “alert + interest” response (Figure 59). While COMPS outperformed the sol-gel training aids, the results proved to be comparable and, with the potential advantages that the sol-gel training aids brings to canine training, including their longevity and ability for dissipation manipulation, further optimization could allow these training aids to be successfully utilized in the field.

Finally, this study investigated the field accuracy of canines. In the Supreme Court case, *State of Florida v. Jardines*, the reliability of detection canines was called into question when it was stated that the active odor of cocaine, methyl benzoate, is produced from common landscaping flowers, such as snapdragons.^{3,60} Since previous literature has proven that it is methyl benzoate, and not actual cocaine that elicits an alert from a detection canine, the question arose as to whether snapdragon flowers could result in a false alert from specially trained narcotics detection canines.⁵⁰ Overall this study

sought to evaluate a detection canine's ability to distinguish between cocaine and snapdragon flowers.

To complete this portion of the study, VOCs from four different snapdragon flowers, Black Prince, Maryland True Pink, Rembrandt, and Twinny Peach were evaluated using HS-SPME/GC-MS and compared to those dissipating from cocaine samples. Results showed that methyl benzoate was, in fact, produced by each snapdragon flower, but was not necessarily the most significant contributor to the flowers' odor profile. In some cases, β -cis ocimene, β -myrcene, 2,6-dimethyl-2,4,6-octatriene, and acetophenone were more prevalent than methyl benzoate, while in other flowers, methyl benzoate and β -cis ocimene contributed equally to the odor profile (Figure 70). While methyl benzoate was the dominant odor for the Twinny Peach snapdragon, this flower, specifically, proved to be the least odiferous.

The persistence over time of the production of methyl benzoate once flowers were cut was determined in order to simulate a floral bouquet/arrangement. Results revealed that, on average, odors only persisted four days after cutting, with some variation depending on the age of the flower at the time of harvesting (Figure 72). Upon learning that it takes about four days for out-of-season snapdragon flowers to be shipped and received by the recipient, flowers may cease to dissipate methyl benzoate at the time of reception.

Snapdragon flowers were presented to 21 canine teams to determine if it would result in a positive alert. Growing, potted plants, and cut flowers were presented to these canine teams. For both potted and cut flowers, canines alerted at a rate of 0%, while alerting 100% of the time to the positive control, cocaine (Figure 73). The box-and-

whisker plot showed that the percent of methyl benzoate contained in cocaine did not overlap with the methyl benzoate produced by any of the snapdragon flowers (Figure 74) and PCA revealed that the odor profiles of the snapdragon flowers were statistically different from cocaine's odor profile (Figure 75). Because of the statistical difference in the dissipating odors from cocaine and snapdragon flowers, certified narcotics detection canines did not positively alert to the snapdragon flowers because of their ability to differentiate between the two pools of odors.

In conclusion, the results obtained from this research project has provided a new tool for detection canine training with the ability for canine handlers to manipulate the dissipation rates of odors presented to their canines. In addition, the reliability of detection canines, in terms of their accuracy in the field has been proven, showing that canines can differentiate from similar odors pools that contain the active odor compound of an illicit material. Both of these results have given insight into the capabilities of detection canines, which ultimately improves their validity in court.

7. REFERENCES

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8. APPENDICES

The appendix contains the temperature and relative humidity changes that occurred within the lab during Tasks 1 and 2 (Table 16 and Table 17). Sol-gel recipes attempted during the optimization process of Task 1 are shown, including the molar ratios between each ingredient (Table 18, Table 19, Table 20, Table 21, Table 22, and Table 23). Finally, the results of all canine trials, for both the sol-gel training aids and the snapdragon flowers are displayed in Table 24, Table 25, and Table 26.

Table 16: Temperature (°C) and relative humidity (%) ranges throughout all Task 2 experiments

Trial	Temperature Range (°C)	Relative Humidity Range (%)
COMPS Dissipation Trial 1	23.594 – 21.593	48.666 – 69.942
COMPS Dissipation Trial 2	23.588 – 21.587	49.783 – 57.613
Sol-Gel Dissipation Trial 1	24.088 – 21.587	46.078 – 70.805
Sol-Gel Dissipation Trial 2	23.588 – 21.587	51.005 – 58.204
Sol-Gel with LDPE 2 mil Bags	22.626 – 19.123	35.439 – 55.355
Sol-Gel with LDPE 4 mil Bags	24.094 – 21.593	46.813 – 63.046

Table 17: Temperature (°C) and relative humidity (%) ranges throughout all Task 3 experiments

Sol- Gel Manipulations	Temperature Range (°C)	Relative Humidity Range (%)
Control (100% PeTMS, Polar Solvent, 5 moles water)	23.084 – 24.584	49.584 – 59.521
100% TMOS Precursor	20.592 – 22.593	33.500 – 53.547
100% TEOS Precursor	21.087 – 22.587	46.699 – 54.633
70% PeTMS and 30% TMOS Precursor	22.587 – 25.588	46.078 – 55.232
60% PeTMS and 40% TMOS Precursor	22.087 – 26.088	44.204 – 57.613
50% PeTMS and 50% TMOS	22.587 – 26.088	42.315 – 55.232
Nonpolar Solvent	23.084 – 25.084	51.675 – 73.262
1 moles of water	22.095 – 24.096	40.129 – 57.290
3 moles of water	22.587 – 26.088	42.315 – 55.232

Table 18: Initial optimization, showing the molar ratios of each ingredient attempted. Recipes highlighted in dark green solicited, while those in red did not solidify over the allotted time period. Those highlighted in light green became more viscous but never fully solidified.

Experiment	TEOS	TMOS	MTMOS	Methanol	Ethanol	NaOH ($\times 10^{-3}$)	NH ₄ OH ($\times 10^{-3}$)	HCl ($\times 10^{-3}$)	TFA ($\times 10^{-3}$)	Water	Time Period
1	1				3.6	1.9				1	Overnight
2	1				3.6	3.6				2	Overnight
3	1				3.6	5.5				3	Overnight
4	1				3.6	7.1				4	Overnight
5		1		3.75		1.8				1	Overnight
6		1		3.75		3.5				2	Overnight
7		1		3.75		5.3				3	Overnight
8		1		3.75		7				4	Overnight
9			1	3.7		1.8				1	Overnight
10			1	3.7		3.4				2	Overnight
11			1	3.7		5.4				3	Overnight
12	1				3.6		1			1	Overnight
13	1				3.6		1.9			2	Overnight
14	1				3.6		3			3	Overnight
15	1				3.6		3.9			4	Overnight
16		1		3.75			1			1	Overnight
17		1		3.75			1.9			2	Overnight
18		1		3.75			2.9			3	Overnight
19		1		3.75			3.8			4	Overnight
20			1	3.7			1			1	Overnight
21			1	3.7			1.9			2	Overnight
22			1	3.7			2.9			3	Overnight
23	1				3.6			0.7		1	Overnight
24	1				3.6			1.3		2	Overnight
25	1				3.6			2		3	Overnight
26	1				3.6			2.6		4	Overnight
27		1		3.75				0.7		1	Overnight
28		1		3.75				1.3		2	Overnight
29		1		3.75				2		3	Overnight
30		1		3.75				2.6		4	Overnight
31			1	3.7				0.7		1	Overnight
32			1	3.7				1.3		2	Overnight
33			1	3.7				2		3	Overnight
34	1				2.7				520	1	Overnight
35	1				2.7				520	2	Overnight
36	1				2.7				520	3	Overnight
37	1				2.7				520	4	Overnight
38		1		2.8					376	1	Overnight
39		1		2.8					376	2	Overnight
40		1		2.8					376	3	Overnight
41		1		2.8					376	4	Overnight
42			1	2.8					371	1	Overnight
43			1	2.8					371	2	Overnight
44			1	2.8					371	3	Overnight
45		1		12.2			543			2	Overnight
46		1		12			7.4			4	Overnight
47		0.9	0.1	12			7.4			4	Overnight
48		0.8	0.2	12			7.4			4	Overnight
49		0.7	0.3	12			7.4			4	Overnight
50		0.6	0.4	12			7.4			4	Overnight
51		0.5	0.5	12			7.4			4	Overnight
52	1				0.56			6.2			3 days
53		1			0.56			6.2			3 days
54		1		4			1.8			1	2 hours
55		1		4			3.6			2	1 hour
56		1		4			5.4			3	1 hour
57		1		4			7.2			4	1 hour
58		1		4		1.8				1	1 hour
59		1		4		3.6				2	1 hour
60		1		4		5.4				3	1 hour
61		1		4		7.2				4	1 hour

Table 19: Sol-gel polymer optimization using a dual catalyst system, showing the molar ratios of each ingredient attempted. Recipes highlighted in dark green solicited, while those in red did not solidify over the allotted time period

Experiment	TEOS	TMOS	MTMOS	Methanol	Ethanol	NaOH ($\times 10^{-3}$)	NH ₄ OH ($\times 10^{-3}$)	HCl ($\times 10^{-3}$)	TFA ($\times 10^{-3}$)	Water	Time Period
62		1		4+1		4		1.8		1	Overnight
63		1		4+2		8.1		3.6		2	15 min
64		1		4+3		12.2		5.4		3	10 min
65		1		4+4		16.2		7.2		4	10 min
66		1		4+1			4	1.8		1	Overnight
67		1		4+2			8.1	3.6		2	immediate
68		1		4+3			12.2	5.4		3	immediate
69		1		4+4			16.2	7.2		4	immediate

Table 20: Sol-gel polymer optimization when varying the amount of solvent added, showing the molar ratios of each ingredient. Recipes highlighted in dark green solicited, while those in red did not solidify over the allotted time period

Experiment	TEOS	TMOS	MTMOS	Methanol	Ethanol	NaOH ($\times 10^{-3}$)	NH ₄ OH ($\times 10^{-3}$)	HCl ($\times 10^{-3}$)	TFA ($\times 10^{-3}$)	Water	Time Period
70		1		4+4		16.2		7.2		4	less than 10 min
71		1		4+5		16.2		7.2		4	less than 10 min
72		1		4+6		16.2		7.2		4	less than 10 min
73		1		4+7		16.2		7.2		4	less than 10 min
74		1		4+8		16.2		7.2		4	less than 10 min
75		1		4+4			16.2	7.2		4	immediate
76		1		4+5			16.2	7.2		4	10 min
77		1		4+6			16.2	7.2		4	10 min
78		1		4+7			16.2	7.2		4	10 min
79		1		4+8			16.2	7.2		4	10 min
80		1		4+20			14	7.2		4	2 hours
81		1		4+15			14	7.2		4	2 hours
82		1		4+10			14	7.2		4	2 hours
83		1		4+5			14	7.2		4	2 hours
84		1		3+5			14	7.2		4	2 hours
85		1		2+5			14	7.2		4	2 hours
86		1		1+5			14	7.2		4	2 hours
87		1		4+1			14	7.2		4	2 hours
88		1		4+2			14	7.2		4	2 hours
89		1		4+3			14	7.2		4	2 hours
90		1		4+4			14	7.2		4	2 hours
91		1		1+3.8			14	7.2		4	2 hours
92		1		2+3.8			14	7.2		4	36 min
93		1		3+3.8			14	7.2		4	38 min
94		1		4+3.8			14	7.2		4	2 hours
95		1		1+3.6			14	7.2		4	< 5 min
96		1		2+3.6			14	7.2		4	< 5 min
97		1		3+3.6			14	7.2		4	< 5 min
98		1		4+3.6			14	7.2		4	< 5 min
99		1		1+3.9			14	7.2		4	2 hours
100		1		2+3.9			14	7.2		4	2 hours
101		1		3+3.9			14	7.2		4	2 hours
102		1		4+3.9			14	7.2		4	2 hours

Table 21: Sol-gel polymer optimization when varying the amount base added, showing the molar ratios of each ingredient. Recipes highlighted in dark green solicited, while those in red did not solidify over the allotted time period. Those highlighted in light green became more viscous but never fully solidified.

Experiment	TEOS	TMOS	MTMOS	Methanol	Ethanol	NaOH ($\times 10^{-3}$)	NH ₄ OH ($\times 10^{-3}$)	HCl ($\times 10^{-3}$)	TFA ($\times 10^{-3}$)	Water	Time Period
103		1		8+2			8.4	3.6		2	30 min
104		1		8+2		8.1		3.6		2	10 min
105		1		4+3.2		13		7.2		4	11 min
106		1		4+3.5		14		7.2		4	5 min
107		1		4+3.7		15		7.2		4	5 min
108		1		4+4		16		7.2		4	5 min
109		1		4+3.2			13	7.2		4	3 hours
110		1		4+3.5			14	7.2		4	51 min
111		1		4+3.7			15	7.2		4	10 min
112		1		4+4			16	7.2		4	7 min
113		1		4+3.31			13.4	7.2		4	25 min
114		1		4+3.25			13.2	7.2		4	1 hr 55 min
115		1		4+3.18			12.9	7.2		4	1 hr 50 min
116		1		4+3.12			12.7	7.2		4	3 hours
117		1		4+3.06			12.4	7.2		4	1 hr 45 min
118		1		4+3.00			12.2	7.2		4	3 hours

Table 22: Sol-gel polymer optimization when varying the concentrations of catalyst stock solutions and implementing the use of a refrigerated centrifuge, showing the molar ratios of each ingredient. Recipes highlighted in dark green solicited, while those in red did not solidify over the allotted time period.

Experiment	TMOS	Methanol	NH ₄ OH (x10 ⁻³)	HCl (x10 ⁻³)	Water	Temp (°C)	Time	Conc Base	Conc Acid
119	1	7+3.8	14	5.42	3	30	3 min	0.09	0.1
120	1	7+3.8	14	5.42	3	5	4 min	0.09	0.1
121	1	7+3.8	14	9	5	30	3 min	0.09	0.1
122	1	7+3.8	14	9	5	5	20 min	0.09	0.1
123	1	7+3.8	14	7.2	4	5	10 min	0.09	0.1
124	1	7+3.8	14	14.4	4	5	Overnight	0.09	0.2
125	1	7+3.8	14	21.6	4	5	Overnight	0.09	0.3
126	1	7+3.8	14	28.8	4	5	Overnight	0.09	0.4
127	1	7+3.8	14	35.9	4	5	Overnight	0.09	0.5
128	1	2+3.8	14	10.7	4	30	Overnight	0.09	0.15
129	1	4+3.8	14	10.7	4	30	8 min	0.09	0.15
130	1	7+3.8	14	10.7	4	30	15 min	0.09	0.15
131	1	2+3.8	14	14.4	4	30	Overnight	0.09	0.2
132	1	4+3.8	14	14.4	4	30	Overnight	0.09	0.2
133	1	7+3.8	14	14.4	4	30	Overnight	0.09	0.2
134	1	4+3.8	14	10.7	4	5	~1 hr	0.09	0.15
135	1	7+3.8	14	10.7	4	5	1 hr 20 min	0.09	0.15
136	1	4+3.8	14	12.9	4	5	Overnight	0.09	0.18
137	1	7+3.8	14	12.9	4	5	Overnight	0.09	0.18
138	1	2+3.8	14	7.2	4	35	10 min	.09 M	0.1
139	1	3+3.8	14	7.2	4	35	10 min	.09 M	0.1
140	1	4+3.8	14	7.2	4	35	10 min	.09 M	0.1
141	1	2+3.8	14	7.2	4	30	10 min	.09 M	0.1
142	1	3+3.8	14	7.2	4	30	10 min	.09 M	0.1
143	1	4+3.8	14	7.2	4	30	10 min	.09 M	0.1
144	1	2+3.8	14	7.2	4	30	10 min	.09 M	0.1
145	1	3+3.8	14	7.2	4	30	10 min	.09 M	0.1
146	1	4+3.8	14	7.2	4	30	10 min	.09 M	0.1
147	1	2+4	14	7.2	4	30	overnight	.085 M	0.1
148	1	3+4	14	7.2	4	30	overnight	.085 M	0.1
149	1	4+4	14	7.2	4	30	overnight	.085 M	0.1
150	1	2+5	14	7.2	4	30	overnight	.085M	0.1
151	1	3+5	14	7.2	4	30	overnight	.085 M	0.1
152	1	4+5	14	7.2	4	30	overnight	.085 M	0.1
153	1	2+4	14	7.2	4	35	overnight	.085 M	0.1
154	1	3+4	14	7.2	4	35	overnight	.085 M	0.1
155	1	4+4	14	7.2	4	35	overnight	.085 M	0.1

Table 23: Sol-gel polymer optimization when implementing the use of a refrigerated centrifuge, showing the molar ratios of each ingredient. Recipes highlighted in dark green solicited, while those in red did not solidify over the allotted time period.

Experiment	TMOS	Methanol	NaOH ($\times 10^{-3}$)	NH ₄ OH ($\times 10^{-3}$)	HCl ($\times 10^{-3}$)	Water	Temp (°C)	Time Period
156	1	4+3.9		16	7.2	4	15	< 6 min
157	1	6+3.9		16	7.2	4	15	7 min
158	1	8+3.9		16	7.2	4	15	13 min
159	1	4+3.7		15	7.2	4	15	15 min
160	1	6+3.7		15	7.2	4	15	20 min
161	1	8+3.7		15	7.2	4	15	25 min
162	1	4+3.4		14	7.2	4	15	34 min
163	1	6+3.4		14	7.2	4	15	41 min
164	1	8+3.4		14	7.2	4	15	58 min

Table 24: Canine trials results: comparison between sol-gel polymer training aids and IFRI COMPS; where “x” signifies a canine alert and “i” signifies canine interest

Team	Date	Sol-Gel (~ 1 mg MB)	Sol-Gel (~ 50 mg MB)	IFRI COMPS	Blank Sol Gel	Cocaine (Positive Control)
A	2/10/2014	i	x	x		x
B	2/10/2014	x		x		x
C	2/10/2014		x	x		x
D	2/10/2014		i	x		x
E	7/16/2014					x
F	7/16/2014					x
G	7/16/2014					x
H	7/24/2014	x	x	x		x
B	7/24/2014	x	x	i		x
I	7/24/2014	x	x	x		x
J	7/31/2014	x	x	x		x
I	7/31/2014	x	x	x		x
K	7/31/2014	x	x	x		x
L	7/31/2014		x	x		x
M	8/27/2014	i	x	x		x
N	8/27/2014	x	i	x		x
O	8/27/2014	x	x	x		x
L	8/27/2014	x	x	x		x
K	9/3/2014	x	x	x	x	x
P	9/3/2014	x	x	x		x
Q	9/3/2014	x	x	x		x
L	9/3/2014	x	x	x		x
C	9/3/2014			x		x
A	9/3/2014	x	x	x		x
Q	9/24/2014	x	x	x		x
R	9/24/2014		x	x		x
S	9/24/2014	x	x	x		x
A	9/24/2014	x	x	x		x
B	9/24/2014	x	x	x		x
Q	9/17/2014		x	x		x
P	9/17/2014		x	i		x
R	9/17/2014			x		x

Table 25: Canine trial results: potted snapdragon flowers; where “x” signifies a canine alert

Team	Date	Twiny Peach	Rembrandt	Maryland Pnk	Black Prince	Cocaine (Positive Control)
A	4/10/2013					x
B	4/10/2013					x
C	4/10/2013					x
D	4/10/2013					x
A	4/15/2013					x
C	4/15/2013					x
D	4/15/2013					x
E	4/15/2013					x
A	4/17/2013					x
C	4/17/2013					x
D	4/17/2013					x
F	4/17/2013					x
G	4/17/2013					x
H	4/17/2013					x
I	4/17/2013					x
A	4/23/2013					x
E	4/23/2013					x
F	4/23/2013					x
H	4/23/2013					x
J	4/23/2013					x
K	4/23/2013					x
L	4/23/2013					x
M	4/23/2013					x
E	5/2/2013					x
H	5/2/2013					x
K	5/2/2013					x
N	5/2/2013					x
F	5/8/2013					x
G	5/8/2013					x
H	5/8/2013					x

Table 26: Canine trial results: cut snapdragon flowers; where “x” signifies a canine alert

Team	Date	Twinny Peach	Rembrandt	Maryland Pink	Black Prince	Cocaine (Positive Control)
H	5/29/2013					x
B	5/29/2013					x
O	5/29/2013					x
P	6/5/2013					x
P	6/5/2013					x
Q	6/5/2013					x
Q	6/5/2013					x
E	6/5/2013					x
R	6/5/2013					x
S	6/19/2013					x
T	6/19/2013					x
U	6/19/2013					x
V	6/19/2013					x
W	6/19/2013					x

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PUBLICATIONS AND PRESENTATIONS

M. Cerreta and K.G. Furton; "Detection Canine Field Accuracy to Flowers Producing Methyl Benzoate, a Cocaine Odorant" (*Submitted, Forensic Science International*)

N.I. Caraballo, M. Cerreta, K. Hentschel, D. Wescott, and K.G. Furton; "An Evaluation of the Volatile Organic Compounds Released from Decomposing Human Remains" (*In Preparation*)

N.I. Caraballo, M. Cerreta, and K.G. Furton "An Assessment on the Influence that Soil has on the Release of Decomposition-Associated Volatile Organic Compounds from Buried Remains" (*In Preparation*)

M. Cerreta and K.G. Furton; Detection; An Assessment of Field Accuracy of Detection Canines Using Flowers that Produce the Same Odorant as Cocaine; Florida Annual Meeting and Exposition (FAME), Tampa, FL; May 2014

M. Cerreta and K.G. Furton; Detection Canine Field Accuracy to Flowers Producing Methyl Benzoate, a Cocaine Odorant; International Forensic Research Institute Symposium; Florida International University, Miami, FL; April 2014

M. Cerreta and K.G. Furton; Detection Canine Field Accuracy to Flowers Producing Methyl Benzoate, a Cocaine Odorant; 66th Annual American Academy of Forensic Sciences (AAFS) Meeting; Seattle, WA; February 2014

K. Beltz, M. Cerreta, and K.G. Furton; The Development of Field Calibrants for Detection Canines; 65th Annual American Academy of Forensic Sciences Meeting (AAFS), Washington, DC; February 2013

M. Cerreta, A. Kabir, and K.G. Furton; The Development of a Novel Sol Gel Polymeric Network for the Molecular Imprinting of Illicit Drugs; Florida Annual Meeting and Exposition (FAME), Tampa, FL; May 2012

M. Cerreta, A. Kabir, and K.G. Furton; The Development of a Novel Sol Gel Polymeric Network for the Molecular Imprinting of Illicit Drugs; International Forensic Research Institute Symposium; Florida International University, Miami, FL; March 2012

M. Cerreta, A. Kabir, and K.G. Furton; The Development of a Novel Sol Gel Polymeric Network for the Molecular Imprinting of Illicit Drugs; PITTCO, Orlando, FL; March 2012

M. Cerreta, A. Kabir, and K.G. Furton; The Development of a Novel Sol Gel Polymeric Network for the Molecular Imprinting of Illicit Drugs; 64th Annual American Academy of Forensic Sciences Meeting (AAFS), Atlanta, GA; February 2012