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Profiling miniaturized separation media by micro infrared spectroscopy

Kelsi Michel Perttula
San Jose State University

PROFILING MINIATURIZED SEPARATION MEDIA BY MICRO INFRARED SPECTROSCOPY

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Kelsi M. Perttula

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by

Kelsi Perttula

APPROVED FOR THE DEPARTMENT OF CHEMISTRY

SAN JOSÉ STATE UNIVERSITY

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Professor Joseph Pesek	Department of Chemistry
Professor Roger Terrill	Department of Chemistry
Professor Marc d'Alarcao	Department of Chemistry

ABSTRACT

PROFILING MINIATURIZED SEPARATION MEDIA BY MICRO INFRARED SPECTROSCOPY

by Kelsi M. Perttula

Separation methods have been miniaturized in recent years. Capillary electrophoresis is now common and other types of analyses are being done on microfluidic chips with capillary channels in lieu of wider column diameters. The surfaces of the media used for separation are chemically modified. Using infrared spectroscopy by a microscope attachment, attempts were made to characterize the surfaces of both capillary channels and modified polymer for microfluidic chips. FTIR microscopy is not common in this type of analysis and is normally used to analyze particles, such as fibers, paint, and other polymer sources. Since modification of miniaturized chemical media is done on a microscopic scale, the FTIR microscope seemed promising for the detection of these changes. However, there was great difficulty in detecting the target material against the background material. Thus, micro-infrared spectroscopy is not an ideal method for characterizing modified separation media, despite its ability to sample small areas. Additionally, a novel medium for microfluidic chips, a material made of polymethylhydrosiloxane (PMHS), was described. PMHS has an advantage over other chip media in that the surface can be chemically modified, similar to capillary electrophoresis.

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CHAPTER 1. INTRODUCTION

Infrared spectroscopy is used to obtain chemical information about various substances. Wavelengths covering the mid infrared (4000 cm^{-1} to 400 cm^{-1}) are used to measure transitions with infrared spectroscopy. However, certain properties are required for infrared absorption to occur. Infrared light of a specific frequency is absorbed at the identical frequency of an asymmetric vibration or resonance of the molecule. During this vibration, the dipole of the molecule must change in order to absorb the infrared radiation[1]. Fourier Transform Infrared Spectroscopy (FTIR) describes how the spectral information in the time domain is converted to the frequency domain. The term FTIR is commonly used interchangeably with infrared spectroscopy.

Infrared radiation triggers two vibrational modes: bending and stretching. Bending vibrations occur at lower energy and are usually part of the "fingerprint" region of an infrared spectrum, below 1500 wavenumbers (cm^{-1}). Stretching vibrations require more energy and occur at higher frequencies, or larger wavenumbers. The region from 4000-1500 cm^{-1} is where most functional group stretching vibrations occur, allowing them to be identified. The stretching vibrations for various functional groups, including terminal alkenes, but also nonterminal alkenes, alkynes, and cyano-group-containing compounds occur reliably in this region. Thus, this is the region that is often used for detecting specific functional groups. The fingerprint region is complex but highly unique. With the help of library searches, this region may be used to identify the whole molecule being analyzed.

An FTIR spectrometer is a somewhat simple instrument compared to other analytical tools. The instrument begins with a high intensity, broadband IR source that is projected into an interferometer, which has a beamsplitter that reflects half of the IR energy to a fixed mirror and half to a moving mirror. The moving mirror scans back and forth to create different path lengths with respect to the fixed mirror[1]. The reflected beams pass through the sample, which may absorb some of the infrared energy if there are bonds of corresponding energy.

The detector then measures the intensity of the infrared light and produces an interferogram. The interferogram is a sum of the waves from the different frequencies with respect to the mirror position. However, this information, which is in the time domain, is not very informative. Upon transforming the interferogram with a Fast Fourier Transform (FFT), the information is converted into a spectrum in the frequency domain. These spectra are used to analyze samples and are compared with other spectra.

Due to the presence of water vapor and carbon dioxide, both infrared absorbing molecules, background samples are necessary for infrared spectroscopic analysis. The ratio of energy from the sample to the background equals the transmittance spectrum that is used for interpretation and comparisons.

Infrared spectroscopy can be used for qualitative information to identify unknown substances as well as relative quantitative information to measure the amount of substance. Although qualitative analysis is the more common use of FTIR analysis,

concentrations can be determined by the Beer-Lambert law, which states that the absorption measured by FTIR is proportional to the concentration of the compound.

$$\text{Absorbance} = \text{Absorptivity } (M^{-1}cm^{-1}) * \text{Pathlength (cm)} * \text{Concentration (M)} \qquad (1)$$

However, for the purpose of these experiments, the FTIR data were primarily used for qualitative analysis, that is, to detect foreign compounds or functional groups on the substrate materials.

FTIR microscopy is most useful for samples that are less than 100 μm in size and for samples that cannot be physically separated. Since the modified analytical media analyzed were small and chemically reacted to the substrates, it seemed FTIR microscopy might be a promising method to analyze these samples.

Typically, FTIR microscopy is used for the analysis of polymers, pharmaceuticals, and forensic science samples. For multilayer polymers, such as vehicle paint, samples can be carved exposing different areas of the paint, and spectra can be compiled for all of the different layers in a short amount of time. The precise area of sampling as well as the maneuverability of the microscope stage makes this an ideal method for these types of samples.

This work was performed at the San Mateo County Sheriff's Office Forensic Laboratory. The instrument setup is most commonly used for the analysis of synthetic fibers in trace evidence cases. The fibers are typically identified with the use of polarized

light microscopy, but the polymer type is often confirmed with FTIR microscopy. Additional types of forensic samples are often analyzed with this instrument, such as vehicle paint and pressure sensitive tapes. Both may have multiple types of polymers, which would be otherwise difficult to analyze. Still, the different polymers must be separated with microsampling to be analyzed as discrete FTIR samples. Suspected narcotics may also be analyzed on this instrument, for ease of sampling and for scenarios where there is a very limited amount of sample. However, FTIR microscopy may also be useful for a variety of other applications in other industries. Pharmaceutical labs and other synthetic laboratories, which may be working on a microscale, would usually prefer not to consume as much sample as would be required with traditional FTIR spectroscopy. FTIR microscopy is a viable alternative for these types of laboratories.

Separation chemistry methods are essential to isolate, purify, and analyze chemicals. Traditional separation chemistry techniques include high-pressure liquid chromatography (HPLC) and electrophoresis. Since their inception, these techniques have been continually improved upon. One somewhat recent improvement is miniaturization.

Capillary electrophoresis has replaced traditional electrophoresis in most applications allowing for far more accurate analyses with extreme precision. Plain fused silica capillaries are the traditional medium for capillary electrophoresis, however, Pesek et al.[2] and others have developed modified capillaries using chemically etched fused silica and modifying the silanols on the surface which can then be further reacted to

attach molecules of varying solubility and chemical properties.[2] The etching occurs in the presence of ammonium hydrogen difluoride (NH_4HF_2) and increases the surface area of the capillary by a factor of one thousand.[2] The silanols are modified through a step called silanization, where the polar silanols of the fused capillary are treated with triethoxysilane in the presence of an acid as a catalyst to produce up to 95% silica hydride groups on the reacted surface. The silica hydride groups are non-polar compared to the silanols, and have value as a high pressure capillary electrophoresis (HPCE) surface on their own.

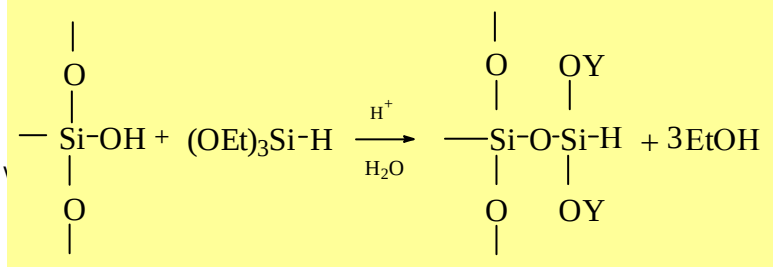

Figure 1. Silanization of silanols.

The next step for modifying the silica hydrides is hydrosilation (see Figure 2). This is accomplished by reacting a hexachloroplatinic acid catalyst or a free radical initiator and an organic compound. The silica hydride intermediate may be reacted with alkenes, alkynes, alcohols, or another electron rich group.

The result of this reaction is the new organic functional group covalently bonded to the silica substrate. Hydrosilation is quite successful in general, but due to steric interactions, 10-50% of the original silica hydrides remain.

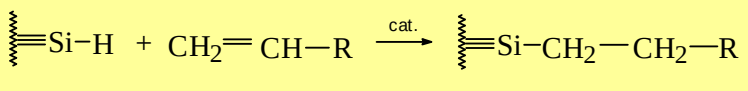

Figure 2. Hydrosilation and the resulting variety of stationary phases.

The unreacted silica hydrides and silanols may not decrease the usefulness of the modified column or capillaries. However, the fact that there may be a significant portion of the IR-absorbing silanols present on the modified surface may further interfere with detection of the modified components with FTIR microscopy. Actually the SiO2 bulk of the capillary will produce a great deal more IR interference than any surface layer.

These modified capillaries broaden the range of types of electrophoresis and HPLC with a capillary, also known as HPCE. The attached molecules change the surface characteristics, which then alter the electroosmotic flow for that particular capillary, or the chemical interactions of the compounds being eluted. Thus, the silanization and hydrosilation reactions on the fused silica allow for different types of compounds to be analyzed on the appropriate type of capillaries.

Another field of chemical analysis miniaturization is rapidly developing. Currently emerging is the continued research and development of microfluidic chips, wherein various chemical reactions and analytical techniques can take place on a space

sometimes no bigger than a business card. Microfluidics, also known as a "laboratory on a chip", has opened countless opportunities to improve chemical analysis on a miniature scale. On a smaller scale, chemical reactions are often more efficient, producing more pure products, more quickly, with less waste. All of these advantages make microfluidics a tool for a vast number of possibilities for chemical analysis, from genetic analysis to synthetic chemistry to HPLC.

First developed by Richard Mathies at UC Berkeley,[3] microfluidic techniques are being used for genetic analysis with miniaturized capillary array electrophoresis microchannel plates. The advantages to the miniaturization of this method are numerous, including decreased volume, greater sensitivity, and faster analysis. In fact, with the radial design of the chip, it is possible to analyze 96 samples simultaneously. Traditional genetic electrophoresis analyzes each sample serially, at a rate of 30 min or more per injection. At the completion of the electrophoresis, the DNA fragments, which have been PCR-amplified with fluorescing dyes, are analyzed with a radial fluorescence scanner. The scanner detects the dyes and compares their electrophoresis times with the internal size standard of known DNA fragments. This miniaturized process is very similar to that which occurs in the macroscale version; however, the capillary version is more sensitive and precise.

Physically, the microfluidic devices may be as small as 150 mm in diameter, with channels as small as 30 μm in diameter. HPLC is another technique that is being miniaturized to exist on a microfluidic chip.

The first microfluidic chips were constructed with microfabrication techniques through a series of masking and etching very similar to the fabrication of microchips used in electrical engineering and computers.[4] Since then, miniaturization has been applied to other chemical methods, including separation techniques to purify other types of analytes. The substrate materials for the chips were further developed, and fused silica and polymers have been explored as microfluidic chip substrates.

Polydimethylsiloxane (PDMS) was developed by Hasselbrink *et al*.[5] as a substrate to make microfluidic chips. This allowed for the manufacturing of moveable valves and other parts within the chip, previously not possible with the microfabrication of fused silica chips.

As discussed previously regarding the modified fused silica HPLC columns, converting the silanols into silica hydrides (Figure 1) on the traditional HPLC cross-linked silica greatly changes the surface charge of the columns and adds a new class of chemicals which may be analyzed on HPLC columns. Furthermore, as seen with the silica capillaries, silica hydride allows for further modification via hydrosilation (Figure 2) to create a variety of HPLC stationary phases.

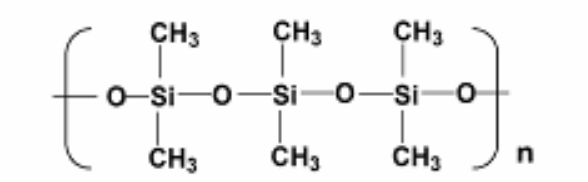

Figure 3. Structure of polydimethylhydrosiloxane (PDMS).

PDMS has many desirable qualities as the raw material for microfluidic chips as it allows for the manufacturing of fairly high-pressure valves and electrokinetic pumps. However, chemical modification is not among its benefits. With two methyl groups covalently bonded to each silicon, PDMS is quite a chemically inert polymer which limits the types of chemicals that can be separated and analyzed on miniature columns made with this material.

By contrast, polymethylhydrosiloxane (PMHS) can be modified, similar to the modifications discussed with fused silica. Since PMHS differs from PDMS by the presence of a hydride at each silicon atom, this material has similarities to fused silica, which has undergone silanization. In this case, most of the silanols have been converted into silica hydrides, which have their own set of unique chemical properties.

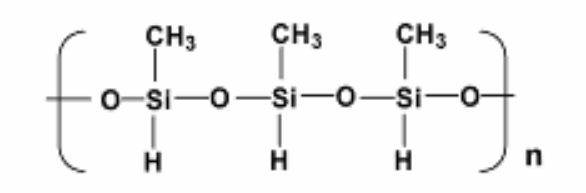

Figure 4. Structure of polymethylhydrosiloxane (PMHS)**.**

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Like the silica hydride silanization product (Figure 1), PMHS may be further reacted via hydrosilation (Figure 2) with a variety of organic moieties, such as the previously mentioned terminal alkenes or other appropriate moieties. Like hydrosilation in fused silica capillaries, this reaction occurs in the presence of hexachloroplatinic acid, which acts as a catalyst.

The resulting modified areas of PMHS could serve as miniature columns which could then allow for the separation and analysis of different compounds, depending on the resulting stationary phase.[6] For example, PMHS that has undergone hydrosilation with 1-octadecene results in a surface with C18 chains bonded to the silicon atoms. Miniature columns like these would be useful for microfluidic chip HPLC of non-polar compounds, as a smaller version of traditional reverse phase HPLC. However, countless possibilities exist with the ability to modify a stationary phase with so many different types of functional groups.

The goal in this work was to detect the resulting structure through targeted analysis of the modified areas of both the capillary and the simulated chip; that is, to determine if the modifications can be detected through the use of Fourier Transform Infrared Microscopy. Analysis of these types of modifications to the capillaries or microchannels can present problems.

Most notably, the substrate material, either fused silica in the case of capillaries or a polymer (PMHS), can mask the chemical signals of the modified surface. Because the presence of the fused silica in the capillaries and compositional polymer raw material for the microfluidic chip present detection challenges, several methods were attempted to determine if these modifications or other chemicals could be detected on these substrates.

Using FTIR to detect modified polymers has been done with attenuated total reflection FTIR spectroscopy (ATR FTIR). Gaboury and Urban used ATR FTIR to quantify silica hydride groups on PDMS treated with plasma.[7] Instead of hydrosilating PDMS to replace the methyl with silica hydrides, Gaboury and Urban treated PDMS with microwave plasma environments of argon and nitrogen gases, which modified the PDMS to acquire silica hydrides instead of only silicon-methyl methyl groups. They made calibration standards of known Si-H modified PDMS to quantify the unknown plasma-treated PDMS. These standards allowed the amount of silica hydride to the modified polymers films to be measured.

It is conceivable that ATR FTIR may be a preferable technique to measure such modifications. ATR has an effective penetration depth of a few hundred nanometers for each reflection. This makes ATR and ideal surface probe, and the multiple passes on the surface also increases the sensitivity for this FTIR technique. Additionally, ATR FTIR techniques often involve less sample preparation, compared to regular transmission FTIR, where over-absorbance can occur so readily.

ATR FTIR is ideal for quantitative analysis of thin films and surfaces as the infrared beam is totally reflected at the interface. Part of the beam also penetrates the surrounding medium, creating an evanescent wave that penetrates in and interacts with the medium as far as a few hundred nanometers.[8]

For bench FTIR, there are FTIR accessories available. For FTIR microscopy, ATR objective lenses are also available for purchase. However, these are optional accessories that are not required for work at the San Mateo County Sheriff's Office Forensic Laboratory; therefore, no such lenses were available for these experiments.

CHAPTER 2. EXPERIMENTAL

For the development of the surface characterization experiments, the first infrared microscopy instrument used was a Thermo-Nicolet (now known as ThermoFisher Scientific; Waltham, Massachusetts) 760 NIC-PLAN. This instrument is located at the San Mateo County Sheriff's Office Forensic Laboratory. At forensic laboratories, the microscope attachment is typically used by trace evidence and, sometimes, narcotic units in order to pinpoint tiny areas on small samples such as fine particles and fibers or limited quantities of narcotics. The attachment gives the user more control over where the FTIR light impinges on the samples. Although the Thermo-Nicolet and ThermoFisher FTIR microscopy configurations are very common in forensic laboratories, Perkin-Elmer makes a competitive FTIR microscope. Lesser-known configurations by Hyperion and Smiths are also available.

In 2008, the San Mateo County Sheriff's Office Forensic Laboratory upgraded the fifteen-year-old 760 NIC-PLAN instrument by replacing the microscope attachment with a ThermoFisher Scientific Continuum microscope (see Figure 5). In both setups, the instrument consisted of an FTIR bench unit with a microscope attachment where the infrared light is focused and directed through adjustable apertures, through a 15× microscope objective, and finally to the stage where samples are manipulated and positioned for FTIR absorption. The sampling area is constrained by the wavelength of infrared light. The sampling dimensions cannot approach the size of the light waves

passing through the apertures. Thus, aperture windows are usually kept to a minimum area of 10,000 nm x 10,000 nm.

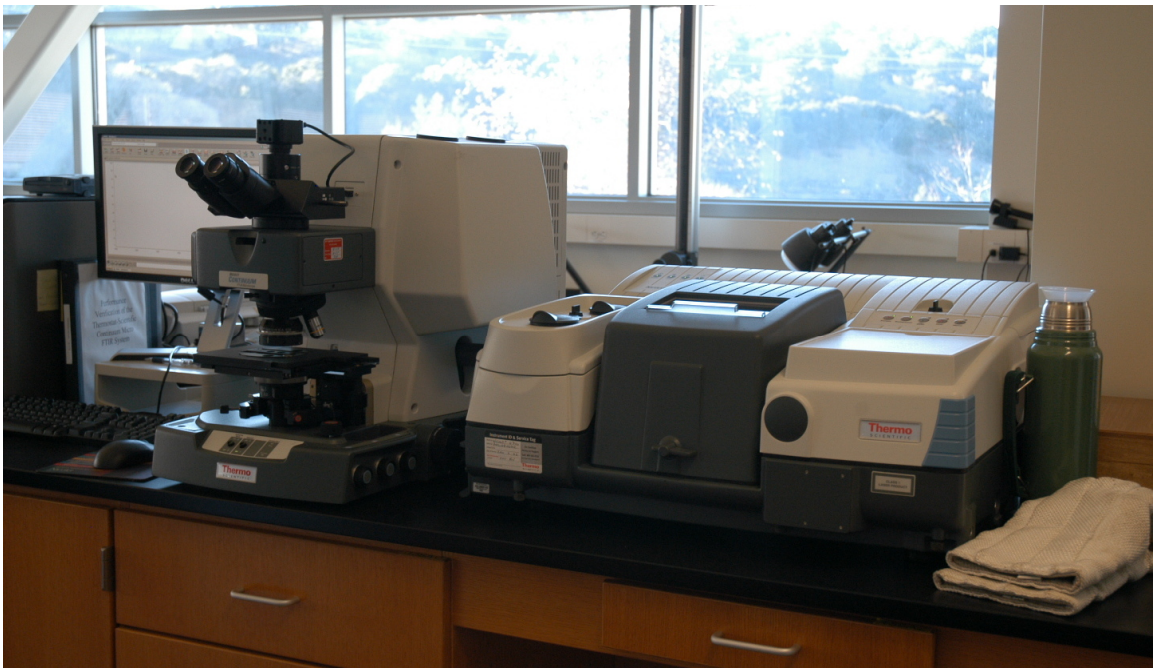

Figure 5. ThermoFisher Scientific Continuum and 6700 Bench Unit.

The upgrade included finer microscope optics and more precise aperture control. Also, the new instrument added the ability to view the sample during FTIR sampling, thus problematic samples could be interrupted and adjusted rather than waiting for all of the scans to be completed.

The aperture sizing in the previous instrument was particularly trouble-ridden, as the manual adjustors had been stripped over years of use and were barely functional.

Control over these apertures is imperative as they direct and limit the infrared light through the area of interest and limit light from absorbing in surrounding areas.

The bench FTIR unit was also upgraded. However, the original FTIR beam splitter and detectors were taken from the older Thermo Nicolet 760 instrument configuration and transferred into the new Thermo 6700 instrument. Thus the sensitivity of the instrument was not expected to be very different. Still, it was believed that these improvements provided easier sample preparation and more optimal collection of data, as well as better quality data due to the improved sampling. Yet this upgrade had minimal effect on our analysis.

Mercury cadmium telluride (MCT) detectors are often used in FTIR analysis and are quite sensitive. In the FTIR bench setup; there is an MCT-A detector, which provides output down to 650 wavenumbers, and an MCT-B detector, which has a 400 wavenumber cutoff. For FTIR microscopy, the MCT-A is used, as it is approximately three times more sensitive than the MCT-B detector. The MCT-A detector must be cooled with liquid nitrogen. However, for applications that require detection in the lower wavenumber range (650-400 cm^{-1}), the MCT-B detector can be used with more scans to compensate for the decreased sensitivity. Due to the small sample size, a less intense optical signal reaches the detector with FTIR microscopy, thus the analysis requires a more sensitive detector. Operation with the MCT-B detector ensures more complete spectra from microscopic chemical samples.

The FTIR microscope can be used in either the reflectance or transmission mode. For transmission alignment, the source IR beam follows a complex path through mirrors and apertures, then though the specimen and condenser, and finally through more mirrors back to the detector. For reflectance alignment, the IR light goes through the same first set of mirrors and aperture and is reflected from the specimen back through the same path to the detector.

Though the setup would seem less complex for reflectance, because IR light is both transmitted and received through the microscope objective lens, less infrared energy can reach the detector. Additionally, the types of samples optimal for reflectance analysis are limited. The sample surface should be flat, and one must consider the infrared absorption of the surface and material below. Lastly, the data usually need manipulation to be compared to FTIR libraries, which are collected in transmittance modes. Thus, transmittance FTIR microscopy is usually the preferred method for data collection and was the method used in most of the experiments done in this study, though a brief series of reflectance experiments were also attempted.

Another difference with FTIR microscopy is that a higher number of scans and higher spectral resolution are desired for the best spectra because the sampling area is so much smaller than conventional FTIR. Also, since the ambient conditions are always fluctuating and the sample area is so small, background samples with an equivalent number of scans are recommended for every sample collection. These requirements together add a significant amount of time to each data collection, but are critical to

obtaining the most precise analysis. For these reasons, the upgrade to the Continuum microscope was very desirable as interrupting faulty analyses could save several minutes.

The increased number of scans in this experimental method theoretically allows for better detection of the materials in question. The signal to noise ratio is proportional to the square root of the number of scans.[1] For example, a spectrum from 100 scans has 10 times better signal to noise compared to one from a single scan.

For these studies, each experiment had a background measurement, and 64 scans were taken for every sample and background sample at a spectral resolution of four wavenumbers. Typical bench FTIR experiments at the laboratory collect 16 or 32 scans at a 16 wavenumber resolution. The spectra were analyzed in the IR light percentage transmitted format, though absorption and transmitted spectra are easily interchangeable with one another by a simple conversion process.

CHAPTER 3. RESULTS AND DISCUSSION

3.1 Initial Experiments

Initial experiments focused on the sample preparation and FTIR microscopy analysis of the modified fused silica capillaries. Analyzed samples included an unmodified bare fused silica capillary, a capillary which has undergone silanization and has 95%+ silica hydrides on the inner diameter, a modified capillary with diol moiety bonded to the silicon atoms, C-18 modified capillaries, and C-18 modified etched capillaries. The etching modified the surface from smooth to jagged which then made the electroosmotic flow more dynamic. Varying the electroosmotic flow allowed for a bigger variety of compounds to be analyzed on the capillary. The goal of the micro-FTIR experiments was to determine if these modifications could be detected with this method of chemical analysis.

First, the polyimide coating, which is typical for capillaries used in electrophoresis, needed to be removed. The coating ensures that the fragile fused silica capillaries are not too easily broken. The capillaries were placed into a 98% sulfuric acid solution. The solution was heated to approximately 100 °C for over 48 hours before the coating began to dissolve.

After the coating had been stripped, the capillaries were frozen with liquid nitrogen. Liquid nitrogen was used to freeze the capillaries to make them rigid and easier to shatter in precise locations. Since the polyimide coating was difficult to remove,

freezing the capillaries ensured that they could be fractured in areas that were clear of the coating.

The stripped capillary sections were crushed into respective petri dishes. A stereomicroscope was used to screen the pieces of capillary for sections that had the ideal shape for analysis. Pieces that were shattered in half in a manner that exposed a sizeable area inside of the capillary were selected for further analysis.

If there was a capillary piece that was bilaterally shattered enough to expose approximately half of the inner diameter of the capillary, it would maximize the area of the modified material and minimize the amount of fused silica for that particular sample. While finding such a piece seems like a fairly simple task, most of the pieces shattered in cross sections. Only a small minority of the pieces were broken in a manner desirable for FTIR microscopy sampling.

Two sections of each type of capillary were selected for FTIR analysis. These were placed on a 1 mm sodium chloride (NaCl) pellet. The sections of capillary were diagrammed on the pellet to track their orientation. These were then sampled on the FTIR microscope using an approximately 10 μm by 20 μm aperture window. Most forensic samples are 10 μm by 10 μm. Because of the linear nature of these capillary samples with minimal width (the modified middle diameter), the longitudinal direction was maximized to ensure that the maximum amount of infrared light passed through the target material.

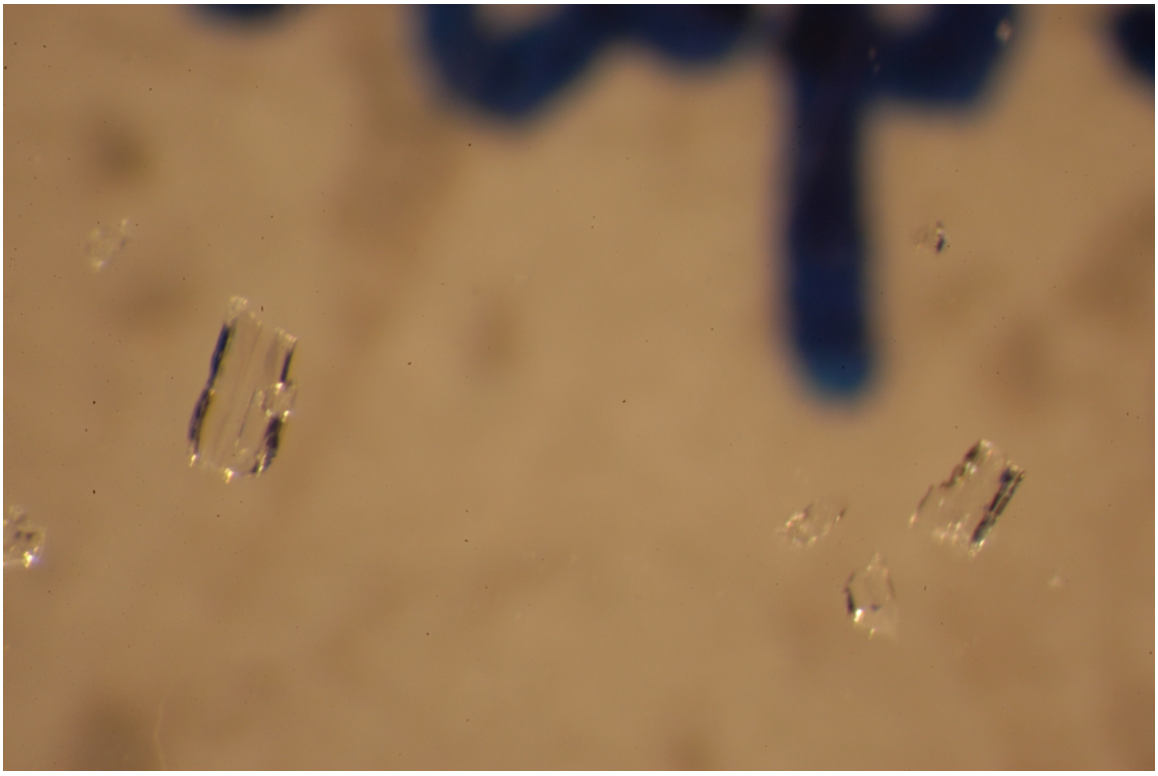

Figure 6. Crushed fused silica modified capillaries at 50× magnification.

As with typical FTIR microscopy analysis, measurements with just the ambient air over the NaCl pellet were taken as a background sample. However, spectra were also collected where the bare capillary was used as the background sample in an attempt to subtract the fused silica. In all cases, no functional groups from the modified capillaries were detected.

In the original NIC-PLAN FTIR microscopy setup, blue light was used to focus the bottom aperture, and green light was used to focus the top aperture. However, with the upgraded Continuum microscope, these different colored lights were not necessary.

The apertures were more easily visualized with aperture shutter panels that were transparent to visible light, just not infrared light. All of the data were collected with 64 scans at a 4 wavenumber resolution, as these are the typical parameters with FTIR microscopy. As seen in Figure 7, the inner diameter width, which has the modified surface, is considerably narrower than the apertures of the microscope.

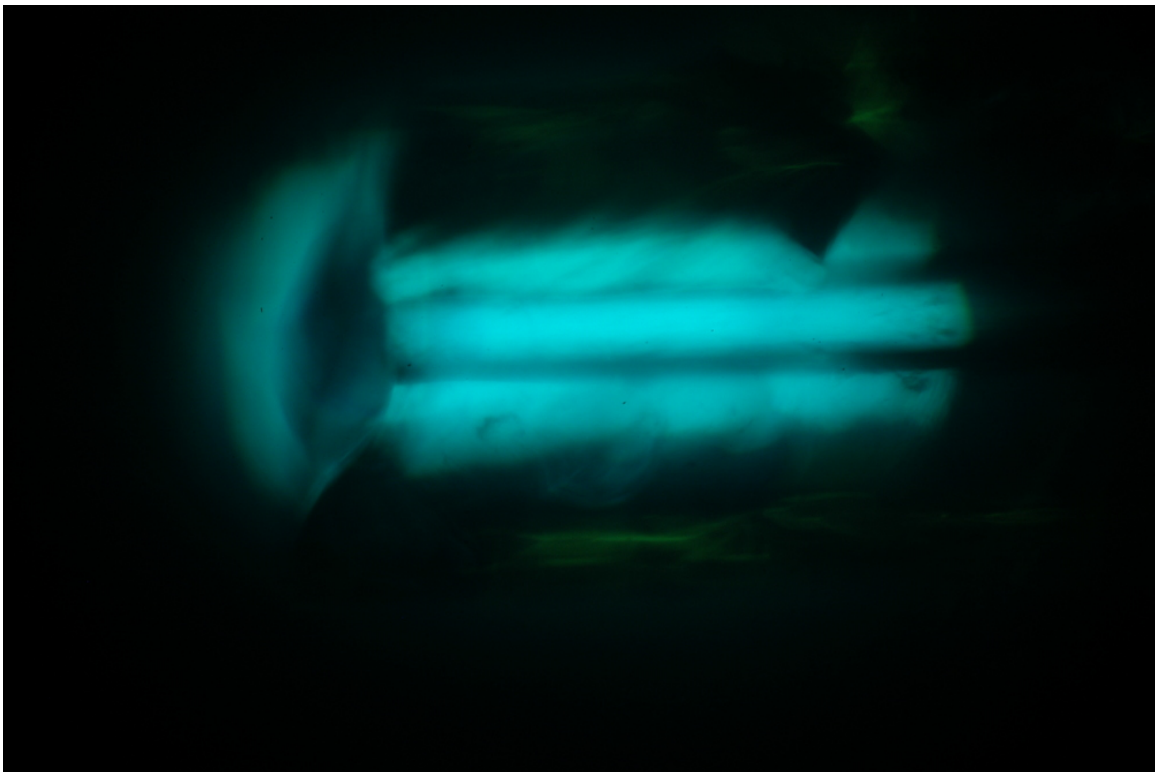

Figure 7. The apertures, focusing lights, and sample on the FTIR microscope.

This size disparity likely caused further masking of any modification to the substrate as the fused silica is strongly IR absorbing. As seen in the spectra below, only fused silica is detected.

Fused silica could be detected with the bare capillary being the background sample. When air was used as the background sample, fused silica was still detected, but it was overly absorbing. However, despite the assumption of a better signal from the modified inner diameter material with a bare capillary background sample, the organic moieties from the diol, C18, and silica hydride modified capillaries were still not detected with FTIR microscopy. In fact, completely negative spectra were seen with the samples that had bare capillary background spectra, apparently from the background and sample material being infrared spectroscopically equivalent. This is illustrated in Figure 8. Though peaks are seen on this negative sample, they are simply noise peaks, and do not contain meaningful data. This result indicated that this approach was not likely to be successful.

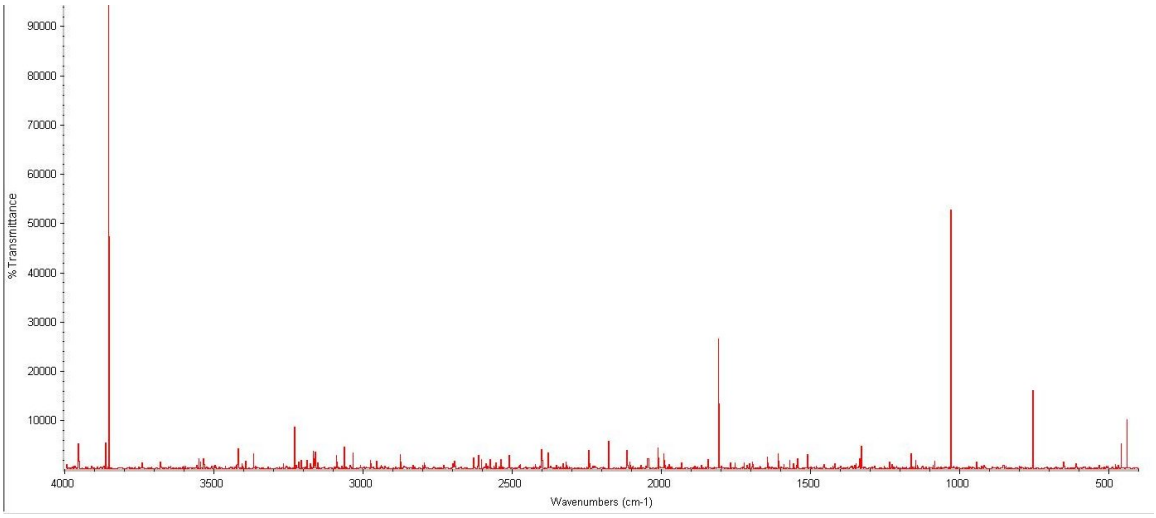

Figure 8. Negative sample due to the background and sample.

The fused silica bare capillary in Figure 9 has a hydroxide absorption peak around 3700 cm^{-1} from the silanols and adsorbed water, as expected.

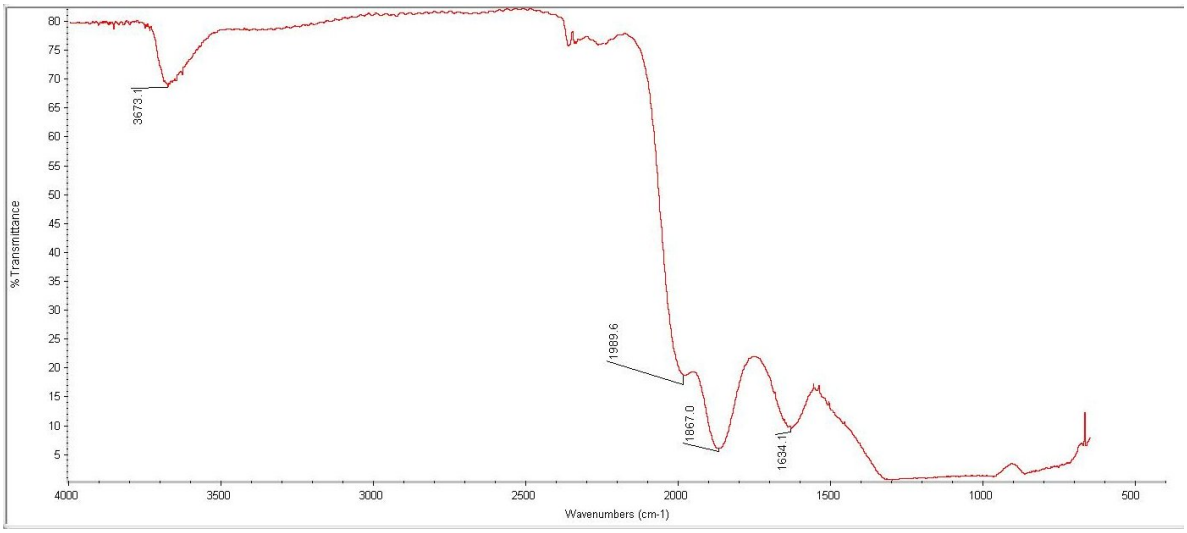

Figure 9. FTIR microscopy spectrum of a bare capillary.

However, the lack of peaks from aliphatic C-H bonds at 2850 cm^{-1} for the diol modified capillary (see Figure 10) demonstrates that fused silica modification is not being detected. An expanded view of the region where these peaks are expected confirms this lack of detection.

At one point, it seemed encouraging that the hydroxyl group was absent in the sample from the silica hydride capillary, however, the IR light absorption for this sample is very uneven, likely due to the subtraction of the background sample, a bare fused silica. Thus, this result was not reproducible.

Subsequent spectra, where the background sample did not completely eliminate any sample signal, were indistinguishable from the bare capillary. However, perhaps

FTIR microscopy was detecting some of the silica hydride at 2250 cm^{-1}, despite the small proportion of these groups compared to silanols.

Likewise, in the spectrum for the C-18 modified capillary (see Figure 12) the silanol peak is absent as in the hydride capillary spectrum (see Figure 11).

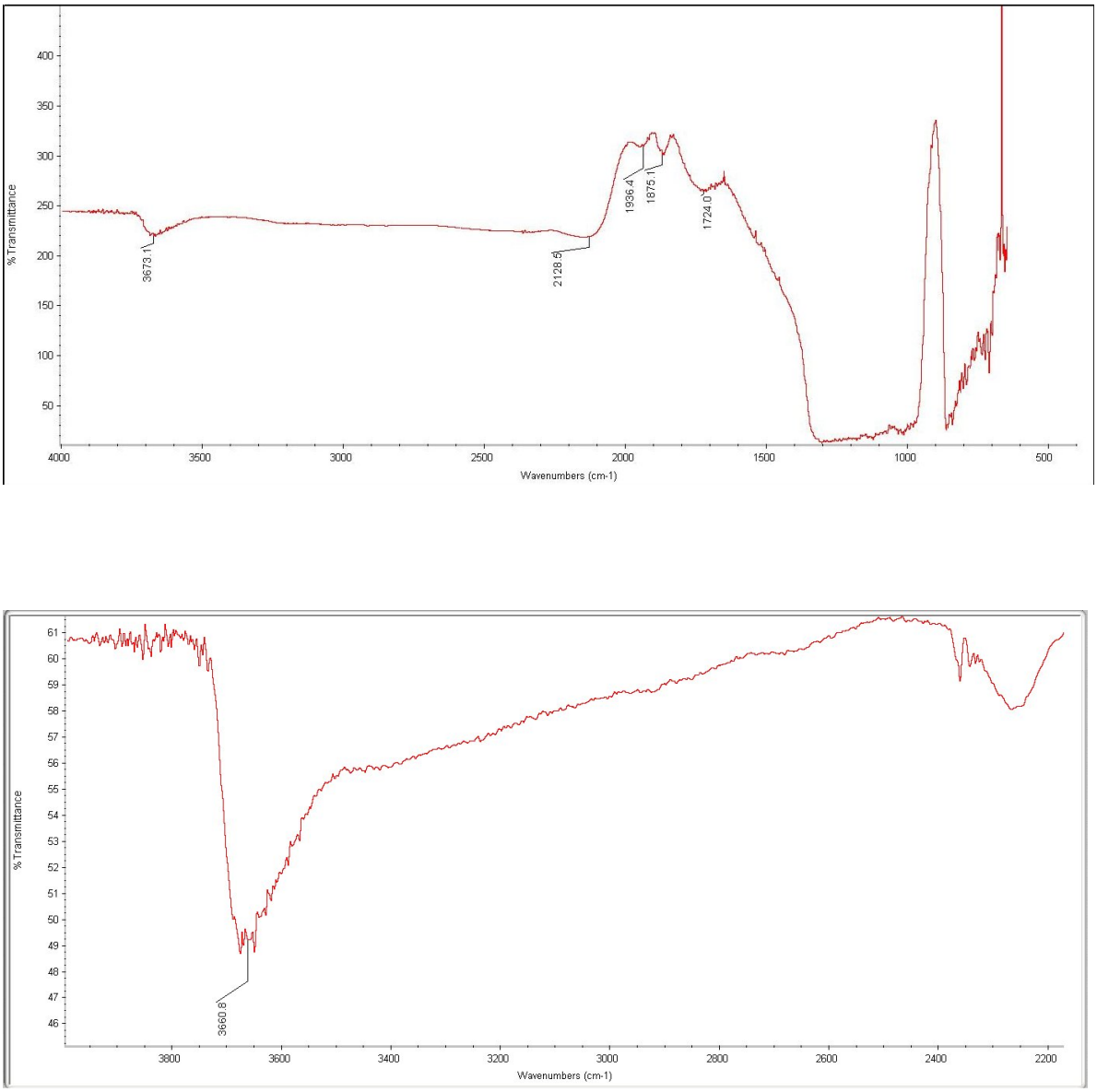

Figure 10. FTIR microscopy spectrum of diol modified capillary and expanded view of where hydrocarbons would be detected.

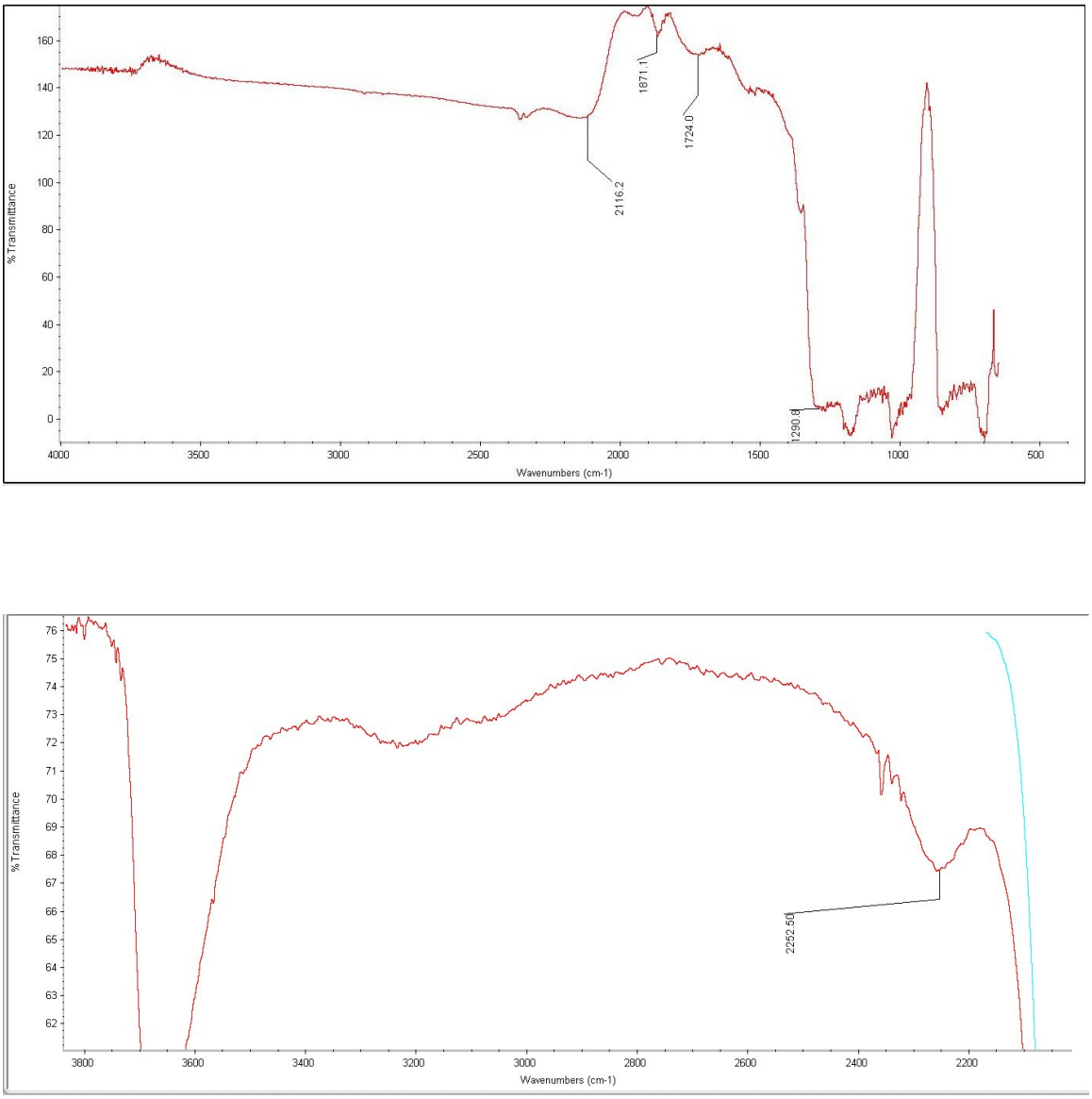

Figure 11. FTIR microscopy spectrum of silica hydride capillary and expanded view of where silica hydrides are expected to be detected.

Once again, the absence of an aliphatic hydrocarbon C-H stretch just below 3000 cm^{-1} indicates the lack of detection of the modifying group by FTIR microscopy.

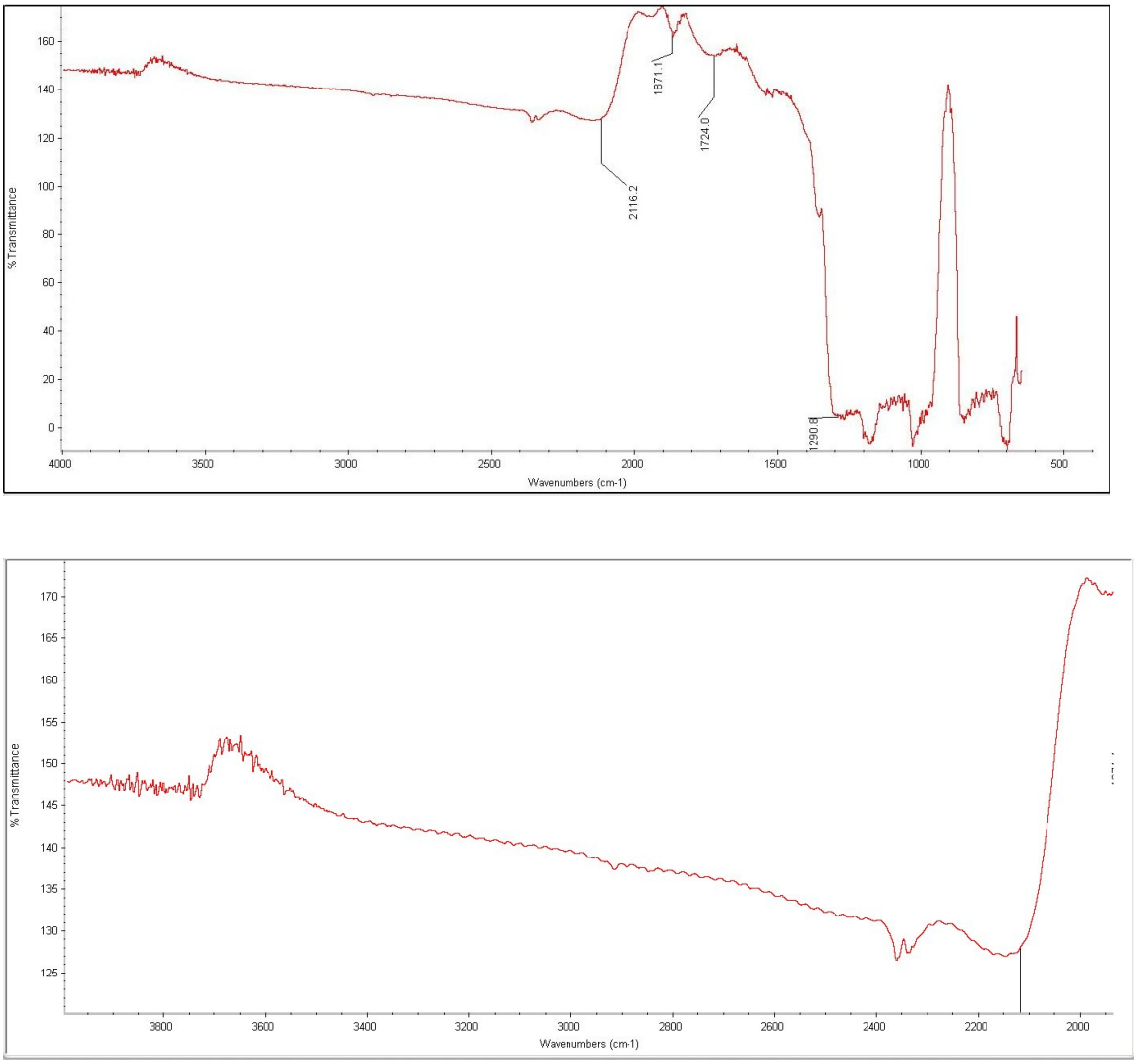

Figure 12. FTIR microscopy spectrum of C-18 capillary and expanded view of where aliphatic hydrocarbons are expected to be detected.

In summary, the results indicate that FTIR microscopy was not successful in detecting the modifying groups from the silanization and hydrosilation reactions of fused silica capillaries. The following table (Table 1) summarizes the results from these experiments.

Table 1. Experiments with various fused silica capillaries

Capillary type	**Substituent (X)**	**Detection with FTIR Microscopy**
bare capillary	-OH	Yes-as expected
silica hydride	-H	No, absence of a 2250 cm^{-1} Si-H stretching band
Diol	$-(CH_2)_6$-CH-(OH) $–CH_2$-(OH)	Not discernably more hydroxyl group absorption compared with than the bare capillary
C18	$-(CH_2)_{17}-CH_3$	No, lack of aliphatic hydrocarbon peaks around 2950 cm^{-1}
C18 etched	$-(CH_2)_{17}-CH_3$	No, lack of aliphatic hydrocarbon peaks around 2950 cm^{-1}

R-X= $(Si-O)_n$-Si-X

3.2 Infrared Microscopy of Raw PMHS Materials

Samples of a copolymer of polymethylhydrosiloxane (PMHS) and polydimethylhydrosiloxane (PDMS) were sampled with the FTIR microscope. As discussed, microfluidic chips consisting of PMHS, though more brittle than chips made of PDMS, offer the possibility of chemical modification to the PMHS surface. Thus, one possibility is to have a PDMS/PMHS copolymer where the strength of PDMS can be used as a support to the PMHS modifiable surface.

A copolymer of these two monomers has been synthesized. Several samples had to be taken as the slices were often too thick and strongly absorbing of infrared light for

FTIR microscopy experiments. A steel FTIR microscopy sample preparation roller further thinned these shavings, and these rolled shavings were successfully profiled with FTIR microscopy.

An overlay of the two polymers is visible in Figure 13. The spectrum for PMHS, in red, can be identified by the presence of the silica hydride peak near 2250 cm^{-1} and the hydrocarbon stretch peaks just below 3000 cm^{-1}. The spectra from different solvent adsorption experiments were compared to the raw PMHS spectrum to contrast any differences from residual solvent (see 3.4).

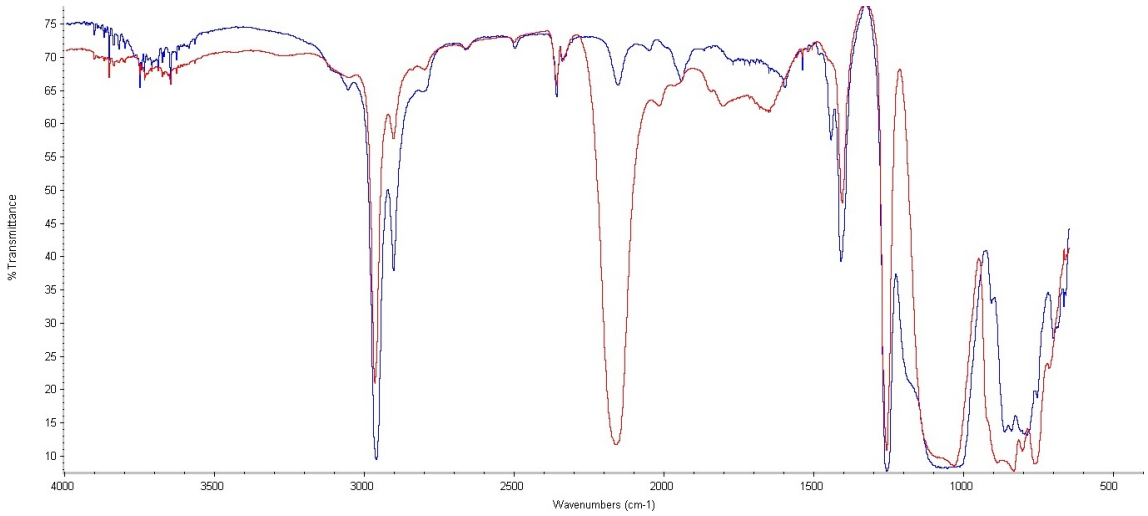

Figure 13. FTIR microscopy spectrum of PMHS and PDMS copolymer.

The spectrum for PDMS, in blue, is characterized by the lack of a prominent silica hydride peak around 2250 cm^{-1} and the increased hydrocarbon stretch below 3000 cm^{-1} from the second methyl group on the silicon.

By contrast, the spectrum for PDMS could be identified by the presence of the silica hydride peak near 2250 cm^{-1} and the aliphatic hydrocarbon stretch peaks just below 3000 cm^{-1}. The spectra from different solvent adsorption experiments were compared to the raw PMHS FTIR microscopy spectrum (Figure 13) to contrast any differences from residual solvent.

3.3 Instrument Upgrade: Thermo-Scientific Continuum and Nicolet 6700 FTIR

As previously mentioned, the NIC-PLAN microscope and Nicolet 760 IR bench unit were somewhat outdated, and their vendor announced that it would be discontinuing support for these instruments. Though the San Mateo County Sheriff's Office Forensic Laboratory had no significant problems with this experimental setup, it was necessary to undertake an upgrade in order to be able to maintain a serviceable FTIR microscope instrument. The FTIR bench unit was also upgraded from the Nicolet 760 to the Nicolet 6700, though the internal IR optics were transferred from the original bench to the new bench unit. Although the instruments are very similar, the new Continuum microscope often provides better data collection of the specimen, as there is better control of the microscope stage, focus, and apertures.

The previously-run modified capillary experiments were repeated on this new instrument to see if the updated equipment produced better results. Very similar results were collected. The fused silica background still dominated the spectra, and the modifying groups were not detected.

In summary, the new instrument did not improve any experimental results. However, as expected, sample preparation and spectra collection were easier due to the instrument upgrade.

3.4 FTIR Microscopy of Wafer Materials

Infrared spectrometry data were collected from copolymer wafers that were investigated for use in microfluidic chips. The copolymer was comprised of PMHS and PMDS fabricated via soft lithography by the Mechanical Engineering Department at San Jose State University in the laboratory of Professor John Lee. These prototype microfluidic chips have test microfluidic channels for the purposes of developing modified channels on the softer PMHS side. The PDMS backing gives greater strength and flexibility, making up for some of the fragility of PMHS.

Absorption experiments were performed on a flake of the PMHS material to measure how well the micro FTIR could detect the presence and evaporation of solvents. Acetone and acetonitrile were placed on the flake of PMHS a few millimeters wide and a few micrometers in thickness. FTIR microscopy spectra were collected at 2 min and 10 min intervals. This was an attempt to detect the solvent in addition to the polymer and, if detected, measure its presence over time while the solvent evaporates.

The presence of the carbonyl stretch from acetone can typically be detected at 1715 cm^{-1}, whereas the nitrile stretch from acetonitrile can usually be observed as a sharp absorption at 2250 cm^{-1}. In the spectra in Figures 14 and 15, peaks are present that indicate the polymer. One peak that is just below 3000 cm^{-1} is suggestive of saturated

carbon hydrogen bonds. The peak around 2165 cm^{-1} can likely be attributed to the silica hydride bond.

Below, in Figure 14, the nitrile peak that one would expect from acetonitrile is nearly masked by the polymer. However, small peaks near 2250 cm^{-1} may be attributed to the nitrile from acetonitrile. Regardless, if some of the solvent was detected, it is difficult to distinguish in the presence of the polymer substrate.

Furthermore, the spectrum indicates that a very thin layer of polymer was sliced for this experiment, as the substrate sample is not over-absorbing as much as many of the polymer FTIR microscopy spectra.

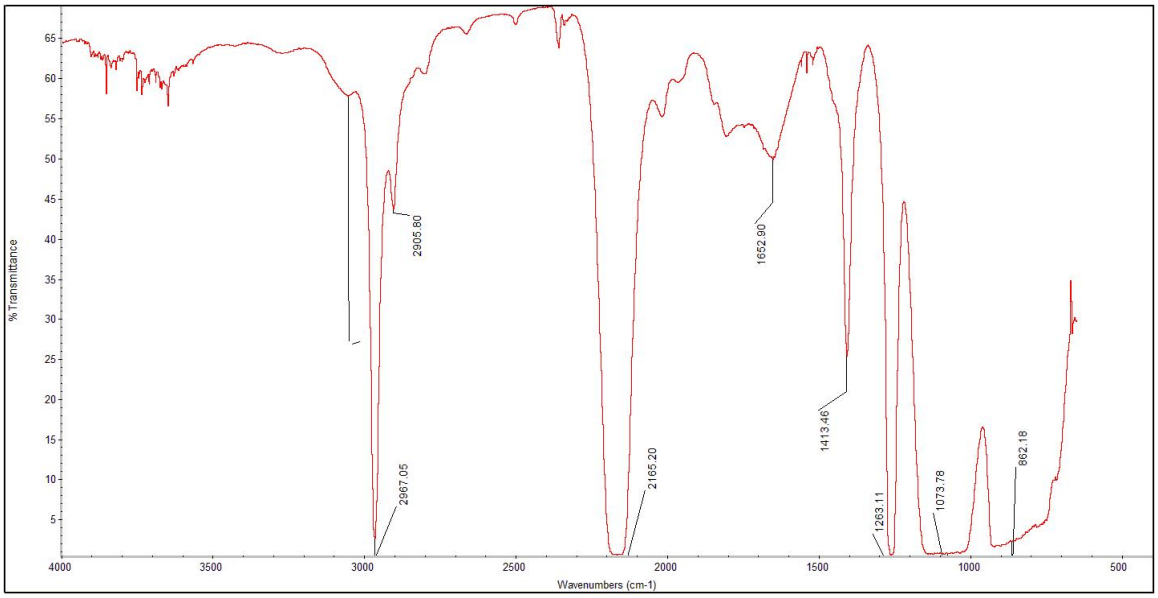

Figure 14. PMHS side of the copolymer with acetonitrile, after 2 min.

A similar sample of PMHS, spotted with acetone, was also analyzed. The spectrum in Figure 15 does not clearly reveal the expected carbonyl peak around 1715

cm^{-1} because the polymer substrate was clearly too thick. This caused several peaks to be off scale. There is a small shoulder to the left of the peak at 1636 cm^{-1}. This may have been due to the presence of acetone. Like the experiment with acetonitrile, it is difficult to be certain that the solvent was detected as the peak size and morphology are affected by the presence of the substrate material. Making polymer flakes small enough for this technique is a challenge, as the FTIR microscope is optimized for microscopic particles, not slabs of polymer.

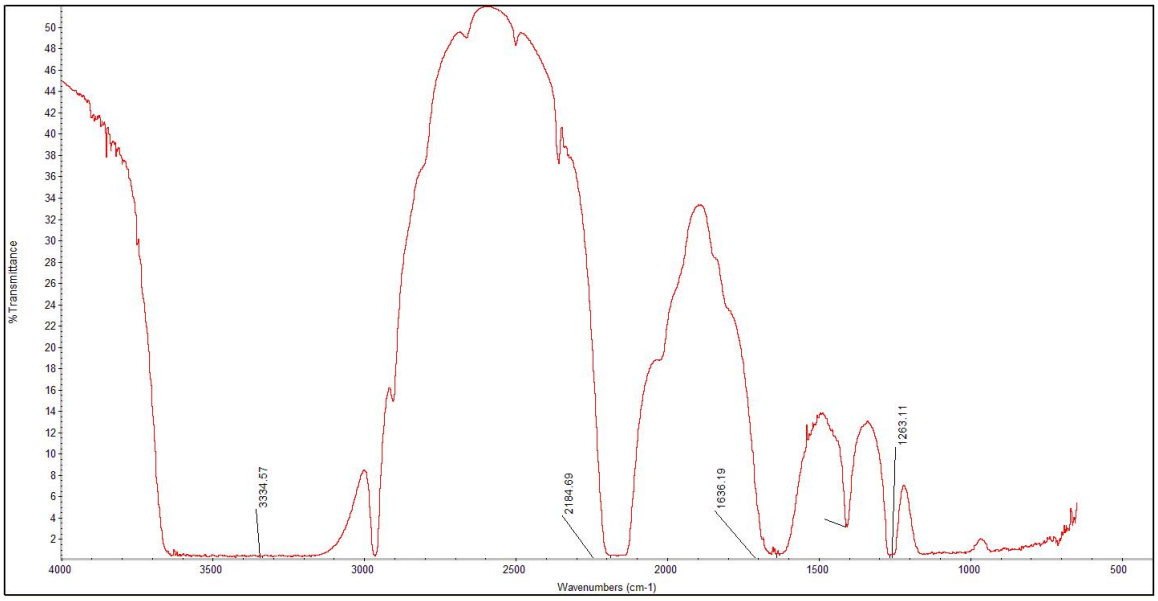

Figure 15. PMHS side of the copolymer with acetone, after 2 min.

However, for the next scan of the same PMHS polymer material with acetone at 10 min (Figure 16), a thinner section of the polymer flake was found. The new spectrum does not have any off-scale peaks. Though a carbonyl peak for acetone is still not readily

observed, like Figure 15, there is a slight shoulder to the 1647 cm^{-1} peak that may be contributed by a carbonyl from acetone.

The absence of definitive peaks indicates that the amount of solvent added, a drop of solvent on a small slice of polymer, was not enough to over come the FTIR signal from the background polymer material. This experiment further adds to the hypothesis that FTIR microscopy does not provide a remarkable way to analyze the modified glass capillaries or future modified microfluidic chips.

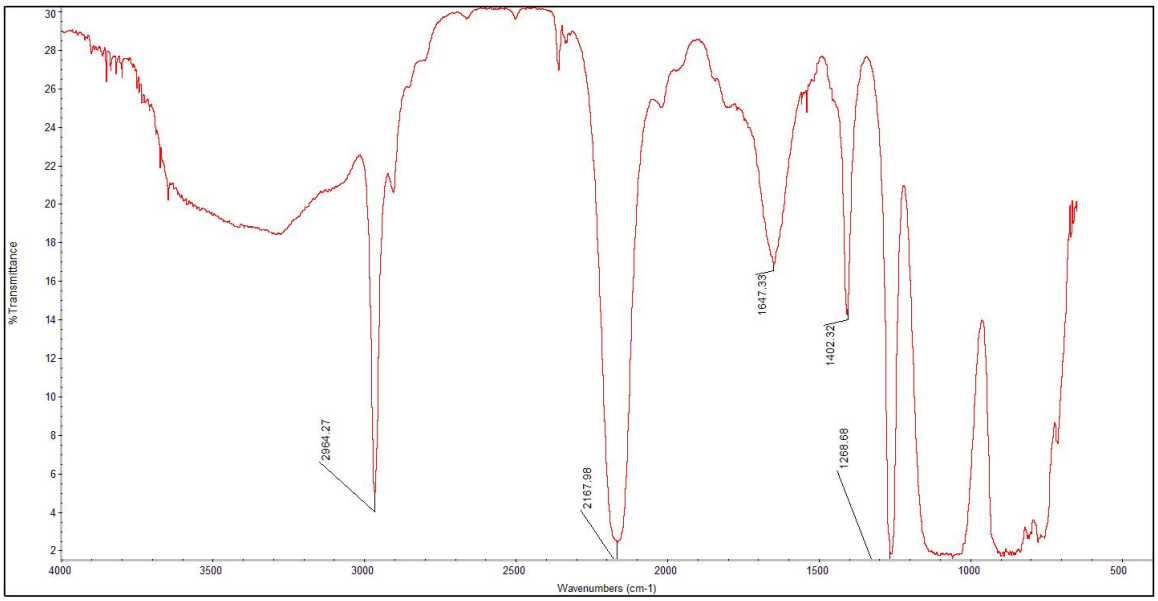

Figure 16. PMHS side of the copolymer with acetone, after 10 min.

Table 2 lists the solvent adsorption experiments and general results. In summary, neither of the solvents was conclusively detected on the thinnest slices of polymer. However, traces of possible functional groups from these solvents may have been present

within two minutes of deposition. Functional groups from the polymer were clearly detected. Thus, one could assume that when the channels are modified in the future, detecting the chemical modifications with FTIR microscopy may not be the most probative method for monitoring such modifications. This is probably due to the polymer microfluidic chip material being very thick compared to the size of the modification, which was thinner than a few drops of a liquid solvent.

Table 2. Wafer adsorption experiment

Polymer	**Adsorption Solvent**	**Detection with FTIR Microscopy**
PMHS A1 2 min	Acetonitrile	Possible very low nitrile peak
PMHS A1 2 min	Acetone	Possible carbonyl peak masked as a shoulder
PMHS A1 10 min	Acetonitrile	No
PMHS A1 10 min	Acetone	Possible carbonyl peak masked as a shoulder

It is also possible that the solvent completely adsorbed to the surface of the polymer. If this happened, it may have occurred in monolayer, much in the way the previously discussed surface modifications purportedly exist. However, peaks from a monolayer would not likely be observed since infrared saturation from the background material is so profound.

3.5 FTIR Microscopy of Modified PMHS Crystals

Raw PMHS crystals, previously reacted with 1-pentyne and cholestryl undecanoate, were available for testing. These represent some of the types of modified PMHS that could be useful in the microchannels of microfluidic chips. Engineering

limits of PMHS preclude manufacture of such modified channels at this point, but research is ongoing to try to overcome this mechanical obstacle.

FTIR microscopy of the modified raw material is likely to detect peaks from the modifying substituent. These samples are comprised of raw material PMHS uniformly hydrosilated on the surface with an organic moiety. Because of the more uniformly reacted surface, it is expected that functional groups from the modifying chemical should be more easily detected with FTIR microscopy, compared to the functional groups from the solvents deposited in the solvent adsorption experiments.

For example, in the solvent adsorption experiments, the goal was to detect one compound, acetone or acetonitrile, on thin slices of PMHS. However, in the raw PMHS crystal experiments, the organic moiety is hypothetically more heterogeneously attached to all the exposed surfaces of PMHS. Thus, it is expected that FTIR microscopy could detect the modifying chemical, 1-pentyne or cholestryl undeconoate.

The hypothetically detectable functional group for 1-pentyne would be the resulting alkene bond after hydrosilation. The resulting pentene group should be characterized by a sharp C-H stretch, just above 3000 cm^{-1}. A carbonyl stretch at approximately 1735 cm^{-1} should be present for the PMHS modified with cholestryl undeconoate.

Sampling of these raw materials with FTIR microscopy was mechanically more difficult than expected. Some of the PMHS modified with 1-pentyne was uncrushed resulting in coarse yellow crystals. The thinnest crystals were selected for FTIR

microscopy, but even these over-absorbed the IR light. After an attempt to crush them with mortar and pestle, the resulting crystals were also too IR absorbing. Another sample of PMHS modified with 1-pentyne was in a fine white powder which was PMHS. This spectrum (Figure 17) was not as over-absorbing as the previous scans.

The reflectance mode (see section 3.6) FTIR microscopy spectrum in Figure 17 is of PMHS modified with 1-pentyne crushed into a fine white power. While it is not an ideal spectrum, some usable data are present. The peak at 3062 cm^{-1} indicates the presence of the alkene, whereas the peaks around 2965 cm^{-1} are characteristic of alkanes. These peaks, along with the peak at 2182 cm^{-1} within the region where silica hydride is detected, are consistent with 1-pentyne hydrosilated on PMHS polymer.

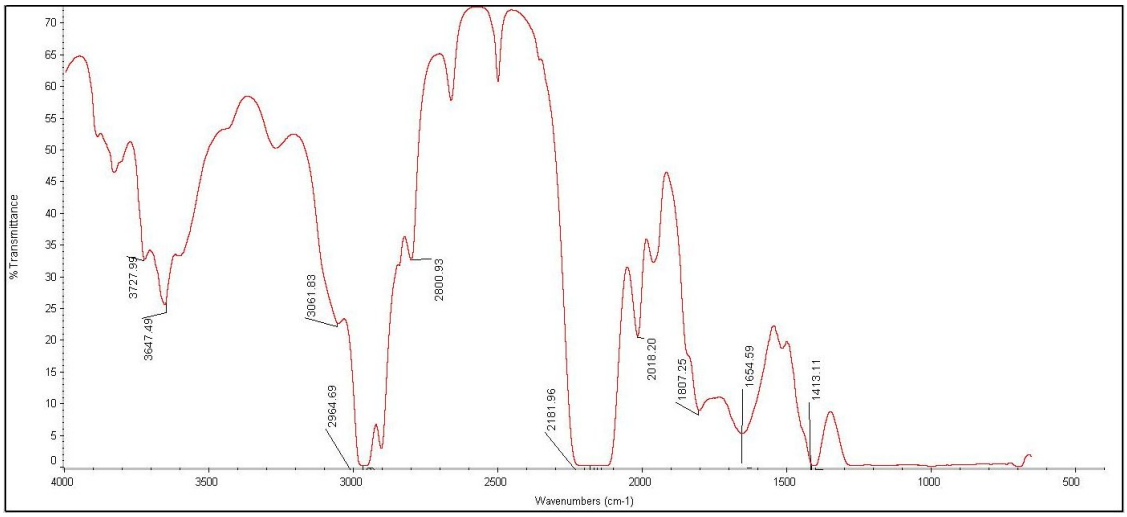

Figure 17. Reflectance mode spectra of PMHS modified with 1-pentyne.

FTIR microscopy analysis confirms that these PMHS crystals were modified. However, this is a fairly predictable result that provides information readily available with regular bench FTIR.

3.6 Reflectance Experiments with Micro FTIR Spectrometry

Due to the typically very small sample size, FTIR microscopy spectra are traditionally collected in the transmittance mode. The infrared light is detected on the opposite side from which it is emitted. However, the reflectance mode is also available on microscope attachments, where infrared light is bounced and scattered off of the sample and detected from the same path from which it was emitted.

Since the substituent of interest was on the surface of the sample, an attempt was made to collect similar spectra of the modified PMHS samples. However, no added information was gathered, and the samples were still overloaded with substrate chemical information.

This is not surprising as only a narrow selection of sample types are ideal for reflectance FTIR microscopy. Opaque samples having an IR-absorbing surface component that otherwise could not be analyzed with transmitted light are needed. However, these experiments did show that the attempts to improve results with the reflectance mode were unsuccessful. The reflectance experiments were a necessary part of exhausting the different possibilities of FTIR microscopy of the preceding types of samples.

CHAPTER 4. CONCLUSIONS

In general, testing for modified substrates with the previously described micro-analytical techniques was not successful. Several experiments confirmed that the substrate material, a polymer, which is often transparent and highly infrared light absorbing, exhibited IR absorption that was too great to be subtracted from samples with the substrate material plus a small amount of modifying chemical substituent group or solvent.

Previous research of organic monolayers details the limits of FTIR spectroscopy when profiling chemical interfaces nanometers in thickness. FTIR profiling of organic monolayers is a relatively new field compared to the analysis of highly crystallized inorganic monolayers.[9]

Infrared external reflection spectroscopy (IR-ERS) can characterize thin films.[9] Plane polarized light at different angles of incidence can be compared to quantitatively measure film thickness. Polarized infrared external reflectance spectroscopy (PIERS) has been used to detect methylene chain monolayers successfully due to the highly crystalized formation and number of methylene units.[10] Crystalized monolayers are denser and more likely detectable than non-crystallized layers. However, spatial limitations of the samples examined in this work limit the ability of the monolayer to crystalize. This limitation may further hinder the detection of infrared spectroscopy for these experiments.

An effort to calculate extinction coefficients (see Table 3) for compounds representing the organic moieties examined, as well as for the solvents examined on PMHS, was made using infrared spectra of known concentration and pathlength.[11] Noise from this instrument ranges on the magnitude of 10^{-4} to 10^{-5}. Thus, the calculated expected absorbance values (see Table 3) are mostly within the range for noise. These calculations corroborate the results observed, which indicate a lack of detection of the modifying organic moiety.

Table 3. Calculated extinction coefficients of compounds to estimate expected FTIR absorbance values

Compound	**Extinction Coefficient ϵ_{cm-1} (L/mol x m)**	**Examined item**	**Estimated Absorbance[b]**
2-decanol[a]	$\epsilon_{2850} \approx 25000$	Diol modified capillary	$A_{2850} \approx 2.6 \times 10^{-4}$
Undecane	$\epsilon_{2850} \approx 7220$	C18 modified capillary	$A_{2850} \approx 1.6 \times 10^{-4}$
Trichlorosilane[a]	$\epsilon_{2250} \approx 4955$	Silica hydride capillary	$A_{2250} \approx 7.4 \times 10^{-4}$
Acetone	$\epsilon_{1725} \approx 76200$	Acetone on PMHS	$A_{1725} \approx 4.1 \times 10^{-4}$
Acetonitrile	$\epsilon_{2300} \approx 25000$	Acetonitrile on PMHS	$A_{2300} \approx 3.6 \times 10^{-6}$

[a] Organic moiety ϵ estimated with similar compound with functional group of interest

[b] Assumed monolayer pathlength by summing bond lengths of moiety or solvent; estimated concentration of 10 M for the diol, undecane, and silica hydride

The effective monolayers prepared by modifying the surface of the capillary and polymer are no more than a few nanometers thick, depending on the moiety. This data also suggests that if any peaks were detected from the acetone and acetonitrile there was a much thicker film than a monolayer.

Data collection with air and air background versus unmodified substrate material showed better spectra for the latter, but no difference between the modified material and the unchanged substrate was detected. This result is likely due to the overwhelming

absorption of the substrate material: fused silica for the capillaries and PMHS in the case of the microfluidic raw material. The spectrum can be improved by using bare capillary as a background rather than air, but the improvements are likely due to the subtraction of the strongly absorbing polymer or fused silica, not because the modifying groups are being detected better. However, this method, as demonstrated in some cases where the substrate absorption amounts were nearly equal, may produce a negative spectrum which was not possible to interpret.

The failure to detect the modifying groups was not entirely unexpected with FTIR microscopy analysis. However, the hypothesis that targeting areas of 10 μm by 10 μm would give a precise enough area of the modifying group that the FTIR signal would be detected was worth testing. FTIR analysis (DRIFT) has been successful in characterizing raw materials of the hydrosilated chemical products, as the products are fairly pure with identifiable functional groups. FTIR microscopy did not improve on the results producible by DRIFT analysis.

Ideally, ready-made PMHS chips with hydrosilation-modified channels would have been analyzed with FTIR microscopy for these experiments. However, the mechanics for making such chips on this polymer alternative have proved more difficult than expected. Thus, fully fabricated microfluidic chips with modified channels could not be tested.

It was also theorized that perhaps the upgraded instrumentation would improve the results. Yet when previous experiments were duplicated, the spectra had no

discernable differences from the original spectra. Thus, outdated instrumentation was ruled out as a reasonable cause for the observed difficulty in detection.

In order to simulate the ability of FTIR microscopy to detect modifications to the polymer, as would have been done had the chips been available, acetone and acetonitrile were deposited onto the polymer. Solvent adsorption experiments and these samples were analyzed with FTIR microscopy for over ten minutes to monitor the presence of the solvent on the polymer.

Spectra when the solvent was present were compared to spectra of the same areas once the solvent had evaporated. The FTIR microscopy technique is ideal for very thin slices of infrared-absorbing materials, such as fine particles like paint chips (which are further sampled and sliced to be microns thick) and single synthetic fibers. The partial capillary sections and the PMHS substrates were simply too thick for this analysis. Worse still, the modified surface or organic solvent was too thin or too small proportionally to be conclusively detected in these experiments.

Finally, modified raw material PMHS that had undergone hydrosilation with an appropriate organic moiety was also tested. Not surprisingly, the expected functional group could be detected. However, this is a significant modification of the original goal, detecting modified microchannels, so its success does not validate FTIR microscopy for the profiling of modified microchannels.

CHAPTER 5. FUTURE WORK

The results of these analyses were generally characterized by the absence of detection of the modifying group or adsorbing solvent. The data reveal reasonable explanations for these results, primarily the large ratio of IR-absorbing background material compared to the modifying groups of the capillaries or the functional groups of the solvents.

The background problem seems quite difficult to overcome for this type of FTIR analysis. One possible improvement is to attach some kind of more heavily IR-absorbing group to the modifying moiety. This group could be something with a large IR absorbance in an area where we otherwise would not expect to see a peak.

Augmenting the modifying moiety may be more straightforward for some of the larger modifying groups *(e.g.,* C18, cholestryl undecanoate) and less with small groups where the chemistry of the modifying group would likely change with the addition of another group (*e.g.*, hydride; smaller alkynes). In all cases, this addition would likely have some effect on the chemical separation properties of the capillary or channel and thus may not be the best course for future experimentation.

There may also exist other instrumentation that would better and more efficiently profile these materials and the corresponding modifications. Scanning electron microscopy or laser ablation MS are two methods that might be successful. But again, they would probably work better on some substituents than others. A long alkane chain

such as C18 or the diol chain would be easier to detect with SEM compared to silica hydrides or smaller alkynes because the former groups may have more elemental information similar to the starting materials. The monitoring of adding of such substituents on such small channels remains a difficult problem to solve. FTIR would have been preferable over some of the proposed methods due to its non-destructive and non-consuming nature. Of course, FTIR is also desirable because of the somewhat easy classification of the products by their characterizing functional groups.

Perhaps some profiling mass spectrometer experimental configurations may be amenable to detecting the modified group. Previously, elemental analysis has been used to detect C18 modified PMHS.[2] With the advent of mass spectrometer setups that do not require the sample to be under vacuum, more types of samples may be examined with instrumentation that reveals elemental composition.

Unfortunately without some of these drastic changes, it is still a challenge to detect modified capillaries and, presumably, future modified microfluidic chips. One possible way to ensure that the modified PMHS lines the channels of microfluidic chips would be to make the entire chip out of the modified PMHS material. Conceivably this could be done by modifying the monomer prior to polymerization.

Unmodified PMHS is already a less desirable material to fabricate microfluidic chips due to its brittle nature compared to the more robust PDMS. Thus making microfluidic chips out of PMHS reacted with, for example, 1-pentyne, may present many more engineering problems than already exist with the challenging material of PMHS. It

is doubtful that modified PMHS would have improved mechanical properties for microfluidic chip fabrication compared to the problematic PMHS. Still, finding a way to fabricate the chips this way may ensure that the properties of the microfluidic channels are what the user expects them to be.

Furthermore, FTIR microscopy of these hydrosilation products at least confirms that the hydrosilation reaction is successful. Thus, if the reaction can occur directly on the PMHS microchannel, there is strong reason to believe that a modified channel would result.

Lastly, due to the limitations of sensitivity of spectroscopy, and the minimal amount of material trying to be detected, ATR FTIR may be worth further exploring forth this type of experiment. With an ATR microscope objective lens, ATR FTIR microscopy may improve sensitivity, resulting in successful detection of the modified surface of both capillaries, and perhaps modified microfluidic channels.

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